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The Development of Plant Conservation in Botanic Gardens and the Current and Future Role of Conservation Genetics for Enhancing Those Conservation Efforts

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Botanic gardens play major roles in plant conservation globally. Since the 1980s, the number of botanic gardens worldwide and their involvement in integrating ex situ and in situ plant conservation has increased significantly, with a growing focus on understanding, documenting, and capturing genetic diversity in their living collections. This article outlines why genetic diversity is important for conservation, and explores how botanic gardens can establish and expand the use of molecular techniques to support their plant conservation efforts.

Keywords: Botanic Gardens; Conservation; Plants; Genetics; Living Collections.

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THE ORIGINS AND DISTRIBUTION OF BOTANIC GARDENS WORLDWIDE

Over the last few decades, the number of botanic gardens and their activities have grown remarkably worldwide. Depending on where and when they were developed, the history and mission of these gardens varies widely. The first botanic gardens of the modern era were established in Europe and were often associated with universities, the earliest of which were "physic gardens" created for the purposes of teaching medicinal plants to medical students. Over the centuries, many botanic gardens were created to serve as public gardens in which the collections were labeled for public education and enjoyment. The traditional research role of these gardens, particularly in Europe and North America, was associated with plant taxonomy, namely discovering, researching, and describing plant species. In the tropical world, the first botanic gardens were created to support the expansion of tropical agriculture, playing a role in introducing and establishing some of the crops that dominated colonial agriculture in tropical countries throughout the world. In the countries that constituted the former Soviet Union, many botanic gardens were established to assist in introducing a wide variety of plant species that could be of value for commercial development. In other countries, such as the United States and New Zealand, many of the earliest botanic gardens were established as public gardens for public enjoyment, and subsequently became "botanic" gardens as their collections and roles expanded[1].

It is therefore difficult to define closely what is a "botanic garden," since they arise from a great diversity of origins and encapsulate many different functions. The most widely used definition of a botanic garden is that adopted by Botanic Gardens Conservation International's (BGCI) in its *International Agenda for Botanic Gardens in Conservation*[2,3]. BGCI

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suggests this definition encompasses the spirit of a true botanic garden:

> *Botanic gardens are institutions holding documented collections of living plants for the purposes of scientific research, conservation, display and education.*

Within the context of this article, we will then use the term "botanic garden(s)" to refer also to arboreta and other specialized plant collections. The term botanical garden(s) is also used synonymously and both are correct.

An early definition of a botanic garden given by the International Association of Botanic Gardens (IABG) in 1963 was ". . . open to the public and in which the plants are labeled" (cited in[2]). However, this definition fails to recognize the complexities of these institutions.

Up to the 1980s, around 800 botanic gardens were known worldwide. These are documented in a series of International Directories of Botanical Gardens[4–7]. In the 1983 edition, 798 botanic garden entries were recorded. The 1990 edition[7] recorded over 1,400 botanic gardens and arboreta. Since then, the number of botanic gardens (many of them newly established) has continued to grow throughout the world; today there are over 3,000 known botanic gardens. The most comprehensive source of botanic gardens and their distribution is provided by BGCI through its "Garden Search" database (https://www.bgci.org/garden_search.php) which currently holds information on over 3,571 botanical institutions worldwide.

The distribution of these botanic gardens is not uniform worldwide, for the most part being inversely related to the richness of the native floras of the countries and regions in which they occur. Thus, tropical regions have few botanic gardens compared with Europe, North America, and the countries of the former Soviet Union, where they have a longer history as part of cultural and scientific traditions. Nevertheless, in some developing countries, the growth in the number of botanic gardens has been dramatic, for example, in Brazil, the number of botanic gardens has increased greatly from 1938 until 2013 (Table 1).

Table 1. Number of botanic gardens in Brazil over the past 80 years.

Year	Number of botanic gardens in Brazil
1938	3
1969	4
1990	11
2000	26
2001	29
2013	36

Sources: Howard, R., Wagenknecht, B.L. & Green, P.S. International directory of botanical gardens. In *Regnum Vegetabile*, first ed., vol. 28 (The International Bureau for Plant Taxonomy and Nomenclature, Utrecht, Netherlands, 1963); Henderson, D.M. & Prentice, H.T. International directory of botanical gardens. In *Regnum Vegetabile*, third ed., vol. 95 (Bohn, Scheltema & Holkema, Utrecht, Netherlands, 1977); Henderson, D.M. *International Directory of Botanical Gardens*, fourth ed. (Koeltz Scientific Books, Koenigstein, Germany, 1983); Heywood, C.A., Heywood, V.H. & Wyse Jackson, P. *International Directory of Botanical Gardens* (Koeltz Scientific Books, Koenigstein, Germany, 1990); Bruni, S. *et al. Directory of the Botanical Gardens of Brazil* (The Brazilian Network of Botanic Gardens Expressao e Cultura, Rio de Janeiro, Brazil, 2000)[8].

THE EVOLUTION OF THE ROLE OF BOTANIC GARDENS IN SOCIETY AND THEIR INCREASING INVOLVEMENT IN CONSERVATION

Prior to the 1980s, the focus of most botanic gardens in operation at that time could be defined primarily as (1) serving as a public amenity, for relaxation and recreation, with limited public educational activities, (2) science and research, particularly in taxonomy, and (3) living collections management, primarily focused on the cultivation of exotic plants. However, since the 1980s, there has been a remarkable renaissance in botanic gardens and they have taken on significantly broader roles in many areas of scientific, horticultural, and educational endeavor, including the following:

- Science and research in taxonomy, genetics, conservation biology, and other disciplines
- Living collections management, including seed and tissue storage
- Greater emphasis on planned collections (accession policies) and more natives grown
- Cultivation for biodiversity conservation (ex situ and in situ; see Box 1)
- Environmental protection and promoting sustainability
- New activities in the management of plants in the wild and in a variety of natural habitats, including ecological restoration, species recovery, and ecosystem services.
- Major programs in public education and environmental awareness
- Strengthened linkages with local communities
- Socioeconomic roles, including social inclusion

It is important to understand the main recent drivers of botanic garden development worldwide. The growth in the environmental movement throughout the world has had a profound impact, with many new botanic gardens needed to address environmental issues, particularly in education, environmental awareness, and biodiversity conservation. Many existing gardens, both public and private, have been converted to become "botanic" for various reasons. There has also been an increasing recognition that botanic gardens are key assets for all countries and most major cities. Many local botanic gardens have been created to support a variety of community needs.

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Box 1. Defining ex situ conservation and its importance.

Ex situ conservation is defined by the Convention on Biological Diversity (CBD) as "the conservation of the components of biological diversity outside their natural habitats." Ex situ conservation of plants is an important technique that has been widely applied to the conservation of biodiversity. It includes the cultivation of living plants in collections, as well as the storage of seeds, spores, tissues, and other propagules in various forms of storage facilities (e.g., seed banks and cryopreservation facilities). The CBD recognized ex situ as an important means for the conservation of the components of biodiversity and urges contracting parties to the Convention (mainly countries) to

*"Adopt measures for the ex situ conservation of the components of biological diversity, preferably in the country of origin of such components;
*Establish and maintain facilities for ex situ conservation of and research on plants, animals and micro-organisms, preferably in the country of origin of genetic resources."

The following are a number of the identified priorities to enhance the practice of botanic gardens in conservation (including in situ and ex situ conservation):

- Better focused and planned approach to ex situ and in situ conservation
- Develop and implement more institutional ex situ and in situ conservation programs
- Collaborate as part of coordinated network approach
- Identify and fill gaps (i.e., to target species that are not currently conserved)
- Research in conservation biology to understand conservation pressures and declines
- Integration of species conservation and ecological restoration
- Understand and manage the basis of genetic diversity at species and population levels
- Enhanced data management on ex situ conservation collections
- Promote more effective and efficient data sharing, to help coordination and achievement of priorities
- Support for ex situ conservation in regions where limited progress has been made thus far

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However, perhaps more than any other factor, the development of botanic gardens has been inspired by a growing recognition of the increasing threat to plant species and their diversity, which is of urgent concern. Many plant species, communities, and their ecological interactions, including the many relationships between plant species and human communities and cultures, are in danger of extinction, threatened by such human-induced factors as climate change, habitat loss and transformation, overexploitation, alien invasive species, pollution, unsustainable agriculture, and other developments (see Box 2). The initiation of conservation programs in botanic gardens began largely based on the recognition that they could be instrumental in leading efforts to prevent the extinction of threatened plant species.

In 1986, a new organization was established by IUCN (the International Union for the Conservation of Nature), which subsequently became independent in the 1990s as BGCI. It has grown to become the network body for botanic gardens. BGCI has greatly influenced and supported the practice of plant conservation through botanic gardens, inspiring the creation or development of many new botanic gardens as well as their roles and activities in plant conservation. In 1999, BGCI prepared and published the International Agenda for Botanic Gardens in Conservation[3,9] now endorsed by hundreds of botanic gardens worldwide. It provides important policy guidance and a framework to guide botanic garden actions in the conservation and sustainable use of plant diversity.

The role of botanic gardens in plant conservation received a further boost with the development and adoption of the Global Strategy for Plant Conservation (GSPC) by the U.N. Convention on Biological Diversity in 2002 (Fig. 1), in which the specific role of botanic gardens in plant conservation was recognized and encouraged (https://www.cbd.int/gspc/strategy.shtml). Indeed, botanic gardens subsequently became key stakeholders, helping to implement the objectives of the strategy and achieve its 16 targets.

The rationale for the GSPC is that plants are a vital component of the world's biological diversity and an essential resource for the planet. It further points out that in addition to the cultivated plant species used for food, timber, and fibers, many wild plants have great economic and cultural importance and potential as future crops and commodities, and even more so as humanity grapples with the emerging challenges of environmental and climate change. Plants play a key role in maintaining the planet's basic environmental balance and ecosystem stability and provide an irreplaceable component of the habitats for the world's animal life[10].

In considering the roles of botanic gardens in plant conservation, it is important to stress the diverse and closely

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Box 2. Major threats to plant species and their habitats.

- Human population growth—population pressure and migrations, urbanization, residential developments, roads, off-road vehicles, changes in land tenure
- Unsustainable agriculture—including unsustainable aspects of agricultural development, involving cash crops, plantations, intensive cattle ranching, overgrazing, slash and burn, and shifting cultivation
- Climate change
- Deforestation—including logging and plantation forestry
- Unsustainable overcollecting—of medicinals, ornamentals, nontimber forest products, fuelwood, charcoal production, resin tapping, and so on.
- Tourism and recreation—including resort and skiing developments, golf courses and leisure activities
- Natural disasters—volcanic eruptions, typhoons, and hurricanes
- Fire, when not a fire-maintained ecosystem
- Mining—mining, mining exploration, oil pipelines, quarrying
- Industrial developments—including waste disposal and pollution
- Invasive species—plant competitors, herbivores, pathogens
- Dams/hydroelectric developments
- Political conflicts—including military operations
- Ecological/biological threats—fragmentation and small plant population inviability
- Salinization and desertification—including soil erosion

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integrated approaches of botanic gardens to conservation. Botanic gardens recognize the importance of applying an integrated approach to their activities in plant conservation by undertaking ex situ conservation based on the best available scientific principles and guidelines, by utilizing germplasm storage methodologies where appropriate, and by supporting the in situ conservation of plant diversity. Today, most botanic gardens highlight conservation as a fundamental part of their mission, and in some cases, the most important part of their mission. For example, the mission of the Missouri Botanical Garden (MBG) is to "To discover and share knowledge about plants and their environment, in order to preserve and enrich life." In MBG's current Strategic Plan (2016–2020) (Fig. 2), the importance of plant conservation is highlighted as follows: By advancing plant conservation and biodiversity, the Missouri Botanical Garden will materially contribute to global human wellbeing."

As an example, some of MBG's conservation priorities defined in this Strategic Plan, which are similar to the conservation priorities of many major botanic gardens worldwide, include

- Maintain and enhance the Garden's leadership role in the biodiversity community through scientific research, conservation, horticulture, economic botany, ecological restoration, and education and link priorities to the goals of the U.N. Sustainable Development Agenda.
- Influence policies at all levels that affect plant science, research, horticulture, and conservation.
- Enhance and maintain world-class living plant collections to increase support for conservation, research, and education.
- Enhance leadership of global policy and research initiatives that are critical to achieving the world's plant conservation efforts.
- Offer continued leadership to the World Flora Online (WFO) initiative and ensure that the WFO becomes an effective means to deliver the results of the Garden's plant systematics research.
- Support and offer leadership to the GSPC, actively working within the Global Partnership for Plant Conservation to achieve GSPC targets.
- Restore and manage the natural landscape at Shaw Nature Reserve to increase the health and diversity of those plant-based communities so as to serve as a local and international model for ecological restoration.
- Continue to develop its Tropicos database and the Living Collection Management System (LCMS) as the Garden's primary means for organizing and disseminating plant knowledge resulting from the Garden's research and horticulture and expand the conservation status coverage within these systems.
- Host internationally significant biodiversity, plant science, and conservation conferences, symposia, workshops,

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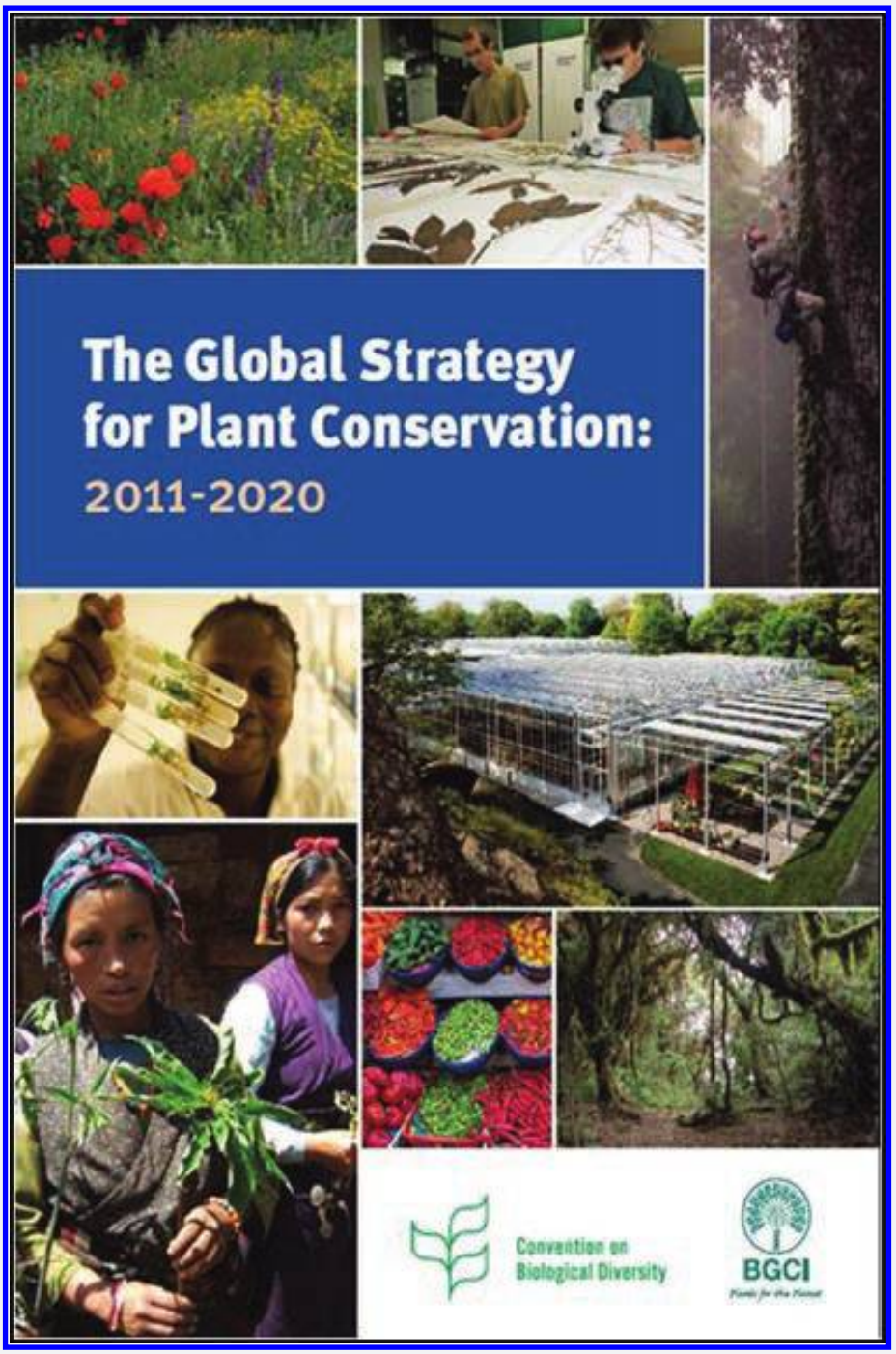

Figure 1. The Global Strategy for Plant Conservation, 2011–2020, provides an agreed international framework for plant conservation worldwide.

and speakers and continue our extensive capacity-building activities.

- Challenge the Garden team to further integrate research, conservation, horticulture, and education, capitalizing on the Garden's most important strengths.
- Apply a multidisciplinary and integrated approach to projects and programs, such as the Garden's work in specific local and global geographic regions, in order to bolster conservation and sustainable ecosystem approaches.

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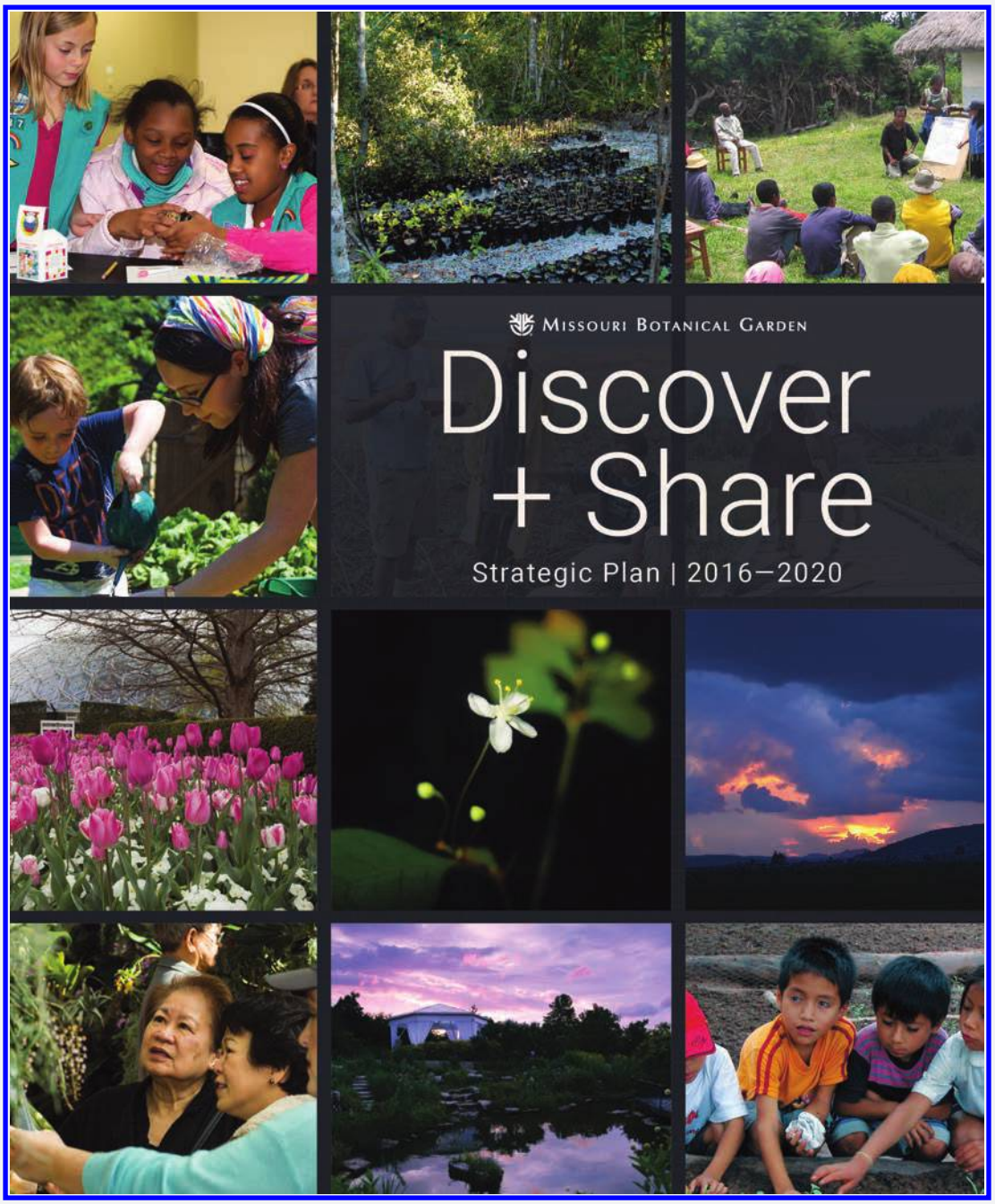

Figure 2. The Strategic Plan of the Missouri Botanical Garden, 2016–2020 provides the institutional direction and response to its institutional commitment to plant conservation locally, nationally, and internationally.

- Collaborate across the divisions of the Garden to educate visitors about the importance of plant conservation and the choices they can make to use resources wisely and make a difference.
- Engage local communities for the purpose of fostering stewardship of plants, biodiversity and the environment in general and to reduce negative human impact on plants and ecosystems.

The need for technical guidance and resources to support developments in plant conservation in botanic gardens has grown over the last few decades as their plant conservation

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activities have become increasingly sophisticated and complex. In 1998, BGCI published *The Darwin Technical Manual for Botanic Gardens*[11] and has recently made available a new online manual "From Idea to Realisation—BGCI's Manual on Planning, Developing and Managing Botanic Gardens" (2016; https://www.bgci.org/resources/2016-bgci-botanic-garden-manual/).

PLANT CONSERVATION THROUGH LIVING COLLECTIONS IN BOTANIC GARDENS

Worldwide, botanic gardens grow a total of more than 6 million living plant accessions, each of which represents one or more individual plants derived from a single collection or location[12]. This illustrates the capacity and potential for botanic gardens to conserve massive amounts of plant germplasm and biodiversity within their collections. The botanic garden community is thus the only international institutional network focused specifically on the conservation of wild plant diversity and can often provide research capacity, horticultural skills, and botanical expertise required for many conservation projects.

A recent analysis of the information in the Plant Search database (https://www.bgci.org/plant_search.php) developed by BGCI, which catalogs the plants held in about a third of the botanic gardens of the world, noted that around 30% of some 350,000 plant species were cultivated in at least one botanic garden[13]. The Plant Search database currently holds 1,397,844 collection records, representing 556,254 taxa (species, subspecies, varieties, cultivars, etc.), at 1,118 contributing institutions (https://www.bgci.org/plant_search.php—accessed 25 March, 2019).

While these figures are impressive, there is still a considerable task for botanic gardens to undertake if the diversity of the world's plants is to be safeguarded, given that around 70% of plant species are not found in living collections. Notably, important gaps exist in the taxa held in botanic gardens; for example, most of the material is housed in botanic gardens located in temperate regions (particularly North America and Europe), and 76% of the species not held as living collections are tropical in origin (it should be noted though that perhaps the living collections in botanic gardens in the tropics may be somewhat underrepresented in the PlantSearch database as many botanic gardens in developing countries in the tropics do not yet have well developed computer-based information systems for their living collections). Collections are also biased toward vascular plants, such that over 50% of vascular genera are conserved, whereas only 5% of nonvascular genera are held in living collections[13].

The eighth target included in the GSPC to be achieved by 2020 is "At least 75% of threatened plants in ex situ collections, preferably in the country of origin, and at least 20% available for restoration and recovery programmes." While substantial progress has been made toward the target over the last decade, it is unlikely that this target will be attained by 2020. If one estimates that there are currently in the region of 100,000 plant species that are threatened worldwide, then this target would suggest that as many as 75,000 species need to be conserved in order to achieve the target. A recent analysis showed that only around 10% of the botanic garden network capacity is devoted to threatened species[13]. To increase the species diversity conserved in botanic gardens, several steps are necessary: (1) existing gardens must attempt to broaden their taxonomic coverage and increase their collections of threatened species, and (2) more botanic gardens need to be established and developed across the world, particularly in those areas where no other institutions are involved in ex situ conservation efforts, such as in many tropical regions. Increasing the extent to which many more plant species can be assessed for their conservation status will undoubtedly increase the number of threatened plants in cultivation because many plants currently grown will in the future be recognized as rare or endangered.

THE CHANGING PRACTICE OF CONSERVATION IN BOTANIC GARDENS UP TO 2000 AND BEYOND

Although botanic gardens have over centuries acquired great skill and expertise in holding and handling plant collections, in the past, relatively few gardens collected or maintained collections (living plants or seed) in a manner satisfactory for conservation purposes. Documentation of provenance has frequently been inadequate and many collections of threatened plants include very small numbers of individual plants or even a single individual, so the extent to which these collections represent the diversity present in the wild is clearly often very limited.

The introduction of computer-based information systems to store and track data on living plant collections has provided the means for botanic gardens to develop efficient new collections databases, recording much more information on their collections than in the past. Up to the 1980s, many botanic gardens record-keeping systems were, at best, recorded in ledgers, accession books, and card indices. Today sophisticated tools for data management are widely used, often incorporating mapping components which allow the storage of accurate coordinates on where individual plants are grown in each garden, or from where they were collected originally. In addition, such computer-based information systems have made possible the tracking of the origins, propagation history, and management regimes and needs for individual plants, helping to ensure that the genetic diversity of different lines in living plant accessions can be tracked accurately.

Over the past few decades, an increasing number of gardens have also reconsidered the role of their collections, making their primary purpose one of conservation. Many botanic gardens have begun banking germplasm, which is

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material collected specifically for conservation purposes[14] and is intended so as to provide an "insurance policy" against plant extinction, safeguard against the loss of genetic diversity, and act as source material for reintroductions[14–18]. Seeds stored in conservation seed banks represent a vast resource of banked germplasm, but can also include the maintenance of pollen, vegetative propagules, and tissue or cell cultures[2]. To be of conservation value, ex situ collections must represent the genetic diversity present in a species or population, and should be appropriately labeled, documented, and cataloged to understand its provenance. Comprehensive and systematic surveys are needed to understand the extent to which the extant genetic diversity has been captured for conservation programs through botanic gardens.

In addition to increasing and managing ex situ collections in such a way to improve their applicability to conservation, the mandate for conservation through botanic gardens has broadened enormously to include plant conservation in situ, the recovery of endangered plants through reintroduction, and the careful management and restoration of plant populations and habitats. The trend is now for conservationists to combine the approaches and techniques of in situ and ex situ conservation in the protection and management of biological diversity in the context of an "Integrated Conservation Strategy"[19,20]. Integrated conservation draws together organizations using different but complementary methods for the conservation of biodiversity. Such methods include land acquisition and management; legal protection; ex situ research and maintenance; plant rescue, reintroduction, and restoration; and public education and awareness. The principal emerging role for botanic gardens is in implementing integrated plant conservation, by marrying species-level research with the protection, management, and restoration of plant communities and ecosystems.

HISTORY AND RATIONALE FOR THE USE OF CONSERVATION GENETICS APPROACHES TO SUPPORT PLANT CONSERVATION

Recently, as part of integrated conservation programs, conservationists have increasingly become aware of the importance of the genetic diversity for the overall conservation of biodiversity. Over the past decades, a wide array of technologies have been developed to measure genetic diversity and investigate the genetic relationships among individuals within a species. With the capability to genotype individuals in populations, researchers have begun to investigate the importance of genetic diversity across all levels of biodiversity. Studies have showed that genetic diversity is positively correlated with fitness at the individual level[21], that population size, genetic diversity, and fitness are positively correlated[22], and that species experiencing declining genetic variation and inbreeding face an increased risk of extinction[23,24]. Additional studies have showed that genetic diversity confers a greater potential for a species to adapt to environmental change[25] and improves ecosystem stability, resilience, and function[26–29]. Other studies have showed that reintroductions that include greater genotypic diversity have a greater rate of persistence and population growth[30–34].

Concurrently, empirical studies have increasingly showed that genetic data can provide important information that is relevant for conservation. Population genetic data provides basic information about the biology of a species, such as its mating system and spatial extent of dispersal or pollination[35–37], which can be important for devising conservation strategies. It is also used to evaluate whether populations have been affected by ecological or demographic processes, such as evaluating the effects of fragmentation on population connectivity[38] or determining whether populations have experienced inbreeding, genetic drift, or population bottlenecks[39]. Many conservation genetics studies in wild species measure how genetic diversity is structured across the landscape and then devise a strategy to conserve populations both in situ and ex situ to maximize the total amount of genetic diversity being protected[40–43]. Genetic data can also be used to determine the uniqueness of species and whether it merits conservation[44–46]. Based on the utility of this information for conservation, genetic data is increasingly being used to guide decisions on population management and conservation to effectively conserve biological diversity in the face of limited resources. Genetics are also being used to guide reintroductions and evaluate their long-term success[47–50].

As botanic gardens have become more involved in undertaking integrated conservation projects, and as recognition increases of the fact that genetic data improves the ability to effectively conserve biological diversity, some botanic gardens have begun developing capacity for conservation genetics and integrating genetic data into their conservation programs. Currently, of the 3,571 botanic gardens listed in the BGCI "GardenSearch" database, 152 (4.3%) report that they conduct conservation genetics research, 337 (9.4%) report that they conduct conservation biology research, and 526 (14.7%) report that they have conservation programs (Source: https://www.bgci.org/garden_search.php.). This certainly is an underestimate of the numbers of botanic gardens with conservation programs and facilities, and in some instances the data presented in "GardenSearch" is probably not fully up to date. Nevertheless, it is clear that currently only a small fraction of the extant botanic gardens have established conservation genetics research programs. These programs are not evenly distributed geographically, as most are found in gardens located in Europe (41), North America (26), and China (9). Although we recognize that not all botanic gardens have the interest or capacity for developing conservation genetics

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Figure 3. The conservation genetics laboratory at MBG. The lab is dedicated to conservation genetics and has the equipment necessary for DNA and RNA extraction, gel electrophoresis, DNA and RNA quantification, polymerase chain reaction (PCR), and next-generation sequencing library preparation. Photo by Christine Edwards

as part of their programs, these figures highlight the opportunities that still exist for botanic gardens to enhance their conservation work through the development of conservation genetics capacity.

In the following sections, we will provide an overview of how to develop a conservation genetics program in a botanic garden based on insights gained through the recent development of one such program in the MBG, which was established in 2014 (Fig. 3). We will overview the general infrastructure required, detail the specific laboratory equipment needed to collect this type of data, discuss the types of molecular data and some of the analytical approaches that can be used to analyze the data to answer conservation questions, and provide some examples of how such data can be used to enhance conservation efforts in botanic gardens. We hope that this article will inspire other botanic gardens to establish their own conservation genetics programs and provide them with an outline of the resources and steps required to do so.

WHAT ARE THE RESOURCES REQUIRED FOR THE DEVELOPMENT OF CONSERVATION GENETICS PROGRAMS IN BOTANIC GARDENS?

Overall, the first and foremost resource necessary to develop conservation genetics capabilities in botanic gardens is a trained staff member to conduct conservation genetics research. The staff member should be trained in laboratory techniques and in the analysis and interpretation of molecular data. This type of training generally comes from experience working in molecular labs in academic settings, such as that obtained through graduate-level research for Masters theses and PhD dissertations. The availability of this type of training is generally geographically restricted, with more training opportunities available in developed countries. To a lesser extent, undergraduate training programs (i.e., research experiences for undergraduates) or internships may provide basic laboratory training, although it is unlikely to provide the full training needed to establish a fully functional genetics program.

The second resource required to develop a conservation genetics program is access to a molecular laboratory, which can be accomplished in several ways. Some botanic gardens (e.g., the MBG) have developed molecular laboratories specifically for research programs in conservation genetics; see Box 3 and following section for a specific description of the facilities and equipment needed to develop a molecular laboratory. Other botanic gardens may have preexisting facilities developed previously for research in molecular systematics that can also be employed for conservation genetics. Another approach is to use molecular facilities at associated universities or research institutions or to collaborate with colleagues that have access to a molecular lab. This may be the most affordable way for institutions to undertake conservation genetics research without investing in a research lab. Given that many botanic gardens share similar missions and research goals, partnerships and collaborations among botanic gardens are an ideal way to improve access to genetic research capabilities. Some larger botanic gardens even provide funding and training opportunities to enable such collaborations; we call on botanic gardens with genetics facilities to strive to develop and expand these types of valuable training opportunities and collaborations in the future. If none of these options is feasible, another alternative is to outsource all laboratory work. At many core laboratories that offer genotyping services, it is possible to outsource all steps, from DNA extraction through to bioinformatics analysis. The advantage of this approach is

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Box 3. Minimum equipment needed to establish a conservation genetics lab.

General lab equipment and supplies:

−20°C freezer and a 4°C refrigerator (for reagent and sample storage)
Magnetic stir and hot plate (for mixing reagents), stir bars, and stir bar retriever
pH meter, electrodes, and pH buffer solutions
Balance to precision of 0.01 g, weigh boats, and small scoops/spatulas
Heated lab water bath (to incubate samples), floating sample holder, and thermometer
Mini centrifuge with changeable rotor (to spin down samples and reagents)
24-sample centrifuge (for DNA extractions or PCR purification kits)
Vortex (to mix samples)
Pipette starter kit including 2, 20, 200, and 1,000 µl volumes, pipette stand, and pipette tips
Multichannel and/or repeater pipettes and tips (optional but **highly** recommended)
Glassware in assorted sizes: graduated cylinders, beakers, and Erlenmeyer flasks
1.5-mL tubes, plastic racks, and sample storage boxes
15- and 50-mL falcon tubes and falcon tube rack
Disposable latex or nitrile gloves
First aid kits, safety goggles, lab coats, chemical spill kits, eye wash, fire extinguisher
Bleach and squeeze bottles for cleaning
Absolute ethanol (for DNA extraction and cleaning)

DNA extraction and quantification:

Silica gel to preserve tissue samples (large bead size preferred for most applications)
Forceps or razor blades for handing samples
Mortar and pestle (for sample grinding)
DNA extraction kits or reagents necessary for CTAB extraction protocols (e.g., 50)
Qubit DNA quantitation starter packs, dsDNA kits and tubes (for sample quantification)

Gel electrophoresis:

Gel electrophoresis chamber, gel molds, and combs
Electrophoresis power supply
UV transilluminator and with safety screen or imaging hood/digital camera (preferred)
Microwave
TBE buffer (either commercially prepared or homemade), large carboy to hold TBE buffer
Agarose, gel loading dye, and DNA ladders
Ethidium bromide or other method of gel staining

PCR equipment:

Access to ice and ice buckets
PCR Thermocycler that holds 96-well plates
96-well PCR plate holders
PCR consumables: 96-well plates, 0.2-mL PCR tubes, strips of 8 0.2-mL tubes, adhesive plate seals
PCR reagents: PCR mastermixes or *Taq*/buffer/dNTPs, ultrapure water

Optional equipment for next-gen applications and more high-throughput sample processing:

Electric bead mill/beads for high-throughput DNA sample grinding (highly recommended)
Vaccum centrifuge with rotors for 1.5-mL tubes and 96-well plates (highly recommended for concentrating samples)
Large refrigerated centrifuge with 96-well plate rotors (for 96-well plate purification kits)
−80°C freezer, dewar for liquid nitrogen, and access to liquid nitrogen (for RNA approaches)

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Box 3. (*Continued*)

Magnetic stand for 96-well plates and magnetic bead purification kits
Access to dry ice for NGS sample shipping
Autoclave (for sterilization of glassware and consumables, preparation of media)
PCR hood (for applications prone to contamination)
Bioanalyzer (for high resolution analysis of sample quality, size, and quantity)
Sonicator (for sample fragmentation for some next-gen approaches)
Application-specific reagents and kits (for next-gen approaches)

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that it involves virtually no laboratory startup costs, but the drawback is that it involves the most expensive per-sample processing charges, which may be unsustainable over the long term.

The final resource needed to develop conservation genetics capacity in a botanic garden is community partnerships. Although community partnerships are not absolutely necessary for conducting conservation genetics projects, they can provide a wealth of services and interactions that can enrich a project and increase the value of the conservation genetics work. For example, donors and community partners may be able to provide financial assistance for the acquisition of laboratory equipment and the infrastructure needed to setup a laboratory, sometimes within the context of specific species conservation projects. Associations with universities may provide access to students who are interested in working on conservation genetics projects to gain research experience. Interactions with land managers, conservation officials, and ecologists can provide important insights into the biology of a target species and help identify the most relevant questions to ask for a conservation genetics study. The agencies tasked with managing a species of conservation concern may provide funding for the project or identify suitable grant opportunities or other sources of funding for projects. Participation by agency partners is also instrumental for most conservation genetic studies because they provide access to populations and issue collection permits. Importantly, buy in from land managers and those tasked with the conservation of a target species is absolutely essential to ensure that the management recommendations derived from a conservation genetics study are implemented to help safeguard biodiversity.

WHAT IS REQUIRED TO DEVELOP A MOLECULAR LABORATORY IN A BOTANIC GARDEN?

To develop an in-house conservation genetics laboratory, the laboratory space must meet a minimum set of requirements. The space should have restricted access for safety purposes and be physically separated from spaces used for other applications, such as dry labs or greenhouses used to process dried plants or soil that might be a source of contaminating DNA. The space should be clean, well lit, and have access to a sink and electrical outlets. The space must contain workspaces, such as tables or counters, and be outfitted with appropriate safety equipment, such as fire extinguishers, first aid kits, safety goggles, and lab coats.

Overall, the most basic functions of a modern conservation genetics lab are to conduct DNA extraction, DNA visualization through gel electrophoresis, and DNA quantification; the basic equipment required for these applications is listed in Box 3. Plant DNA extractions can be conducted using commercial kits, which have the advantage of requiring only the most basic lab equipment (i.e., pipettes and centrifuges), but have the drawbacks of having high per-sample costs and variable success rates, such that the DNA extractions derived from these kits need to be tested for their success in downstream applications. At MBG, we generally find that we obtain the greatest DNA concentrations from plant samples for the lowest cost using CTAB/chloroform DNA extraction protocols[51], which require an externally vented fume hood. For more high-throughput DNA extraction capabilities, we also recommend bead beaters for sample grinding, as this represents a huge time savings relative to manually grinding each sample. In addition, a molecular lab generally should have the equipment needed to visualize DNA through gel electrophoresis and to quantify DNA concentrations because even when outsourcing all downstream sample processing, most core facilities have specific requirements for DNA quality and concentrations. In the United States, the cost of the basic equipment to conduct DNA extraction, gel electrophoresis, and DNA quantification is in the range of $10,000–$15,000.

Although not absolutely essential, the capability to conduct polymerase chain reactions (PCRs) is highly desirable in a conservation genetics lab, as most modern genetic analysis approaches involve PCR. Even though thermocyclers for PCR are costly ($3,000–6,000), they provide the ability to perform

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in-house genotyping reactions and next-generation DNA sequencing library preparation, which dramatically reduces the per-sample cost of genotyping; the costs of purchasing a thermocycler would typically be recovered after conducting a small amount of genotyping. If a large number of PCR reactions are going to be conducted in the lab, we also highly recommend a multichannel/repeating pipette to reduce pipetting errors (Box 3).

Depending on the conservation question to be addressed, some specialized equipment may also be needed. For example, for applications involving RNA such as gene expression analysis, ultracold –80°C freezers and access to liquid nitrogen are recommended to preserve RNA sample quality. A sonicator may be necessary for some next-generation DNA sequencing (NGS) applications (Box 3), and a bioanalyzer is useful for obtaining high-resolution information about NGS libraries; however, both of these can be outsourced to large core facilities at universities or commercial laboratories.

For nearly all average-sized laboratories, it is not advisable to purchase DNA sequencers; instead, we recommend using large core facilities at universities or commercial laboratories, as they provide high-quality genotyping services at low costs. Use of core facilities is recommended for several reasons: first, these large core facilities are able to offer highly affordable prices because the large number of samples they process allows them to obtain bulk discounts on reagents. Use of core facilities eliminates the large start-up expense required to purchase a sequencer, particularly since the per-sample discount involved with running the samples in-house would not be recovered given the volume of samples analyzed in an average lab. Furthermore, DNA sequencing technology evolves rapidly, and using core facilities prevents purchasing a machine that may quickly be outdated. Moreover, because conservation genetics analyses may use a variety of technologies for DNA analysis, utilizing core facilities allows flexibility in the technology that can be utilized.

Finally, the last essential piece of equipment necessary for a genetics lab is a computer with Internet access. A computer is necessary for placing orders at core facilities to process genetic samples. The resulting genetic data is delivered electronically, frequently through secure websites. The scoring and analysis of genetic data is also accomplished completely via computers. Although data analysis of most conventional markers can be accomplished using a standard desktop computer, it should be noted that analysis of NGS data may require additional computational resources because of the large amount of data produced. Many programs to analyze NGS data work only on Linux or Mac operating systems and require large amounts of memory, and the analysis of some large NGS data sets may only be possible using a high-performance computing cluster. Since most botanic gardens lack a computer cluster, this further highlights the importance of community partnerships, as universities commonly have such resources.

TYPES OF MOLECULAR MARKERS USED FOR CONSERVATION GENETICS RESEARCH

A wide variety of molecular markers have been employed for conservation genetics research, to the extent that it is outside of the purview of this review to cover them all. Many of these markers have been described extensively in other reviews[52–59], such that here we will outline only a few of the most important ones employed for conservation genetics, as well as the trends for their use over time.

The markers described here can be divided into two major categories: codominant and dominant. For **codominant markers**, both alleles of a heterozygote are observable and can be scored independently. Codominant markers can therefore be used to test for deviations from Hardy–Weinberg equilibrium (HWE), which is important for detecting inbreeding, genetic bottlenecks, and other information relevant for conservation. Codominant markers include allozymes, microsatellites, and single nucleotide polymorphisms (SNPs; see below for a description of each). **Dominant markers** are those characterized by analyzing their presence or absence, such that homozygous dominant (or "present") is indistinguishable from a heterozygote. The Hardy–Weinberg equation must therefore be used to obtain the frequency of heterozygotes of dominant markers, with the assumption that the population is under HWE. Since this assumption is frequently not true for many species, we therefore recommend using codominant markers whenever possible. Dominant markers include restriction fragment length polymorphisms (RFLPs), RAPDs, Inter simple sequence repeats (ISSRs), and AFLP.

The first type of molecular marker widely employed for conservation genetics studies was allozymes. Allozymes are protein polymorphisms encoded by nonsynonymous amino acid substitutions, which cause differences in their charge that are detected by conducting gel electrophoresis to observe differences in the way they migrate[60]. Some advantages of this type of marker are that it is codominant, relatively simple to use, and cost effective. One important drawback of allozymes is that they generally demonstrate very low levels of variation within populations because they have a selective constraint due to their important function in the cell. Allozymes were the primary marker used for population genetics until the 1980s, and although they are still currently used for population genetics, their use is declining because newer DNA-based approaches demonstrate greater polymorphism and ability to resolve patterns of genetic diversity and structure.

The next markers that became popular for conservation genetics is RFLPs. This approach involves digestion of

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DNA by restriction enzymes, which cut DNA at specific DNA recognition sites, followed by gel electrophoresis. Variation in RFLP markers is caused by mutations in the restriction enzyme cut site. Currently, RFLP is most frequently used in combination with PCR (i.e., PCR–RFLP). In this approach, a DNA region is amplified using PCR (frequently using "universal" molecular markers), followed by digestion using restriction enzymes and gel electrophoresis[59]. Although important in the 1980s and 1990s, the use of RFLPs has declined over time, largely replaced by other DNA-based technologies.

The next two approaches are dominant, DNA-based markers that involve the analysis of multiple loci simultaneously in a single genotyping reaction. ISSRs involve PCR reactions using primers targeted to repetitive DNA regions called simple sequence repeats (SSRs; e.g., CACACACAC)[59]. Thus, the PCR reaction simultaneously amplifies multiple DNA regions that are located between SSRs. AFLPs involve digestion of DNA using restriction enzymes followed by a series of PCR reactions to amplify DNA fragments resulting from the digestion[61,62]. For both ISSRs and AFLPs, the results are visualized using gel or capillary electrophoresis. The benefits of these approaches are that they are cost-efficient, relatively easy to use, and can be applied to a species with no prior knowledge of their genome. The main drawbacks are that they are dominant, anonymous markers, such that the processes that cause variation in them may not be known. They may also suffer from problems with homology and convergence.

SSRs (also known as microsatellites) are repetitive DNA regions (e.g., CACACACA) located throughout nuclear DNA. SSRs generally demonstrate high rates of variation that is thought to be attributable to errors in DNA polymerase during cell division that may add or remove repeats, resulting in variation in the size of the repeat region. To develop SSR loci for genotyping, a DNA sequence that includes an SSR and its flanking regions is necessary, from which PCR primers are designed in the flanking regions to amplify the SSR. To genotype SSRs, PCR along with capillary electrophoresis is used to determine the size of the PCR fragments in each individual. Because SSRs are located in regions of the genome that have high mutation rates, microsatellites can often be used only in a target species, or sometimes in a group of closely related species. The benefits of SSRs are that they are codominant, have high mutation rates that provide high resolution of genetic diversity and structure, and are species-specific so they have low rates of contamination and are highly repeatable. These attributes have made them very popular in the field of conservation genetics and they are still widely employed to this day.

One drawback of SSRs is that they require prior knowledge of the genome in order to develop species-specific PCR primers that flank an SSR. This was a particularly a problem during their early use during the 1990s and 2000s, as developing SSR loci involved time-consuming steps including hybridization to a probe, followed by extensive PCR, cloning, and Sanger DNA sequencing[63]. This is now less of an impediment, as identifying SSR loci is easily accomplished though high-throughput NGS of genomic DNA; see[64] for a recent review of current ways to identify SSR loci using NGS. Another drawback of SSRs is that they require extensive time in the laboratory, making it possible to genotype only a limited number of loci (often <20); however, because they demonstrate high levels of allelic diversity per locus, relatively few SSR loci are generally needed to resolve genetic variation[65].

Another marker used in conservation genetics is DNA sequences. Originally, Sanger DNA sequencing approaches were used to sequence one or a few DNA regions, such as plastid or nuclear ribosomal DNA regions. These early analyses generally provided poor resolution in plants at the intraspecific level, but they were often able to resolve the evolutionary relationships among closely related species. With the advent of NGS approaches, it is now possible to generate data sets spanning whole organellar genomes or hundreds to thousands of nuclear DNA regions. With the added resolution of NGS approaches, these studies are becoming increasing useful for understanding the evolutionary origins of rare species and determining whether a rare species is distinct from close relatives and therefore deserving of protection. DNA sequencing using both Sanger and NGS approaches is still widely employed for phylogeny reconstruction and can be useful for answering conservation questions.

Also based on DNA sequencing, SNPs are simply a nucleotide position that varies within a population or species; for example, some individuals in a population may have a T at site, others may have a C, and others may be heterozygous (T/C). SNPs are located throughout the genome and are the most abundant type of polymorphism[59]. Prior to the advent of NGS, SNP genotyping required the development of extensive genetic resources in order to identify SNPs and design assays that could be used to genotype them. However, since the advent of NGS, it has become much more simple to conduct SNP genotyping without prior development of genomic resources. With sufficient funding, it is now possible to conduct whole genome sequencing (WGS) to quantify SNP variation, although this approach is quite costly, putting it out of reach for most conservation genetic studies.

In response to the cost limitations imposed by WGS, several approaches have been developed to assay SNPs across a reduced subset of the genome, thereby enabling the generation of genome-wide SNP genotype data at a greatly reduced cost relative to WGS. These approaches, often termed reduced-representation sequencing approaches, include restriction-associated DNA sequencing (RAD-seq) and genotyping by sequencing (GBS). Both of these approaches involve digestion of DNA using restriction enzymes, followed

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by NGS sequencing of the DNA regions flanking a restriction site. Many different variations of RAD-seq and GBS have developed[66–71], and the relative merits of these approaches have been reviewed extensively elsewhere[59,72–74]. The advantages of these reduced-representation DNA sequencing approaches are that they frequently generate genotype data across thousands of SNPs distributed across the genome, which provide greater resolution of genetic diversity and structure and are suitable for a large number of evolutionary analyses. The drawbacks are that analysis of such large-scale data sets requires extensive computational skills and infrastructure that are lacking at many institutions. This again highlights the importance of partnerships and training to gain the skills and access to the resources necessary for such intensive analysis.

At MBG, we most frequently employ SSR or SNP markers to genotype individuals for conservation genetics studies. The type of marker that we select for a study generally depends on the size of the project, the amount of money available, and the question to be answered. Because of their affordability and low computational demands, we employ SSRs in studies involving a large number of individuals (i.e., >400), as very large SNP data sets become computationally intractable at this size. SSRs are also beneficial for parentage analysis because they are highly polymorphic, often demonstrating dozens of alleles per locus, which can provide high resolution to differentiate among potential parents. Another application for which SSRs are useful is for determining the basic life history traits of species for which no previous genetic study has been conducted. For example, SSRs are particularly useful for detecting polyploids, as the maximum number of alleles present in an individual can provide a preliminary idea of the ploidy of the species. However, we generally employ RAD-seq for most other studies because the large number of SNP markers generated by this approach is suitable for a broad range of analytical applications; for example, in addition to understanding genetic diversity and structure, the DNA sequences can be analyzed phylogenetically to provide important insights into the origins, evolutionary history, and distinctiveness of endangered species[75,76]. SNP data can also be used to understand patterns of adaptive genetic variation[77] and provide high resolution for modeling of demographic scenarios[78]. As such, we feel that the future of conservation genetics lies in the analysis of large-scale SNP datasets generated using either reduced-representation approaches or eventually through WGS as it becomes more affordable.

ADVANCES IN ANALYSIS OF GENETIC DATA TO ANSWER MANY QUESTIONS RELEVANT FOR CONSERVATION

The genetic data generated by genotyping of molecular markers can be analyzed in a wide variety of ways that are useful for conservation. In some cases, the specific computer programs and metrics used to analyze genetic data may differ according to the type of molecular marker used or whether it is dominant or codominant, but overall the general categories of analysis are similar across data types. This list is meant to provide a general overview of the some of the main categories of genetic data analyses that can be used to help address conservation questions, but is by no means a comprehensive list of all of the programs and approaches that are available for the analysis of genetic data.

The first category of data analyses is basic genetic diversity summary statistics, which were among the first metrics used to analyze population genetic data. For all types of markers, summary statistics provide basic metrics of genetic diversity, which can be used to compare populations and provide insight into whether specific populations may have experienced declines in genetic diversity. For dominant markers, the basic diversity metrics include the number of polymorphic loci per population, the percent polymorphic loci, allelic diversity (for markers demonstrating multiple alleles per locus) and the Shannon information index, or expected heterozygosity (H_E) assuming HWE.

Additional information about the life history strategy of a species and the factors affecting genetic diversity in populations can be gained from the analysis of codominant markers. Metrics include H_O, simply the proportion of heterozygotes in a population, expected heterozygosity (H_E), which is the heterozygosity expected given the observed allele frequencies in a population assuming HWE, and the inbreeding coefficient (F_{IS}), which summarizes the relationship between H_O and H_E. F_{IS} can range between 1 and –1, with positive values found in individuals with $H_O < H_E$, often indicating inbreeding, and negative values found in individuals with $H_O > H_E$, likely indicating assortative mating or self-incompatibility. A similar analysis is the assessment of assess deviations from HWE, with heterozygote deficiencies resulting from inbreeding, null alleles, or a Wahlund effect, and heterozygote excesses resulting from nonrandom mating (such as in self-incompatible species) or through a recent genetic bottleneck. Clearly, all of these metrics are highly useful for understanding life history strategies of plants (i.e., a selfing or self-incompatible mating system) and detecting whether the genetic diversity in specific populations may be affected by inbreeding or genetic bottlenecks.

A wide variety of metrics have also been developed to understand the structuring of genetic variation, which is useful for devising conservation strategies to protect genetic variation both in situ and ex situ. The classic metrics used to understand population structure are Wright's F-statistics[79] and AMOVA[80]. The commonly used F_{ST} measures the fixation of allele frequencies in populations, which is affected by the extent of migration among populations. It can be used to measure the overall fixation of alleles across all populations or to compare population pairs. Subsequently, a range

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of metrics have been developed to measure pairwise genetic differentiation among populations[81,82]. AMOVA is based on the same theoretical framework as Wright's F-statistics, but it is used to partition variance across hierarchical levels, such as the variation within individuals, among individuals in a population, and among populations. This information can also be used to understand life history traits, with high among-population variation indicating species with a low propensity for migration (i.e., self-fertilizing species with gravity dispersed seeds), and high within-population variation indicating species that have a greater propensity to migrate (i.e., outcrossing, wind-pollinated species). These approaches are very useful for identifying the full extent of genetic diversity in a species and how it is structured geographically so that strategies can be devised to protect this variation; these parameters may be particularly important for designing ex situ conservation strategies in botanic gardens.

In addition to traditional approaches to measure genetic structure, a variety of approaches were developed that have dramatically expanded our ability to understand patterns of genetic structure. An important advance in population genetics was the development of the computer program STRUCTURE[83–85], which is useful because it analyzes patterns of population structure and admixture without the need to designate populations a priori. This makes it useful for detecting cryptic population structure and for identifying hybrids. The results from STRUCTURE can be validated using other approaches that also do not require designation of populations to analyze population structure, such as analyses of pairwise genetic distance among individuals or ordination approaches like principal components analysis (PCA). Other approaches have been developed that incorporate spatial data to specifically analyze the spatial extent of genetic structure or isolation by distance[86]. All of these approaches are generally used along with F_{ST} and AMOVA to develop of strategies to protect genetic variation. When STRUCTURE or PCA is used at the interspecific level, they can also be used to determine whether an endangered species is genetically distinct from its close relatives, which is necessary to ensure that botanic gardens are devoting their conservation resources to protect and conserve truly unique species.

Another category of analytical approaches that can be useful for conservation are those that involve modeling to understand the demographic history of populations, including programs such as IMa, diyABC, and ∂a∂l[78,87,88]. Although the parameters that can be tested vary among programs, some parameters that they measure include the order and timing of divergences among populations, whether migration has occurred among populations in the process of divergence, whether populations have experienced population bottlenecks or expansions, and whether there is evidence for selection. These program are useful for conservation because they can help identify the factors that have had important effects on populations over the course of their history; for example, they can be used to understand how long a population has been isolated from relatives to determine whether it may be a unique species, or identify whether populations show low genetic diversity because of past genetic bottlenecks.

Other important advances include parentage analysis[89], which can be used to determine the parents of seedlings, which has applications for designing reintroductions to minimize inbreeding among siblings and ensure that they have high genetic diversity. Another recent advance in statistical analysis that may be particularly important for designing reintroductions is the ability to identify potentially adaptive genetic loci, which was reviewed recently[77]. If particular populations demonstrate potentially adaptive differences, then this information could be used to target source populations for reintroductions that would be the most well adapted to the reintroduction site.

CASE STUDIES FROM MBG—HIGHLIGHTING THE PRACTICAL APPLICATION OF CONSERVATION GENETICS TO HELP SET CONSERVATION PRIORITIES, ESTABLISH CONSERVATION TARGETS, AND CONDUCT SPECIES RECOVERY

The first example of a species for which we have used genetic data to dramatically enhance conservation efforts is *Dracaena umbraculifera*[90]. The project began because of a plant labeled as *D. umbraculifera* in the living collections at MBG. *D. umbraculifera* was described in 1797 from a cultivated plant attributed to Mauritius, but repeated surveys were unable to relocate it, and it was listed by IUCN as extinct; however, the BGCI PlantSearch database showed that 18 botanic gardens had accessions of the species, one of which was at MBG. We therefore began a study to understand (1) where *D. umbraculifera* originated, (2) what species are its closest relatives, (3) whether it is indeed extinct in the wild, and (4) whether the botanic garden accessions are correctly identified and have some conservation value. We reconstructed the phylogeny of *Dracaena* from the western Indian Ocean, including *Dracaena* species in Madagascar and Mauritius, plants in botanic gardens, and two positively identified individuals of *D. umbraculifera*, one of which is a living plant in a private garden. Phylogenies revealed that *D. umbraculifera* is more closely related to *Dracaena* from Madagascar than Mauritius. Anecdotal information also indicated that the living plant confirmed to be *D. umbraculifera* was from Madagascar; we therefore conducted a field expedition to Madagascar, where we located five wild populations of *D. umbraculifera* (Figs. 4 and 5). Locating this species was instrumental for ensuring that it is effectively conserved. For example, even though the species is rare and is still critically endangered as the result of deforestation, we have collected seeds and cuttings from

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Figure 4. A flowering plant of Dracaena umbraculifera. D. umbraculifera was thought to be extinct and then was rediscovered following a phylogenetic analysis that showed that it was native to Madagascar, not Mauritius as was previously thought. Photo by Patrice Antilahimena

Figure 5. The growth habit of Dracaena umbraculifera. Photo by Patrice Antilahimena

Figure 6. Dracaena umbraculifera being propagated from cuttings at Parc Ivoloina for ex situ conservation. Photo by Peter Wyse Jackson

highly threatened, unprotected populations and are growing it in ex situ collections in Madagascar (Figs. 6 and 7), with the eventual goal of reintroducing it into protected sites.

Another example of how genetic data can contribute to understanding the life history of an endangered species and facilitate the conservation of its genetic diversity is in *Polygala lewtonii*, an endangered, amphicarpic plant endemic to a small region of central Florida the United States (Fig. 8)[37]. *P. lewtonii* has a mixed mating system with three types of flowers: (1) aboveground, chasmogamous flowers (i.e., open-pollinated; CH), (2) aboveground, cleistogamous flowers (i.e., closed, selfing; CL), and (3) CL flowers on belowground stems (amphicarpy). Aboveground seeds are ant-dispersed, whereas belowground seeds are spaced across the length of the rhizome. In this study, we collected individuals of *P. lewtonii* at both range-wide and fine geographic scales and genotyped them at 11 microsatellite loci. We analyzed patterns of genetic diversity and structure to understand (1) the predominant mating system (selfing or outcrossing), (2) how genetic variation is structured across the landscape, and (3) the optimal strategy to conserve the full range of genetic variation. The results of the study indicated that *P. lewtonii* reproduces predominantly by selfing or biparental inbreeding, with a very limited amount of reproduction occurring through outcrossing. We found very fine-scale patterns of genetic structure, indicating that some gene flow is occurring among aboveground CH flowers but both pollen and outcrossed seeds are moving limited distances (maximum of 0.5 km). Because genetic variation is structured at a fine spatial scale, we concluded that it will be necessary to protect as many populations as possible to fully conserve the genetic variation in *P. lewtonii*. We are currently working to devise a strategy

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Figure 7. Plants of Dracaena umbraculifera growing in ex situ cultivation in Madagascar. After rediscovery of the species, seeds have been collected from unprotected populations. Plants are being grown for future reintroductions into protected sites. Photo by Chris Birkinshaw

Figure 8. The showy, aboveground chasmogamous flowers of Polygala lewtonii. Results of genetic analyses show that the showy flowers do not show high rates of outcrossing and that most reproduction arises from self-fertilization or inbreeding. Photo by Carl Weekly

to conduct conservation seed banking to effectively conserve the genetic diversity of the species in ex situ collections. The genetic data from this study is also being used to support a broader integrated conservation program for this species, which is being coordinated by the Center for Plant Conservation, a network of botanic gardens involved in conservation of imperiled North American species.

Another example is genetic work to facilitate the conservation and reintroduction of *Ziziphus celata*, an endangered, long-lived shrub endemic to the Lake Wales Ridge of central Florida[91]. Genotyping of *Z. celata* revealed that it is clonal, that most populations contain only a single genotype, and that very few individuals existed[92,93]. Research also revealed that it is self-incompatible and contained only a few mating types[94]; wild populations therefore suffer from mate limitation and do not reproduce sexually. Using genetic information, an ex situ population containing cross-compatible genotypes was established to promote sexual reproduction, which has subsequently produced seedlings that are being used to augment uniclonal populations and conduct reintroductions in publicly protected sites (Fig. 9)[95]. To guide these reintroductions and further understand the biology of the species, we have conducted parentage analysis of seedlings used in augmentations/reintroductions to understand (1) whether genotypes contributed equally as both pollen donors and pollen recipients, (2) the overall contribution of each set of parents to each reintroduction and how it changed over time, (3) the levels of genetic diversity of each reintroduction and how it changed over time, and (4) what the necessary steps are to increase the genetic diversity and representation of parents in each reintroduction to ensure high mate availability and reduce the likelihood of inbreeding once they reach reproductive stages. We genotyped over 1,000 seedlings from nine reintroductions and identified parents for each individual. We found that only six reproductively mature genotypes produced virtually all seedlings and that parents served equally well as pollen recipients, but only two genotypes were pollen donors in 92% of seedlings, suggesting that some genotypes have low pollen viability. Many early reintroductions contained offspring from only a few of the possible parents and were dominated by one full-sib group. More recent reintroductions contained all possible full-sib groups in more equal proportions, likely because more genotypes were reproductive in the ex situ breeding population. Genetic diversity was similar across reintroductions because they are derived from offspring of the same six founder individuals. These results are being used to augment existing reintroductions to balance the relative proportions of full-sib groups, which will help promote sexual reproduction and avoid inbreeding, thereby improving the chances of their long-term success.

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Figure 9. Caged individuals of Ziziphus celata that were reintroduced experimentally. The parentage of these individuals was assessed prior to being transplanted. The genetic data was used to structure the individuals so that they were placed next to individuals with different parents to avoid long-term inbreeding. Photo by Christine Edwards

CONCLUSIONS AND FUTURE PERSPECTIVES

The development of plant conservation practices through botanic gardens over the last three decades has transformed our ability to safeguard plant species diversity worldwide. Increased focus on formulation of shared policies, priority setting, coordination of plant conservation action between botanic gardens at national and international levels, capacity building, plant collection documentation systems, and drawing many more botanic gardens into the plant conservation community has had a profound impact on the effectiveness of botanic garden work in plant conservation.

The growth in the networks of botanic gardens at all levels, promoting plant conservation as a primary "cause" for all botanic gardens has ensured that many botanic gardens have become champions and excellent practitioners in the field. The establishment and development of BGCI since 1985 has further stimulated the development of policies and new approaches by botanic gardens to plant conservation. This has included the expansion of efforts beyond a previous emphasis on the cultivation of general living collections to ones that are targeted toward plant conservation and include new involvement in in situ conservation efforts, including species recovery, ecological restoration and the identification, documentation, and management of important sites in nature for plant species diversity.

To address the ongoing challenges in addressing plant conservation needs, botanic gardens must continue to advance and refine their approaches to conserving plant diversity. With the advances that have been made in understanding the diversity and levels of endangerment of plant species, along with the increasing level of threat that many plant species are facing, we feel that general recognition of the need to conserve genetic diversity will increase in the future. One area where we envision that botanic gardens may make important conservation advances is in the field of conservation genetics. While there are certainly many more areas where botanic gardens can increase their effectiveness and contributions to biodiversity, we believe that growing

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and supporting the need for new capacity, facilities, and programs in conservation genetics in botanic gardens will help to achieve significant progress in safeguarding the world's plant species. To accomplish this, conservation genetics programs must change from being something found generally only in the largest botanic gardens to something that is a commonplace component in conservation programs. Although this is an ambitious goal, we believe it is an achievable one if botanic gardens work individually and collectively to make it a priority for future investment.

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