Assessing Status, Capacity, and Needs for the Ex Situ Conservation of the Hawaiian Flora[1]

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Abstract: The flora of the Hawaiian Islands has one of the highest rates of endemism in the world, and over half of all taxa are at risk of endangerment or extinction. When in situ management alone cannot protect plant populations, maintaining viable germplasm using ex situ storage methods will prevent species extinctions. Germplasm collections with high conservation value are genetically diverse, representative of taxa and populations, and have a well-documented history in cultivation. Ex situ facilities and conservation agencies were surveyed to determine if existing ex situ capacity was sufficient to represent Hawai‘i's species of conservation importance (SCI) and to identify limiting factors. SCI were defined and their representation in ex situ collections quantified, the number of wild plants and populations were estimated, and the attempted ex situ methods were recorded. There are 724 SCI, 528 of which are located in at least one facility. Sixty-four percent of the secured taxa are represented by collections from only 10% or fewer of the wild plants. Seed banks have secured more SCI, and with better in situ representation, than any other ex situ method. Seventy-eight percent of SCI have seeds with long-term storage potential. Existing seed storage facilities are currently inadequate for representing all SCI and should be expanded. SCI with low long-term potential in conventional seed storage can be represented in cryopreservation, micropropagation facilities, nurseries, and botanical gardens. Recommendations include establishing a network to coordinate collections, improve data management, and draft conservation plans with ex situ collection goals. This type of assessment can be applied to other regions that do not have a unified and consistent method of tracking ex situ representation.

Conserving plant biodiversity and preventing the extinction of threatened taxa require two approaches: (1) in situ management to preserve habitats, and (2) ex situ techniques to secure and maintain viable propagules in a "genetic safety net" for future restoration and recovery efforts (Barrett and Kohn 1991, Havens et al. 2004). Well-documented, genetically diverse germplasm collections provide insurance against the extinction of threatened flora. Ex situ collections serve as a propagule source for recovery efforts and habitat restoration, where additional propagules can be collected to refresh ex situ collections as they age (Cochrane et al. 2007, Guerrant and Kaye 2007). There are concerns that increasing ex situ services may divert resources from ongoing in situ management and restoration (Hamilton 1994). These concerns can be placated by emphasizing that ex situ collections (1) are part of an overall strategy to conserve plants and their habitats; (2) are a propagule source for conducting experimental, research-driven restoration projects that carry a risk of failure (see Guerrant and Kaye 2007, Menges 2008); and (3) provide time and a safe location

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to maintain representation of populations as habitat protection and threat management are conducted (Li and Pritchard 2009, Volis and Blecher 2010). There is also a misunderstanding that once collections are secured, collectors and managers may assume that no further maintenance is needed and their collections are indefinitely secured. Subsequent collections may be needed when viability of the initial collections declines or if initial collections are not adequately representative of a taxon or population (Hamilton 1994).

Ex situ conservation utilizes several methods for propagule collection, propagation, and representation of sufficient genetic diversity (Husband and Campbell 2004, Volis and Blecher 2010). This work requires technical expertise regarding when and how to collect viable propagules. It also requires advanced horticultural and biological techniques to establish and maintain viable propagules. Initial collections may be necessary for conducting research to determine the best propagules and storage conditions for each species. Seed storage is the most cost-effective method to preserve germplasm. It is less detrimental when collecting from wild plants and captures a higher level of genetic variation in a single collection than other ex situ methods (Havens et al. 2004, Li and Pritchard 2009). When viable, desiccation-tolerant seeds, spores, or pollen cannot be obtained, collectors can harvest clonal material; desiccation-sensitive seeds, spores, and pollen; or immature seeds to be secured using micropropagation techniques or cryopreservation. If these methods fail, horticulturists can propagate and maintain plants in nurseries and botanical gardens from seed or vegetative plant material. Once protocols are established, accurate inventories with standardized provenance data are essential to preserve the genetic diversity of a flora (Rae 2011). A database of inventories and research will help prioritize future in situ collections and prevent use of ex situ techniques that may not be viable long-term storage options for particular species (see James 2004). Ultimately, planning and coordinating both in situ and ex situ efforts will require a system for organizing biological data, conservation needs, and ex situ inventories (Badley et al. 2004, Rae 2011).

Rare or threatened floras are primary candidates for strong ex situ conservation programs (Barrett and Kohn 1991), and the preservation of plant diversity has economical, environmental, and health benefits (Li and Pritchard 2009). The flora of the Hawaiian Islands has one of the highest rates of endemism in the world (89% for angiosperms, 71% for pteridophytes), with over half of all taxa at risk (Wagner et al. 1999, Sakai et al. 2002, Palmer 2003). Approximately 10% of the flora is extinct (Wagner et al. 1999), and over 30% of the flora is federally listed as threatened or endangered (out of 1,360 species [Imada 2012]). Reasons for the decline of native species are numerous, centered around the introduction of invasive species, including plants, invertebrates, and vertebrates, which can cause vast habitat degradation (Diong 1982, Loope et al. 1988, Cuddihy and Stone 1990, Loope 1992, Joe and Daehler 2008, Athens 2009, Weller et al. 2011). Conservation programs across Hawai‘i are engaged in all methods of ex situ conservation and research including greenhouse propagation, tree and field nurseries, living collections in botanical gardens, micropropagation, cryopreservation, and seed banking. Federal, state, and county agencies; nonprofit organizations; community groups; and private individuals are involved in plant conservation. The species most in need of ex situ representation by these conservation agencies are referred to here as “species of conservation importance” (SCI).

The goal of this research was to conduct a statewide inventory and assessment of programs to participate in a potential statewide ex situ network to conserve native Hawaiian flora. The specific questions addressed by this work included the following:

- How well do the existing ex situ collections represent the Hawaiian flora?
- What is the current ex situ capacity of facilities in Hawai‘i?
- What are limiting factors that prohibit ex situ capacity building?

- Would a plant conservation network facilitate an increase in the ex situ capacity in Hawai'i?

To address these questions, we visited facilities and interviewed conservation experts to determine the current status of ex situ conservation in Hawai'i, the limiting factors to building ex situ capacity, and the benefit of creating a statewide ex situ conservation network.

MATERIALS AND METHODS

Species of Conservation Importance

The list of SCI includes U.S. federally listed endangered, threatened, and candidate taxa; all species on the Hawai'i Plant Extinction Prevention (PEPP) list with fewer than 100 wild plants or rare on specific islands; IUCN Red List vulnerable, endangered, or critically endangered species; and species listed by the State of Hawai'i as important habitat or community-dominant species (Mitchell et al. 2005, U.S. Fish and Wildlife Service 2010). Taxonomic nomenclature followed the most recently revised classifications (Imada 2012, Wagner et al. 2012). Extinct taxa without ex situ representation were removed from the list, because no propagules exist that could be stored. The number of known individuals and/or populations was compiled for each SCI from information provided by the U.S. Fish and Wildlife Service (updated 2 March 2011) and updated during interviews with local botanists. The estimates for the number of plants do not include outplanted individuals, but some may include immature plants. The distribution by island for each taxon was obtained from Wagner et al. (1999, 2012). SCI were further grouped by rarity and listed as threatened (SCI that are federally listed, on the Hawai'i PEPP list of species with fewer than 100 known individuals or rare on specific islands, and any other species with 150 individuals or fewer remaining), potentially threatened (between 150 and 1,000 wild plants and not threatened), or common (>1,000 wild plants and not threatened).

Determination of Ex Situ Representation

Twenty-three facilities provided an inventory of their collections (Table 1). We determined ex situ representation for each taxon by calculating the percentage of wild plants (threatened and potentially threatened SCI) or populations (common SCI) represented at each facility. Exact wild plant representation was not apparent on most inventories; therefore in those instances each accession was recorded as originating from a different wild plant. The species that were present on at least one inventory were classified as "secured" taxa, and SCI not present on any inventory were "unsecured" taxa. Each facility received a score for each SCI based on how representative its collection is of the extant wild plants. Collections were ranked on a five-point scale: (1) 1%–10% of the wild plants potentially represented; (2) 11%–49%; (3) 50%–84%; (4) 85%–100% with less than 50 seeds, three explants, or three plants representing each wild plant; and (5) 85%–100% with greater than or equal to 50 seeds, three explants, or three plants representing each wild plant. Targets for replication of representation for a score of "5" were based on mitigation requirements for genetic storage for the U.S. Army Garrison-Hawai'i (Mākua Implementation Team 2003).

Determination of Seed Banking Potential

To determine the limiting factors to increasing ex situ representation, we visited known facilities and interviewed botanists/collectors, facility managers, and conservation directors. Based on interviews, site visits, and ranking of SCI representation, a list of ex situ facilities most suited to secure the remaining collections was proposed. A preferred ex situ method was determined for each SCI, with preference given to the most cost-effective method in this order: seed banks, micropropagation, nurseries, and then botanical gardens (O'ahu Army Natural Resource Program [OANRP] 2006, Li and Pritchard 2009). The potential for seeds of each taxon to be stored conventionally (–18°C and 20% relative

TABLE 1

Ex Situ Facilities That Provided Inventories

Name	Location	Micro-propagation	Seed Bank	Nursery	Garden
Amy Greenwell Ethnobotanical Garden[a]	Hawai‘i			X	X
Div. of Forestry and Wildlife-O‘ahu[b]	O‘ahu			X	
D. T. Fleming Arboretum[a]	Maui				X
Haleakalā National Park	Maui		X	X	
Hawai‘i Island Native Seed Bank[a]	Hawai‘i		X		
Hawai‘i Volcanoes National Park	Hawai‘i		X	X	
Honolulu Botanical Gardens[b]	O‘ahu			X	X
Kōke‘e Rare Plant Facility	Kaua‘i			X	
Lā‘au Hawai‘i[b]	O‘ahu			X	
Leeward Community College[b]	O‘ahu			X	X
Lyon Arboretum[a]	O‘ahu	X	X	X	X
Maui Nui Botanical Garden[a]	Maui			X	X
Native Nursery LLC	Maui			X	
USDA-National Center for Genetic Resources Preservation	Colorado	X	X	X	
National Tropical Botanical Garden[a]	Kaua‘i		X	X	X
O‘ahu Army Natural Resources Program[a]	O‘ahu		X	X	
Olinda Rare Plant Facility[a]	Maui			X	
Pahole Rare Plant Facility[b]	O‘ahu			X	
UC-Irvine[b]	California		X	X	
‘Ulupalakua Ranch	Maui			X	
U.S. Army-Pōhakuloa Training Area	Hawai‘i		X	X	
Volcano Rare Plant Facility[a]	O‘ahu		X	X	
Waimea Arboretum[b]	O‘ahu			X	X

Note: Although some facilities may have more than one ex situ type (i.e., seed bank and nursery), some facilities may have provided an inventory for only one type.

[a] Indicates facility visited for survey.

[b] Indicates visited within 2012 before survey.

humidity [Walters 2004]) in seed banks was classified as “high,” “low,” or “unknown.” This was determined by reviewing viability tests conducted at seed banks in Hawai‘i. Taxa were classified to have “high” storage potential if seeds had been desiccated and remained viable in cold storage (4°C or −18°C). If seeds were not viable after desiccation, the taxon was classified as “low.” The storage potential for each SCI that has not been tested at those facilities was classified using information on that taxon from the Royal Botanical Gardens-Kew Seed Information Database (Royal Botanic Gardens Kew 2008). All SCI with no information on seed storage potential were classified as “unknown.” Taxa with “low” or “unknown” storage potential need to be studied and likely maintained with ex situ storage methods other than conventional seed banking until determined otherwise.

Limiting Factors to Increasing Ex Situ Capacity and Determination of Creating an Ex Situ Network

All staff at the ex situ facilities and conservation programs were asked a series of standardized questions depending on whether or not they were employed at an ex situ facility or a conservation program and/or collecting agency (Supplemental Appendix S1, available online from www.BioOne.org). In addition to quantifying the ex situ representation of SCI, questions focused on determining if and how programs utilized ex situ goals and what were the limiting factors to increasing their plant conservation efforts. Interviewees were also surveyed for their interest in participating in a plant conservation network and were asked whether there was a potential benefit to integrating ex situ facilities with collection and recovery efforts in a coordinated network. Other questions were directed toward determining current data management methods; the degree of interaction among conservation programs, collectors, ex situ services; and how access to ex situ services can be improved. All responses were quantified and ranked according to prevalence.

RESULTS

There are 724 SCI, which represents 54% of the flora (Supplemental Appendix S2, available online from www.BioOne.org). Their distributions range from all major islands to endemism on a single island, and estimates of in situ wild plants range from zero to thousands. There are 528 (73%) SCI secured with some level of representation. Eighteen taxa that are extinct in the wild have at least one founder represented. Sixty-four percent of the 528 secured taxa are represented by collections from 10% or less of the known wild individuals. Nine percent of the secured taxa are represented by 85%–100% of the known wild plants (Figure 1). Half of the secured SCI are represented at only one or two ex situ facilities. Thirty-seven of the 196 unsecured species are estimated to have less than 50 wild plants remaining in situ. Of the 169 secured taxa with less than 50 wild plants, 29% are represented at only one ex situ facility, and 22% are represented ex situ by ≤10% of the remaining wild plants. There are 522 species

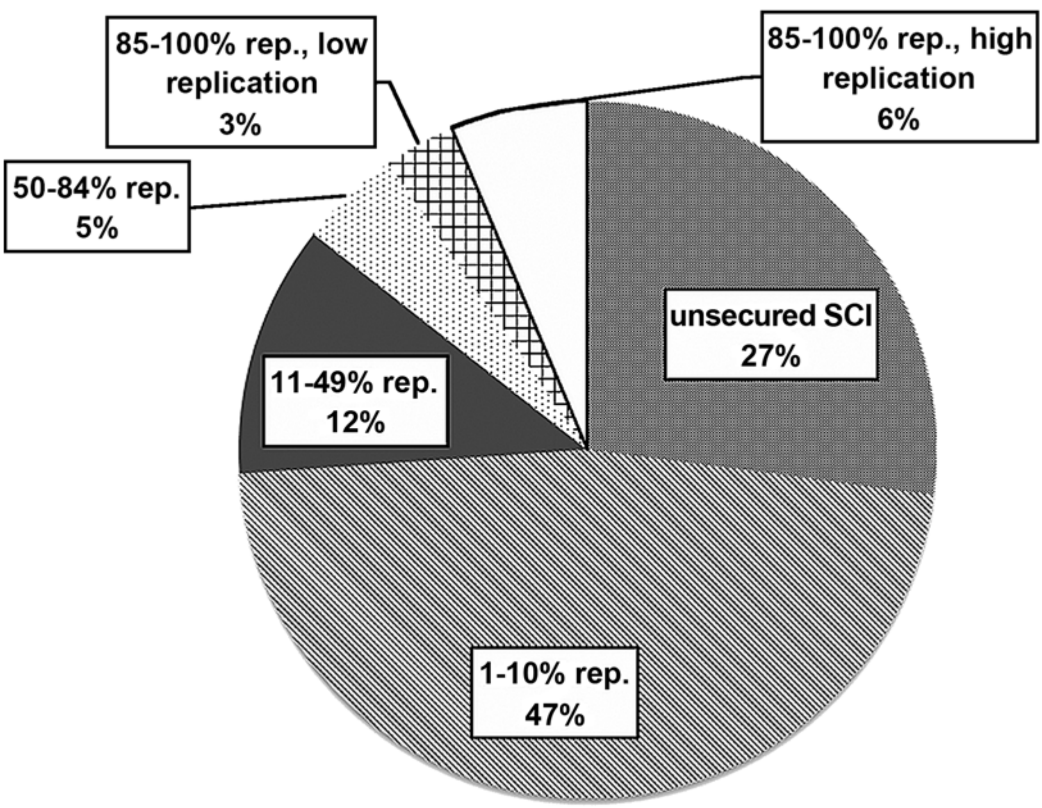

Figure 1. Ex situ representation of all 724 species of conservation importance (SCI). SCI are grouped by how representative they are of the wild plants: 0% (unsecured), representative of 1–10%, 11–49%, 50–84%, and 85–100% of the wild plants. Representation at 85%–100% was further split between SCI that have high (≥50 seeds, three explants or plants) and low (<50 seeds, three explants or plants) replication of propagules in storage.

considered threatened, 51 species considered potentially threatened, and 151 considered to be common.

Ex Situ Facilities

Of the five micropropagation laboratories surveyed, only one is active in the conservation of native Hawaiian flora. One other laboratory expressed interest in conducting research but is a teaching laboratory not staffed as a storage facility. There are currently four seed banks in the state with various capacities for seed storage preparation and viability testing. In addition, several facilities store seeds (short-term and long-term) without applying internationally accepted storage preparation methods or viability testing (Smith et al. 2004). There are 15 nursery and eight garden facilities that maintain living collections of SCI.

Seed banks represent more SCI than any other ex situ method (48% SCI in seed banks, 15% in micropropagation, 37% in nurseries, 41% in botanical gardens). Furthermore, although approximately 20% of the collections in both seed banks and micropropagation are highly representative of the remaining wild plants (≥85% of plants represented), seed banks have over three times as many SCI stored with this high level of representation (Figure 2). The majority of SCI, 78%, have "high" seed bank storage potential. The proportion of unsecured species that have "high" storage potential is 71%, suggesting that the majority of these taxa can be stored in seed banks. Ultra-low temperature storage or cryopreservation practices are currently being researched at two seed-banking facilities, and protocols have yet to be established for Hawaiian taxa.

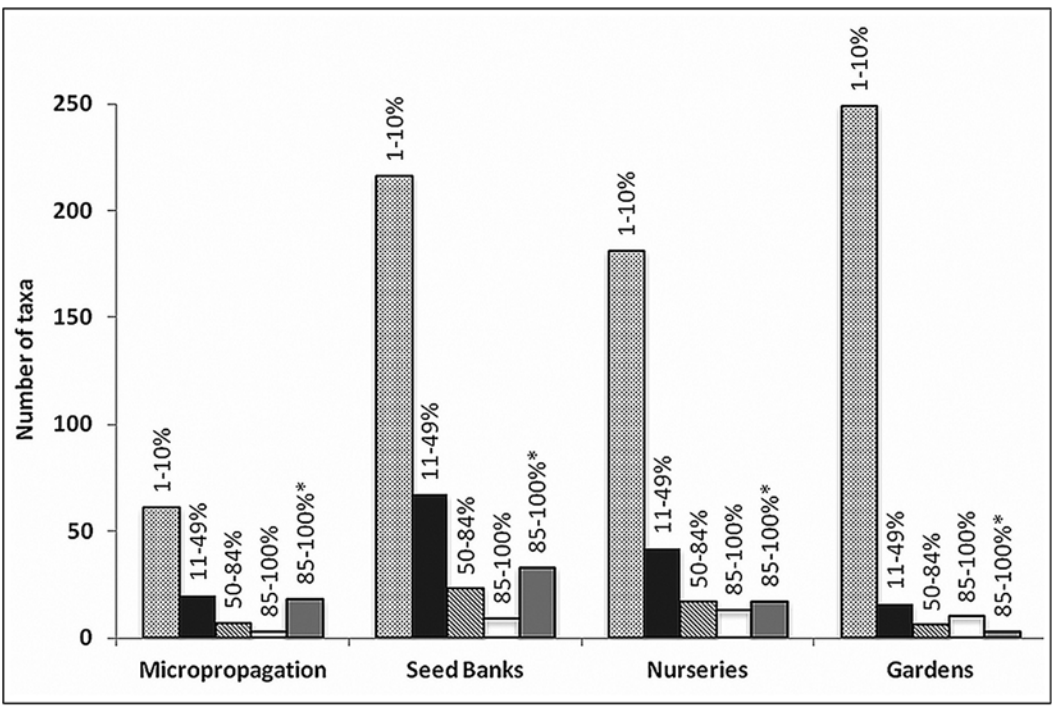

Figure 2. Number of taxa grouped by percentage of founder representation by ex situ type. Representation was determined by the number of wild plants represented in the collection with the largest number of wild plants by ex situ type. *, high replication (≥50 seeds, three explants or plants) of propagules in storage.

Interviews

There were 24 ex situ facility managers and 19 individuals from conservation programs interviewed. Lack of funding was the most frequent limiting factor to sustaining and increasing collections, indicated by 81% of programs. A lack of adequate funding for helicopter transport, staff, and infrastructure improvements was listed as the area most needing additional funds. Sixty percent of interviewees (26 out of 43) noted a lack of data management, database use, and collaboration to be a major obstacle in securing SCI ex situ, and this was the second most common obstacle noted. Only three programs had ex situ goals for specific SCI. These three programs also are the only programs to keep track of maternal lines from wild plants, through ex situ collections to in situ outplantings. The third most common limiting factor (47%) was the lack of trained staff to conduct both in situ and ex situ actions and services, such as collecting, propagating, or preparing seeds for storage. The fourth most frequently noted obstacle (42%) was the lack of ex situ facilities on their islands and the communication with existing facilities. Seventy-five percent of ex situ facilities have little to no space and infrastructure to expand. Other limiting factors included the lack of space for expanding ex situ services (40%), the lack of sanitation protocols for transporting propagules among facilities (23%), and in situ limitations such as the lack of suitable outplanting locations or difficulty in securing viable propagules for propagation (21%). Staff at the State of Hawai‘i's Division of Forestry and Wildlife indicated a need for seeds of common species to be readily available for both postfire seed-sowing restoration efforts and watershed protection. All ex situ facilities and conservation programs indicated that a coordinated network of ex situ facilities would enhance services and benefit their programs.

DISCUSSION

Years of diligent collections and maintenance by Hawai‘i's conservation programs has led to ex situ representation of 73% of the 724 identified SCI, a substantial achievement. This effort is successful compared with the progress of North America, which as of 2010 had 39% of its threatened flora represented ex situ (Kramer et al. 2011). Securing the 196 SCI that are not represented in ex situ collections, in order of rarity, should be a priority. Another priority should be securing ex situ collections that are better representative of the genetic variation in the remaining populations (Hamilton 1994, Brown and Marshall 1995, Havens et al. 2004, Volis and Blecher 2010). Only 26% of SCI are potentially represented by more than 10% of wild plants, and this is not adequate. Seed storage is the most efficient way to represent taxa with genetically diverse collections and costs only 1% of the costs to maintain plants in their native in situ habitats (Li and Pritchard 2009). Increased access to this service should be the priority for conservation programs statewide.

Hawai‘i's seed-banking capacity can increase with financial investments to obtain the staff, infrastructure, and equipment for conducting research and developing protocols to properly germinate, dry, and store seeds. Seed banking should be promoted as a necessary component of land conservation and plant recovery and reintroduction plans (Cochrane et al. 2007). It is a service that could be financially compensated in written contracts and included as a component of grants applied for by the conservation programs that rely on these services. Hawai‘i's seed-banking capacity should also expand to include cryopreservation, especially for species with seeds with low seed-banking potential (i.e., recalcitrant or desiccation-sensitive seeds), because these services are considered the most cost-effective and practical long-term ex situ storage option for species with seeds that cannot be stored conventionally (Li and Pritchard 2009, Pence 2013).

Increased collaboration of the existing seed banks in Hawai‘i could help increase their current capacity without much increase in funding. This network of seed banks would be most effective if connected to in situ collectors, natural resource managers, and all other ex situ facilities. A network in Hawai‘i could also coordinate research efforts with the

U.S. Department of Agriculture-Agricultural Research Service (USDA-ARS) National Center for Genetic Resources Preservation (NCGRP), which serves as the national seed storage research center. Increased emphasis could be placed on collections of common (not rare) SCI that could be used in the case of catastrophe (fire, hurricane) and to restore habitats, a need identified by conservation directors for the State of Hawai‘i's Division of Forestry and Wildlife. This effort would align with the mission of the Bureau of Land Management's Seeds of Success program (SOS) and the Royal Botanic Gardens, Kew Millennium Seed Bank (MSB), which has established protocols and collection goals for common native species. SOS currently has over 13,000 seed collections from common species throughout the nation <http://www.nps.gov/plants/sos/> (Byrne and Gordon 2009).

Micropropagation services are critical for species that do not have seeds that can be stored conventionally in seed banks (Pence 2013). In vitro protocols have been developed for approximately 300 SCI (N. Sugii, pers. comm.). Species in the Campanulaceae, Lamiaceae, and Gesneriaceae families (Sugii 2011); pteridophytes and bryophytes (Sugii 2011); Orchidaceae (Pence 2004, Ballesteros et al. 2012); and species that cannot be stored in conventional seed storage are best suited to this method for long-term storage.

Nurseries play a crucial role in producing plants for restoration projects and holding living collections of rare taxa as part of breeding collections. The system of mid-elevation nurseries on most main islands serves many plant conservation programs and is mostly underutilized. The lack of a facility to propagate collections on Moloka‘i provides the greatest opportunity to expand these services. Another opportunity to expand ex situ nursery services would be to establish a nursery on Hawai‘i Island at a lower elevation that could be used to grow plants for dry-forest restoration. Sanitation protocols should be developed that could be adopted by facilities statewide to aid in the transfer of plants without pests or pathogens.

The role of botanical gardens in conservation efforts worldwide could expand (Sarasan et al. 2006, Long et al. 2010). Few collections of native plants at botanical gardens are of high conservation value due to unknown provenance or having been cultivated from only a single founder. Conservation programs could identify taxa that are good candidates to be maintained in botanical gardens. Good candidates would be taxa that are long-lived, in need of controlled breeding or exposure to ambient pollination, have research needs that would benefit from a living collection, will not interbreed with related species, have high ornamental value, and are culturally significant (see Oldfield 2009). A limiting factor to establishing collections in botanical gardens is the overlap of habitat conditions in the gardens with that of the wild plants.

Formalizing an Ex Situ Network in Hawai‘i

Plant conservation networks have brought together collectors and ex situ facilities in other regions and should serve as models for a similar network in Hawai‘i. Seed-bank networks such as GENMEDOC, which includes the West Mediterranean islands; The European Native Seed Conservation Network, which coordinates native seed conservation within Europe (Oldfield 2009); and the Australian Seed Bank Partnership have successfully increased collaboration and promoted the exchange of technical information within their regions. The New Zealand Plant Conservation Network and the Australian Plant Conservation Network have both worked to improve the practice of plant conservation and the efficiency in achieving outcomes (Godefroid et al. 2011, Sawyer 2013). They have produced seed collection, processing, curating, and storage manuals for seed banking and created a venue for meetings, workshops, and exchange visits for partners to share experiences, ideas, and protocols. MSB is the largest ex situ plant conservation project in the world and is managed by the Royal Botanic Gardens, Kew. It has a large network of collectors around the world with the goal of securing 25% of the world's flora by 2020. Recently MSB has hired a coordinator to oversee increasing collaborations and collections from the Pacific region, and Hawai‘i has begun discussions about using MSB protocols and seed-banking research and storage facilities. The possibility of a plant conservation network in Hawai'i is appealing to MSB, which favors a formal agreement with one program to represent the state's collections (P. Giovannini, pers. comm.).

Hawai'i's informal network of programs has been working together to share expertise and facilitate plant conservation. To strengthen and formalize this network, an entity should be identified or created that can represent the consortium of involved programs, fund network projects and infrastructure improvements, and facilitate progress toward their shared goals. This entity should

- coordinate propagule transfer between collectors and ex situ facilities;
- standardize curation of SCI and maintain a statewide database;
- develop and implement standardized protocols for collection, propagation, and storage methods;
- increase communication between programs via meetings, workshops, published protocols, and news updates;
- develop and implement sanitation protocols for transferring plants between facilities;
- identify research needs for rare plant conservation;
- write a comprehensive conservation plan for SCI including ex situ goals;
- identify unsecured taxa despite repeated collections and propagation attempts.

Comprehensive Strategic Plan

Conservation goals are necessary for adequately representing plants with ex situ collections. Goals should be adopted that are reflective of the current literature in capturing genetic diversity within plant populations (Offord and Meagher 2009, Sawyer 2013). Targets from the 2020 Global Strategy for Plant Conservation (Havens et al. 2004) should be adopted in Hawai'i. In particular, goals for conserving global plant diversity with ex situ methods (Target no. 8 of the Global Strategy for Plant Conservation) were revised in 2011 to secure 75% of the world's threatened plant species in ex situ collections and 20% with propagules available for restoration programs by the year 2020. Of Hawai'i's 522 threatened SCI, 379 (73%) are represented to some degree. This is very close to the 75% goal, but over half of the collections are from less than 10% of the known wild plants. Increasing the genetic diversity within collections has been identified as a need in the 2010 North American ex situ assessment as well (Hird and Kramer 2013).

By continuing to develop the existing facilities on each island and improving access to all ex situ services across the state in the ways suggested here, Hawai'i will be better able to meet the challenge of securing propagules from its unique flora. This type of assessment can be applied to other regions that do not have a unified and consistent method of tracking ex situ representation. The benefit would be to prioritize species in need of ex situ representation and identify adequate and cost-efficient methodology in ex situ storage and limiting factors to expanding capacity.

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