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CAN A BOTANIC GARDEN CYCAD COLLECTION CAPTURE THE GENETIC DIVERSITY IN A WILD POPULATION?

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Premise of research. Conservation of plant species often requires ex situ (off-site) cultivation of living collections. Cycads constitute the most imperiled major group of plants, and ex situ collections are an important part of conservation planning for this group, given seed recalcitrance, difficulties with tissue culture, and ongoing in situ threats. Very little is known about the genetics of ex situ conservation collections of cycads. Thus, this study seeks to illuminate how well an ex situ collection of a cycad can capture the diversity in a wild population.

Methodology. A model species, *Zamia decumbens*, was chosen on the basis of geographic isolation and detailed census knowledge, which allowed near-total sampling of in situ plants. Overall, 375 in situ plants were compared to 205 ex situ plants via 10 microsatellite markers.

Pivotal results. Genetic-distance analysis shows high fidelity of the ex situ collections to their in situ source populations as well as clustering of ex situ progeny by accession and strong identity with their respective mother plants. Structured resampling of allele capture from the in situ populations by the ex situ collections shows that allele capture increases as number of ex situ plants maintained increases, but with a diminishing rate of increase.

Conclusions. These data demonstrate that botanic garden collections can better conserve the genetic diversity of in situ cycad populations if four recommendations are followed: (1) use the species biology to inform the collecting strategy; (2) manage each population separately; (3) collect and maintain multiple accessions; and (4) collect over multiple years.

Keywords: conservation genetics, ex situ conservation, living collections, microsatellite, *Zamia decumbens*.

Introduction

Ex Situ Conservation at Botanic Gardens

In recent decades, botanic gardens have enthusiastically adopted conservation as a major mission area. This is readily seen not only in the mission and purpose statements of gardens but also in major guiding documents for the community, including the Global Strategy for Plant Conservation (GSPC) of the Convention on Biological Diversity (Sharrock 2012). Among the formal targets of the GSPC are benchmarks for ex situ (off-site) conservation, which specify that 75% of threatened taxa be conserved ex situ and made available for reintroduction. Botanic gardens are increasingly focused on ex situ conservation (Raven and Havens 2014), and guidelines for creating genetically diverse ex situ collections are available, especially for germplasm banking (Guerrant et al. 2004, 2014). However, these are largely based on crop resource genetics models and are focused on crops and crop wild relatives (e.g., Marshall and Brown 1975; Cohen et al. 1991). Major genetic issues in ex situ conservation strategy deal with potential inbreeding depression (Hedrick and Kalinowski 2000), loss of alleles through genetic drift (Brown and Briggs 1991), and selection (Schaal and Leverich 2004) under the ex situ environment.

In addition to germplasm banking, living plant collections can also contribute to ex situ plant conservation (Dosmann 2006; Cibrián-Jaramillo et al. 2013). Here, we distinguish between germplasm collections, such as seed and tissue cultures (which are live), and "living collections," which are horticulturally or arboriculturally maintained plants accessioned into the holdings of a botanic garden—i.e., the flora cultivated on the grounds of an arboretum. These living collections are particularly important for ex situ conservation of exceptional species (i.e., species that cannot be conserved ex situ through standard seed- or tissue-banking protocols; Pence 2011). In recent years, progress has been made in investigating the best

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genetic ex situ conservation strategies for living plant collections, particularly for threatened exceptional species that are not considered economically critical (reviewed in Cibrián-Jaramillo et al. 2013)

Cycads: A Flagship Group for Ex Situ Conservation

Cycads (order Cycadales) are the most threatened major group of plants in the world (Baillie et al. 2004); the majority of the 331 extant species (Osborne et al. 2012) are listed on the IUCN Red List of threatened species, and more than 75% are threatened with extinction (Gilbert 2010). Cycad species are of great research interest because they represent a lineage that is Paleozoic in origin and was once much more diverse and widespread but has extant diversity that is Cenozoic in origin (Nagalingum et al. 2011; Salas-Leiva et al. 2013). Given their long history, living cycads are often used in teaching collections at botanic gardens and universities.

Cycads are highly sought after by collectors throughout the world. They are also highly ornamental and often drought tolerant, making them very desirable for environmental horticulture. Their desirability, combined with their very slow growth rates and challenges in propagation (Calonje et al. 2011), results in high prices in the marketplace (Kay et al. 2011). This high price drives one of the major threats to extant cycads: overcollection of wild plants for the horticulture trade (Donaldson 2003). Habitat destruction (Calonje et al. 2013) and invasive insect species (Marler and Moore 2010; Magellan et al. 2013) are the other significant threats.

The IUCN Species Survival Commission's Cycad Specialist Group has developed a plan for cycad conservation (Donaldson 2003), with the overall objective of conserving these species in the wild while ensuring that ex situ collections are available to complement in situ conservation strategies (Walters 2003). This is due to the speed at which many populations are declining in the wild (e.g., Marler 2012), combined with the challenges in reversing that decline in habitat.

Biological and horticultural factors also support ex situ living collections of cycads as the immediate appropriate response to conservation threats. First, whereas cycad pollen can successfully be cryopreserved (Osborne 1989), large-scale seed banking has not yet been possible with cycads, as their seeds are considered recalcitrant (e.g., Woodenburg et al. 2007), although work in this area is ongoing (Pritchard et al. 2011; Berjak and Pammenter 2014). While tissue-culture methods have been developed to successfully recover plantlets via somatic embryogenesis for different cycad genera (Chavez et al. 1998; Chavez and Litz 1999), successful plantlet acclimation to ex vitro conditions remains elusive (P. Moon, personal communication). Furthermore, low germination rates, low seedling survival rates, and long generation times can make in situ cycad population recovery slow (Raimondo and Donaldson 2003), whereas knowledge of appropriate conservation horticulture for these species facilitates rapid ex situ protective cultivation (Calonje et al. 2010; Murphy et al. 2013). In addition, in some cases obligate pollinators have been thought to be reduced or extirpated (e.g., Vovides et al. 1997), making natural in situ recovery virtually impossible. Four cycad species, including the famous *Encephalartos woodii*, are extinct in the wild (IUCN 2013) and thus already fully dependent on ex situ cultivation. Given all of these circumstances, ex situ collections are especially important to the survival of this plant group.

Population Genetics of Cycads

Given the conservation concerns in this group, population genetics of cycads has seen focused study in recent years. As one example, González-Astorga et al. (2008) compared imperiled *Dioon* species and found inbreeding in some species yet excess heterozygosity in others. Placing such results in a context, their broader review of cycad population genetic studies (8% of taxa) showed that observed heterozygosity is generally lower than expected in this group (González-Astorga et al. 2008). This is consistent with inbreeding, perhaps because of the documented decline of many cycad populations. For example, *Cycas micronesica* shows low to moderate inbreeding within populations (Cibrián-Jaramillo et al. 2010), and this once-numerous taxon has undergone severe recent decline. But perhaps low heterozygosity does not indicate imperilment for cycads. For example, a recent study of the narrow insular endemic *Zamia lucayana* found two of the three extant populations to have low observed heterozygosity and all populations to have moderate inbreeding (Calonje et al. 2013). Whereas this species is listed as endangered (IUCN 2013), recent direct negative effects from overcollection, habitat destruction, or invasive species were not observed (Calonje et al. 2013).

Despite the increased research attention on in situ cycad conservation genetics, thus far only a single study (Da Silva et al. 2012) has examined the genetics of an ex situ cycad collection, using amplified fragment length polymorphism markers. That innovative study found that the ex situ holdings of *Encephalartos latrifrons* at Kirstenbosch Botanical Garden reflected the in situ diversity and even held a genotype group no longer found in situ. But, given that the Da Silva et al. (2012) study involved a single South African species, and as noted in another recent work focusing on Caribbean island cycads (Meerow et al. 2012), perhaps it is as yet too early to make generalizations regarding population genetics of all cycads.

Need for and Feasibility of This Study

Given the limited ability to generalize on the basis of previous cycad population genetics studies, the urgency of cycad conservation, and the need for genetically diverse ex situ collections, there is a clear need for targeted genetic evaluation of ex situ collections of cycads. Such an assay will inform and refine conservation work, as outlined in the Cycad Action Plan (Donaldson 2003). Comparing the population genetics of ex situ collections with that of their wild source populations can provide direct insight into the effectiveness of such collections at capturing and representing in situ genetic diversity (Griffith et al. 2011). This is the motivation for our study.

Following Meerow et al. (2012) and related studies, molecular markers now developed for *Zamia* have allowed conservation genetic investigation in this genus (e.g., Calonje et al. 2013). Thus, this presents an opportunity to further develop an understanding of botanic garden collection genetics (cf. Namoff et al. 2010).

Material and Methods

Model System

For our case study, the effectiveness of ex situ conservation protocols at the Montgomery Botanical Center (MBC; Coral Gables, FL) in capturing in situ genetic diversity were examined. MBC cycad collections have been structured with the goal of maximizing genetic diversity at the population level (Husby et al. 2007). This strategy was devised in view of the well-understood negative influence on conservation of low genetic diversity, due to inbreeding depression (Schemske et al. 1994; Frankham 1995), and loss of alleles through genetic drift in small collections (Gale and Lawrence 1984). Estimates of genetic diversity from allozyme data on Caribbean *Zamia* (Walters and Decker-Walters 1991) informed the current population-based collecting protocol at MBC, which seeks to curate at least 15 plants from each population, derived from at least five mother plants.

The sinkhole cycad *Zamia decumbens* was selected as a model species for this investigation (fig. 1). This species is known from a limited area of the Maya Mountains in southern Belize and is currently considered critically endangered (IUCN 2013). At the time of its description (Calonje et al. 2009), the species was known from two main populations of 234 and 183 plants, restricted to two limestone sinkholes separated by 7 km, and a few scattered hilltop populations of no more than 12 plants each. The remote, isolated locations preclude any potential introgression of other *Zamia* spp. from horticulture or in situ plants. Also, the feasibility of a genetic assay using this species with molecular markers from ongoing *Zamia* research (e.g., Meerow et al. 2012) was demonstrated in a pilot screen.

Sampling Protocol

This study is focused on the two sinkhole populations and compares these wild plants to cultivated plants in MBC ex situ collections derived from these populations. The two sinkhole populations were selected for this analysis because they represent discrete populations with every adult individual known and tagged. No intermediate populations between these two sites have been found in extensive surveys of surrounding forest habitat. This allows for extensive, near-total sampling for this assay. These two in situ populations, here called Sinkhole 1 (SH1) and Sinkhole 2 (SH2), were compared to living collections developed from seeds collected during fieldwork in 2010. The ex situ plants are curated as separate accessions, defined as collections derived from single, separate mother plants (three accessions from SH1, four accessions from SH2; see table 1).

DNA Microsatellite Data

DNA isolation, PCR amplification, and subsequent visualization of simple sequence repeat fragments follow protocols described by Meerow and Nakamura (2007). We used 10 DNA microsatellites for this analysis, which were developed for Caribbean *Zamia* studies: Zam28, Zam33, Zam53, Zam59, Zam60, Zam61, Zfg23, Zfg25, Zfg32, and Zfg33 (Meerow et al. 2012).

Population Genetic Assay and Structured Resampling

Comparative estimates of genetic distance (Nei 1978) and multivariate analysis of genetic distance (following Orloci 1978; Huff et al. 1993) were implemented in GenAlEx, version 6 (Peakall and Smouse 2006). To assay the degree of diversity captured via the current population-based collecting protocol, the amount of allele capture was compared between the in situ populations and the ex situ collection, on the basis of protocols developed by Namoff et al. (2010). The sample size of the *Zamia decumbens* ex situ collection ($n = 205$ for the two populations together; table 1) was much larger than many garden collections; the protocol at MBC calls for a planting of 15 plants per population, or 30 total plants in this case. Therefore, resampling of the collection data, without replacement, to obtain randomly selected model populations (hereafter referred to as "resamples") was performed. These resamples were composed of randomly selected entire accessions (plants from one mother; i.e., half-sibling cohorts). The resamples were structured to include one to seven accessions and 1–205 individuals (i.e., encompassing the entire range of the current ex situ collection). Estimates of genetic capture for these random samples were made by comparing each resample to the population via GenAlEx and comparing the proportion of private alleles to the total of alleles in the population and the resample. Allelic capture was modeled as a function of number of individuals in the collection, with a saturation growth rate model (genetic capture $= (m \times \text{collection size})/(r + \text{collection size})$) that was originally developed on the basis of saturation kinetics of enzyme reactions but applies to other biological processes (Ware et al. 1980), and was fitted in CurveExpert Professional (Hyams 2014) using least squares. It expresses a law of diminishing returns. In this model, the parameter m is the maximum possible genetic capture (100%), which is fixed, and r controls the rate at which the model approaches the maximum capture (this parameter is used for the least squares fit to the data). This type of model has the advantage of providing a good fit to the data while also taking into account the known constraints of the model system: a 100% asymptote ($a = 100$) and an origin at 0 (0% genetic capture when there are no plants in the collection).

Results

Distance Analysis

Genetic-distance analysis shows a high degree of fidelity to its source population for each ex situ collection. The ex situ collections from SH1 and SH2 show the closest identity with their source populations, and the two in situ populations show greater distance from each other and from the other sinkhole's ex situ collection (table 2). Multivariate analysis of genetic distance by individual plants also shows a clear distinction between SH1 and SH2 and high identity of the ex situ collections with their respective source populations (fig. 2).

Genetic Capture by Ex Situ Collection

Including all available accessions, the ex situ collections capture 70.0% of the alleles in SH1 and 73.6% of the alleles

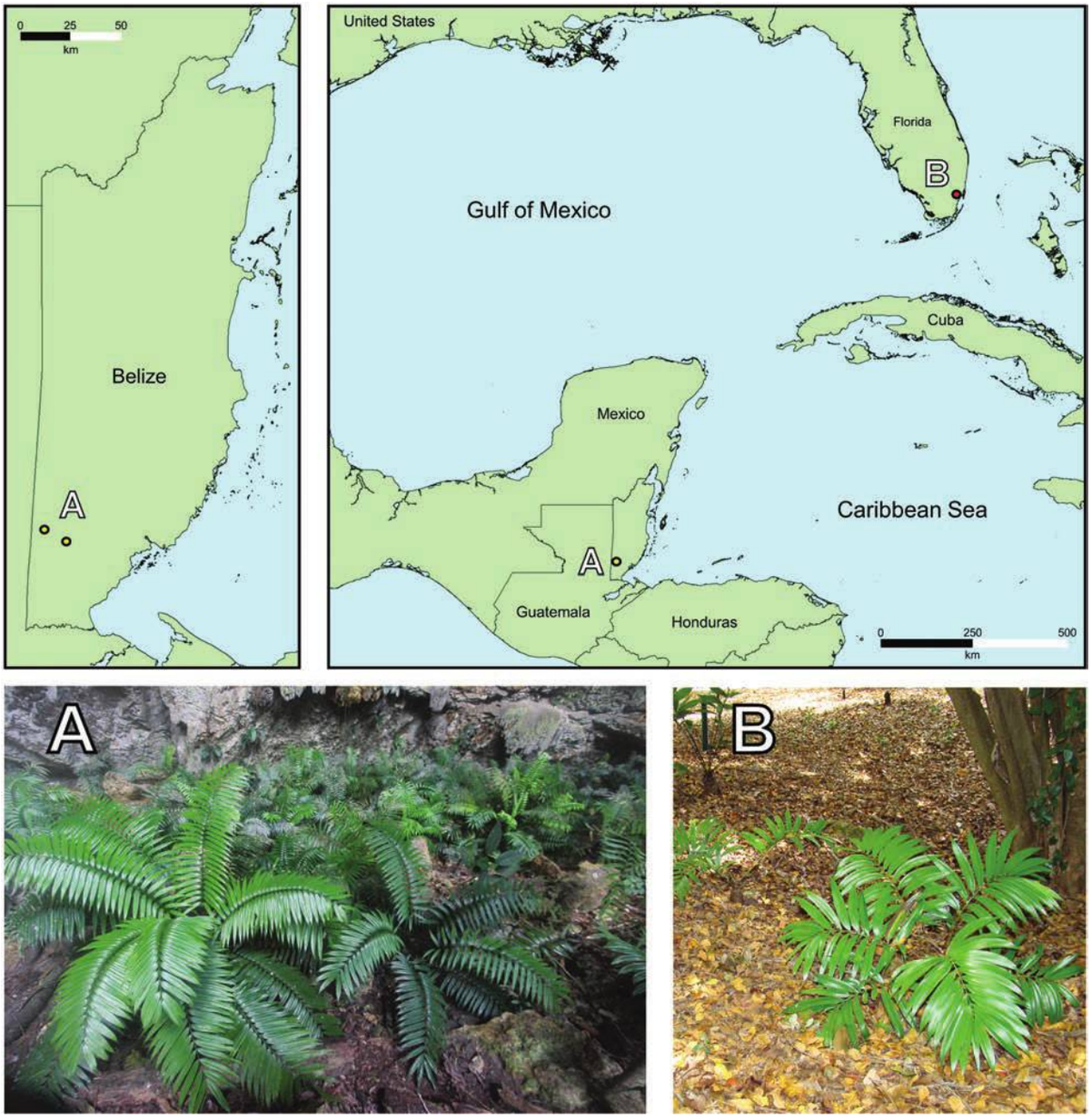

Fig. 1 Model system used in this study. The two largest *Zamia decumbens* populations are restricted to two large limestone sinkholes in southern Belize (location A in top panels). These in situ plants (*A*) were compared to ex situ plants at Montgomery Botanical Center (*B*; location B in top-right panel), which were grown from seed collected in those sinkholes in 2010.

in SH2, if treated separately, and 77.6% of the alleles if all ex situ plants are compared to the two sinkhole populations combined (fig. 3). Structured resampling of allele capture by collection size shows an increase in genetic capture as collection size increases (table 3; fig. 4). Collections composed of a single accession (seven possible iterations, 14–46 individual plants) captured between 27.63% and 56.57% of the alleles in the in situ populations, while the full ex situ collection of 7 accessions (one possible iteration, 205 plants) captured 77.63% of the in situ alleles. A proportional decrease in the rate of increase is also indicated through a "diminishing-returns" relationship (fig. 4; $r^2 = 0.585$), estimated as genetic capture = (100 × collection size)/(46.7155 + collection size).

Discussion

Insights and Recommendations from This Model System

The title of this article asks a straightforward question: can a botanic garden cycad collection capture the genetic diversity

Table 1

Sampling Structure for *Zamia decumbens* Populations Used in This Study

Code	Source	Type	*N* plants
Plants from Sinkhole 1 population:			
SH1	Sinkhole 1	In situ	195
SH1-101	Accession 101	Ex situ	46
SH1-103	Accession 103	Ex situ	34
SH1-108	Accession 108	Ex situ	14
Total ex situ			94
Plants from Sinkhole 2 population:			
SH2	Sinkhole 2	In situ	180
SH2-027	Accession 27	Ex situ	21
SH2-031	Accession 31	Ex situ	31
SH2-085	Accession 85	Ex situ	31
SH2-135	Accession 135	Ex situ	28
Total ex situ			111

Note. "In situ" is defined as wild plants in naturally occurring populations; "ex situ" is defined as plants in cultivation in the garden collection, grown from seed collected in the wild.

in a wild population? To our knowledge, this study represents the most intensive comparison of an ex situ plant collection with its source population in terms of sampling depth, considering the number of individuals examined versus the number of individuals known to exist in the wild. Thus, this model can provide direct insights on the efficacy of ex situ collections in conserving diversity for cycads and can also help inform such work for other plant groups. While guidelines on numbers of propagules and collections offer a necessarily pragmatic and practical starting point (e.g., Seeds of Success 2012), perhaps a unified collecting protocol will not best serve the goal of high genetic capture in all cases (Guerrant et al. 2014). The findings presented here are consistent with what is known about the biology of *Zamia decumbens* and cycads in general. A general summary of these findings could be framed thusly: the biology of the target species must be carefully considered in developing a strategy for ex situ cultivation. Here, we frame our discussion in this way (biology informs strategy), considering our case study of *Z. decumbens*. In this way we can begin to answer the basic question posed by the title of this article.

Different species may require different sampling protocols if genetic diversity is the collecting goal. A similar assay for a palm, *Leucothrinax morrisii* (Namoff et al. 2010), showed that a collection of 59 plants captured more than 90% of the genetic diversity in a single population and that a collection of 15 plants from three accessions was sufficient to recover a mean of 83% of alleles in the population. Employing the same three-accession collecting protocol for *Z. decumbens* could potentially recover 70% of the alleles in a single population, considering SH1 alone (fig. 3). However, a much larger number of individuals (94) contribute to this level of genetic capture. Treating the two wild populations together, seven ex situ accessions totaling 205 plants captured only 78% of the wild alleles. Thus, a difference in the rate of genetic capture as sampling increases is seen between *Zamia* and *Leucothrinax*. *Leucothrinax* is monoecious and panmictic and flowers annually and abundantly (Lewis and Zona 2008; Namoff et al. 2010). In contrast, *Z. decumbens*, like all cycads, is dioecious, and on the basis of our in situ observations in 2008, 2010, and 2014, only a small proportion of adult-sized plants within these populations are reproductive at any given time. So, in response to our basic question, ex situ collections can capture genetic diversity of cycads if mother-plant sample sizes are adequate, but cycads may require larger numbers of individuals than other plant groups.

Clear differentiation between the two sinkhole populations is demonstrated in both the genetic-distance matrix (table 2) and the multivariate analysis of individual genetic-distance data (fig. 2). In addition, strong identity of the ex situ accessions with their source populations is indicated by the same analyses. For ex situ collections management, this is important information that confirms that collecting from separate populations is crucial to represent the full range of in situ diversity of a species adequately. This can also highlight the necessity of keeping plants from different populations separately curated in the garden setting (Krishnan et al. 2013). This is consistent with management conclusions for another imperiled cycad, *Cycas micronesica*, based on microsatellite data (Cibrián-Jaramillio et al. 2010). For cycads, spatial separation in a garden setting is not typically needed because of obligate insect pollinators, but in places where these pollinators are present, exclusion measures might be needed to ensure continued fidelity of ex situ seed-propagated plants to source populations (Calonje et al. 2011). For long-lived, long-generation-time plants such as cycads, rapid genetic drift from such ex situ propagation is not as great a concern as it is for annuals and other quickly maturing species (Rucińska and Puchalski 2011). In response to our basic question, a botanic garden collection of a cycad can capture the genetic diversity of a wild population, but accessions derived from distinct wild populations should be managed separately.

The importance of collecting from multiple accessions is shown by the increase in genetic capture with increasingly larger sample size (table 3; fig. 4), but it is especially well illustrated by the multivariate analysis (fig. 2). Individual ex situ plants cluster by genetic distance around the female in situ plant (i.e., mother plant) from which they were collected. Thus, any one accession does not appear capable of representing the entire genetic space occupied by these in situ populations, but taken together, multiple accessions can overlap with a greater amount of the genetic variation seen in the wild. To consider the effect of multiple accessions in the context of our basic question, botanic garden cycad collections have a better chance of capturing the in

Table 2

Genetic Distance among In Situ Populations and Ex Situ Collections of *Zamia decumbens*

	Sinkhole 1 (SH1)	SH1 ex situ	Sinkhole 2 (SH2)	SH2 ex situ
Sinkhole 1	0	.034	.129	.123
SH1 ex situ		0	.166	.161
Sinkhole 2			0	.012
SH2 ex situ				0

Note. "Genetic distance" is defined as Nei's (1978) genetic distance.

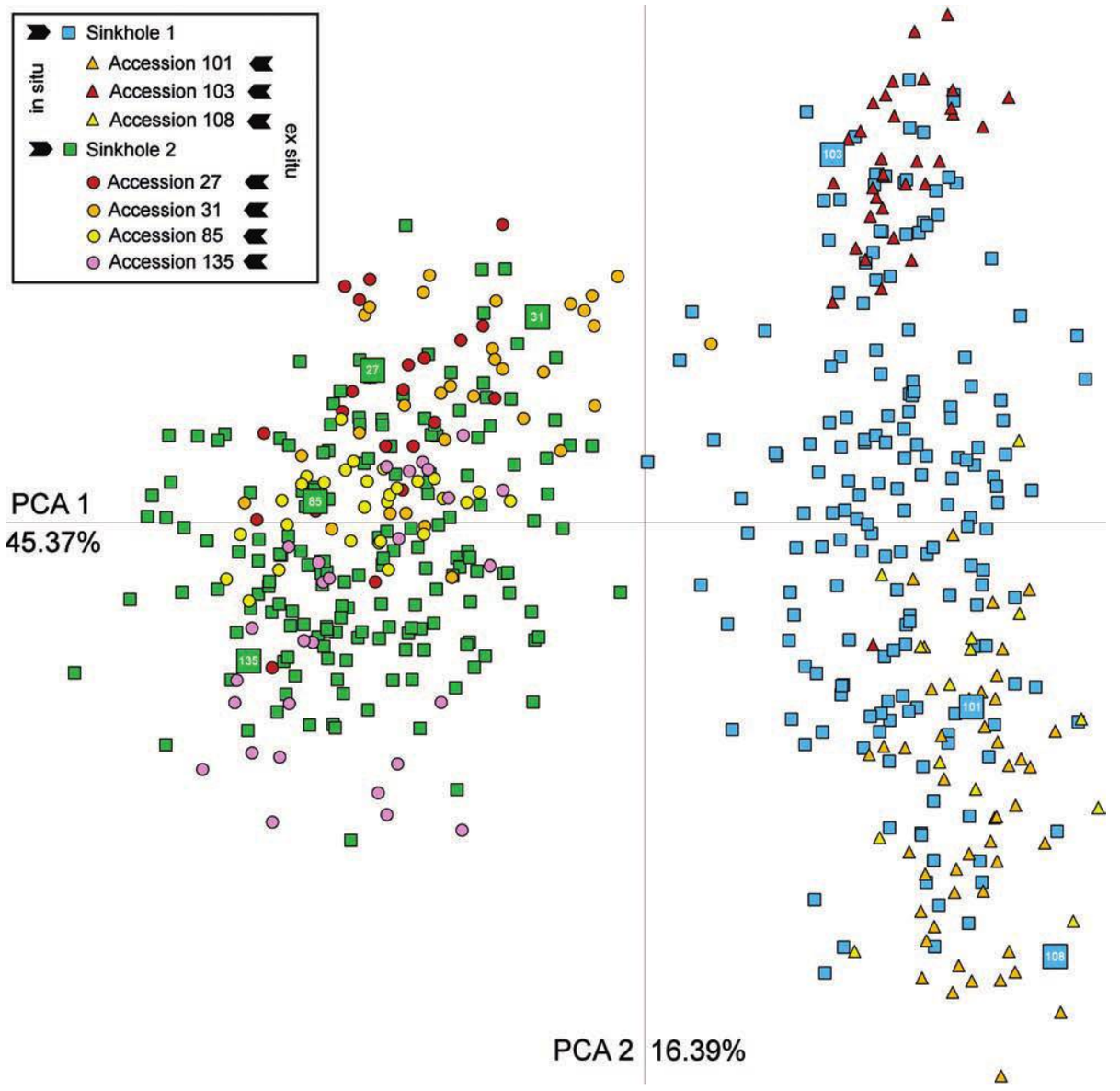

Fig. 2 Multivariate analysis of genetic-distance data for all *Zamia decumbens* plants in the study ($n = 580$; see table 1). The first two principal-components axes (PCA 1 and PCA 2) are indicated, with the percent of variation explained by each. Each point represents an individual plant. Squares indicate in situ (wild) plants, triangles indicate ex situ (botanic garden) plants from Sinkhole 1 (collected as seed), and circles represent ex situ plants from Sinkhole 2. Most genetic distance is explained by axis PCA 1, which completely separates the two in situ populations. Ex situ collections show high fidelity to the in situ population from which they were collected. In addition, the ex situ collections appear to cluster (by accession) around the mother plants from which they were collected (indicated by numbered larger square points).

situ genetic diversity of a population if multiple accessions are collected and curated.

This need for multiple accessions also suggests that collections in multiple years could yield greater genetic capture. The seven ex situ accessions included in this study represent the only seven female plants with mature cones found in 2010, out of 417 plants in the two sinkhole populations. Our population survey that year indicated that few male cones were produced in the preceding pollination season, meaning that the number of male plants contributing allelic diversity to the female cones we sampled was likely quite low. This very infrequent reproduction has been observed in other cycads, including other rainforest *Zamia* (Clark and Clark 1987, 1988). Seed collection of an entire year's output has very little effect on long-term popula-

tion health relative to loss of adult plants (Raimondo and Donaldson 2003). The ex situ collections studied here, as seed-grown plants collected in 2010, are derived from seven female plants pollinated with pollen from an unknown but likely low number of male plants. Thus, the ex situ collection has a very limited potential parentage relative to the entire population size. Thus, for *Z. decumbens*, the number of reproductive individuals in any given year is much lower than the total population size. Perhaps this can partially explain how relatively large numbers of ex situ plants are needed to reach only 78% capture of the in situ alleles, while the prior *Leucothrinax* study achieved more than 90% allele capture with only 40 or more ex situ plants (Namoff et al. 2010). Thus, the last consideration of our basic question concludes that a botanic garden cycad collection might best capture in situ genetic diversity by carefully considering the phenology of in situ populations and planning for collecting trips over multiple years if needed.

Limitations of This Model System and Future Directions

The assay detailed here represents a good step toward understanding the efficacy of ex situ cycad collections in capturing in situ genetic diversity. Namoff et al. (2010) set forward limitations of their model system using *Leucothrinax*, noting that studies of dioecious or highly restricted species could add further information. Our study helps address both of those areas, but there remain many other important life-history factors that can influence successful ex situ conservation work. Among cycads and even among *Zamia* species, there remains considerable variation in reproductive output and phenology, and the model presented here may not fit in all cases. For example, Caribbean species of *Zamia* appear to cone in a higher proportion and more often than *Zamia* species from rain forests (Negrón-Ortiz et al. 1996). Future examination of a cycad species with such life-history traits would further inform the main question of this article.

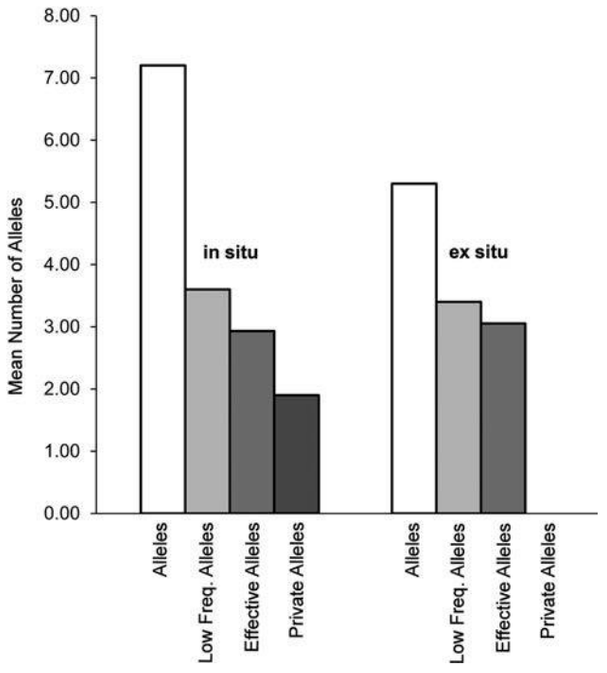

Fig. 3 Summary of alleles for Sinkhole 2 in situ and ex situ plants. Low-frequency alleles are defined as those with <5% frequency. For these two groups, the number of alleles observed in situ (7.2) minus the private alleles observed in situ (1.9) is equal to the alleles observed ex situ (5.3). Thus, the ex situ collection captured 73.6% of in situ alleles. No private alleles were observed in the ex situ collection. Sinkhole 1 shows a similar pattern, with no ex situ private alleles and 70.0% allele capture, as does analysis with the two wild populations combined, with 77.6% allele capture (graphs not shown).

Table 3

Allele Capture by Number of Accessions

No. accessions	No. plants	Mean genetic capture (%)
1	14–46	41.16 ± 12.7
2	35–80	56.07 ± 10.2
3	80–111	67.98 ± 1.8
4	94–142	70.78 ± 4.5
5	111–170	73.38 ± 3.0
6	159–191	76.33 ± 1.0
7	205	77.63

While ex situ conservation collections are often critical to achieve conservation goals, an integrated framework of other needs is well known (Havens et al. 2014). One separate but closely relevant aspect concerns the potential for reintroduction of these ex situ plants. As noted above, future reintroduction is an important goal of ex situ work (Sharrock 2012). Reintroduction potential is highlighted as an important indicator for conservation value of plant collections (Cibrián-Jaramillo et al. 2013), and one important study in this area looked at the potential for garden collections of a critically endangered cycad to augment in situ populations (Da Silva et al. 2012). In our study, the basic question defined our goal as evaluating genetic capture, which is the fundamental basis for ex situ conservation and for future reintroduction success. Our ex situ plants derived from fieldwork in 2008 and afterward are not yet reproductively mature, so reintroducible propagules from these collections are not yet available. However, future examination of the reintroduction potential for that new generation of ex situ plants will be important information to help close the loop.

Thus, as illustrated by this study, genetic information has great potential to inform ex situ conservation collections management. But the limitations discussed here prompt caution against overstating the utility of genetics for plant conservation. Again, assay of the type described here must be considered in the context of specific biology (Guerrant et al. 2014). And finally, while conservation genetics study certainly provides much greater insight into ex situ collections management, it cannot replace the basic work of the botanist, curator, propagator, or horticulturist—it remains essential to integrate conservation genetics with other data and with tangible effort.

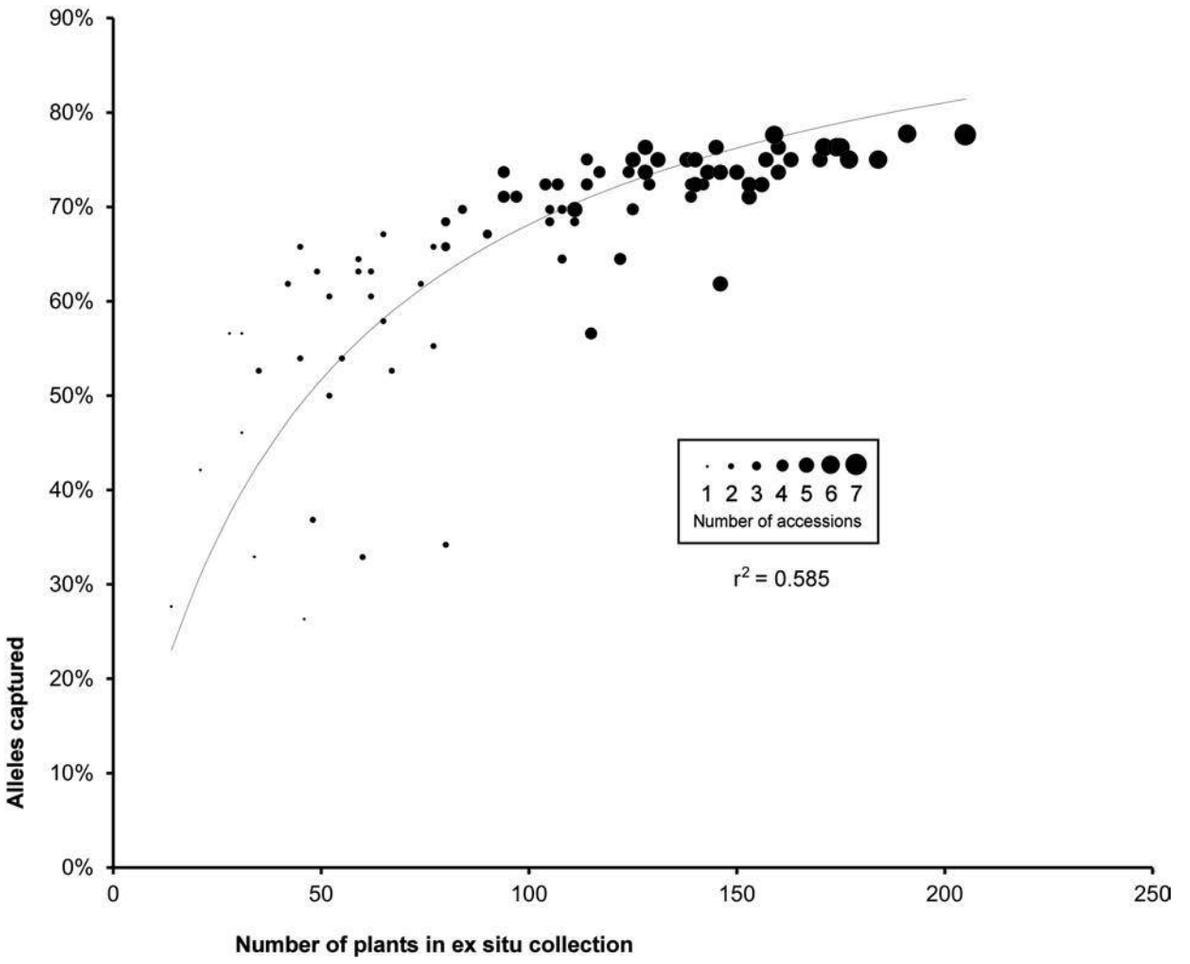

Fig. 4 Ex situ collection modeling for *Zamia decumbens*: the percentage of alleles captured in random resamples of the ex situ collection, by total number of individual plants in the collection and by number of accessions (i.e., half-sibling groups). A single-accession collection (smallest points) would capture between 27% and 57% of in situ alleles, while the entire ex situ collection (7 accessions, 205 plants) captures 78% of in situ alleles.

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