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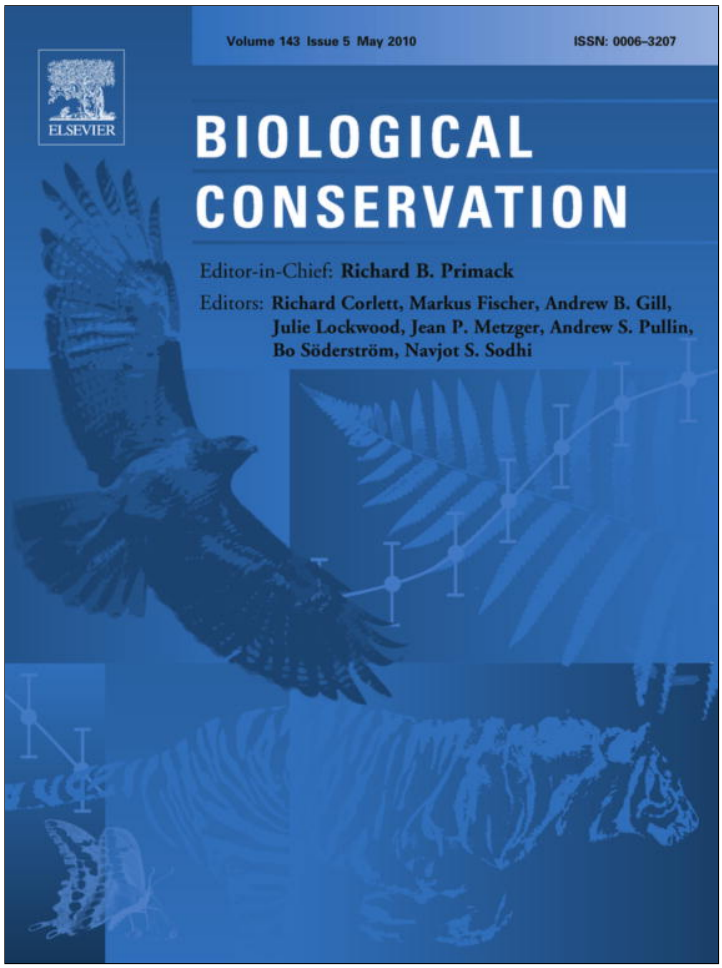

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How well does a botanical garden collection of a rare palm capture the genetic variation in a wild population?

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ABSTRACT

Conservation is increasingly central to the botanic garden mission. Living plant collections are important components of conservation. Critical evaluation of living conservation collections with population genetic analysis can directly inform *ex situ* conservation strategy. Here, we quantify the degree of genetic variation captured through a population-based collection protocol, and explore optimal sampling for *ex situ* conservation. An extensive living collection derived from one population of *Leucothrinax morrisii* (Arecaceae) provided a model system. We compared 58 specimens from the *ex situ* collection with 100 individuals from throughout the parent population via 6 ISSR loci. Random bootstrapped resamples of the data were made to model differently structured *ex situ* collections. Mean diversity (He) differed little between the collection (0.204) and the population (0.216), and genetic distance (D) was very close (0.036). Very few private alleles were found between the collection and the population. Allelic capture, as measured by percent of private alleles, was greater than 94%. Resampled collections of different sizes captured from 48% to 94% of alleles. Pairwise comparison of bootstrapped resamples suggests that increasing the representation of half-sibling groups does not significantly increase allele capture. Increase in allele capture with increasing sample size is greatest at low resample sizes, and showed diminishing returns as resample size increased. No appreciable increase in allele capture was gained through maintaining different half-sibling groups. These data inform sampling for *ex situ* conservation purposes, and recommend sample sizes of at least 15 individuals, with the upper limit based on resources.

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1. Introduction

1.1. Ex situ botanic garden conservation

Botanic gardens often work to cultivate rare plant species for the purpose of *ex situ* conservation (Dosmann, 2006). Strategies for conserving living plants vary among and within garden collections (Wyse Jackson and Sutherland, 2000; Anonymous, 2002; Havens et al., 2004a, 2004b; Farnsworth et al., 2006). Thorough modeling for crop resource planning concluded that a living collection of at least 20 plants is desirable (Gale and Lawrence, 1984). Modeling an effective sample for conservation purposes demonstrates that larger collections conserve more genetic diversity (Lawrence et al., 1995a,b). As understanding of the importance of genetic diversity has increased, more botanic gardens have sought to maximize their collections genetic diversity by preserving multiple individuals from several populations, as 'seed orchards,' 'seed colonies,' or 'field genebanks' (Valois, 1994; Vaxevanidou et al., 2006). It can be difficult to estimate the conservation value of an *ex situ* collection (Schall and Leverich, 2004). Direct evaluation of conservation value is not often performed.

1.2. Conservation of palms (Arecaceae): a model group

The palm family provides a model group with a robust context of conservation work. Due to the economic and ecological importance of these plants, palm conservation work has been underway for some time, including assessments of conservation status (Moore, 1977; Dransfield et al., 1988; Henderson et al., 1990; Johnson, 1996; Pintaud et al., 1999; Zona et al., 2007), *ex situ* living collections (Dowe, 1993), and development of reintroduction strategies (Lippincott, 1995; Maschinski and Duquesnel, 2007). Recommendations for palm conservation often include *ex situ* botanic garden cultivation (Dransfield and Beentje, 1995; Johnson, 1996), and botanic gardens often cite the conservation value of

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palm collections (Wyse Jackson et al., 1990; Chakraverty and Basu, 1994; Lambert, 1994). The implications of international policy on palm conservation collections have also been studied (Zona, 2001). Survey of *ex situ* palm conservation discussed the conservation merits of botanic garden collections versus *in situ* strategies (Maunder et al., 2001). This work concluded that one economical approach would be to establish seed orchards proximal to native threatened palm species. This strategy of complementary *in situ* and *ex situ* work continued to be recommended thereafter (Maunder et al., 2002). Recent studies have been critical of field genebank conservation strategies for palms, citing the difficulty of establishing clear relationships between phenotype and conservation of genetic diversity (van Leeuwen et al., 2005).

As there is broad context and history of conservation work in the palm family, and especially for the role of *ex situ* collections in this work, the Arecaceae is an appropriate model system for close examination of conservation efficacy.

1.3. Population genetics of palms

A variety of population genetic questions have been explored in Arecaceae, providing a robust background for conservation strategies. Previous studies have been based on molecular markers such as Random Amplified Polymorphic DNA (RAPD) (e.g., Dowe et al., 1997), allozymes (Hayati et al., 2004), and microsatellites (e.g., Cole et al., 2006). Shapcott (1998) demonstrated low genetic variation in the rare *Ptychosperma bleeseri*, and highlighted the threat of genetic swamping via ornamental palm production. Further investigation by Shapcott (1999) implied a high degree of inbreeding in native *Pinanga* species in Borneo. Meerow et al. (2002) compared the genetic diversity between and among coconut palm cultivars in Florida. González-Pérez et al. (2004) compared the genetics of native *Phoenix canariensis* with introduced *Phoenix dactylifera*, and palms of intermediate morphology. The data here suggest that the *P. canariensis* populations (of conservation concern) are of recent descent from widespread cultivars of *P. dactylifera*. Bacon and Bailey (2006) highlighted the importance of accurate taxonomic circumscription in palm conservation, using *Chamaedorea alternans* as an example. With specific regard to conservation issues in palms, Shapcott et al. (2009), using population genetic data, gave robust evidence that consideration of local provenance varies in importance for *Livistona carinensis* conservation efforts. In that study, palms from within Djibouti varied little, whereas *L. carinensis* of Somali and Yemeni provenance differed significantly. This informs *ex situ* efforts in plant conservation directly, highlighting the need for population-level curation of conservation collections.

1.4. Assessing a current conservation protocol

The current study seeks to empirically test how much genetic variation within a plant population is captured through a population-based collection protocol, thereby critically evaluating an established *ex situ* conservation strategy. Comparing levels of genetic variation among plants in an *ex situ* collection to the levels of variation within the parent population, the current study addresses a concern not previously investigated: although a population-based collection protocol enhances the potential diversity of the collection, it is not known how effectively or efficiently this is achieved. Critical evaluation of actual *ex situ* collections with population genetic methods could greatly inform and guide planning and strategy.

Patterns of genetic variation in populations of *Coccothrinax argentata* from South Florida have been recently assayed (Davis et al., 2007). This study identified a set of eleven inter simple sequence repeat (ISSR) loci which show genetic differentiation between populations from the Florida Keys and the southern extreme of the Florida Peninsula. This recent technical advance (Davis et al., 2007) allows for direct assay of conservation collections for genetic capture. The current study employs this data type, with automated data collection methods, and traditional and novel population genetic assays to investigate and model the genetic capture of an *ex situ* conservation collection relative to its parent population. This work aims to provide information useful for planning conservation collections. For broad applicability, this project focuses on two parameters, sample size and breadth of accessions.

2. Materials and methods

2.1. Model system

For the current case study, the current *ex situ* conservation protocols at Montgomery Botanical Center (MBC; Miami, Florida, USA) were examined. MBC palm 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-known negative influence on conservation due to inbreeding depression (Schemske et al., 1994; Frankham, 1995) and genetic drift in small collections (Gale and Lawrence, 1984), and is based on consequent conservation management recommendations (Johnson, 1996).

The Keys Thatch Palm, *Leucothrinax morrisii.* was employed as a model taxon for this study (Fig. 1). This species is widespread and common on Caribbean islands (Zona et al., 2007; Lewis and Zona, 2008). Although *L. morrisii* is of least conservation concern as a species (Zona et al., 2007), it is listed as endangered by the State of Florida, USA, where it occurs in geographically limited populations on the lower Florida Keys (Coile and Garland, 2003). *L. morrisii* is uncommon in the nursery and landscape trade (Spanner, 1997), reducing the chance that introgressed genes from nursery stock will have entered the wild population (Shapcott, 1998).

2.2. Sampling protocol

Two groups were compared. One group represented a botanic garden collection and the other group represented the native parent population (Fig. 1). For the botanic garden, an *ex situ* conservation collection was studied. This collection was derived from a seed collection of *L. morrisii* at Big Pine Key (Florida). The *ex situ* collection included 58 plants, from seed collected from 11 individuals in 1998. These 58 plants are hereafter referred to as 'the collection'. The other group studied was the wild population from which the garden collection was derived. In the wild population, 100 individuals from throughout the range of the species in Big Pine Key (hereafter referred to as 'the population') were sampled for comparison, in 2007. On Big Pine Key, this species is abundant in undisturbed areas. The individuals were sampled from 5 sites of roughly two hectares each along a transect running northwest-southeast, for a distance of 4 km (Fig. 1). The Big Pine Key population was continuous and moderately dense along this transect. Herbarium vouchers were deposited at FTG.

2.3. DNA extraction and amplification

For DNA analysis, approximately 1 g of leaf tissue was cut from each individual. DNA was extracted from fresh material using the Qiagen DNeasy protocol (Qiagen, Ltd.), following the manufacturer instructions. Genetic variation in the collection and the population was assayed via DNA using fluorescently-labeled intersimple sequence repeat (ISSR) data (Wolfe et al., 1998 Williams et al.,

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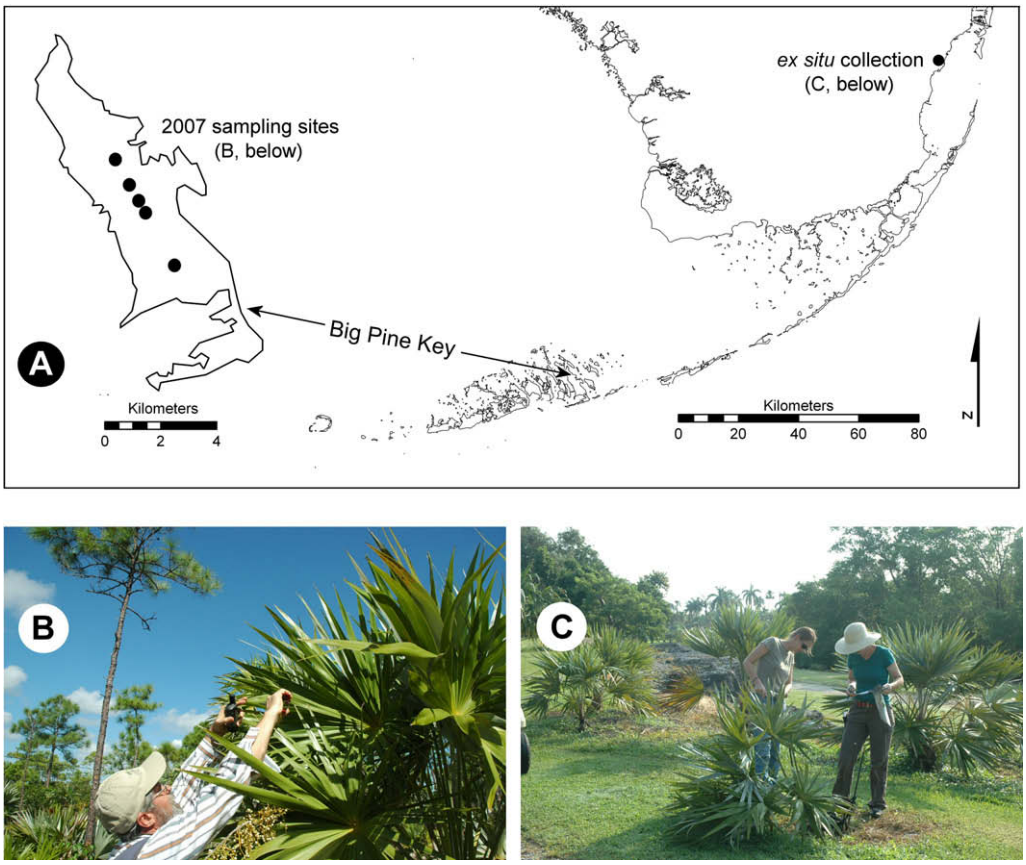

Fig. 1. Model system and species employed for this study, *Leucothrinax morrisii* (H. Wendl.) C. Lewis and Zona. A. Location of wild population and study transect on Big Pine Key, Florida. B. The insular population studied (the "population") grows on Pine Rockland habitat. C. *Ex situ* conservation collection maintained in Miami, Florida (the "collection"), derived from seed collected on Big Pine Key in 1998. Curatorial data regarding half-sibling group informed the structured bootstrapping employed in the study.

1990; Wolfe and Liston, 1998; Cariaga et al., 2005). Loci, and ISSR amplification follow Davis et al. (2007). Six labeled ISSR primers were used from University of British Columbia Primer Set #9: NED815 (5′ CTC TCT CTC TCT CTC TG), FAM819 (5′ GTG TGT GTG TGT GTG TA), HEX848 (5′ CAC ACA CAC ACA CAC ARG), NED810 (5′ GAG AGA GAG AGA GAG AT), FAM817 (CAC ACA CAC ACA CAC AA), and HEX825 (ACA CAC ACA CAC ACA CT).

Amplifications were carried out in 25 μl reactions containing: 1 μl total genomic DNA, buffer (1× thermophilic DNA buffer from Promega), 2.5 mM of $MgCl_2$, 1 mM of each dNTP, 1,0 μM of primer and 1.0 units of Taq DNA polymerase (Promega). Amplifications were achieved in a thermal cycler (MJ Research PTC-200) programmed for 39 cycles with the following temperature profile: initial denaturing stage (3 min at 95 °C), 1 min at 95 °C, 1 min annealing temperature °C, 2 min at 72 °C. Cycling was concluded with a final extension at 72 °C for 7 min. Optimal annealing temperatures were determined using a temperature gradient. Amplification products were visualized using a 1.5% agarose gel.

2.4. Visualization

Fluorescently labeled PCR products were multiplexed and separated on an ABI 3700 Genetic Analyzer (Applied BioSystems, Florida International University, DNA Core Facility) using capillary instrumentation. Samples were run against internal standard Mapmarker1000 (Eurogentec). Alleles were automatically scored using GeneMarker (Softgenetics), and were then manually checked and adjusted.

2.5. Population genetic assay and structured bootstrapping

Comparative estimates of mean genetic diversity (He), genetic distance, and % polymorphic loci were performed 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 from the population to the collection was compared. The sample size of the *L. morrisii* collection ($n = 58$) was larger than many garden collections. Therefore, resampling the collection data, with replacement, to obtain randomly selected model populations (hereafter referred to as 'resamples') was performed. These resamples were composed of randomly selected entire half-sibling cohorts (=plants from one mother; i.e. accessions). The resamples were structured to include from 1 to 11 accessions and from 1 to 58 individuals. Estimates of genetic capture for these random samples were made by comparing each bootstrapped resample to the population via GenAlEx version 6 (Peakall and Smouse, 2006).

2.6. Critical examination of conservation protocol

The resample data was evaluated for the relative effects of increasing the number of individual and increasing the number of accessions (=half-sibling groups). Allelic capture was modeled as a function of number of individuals in the collection using two approaches, logarithmic and logistic. The logarithmic model was fitted using least squares in Microsoft Excel (Microsoft Corporation, 2003). The 3-parameter logistic model, which takes into account the bounded nature of allelic capture (Meyer et al., 1999),

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was fitted by least squares using Loglet Lab 3.0 (Rockefeller University, 2009). Value of increasing accessions was evaluated by pairwise comparisons. Resamples of the same sample size but with different numbers of accessions were compared regarding change in allele capture. The differences in allele capture between higher and lower accession numbers within each sample size were tabulated and a 95% confidence interval for overall allele capture change with increased accession representation was calculated via JMP 7.0.2 (SAS Institute, Inc., 2007).

3. Results

Visualized and scored ISSR banding patterns yielded a total of 114 loci. Summary statistics describing the ISSR data are found in Table 1. The collection and population had similar diversity metrics. For the collection, mean He was found to be 0.204 ± 0.17, and for the population, mean He = 0.216 ± 0.17. We recovered 78.95% polymorphic loci for the collection, and 81.14% for the population. Nei's Genetic Distance between the collection and the population was 0.036 (Identity = 0.965). Similarly, Nei's Unbiased Genetic Distance was 0.032.

A small number (6.54%) of the alleles in the population were private alleles not recovered in the collection, and 3.84% of the alleles in the collection were private alleles. Fig. 2 shows summary comparative banding pattern data between the collection and the population. The overall banding pattern data were homogenous with respect to the number of bands, number of low frequency bands, and number of private alleles (Fig. 2).

Mean percent allelic capture increases as the number of accessions increases (Table 2). The increases in allelic capture are generally less significant as the total number of accessions (and individuals) increases; i.e. greater gain in mean percent allelic capture is obtained by increasing from 1 to 2 accessions (increase over 10%) than from 10 to 11 accessions (increase <1%).

As the collection size increases, the percent allelic capture also increases (Fig. 3). Percent capture of alleles ranged from 48.6% for a single accession of one individual, to 93.5% for the complete collection of 58 individuals from 11 accessions. As the number of individuals and accessions increases, the increase in allelic capture decreases. Because single individuals contained approximately half of the population alleles, the fitted models were shifted to take this into account (the logarithmic model was shifted up and the logistic model shifted left). The logarithmic fit accounted for 83% of the variability in allelic capture, whereas the logistic model accounted for 80% of the variability.

The effect of accession representation is shown in Fig. 4, which plots the change in allele capture for resamples of the same size with different accessions. No significant linear trend is observed in the plot ($p = 0.83$), although the 95% confidence interval for overall allele capture across sample sizes is slightly positive (0.45107%, 3.11338%).

A generalized summary for this model system is that increasing the individuals in the garden collection increases the capture of genes from the parent population (Fig. 3). The rate of increase in genetic capture diminishes as the number of individuals in the collection increases. Structuring this garden collection to include offspring from multiple maternal plants in the wild population did not result in an appreciable increase in genetic capture (Fig. 4).

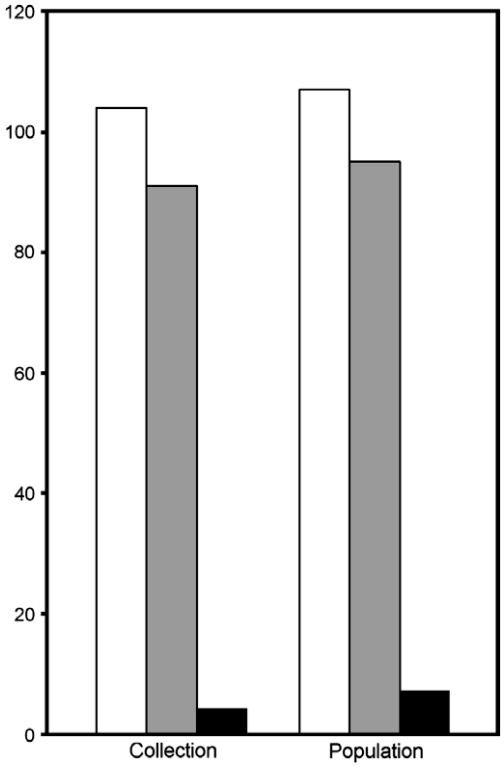

Fig. 2. Summary of alleles (=ISSR bands) recovered in complete collection and population. White = total bands recovered; gray = low (<5%) frequency alleles recovered; black = private alleles recovered. A X^2 test for homogeneity between the collection and the population for a contingency table (JMP 7.0.2; SAS Institute Inc., 2007) gave no evidence to refute the null hypothesis that the populations are homogeneous with respect to the 3 allele categories: p-value = 0.3670.

Table 2
Modeled mean percent allelic capture by number of accessions in collection.

Accessions	*n* plants	Mean allelic capture (%)
1	1–13	68.39
2	4–19	79.44
3	10–22	83.77
4	13–28	85.39
5	11–31	85.64
6	19–39	86.41
7	33–45	87.51
8	36–48	87.77
9	42–54	91.42
10	45–57	92.69
11	58	93.46

Table 1
Summary statistics for ISSR data.

	Collection	Population
Sample size (*n*)	58	100
Mean diversity (He)	0.204 ± 0.17	0.216 ± 0.17
Polymorphic loci	78.95%	83.33%
Nei's genetic distance	0.036	
Nei's genetic identity	0.965	
Nei's unbiased genetic distance	0.032	
Nei's unbiased genetic identity	0.968	

4. Discussion

4.1. Insights from this model system

Assessment of genetic diversity of native *Leucothrinax* provides basic insight into the natural history and population genetic structure of this species, and offers further information to complement existing studies in Arecaceae (Shapcott, 1999; Meerow et al., 2002; González-Pérez et al., 2004). The data gathered by this study

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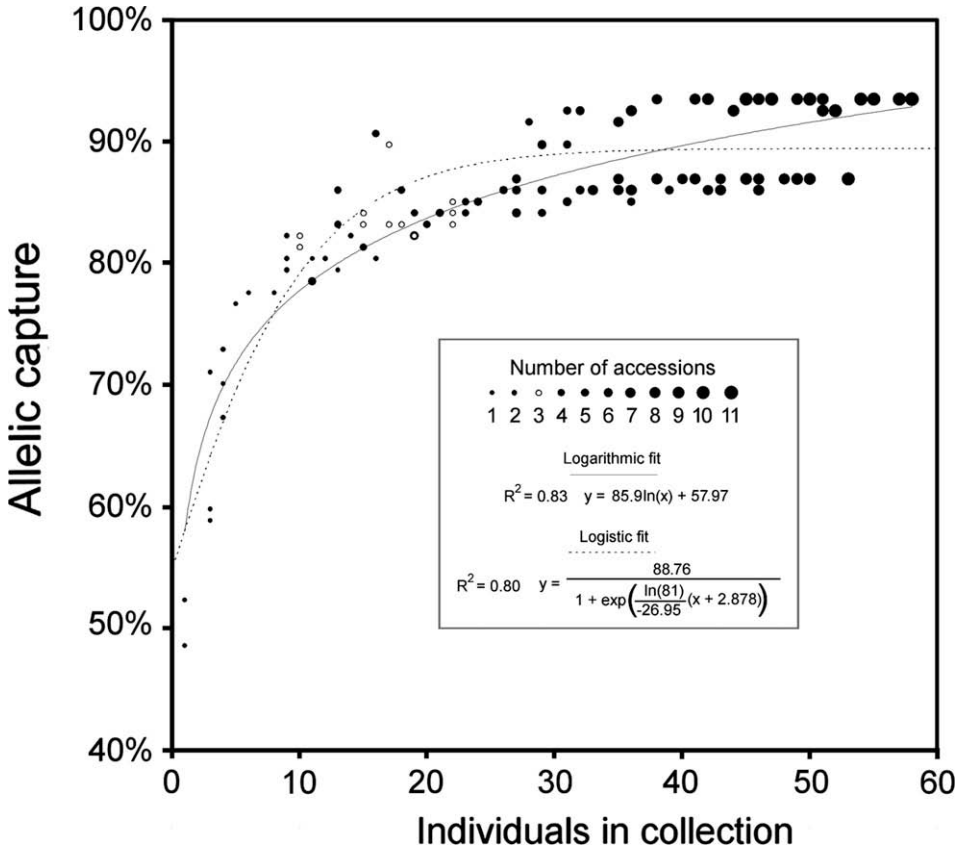

Fig. 3. *Ex situ* collection modeling:% allelic capture for random resamples of collection, by total number of individuals in collection and by number of accessions (=half-sibling groups). Two models were fit to the data, one that best accounts for allelic capture in the short term (logarithmic model) and one that reflects the long-term bounded nature of the phenomenon (logistic model). Resamples comprised of 3 accessions are marked as open circles to evaluate a specific conservation protocol (see text).

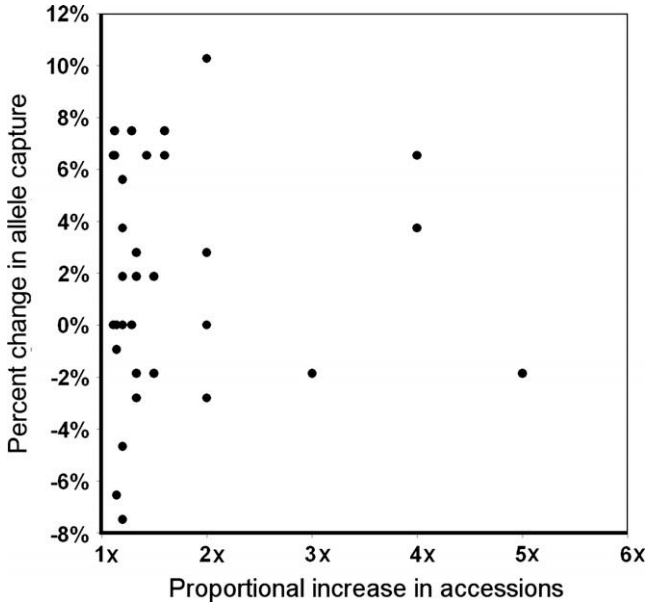

Fig. 4. Effect of increasing accession representation; plot of pairwise comparisons of resampled collections of the same size with different numbers of accessions represented. A greater proportional increase in accessions does not appear to enhance allelic capture over smaller proportional increases (no significant linear trend, $p = 0.83$). The 95% confidence interval for percent increase in allelic capture across proportional increases in accessions (0.45107%, 3.11338%) does not include zero, indicating that on average increasing accessions is slightly beneficial. However, gains in allele capture from adding accessions are much less significant than gains from increasing the overall number of individuals (Fig. 3), regardless of maternal parent.

provides additional findings for broader studies of palm conservation genetics.

The degree of genetic diversity found via this assay is consistent with what is known for many palm populations, with 81.14% polymorphic loci for this *Leucothrinax* population. This degree of diversity may be on the higher end for palms of conservation concern. For example, *L. carinensis* populations often have no recoverable genetic diversity, and for the entire species, only an average of 6.17% polymorphic loci over nine populations (Shapcott et al., 2009). Dowe et al. (1997) found *Carpoxylon macrospermum* polymorphic loci to run between 2% and 4% via RAPD analysis; while *C. acuminata* showed 27–55% polymorphic loci via isozymes (Shapcott, 1998). At the higher end, 91% polymorphic loci were recovered for *Pinanga dumetosa* (Shapcott, 1999). Review of 65 rainforest palm species showed 38% polymorphic loci on average for this group (Shapcott, 2002), whereas woody plants in general average around 50% polymorphic loci (Hamrick and Godt, 1989). *Coccothrinax argentata*, a close relative of *Leucothrinax* (Lewis and Zona, 2008), shows a similar level of polymorphic loci, at 77% (Davis et al., 2007). So, for a single palm population, the *Leucothrinax* population assayed for this study has a high relative level of diversity, which allows for a rigorous test of allelic capture through the current study method. Likewise, the percent polymorphic loci found in *Leucothrinax* is within the reported range for palms, which enhances the applicability of this study for other species.

Bringing plants into protective cultivation is a central aspect of botanic garden conservation work (Anonymous, 2002), and this mission is the focus of the investigation presented here. The current study is designed to inform potential protocols for botanic garden conservation efforts. To maximize applicability to the broad diversity of botanic garden skill sets, emphases, and resources, we

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focus here on sample size and accession breadth, as these are basic collecting parameters which easily translate among all plant groups. Thus, two basic questions are approached here. First, what is an optimal number of plants to maintain in a botanic garden to conserve a single population? Second, are seed collections from multiple maternal plants essential to conserve a population?

The results here support and confirm that increasing the number of plants in the collection will enhance the degree of genetic diversity captured (Guerrant et al., 2004). Also, increasing the accession breadth within a collection will increase the genetic diversity captured within the target population (Table 2; Fig. 3). Since we are using these data to evaluate botanic garden conservation protocols, of specific use here is the efficiency of the model system, given resource limitations for cultivating living collections. For this model system, greatest increase in captured allelic diversity is gained first by maintaining a single specimen, and then by increasing the collection size above a single specimen. Exploring the data for a single accession (i.e. one half-sibling group) collection is informative here. Single-individual collections capture an average of 50% allelic diversity, while increasing this number above 10 will yield a mean allelic capture of 80% (Fig. 3, smallest sized points).

Increase in the number of accessions (half-sibling groups) maintained correlates with increased capture of allelic diversity, although the relationship is potentially confounded with the increasing number of individuals. By controlling for number of individuals and varying numbers of accessions, the overall effect of larger accession counts shows a slight increase in allelic capture (Fig. 4). The effect of increased accession representation is statistically positive, but is dwarfed in magnitude by the effect of simply increasing the number of individuals in the population. This suggests a relatively high degree of allelic diversity among individuals within half-sibling groups as compared to among half-sibling families, as has been found for some plant species (Bin and Wan-chun, 2004), suggesting a high differentiation of paternity within each family (Young and Brown, 1999). For populations with different apportioning of genetic diversity, such as those with many full siblings due to limited paternity, increasing the number of accessions would be very beneficial and thus remains a wise strategy when the population genetic structure is unknown.

Evaluating the specific *ex situ* protocol offers additional information. The collections policy under which these plants are maintained (Husby et al., 2007) calls for a collection of at least 15 plants, preferably from 3 accessions, for purposes of maintaining genetic diversity. In terms of collection size, resamples of 15 plants capture an average of 82.87% of the allelic diversity in the population. Maintaining 3 accessions similarly captures an average of 83.77% population allelic diversity ($n = 11$ resamples; Fig. 2). So, for this model system, the current *ex situ* collections protocol achieves over 80% allelic capture. Increasing the sample size and accessions maintained will increase the allelic capture, but diminishing returns are increasingly seen above 3 accessions (Fig. 3). The logarithmic fit and logistic fit both offer good approximations of expected allelic capture given a collection of a certain size. The logarithmic fit and logistic fit models peak in effectiveness relative to increased collections size between 20 and 30 plants.

A similar study provides a comparative context. Thorough examination of *ex situ* collections of *Elaeis guineensis* (Hayati et al., 2004) determined that over a broad pan-African survey of germplasm collections, polymorphic loci averaged 54%. This study tested the relatedness of 26 different collections (Nei, 1978), and found that two populations were virtually identical ($D = 0.00$), and the greatest difference was between collections in Madagascar and Sierra Leone ($D = 0.568$). The mean D value was 0.113, with an overall correlation between geography and genetic distance (Hayati et al., 2004). Thus, the findings for the *Leucothrinax* collection show a very close relationship between this collection and its population ($D = 0.036$), closer than average for the Hayati et al. (2004) work, but within the range of reported D values for that study. So, the conservation protocol examined here is functioning as proposed with regard to representing genetic diversity within an *ex situ* collection.

4.2. Limitations of the model system

The model system employed for this study has broad application. However, further work of this type with *ex situ* collections possessing a variety of life histories would be further informative. *L. morrisii* is a monoecious, long-lived perennial, and the parent population studied here is confined to an island (Big Pine Key, Florida, USA). The Big Pine Key population is abundant and seedling recruitment appears robust. Palms of hapaxanthic life history (Dransfield et al., 2008), dioecious plants, palms subjected to a known population bottleneck (Maschinski and Duquesnel, 2007), palms under extirpation threat (Cibrian-Jaramillo et al., 2009), or continuously and widely distributed species (Gapare et al., 2005) may show divergent results from those presented here.

The assay method employed here, ISSR banding pattern data, has been commented on as prone to interpretive error when scored manually from agarose gels (Crawford and Mort, 2004). In the current study, automated scoring of the ISSR data via capillary sequencer reduces ambiguity in scoring and allows for more robust inferences (Mort et al., 2003; Archibald et al., 2006).

With regard to the collection modeling, although the logarithmic model produced a better overall fit to the data ($r^2 = 0.83$ vs. $r^2 = 0.80$) it did not reflect the bounded nature of allelic capture (which cannot exceed 100%), so it would eventually depart from the data as more individuals are added to the collection. This can be seen in the steady increase of the logarithmic function even when the allelic capture has leveled off. To better reflect the bounded nature of allelic capture, a logistic model was fit to the data, with a ceiling of 0.8876 to best fit the data up to 60 individuals in the collection. This model explained slightly less of the variability in the data in the short run, but this difference would reverse if the simulation were to continue beyond 60 individuals in the collection. For purposes of collections planning, these two functions give similar estimates of appropriate collections depth for *ex situ* conservation purposes.

4.3. Recommendations for the future

Understanding effective sampling structure needed to capture significant variation for living plant conservation collections informs future planning for viable *ex situ* botanical populations. The current study specifically informs workers interested in monoecious palms, but broadly informs collections planning for long-lived perennial plants, which encompasses the majority of gardens and arboreta. Based on the current work, single-specimen collections could potentially hold significant conservation value, by capturing around half of the targeted alleles. However, based on the economics of botanic garden operation, maintaining a single-specimen collection may not be the best use of limited resources. Furthermore, redundancy in living collections mitigates against loss by unplanned circumstances (Griffith et al., 2008). Maintaining collections of multiple individuals increases the allelic capture and conservation value of the collection (Fig. 3).

In this model system, we determined that increasing sample size gives the greatest gain in allele capture (Fig. 3), and increasing accession breadth does not significantly add to allele capture (Fig. 4). The results presented for this particular model system are consistent with recommendations to conserve collections numbering around 15 plants, and preferably of multiple half-sibling groups.

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This strikes a good balance between the rapid increase in allelic capture seen for increasing a collection from 1 to 10 plants, and the eventual saturation of the allelic capture curve with sampling above 30 individuals (Fig. 3).

Further studies of *ex situ* collections with other life histories, including dioecious species, hapaxanthic or monocarpic species, or species with very short or very long generation times, will be important to see the breadth of applicability of these data for planning purposes. In the future, with increasing efficacy of obtaining genetic data, analyses of the type presented here may become a routine part of *ex situ* collections management. At the very least, these modeling approaches will be desirable for plants of very limited population sizes or other conservation concerns.

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