Conservation Genetics of Breadfruit: Using Microsatellite Primers to Determine Genetic Diversity

Introduction

Much work has been done in an attempt to gather information about the genetic diversity and the history of the human mediated migration of breadfruit (*Artocarpus altilis*, Moraceae) and its wild relatives (Fig.1). Breadfruit is a staple starch crop in Oceania, but its use on some islands has declined following WWII, which ushered in western products. This combined with threats from global climate change make the conservation of the genetic diversity is crucial. The domestication of this plant by humans over millennia has spawned more than 100 cultivars, most of which are sterile and seedless. The evolution of breadfruit into a crop that is infertile follows a shift from being utilized for its seeds to being a source of starch. This shift coincides with the human mediated pattern of dispersal of breadfruit. Humans transported breadfruit through root cuttings and vegetatively propagated it for millennia because seeds remain viable for only a few weeks. Zerega et al (2004) has shown that Melanesian and Polynesian cultivars were domesticated from the New Guinean *A. camansi* and human mediated dispersal followed an easterly trajectory from New Guinea with seeded cultivars in Melanesia grading into seedless cultivars in Polynesia (Fig.2). In Micronesia, *A. camansi* derived breadfruit has hybridized with another wild relative *A. mariannensis*. Hybrids often exhibit characteristics of both of their parental species leaf color, texture, shape, and differences in taste. Despite the fact that breadfruit is predominantly vegetatively propagated, a great deal of morphological diversity exists. However, less is known about the underlying genetic diversity. With molecular tools we can now assess the genetic variation between the different cultivars and wild relatives. To do this, we have acquired leaf samples from the world's largest breadfruit germplasm that maintains approximately 220 trees from 18 island groups in the south Pacific. This garden, part of the National Tropical Botanic Garden and the Breadfruit Institute, is located in Hawaii. We ultimately seek to use microsatellites, a DNA fingerprinting method, in an applied manner to assess the genetic diversity of the collection and uniquely identify cultivars using only leaf tissue. This will be a much faster and more reliable means of identifying cultivars compared to using morphological characters that often require trees to reach maturity (over 5 years). This information will be useful in the future management of the collection. The present study employs a subset of the collection to evaluate the effectiveness of microsatellites in breadfruit for this purpose.

Figure 1

Figure 2

Hypotheses

1) Microsatellites can be used to detect genetic variation in very closely related breadfruit cultivars.
2) Genetic variation is higher in the wild progenitor species of breadfruit than in breadfruit cultivars.
3) Genetic variation will differ among breadfruit cultivars from different regions within Oceania. We hypothesize that the fertile Melanesian cultivars and the hybrid Micronesian cultivars will have greater genetic diversity than sterile Polynesian cultivars.
4) Breadfruit cultivars are more closely related to one another than they are to its wild progenitor species.

Addressing these hypotheses will set the framework more specific investigations using new microsatellite primers. In the future it would also be our goal to develop microsatellite profiles to uniquely identify different cultivars for management and distribution purposes.

Abstract

Abstract

The purpose of this study was to explore the genetic diversity of different cultivars of breadfruit compared to their wild progenitors. Humans have been moving breadfruit between the islands of Oceania for centuries and the domestication and propagation of the plant via transport of root cuttings has resulted in many different breadfruit cultivars. Microsatellites, a DNA fingerprinting technique, were used to assess genetic diversity of a subset of samples from the world's largest breadfruit germplasm collections.

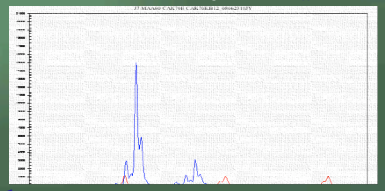
The data suggest that microsatellite primers can be used to differentiate between very closely related breadfruit cultivars. Data also suggest that cultivars exhibit as much or more genetic diversity than their wild progenitors and that cultivars from the same region are more closely related to one another than to cultivars from other regions. The current study is preliminary with only small sample sizes as methods are still being optimized . In the future methods should be optimized and a lager set of samples will be able to be reviewed in order to make better conclusions.

Methods

For this investigation, we used a subset of the National Tropical Botanic Garden collection of breadfruit. We used 16 samples of *A. altilis*, 5 *A. mariannensis*, and 4 hybrids. Leaf samples were stored in an 80ºC freezer with silica gel. DNA extractions were performed using a Fast DNA kit from MP Biomedical (Solon, OH) following standard protocol. Gel electrophoresis was run with each of the extracted DNA samples to ensure the presence of genetic material as well as the appropriate concentration and quality. Next, PCR reactions were performed using primers for microsatellite regions MAA60 and MAA135. These primers were specifically designed for *A. altilis* and the forward primer was labeled with an M13 tag. An M-13 tag primer was also included in the reaction to fluorescently label the microsatellites. The PCR was run using standard protocol (Fig 3). The amplified microsataellite fragments were then run on the Beckman CEQ 8000 Genetic Analysis System following standard protocol (Fig 4). Data was analyzed for genetic variation by calculating heterozygosity (total # of hererozygotes/ total # of samples), allele richness, and allele frequencies in cultivated breadfruit vs. a wild progenitor species as well as between breadfruit cultivars from the major geographic regions of Oceania (Melanesia, Polynesia, Micronesia). The program FSTAT was used for the above analyses. Additionally, PAUP (Swofford 2001) was used for neighbor joining analysis to look at the relationships among individuals. Due to the fact that conditions for amplifying breadfruit microsatellites are still being optimized, the numbers of samples that were successful was small. Thus this study represents only a preliminary investigation of the hypotheses.

Figure 3

Figure 4



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Results

Table 1

	H at Locus MAA60	H(e) at Locus MAA60	Allele Richness	H at Locus MAA135	H(e) at Locus MAA135	Allele Richness
Cultivated	(14) 0.428	0.879	5.336	(7) 0.285	0.857	3.084
Wild- *A. mariannensis*	(4) 0.250	0.833	4.000	(2) 0.500	0.500	2.000
Wild- *A. camainsi*	N/a	N/a	N/a	(2) 0.500	1.000	3.000
Polynesian breadfruit	(9) 0.333	0.750	3.195	(5) 6.00	0.875	3.195
Melanesian breadfruit	(3) 0.667	0.830	4.000	(2) 0.500	1.000	3.000
Micronesian breadfruit	(4) 0.500	1.000	4.393	(6) 0.333	0.867	2.999

Table 1 shows the heterozygosity of the several populations. These results are preliminary due to the lack of data that could be colleted on some samples (such as *A. camansi*), restricting the loci and sample sizes that could be completely evaluated. True heterozygosity (H) was calculated for each population as well as projected using the Hardy Weingburg formula (He). H is higher for cultivated than wild. Melanesian breadfruit shows highest values followed by Micronesian and Polynesian breadfruit.

Allele frequencies were also calculated. Several alleles were common within groups and several were rare. There were alleles common to different groups of breadfruit. Number of individuals per group are noted under heterozygosity in parenthesis.

Discussion

Hypothesis 1: Microsatellites can be used to detect variation in very closely related breadfruit cultivars.

For Locus MAA60, 6 of 14 breadfruit cultivars share the same genotype. The remaining 8 breadfruit cultivars each have a unique genotype. Among these 8, some are seedless Polynesian cultivars that share identical isozyme profiles (Ragone 1997) and are morphologically nearly identical. Therefore, microsatellites can detect variation among very closely related breadfruit cultivars. With increased sampling and additional microsatellite regions, it may be possible to uniquely identify all cultivars which will allow for the identification of gaps and redundancies in the collection and aid in effective germplasm management.

Hypothesis 2: Genetic variation is higher in wild vs. cultivated species.

Measured by both heterozygosity and allele richness, the data suggest that genetic diversity is greater among cultivated breadfruit than at least one of the wild progenitors (*A. mariannesis*). This could suggest that breadfruit was domesticated from a large, genetically diverse pool of progenitors and has since maintained much of its diversity. This could also be due to a smaller sample size of the wild progenitor in this analysis. In both the wild and the cultivated groups expected heterozygosity was much higher than observed, indicating that the members of the group deviate from the expectations of the Hardy-Weinburg equilibrium, which would be expected for a cultivated plant that is vegetatively propagated.

Hypothesis 3: Genetic variation will differ among breadfruit cultivars from different regions within Oceania, with Micronesian and Melanesian groups tending to be more diverse.

Both the heterozygosity and allele richness based on locus MAA135 would suggest that Polynesian cultivars are the most diverse. This is interesting because Polynesian breadfruit is vegatatively propagated, a method known to decrease genetic diversity. This suggests that Polynesian breadfruit was domesticated from a large gene pool and has maintained much of its original diversity. However, the differences in allele richness between each o the regions were not high. Additionally, allele richness and observed heterozygosity at locus MAA60, indicate that Melanesian and Micronesian breadfruit displayed much higher levels of diversity than Polynesian breadfruit, supporting the hypothesis... More data is clearly needed in order to better understand the diversity of these groups.

Hypothesis 4: Breadfruit cultivars are more closely related to one another than they are to its wild progenitor species.

Neighbor joining analysis suggested cultivated Melanesian and Polynesian breadfruits are generally most closely related to one another and to *A. camansi* and cultivated hybrids are more closely related to one another and to *A. mariannesis*. However, cultivated breadfruit as a whole are not more closely related to one another than they are to the wild progenitors as the wild progenitors are nested within clades of cultivated breadfruit. This suggests that based on the locus tested here, the common ancestry of all these species is still strong and cultivars have not evolved much under domestication. However, this is based on a limited sample size of a single microsatellite region and increased sampling could reveal additional info.

References

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