Micronesica 2021-03: 1–10

Genetic diversity of *Serianthes nelsonii* on Guam and Rota*

SAEKO KATOH[1], KOJI TAKAYAMA[2,3], HIDETOSHI KATO[1], MARI MARUTANI[4], NORIAKI MURAKAMI[1], MARIO MARTINEZ[4,5], JAMES MANGLONA[6], JOSEPH TUQUERO[4] & JAMES MCCONNELL[4†]

[1]*Makino Herbarium, Tokyo Metropolitan University, Hachioji, Tokyo, Japan*

[2]*Museum of Natural and Environmental History, Shizuoka, Japan*

[3]*Present address: Graduate School of Science, Department of Botany, Kyoto University, Kyoto, Japan*

[4]*Agricultural and Life Science Division, College of Natural and Applied Sciences, University of Guam*

[5]*Present address: U.S. Navy, Naval Facilities Engineering Systems Command Marianas, FPO AP, PSC 455, Box 195, Santa Rita, GU 96540, USA*

[6]*Department of Lands and Natural Resources Rota, the Commonwealth of Northern Mariana Islands*

Abstract— *Serianthes nelsonii* Merr. is an endemic leguminous tree found in limestone forests of Guam and Rota in the Mariana Islands. It is an endangered species. Plant recovery efforts have focused on plant surveys, phenological studies, seed propagation, and field out-plantings. This study was conducted to understand the genetic structure of *S. nelsonii* populations on Guam and Rota for the conservation program utilizing genetically unique germlines of the species. Samples were obtained from wild Rota trees, outplanted Rota trees, the lone Guam *S. nelsonii* mother tree and its seedlings. A genome-wide multiplexed inter-simple sequence repeat (ISSR) genotyping by sequencing (MIG-seq) analysis was used for single-nucleotide polymorphisms (SNPs) detection. The STRUCTURE analysis showed a clear genetic differentiation between plants from the two islands, between Rota wild trees and Rota out-planted tree populations, and among individuals from Rota out-planted trees. Lower genetic diversity in the Guam seedlings population was observed, which might have been due to the selfing of the Guam mother tree. This study provides the first genetic information on *S. nelsonii* in Guam and Rota. *In-situ* protection and *ex-situ* establishment of *S. nelsonii* populations should consider genetic data in developing a conservative management plan.

Introduction

Serianthes nelsonii Merr. (Family: Fabaceae) is one of the largest endemic leguminous trees found on Guam and Rota in the Mariana Islands. Taxonomically, *S. nelsonii* is placed in the subfamily Mimosoideae, tribe Ingeae (Frosberg, 1960). The Legume Phylogeny Working Group (LPWG) recently classified the genus *Serianthes* in Fabaceae- Caesalpinioideae-mimosoid clade (2017). The record of the chromosome number of *Serianthes* was shown for *S. kanehirae* as 2n=26.

* Citation: Katoh, S., K. Takayama, H. Katoh, M. Marutani & N. Murakami, M. Martinez, J. Manglona, J. Tuquero & J. McConnell. 2021. *Genetic diversity of Serianthes nelsonii on Guam and Rota*, *Micronesica* 2021-03, 10 pp. Published online 31 December 2021.
http://micronesica.org/volumes/2021

† Corresponding author: mcconnell@triton.uog.edu

However, no chromosome number and ploidy level records are recorded for *S. nelsonii* (Goldblatt,1981). Tree heights of over 20 m and trunk diameters of almost 2 m have been reported in Guam and Rota, with a record height of up to 36 m and diameters of nearly 2 m (U.S. Fish and Wildlife Service 1994). In Guam, *S. nelsonii* is called 'håyun lågu,' meaning 'foreign tree' (Kanehira 1933) or 'tree from the north' (U.S. Fish and Wildlife Service 1994). In Rota, *S. nelsonii* is called 'tronkon guafi,' (Moore, 1981), meaning a fire tree, and very seldomly 'tronkon fia' (E. Hocog, personal communication).

Guam lies between 13.2°N and 13.7°N and between 144.6°E and 145.0°E and has an area of 549 km^2, while Rota has an area of 85 km^2 and lies between 14.2°N/145.2°E, about 50 km northeast of Guam. *S. nelsonii* was placed on the endangered species list on March 1987 by the U.S. Fish and Wildlife Service and is currently designated as a critically endangered plant by the International Union for Conservation of Nature (IUCN) (U.S. Fish and Wildlife Service 1987; Wiles et al. 1996; Wiles & Williams 2017). Currently, *S. nelsonii* is found in a restricted habitat in primary limestone forest, with a few individuals also present in secondary forest. *Serianthes nelsonii* is believed to be a primarily self-pollinated plant. Possible means of cross-pollination and seed dispersal are unknown, although Marianas fruit bats, *Pteropus mariannus* Desmarest, occasionally feed on flowers of *S. nelsonii* and may assist in pollination. Commonly poor seed dispersal under natural conditions results in wild seedlings only under mother tree crowns (Wiles et al. 1996).

In Guam, six mature trees were recorded in the wild in the early 1970s, but only one adult tree is known to survive in 2019. Likewise, the number of wild trees of the Rota population has decreased from 121 in 1992 (Wiles et al. 1996) to 33 in 2014 (U.S. Fish and Wildlife Service 2016). A recovery plan for *S. nelsonii* was published in 1994. Among the requirements to down-list the species from "endangered" to "threatened" status is the goal to have two populations with 500 mature individuals on each island (U.S. Fish and Wildlife Service 1994). Some studies and efforts to prevent herbivore damage and determine the causes of low survival rates of seedlings and juveniles have been performed without genetic studies (U.S. Fish and Wildlife Service 2016; Marler & Cascasan 2015; Marler & Musser 2015). There are many examples of failed or ineffective conservation programs implemented without obtaining genetic information (Kaneko et al. 2013). Although Marler et al. (2015) suggested sudden seedling mortality might be caused by inbreeding depression, there was no genetic evidence of inbreeding depression in their report. For the plant recovery plan of *S. nelsonii*, genetic information is essential (U.S. Fish and Wildlife Service 1994).

There are few genetic analysis methodologies available for species with minimal populations and population sizes similar to *S. nelsonii*. To overcome this problem, we conducted genome-wide single-nucleotide polymorphism (SNP) detection using "multiplexed inter-simple sequence repeat (ISSR) genotyping by sequencing" (MIG-seq) (Suyama & Matsuki 2015). This method is a microsatellite-associated DNA sequencing technique. ISSRs are amplified by multiplex polymerase chain reaction (PCR) using MIG-seq primers designed to anneal to the 3' ends of repetitive motifs, including two bases of anchor sequences. Suyama & Matsuki (2015) have shown that the MIG-seq method is applicable for low-quality DNA and when there are fewer markers in ecological and conservation genetic studies, including genetic differentiation among individual plants and populations. For example, Kusuma (2019) successfully applied the MIG-seq method for seedling selection for *ex situ* conservation of critically endangered tree species, *Vatica bantamensis* (Hassk.) Benth & Hook. ex Miq. in Indonesia. Similarly, Sakaguchi et al. (2020) used the MIG-seq method to reveal the phylogenetic origin of *Magnolia pseudokobus,* extinct in the wild, as being conspecific with *M. kobus* and suggested to name *M. kobus* f. *pseudokobus,* which was generated by a spontaneous mutation. MIG-seq was also used successfully to obtain historical genetic information from museum collections having deteriorated DNA materials (Strijk et al., 2020; Eguchi et al., 2020).

The present study was conducted to assess the genetic similarity or dissimilarity of *S. nelsonii* populations on Guam and Rota using the MIG-seq method.

Materials and Methods

Sample Collection

Leaves of *S. nelsonii* were sampled from both Guam and Rota for DNA extraction (Fig. 1). In Guam, samples were collected from the one known remaining mature tree (labeled as 'Guam mother tree') and 11 seedlings within 30 m of the mother tree (labeled as 'Guam seedlings') in Ritidian (13.65°N/144.86°E) in December 2016 (Fig. 1A). The Guam mother tree seedlings originated from the tree given that broader natural seed dispersal doesn't occur. The Guam mother tree was treated as one population in our genetic analysis. Leaf samples were collected in Rota in January 2017 (Fig. 1B). There were nine wild mature plants (labeled as 'Rota wild trees'), including one tree in I Chego/Saligai, two trees in Guayaungan/Sisiao, and six trees in Ca'an.

Additionally, leaf samples of 11 out-planted trees in Rota were collected from eight trees at Talo and three trees at As Igua (labeled as 'Rota out-planted trees'). Parents of 'Rota out-planted trees' were located at either the Guayaungan area or Isang near private property near the Guayaungan area (J. Manglona, unpublished data). Table 1 summarizes sampling locations of *S. nelsonii* population on Guam and Rota. The phenological stage of each out-planted tree in Rota is classified as either mature or immature. For the genetic analysis, collected leaves from individual trees at each site were placed in a labeled small paper bag. All paper bags were placed in a sealed plastic bag with silica gel before DNA extraction.

DNA Extraction and Analysis

According to the manufacturer's protocol, total DNA was extracted from silica-gel-dried leaf samples using the QIAGEN DNeasy Plant Mini kit (Qiagen, Hilden, Germany). Multiplexed inter-simple sequence repeat (ISSR) genotyping by sequencing (MIG-seq) was used for single-nucleotide polymorphism (SNP) detection according to the procedure by Suyama & Matsuki (2015). Approximately 12.5 pM of the library was used for sequencing on an Illumina MiSeq Sequencer (Illumina, San Diego, CA, USA), using a MiSeq Reagent Kit v3 (150 cycles, Illumina). Low-quality reads and primer sequence reads were removed from the raw sequencing data using the FASTX toolkit (http://hanno nlab.cshl.edu/fastx toolkit/) and TagDust (Lassmann et al. 2009). All remaining reads were trimmed to 63 bases. Before the *de novo* assay, samples with fewer than 50,000 reads were removed, and 32 samples were used for SNP detection. SNPs were called using Stacks1.48 (Catchen et al. 2013). The minimum depth option for creating a stack was set to 3 (-m 3), and default settings for other options were used. Four parameters of Populations in Stacks were used to select SNPs for population analysis. The parameters were set for minimum percentage of samples in a population (-r 0.75), minimum number of population in a locus (-p 1), minimum minor allele frequency (-min_maf 0.04), and maximum observed heterozygosity (-max_obs_het 0.6).

The F_{IS} value for each SNP within each population was calculated using Populations. SNPs with F_{IS} values less than -0.75 or more than 0.75 were removed to exclude SNPs that might be located on the organelle genome and rich in null alleles. Pairwise R^2 values for each SNP pair were calculated by Hploview 4.1 (Barrett et al. 2005), and if a value was higher than 0.5, an SNP locus with a lower genotyping rate was removed to exclude linkage between SNPs. The number of polymorphic SNPs and the genetic diversity indices, observed heterozygosity (H_O), expected heterozygosity (H_E), and fixation index (F_{IS}) were also calculated using Populations in Stacks.

Population genetic structure was estimated by admixture analysis using the software Structure 2.3.4. (Pritchard et al. 2000). The allele frequency correlation and ancestry admixture models were used on the complete set of data containing all four populations. Under this model, it is assumed that the *K* populations represented in our sample have each undergone independent drift away from the hypothetical "ancestral" population. The estimated *F* values were also calculated. For all analyses, 100,000 burn-in steps and 100,000 replicates were used with at least ten replicates for values of *K*

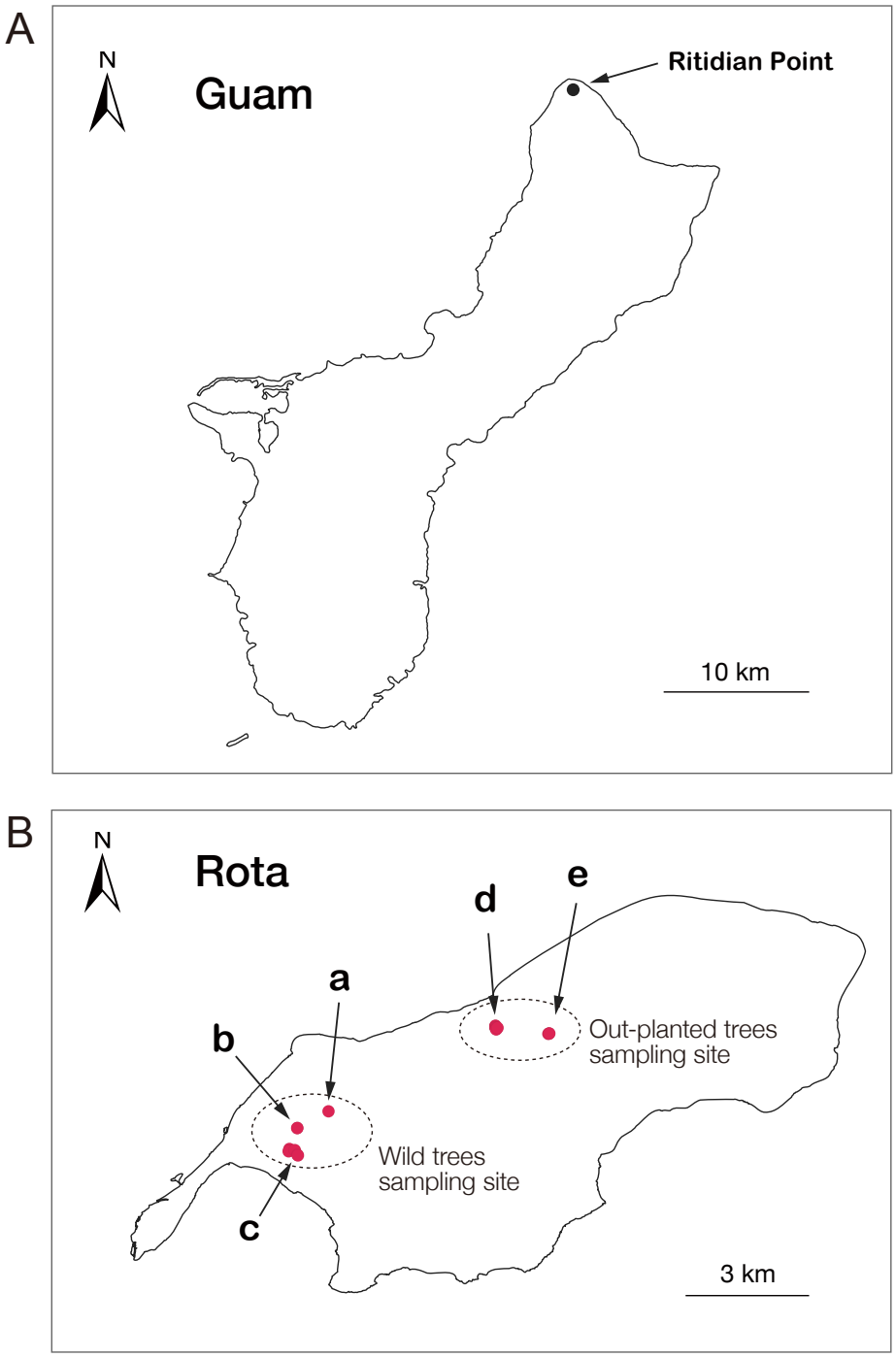

Figure 1. Sampling sites for *Serianthes nelsonii* in this study. A. Sampling site of the *S. nelsonii* mother tree and seedlings in Ritidian, Guam. Seedlings were found under the mother plant. B. Sampling sites of *S. nelsonii* on Rota indicating three mature plant sites (a. I Chego/Saligai; b. Guayaungan/Sisiao; and c. Ca'an) and two out-planting sites (d. Talo and e. As Igua).

Table 1. Location of *Serianthes nelsonii* population of the genetic study in Guam and Rota.

Population	Location	Plant No.	Latitude	Longitude	Elevation (m)
Guam:					
Mother tree	Ritidian Point	Guam 01	13.649°N	144.863°E	155
Seedlings	Ritidian Point	Guam 02-12	13.649°N	144.863°E	155
Rota:					
Wild trees	a. I Chego/Saligai	Rota 12	14.150°N	145.165°E	296
	b. Guayaungan/Sisiao	Rota 13	14.146°N	145.160°E	339
		Rota 14	14.146°N	145.160°E	339
	c. Ca'an	Rota 15	14.143°N	145.158°E	289
		Rota 16	14.143°N	145.158°E	287
		Rota 17	14.143°N	145.158°E	285
		Rota 18	14.143°N	145.159°E	295
		Rota 19	14.142°N	145.160°E	281
		Rota 20	14.142°N	145.160°E	277
Out-planted	d. Talo	Rota 05 M[z]	14.170°N	145.206°E	114
		Rota 07 M	14.170°N	145.206°E	114
		Rota 01 I	14.170°N	145.206°E	110
		Rota 02 I	14.170°N	145.206°E	111
		Rota 03 I	14.170°N	145.206°E	112
		Rota 04 I	14.170°N	145.206°E	113
		Rota 06 I	14.170°N	145.206°E	114
		Rota 08 I	14.170°N	145.207°E	111
	e. As Igua	Rota 09 M	14.169°N	145.216°E	166
		Rota 10 M	14.169°N	145.217°E	165
		Rota 11 M	14.169°N	145.217°E	166

[z] Phenological stage of out-planted trees: M=mature, I=immature.

ranging from 1 to 8, where *K* is the number of genotypic groups. The optimal *K* was chosen using the delta*K* method (Evanno et al. 2005) with Structure Harvester (Earl & vonHoldt, 2012). "STRUCTURE" is the robust clustering method in population genetics, especially for mixed-ploidy populations (Stift et al., 2019). It was the best method for *S. nelsonii* lacking any information on ploidy level.

Results

Genetic Diversity

The total and the average number of reads obtained from 32 individuals were 3,272,264 and 102,258, respectively. Table 2 presents the results of the genetic study of two populations of *S. nelsonii* in Guam and two populations from Rota. The total of 178 SNPs was detected for all four populations after SNP selections. The number of polymorphic SNPs (*S*) within populations ranged from 40 to 67 in Guam and 130 to 148 in Rota (Table 2).

The observed (H_O) and the expected (H_E) heterozygosity of the "Rota wild trees" (RWT) population were higher than those of the "Guam seedlings" (GST) population (t=-4.95218, $p < 0.0001$ for H_O and t=-4.8931, $p < 0.0001$ for H_E). Similarly, the heterozygosity was greater with the "Rota out-planted trees" (ROT) population than the GST population (t=-6.09925 $p< 0.0001$ for H_O and t=-6.20368, $p< 0.0001$ for H_E). The H_O and H_E between RWT and ROT populations were not significantly different (t= -1.16523, $p= 0.1224$ for H_O, and t= -1.30453, $p= 0.0964$ for H_E). Although population genetic diversity indices were calculated for the "Guam mother tree" (GMT), the indices should be considered to represent inner-individual genetic diversities of the tree, since this population site consisted of only one individual and do not apply to basic population genetic theories.

Genetic Structure

From the results of admixture analysis, the number of genetic clusters $K= 2$ was most supported, and $K = 5$ was the secondary supported value to our data based on the delta*K* method (Evanno et al. 2005). For every *K*, a small amount of admixture from other ancestries was observed in the 'Guam mother tree' (Fig. 2). For $K = 2$, most of the individuals in both Guam and Rota showed single but clearly different ancestries. When $K = 3$, both the RWT population and ROT population were dominated by single ancestries. For $K = 4$ or 5, while the RWT population had one dominant ancestry, the ROT population was divided into two clusters with different ancestry. Individuals from Talo showed admixed ancestry, but trees from As Igua showed a single ancestry. For all numbers of clusters (*K*), *F* values of the major cluster of Guam (yellow; 0.579-0.599) were higher than the *F* values of the clusters of Rota (purple, 0.187-0.342; green, 0.308-0.378; grey, 0.393-0.400) except blue (0.879).

Discussion

Genetic Differentiation Among Populations

According to the STRUCTURE analysis, *Serianthes nelsonii* plants examined from Guam and Rota were genetically differentiated by different parental ancestries. Further, the STRUCTURE analysis indicated genetic diversity between the RWT population and the ROT population (K=3 to 5 in Fig. 2). Although RWT had one dominant ancestry, the ROT showed several ancestries, some of which were unique ones not found in RWT. Parent(s) of ROT died, perhaps due to several tropical storms, and they were not from the mature RWT population examined in this study. The result implies that the ROT population inherited genetic materials from "lost" parent tree(s), which had different genetic clusters from the existing RWT population. The RWT population was comprised

Table 2. Genetic variation of *Serianthes nelsonii* in Guam and Rota.[Z]

Population	*N*	*N′*	SNP	*S*	Private	Variant positions		
						H_O	H_E	F_{IS}
Guam:								
Mother tree	1	1	178	40	0	0.213	0.106	0.000
Seedlings	11	10.3	178	67	17	0.125	0.142	0.056
Rota:								
Wild trees	9	8.2	178	130	18	0.230	0.239	0.060
Out-planted trees	11	10.2	178	148	30	0.255	0.266	0.054

[Z] *N* = number of plants in a population; *N′* = mean number of genotyped individuals; SNP = total number of SNPs detected; *S* = number of polymorphic SNPs; Private = number of private SNPs within a population; H_O = observed heterozygosity; H_E = expected heterozygosity; F_{IS} = fixation index.

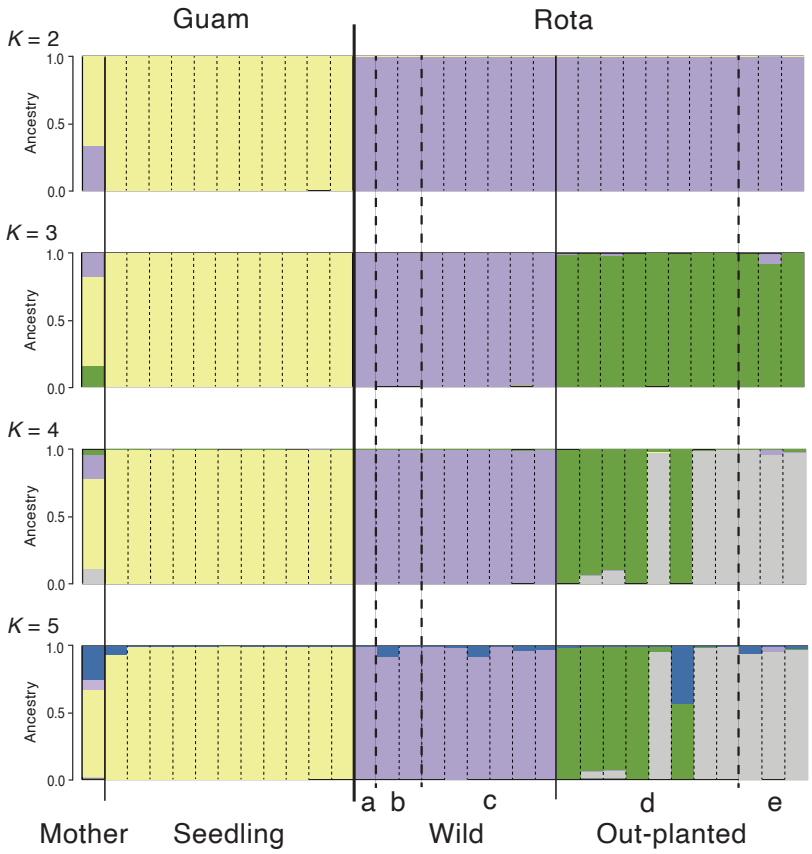

Fig. 2. Individual-based genetic structure elucidated by admixture analysis (STRUCTURE) and distributions of genetic ancestry when the number of clusters ranged from *K* = 2 to 5. Each bar represents an individual of *Serianthes nelsonii* in the study. Different colors represent different genetic ancestry groups. The y-axis aids in estimating the percentage of each genetic ancestry group within an individual. Abbreviations of Rota population indicate sampled locations: a. I Chego/Saligai; b. Guayaungan/Sisiao; c. Ca'an; d. Talo; and e. As Igua.

of a homogenous genetic composition, and suggests that individuals in this population are possibly descendants of one or fewer genetically similar ancestors that once lived in the vicinity. Most ROT showed one dominant cluster within an individual plant, indicated by either green or grey in Fig. 2.

In Guam, 11 individuals from the seedlings (GST site) were nearly all homogenous with one dominant cluster (yellow in Fig. 2), which is also a dominant genetic segment in the Guam mother tree (GMT). Although the GMT showed an admixture of several ancestries, some of which are also shown in trees from Rota, the Guam seedlings did not maintain genetic diversities found in the mother tree. The high value of *F* of the Guam dominant component (yellow) also supported this assumption because a higher *F* value points to genetic drift that may cause loss of genetic diversity with a reduction of population size (Falush et al. 2003).

The genetic diversity within the GMT is not maintained in individuals of the GST population. Instead, seedlings only carried one type of genetic component found in the GMT, which was not found in the two Rota populations. Although Guam and Rota share a cluster at K=2-5, a historical mechanism is unknown. Further study is needed to increase understanding of the genetic population structure of *S. nelsonii* on Guam and Rota. DNA data from more specimens, including herbarium collections and any other heretofore unknown wild plants should be included.

DECLINING GENETIC DIVERSITY

In Rota, a rapid decrease from 121 to 33 trees over 20 years and a reduction of the distribution range were reported in 2016 (U.S. Fish and Wildlife Service). A rapid decrease in the population size of the species has caused a strong genetic drift, a process of relentless loss of genetic variation (Kliman et al. 2008). Further, inbreeding decreases heterozygosity, and the rate of loss is much faster with smaller populations than larger populations (Ellstrand & Elam 1993). Recurrent viable seed production by the sole *S. nelsonii* on Guam assumes that this tree is self-pollinating. Self-pollination may reduce the heterozygosity in the Guam mother tree's offspring.

The observed and expected heterozygosity (H_O and H_E) of both RWT and ROT populations were higher than those of the GST population (Table 2). The numbers of polymorphic sites and private SNPs of two Rota populations were also greater than the GST population. The lower genetic diversity of the GST population is possibly the result of the selfing of the GMT since there were no other mature trees found in Guam in the vicinity of the seedling population site. The 17 private SNPs obtained within the GST population might suggest unknown adult trees in the wild; however, it is improbable based on our intensive field surveys and no reports from previous studies. We assume that the 17 private SNPs were due to missing data in the MIG-seq. It is possible that these 17 private SNPs could also be found in the Guam mother tree if we could have obtained more data via MIG-seq analysis.

Population extinctions and decreases in population sizes may account for the low genetic diversity. It is hypothesized that genetic diversity increases with larger population size (Hague and Routman 2016; Hansen and Wesche 2006). Because Rota has a larger habitat area and a greater number of wild individuals than Guam, Rota populations showed higher genetic diversities. However, a rapid decrease in population size on both islands would cause the decline of genetic diversity. The expected heterozygosity is much lower for selfing plants and endemic plants than both outcrossing plants and widespread plants (Hamrick and Godt 1990).

In summary, this study demonstrates genetic variation among all populations of *S. nelsonii* in Guam and Rota. *Ex situ* establishment of these endangered plant populations will require conservation management to control the gene flow between island populations. Additional genetic studies are needed to advance the understanding of the biodiversity in *S. nelsonii* populations. There is an urgent need to obtain DNA data from available herbarium specimens and search for other mature plants hidden in the forests in Guam and Rota to better define the genetic population structure of *S. nelsonii* in the Marianas.

Acknowledgements

We would like to thank James Bamba, Gregorio Borja, and Jason Andrew for collecting plant samples. This study was supported by USDA-NIFA Hatch McIntire-Stennis Accession no. 1005273, the Grant-in-Aid for Scientific Research No. JP17H04609 to NM, and Department of Lands and Natural Resources, Saipan and Rota of Commonwealth of Northern Mariana Islands. Permission to access the mature tree on Guam was granted through an existing cooperative agreement with UOG and the Department of Defense (U.S. Navy, Joint Region Marianas). The study was conducted under USFWS Permit No. TE78405C "Native endangered and threatened species recovery endangered and threatened plants."

References

Barrett, J. C., B. Fry, J. Maller & M. J. Daly. 2005. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 21: 263-265.

Catchen, J., P. A. Hohenlohe, S. Bassham, A. Amores & W. A. Cresko. 2013. Stacks: an analysis tool set for population genomics. Molecular Ecology 22: 3124-3140.

Earl, D. A. & B. M. vonHoldt. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources 4: 359-361.

Eguchi, K., E. Oguri, T. Sasaki, A. Matsuo, D.D. Nguyen, W. Jaitrong, B. E. Yahya, Z. Chen, R. Satria, W.Y. Wang &Y. Suyama. 2020. Revisiting museum collections in the genomic era: potential of MIG-seq for retrieving phylogenetic information from aged minute dry specimens of ants (Hymenoptera: Formicidae) and other small organisms. Myrmecological News 30:151-159.

Ellstrand, N.C. and D.R. Elam. 1993. Population genetic conservation of small population size: Implications for Plant Conservation. Anna. Rev. Ecol. Syst. 24:217-242.

Evanno, G., S. Regnaut & J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology 14: 2611-2620.

Falush, D. Stephens, M., J.K. Pritchard. 2003. Inference of population structure using multilocus genotype data: Liked loci and correlated allele frequencies. Genetics 164:1567-1587.

Frosberg, F. R. 1960. Serianthes Benth. (Leguninosae-Mimosoideae-Ingeae). Reinwardtia 5:293-317.

Goldblatt, P. 1981. Chromosome numbers in legumes II. Annuals of the Missouri Botanical Garden 68:551-557.

Hague, M. T. J. & E. J. Routman. 2016. Does population size affect genetic diversity? A test with sympatric lizard species. Heredity 116: 92-98.

Hansen, I. & K. Wesche. 2006. Relationships between population size, genetic diversity and fitness components in the rare plant Dictamnus albus in Central Germany. Biodiversity & Conservation 15: 2249-2261.

Hamrick, J.L. and M.J.W. Godt. 1990. Allozyme diversity in plant species. In: Brown, A.H.D., M.T. Clegg, A.L. Kahler, and B.S. Weir. (Eds.) Plant Population Genetics, Breeding and Genetic Resources. Sinauer, Sunderland, MA, pp. 43-63.

Kanehira, R. 1933. Flora Micronesica. Inoue Shoten (published the reprint in 1982) Tokyo Japan.

Kaneko, S., T. Abe & Y. Isagi. 2013. Complete genotyping in conservation genetics, a case study of a critically endangered shrub, Stachyurus macrocarpus var. prunifolius (Stachyuraceae) in the Ogasawara Islands, Japan. Journal of Plant Research 126: 635-642.

Kliman, R., B. Sheehy & J. Schultz. 2008. Genetic Drift and Effective Population Size. Nature Education 1(3):3.

Kusuma YWC, Ariati SR, Risna RA, Mitsuyuki C, Suyama Y, Isagi Y. 2019. Seedling Selection Using Molecular Approach for Ex Situ Conservation of Critically Endangered Tree Species

(Vatica bantamensis (Hassk.) Benth. & Hook. ex Miq.) in Java, Indonesia. Tropical Conservation Science. doi:10.1177/1940082919849506.

Lassmann, T., Y. Hayashizaki & C. O. Daub. 2009. TagDust: a program to eliminate artifacts from next generation sequencing data. Bioinformatics 25:2839–2840.

Legume Phylogeny Working Group (LPWG). 2017. A new subfamily classification of the Leguminosae based on a taxonomically comprehensive phylogeny Taxon 66:44-77.

Marler, T. E. & A. N. Cascasan. 2015. Number of emerged seedlings and seedling longevity of the non-recruiting, critically endangered Håyun lågu tree Serianthes nelsonii Merr. (Fabales: Legminosae) are influenced by month of emergence. Journal of Threatened Taxa 7: 8221-8225.

Marler, T.E., A.N. Cascasan & J.H. Lawrence. 2015. Threatened native trees in Guam: Short-term seed storage and shade conditions influence emergence and growth of seedlings. HortScience, 50, 1049–1054.

Marler, T. & C. Musser. 2015. Potential stressors leading to seedling mortality in the endemic Håyun lågu tree (Serianthes nelsonii Merr.) in the island of Guam. Tropical Conservation Science 8: 738-744.

Moore, P.H. 1981. Field and Garden Plants of Guam. College of Agriculture and Life Sciences, University of Guam 182p.

Pritchard, J. K. M. Stephens & P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155: 945-959.

Safford, W. E. 1905. The Useful Plants of Island of Guam. Bulletin of the United States National Museum 9: 416p.

Sakaguchi, S. K. Nagasawa, Y. Umetsu, J. Nagasawa, S. Ichikawa, S. Kinoshita, K. Hiratsuka, Y. Suyama, Y. Tsunamoto, Y. Isagi & H. Setoguchi. 2020. Phylogenetic origin of Magnolia pseudokobus (Magnoliaceae), a rare Magnolia extinct in the wild, revealed by chloroplast genome sequencing, genome wide SNP genotyping and microsatellite analysis. Journal of Forest Research DOI: 10.1080/13416979.2020.1767268

Stift, M., F. Kolar & P. Meirmans. 2019. STRUCTURE is more robust than other clustering methods in simulated mixed-ploidy population. Heredity 123:429-441.

Strijk, J. S., T.B. Hoàng, V.N. Nguyen, J.T. Pereira, J.W.F. Slik, R. S. Sukri, Y. Suyama, S. Tagane, J. J. Wieringa, T. Yahara, D. D. Hinsinger. 2020. Museomics for reconstructing historical floristic exchanges: Divergence of stone oaks across Wallacea. PLoS One 15(5):e0232936.

Suyama, Y. & Y. Matsuki. 2015. MIG-seq: an effective PCR-based method for genome-wide single-nucleotide polymorphism genotyping using the next-generation sequencing platform. Scientific Reports 5:16963.

U.S. Fish and Wildlife Service. 1987. Endangered and threatened wildlife and plants; determination of endangered status for Serianthes nelsonii Merr. (hayun lagu or tronkon guafi). Federal Register 52: 4907-4910.

U.S. Fish and Wildlife Service. 1994. Recovery plan for Serianthes nelsonii. U.S. Fish and Wildlife Service, Portland, Oregon. 60p.

U.S. Fish and Wildlife Service. 2016. 5-year review of Serianthes nelsonii. Short Form Summary. U.S. Fish and Wildlife Service, Pacific Islands Fish and Wildlife Office, Honolulu, Hawai'i. 12p.

Wiles, G. J., I. H. Schreiner, D. Nafus, L. K. Jurgensen & J. C. Manglona. 1996. The status, biology, and conservation of Serianthes nelsonii (Fabaceae), an endangered Micronesian tree. Biological Conservation 76: 229-239.

Wiles, G. & E. Williams. 2017. Serianthes nelsonii. The IUCN Red List of Threatened Species: e.T30437A98715973. http://dx.doi.org/10.2305/IUCN.UK.2017-3.RLTS.T30437A98715973.en . Downloaded on 06 February 2018.

Received 17 May. 2020, revised 02 Aug. 2021.