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Conservation Genomics of the Endangered Virgin Island Boa (*Chilabothrus granti)*

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Abstract

The Virgin Islands Boa (*Chilabothrus granti*) is a species of conservation concern and is restricted to the Puerto Rico Bank, which encompasses the island of Puerto Rico and the Virgin Islands Archipelago to the east. The species is endangered because of increased anthropogenic activity, invasive rat populations, feral cat predation, and threats to habitat stability related to climate change. I obtained 96 samples of Virgin Island boas from researchers studying the species over the last 20 years, representing samples from a total of four island populations. I extracted and purified the DNA, then amplified and sequenced each sample at the mitochondrial gene cytochromeB. I also sent samples for genome-wide 3RAD genotyping-by-sequencing. From the mitochondrial dataset I estimated six haplotypes, with each island having only a single haplotype present except for a translocated population in the USVI. For the 3RAD dataset I used a depth of sequencing plot to examine data quality, then used Discriminant Analysis of Principal Components to visualize population genetic relationships. Together these data show that the VI boa retains less genetic diversity as a species compared to almost all other snakes in the Caribbean with the particularly troubling lack of mtDNA diversity in remaining island populations. Despite the fact that a translocated population retains higher levels of genetic diversity, I expect that this will decline through time owing to strong genetic drift in the small population. These data will be useful for designing genetically-informed conservation strategies for the species.

1. Introduction

The Virgin Island Boa (*Chilabothrus granti)* also known as the Puerto Rican Tree Boa (Figure 1), is a small, nocturnal arboreal snake endemic to the Greater Antilles region; specifically the Puerto Rico Bank, comprising the island of Puerto Rico and the Spanish, US, and British Virgin Islands. Over the past 50 years, populations of this snake have shrunk dramatically, and their historical range has been drastically reduced [1]. Protection of the Virgin Island (VI) Boa is listed under two United States Federal programs for protection: the Endangered Species Act of 1973 (joined in 1979) and the Coastal Management Act (1972), as well as holding international protections under the International Union for Conservation of Nature (IUCN) and the Convention on International Trade in Endangered Species (CITES, Appendix I) [2]. Found in mostly subtropical forests, with a high level of tree canopy connection, surplus of lizard prey (mostly *Anolis*), and residing at low elevations, this endangered species' preferred habitat has been all but taken over by urbanization or made untenable by invasive species. Threats to this species include: habitat fragmentation, invasive predatory rats (*Rattus rattus*), feral cat populations (*Felis catus*), human anthropogenic activity, and climate change [2,3,4]. A major concern for conservationists studying this species is obtaining enough samples to understand the scope of the genetic diversity within and among populations. Because of the difficulty in finding this species in the wild, most specimens previously acquired were collected from snakes found after death near human settlements or from small island populations. The last remaining wild populations of *Chilabothrus granti* are found on the islands of St. Thomas, Cayo Diablo, Cayo Ratones, Culebra, Tórtola, VI Cay, and Rio Grande [2,3].

Among these known populations, there are thought to be between 1200-1500 total boas left in the wild [1], although it is predicted that this number has further dropped within recent years. The largest population of the Virgin Island

Boa population had previously been found on St. Thomas. As development of the island and deforestation have fragmented the remaining habitat in this region, these numbers have continued declining. The population found on the island of St. Thomas is thought to have a lower long-term survivability than that of other populations because of the island's continuing development, further fragmenting their habitat. Between the years 2005-2011, a study conducted on the island of St. Thomas surveyed the population resulting in no live boa specimens ever found under survey effort [2]. The population residing on Cayo Diablo is thought to be at the island's carrying capacity and has been reported to be one the densest population of naturally occurring boas found, with 100-150 individuals per hectare. Due to low elevation and small size of the island however, this island is extremely vulnerable to the effects of climate change, invasive predators and sea level rise [2]. Indeed, a population of about 500 boas occurred on Cayo Ratones, and surveys in 2018 revealed that this population was likely extirpated following the passage of hurricane Maria and the recolonization of the island by rats. That population represented one third of the census population size of the entire species. Greater communication and collaboration with the British Virgin Islands is needed to confirm the presence or absence of the Virgin Island Boas on Tórtolaand any nearby islands. Tórtola is a large island, but nothing is known regarding snake density or distribution there, as no focused surveys have ever been conducted. It is important to note that these populations are very vulnerable to any changes to their environment, due to their small numbers as well as the species' overall lack of genetic diversity [2].

Figure 1. Virgin Islands Boa (*Chilabothrus granti*) from the US Virgin Islands. Photo by R. Graham Reynolds.

The low levels of genetic diversity of this species can be directly attributed to habitat fragmentation, habitat loss, and reduced gene flow within populations. With the growing intensity of weather phenomena influenced by climate change, the survival of these populations has increasingly become even more threatened. With some of the largest hurricanes to date occurring in the past 10 years, islands in this region are repeatedly under threat of destruction. Sea level rise is another concern for this species because of their preferred habitat, which consists of low elevation areas. Not only does rising sea level have implications of a lowered survivability among these current populations, but it can also affect the outcome of future reintroduction projects. Strong waters and winds have also historically helped rat populations become reestablished on islands where previous extermination processes, costing millions, had occurred [5].

Thus, it is important to determine the level of genetic diversity among mitochondrial and nuclear DNA in endangered species to better guide the preservation of diversity among the genepool. Without proof of the limited diversity, it is hard to generate support for the species in aims of conservation. We can further use these data to measure the change in genetic diversity over time and sequences of specific individuals could be used to optimize breeding pairs that would maximize the genetic diversity in offspring. Future applications could help to prioritize which individuals should be released on certain islands or locations to prevent a barrier against further inbreeding; as well as providing evidence for continued protection under the Endangered Species Act.

Although practices that allow the animals to reproduce on their own are typically favored, it was decided that reintroduction and captive breeding of these snakes was vital for conserving what genetic diversity was left in the species in 1985. In 1986, in collaboration with the U.S. Fish and Wildlife Service, the Puerto Rico Department of Natural and Environmental Resources and the Virgin Island Fish and Wildlife Service, the Toledo Zoo had the first successful captive breeding of the Virgin Island Boa. It was from here that the Species Survival Plan was published in 1990. Islands were scouted in preparation, characterized by their suitability for translocation and reintroduction. Factors determining suitability included island size, availability of prey, protected status of the island, prevalence of invasive predators, and suitable habitat were all considered during vetting of the prospective relocation destination [4]. More captive snakes were bred for the future release and rat extermination on VI Cay was implemented. Snakes were assessed on their ability to survive in the wild prior to releasing 42 total captive born snakes, spanning three different age classes, from seven different zoos on VI Cay between 1993-1995. Post release, a ten year study was conducted to assess the snake population's overall well-being, confirming that the original 42 snakes released had increased to a 170 strong population [4]. More recent surveys in 2018 done by Reynolds et al. detected only 20 boas over the span of 2 nights, resulting in an estimate of 26 - 33 total boas across the island. The drastic drop in population over a short period of time only adds to the urgency of maintaining this diversity.

The importance of assessing release sites prior to reintroduction and having an initial population with a high survivability and resistance is vital to the success of reintroduced populations in the future. Because populations have been evolving separately in their isolated populations, some conservationists suggest against breeding snakes from the separate populations in effort to diversify the gene pool; this ideology comes from not knowing the long term effects of out-breeding depression on the fitness of the progeny left behind. Issues surrounding reintroduction include: finding viable habitat, exterminating rats from these habitats, unpredictability in climate change, and finding sufficient prey source for introduced populations. The steadily declining populations and fear of further inbreeding however, made translocation and reintroduction a better solution than no intervention at all. The Cayo Ratones reintroduction was initiated with the release of 28 captive-born boas from seven different zoos and was later supplemented with 13 additional boas for a total of 41 individuals. By 2004, the population had grown to an estimated 500 boas [2]. Unfortunately, since 2004, Cayo Ratones has been recolonized by rats, and no boas were found during a 3-night transect survey in 2018. Although VI boas are difficult to find, other reports have concluded that the Virgin Island Boa is no longer found at this location.

Figure 2. Range of the Virgin Islands Boa (*Chilabothrus granti*).

What is known about the genetics of this species has been minimal at best. Prior to this study, Rodriguez-Robles et al. (2015) sampled the five islands of Cayo Diablo, Tortola, Culebra, St. Thomas and Puerto Rico; using Cyt b, ND4, tRNAs, and concluded that mtDNA markers from samples in Puerto Rico, Cayo Diablo, and St.Thomas had no nucleotide diversity within the populations but that each island had its own distinct haplotypes [7]. The samples from Culebra and Tortola however, identified private mtDNA haplotypes at each location. In using a multilocus approach for this project as well, we are able to obtain a higher genetic sample size in spite of having a low number of individuals for sampling. Rodriguez-Robles et al. further concluded that five of the seven nuclear genes observed in their study were found to be highly conserved, with only two of the seven nuDNA loci exhibiting variation among the species [3]. Reynolds et al. (2015) obtained a different sample set from St. Thomas, Cayo Diablo, and Puerto Rico, finding a single mtDNA haplotype apparently unique to each island and that sample sites had a maximum separation of four mutational steps [2]. It was observed that only a single step distinguishes Río Grande and Cayo Diablo sequences, with the Río Grande population exhibiting the highest allelic richness. Because of this assessment it is assumed that the amount of gene flow between snake populations, even those found on the same island, is minimal to nonexistent when habitat is not continuous. In 2015, Reynolds et al. developed nine species specific primers for identifying polymorphic microsatellite loci [2]. These primers are important for future conservation and analysis because the ability to pull alleles from a resequenced section of the genome will be a lot cheaper and faster in the future. But, the ability to examine single nucleotide polymorphisms (SNPs) across the genome would capture a more detailed look at population genetic differentiation.

Reintroduction and all future captive breeding should be closely monitored to ensure proper measures are being taken to maximize genetic diversity within the species [2]. The purpose of this study is to define the scope of the diversity among several important populations of the endangered Virgin Island Boas through analyzing mtDNA and nuclear DNA. It is with this information and in collaboration with the Asheboro Zoo (North Carolina) and US Fish and Wildlife Service (Puerto Rico) that we hope to maximize success of future captive breeding and population management. The most recent genetic findings support a strategy of converging reproductively isolated populations from within each island occupied by VI boas and allow them to interbreed in a new location. The previous lack of genetic and occurrence data at an appropriate scale made it difficult to approach populations in this way, but the data generated should give us a more comprehensive look at the species, as well as direction for the best way to manage individual populations on islands. I proposed two hypotheses. 1) If islands are genetically divergent, then unique alleles should be found on each island. Alternatively, 2) if alleles are widespread, then islands should show evidence of mixed alleles (Fig. 3). The importance of distinguishing the amount of genetic diversity, the habitable range currently occupied, as well as possibilities for future captive breeding/ reintroduction -are vital for this species' survival.

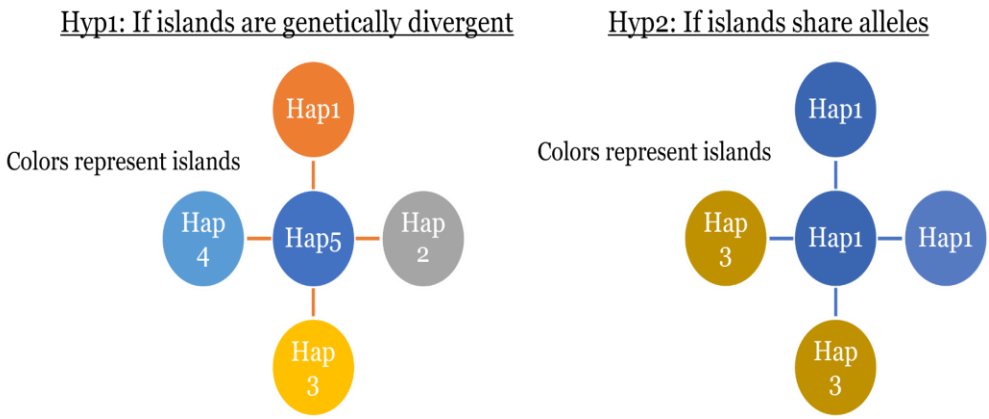

Figure 3. Hypothetical arrangement of alleles on islands. Islands might be expected to harbor unique alleles, or to be a mixture of extant genetic diversity in the species. The first hypothesis predicts that if islands do not share haplotypes, the islands would only be represented by one circle. In the second Hypothesis: is Islands shared haplotypes multiple island circles would have the same color.

2. Methodology

2.1 Sample Collection

Samples for this study encompass nearly 20 years of effort on the part of researchers studying the Virgin Islands Boa. Samples from St. Thomas were collected by P. Tolson, N. Angeli, R. Platenberg, and others. Samples from Puerto Rico were collected by G. Reynolds, A. Puente, and JP Zegarra. Samples from Cayo Diablo were from individuals collected and housed at the Toledo Zoo in the late 1990s by P. Tolson. Samples from VI Cay were collected by D. Smith and G. Reynolds.

All samples consist of tissue or scale clips removed from individual snakes and stored in either 95% EToH or RNALater(R) buffer. Samples were cryo-stored long-term in -80 degree C freezers. For road-killed snakes collected, a small piece of muscle tissue or liver was removed. For live snakes, either a portion of the tail (<5 mm) or 3-5 ventral scale clips were taken using antiseptic surgical techniques. Any boa found with a clipped or damaged tail tip was not sampled to prevent repeated sampling, and live boas were returned to the exact capture location within 24 hours of sampling. All methods described here have been approved by multiple IACUC permits, including most recently for the Reynolds Lab by UNC Asheville's IACUC [1].

2.2 Mitochondrial Sequencing Analyses

DNA samples were extracted using a Qiagen Wizard SV DNA extraction kit after thawing and spinning each sample on a Vortex Genie 2™ to ensure they were well mixed. For each sample I pipetted 5 ul of each DNA sample, 12.5 ul of Green MasterMix, 2.5 ul of F primer and 2.5 ul of R primer, as well as 2.5 ul of ddh_2O. This mixture was then spun on an OHAUS Frontier™ 5306 to ensure homogeneity. The primers used were specific to target the entire coding region of the cytochrome B locus. I loaded samples on a SimpliAmp thermocycler to amplify the cytB region of the mtDNA using a polymerase chain reaction (PCR) with an annealing temperature of 46 degrees . I then tested PCR products via gel electrophoresis using TAE and GelGreen(R) agarose tabs.

PCR products were sent to the North Carolina State Genomics Laboratory for clean-up (using Exo-SAP^R) and sequenced in both directions on an ABI 3500 Sanger sequencer using Big Dye(R) chemistry. Sequences were returned as .abi files, which I loaded into Geneious^R for clean-up and processing. I created contigs for each forward and reverse sequence from an individual, then manually aligned and trimmed contigs to the coding region of the cytochrome B gene. I paired newly-sequenced samples with an existing dataset from Reynolds et al. (2015) that included 24 sequences, and created a final alignment of all available sequences [2].

To examine the mtDNA data set I used R V. 4.1.1 [6] implemented in R-studio V. 1.1.442 [7]. I first installed the packages ape [8] and pegas [9] to read in the FASTA file using *read.dna()* from the package ape. I then applied the code *haplotype()* from the pegas library to collapse the individuals into distinct maternal haplogroups. From there, I was able to construct a haplotype network using the code *haploNet()* from the pegas package. This organized the data into a visual representation of genealogical relationships among the individuals, and showed the genetic distance and mutational steps between distinct haplotypes. I was able to determine the nucleotide diversity among samples using *nuc.div()* and then calculated the haplotype diversity with *hap.div()* both from the pegas package. Eighty-eight samples were generated containing 1,100 base pairs of Cytochrome b were generated.

2.3 RAD-Seq Genotyping Analyses

A subset of 96 samples were selected to represent all the sampling populations, with emphasis on St. Thomas and VI Cay. DNA extracts from these samples were quantitated using a QuBit(R) 3 with the BS (broad spectrum) kit.

Extracts were then sent to Tangled Bank Conservation (Asheville, NC) for triple-digest RADseq (3RAD) library preparation [10]. 3RAD consists of using two restriction enzymes to cleave whole genomic DNA at specific cut sites, and inclusion of the third restriction enzyme cleaves adapter-dimers to improve library quality. The samples are digested in a reaction buffer with the three enzymes, then the DNA fragments are ligated to iTru adapters and internal barcodes with sequences specific to each individual. Barcoded samples are then pooled and PCR amplified with iTru5 and iTru7 primers, purified, and then size-selected using AmPure Beads. Size-selected and cleaned libraries were then sent to GENEWIZ Inc. (Brooks Life Sciences, South Plainfield, NJ, USA) for 150bp paired-end sequencing on an Illumina Hi-Seq^R platform in a single lane. Resulting fastq files were then batched through Tangled Bank

Conservation's proprietary ipyrad 0.7.28 (Eaton 2014; Eaton and Overcast 2018) bioinformatics pipeline to yield a VCF file containing genotypes for each sample [11,12]. Genotypes consist of single nucleotide polymorphisms (SNPs) called for each locus represented in the dataset, and missing data result from either missing loci for a sample and/or missing allele calls for a sample at a locus. A total of 83 samples of the 96 sent were usable for our dataset.

I loaded the resulting VCF file into R using the function *read.vcfR()* in the package vcfR where I examined the quality of the data using a depth of sequencing plot with the code *extract.gt()* also from the package vcfR, to ensure the coverage across loci was > 5X [13]. I converted the VCF file to a genlight object using the function *vcfR2genlight()* and then converted the file to a genotype based genid object using the function *df2genid()* in the package adegenet [14,15] . During this conversion some individual data files were omitted due to missing data. I was able to view the individual samples dropped from the dataset with the code *propTyped()* in the package adegenet. After proofing the dataset I ended up with 83 genotypes of nuclear DNA consisting of 83,000 single nucleotide polymorphisms (SNPs) per sample. I then converted the genotype matrix to a *hierf* object while attaching a population configuration file to identify which genotypes belong to which population using the function *genid2hierfstat()* in the package adegenet. From here I was able to generate a genetic distance matrix to estimate evolutionary divergence based on allele frequency differences using the function *genet.dist()* in the package hierfstat [16] and visualization by exporting a matrix using the function *write.table()*. To estimate divergence among grouping and genotypes I implemented a multivariate statistical method called Discriminant Analysis of Principal Components (DAPC) within the package adegenet and the function *dapc()*. From this analysis I manipulated the data with the codes: *scatter(), assignplot()* and *compoplot()* all within the package adegenet, to visualize the data. We then calculated the inbreeding statistics on an individual sample basis using the function *inbreeding()* in the adegenet package [14] . This statistic is a measure of the means of 100 generated samples in an estimate of *F* for the likelihood of a sample being homozygous or heterozygous for a given genotype across loci; *F* being the probability that matching alleles are inherited from a common ancestor.

3. Results

3.1 mtDNA

I obtained 1,100 bp of sequence data for cytB from each of the 88 retained boa samples from four island populations. From these samples I found 6 distinct haplotypes (Table 1). Haplotype I was the most frequent, with 65 boas, and is found in two of the populations on St. Thomas (STT) and Virgin Island Cay (VICay). Haplotype II was limited to the Puerto Rico population with only 7 boas. Haplotype III was only present in Cayo Diablo with 5 boas. Haplotype IV had 5 boas in the VICay population, and V and VI each had one boa represented within VICay. The majority of the diversity among mitochondrial haplotypes is located within VICay, encompassing 4 out of the 6 distinct haplotypes. It is important to distinguish that although there are 6 haplotypes, the individuals with these rarer haplotypes are not guaranteed to have bred or survived long enough to be able to pass on their variability.

Table 1. Haplotype table distinguishing the number of boas from each of the 4 populations within a distinct haplotype. Six total haplotypes were derived from the cytochrome b loci of the mitochondrial genome of 88 boa samples

Haplotype Number	CayoDiablo	PuertoRico	STT	VICay
I	0	0	21	44
II	0	7	0	0
III	5	0	0	0
IV	0	0	0	5
V	0	0	0	1
VI	0	0	0	1

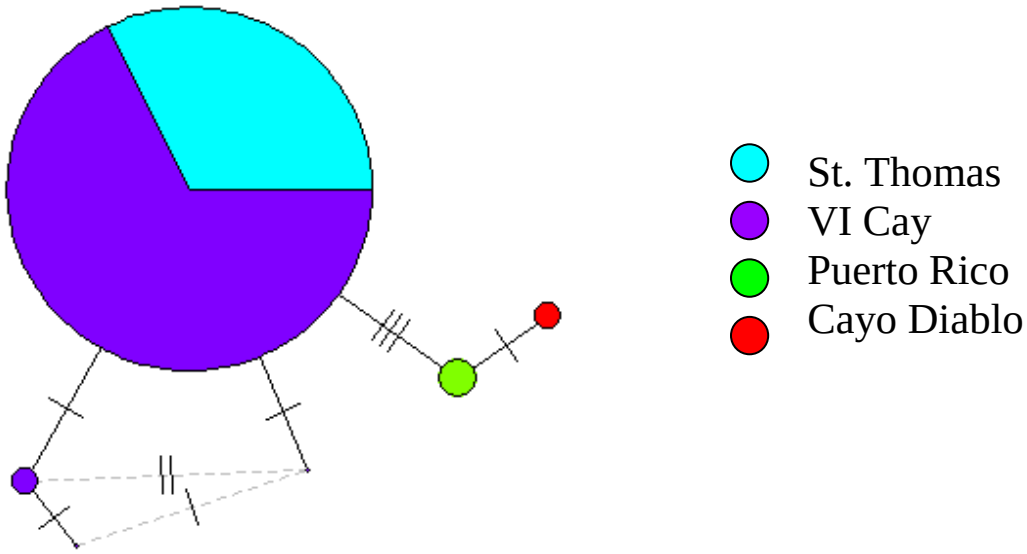

Figure 4. Haplotype network depicting the 6 unique haplotypes derived from the data. Size of the circle indicates the number of samples with that haplotype and color represents the different populations by island. Lines signify a direct evolutionary relationship between haplotypes, while mutational steps between haplotypes are represented by tick marks.

With these same mtdna data, we get a better representation of this information with a haplotype network (Fig. 4) As seen by the large size, the majority of the samples are within Haplotype I composed of STT and VICay individuals. VICay also has three other distinct haplotypes present but with minimal representation. Puerto Rico and St. Thomas also have their own unique haplotypes present within those populations. The VICay population is most divergent from the Cayo Diablo population with 6 mutational steps between them. The Cayo Diablo haplotype is one mutational step divergent from the Puerto Rico population and the Puerto Rico haplotype is three mutational steps divergent from the most common haplotype. Two of the VICay haplotypes IV and V are each a single mutational step from Haplotype I,

while haplotype VI, also from VICay, is three mutational steps from Haplotype I. The dashed lines show a less confident relationship pattern that was predicted.

3.2 Nuclear DNA RADseq

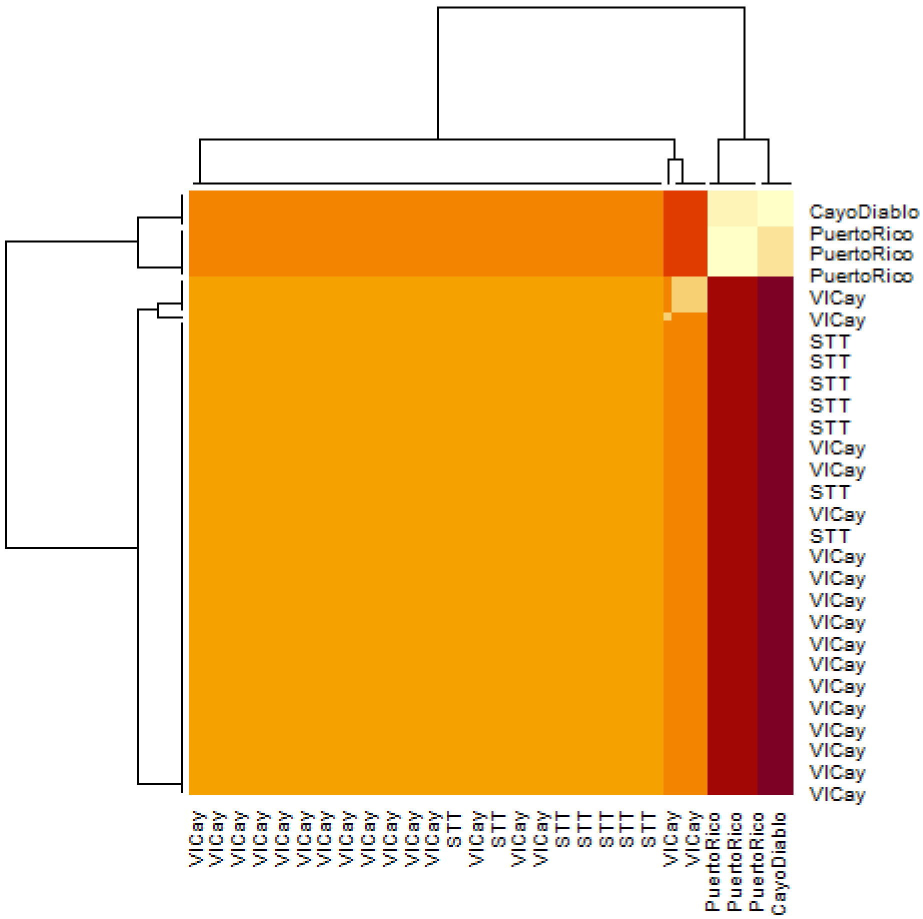

Figure 5. A genetic distance matrix displaying relatedness of samples across other samples. Lighter colors indicate more similarity, and darker colors represent more variation. The phylogeny above and to the side of the graph shows how individuals should be grouped based on their relatedness to each other.

The genetic distance matrix (Fig. 5) I then generated shows that samples within an island population are most related to each other except for the case of VICay and STT samples. A subset of VICay and STT samples form their own separate branches on the proposed phylogeny, while Cayo Diablo and Puerto Rico samples have separate branches as well. VICay samples are most distantly related to the Cayo Diablo population and then the Puerto Rico population. The VICay population having the most diverse representation of haplotypes among populations shows how successful the reintroduction of Virgin Islands boas to this island has helped to conserve genetic diversity from across islands.

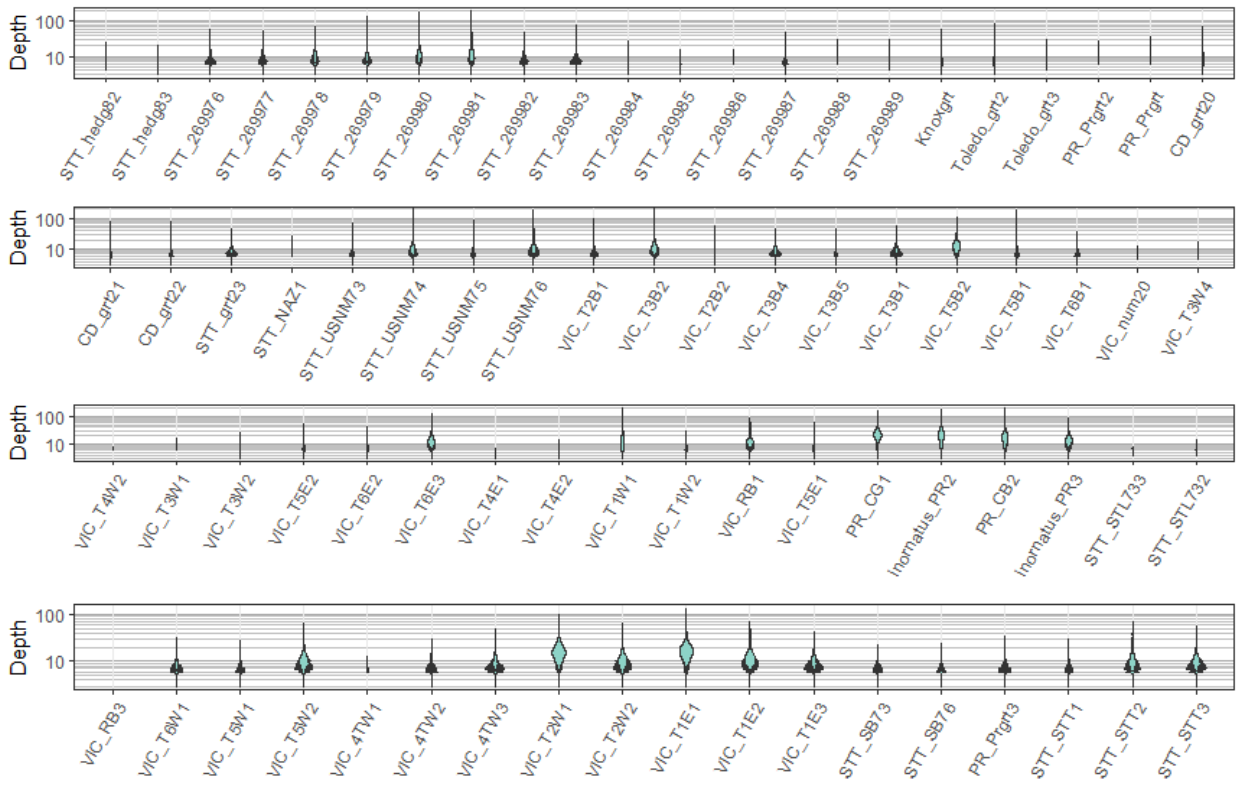

Figure 6. Depth of sequencing plot showing amount of coverage across loci for each sample. Individuals were dropped if they did not have at least 5X coverage

For whole genome sequences, I first generated my depth of sequencing plot (Fig. 6) Using the 3RAD-Sequencing I used 83,000 single nucleotide polymorphisms (SNPs) per sample. Answering the question: How much coverage per loci per individual do we have? From this I was able to see which individuals did not have enough coverage to then be used in the Discriminant Analysis of Principal Components. Only two of the 83 samples from VICay were dropped from the dataset: VIC_RB3 and VIC_T4W2.

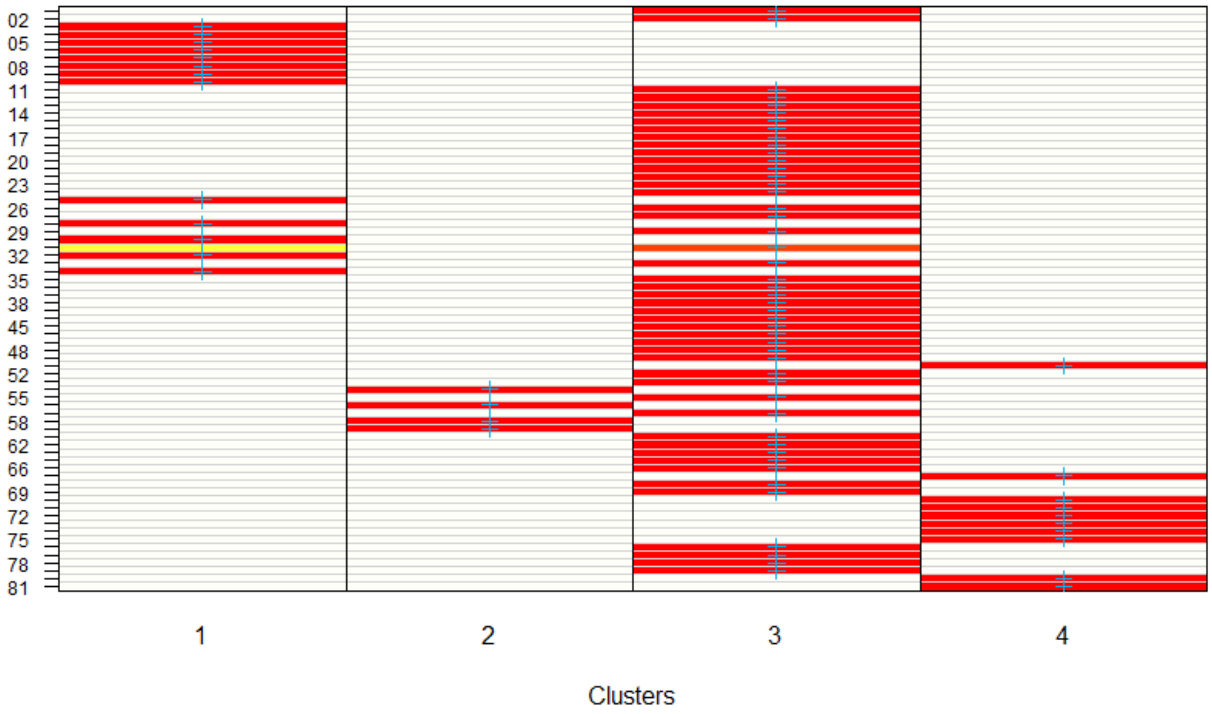

Figure 7. Assignplot forming clusters of samples based on their nuclear genome similarities, red indicating a high confidence level in the assignment to a group, and yellow representing less confidence in placement. The y axis represents each sample creating a row and each column represents a cluster.

In the Assignplot (Fig. 7), four distinct groups were created, with the majority of individuals being placed in high confidence, and only a single individual being placed with less confidence. Most of the samples resided in the third cluster, and the least represented cluster was number 2. From there I ran a multivariate statistical analysis on the same data using Scatterplot (Fig. 8). We wanted to know if islands grouped separately or if samples from within each group consisted of multiple islands. In correspondence with our (.str) file that was used for this analysis, we see that numbers 31-59 on the y axis are representatives from the VICay population. From this, we were able to see that individuals from VICay were represented in 3 of the 4 clusters: in groups 1, 2 and 4 within the 95% confidence interval. The other islands, however, were limited to just a single cluster. This outcome reflects the data generated in the mtDNA analysis and can be directly attributed to the translocation efforts on VICay.

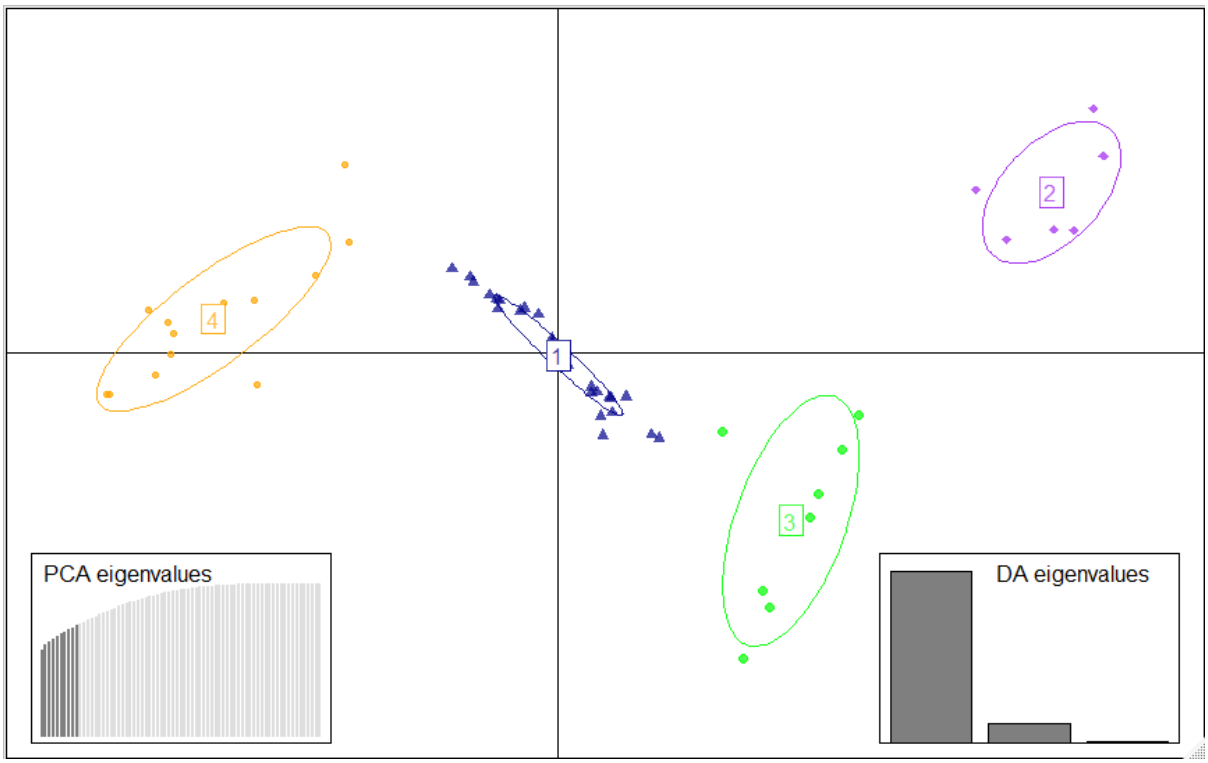

Figure 8. A Two dimensional representation of variance, with the first axis representing the most amount of variance and the second axis representing the remaining variance in the nuclear genome. Each group is represented by a different color and a 95% confidence interval circle. Space between points indicates genetic distance between individuals.

This reflected what we had anticipated of VICay, but also reinforced what we expected of other islands holding less genetic diversity. Group 1 seems to have the most consistent variance among samples along the first axis of variance and the smallest confidence interval circle. Group 2 as shown in the assignplot had the least amount of individuals per cluster while groups 3 and 4 were relatively similar in number.

4. Discussion

The relative haplotype and nuclear genetic diversity we see specifically in VICay is due to the successful reintroduction of Virgin Islands boas to this island. Because of the separation of land masses by water, generated during the pleistocene, islands accumulated genetic differences over time. The reintroduced individuals to VICay are from multiple islands and therefore have a more diverse representation of the entire species. The mtDNA showed that 6 different haplotypes are present within the species and the nucDNA resulted in 4 genetically diverse groups among samples. As shown, islands without any interactive management practices have very limited haplotype diversity in the mtDNA. All other islands that had not had reintroduction were limited to a single distinct haplotype per island and a singular nuclear genomic cluster. Without the continued management of these populations, the allelic frequencies we see now will not continue to persist. The St. Thomas population has rapidly declined over recent years, and the outlook for the species here is less than favorable. We see the persistence of the STT haplotype on VICay however because of the individuals bred and captivity over 20 years ago. Haplotype I most likely originated from STT but occurs in high abundance on VICay now because of human introduction. We see that the most divergence lies within the comparison of Puerto Rico and Cayo Diablo populations to VICay's populations. It is thought that with the loss of key food species on VICay, the survivability of these snakes is likely to be further impacted. Based on our data, it seems that these populations have been experiencing genetic relaxation for many generations now and have likely lost much of their previous diversity. Because of natural selection, evolution, and just pure chance, individuals that contain the different

haplotypes are not guaranteed to reproduce and pass on their genes. If unmanaged these populations will continue to lose diversity at increasingly higher frequencies until fixation. Management is an important aspect of conservation in this species because having such small population sizes increases the rate of population genetic relaxation over each generation. Prior research done showed similar results of limited variation among the species in the mtDNA [2,3] , the data generated here, however, shows a much clearer picture of the diversity among populations for the entire species. Prior to this study, there was no comprehensive analysis for the species. Rodriguez-Robles had discovered no nucleotide diversity within the populations but that each island had its own distinct haplotype [3] ; while our work, using a larger sample group, showed that there was more nuDNA diversity than initially thought.

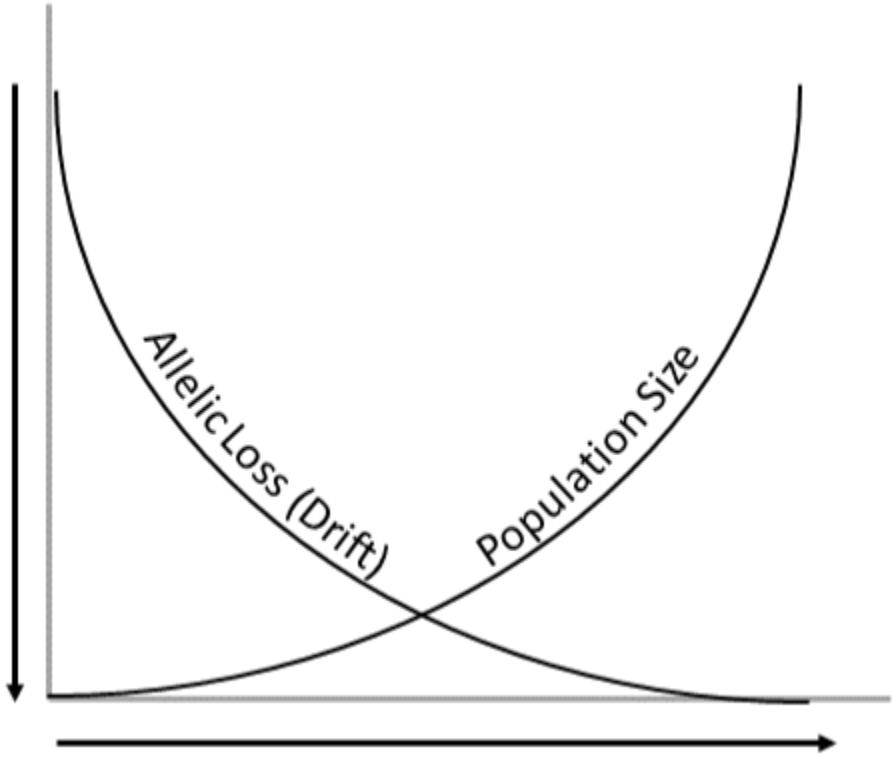

Figure 9. A graph depicting a genetic drift simulation with the amount of allelic loss and population size over time. An emphasis on the point of intersection where there is an equilibrium between the two.

The figure above shows the expectation of the strength of genetic drift relative to population size. At small population size, drift is extremely strong and alleles (particularly rare alleles) are lost from the population every generation. The longer the population stays small, the more genetic diversity is lost owing to drift (the "bottleneck effect"). If the population size grows, the strength of drift declines because more individuals are contributing to the gene pool.

The genetic drift simulation above shows how smaller population sizes decrease the chance in an individual finding a mate. Because of this, the rate of alleles dropped within our species population can be predicted to become higher with each new generation being born. Currently, work is being done at the Asheboro Zoo in North Carolina, with goals of being able to make informed decisions on captive breeding pairs in efforts to maximize allelic frequency potentials of the species. Not only is this approach the best chance of preserving the species to date, having a captive population also allows for us to maintain a certain number of these boas to prevent them from a possible single incidence of extinction. With the predicted impacts of climate change being most severe on coastal and island communities, the already limited habitat of this species will continue to shrink; and although work has been done to mitigate rat population influence, a single strong storm surge could undo years of work and decimate island habitats. The threats to this species are ever prevalent, but the data we have generated will help to create genetically informed conservation strategies.

5. Acknowledgments

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6. References

1. Reynolds, R. G., & Henderson, R. W. (2018). Boas of the World (superfamily Booidae): A checklist with systematic, taxonomic, and conservation assessments. *Bulletin of the Museum of Comparative Zoology*, *162*(1), 1–58. https://doi.org/10.3099/mcz48.1
2. Reynolds, R. G., Puente-Rolón, A. R., Platenberg, R., Tyler, R. K., Tolson, P. J., & Revell, L. J. (2015). Large divergence and low diversity suggest genetically informed conservation strategies for the endangered Virgin Islands boa (chilabothrus monensis). *Global Ecology and Conservation*, *3*, 487–502. https://doi.org/10.1016/j.gecco.2015.02.003
3. Rodríguez-Robles, J. A., T. Jezkova, M. K. Fujita, P. J. Tolson, and M. A. García. 2015. Genetic divergence and diversity in the Mona and Virgin Islands Boas, Chilabothrus monensis (Epicrates monensis) (Serpentes: Boidae), West Indian snakes of special conservation concern. Molecular Phylogenetics and Evolution 88:144-153.
4. Tolson, P.J., Garcia, M.A., and J. J. Pierce. 2008. Re-introduction of the Virgin Islands boa to the Puerto Rico Bank. Pp. 66-69 In: P. Soorae (Ed.) Global Re-introduction Perspectives: re-introduction case-studies from around the globe. IUCN/SSC Re-introduction Specialist Group, Abu DhabiUAE. Viii+284p.
5. Savidge, J.A., Hopken, M.W., Witmer, G.W., Jojola, S.M., Pierce, J.J., Burke, P.W. and Piaggio, A.J., 2012. Genetic evaluation of an attempted Rattus rattus eradication on Congo Cay, US Virgin Islands, identifies importance of eradication units. Biological invasions, 14(11), pp.2343-2354.
6. R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.
7. RStudio Team (2021). RStudio: Integrated Development for R. RStudio, PBC, Boston, MA URL http://www.rstudio.com/.\
8. Paradis E, Schliep K (2019). "ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R." Bioinformatics, 35, 526-528.
9. Paradis E (2010). "pegas: an R package for population genetics with an integrated–modular approach." Bioinformatics, 26, 419-420.
10. Bayona-Vásquez, N. J., Glenn, T. C., Kieran, T. J., Pierson, T. W., Hoffberg, S. L., Scott, P. A., et al. (2019). Adapterama III: quadruple-indexed, double/triple-enzyme RADseq libraries (2RAD/3RAD). PeerJ 7:e7724.
11. J. Catchen, P. Hohenlohe, S. Bassham, A. Amores, and W. Cresko. Stacks: an analysis tool set for population genomics. Molecular Ecology. 2013. [reprint]
12. J. Catchen, A. Amores, P. Hohenlohe, W. Cresko, and J. Postlethwait. Stacks: building and genotyping loci de novo from short-read sequences. G3: Genes, Genomes, Genetics, 1:171-182, 2011. [reprint]
13. Knaus BJ, Grünwald NJ (2017). "VCFR: a package to manipulate and visualize variant call format data in R." Molecular Ecology Resources, 17(1), 44–53. ISSN 757, http://dx.doi.org/10.1111/1755-0998.12549.
14. Jombart T (2008). "adegenet: a R package for the multivariate analysis of genetic markers." Bioinformatics, 24, 1403-1405. doi: 10.1093/bioinformatics/btn129.
15. Jombart T, Ahmed I (2011). "adegenet 1.3-1: new tools for the analysis of genome-wide SNP data." Bioinformatics. doi: 10.1093/bioinformatics/btr521.
16. Goudet, J. (2005) Hierfstat, a package for R to compute and test hierarchical F-statistics. Molecular Ecology Notes. 5: 184-186