

Assessing the Genetic Impact of Headstarting and Potential for Mitigation in the Jamaican Iguana, *Cyclura collei*

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Introduction

The Jamaican iguana, *Cyclura collei*, was once common and is now represented in the wild by a single small population in the Hellshire Hills (see Figure 2). The population is now listed as Critically Endangered by the IUCN Red List of Threatened Species, but for nearly half a century the Jamaican iguana was feared to be extinct.^[1] The Hellshire Hills population was discovered in 1990 when a pig hunter's dog captured an adult specimen in this remote southeastern region of Jamaica. Two active nesting sites were eventually discovered (see Figure 3) but it was determined that several factors were causing a continued decline in this last surviving population. The major threats included predation by feral mammals and habitat destruction by charcoal burners.^[2] Research indicated that if this trend was not reversed extinction of this small population was likely. A headstart program was initiated to rear young until they were large enough to fend off invasive mammalian predators. Since 1991, hatchlings have been collected annually and raised at the Hope Zoo in Kingston. In the case of the Jamaican iguana, the program appears to have been successful, and the number of breeding individuals in the Hellshire Hills is now increasing. However recent genetic research has shown that the population has lost genetic variation which is causing heterozygosity levels to decline (see Figure 4). If left uncorrected, these factors could lead to increased inbreeding depression, the decline of the population, and may potentially lead to extinction. Our current goal is to determine whether this loss of genetic variation can be mitigated or even reversed through genetic monitoring and selection of individuals for inclusion in the headstart program.

Methods

- Blood samples have been collected from wild caught hatchlings of *Cyclura collei* since 1991.
- Samples collected between 1991 and 2009 were used for this study.
- The DNA was isolated from the blood samples using an ABI 6100 Nucleic Acid Prepstation, with the NucPrep™ gDNA Isolation kit according to the recommended protocol (Applied Biosystems, Foster City, CA).
- Microsatellites, highly variable molecular markers, originally developed for other species of *Cyclura* were screened for variability in *Cyclura collei*.
- Allele frequencies and degree of genetic variability, heterozygosities, in each annual cohort of hatchlings and wild caught adults were estimated for comparison.
- A hierarchical AMOVA was used to determine if allele frequencies in hatchlings collected prior to the release of headstarted iguanas differed significantly from that observed in the years following the first set of releases.
- The probability of producing heterozygous offspring, assuming random mating, and given allele frequencies in the post-headstart populations were calculated for each individual in the study as a breeding value.
- The average breeding value of post-headstart individuals was set to zero, and reflect the proportion of loci that are expected to be heterozygous beyond that of an average individual in the post-headstart population.

Results

- Twelve of 30 microsatellite loci developed for three other species amplified and were variable in *C. collei*.
- DNA was successfully isolated from a total of 375 individuals, including 11 captive individuals currently in US zoos, and these were genotyped via fragment analysis for six informative loci.
- Fragment and data analysis from the time span of 1991 to 2009 displayed a slight decline in average heterozygosity in the individuals collected before and after the initiation of headstart iguana releases.
- Hatchlings collected before the release of headstart iguanas showed an average heterozygosity level of 0.450, while those in the post-headstart group displayed a decrease, with an average of 0.391.
- The proportion of genetic variation distinguishing the two groups was 13%, four times greater than the degree of variation that discerns cohorts prior to or following releases from the headstart program.
- Analysis of Molecular Variance (AMOVA) confirmed the statistical significance of the decline in genetic variability.
- Breeding values were calculated for pre-headstart (1991-1996) and post-headstart (2002-2009) individuals.
- Post-headstart breeding values were adjusted to have a mean of zero (S.D. 0.06).
- The pre-headstart group displayed an average breeding value of 0.08 (S.D. 0.089).
- The US zoo captive population displayed an average breeding value of 0.04 (S.D. 0.057).



Figure 1: A subadult headstarted *Cyclura collei* just after release.

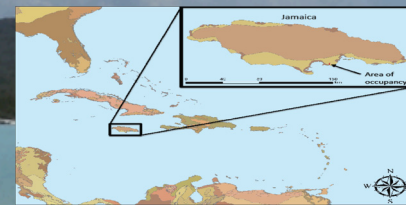


Figure 2: Map of the Caribbean with an inset of the island of Jamaica showing the location of the Hellshire Hills and the last remaining population of *Cyclura collei*.



Figure 3: *Cyclura collei* are released at one of the two major nesting sites.

References

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- Wilson, B.S., Alberts, A.C., Graham, K., Hudson, R.D., Kerr Bjorkland, R., Lewis, D., Lung, N.P., Nelson, R., Thompson, N., Kunna, J., and Vogel, P. 2004. Survival and reproduction of repatriated Jamaican iguanas: headstarting as a viable conservation strategy. In: A.C. Alberts, R.L. Carter, W.K. Hayes and E.P. Martins (eds), Iguanans: Biology and Conservation, pp. 220-231. University of California Press, Berkeley, California.

This work was supported by the Mohamed bin Zayed Conservation Fund and the Fresno Chaffee Zoo.



Discussion

- Iguana hatchlings were first collected in 1991 for headstarting and adults were not released until March 1996, giving a baseline for heterozygosity levels that the surviving iguanas maintained prior to the release of headstart individuals.
- A comparison of heterozygosity, by cohort, with individuals collected before and after the release of headstart iguanas shows a decline after their release, suggesting the program has resulted in a loss of genetic variation in the population (see Figure 4). This loss of variation is accredited to a few alleles being detected at very low frequencies in more recent years.
- Analysis of Molecular Variance (AMOVA) shows that a statistically significant amount of the total genetic variation in our samples distinguishes individuals collected before and after the release of headstarted iguanas, indicating that the loss of genetic variation is significant (see Figure 5).
- Breeding values for heterozygosity of both pre-headstart and post-headstart groups were compared to see how genetic variation had been lost in the population (see Figure 6).
- 11 individuals tested from the captive US zoo population have an average breeding value of 0.04, indicating their relative representation of mirroring the diversity of the wild population.
- In 2009, 14 individuals were picked at random for the headstart program, and the remaining hatchlings were released. Average breeding values for headstart iguanas collected in 2009 was -0.014 (S.D. 0.049). In the 2009 cohort, 24 of the 77 hatchlings sampled exhibited breeding values greater than zero indicating that their offspring would be more heterozygous on average relative to the current breeding population (see Figure 7). This research suggests that the observed loss of genetic variation may potentially be mitigated or even reversed if the headstart program selected individuals based on their genetic composition.

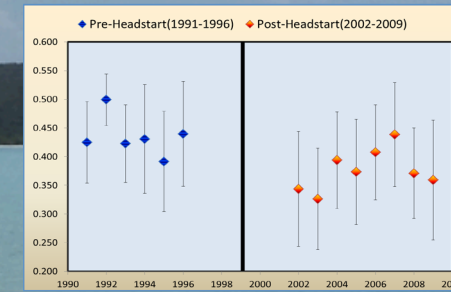


Figure 4: Heterozygosity comparison of individuals collected before and after the release of headstart iguanas. Average Heterozygosity: Pre: 0.450 Post: 0.391

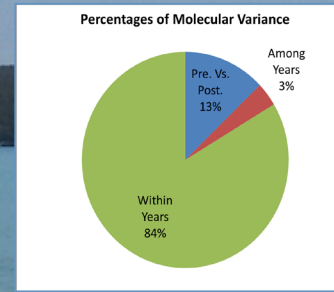


Figure 5: Molecular Variance among hatchlings within years vs. among years and grouped by the years before and after the first set of headstart releases (p<0.01).

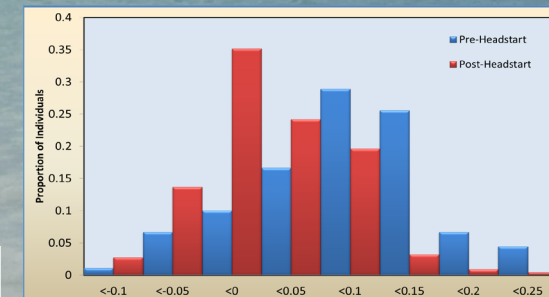


Figure 6: Breeding values of heterozygosity comparing Pre-Headstart to Post-Headstart individuals.

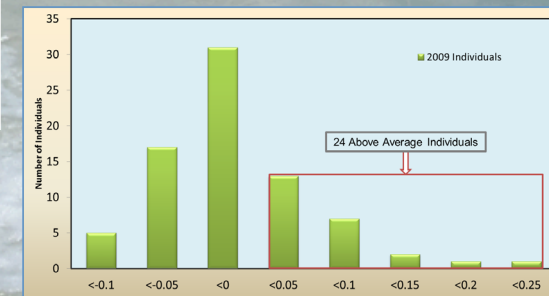


Figure 7: Breeding values of 2009 individuals (24 individuals with values above average).