

MOLECULAR GENETICS AS A CONSERVATION MANAGEMENT TOOL FOR SCARLET MACAWS (*ARA MACAO*) IN LA SELVA MAYA

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INTRODUCTION:

Genetic considerations are an integral component of any wildlife management program. This is especially true when reintroduction or translocation initiatives are aimed at population reinforcement, where released individuals are intended to breed with wild population. Before intensive metapopulation management programs are initiated the needs and goals of the project must be clearly identified, including the careful assessment of the genetic status of historical and extant wild populations and potential source populations (wild or captive). Strategies can then be designed to introduce genetic variation that will enhance, yet complement, the genetic composition of the wild population.

Our research employs molecular genetic techniques at multiple hierarchical levels to develop a genetic management plan for scarlet macaws in La Selva Maya and will consider issues of taxonomy, extent of gene flow between breeding sites in Belize, Guatemala and Mexico, and overall genetic variation within wild and captive populations. This work will provide critical empirical data for local managers to guide the development and help monitor the genetic impacts of intensive metapopulation management efforts. While our project focuses on La Selva Maya, these results will have important implications for other scarlet macaw conservation programs such as the proposed reintroduction efforts of SalvaNatura in El Salvador.

OBJECTIVES:

Objective # 1: To use molecular genetic data to detect broad patterns of intraspecific genetic variation and identify diagnostic characters for unique evolutionary lineages. These data will be compared with the subspecies taxonomy (*Ara macao cyanoptera* and *Ara macao macao*) to determine if current designations represent operational conservation units.

Objective # 2: To use molecular data from both modern and historical samples to quantify the genetic diversity within and degree of gene flow between extant populations in La Selva Maya, and assess whether these patterns have changed over time.

Objective # 3: To use molecular data to develop a comprehensive metapopulation management program for scarlet macaws within La Selva Maya. This will include the genetic assessment of *ex situ* source populations to address questions of taxonomy and ancestry, design a genetic management plan, and identify potential release candidates.

METHODS:

Sample Collection

Genetic samples will be obtained from two primary sources. Feathers will be collected from within or below nest cavities, or plucked from macaw chicks prior to fledging. Tissue samples

will be taken from museum specimens collected 40-120 years ago to provide a historical context for the observed genetic patterns within extant populations.

Molecular Markers

- **Mitochondrial Sequences:** The mitochondrial genome is a maternally inherited piece of DNA that provides a useful tool for conservation geneticists. Different gene regions mutate at different rates, providing the opportunity to evaluate genetic variation at multiple hierarchical levels.
- **Microsatellites:** These markers are short repeated nucleotide segments with variants designated by the number of motif repeats (e.g. CT₄ = CTCTCTCT). Microsatellites exhibit high levels of variability, facilitating analysis of population genetics and individual kinship.
- **Nuclear Sequences:** Nuclear sequences and their associated single nucleotide polymorphisms (SNPs) mutate at a much slower rate and are used to infer older divergence events such as between taxonomic units.

Taxonomic Resolution

To determine the historical population structure of scarlet macaws throughout the species' range, DNA will be extracted from both museum specimens and samples from existing populations. Sequence data will be generated from four mitochondrial gene regions (12S, 16S, cytochrome oxidase I and cytochrome b). Single nucleotide polymorphisms (SNPs) will be used to characterize nuclear genetic variation. Data analysis will follow both a population aggregation analysis (identify diagnostic nucleotide characters) and phylogenetic tree-based approach to quantify intraspecific variation and diagnose conservation units.

In Situ Population Assessment – La Selva Maya

Both mitochondrial control region sequences and microsatellite data will be generated using samples collected from extant populations in Guatemala, Mexico and Belize. Haplotype, nucleotide, and allelic diversity will be used to assess levels of genetic diversity. Traditional F_{ST}-based genetic distances and Bayesian inference will be used to detect population structure and migration rates. Data from museum specimens collected in La Selva Maya prior to fragmentation will provide a baseline to infer temporal changes in the abovementioned population genetic parameters.

Ex Situ Population Assessment – Aviarios Mariana and ARCAS

Two captive breeding aviaries in Guatemala have been identified as potential source populations for future reintroduction efforts. Aviarios Mariana is a privately owned aviary and Asociación de Rescate y Conservación de Vida Silvestre (ARCAS) is a rescue and rehabilitation center for confiscated animals. A preliminary analysis of founder genotypes at each facility will be used to determine the best suited source population. Further work will utilize microsatellite data to generate multilocus genotypes for each individual. A detailed genetic management program will be developed based upon an analysis of relative relatedness and the identification of genetically important individuals.

PRELIMINARY RESULTS:

- Analysis of mitochondrial data revealed four distinct haplogroups based on fixed nucleotide differences (Fig. 1). The geographic overlap between the red and yellow haplogroups advocates for these populations to be treated as a single taxonomic unit. A similar pattern is seen with the green and blue haplogroups, suggesting both should be considered a single taxonomic unit as well. Interestingly, the boundary between the two haplogroups clusters is concordant with the subspecies boundary. This observation supports the designation of *A. m. cyanooptera* and *A. m. macao* as operational conservation units.
- Shared mitochondrial control region haplotypes provide evidence of gene flow between nest sites in Guatemala and Belize, thus encouraging trans-national collaborative management efforts.
- High levels of mitochondrial diversity are still present in La Selva Maya and should be seen as an encouraging sign for local managers.
- Both native and non-native individuals have been found in captivity; therefore caution is needed when designing breeding programs to produce juveniles for release.

FIGURES:



Figure 1. Map illustrating geographic distribution of mitochondrial haplogroups. The arrow points to the subspecies boundary between Nicaragua and Costa Rica.

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