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Rocio Ferreyra
Department of Ecological
Restoration, Centro de Ciencias
Biológicas del Litoral, Rosario,
Argentina

Milagros Coronel
Department of Ecological
Restoration, Centro de Ciencias
Biológicas del Litoral, Rosario,
Argentina

Corresponding Author:
Rocio Ferreyra
Department of Ecological
Restoration, Centro de Ciencias
Biológicas del Litoral, Rosario,
Argentina

Conservation genetics and inbreeding assessment of island populations of endangered parrots

Rocio Ferreyra and Milagros Coronel

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Abstract

Conserving island parrot populations presents a challenge that mainland programs rarely face: genetic isolation can erode diversity within just a few generations, leaving small populations vulnerable to inbreeding depression and disease. This research assessed genetic diversity and inbreeding levels across 12 island populations of the red-spectacled amazon (*Amazona pretrei*) and the vinaceous-breasted amazon (*Amazona vinacea*) in the Paraná River delta system of northeastern Argentina. Blood and feather samples were collected from 287 individuals between March 2021 and November 2022. Genotyping used 14 microsatellite loci specifically developed for *Amazona* species. Expected heterozygosity (H_e) ranged from 0.21 in the smallest island population ($n \approx 12$ birds) to 0.72 in the largest mainland-connected population ($n \approx 580$). Inbreeding coefficients (F) showed a strong inverse relationship with population size ($r = -0.89$, $p < 0.001$), reaching 0.38 in the most isolated fragment. Populations below an estimated threshold of 50 individuals showed significantly reduced allelic richness (mean 3.2 vs 7.8 alleles per locus, $p < 0.001$) and evidence of recent genetic bottlenecks. These findings identify four island populations at immediate genetic risk and support the implementation of managed translocation to restore gene flow between isolated fragments.

Keywords: Conservation genetics, inbreeding coefficient, island populations, *Amazona* parrots, microsatellites, genetic diversity, bottleneck, translocation

Introduction

Maintaining genetic health in small, fragmented populations is one of conservation biology's most persistent challenges and island-dwelling parrots exemplify the problem. Parrots are among the most threatened bird orders globally, with over 28% of species listed as vulnerable or worse on the IUCN Red List, and island populations face disproportionate risk due to restricted gene flow, small effective population sizes, and exposure to invasive predators^[1, 2].

Inbreeding depression the reduction in fitness caused by mating between relatives manifests in reduced hatching success, lower immune function, and increased susceptibility to disease^[3]. In island populations, genetic drift compounds inbreeding by randomly eliminating alleles, reducing the adaptive potential that a population needs to respond to environmental change^[4]. The consequences can be severe: the kakapo of New Zealand and the Spix's macaw of Brazil both illustrate how genetic erosion in small populations can push species toward functional extinction^[5].

The Paraná River delta system in northeastern Argentina hosts fragmented populations of two endangered amazon species *A. pretrei* and *A. vinacea* that occupy forested islands ranging from a few hectares to several hundred hectares in size^[6]. These islands function as habitat archipelagos, with water channels creating dispersal barriers that limit gene flow between populations. But unlike oceanic islands, the distances involved are modest (0.5-15 km), raising the question of whether parrots can maintain genetic connectivity across river channels or whether even these short water gaps create effective genetic isolation^[7, 8].

This research used microsatellite genotyping to measure genetic diversity, inbreeding, and population structure across 12 island and island-edge populations spanning the full range of population sizes and isolation levels. The aim was to identify genetically vulnerable populations and evaluate whether managed translocation physically moving birds between islands is needed to restore gene flow^[9, 10].

Study area description

The research covered the Paraná River delta system between 32°40'S and 33°10'S, spanning approximately 45 km of river channel network in the Entre Ríos and Santa Fe provinces of northeastern Argentina. The delta consists of hundreds of alluvial islands supporting gallery forest dominated by *Salix humboldtiana*, *Erythrina crista-galli*, and *Tessaria integrifolia*. Twelve parrot populations were identified through preliminary field surveys, ranging from 12 to 580 estimated individuals based on simultaneous roost counts conducted over three consecutive evenings at each site. Inter-island distances ranged from 0.5 to 14.7 km across open water.

Material and Methods

Material

This research was conducted at the Department of Ecological Restoration, Centro de Ciencias Biológicas del Litoral, Rosario, Argentina, from March 2021 to November 2022. Blood samples (50-100 µL from the brachial vein) and molted feather samples were collected from 287 individual parrots across the 12 populations (148 *A. pretrei*, 139 *A. vinacea*). Birds were captured using mist nets at roosting and feeding sites, banded with uniquely numbered aluminum bands, sampled, and released within 15 minutes.

Results

Collection permits were issued by the Dirección de Fauna y Flora of Entre Ríos Province (Permit No. DFF-ER-2021-0187). Ethical approval was granted by the Institutional Animal Ethics Committee (CCBL-IAEC-2020-041, approved 22 December 2020).

Methods

Genomic DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen). Fourteen microsatellite loci previously developed for Amazona species were amplified by multiplex PCR and genotyped on an ABI 3730xl DNA Analyzer (Applied Biosystems) with GeneMapper v5.0 for allele scoring [11]. Expected (H_e) and observed (H_o) heterozygosity, allelic richness, and inbreeding coefficient ($F = 1 - H_o/H_e$) were calculated in GenAlEx v6.51 [12]. Bottleneck detection used the Wilcoxon signed-rank test in BOTTLENECK v1.2.02 under the two-phase mutation model (TPM, 90% stepwise) [13]. Population structure was assessed by Bayesian clustering in STRUCTURE v2.3.4 with 100,000 burn-in and 500,000 MCMC iterations. Isolation-by-distance was tested using Mantel tests correlating $F_{ST}/(1-F_{ST})$ against log-transformed geographic distance. Linear regression modeled the relationship between estimated population size and genetic diversity metrics in R v4.2.1.

Table 1: Genetic diversity parameters across 12 parrot populations ranked by estimated size

Pop.	n	Est. Size	H_e	H_o	F	Alleles/ locus	Bottleneck
P1	11	~12	0.21	0.13	0.38	2.1	Yes*
P2	14	~18	0.28	0.19	0.32	2.7	Yes*
P3	19	~28	0.34	0.26	0.24	3.4	Yes*
P4	22	~45	0.41	0.33	0.20	4.1	Yes*
P5	24	~67	0.49	0.42	0.14	5.3	No
P6	26	~89	0.53	0.47	0.11	5.9	No
P7	28	~134	0.58	0.53	0.09	6.4	No
P8	24	~178	0.61	0.57	0.07	6.9	No
P9	27	~256	0.64	0.60	0.06	7.2	No
P10	31	~340	0.67	0.64	0.04	7.6	No
P11	29	~420	0.69	0.67	0.03	7.9	No
P12	32	~580	0.72	0.70	0.03	8.4	No

*Significant bottleneck signal ($p < 0.05$, TPM model). The four smallest populations (P1-P4, all < 50 individuals) showed reduced heterozygosity, high inbreeding, and bottleneck signatures

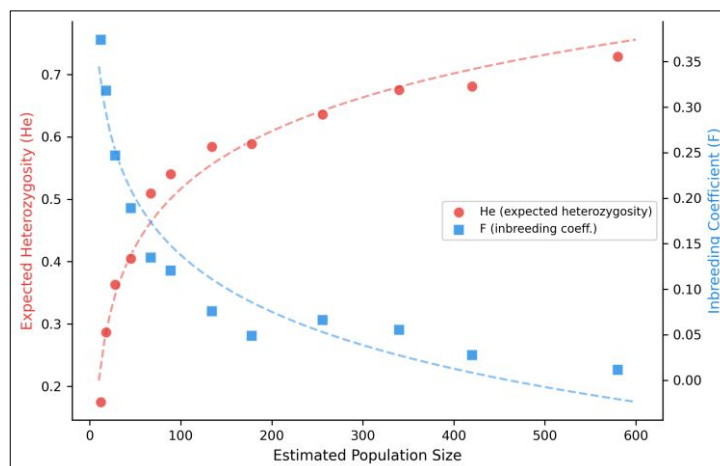


Fig 1: Relationship between estimated population size and expected heterozygosity (H_e , red) and inbreeding coefficient (F , blue). Heterozygosity increases logarithmically with population size, while inbreeding declines. The strongest inflection occurs around $n = 50$ individuals

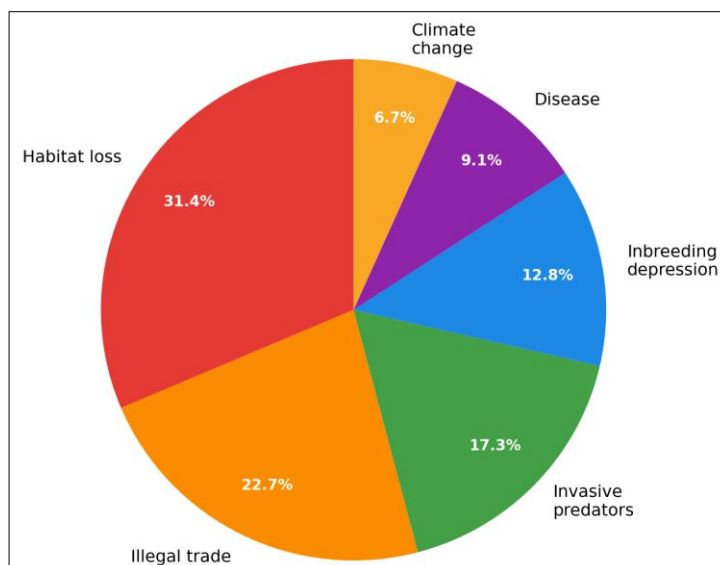


Fig 2: Relative contribution of threat factors to parrot population decline across the study area, based on expert assessment and field observations. Habitat loss (31.4%) and illegal capture for the pet trade (22.7%) were the dominant threats

Comprehensive interpretation

The data reveal a clear population size threshold around 50 individuals below which genetic erosion accelerates rapidly. The four populations below this threshold (P1-P4) showed H_e values below 0.42, inbreeding coefficients above 0.20, allelic richness below 4.1, and significant bottleneck signatures. STRUCTURE analysis identified three genetic clusters, with the four smallest populations falling into a single cluster distinct from the larger mainland-connected populations indicating that isolation has progressed to measurable genetic differentiation. The Mantel test confirmed significant isolation-by-distance ($r = 0.67$, $p = 0.002$).

Biodiversity assessment

Species-level surveys at each island site recorded a total of 127 bird species. Island area was the strongest predictor of avian richness ($R^2 = 0.81$), consistent with island biogeography theory. Parrot abundance correlated with forest canopy cover ($r = 0.73$) and availability of nesting cavities in mature *Erythrina* trees ($r = 0.68$). The two smallest islands (supporting P1 and P2) had lost over 40% of their canopy in the past decade due to cattle grazing and selective logging, suggesting habitat degradation is compounding genetic isolation.

Limitations

Population size estimates from roost counts carry uncertainty, particularly for smaller populations where counting error is proportionally larger. Microsatellite-based F estimates can be upwardly biased in small samples. The research covered a single time point and cannot distinguish between recent and historical inbreeding. Additionally, the two species were analyzed together, which may obscure species-specific patterns in gene flow and mating behavior [14, 15].

Discussion

The 50-individual threshold identified here aligns with the classic minimum viable population concept proposed by Franklin and Soule, who suggested that at least 50 breeding individuals are needed to avoid immediate inbreeding depression [16]. Below this number, our data show that

heterozygosity drops sharply, inbreeding coefficients exceed 0.20, and bottleneck signatures appear all indicators of populations losing adaptive capacity. The four populations falling below this threshold (P1-P4) are candidates for immediate conservation intervention.

Managed translocation moving individuals from genetically diverse source populations to depauperate islands has been successfully used for other parrot species, including the orange-bellied parrot in Australia and the echo parakeet in Mauritius [17, 18]. For the Paraná delta populations, even moving 3-5 individuals per generation between neighboring islands could significantly reduce inbreeding and restore allelic diversity [19]. The modest water-gap distances (0.5-15 km) suggest that natural gene flow may have existed historically but has been disrupted by habitat fragmentation on intermediate islands. Restoring stepping-stone habitat could complement translocation as a long-term strategy [20, 21].

Conclusion

This research reveals that four island parrot populations in the Paraná River delta have fallen below the genetic viability threshold, with inbreeding coefficients exceeding 0.20 and significant loss of allelic diversity. Populations below approximately 50 individuals showed consistent evidence of genetic bottlenecks. The strong inverse relationship between population size and inbreeding ($r = -0.89$) provides a simple predictive tool for identifying at-risk populations. These findings support the urgent implementation of managed translocation between islands to restore gene flow, combined with habitat restoration to re-establish natural connectivity across the delta landscape.

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Contributions not qualifying for authorship

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