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SHORT COMMUNICATION

Genetic patterns and conservation of the Scarlet Macaw (*Ara macao*) in Costa Rica

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Abstract Once widely distributed throughout the lowland forests of Costa Rica, scarlet macaws *(Ara macao*) have been reduced to two major, geographically separated, populations along the Pacific slope. Past demographic declines raise conservation concerns regarding the detrimental effects of population fragmentation. This investigation aimed to evaluate the current status of scarlet macaws along the Pacific slope by examining levels of genetic variation and patterns of genetic structure within and among remnant populations. Statistical analyses using multilocus genotypes revealed strong differentiation between Central and South Pacific populations, suggesting local geographic barriers have historically restricted gene flow between these localities. High genetic diversity suggests neither population suffers from genetic erosion, likely resulting from relatively large population sizes and high dispersal capacity and longevity. However, evidence of disequilibrium within the Central Pacific population infers anthropogenic threats have disrupted natural population dynamics. These results advocate on focusing available resources on habitat restoration and nest protection, as a means to assist in reestablishing demographic stability and maintain the genetic health of wild scarlet macaws in Costa Rica.

Keywords *Ara macao* · Scarlet macaw · Fragmentation · Genetic structure · Conservation

Background

Scarlet macaws (*Ara macao*) inhabit Neotropical lowland forests from southeastern Mexico to northern Bolivia, and are known to undergo daily (>20 km; Myers and Vaughan 2004) and seasonal movements (Stiles and Skutch 2007). As with most psittacids, this species faces a variety of anthropogenic threats throughout its range (Snyder et al. 2000). In Costa Rica, the scarlet macaw suffered a 37 % reduction in its original estimated forest habitat of 42,501 km^2 between 1940 and 1977 (Vaughan 2011). Moreover, nest poaching severely affects scarlet macaw populations countrywide (Vaughan 2002; Dear et al. 2010). As a result, this once abundant species now exists scattered across the landscape in isolated forest fragments (Fig. 1). Along the Pacific slope, scarlet macaws are restricted to two primary remnant populations in the Central (450–500 individuals) and South (~800–1000 individuals) Pacific regions. Land management greatly differs between sites, with important implications for scarlet macaws. Despite losses by selective logging, approximately half (40.4 %) of forest cover in the South Pacific is legally protected (ELAP-UCI 2005). Conversely, the Central Pacific landscape is highly fragmented and dominated by crops and small forest patches (<40 ha), with isolated large forest patches (>200 ha) having protected status (Myers and Vaughan 2004).

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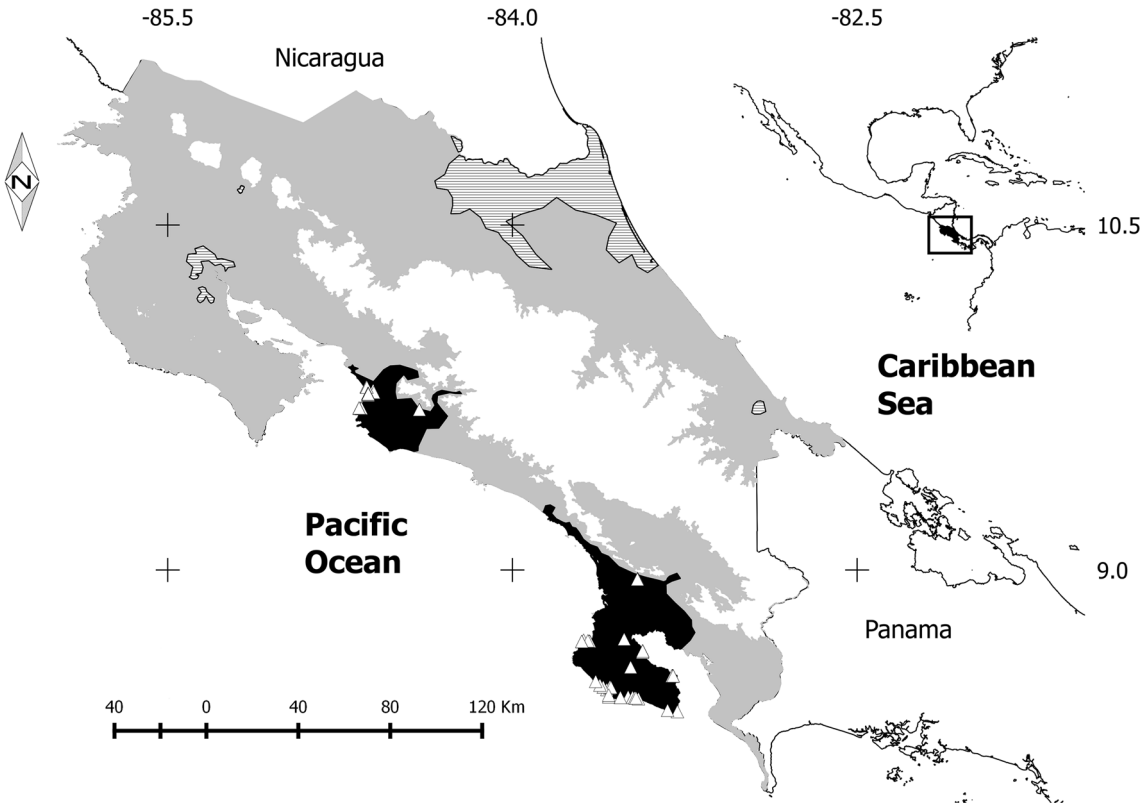

Fig. 1 Current (*black*) and historical (*grey*) distribution of the scarlet macaw in Costa Rica. *Solid areas* correspond to major populations and *lineated areas* are remnant populations. Non-invasive sample collection (2006–2011) sites are shown as *white triangles*

Consequently, conservation concerns have arisen about possible impacts of fragmentation and demographic declines on the genetic health of these remnant populations (Dear et al. 2010), specifically erosion through genetic drift, loss of heterozygosity and changes in the allelic frequencies (Amos and Blamford 2001; Allendorf and Luikart 2007). Only one previous study (Nader et al. 1999) has attempted to quantify genetic variation among Costa Rican scarlet macaws; while finding considerable levels of nuclear diversity, their dataset was limited to 16 confiscated and captive individuals, thus providing minimal insights into genetic status of wild populations. Adding a sense of urgency, recent phylogeographic analyses identified scarlet macaws along the Pacific slope of lower Central America as a distinct population segment of the *A. m. macao* lineage (Schmidt 2013). Coupled with relatively large census estimates, the Central and South Pacific regions in Costa Rica are considered two of the most important scarlet macaw populations in Mesoamerica (USFWS 2012).

This study aimed to assess whether recent habitat fragmentation has affected the scarlet macaw's genetic variability and lead to population structure along the Pacific slope of Costa Rica. This information would be used to better allocate resources and guide local conservation management decisions.

Materials and methods

Sample collection and DNA extraction

Scarlet macaw feather and fecal samples were collected in the Central Pacific (CP, in 2006 and 2011: feathers $n = 52$; feces $n = 58$) and South Pacific (SP, in 2007 and 2011: feathers $n = 60$; feces $n = 58$) regions of Costa Rica. Feathers were collected directly from the ground and kept in paper envelopes whereas fecal samples were collected on plastic sheets placed below foraging or roosting trees and stored in 16 ml vials containing desiccating silica beads (Wasser et al. 1997). Genomic DNA was extracted from feathers using the DNeasy Blood & Tissue Kit (Qiagen), with modifications (Gebhardt and Waits 2008). For feces, the QIAamp DNA Stool Mini Kit (Qiagen) was used based on the modifications proposed by Chaves et al. (2010).

Microsatellite genotyping and data analysis

Extracted DNA was used to amplify seven microsatellite loci originally developed by Caparroz et al. (2003) and Russello et al. (2001, 2005), using primers redesigned by Gebhardt and Waits (2008) to improve amplification

success when working with non-invasive samples. PCR reactions followed published protocols using the Multiplex PCR Kit (Qiagen), with exception of AgGT19 being amplified in singleplex due to problems during multiplex PCR runs. Amplified PCR products were separated in an ABI 3130 Genetic Analyzer (Applied Biosystems) and alleles were called with GENEMARKER (Softgenetics). Each PCR reaction was run in triplicate and final genotypes were called when two of three repetitions yielded consistent results. For posterior analyses, only samples with convincing genotypes for ≥6 loci were included.

The program GIMLET (Valière 2002) detected identical genotypes (probability >90 %) and null alleles and genotyping errors were inferred using MICRO-CHECKER (Van Ooseterhaut et al. 2004). GENEPOP (Rousset 2008) identified departures from the Hardy–Weinberg Equilibrium (HWE) and presence of linkage disequilibrium (LD) among loci. P-values were calculated with the following MCMC parameters: 10,000 dememorizations, 1000 batches and 10,000 iterations per batch and results adjusted with the Bonferroni correction (Rice 1989). Allele richness, private allele richness, and observed and expected heterozygosity were calculated using GENALEX (Peakall and Smouse 2012).

Population structure was inferred by estimating the most probable number of genetic clusters (K) with the Bayesian algorithm implemented in the program STRUCTURE (Pritchard et al. 2000; Falush et al. 2003). Simulations were run using an admixture model with correlated allelic frequencies between populations and including sampling location information. The parameters for the runs were adjusted for $1 < K < 5$ with a burn-in of 500,000 and 1,000,000 MCMC replications after burn-in and with 10 independent repeat runs for each K. The rate of change of the maximum likelihood function (ΔK) with respect to K, as proposed by Evanno et al. (2005) was employed to determine the optimal number of clusters. The null hypothesis of no genetic differentiation between populations was tested in GENALEX. A hierarchical analysis of molecular variance (AMOVA; Excoffier et al. 1992) was used to determine the partition of the genetic diversity variance based on the estimation of pairwise F_{ST} (Weir and Cockerham 1984), performing 9999 iterations and fixing the test significance at 0.05.

Results and discussion

Genetic structure and differentiation

After excluding identical genotypes (CP: $n = 15$; SP: $n = 12$) and samples with missing data or that failed to produce PCR products ($n = 105$), the final sample size was 96 (CP: $n = 41$; SP: $n = 55$). Within the final microsatellite matrix, we found evidence of significant population structure among scarlet macaws in Costa Rica. The value of the AMOVA-estimated F_{ST} was low but highly significant ($F_{ST} = 0.048$, $p < 0.01$, Table 1). Bayesian analyses further confirmed this apparent genetic structure (Fig. 2), where the maximum likelihood mean value was highest at $K = 2$. Likewise, a large number of private alleles were present within both populations (9 in the CP and 19 in the SP).

Uncovering high levels of differentiation was surprising because the geographic distance between populations is relatively small (~80 km), especially for a highly mobile species such as the scarlet macaw. Given the strength of the differentiation signal, it is unlikely the observed genetic distance between CP and SP populations resulted from recent habitat fragmentation events. Insufficient time has elapsed since large-scale land conversion began in Costa Rica for genetic drift to produce such strong genetic signatures, given the scarlet macaw's longevity and high dispersal capability. It is possible, then, that landscape features may have acted as historical barriers along the Pacific slope of Costa Rica. Scarlet macaws show a strong preference for lowland humid forests, locally staying below ~760 masl (Stiles and Skutch 2007). The geographic positioning of the Central Cordilleras result in pockets of low elevation areas that are large enough to support macaw populations in the Central and South Pacific regions. However, lowland habitats in intervening areas are restricted to a 5–10 km wide strip of land, due to the close proximity of montane areas to the coast (Fig. 1), acting as an effective barrier to dispersal. Schmidt (2013) found similar patterns of restricted gene flow and population genetic structure associated with significant changes in topography over small geographic spaces.

Genetic variability

Diversity indices revealed moderate to high levels of molecular variation among scarlet macaw in Costa Rica (Table 2). Measures of nuclear diversity reported for the species in Brazil [$He = 0.741$, $A = 8.4$; (Presti et al. 2011)] and northern Central America [$He = 0.696$, $A = 7.11$; (Schmidt 2013)], using the same microsatellites as here, are very similar to our reported values, indicating high genetic variation may be an inherent characteristic of the scarlet macaw. This observation is consistent with other studies showing more widespread and generalist species exhibit higher genetic diversity relative to more restricted and specialized macaw species (Caparroz et al. 2001; Presti et al. 2011, 2015).

While our findings are based on a small number of nuclear loci, these data imply demographic declines have not yet produced significant loss of molecular diversity.

Table 1 Analysis of molecular variance (AMOVA) and fixation index (F_{ST}) for 96 scarlet macaw individuals from two populations in Costa Rica

Source of variation	d.f.	Sum of squares	% of variation	F_{ST}
Within populations	1	13.552	4.7	
Between populations	190	447.745	95.3	0.048*

* $P = 0.00$

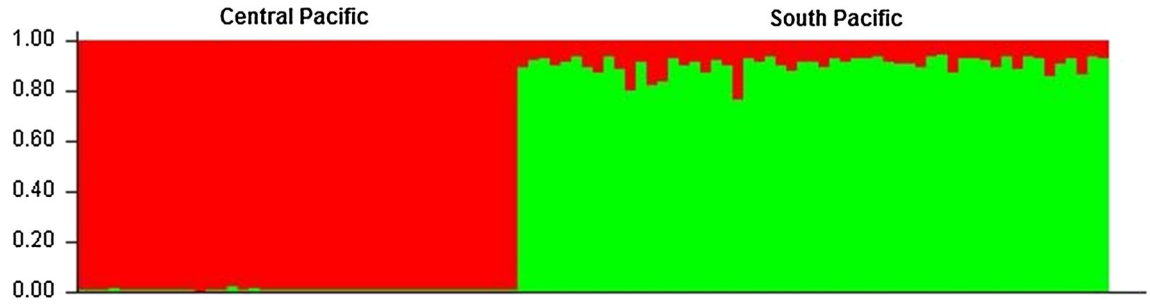

Fig. 2 Individual membership coefficient (Q) plot for scarlet macaws in Costa Rica, estimated for K = 2, evidencing two genetic clusters: Central Pacific and South Pacific

Table 2 Genetic variability of scarlet macaw populations in Costa Rica

Population	*n*	Locus	*A*	P_A	H_O	H_E
Central Pacific	41					
		UnaCT21	9	2	0.75	0.82
		UnaCT43	9	2	0.78	0.82
		UnaCT74	8	1	0.71	0.69
		UnaCT41	3	0	0.20	0.28
		AgGT17	8	0	0.85	0.81
		AgGT21	6	3	0.54	0.59
		AgGT19	4	1	0.43	0.41
Mean			6.71	1.33	0.61	0.63
South Pacific	55					
		UnaCT21	9	2	0.69	0.73
		UnaCT43	10	3	0.80	0.84
		UnaCT74	9	2	0.78	0.73
		UnaCT41	5	2	0.36	0.43
		AgGT17	13	5	0.76	0.85
		AgGT21	6	3	0.70	0.75
		AgGT19	5	2	0.48	0.49
Mean			8.14	2.50	0.65	0.68

n sample size; *A* number of alleles; P_A number of private alleles; H_O observed heterozygosity; H_E expected heterozygosity

Relatively large population sizes (Dear et al. 2010), high dispersal (Myers and Vaughan 2004), and longevity (Bourke et al. 2010; Brouwer et al. 2000) may have helped buffer scarlet macaw populations against genetic erosion. In South America, the scarlet macaw also shows high genetic variability despite population reductions, possibly because of intense gene flow across widespread lowland habitats (Oliveira-Marques 2010). Similar patterns have been seen across other species with comparable distributions and life history traits (Hailer et al. 2006; Lerner et al. 2009).

It is important to note, however, differences in land management may be influencing population stability for the Central and South Pacific populations. No microsatellite anomalies were recovered within the SP dataset, possibly reflecting a larger population size and greater habitat protection in the South Pacific region. Conversely, several deviations were noted among CP genotypes. Highly fragmented landscapes in the Central Pacific region, due to intense human activities, may be disrupting the equilibrium status of this population. Specifically, significant linkage disequilibrium was observed among four loci, departures from HWE for two loci, along with presence of null alleles for UnaCT41. Changes in population size may result in higher incidence of linkage disequilibrium (Noonan et al. 2006), heterozygote deficits, and loss of low frequency alleles (Allendorf and Luikart 2007).

Conservation implications

This study found genetic erosion is not an eminent threat to the two major scarlet macaw populations in Costa Rica, however evidence of historical differentiation advocates treating each population as a separate management unit. Our work further underscores the importance of mitigating anthropogenic threats to reinforce demographic stability

and maintain genetic integrity for these evolutionarily important populations. Therefore, we highly recommend that available resources be directed strictly towards the protection and restoration of this species' habitat within each management unit to reinforce local genetic connectivity. Additional efforts should focus on eliminating nest poaching. Although popular in Costa Rica, captive releases are not immediately necessary to increase genetic variability or population numbers, diverting vital resources away from more critical conservation actions. Lastly, though the observed genetic variability reveals geographic isolation has not produced profound negative consequences for both populations, the current situation is highly fluid. Long-term studies as well as monitoring programs are needed to continually assess the spatio-temporal changes potentially occurring within these populations.

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