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Population genetics of the endangered Crowned Solitary Eagle (*Buteogallus coronatus*) in South America

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Abstract

The Crowned Solitary Eagle (*Buteogallus coronatus*) is one of the rarest and most severely threatened birds of prey in the Neotropical region. We studied levels of neutral genetic diversity, population structure and the demographic history of the species using 55 contemporary samples covering a large fraction of the species range, which were genotyped at 17 microsatellite loci. Our results indicated genetic homogeneity across the sampled regions, which may be explained by a high dispersal capability of Crowned Solitary Eagles resulting in high gene flow or relatively recent population expansion. Further demographic tests revealed that the species has experienced a recent demographic reduction, but inbreeding was not detected. The existing connectivity between geographically separated populations may have buffered the negative effects of the demographic bottleneck. Alternatively, the demographic reduction may be too recent to detect a genetic signature due to the long generation time of the species. Potential conservation strategies, including the possibility of translocations of individuals, are discussed.

Key words: Population genetics; bottleneck; genetic structure; birds of prey; conservation.

Introduction

The Crowned Solitary Eagle (*Buteogallus coronatus*) is one of the rarest and most severely threatened birds of prey in the Neotropical region. Its range extends from southern Brazil to northern Patagonia, where it inhabits a variety of forested habitats, including woodlands and other savanna-like landscapes (Ferguson-Lees and Christie 2001; Fig. 1). The species is listed as endangered under the IUCN Red List with a declining world population estimated at less than one thousand reproductive individuals (BirdLife International 2016). Reduced population size and range contraction of Crowned Solitary Eagles is suspected to be human induced, including habitat loss (Bellocq et al. 1998; Fandiño and Pautasso 2014), electrocution (Maceda 2007), as well as shooting (Sarasola and Maceda 2006; Sarasola et al. 2010).

Possibly, because Crowned Solitary Eagles occurs in low densities in remote and barely explored areas, little is known about the biology of the species and no information exists on the demography and population connectivity between geographic regions. Likewise, there is a lack of knowledge on the extent to which population decline and range contraction (Fandiño and Pautasso 2014) have affected levels of genetic diversity in this species.

To evaluate the genetic status of Crowned Solitary Eagles, we collected samples covering a large fraction of the species' geographic distribution. We estimated the levels of neutral genetic variability and investigated whether these levels have been affected by population reduction. We also explored the existence of population genetic structure among the sampled individuals and discussed potential implications for the conservation of the species.

Methods

65 **Sampling and microsatellite genotyping**

66 A total of 69 samples was collected across a latitudinal gradient of 1400 km covering
67 three Neotropical semiarid biomes (i.e., Espinal, Monte Desert and Chaco; Fig 1) and
68 two out of three areas suggested as important for the conservation of the species in
69 Argentina (Mendoza and La Pampa; Bellocq et al. 2002).

70 Samples were obtained from wild individuals at breeding territories. We took
71 blood samples from fledglings and/or collected naturally shed feathers from breeding
72 adults at the nesting sites (n= 53). We also used samples from captive birds (adults)
73 from zoos and wildlife rescue centers, only when the recovery location of individuals
74 was known (n= 16).

75 Samples were genotyped at 17 microsatellite loci (Table 1; see Supplementary
76 File and Andris et al. 2012 for information about the markers and PCR protocols). PCR
77 products were run on an ABI PRISM 3130xl DNA sequencer (Applied Biosystems) and
78 allele size was determined using the Genescan 500-LIZ size standard and Genemapper
79 version 4.0 (Applied Biosystems).

80 Before conducting subsequent genetic analyses, we searched for DNA replicates
81 i.e. feathers from different locations and/or collected in different years that might belong
82 to the same individual, and which may bias allelic frequencies. To this end, we
83 performed identity analyses in CERVUS. These analyses revealed 14 resampled
84 individuals (out of n= 69), which were removed from population analyses (none of the
85 resampled individuals changed the geographic location among sampling events).
86 Further, we inferred paternity using CERVUS (Marshall et al. 1998; Kalinowski et al.
87 2007) and genetic relatedness was estimated with ML-Relate (Kalinowski et al. 2006),
88 which allowed the identification of closely related individuals (e.g. full sibs or parent-

offspring) in the population. See Supplementary File for further details on these analyses.

Overall, the final sample size was 55 different samples collected across an area of 250,000 km^2 (Fig. 1).

Data analyses

Genetic diversity and microsatellite analysis

The number of alleles and the expected and observed heterozygosity per locus were calculated using the software GIMLET v. 1.3.3 (Valière 2002). To determine the minimum number of loci necessary for individual discrimination, we calculated the cumulative probability of genotype identity (PID) between unrelated individuals and full siblings for different sets of loci in GIMLET.

Tests for deviations from Hardy-Weinberg and linkage equilibrium were performed in Genepop 4.0 (Raymond and Rousset 1995) and subsequently adjusted with a Benjamini-Yekutieli correction (Narum 2006).

Patterns of gene flow among populations

The extent of genetic differentiation (pairwise F_{ST}) between the Mendoza and La Pampa areas, the two most extensively sampled populations (Fig. 1), was estimated using the program GENETIX (5,000 permutations were used to assess significance; Belkhir et al. 2004). The remaining study populations were not included in this analysis due to low sample sizes ($n < 4$ in each region).

We further explored the existence of genetic structure in our data set using STRUCTURE 2.3.4 (Pritchard et al. 2000). Four independent runs (k= 1-4), with twenty replicates for each K, were run to estimate the true number of genetic clusters of individuals (K). Simulations were performed with a 10^5 burn-in period followed by 10^6 MCMC repeats after burn-in and assuming the admixture model and correlated allele

frequencies. To find the most appropriate K value, we followed the Evanno method based on the rate of change of the likelihood function with respect to K (see Evanno et al. 2005), as implemented in Structure Harvester (Earl and VonHoldt 2012).

We also explored the partition of the total genetic variation, based on a Principal Coordinates Analysis (PCoA), in GenAlEx 6.5 (Peakall and Smouse 2012).

Population demography, inbreeding and relatedness

To test for recent declines in population size, we used BOTTLENECK 1.2.02 (Piry et al. 1999). Heterozygosity excess was tested using Wilcoxon and Sign tests (based on 1,000 replications), under both the Infinite Allele Model (IAM) and the two-phase model (TPM; 95% stepwise mutation model with 5% multi-step mutations and a variance among multiple steps of 12; (Di Rienzo et al. 1994; Piry et al. 1999). We used NeEstimator V2.01 (Do et al. 2014) to estimate the contemporary effective population size (N_e) from our sample based on two different methods (linkage disequilibrium LD described by Bartley et al. 1992 and heterozygosity excess HE described in Luikart and Cornuet 1999) that use one point sample of individuals.

The level of inbreeding in the population was examined through the inbreeding coefficient (F_{IS}) calculated in GENETIX (Belkhir et al. 2004). Significance of F_{IS} was determined by 10,000 iterations of bootstrapping over loci. Mean relatedness within the population was estimated in GenAlEx, using Queller & Goodnight's R estimate (1989). To test whether the geographic distance between the samples was correlated with their pairwise relatedness, we performed a Mantel test (Legendre and Legendre 1998) in GenAlEx. Geographic origins for DNA samples were obtained at the breeding territories for wild birds and for sites of bird collection for captive birds. Significance of the autocorrelation coefficient was tested by resampling methods using N= 10,000 randomizations.

Results

Genetic diversity

We found no evidence for a deviation from Hardy-Weinberg equilibrium in the analyzed loci, except in IEAAAG15 and Hf-C1E8, which were discarded from subsequent analyses (Global test; P= 0.1330). No pairs of loci showed significant linkage disequilibrium after multiple test correction (Table 1).

Mean expected heterozygosity for the whole sample size over the 15 loci was 0.51, while observed heterozygosity was 0.47 (Table 1). For the 15 polymorphic microsatellite loci used in this study, the probability of identity (PID) for unrelated individuals was very low (1.15^{-10}), while the probability of identity was sufficient for the identification of siblings ($PID_{sibling} = 1.02 \times 10^{-4}$).

The analyses of parentage and relatedness revealed that 11 samples were closely related individuals (i.e. full sibs or parent-offspring). In such cases, the offspring samples and one randomly chosen individual from each full sib pair were excluded from all the analyses described below.

Population structure

F_{st} values indicated a lack of differentiation between Crowned Solitary Eagle populations from La Pampa and Mendoza (F_{st}= 0.006; p= 0.296). The absence of significant genetic differentiation among the collected samples was corroborated by other analyses since: 1) The Bayesian cluster analyses in STRUCTURE showed the highest posterior probability at K=1, suggesting the existence of a single genetic cluster for all individuals and 2) PCoA showed that all individuals clustered together, with no structure (Percentage of variance: Coordinate. 1 = 16.4%, Coordinate 2 = 8.8%; Fig. 2).

Further, pairwise relatedness between individuals was not associated with the geographic distance between them (Mantel test: R= 0.04, P= 0.32).

Population Demography

Significant excess of heterozygosity was detected under both the infinite alleles model (Wilcoxon test: p< 0.001; Sign test: p= 0.03) and the two-phase model (Wilcoxon test: p= 0.013; Sign test: p= 0.04), indicating that the population has experienced a recent genetic bottleneck. This is supported by a low estimate of effective population size (N_e= 50; 95% CI= 30 – 107) based on the LD method, while little power was obtained using the HE method (Ne=infinite; 95% CI= 19 – infinite). N_e values remained similar after excluding rare alleles with frequency of either 0.02 or 0.01. The inbreeding coefficient F_{IS} of the population was negative and not significant (5,000 permutations: F_{IS} = -0.005; P= 0.612).

DISCUSSION

This is the first attempt to study the population structure and demography of the Crowned Solitary Eagle in order to evaluate the genetic status of this endangered species. No evidence of population genetic structure was found, but we can report the existence of a recent genetic bottleneck, possibly, as a result of the reduction that Crowned Solitary Eagles have experienced in both range and population size (Sarasola and Maceda 2006; Sarasola et al. 2010; Fandiño and Pautasso 2014).

Despite population decline, which entailed local extinctions in part of the species' range (e.g. Uruguay; Alvarez 1933), our genetic data suggest that Crowned Solitary Eagles at the Neotropical semiarid biomes (ca. 50% of the species range; BirdLife International 2016) constitute a single genetically panmictic population. It is

192 possible that the high dispersal capability of Crowned Eagles is buffering (e.g. through
193 an interchange of breeders) the genetic divergence among populations by
194 geographically connecting separated individuals since gene flow, even if limited, may
195 counteract the genetic negative effects of habitat fragmentation (Alcaide et al. 2009).
196 Although samples from central Argentina dominated our dataset and thus, our survey
197 might not be sufficiently powerful to comprehensively assess the level of isolation of
198 northern areas, it should be noted that no evidence of genetic differentiation among
199 samples from central Argentina and the remaining study areas was found. In addition,
200 geographic and genetic distances were unrelated indicating that eagles in close
201 proximity were as genetically similar as those located far away from each other.
202 Inbreeding, a major threat associated with demographic reductions (Hedrick and
203 Kalinowski 2000; Keller and Waller 2002), was not detected. Assuming that the
204 population decline reduced genetic diversity, and given that allelic diversity is reduced
205 faster than heterozygosity after a bottleneck, the lack of inbreeding may indicate that the
206 demographic decrease is too recent to detect an inbreeding signature in the population.
207 It is possible that the long generation time and slow population turnover of Crowned
208 Solitary Eagles (expected age at first breeding of 4-6 years and an lifespan of at least 20
209 years based on information available for other large eagle species; Newton 1979), may
210 have reduced the impact of the demographic bottleneck.
211 The estimated effective population size (N_e= 50, 95% CI= 30-107), a key
212 parameter for the assessment of a population viability, indicates that the Crowned
213 Solitary Eagle population in central and western Argentina must be small and thus, very
214 vulnerable. A loss of genetic variability and inbreeding, associated with small N_e, may
215 compromise the long-term viability of the species by reducing the capacity of
216 individuals to deal with stochastic environmental perturbations. However, given the

high human-related mortality registered in the study area (Sarasola and Maceda 2006), the low effective population size suggested here is of special concern in the short term.

It is important to note that the lack of genetic structure or inbreeding found here do not imply the absence of threats to the Crowned Solitary Eagle. Low human densities in arid and semi-arid habitats and yet unnoticeable effects of recent habitat loss may buffer the effects of human-persecution and range reduction on the species genetics. Future work on the Crowned Solitary Eagle should assess the existence of genetic structure in the whole range of the species. Also, further analyses including historical samples are needed to assess the genetic impact of the demographic reduction experienced by the Crowned Solitary Eagle.

This first assessment of the population genetics of Crowned Solitary Eagles is especially valuable for management actions taken for the species. The absence of genetic clusters found among the Crowned Solitary Eagles suggest that the western populations of the species may be considered as a single management unit. Accordingly management activities may include captive-breeding and rehabilitation of individuals aiming to reinforce wild populations and maintain the level of diversity at the whole range of the species. However, given the high mortality rate of Crowned Solitary Eagles caused by anthropogenic factors, complementary conservation actions (legal protection and the counteraction of the most important mortality factors such as electrocution and shooting) should be taken to ensure the viability of the species.

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Figure 1. Distribution of DNA sampling locations of the Crowned Solitary Eagle and extent of the three main semiarid biomes covered in this study following Cabrera (1976). Polygons indicate populations from the Mendoza and La Pampa areas following the delimitation proposed by Bellocq et al. (2002). Inset map shows the sampling area at a larger scale (solid polygon) and the distribution range for the species (light grey shaded) according to the IUCN (BirdLife International 2016)

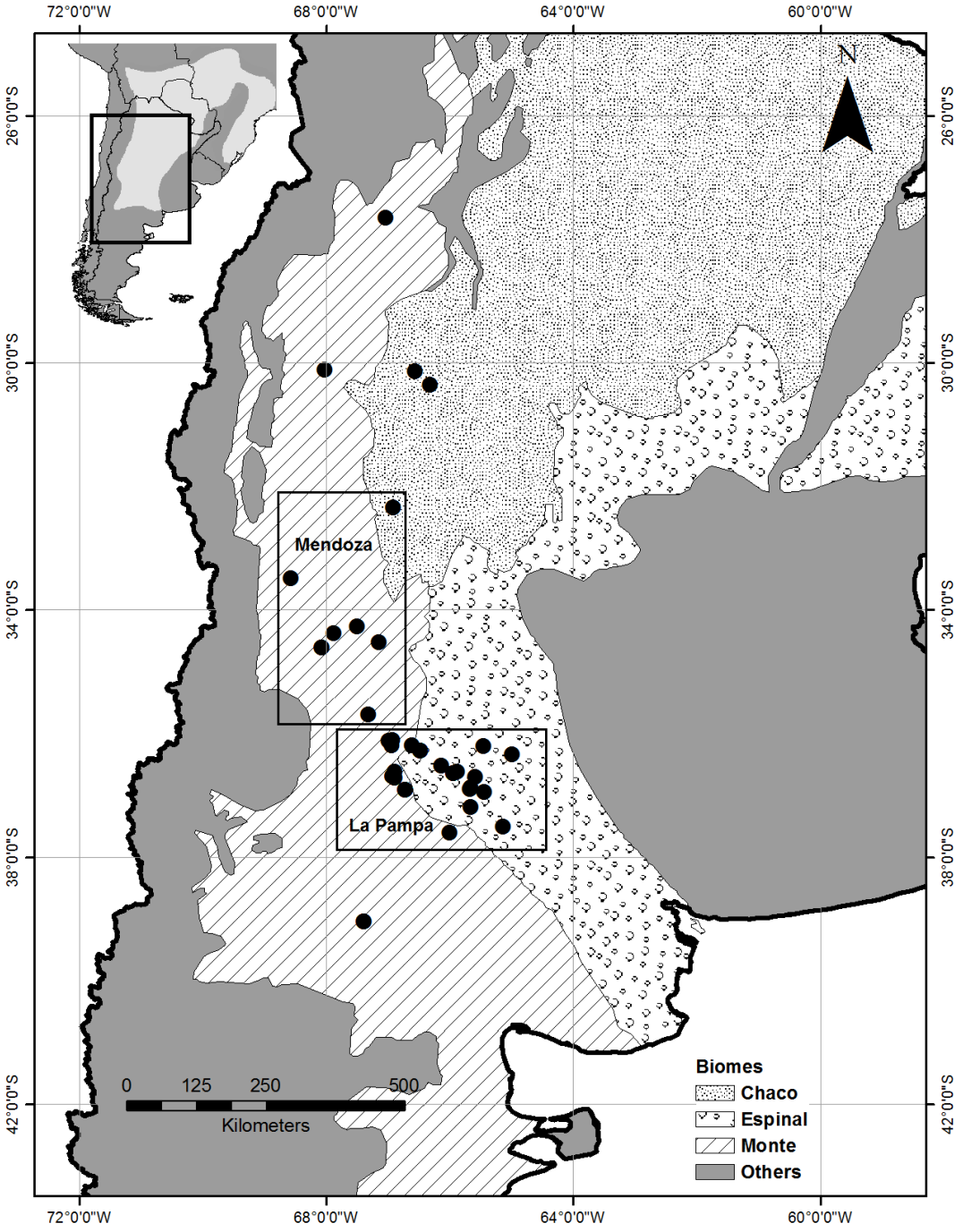

Figure 2. Principal Coordinate Analysis of individual genotypes obtained across the Crowned Solitary Eagle distribution in southern South America covering the Monte Desert, the Chaco and the Espinal biomes. Percentages of variance are 16.4% (Coord. 1) and 8.8% (Coord. 2).

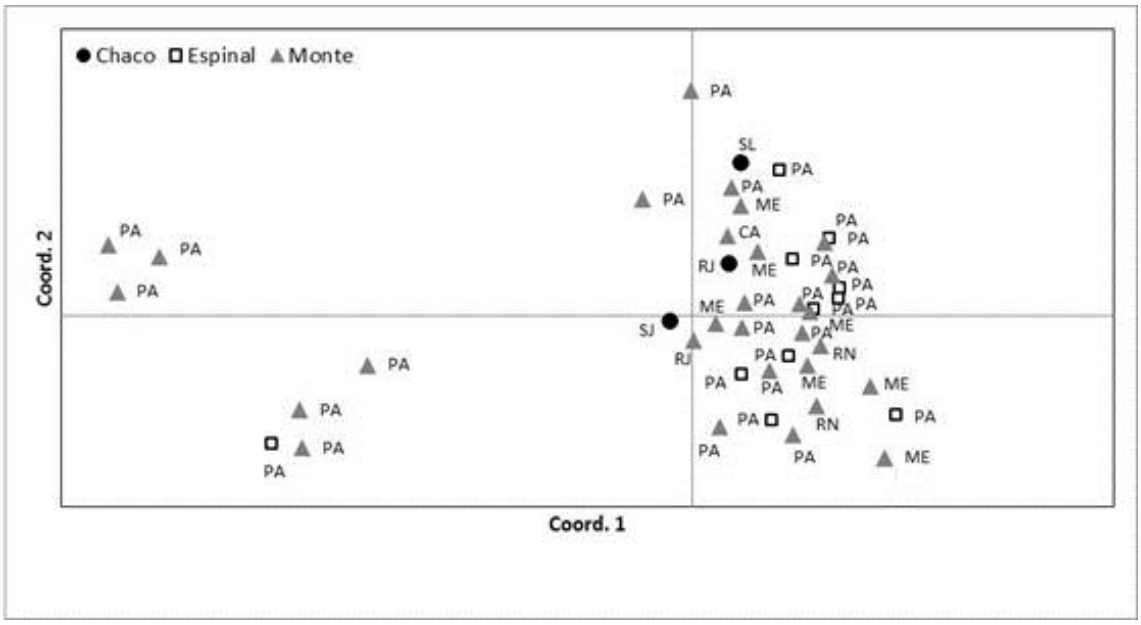

Table 1. Summary data for the seventeen microsatellite loci used: GenBank Accession number, annealing temperature in PCR (Ta), number of alleles (k), observed heterozygosity (H_O) and expected heterozygosity (H_E). Raptor species for which the marker was developed and the source are detailed in Andris et al. 2012.

Locus	**Gene bank Number**	**Ta (ºC)**	**k**	H_O	H_E
BswB234w	JQ309945	56	6	0.39	0.44
BswB111aw	JQ309946	60	2	0.34	0.27
BswD220w	JQ309947	56	5	0.77	0.77
BswD107w	JQ309948	56	9	0.82	0.85
BswA317w	JQ309960	56	4	0.3	0.3
BswA302w	JQ309961	56	2	0.39	0.31
NVHfr206	JQ309958	56	3	0.43	0.5
IEAAAG04	JQ321581	56	6	0.73	0.72
IEAAAG15*	JQ309959	56	2	0.07	0.02
Hal04	JQ309957	56	7	0.43	0.63
Hal09	JQ309956	56	3	0.59	0.51
Hal10	JQ309955	56	3	0.36	0.46
Bbu42	JQ309954	56	9	0.68	0.72
Bbu46	JQ309953	56	7	0.64	0.67
Hf-C1E8*	JQ309952	53	4	0.16	0.59
Hf-C3F2	JQ309951	56	4	0.57	0.5
Hf-C5D4	JQ309950	56	2	0.27	0.31
Average				0.47	0.51

* Loci showing significant departure from Hardy-Weinberg equilibrium and removed from further analyses.