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RESEARCH ARTICLE

Genetic structure reveals management units for the yellow cardinal (*Gubernatrix cristata*), endangered by habitat loss and illegal trapping

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Abstract The yellow cardinal, *Gubernatrix cristata*, is an endangered passerine from southern South America. Populations are declining due to the loss of their natural habitat, which has caused a fragmented distribution, and the continuous extraction of individuals from the wild, mainly males, to sell them as cage birds. In this study, we assess the genetic variability of remaining yellow cardinal's populations and determine whether these populations represent independent management units. We found that the degree of geographic isolation of the remaining populations parallels the genetic differentiation of these populations for both mitochondrial and nuclear markers, and supports the delimitation of four management units for the yellow cardinal (three in Argentina and one in Uruguay). Assignment tests showed that geographic genetic differentiation can be used to assign seized individuals from illegal pet trade to their original populations and thus manage their release.

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Introduction

Habitat fragmentation is one of the main drivers of species loss (Fahrig 2003; Pereira et al. 2010; Rands et al. 2010) as it leads to population isolation and contraction (Dobson 1996). The reduction in number of individuals jeopardizes the viability of populations by diminishing the capacity of these populations to respond to changes in the short (demographic and environmental stochasticity) and the long term (climate change and other changes in selective pressures) (Sambatti et al. 2008). This decrease in the ability to respond to changes is strongly related to the loss of genetic variability due to genetic drift and/or inbreeding (Allendorf and Luikart 2007). The use of genetics in the management of threatened species in the wild is crucial for preserving current diversity to ensure future adaptive potential, i.e., the ability of a species to adapt to changing environments (Mayr 1963; Allendorf and Luikart 2007). Currently, it is well recognized that the maintenance of genetic diversity of endangered species is critical to ensure long-term survival of such species (Hedrick and Kalinowski 2000; Spielman et al. 2004; Frankham et al. 2010). Thus, genetic studies can help identify priority groups in order to conserve the greatest proportion of the original genetic variation (Ryan 2006). One approach to achieve this goal is the identification of management units, composed of demographically independent populations with substantial differences in allelic frequencies of several loci (Moritz 1994).

The yellow cardinal, *Gubernatrix cristata* (hereafter cardinal), is an endemic passerine from southern South America and the only representative of the monotypic genus

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Gubernatrix. It is included in the family Thraupidae along with other tanagers such as *Diuca, Paroaria* and *Lophospingus* (Campagna et al. 2011; Barker et al. 2013; Burns et al. 2014). In the past, this species was widely distributed in the thorny deciduous shrubland forests of central Argentina (Espinal region), most of Uruguay and part of southern Brazil (Ridgely and Tudor 2009). However, the continuous extraction of individuals from the wild, mainly males, to commercialize them as cage birds for over a century (Pessino and Tittarelli 2006; BirdLife International 2016), coupled with the loss of their habitat due to agriculture and livestock-production activities, have caused a marked decline in range and population size. For these reasons, the species is currently categorized as endangered (BirdLife International 2016). Nowadays, it has a fragmented distribution with the main populations located in Argentina. According to BirdLife International (2016) its current global population is around 1000–2000 individuals with a clear downward trend. In Uruguay there are about 300 individuals (Azpiroz et al. 2012), with only a few records of individuals in Brazil (Martins-Ferreira et al. 2013).

Endangered species need strategic management measures to guarantee their viability in the short and long-run (Neel et al. 2012). Depending on the particular threats faced by the species, management actions have to be defined and implemented. Therefore, a good knowledge of remnant wild populations is required. Information about the genetic structure through the entire range of yellow cardinals can help set priorities for the protection of particular areas and inform decisions for releasing seized animals from the illegal trade.

In this study, we analyze how intraspecific genetic diversity is geographically distributed in the remaining natural populations of the yellow cardinal and whether these populations represent independent demographic units as a result of habitat fragmentation and the decline in population numbers. We assess genetic variability of populations in mitochondrial and nuclear (microsatellites) molecular markers and determine genetic differentiation between populations to identify management units for this species. We also analyze if markers allow the assignment of confiscated individuals to their population of origin, which could be used as an instrument for management decisions in this endangered species.

Materials and methods

Sample collection

We collected samples from a total of 110 yellow cardinals from five different populations representing the entire present geographic distribution of the species (except for Brazil where only a few individuals were sighted in the last years). We named these five populations with the names of the provinces—or country—were sampling took place, from northeast to south (Fig. 1): Uruguay (U, n = 30), Corrientes (C, n = 30), San Luis (SL, n = 7), La Pampa (LP, n = 18), and Río Negro (RN = 25). Sampling in Corrientes covered two sub-regions (C1, n = 12; C2, n = 18), in La Pampa three sub-regions (LP1, n = 8; LP2, n = 5; LP3, n = 5) and in Río Negro two sub-regions (RN1, n = 8; RN2, n = 17). Sub-regions within main sampling regions were separated by approximately 100 km (Fig. 1). We captured individuals with mist nets during four reproductive seasons (September-December 2011–2014). We took blood samples (20–30 µL) via brachial vein puncture and stored them in lysis buffer (100 mM Tris pH 8, 10 mM NaCl, 100 mM EDTA and 2% SDS) at room temperature. For 20 individuals from Uruguay that were kept in captivity, we plucked five to eight feathers per individual.

Genetic analysis

We extracted DNA from blood and feather samples using Qiagen extraction kit (Hilden, Germany) and amplified a fragment of the mitochondrial control region using primers LCR3 and H1248 (Tarr 1995). We performed polymerase chain reaction (PCR) amplifications in a total volume of 25 µl with 50–100 ng of total genomic DNA template, 0.2 µM of both forward and reverse primers, 0.2 mM of each dNTP, 2.5 µl of 10× PCR buffer (Invitrogen), 2.25 mM $MgCl_2$, and 0.1 U Taq DNA Polymerase. Reaction cycles followed 2 min of hot start at 94 °C; then 35 cycles of denaturation for 30 s at 94 °C, 30 s at 55 °C annealing temperature, and 60 s at 72 °C, and a final extension for 8 min at 72 °C. Amplification products were sequenced in an ABI 3130 XL (Applied Biosystems, Foster City, CA, USA) sequencer using ABI Big Dye™ Terminator Chemistry.

We edited and aligned sequences using Bioedit v7.2.0 (Hall 1999) and estimated population-level diversity by assessing the number of haplotypes and polymorphic sites, the number of private haplotypes (i.e., haplotypes occurring in only one region), haplotype diversity H (i.e., the probability that given two randomly chosen haplotypes, these differ from one another, Nei 1987), and nucleotide diversity Pi (i.e., the average number of nucleotide differences per site between two randomly chosen DNA sequences, Nei and Li 1979). We calculated parameters using DNAsp 5.10.01(Librado and Rozas 2009).

For microsatellite genotyping we used ten pairs of primers specific to this species that were designed by Martins-Ferreira et al. (2010). We performed amplification reactions in 25 µl reaction volumes using 50–100 ng total genomic DNA template, 0.2 µM of both forward

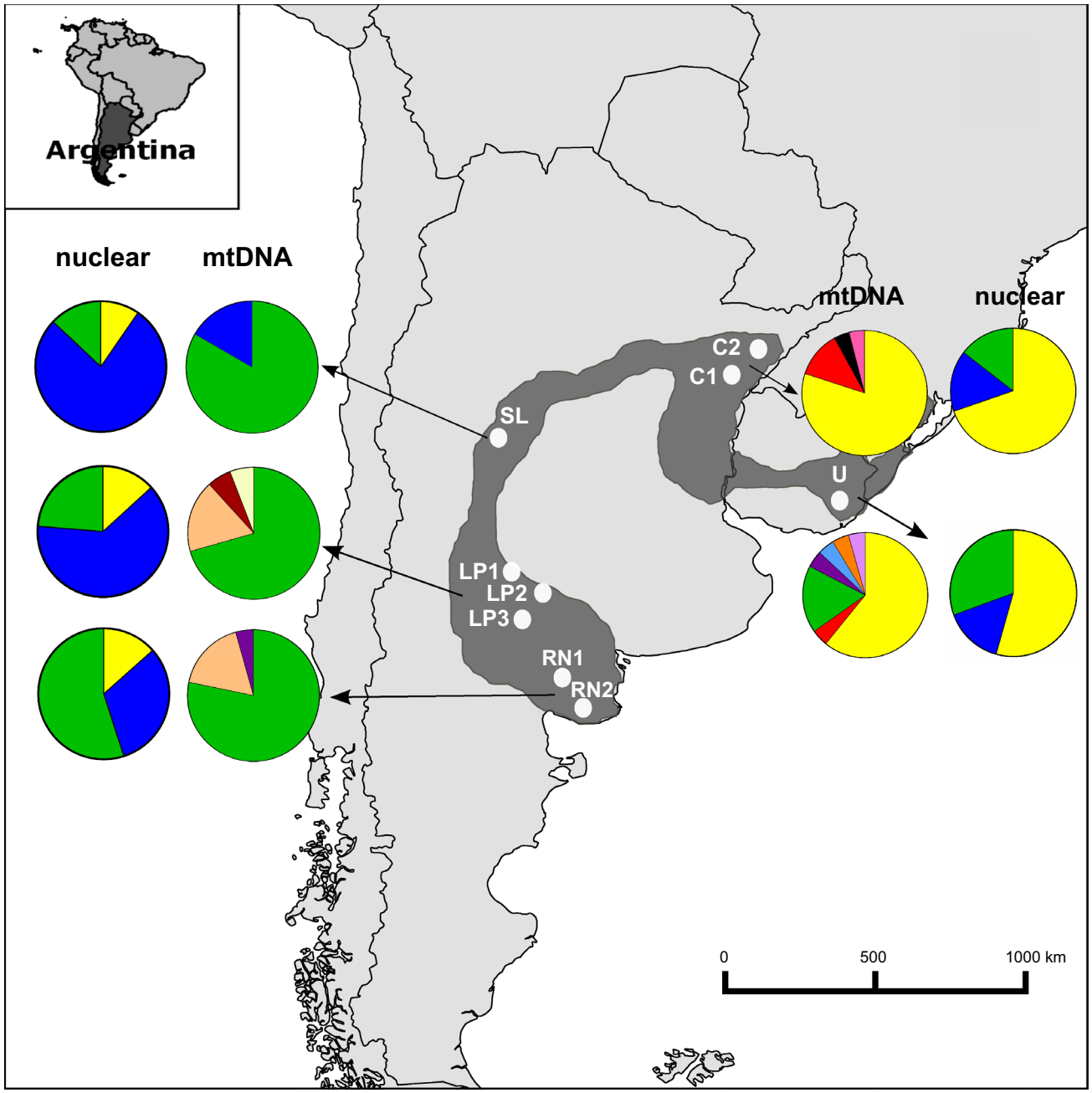

Fig. 1 Map showing the historic distribution of yellow cardinals (*grey* area) in South America and sampling locations of current populations: Uruguay (U; 33°53′54″S, 54°44′33″W), Corrientes (C1; 29°12′6″S, 58°15′41″W and C2; 28°39′22″S, 57°26′4″W), San Luis (SL; 32°25′10″S, 66°53′27″W), La Pampa (LP1; 36°12′15″S, 65°05′58″W, LP2; 36°38′6″S, 64°17′48″W and LP3; 37°42′52″S, 64°46′14″W) and Rio Negro (RN1; 40°3′9″S, 64°8′38″W and RN2; 40°24′28″S, 63°40′54″W). *White dots* indicate different sampling areas. Graphs show each population's haplotypes and the frequency distribution of microsatellite clusters. For mtDNA: H1 *yellow*, H2 *red*, H3 *black*, H4 *pink*, H5 *green*, H6 *light orange*, H7 *dark red*, H8 *light yellow*, H9 *violet*, H10 *light blue*, H11 *orange*, H12 *light violet* and H13 *blue*. For microsatellites the three clusters are shown in *yellow, green* and *blue* (Color figure online)

(fluorescence-labelled at 5′-end with FAM, VIC, PET and NED) and reverse primers, 0.2 mM of each dNTP, 2.5 µl of 10× PCR buffer (Invitrogen), 2.25 mM $MgCl_2$, and 0.1 U Taq DNA Polymerase. All primers were successfully amplified in multiplex PCRs. Reactions cycles followed 5 min of hot start at 94 °C; then 40 cycles of denaturation for 30 s at 94 °C, 60 s at annealing temperature 55 °C, and 45 s at 72 °C, and a final extension for 10 min at 72 °C. We sized PCR products using the above-mentioned sequencer and determined allele sizes with Peak Scanner v1.0

(Applied Biosystems). We analyzed genotypes in order to detect errors due to presence of null alleles, stuttering and allelic dropout using Microchecker v2.2.3 (Oosterhout et al. 2004). We assessed absolute and effective number of alleles and private alleles with GenAlEx 6 (Peakall and Smouse 2006) and calculated allelic richness with FSTAT v2.9.3.2 (Goudet 2002). We used GenPop v4.0.10 (Raymond and Rousset 1995) to calculate the inbreeding coefficient (F_{IS}). We calculated deviations from Hardy–Weinberg equilibrium for each population and each locus with a Markov chain method (Guo and Thompson 1992) as implemented in Arlequin v3.5.1 (Excoffier and Lischer 2010). This program was also used to test for linkage disequilibrium for each pair of loci within all populations. We assessed significance of linkage disequilibrium tests after applying a Bonferroni correction to account for multiple comparisons (Rice 1989).

Population genetic structure

We assessed genetic divergence among regions/sub-regions by an analysis of molecular variance (AMOVA; Excoffier et al. 1992) using Arlequin v3.5.1 (Excoffier and Lischer 2010) for both nuclear and mitochondrial markers.

We also estimated the number of populations (k) using a Bayesian clustering approach with STRUCTURE v2.3.3 (Pritchard et al. 2000). Taking the multi-locus genotype of each individual into account, STRUCTURE probabilistically assigns each individual to one or more clusters. We simulated 1–6 populations and assumed correlated allele frequencies, as recommended for distinct populations that are closely related (Porras-Hurtado et al. 2013), and an admixture model. By doing so, we allow parts of an individual's genome to originate from more than one cluster (admixture). This is the most conservative option when the extent of gene flow and recombination is *a priori* unknown (Falush et al. 2003). We ran simulations under a burn-in of 200,000 steps and a data collection period of 1,500,000 iterations. Each simulation was replicated ten times. To select the most likely number of groups without an *a priori* sample identification we analyzed the simulation with the highest likelihood and also used the Δk method of Evanno et al. (2005). We used the online program STRUCTURE Harvester to plot likelihood values and Δk (Earl and von Holdt 2012), and CLUMPP software (Jakobsson and Rosenberg 2007) to average the estimated cluster membership coefficient matrices of the multiple runs of STRUCTURE using FullSearch algorithm. The output from CLUMPP was visualized using the program DISTRUCT (Rosenberg 2004).

We analyzed if the clusters determined with STRUCTURE correlated with the presence of frequent haplotypes using a Chi square replicated goodness of fit test. As nuclear and mitochondrial loci are physically unlinked, a non-random association provides the possibility of inferring migrating individuals (Wiemann et al. 2010).

To test whether genetic differences are correlated with geographic distance, we performed Mantel tests for both genetic markers using population pairwise F_{ST} values and geographic distance (in km) as implemented in GenAlEx 6 with 9999 permutations. We measured geographic distances between adjacent sub-populations linearly with Google Earth and distances between more distant sub-populations were calculated as the sum of measured distances (e.g. distance LP2_C = (LP2_LP1) + (LP1_SL) + (SL_C)). This reflects the assumption that dispersal will occur along the U-shaped distribution range of the species (Fig. 1).

We performed assignment tests to study how accurately STRUCTURE could determine the origin of unknown individuals. To do this, we performed five internal consistency tests in order to test if individuals with known location information would be successfully assigned to the correct group. For each of the five trials one individual from each management unit was randomly selected and defined as having unknown-group affiliation. In total, we tested 15 individuals. Simulations were run under conditions described above (burn-in 200,000, MCMC 1,500,000) with three replicates.

The program Bottleneck (Piry et al. 1999) was used to determine if any of the populations had gone through a recent bottleneck, which is expressed by an excess of heterozygosity compared to the heterozygosity expected under drift-mutation equilibrium (Nei et al. 1975; Maruyama and Fuerst 1985). For each population and locus we computed the distribution of the heterozygosity expected from the observed number of alleles given the sample size, under the assumption of mutation-drift equilibrium. This distribution was obtained through simulating the coalescent process of the genes under the two-phase model of microsatellite mutation (TPM; Di Renzo et al. 1994). Analyses were performed using 1000 replications; 70% of the mutations were set as single-step and 30% were set as multi-step (Piry et al. 1999). We tested if populations displayed an excess of expected heterozygosity compared to the heterozygosity expected under drift-mutation equilibrium using a sign test and a mode-shift test (Luikart et al. 1998).

Results

Genetic variability

We obtained 736 bp sequences of the mtDNA control region for 94 yellow cardinals and distinguished 11 polymorphic sites in 13 different haplotypes (GenBank Accession No.: KT878880-KT878892). There were two

frequent haplotypes, H1 (34%) and H5 (41%), while the other haplotypes occurred at low frequencies (Fig. 1). Diversity indices varied among geographic regions (Table 1). U and LP showed the highest diversity values and SL and C the lowest. Each region, except the southernmost RN, exhibited private haplotypes (U: H10, H11 and H12; C: H3 and H4; SL: H13 and LP: H7 and H8; Fig. 1).

We genotyped 102 cardinals at 10 microsatellite loci. All loci were highly polymorphic, with 5 (at loci E2 and G10) to 17 alleles (at locus F2). There was no evidence of dropout of large alleles, genotyping errors, or null alleles at any locus, nor evidence of linkage disequilibrium between any pair of loci. Eastern populations (C and U) displayed departure from genotype proportions expected under Hardy–Weinberg equilibrium for 6 out of 10 loci, which might be biologically relevant and could indicate either further subdivision within the areas or inbreeding. We analyzed these possible explanations and found no evidence of genetic structure between C1 and C2 ($F_{ST}=0.02$, $p=0.34$), thus discarding a Wahlund effect, but found that both populations showed the highest inbreeding coefficients (F_{IS} values: $U=0.24$, $C=0.28$, vs. 0.08–0.16 in the other populations). The population LP showed the highest values for the diversity parameters measured at the microsatellite loci (Table 2).

Population genetic structure

Analysis of molecular variance (AMOVA) revealed significant differences between regions (mtDNA $\Phi_{CT}=0.27$, $P=0.047$; microsatellites $F_{CT}=0.04$, $P=0.004$). Most genetic variation occurred within sub-regions (mtDNA 66%, microsatellites 93%), while the smallest fraction of the variance was due to divergence among sub-regions within regions (mtDNA 7%, microsatellites 3%). We found evidence of significant differentiation among sub-regions within regions for microsatellites ($F_{SC}=0.04$, $P=0.012$) but not for mtDNA ($\Phi_{SC}=0.10$, $P=0.078$). Thus, we did not further analyze sub-region variation for mtDNA data.

Pairwise differences among regions showed no genetic differentiation among western regions SL, LP and RN at the mtDNA (Table 3) and therefore we recognized three distinctive groups (U, C, SL_LP_RN). There was a strong differentiation between eastern (U and C) and western (SL_LP_RN) populations ($\Phi_{STs}=0.16$–0.70, $P<0.005$, Table 3). H1 was the most frequent haplotype in the eastern extreme of the distribution and was not observed in any sample from the west while H5 was the most frequent haplotype in the west and rare in the east (Fig. 1; Table 1). Differentiation between eastern regions U and C was also significant ($\Phi_{ST}=0.1$, $P=0.003$).

Microsatellite data indicated moderate differentiation between eastern and western populations ($F_{STs}=0.05$–0.1,

Table 1 MtDNA diversity measures by region (*U* Uruguay, *C* Corrientes, *SL* San Luis, *LP* La Pampa, *RN* Rio Negro)

	N	Haplotype diversity (H)	Nucleotide diversity (Pi) %	Most common haplotypes	Number and frequency of private haplotypes
U	23	0.62	1.27	H1 (61%)	3 (13%)
C	25	0.36	0.52	H1 (80%)	2 (8%)
SL	6	0.33	0.45	H5 (83%)	1 (17%)
LP	17	0.49	1.16	H5 (71%)	2 (12%)
RN	23	0.37	0.91	H5 (79%)	0

Table 3 Pairwise population fixation indices (Φ_{ST}) calculated from haplotype diversity at a 736 bp mtDNA control region fragment for yellow cardinals belonging to four regions of Argentina (*C* Corrientes, *SL* San Luis, *LP* La Pampa, *RN* Rio Negro) and Uruguay (U)

	U	C	SL	LP	RN
U					
C	**0.102**				
SL	**0.338**	**0.695**			
LP	**0.165**	**0.460**	0.061		
RN	**0.250**	**0.555**	0.038	−0.027	

Significant values ($P<0.005$) are shown in bold

Table 2 Microsatellite diversity measures by region (*U* Uruguay, *C* Corrientes, *SL* San Luis, *LP* La Pampa, *RN* Rio Negro) and for the total population

	n	PA	HO (±SD)	HE (±SD)	Na	Ne	AR
U	26	3	0.582 ± 0.089	0.639 ± 0.062	5.6	3.2	4.2
C	26	5	0.485 ± 0.082	0.661 ± 0.037	5.6	3.4	4.2
SL	7	4	0.643 ± 0.091	0.639 ± 0.081	4.8	3.8	4.9
LP	18	15	0.643 ± 0.072	0.748 ± 0.039	7.5	4.6	5.5
RN	25	5	0.500 ± 0.082	0.640 ± 0.051	6.5	3.1	4.4
TOTAL	102	32	0.571 ± 0.037	0.665 ± 0.025	6	3.6	

n number of samples, *PA* Private alleles, *HO* Average observed heterozygosity, *HE* Average expected heterozygosity, *Na* Average number of alleles across loci, *Ne* Average number of effective alleles across loci, *AR* Average allelic richness (based on sample size SL = 7)

Table 4) and a low differentiation among western regions SL, LP and RN. Interestingly, LP1 and LP2 were not different from SL, while LP3 was not different from RN, thus subdividing the populations of La Pampa in a northern and a southern group (Table 4; Fig. 1).

For the bayesian analysis of microsatellite data with STRUCTURE, Δk was maximized at k = 3. Most of the individuals had more than 50% assignment probability to one cluster (Fig. 2). There was a tendency towards a more frequent assignment of individuals from eastern regions U and C to the yellow cluster, of individuals from SL and north of LP to the blue cluster, and of individuals from southern LP and RN to the green cluster (Fig. 2).

The frequency of the most common haplotypes correlated with microsatellite clusters, as detected by a Chi square replicated goodness of fit (Pearson $\chi^2 = 45.48$, $P < 0.001$, Table 5). We detected an association of haplotype H1 to the yellow cluster and haplotype H5 to the blue and green clusters. Detailed inspection of the few individuals with a microsatellite cluster assignment not fitting to the majority of its geographic population (N = 34) revealed that two individuals from Uruguay also carried a mtDNA haplotype not frequently found in their geographic region (Fig. 2) and could thus represent possible migrants (from west to east of the cardinals' range).

We found evidence of a positive significant correlation between geographic distance and genetic differences in cardinals for microsatellites (mantel test r = 0.551, $P = 0.003$, Fig. 3a) and mtDNA data (mantel test r = 0.467, $P = 0.02$, Fig. 3b).

Internal consistency tests that assigned individuals of known origin back to their groups were highly consistent. All individuals were correctly assigned to their

Table 4 Pairwise population fixation indices (F_{ST}) calculated from nuclear data of yellow cardinals illustrating differences between populations of eight zones of Argentina and Uruguay

	U	C1	C2	SL	LP1	LP2	LP3	RN1	RN2
U									
C1	**0.059****								
C2	**0.056****	0.016							
SL	**0.097****	**0.088****	**0.065***						
LP1	**0.086***	**0.091****	**0.085****	0.014					
LP2	**0.081***	**0.085****	**0.075***	0.045	0.038				
LP3	**0.049***	**0.063***	**0.052***	**0.065****	**0.056****	**0.063***			
RN1	**0.089****	**0.094****	**0.085****	**0.078****	0.033	**0.087***	0.031		
RN2	**0.072****	**0.093****	**0.089****	**0.073****	**0.074****	**0.115****	0.018	0.030	

Significant values are shown in bold (*$P < 0.05$; **$P < 0.005$)

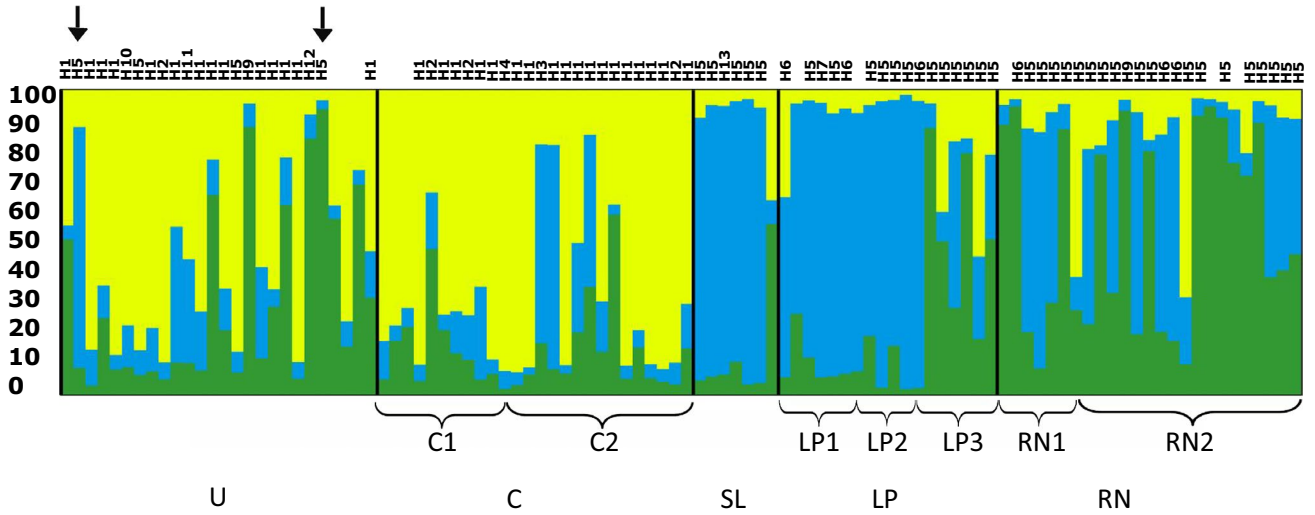

Fig. 2 Bayesian clustering analysis for k = 3 including 102 *yellow* cardinals. Each *vertical line* represents one individual, with the proportion of assignment to each one of the three clusters highlighted by the *different colors* in the column. Geographic region (and sub-region) of each sampled individual are indicated on the *x-axis* (*U* Uruguay, *C* Corrientes, *SL* San Luis, *LP* La Pampa and *RN* Rio Negro). *Arrows* indicate possible migrants (Color figure Online)

Table 5 Association between all haplotypes shared between at least two regions and the highest assignment probability to cluster 1, 2 or 3

	H1	H5	H6	H9	H2	Total
Cluster 1 *(yellow)*	22	6	0	0	4	32
Cluster 2 *(blue)*	4	9	3	2	0	18
Cluster 3 *(green)*	3	24	3	0	0	30
All groups	29	39	6	2	4	80

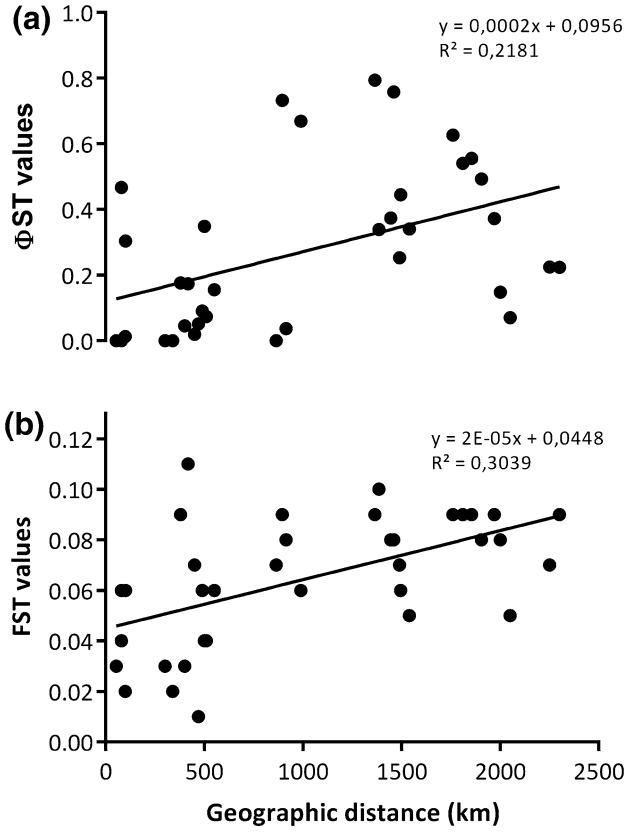

Fig. 3 Correlation between genetic and geographic distance for **a** microsatellite and **b** mtDNA data. *Dots* represent pairwise F_{ST} values and geographic distances among the sampled sub-populations. Significance was evaluated with a Mantel-test

management unit, with a maximum variation in membership of 2% compared to original average runs.

The sign test executed with Bottleneck showed no support for a recent genetic bottleneck in any population ($P > 0.05$). Additionally, the mode-shift test did not detect any mode shift in the frequency distribution of alleles for any of the populations.

Discussion

This study represents the first geographically comprehensive study of the yellow cardinal's global population and provides insights into its genetic diversity and distribution. We found that the geographic isolation among populations parallels the genetic differentiation found in both mitochondrial and nuclear markers.

Genetic diversity

Genetic diversity differed among populations. In general, the Uruguayan population (U) and LP were those with the highest diversity values, while SL and C showed the lowest ones. Haplotype diversity (H) was low (i.e., below 0.5, Grant and Bowen 1998) for all populations except for Uruguay. Such a decreased genetic variability may result from a bottleneck or a small population size (Nei et al. 1975). Given the small number of individuals observed in SL and the low number of locations where the yellow cardinal is found in C, it is likely that both factors are affecting genetic diversity. Statistical analyses did not, however, show evidence of genetic bottlenecks, which might be related to the sensibility of these tests to low population sizes (Peery et al. 2012), as well as the lack of complete isolation between different populations. The decline in population size due to habitat fragmentation and to the removal of individuals for illegal pet trade could have generated a bottleneck, which is reflected in the low genetic variability. Given the persistence of threats over time, populations may have not yet recovered from the presumed population decline. Martins-Ferreira et al. 2010 studied the genetic variability of this species in some regions and found, despite the lower sample size, a greater diversity than we did. We probably found a consistently lower number of alleles than the number presented in their study because their approach (using one sample per site) systematically overestimates diversity. Also, they did not study any Argentinean population in the field, but instead analyzed eight tissue samples from museums, which could reflect ancestral diversity, supporting the existence of recent bottlenecks.

Population genetic structure

Information on population genetic variability of endangered species is relevant for conservation (Frankham 2010; Haig et al. 2011). Both mitochondrial and microsatellite markers showed genetic structure in the yellow cardinal, although differentiation in microsatellites was lower than in mtDNA. This might arise as a consequence of their

different way of inheritance and their effective population size (Wayne and Morin 2004), posing more genetic drift on mtDNA and thus resulting in a greater differentiation at this marker (Ballard and Whitlock 2004). Alternatively, the lower differentiation in microsatellites compared to mtDNA could arise from frequent releases of seized males in different areas. Moreover, microsatellites usually present a higher polymorphism than mtDNA making them more effective for population studies in relatively recent evolutionary times (Estoup et al. 2002). This could explain the finer resolution of microsatellites in our study (three distinct groups, as compared to two groups for mitochondrial DNA). The differentiation between eastern and western regions in the mitochondrial marker may reflect the evolutionary history of the species, which could be related to the Parana-Paraguay basin geographical barrier. This barrier has affected the genetic structure of many other species of birds as well (Short 1975; Campagna et al. 2014).

On the other hand, habitat continuity in the past may have allowed for gene flow to occur between the southern regions. However, genetic analyses using microsatellite markers (which provide information on the most recent evolutionary history) revealed that individuals of these regions are genetically differentiated. Specifically, they show a differentiation between populations in the west, which are divided into two units (RN and south of LP and north of LP and SL). This differentiation could be due to the recent fragmentation of the natural habitat, which led to the isolation of populations.

Bayesian analyses support the differentiation found for the mitochondrial marker between eastern and western populations in Argentina, and also reveals the subdivision of western populations. Based on this evidence, we can identify four genetically distinct groups of the yellow cardinal, three in Argentina and one in Uruguay. Yellow cardinals from eastern Argentina (C) behave as a panmictic unit, while Argentinean western populations are divided into a northern and a southern group. Although STRUCTURE may not reliably group individuals into clusters when populations follow an isolation-by-distance pattern like the one we found in this study, we are confident of the recognized number of management units, as they were also supported by AMOVA analyses. The isolation-by-distance pattern suggests a decrease in genetic similarity with increasing geographic distance and might imply that a major factor generating population structuring is the limited movement of individuals. However, habitat discontinuities could also act as barriers to gene flow by increasing the amount of differentiation for those populations in which a physical boundary (lack of habitat) limits the effective dispersal of cardinals. Even though this explanation is in agreement with the known fact that habitat loss is one of the main threats for this species, it still has to be determined whether physical barriers are preventing dispersal among populations, which would be critical for determining release sites of individuals confiscated by government authorities.

Interestingly, we found two specimens in the Uruguayan population that exhibited affinities to individuals from southern Argentina both in mtDNA and microsatellites (arrows in Fig. 2). Here we have to recall that the analyzed specimens from Uruguay are of captive origin. As migration over this distance is very unlikely, these two specimens probably constitute events of illegal animal trade across the countries.

Conservation implications

More than 95% of the global population of the yellow cardinal inhabits Argentina and Uruguay. Our results support the delimitation of four management units for the yellow cardinal (three in Argentina and one in Uruguay). This information is crucial for planning conservation actions aimed at maintaining genetic variability and minimizing the risk of extinction of the species (Waples 1991). As shown by the assignment tests, the genetic differentiation between regions can be used to assign the origin of seized individuals and plan their release, provided sanitary conditions are met. This tool is extremely valuable and has been used in other cases of birds and turtles that suffer from illegal captures (Faria et al. 2008; Schwartz and Karl 2008; Fernandes and Caparroz 2013; Presti et al. 2015). Although the greatest challenge is working in collaboration with the authorities in order to stop the main threats that the species is facing, our results may be used as a tool for the quick release of confiscated individuals and to delineate a conservation plan aimed at preserving the present genetic diversity of the species.

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