Short communication

Genetic divergence among Snail Kite subspecies: implications for the conservation of the endangered Florida Snail Kite *Rostrhamus sociabilis*

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Figure 1. Geographic range for each of the three recognized Snail Kite subspecies. Black dots represent sample locations (see Table S1 for sample information).

Estimating genetic divergence among populations is crucial for the conservation of many threatened and endangered species (Allendorf & Luikart 2007). Many management programs are concerned with resolving phylogenetic and taxonomic uncertainties among taxa in order to prioritize conservation efforts below the species level (e.g. subspecies, evolutionary significant units), which is important because much existing taxonomy may not reflect the underlying genetic diversity (Moritz 1994, Crandall *et al.* 2000). Unfortunately, basic information on population genetic divergence is lacking for many threatened species, hindering conservation and management actions such as translocations and reintroductions (Moritz 1999).

The Snail Kite *Rostrhamus sociabilis* is a medium-sized hawk that feeds mainly on freshwater snails and is considered a wetland-dependent species (Beissinger 1988). Three Snail Kite subspecies are currently recognized based on morphometrics (Sykes *et al.* 1995): (1) *Rostrhamus sociabilis plumbeus*, distributed in peninsular Florida and Cuba; (2) *Rostrhamus sociabilis major*, distributed in Mexico, Guatemala and Belize; and (3) *Rostrhamus sociabilis sociabilis*, distributed in Nicaragua, Costa Rica, Panama and South America (Fig. 1). The separation of subspecies based on size variation alone has been questioned (Sykes *et al.* 1995). Prior to this study, information on the genetic divergence among

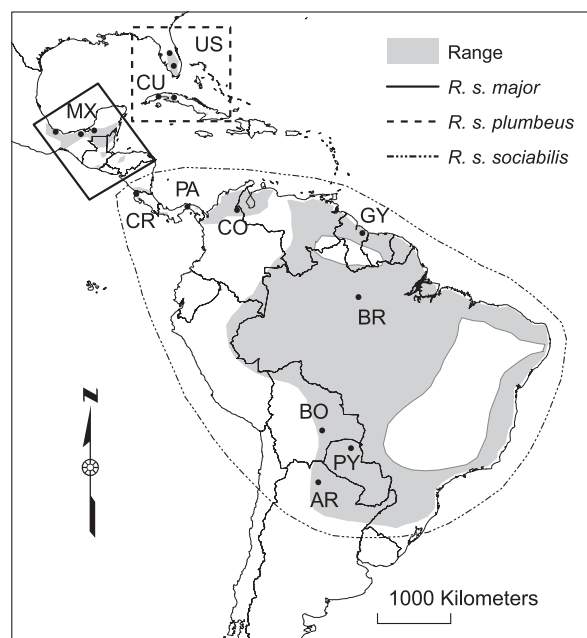*Corresponding author.
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Snail Kite subspecies was lacking, which is needed in light of the endangered status of the population of *R. s. plumbeus* in Florida.

The Florida population of *R. s. plumbeus* was listed as federally endangered in 1967 due to severe population declines following wetland destruction of the Florida Everglades beginning in the 1930s (USFWS 1999). After a brief period of favourable conditions, the Florida Snail Kite population remained relatively stable and increased during the mid-1990s (Martin *et al.* 2007). However, the population has recently declined dramatically, beginning in 1999 and has not recovered since (Martin *et al.* 2007). Although current population estimates (approximately 1600 birds, Martin *et al.* 2007) do not suggest that demographic stochasticity is of immediate concern, random loss of genetic diversity due to small population size may constitute a threat for the Florida Snail Kite, especially if decline continues, which matrix population models predict (Martin 2007).

Given the limited information about Snail Kites outside of Florida, it remains unclear if other populations represent different evolutionary trajectories based on the criterion of genetic 'exchangeability' (*sensu* Templeton 1989). If gene flow among populations is low, loss of adaptive genetic diversity could be a serious concern for the viability of the Florida population. Given the projected population decline of the Florida Snail Kite, management considerations to mitigate loss of genetic diversity (e.g. captive breeding, supplemental translocation) may need to be considered. However, translocation of individuals among populations

can have detrimental consequences when populations are not genetically and ecologically exchangeable (Crandall *et al.* 2000). Therefore, this paper has two main objectives: (1) to estimate the level of genetic divergence among Snail Kite subspecies using mitochondrial DNA (mtDNA), and (2) to discuss the relevance of our finding to the conservation of the Florida Snail Kite.

METHODS

Taxon sampling

Samples encompassed the Snail Kite range, but were limited due to CITES II listing of Snail Kites. Twenty-five samples (12 *R. s. plumbeus*, three *R. s. major*, 10 *R. s. sociabilis*) were collected throughout the distribution range of the three subspecies using museum and modern samples (Fig. 1, Table S1). A sample from the closely related Slender-billed Kite *Rostrhamus hamatus* (Ridgely & Gwynne 1989) was used as an outgroup for phylogenetic analysis.

DNA sequencing

DNA extraction from modern samples was performed using a PUREGENE Genomic DNA Purification Kit (Gentra Systems). For museum samples, DNA was extracted using a QIAamp DNA Micro Kit (Qiagen). DNA extraction and PCR setup for museum samples were conducted in an ancient DNA laboratory. Negative controls were performed for all extractions and amplifications. Portions of Domain I and Domain III of the mtDNA control region were amplified in a 50 L PCR using various primers (Table S2). We also sequenced ND2 and cytochrome-*b* from an *R. s. plumbeus* and a *R. s. sociabilis* sample; when compared these regions exhibited little to no variation and were not examined further. PCR products from the modern extractions were cleaned using a polyethylene glycol (20%): NaCl (2.5 M) precipitation. Museum PCR products were cleaned using a Perfectprep Gel Cleanup kit (Eppendorf). Purified PCR products were sequenced in both directions using the PCR primers with an ABI Prism 3100-Avant genetic analyzer (Applied Biosystems) and edited in SEQUENCHER 4.5 (Gene Codes Corporation). Sequencing of PCR products and careful examination of chromatograms from both strands should allow detection of PCR and sequencing errors (Barry *et al.* 2003), ensuring haplotypes were correctly identified. In addition, we re-amplified and sequenced a subset of sequences an additional time to further check for sequencing errors in rare haplotypes.

Data analysis

Domain I and III sequences were concatenated for all analyses. We used DNASP 4.10.9 (Rozas *et al.* 2003) to determine overall and subspecies haplotype diversity (h), nucleotide diversity (π), average number of nucleotide differences (k)

among haplotypes and F_{ST} (Hudson *et al.* 1992) among subspecies. Evolutionary relationships were estimated using maximum parsimony (MP) and maximum likelihood (ML) in PAUP* 4.0b10 (Swofford 2002). The appropriate model for ML analyses was determined using the AIC criterion in MODELTEST 3.6 (Posada & Crandall 1998). For MP and ML, a heuristic search with 100 random addition replicates was performed. Support was assessed using 100 pseudo-bootstrap replicates, each with 10 random additions of taxa. To investigate geographic genetic structure we constructed an unrooted parsimony network of haplotypes using TCS 1.21 (Clement *et al.* 2000).

RESULTS

The sequence alignment contained 861 bp of the mtDNA control region, covering Domains I (360 bp) and III (501 bp). Among Snail Kite samples, there were 10 haplotypes defined by seven polymorphic sites (four were parsimony-informative) (Table S3). There were no transversions, suggesting recent polymorphism in Snail Kites. Only a single haplotype was found in *R. s. major*, though only three *R. s. major* individuals were sampled. This haplotype was also found in three (25%) *R. s. plumbeus* samples, whereas all remaining *R. s. plumbeus* samples shared a haplotype not found in the other two subspecies. Eight of the 10 haplotypes were represented by *R. s. sociabilis*, none of which was found in *R. s. plumbeus* or *R. s. major*.

Haplotype diversity for Snail Kites was high ($h = 0.823 \pm 0.057$ sd), whereas nucleotide diversity was low ($\pi = 0.00222 \pm 0.0011$). Both haplotype and nucleotide diversity were greatest within *R. s. sociabilis* ($h = 0.956$; $\pi = 0.00269$) compared to *R. s. plumbeus* ($h = 0.409$; $\pi = 0.00049$) and *R. s. major* ($h = 0$; $\pi = 0$). The average number of nucleotide differences (k) among all Snail Kite samples was 1.773; being lowest between *R. s. plumbeus* and *R. s. major* ($k = 0.750$), intermediate between *R. s. major* and *R. s. sociabilis* ($k = 2.300$), and highest between *R. s. plumbeus* and *R. s. sociabilis* ($k = 2.600$). Statistically significant genetic differentiation was found among all subspecies ($F_{ST} = 0.54608$, $P < 0.05$).

MP analysis produced 520 most parsimonious trees (Length = 74; Consistency Index, excluding uninformative sites = 0.5714), whereas ML produced seven equally likely trees (–ln 1508). Although both MP and ML revealed similar topologies, the ML tree had higher nodal resolution (Fig. 2; MP tree not shown). We obtained low bootstrap support due to low sequence variation within our dataset; however, consensus among the seven ML trees revealed that some nodes were found consistently. The ML consensus tree indicated that none of the Snail Kite subspecies exhibited reciprocal monophyly and that there was little divergence among taxa and populations.

A maximum of four mutational steps was found among the haplotypes revealing shallow sequence divergence
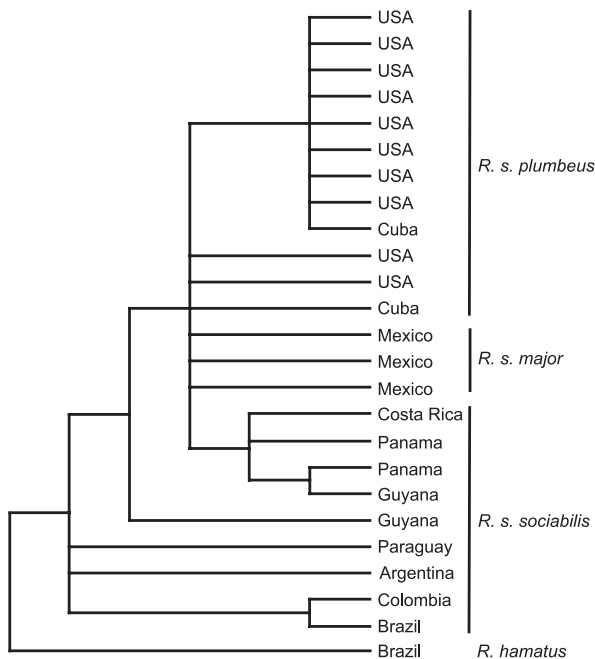
Figure 2. Majority Rule consensus tree of seven maximum likelihood (ML) trees obtained from analyses of mtDNA control region haplotypes in Snail Kites. Samples are labelled according to the country in which they were collected.

Figure 3. Parsimony haplotype network showing the relationships between the 10 control region haplotypes found for the Snail Kite in this study (outgroup excluded). The number of line segments between haplotypes represents the number of mutations that have occurred. All *Rostrhamus sociabilis sociabilis* haplotypes are in the box.

among haplotypes (Fig. 3). Most *R. s. sociabilis* samples from the southern part of South America (i.e. Bolivia, Paraguay and Argentina) were two to three mutational steps away from the remaining subspecies, whereas many *R. s. sociabilis* samples from Central America (i.e. Costa Rica and Panama) and the northern part of South America (i.e. Guyana and Colombia) were only one to two steps away from *R. s. plumbeus* and *R. s. major* haplotypes.

DISCUSSION

Overall, Snail Kites exhibited similar haplotype diversity to that observed in other raptors (Roques & Negro 2005, Shephard *et al.* 2005, Johnson *et al.* 2007). Only two of 10 haplotypes were found in *R. s. plumbeus*, and only a single haplotype was detected in *R. s. major*. In contrast, eight haplotypes (80%) were recovered in *R. s. sociabilis* despite sampling fewer individuals. This finding suggests that both *R. s. plumbeus* and *R. s. major* exhibit low genetic variation, although additional samples from *R. s. major* may reveal greater variation than our limited sampling indicated.

Higher haplotype diversity in *R. s. sociabilis* might be partially attributed to the larger geographic range of this subspecies. Moreover, the phylogenetic and network analyses suggest that *R. s. sociabilis* contains the oldest Snail Kite lineage: *R. s. sociabilis* haplotypes represent the basal clade on the phylogenetic tree and are clustered in the
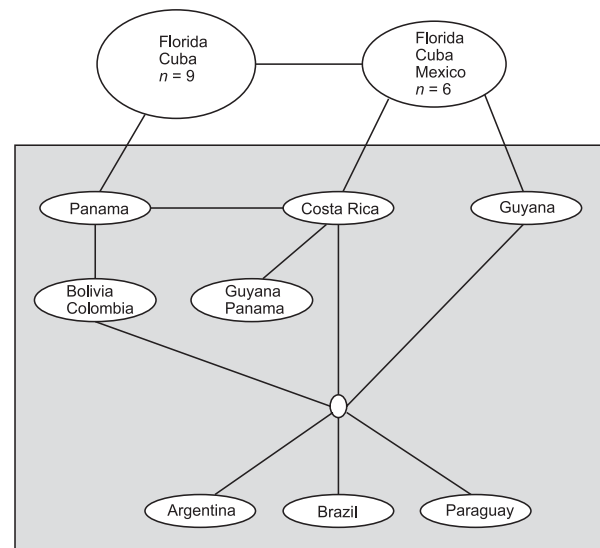
haplotype network. This suggests that Snail Kites may have radiated from South America into Central America, Cuba and Florida. Range expansions involving population bottlenecks may create decreasing genetic diversity as distance from the source population increases (Hewitt 1996), which could explain the lower haplotype diversity in *R. s. major* and *R. s. plumbeus*. This phenomenon could be pronounced in *R. s. plumbeus*, whose geographic range constitutes the periphery of the species' geographic range.

None of the *R. s. sociabilis* haplotypes was present in *R. s. plumbeus* or *R. s. major*, which suggests no gene flow between *R. s. sociabilis* and either *R. s. plumbeus* or *R. s. major*. In contrast, we found that *R. s. plumbeus* from both Florida and Cuba shared a haplotype with *R. s. major*, indicating no genetic differences between these subspecies in our dataset. Observations that Snail Kites disperse outside their documented range (e.g. putative *R. s. major* individuals have been observed in Texas; Sykes *et al.* 1995) further suggest there may be gene flow between *R. s. major* and *R. s. plumbeus*. Given this, and the close proximity between Florida and Cuba, it is not surprising that differences were not found between the Florida and Cuba populations of *R. s. plumbeus*.

Conservation and management implications

Beginning in the 1930s and through the mid-1960s, Florida Snail Kites experienced severe population declines (Sykes *et al.* 1995). Count surveys suggested that the Florida

population numbers might have plummeted to less than a few 100 individuals during this time period (reviewed in Sykes *et al.* 1995). Robust estimates for population size from 2002–5 were approximately 1400 individuals (Martin *et al.* 2007). These numbers suggest that the Florida Snail Kite population may have undergone a population bottleneck starting in the early 20th century. However, at present there are no observable phenotypic traits unique to the Florida population that might be linked with loss of genetic diversity and possible inbreeding depression.

If gene flow into Florida is limited, additional population declines may lead to reduced genetic diversity via low effective population sizes and genetic drift. If this happens, captive breeding and supplemental translocations are potential management strategies (Tallmon *et al.* 2004, Russello & Amato 2007). Although translocations have assisted in the recovery of some endangered species (Tallmon *et al.* 2004), collecting sufficient information on both the genetic and ecological exchangeability among populations is critical before translocations are considered (Crandall *et al.* 2000, Fraser & Bernatchez 2001). Currently, there is limited information on behavioral and demographic differences (i.e. ecological exchangeability) among Snail Kite populations (but see Beissinger *et al.* 1994, Tanaka *et al.* 2006). Our findings suggest that *R. s. plumbeus* and *R. s. major* may be more genetically exchangeable than either is to *R. s. sociabilis*. Thus, if supplemental translocations are considered, individuals from Cuba or *R. s. major* are likely to be most successful. Before this step is taken, however, additional research to confirm these results using nuclear DNA is needed, and further research to assess ecological exchangeability will need to be conducted.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Subspecies, source, catalog number, collection date, location collected, sample type, and GenBank accession numbers for the 26 samples used in this study.

Table S2. Primers for the mtDNA control region of the Snail Kite *Rostrhamus sociabilis*.

Table S3. Haplotypes (H), number of each subspecies per haplotype, and country of origin (in parentheses) of the 10 mtDNA control region haplotypes found in the Snail Kite.

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