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NONINVASIVE ESTIMATION OF MINIMUM POPULATION SIZES AND VARIABILITY OF THE MAJOR HISTOCOMPATIBILITY COMPLEX IN THE ANDEAN CONDOR

MIGUEL ALCAIDE[1,2,5], LUIS CADAHÍA[1,3], SERGIO A. LAMBERTUCCI[4], AND JUAN J. NEGRO[1]

[1]*Estación Biológica de Doñana (CSIC), Avda. Américo Vespucio s/n 41092, Sevilla, Spain*
[2]*Department of Organismic and Evolutionary Biology, Harvard University, 26 Oxford Street, Cambridge, MA 02138*
[3]*Centre for Ecological and Evolutionary Synthesis (CEES), P. O. Box 1066, Blindern, NO-0316, Oslo, Norway*
[4]*Laboratorio Ecotono, INIBIOMA-CONICET, Quintral 1250, 8400, Centro Regional Universitario Bariloche, Universidad Nacional del Comahue, San Carlos de Bariloche, Argentina*

Abstract. Estimating indices of abundance of threatened species is crucial to preserving biodiversity. Over the last few decades, noninvasive genetic sampling has proven to be a more straightforward and less expensive approach than capture–mark–recapture analyses. In particular, molted feathers have become extremely popular for the monitoring of bird populations. Diagnostic molecular markers such as microsatellites, however, are still not available for many avian species of conservation concern. Highly polymorphic genes of the major histocompatibility complex (MHC), on the other hand, have become reasonably accessible during the last few years. We tested the suitability of MHC profiles as DNA fingerprints to assist the identification of individuals of a scavenger difficult to monitor through traditional approaches, the Andean Condor (*Vultur gryphus*). To achieve this aim, we isolated polymorphic and putatively functional genes of MHC class I (exon 3, 6 alleles) and MHC class IIB (exon 2, 11 alleles). Single-strand conformational polymorphism and direct sequencing of MHC genes, combined with molecular sexing and inference of age class from feather color, allowed us to identify 80 different individuals from 110 molted feathers collected at roost sites. Inferred sex and age ratios were concordant with previous studies relying on direct observations. Among adults, the number of males was double that of females; among juveniles, this ratio was inverted. Besides providing valuable data regarding genetic variation at functionally important genes related to resistance to pathogens, we demonstrate additional potential of polymorphic MHC loci beyond their well-known role in evolutionary ecology.

Key words: abundance, adaptive variation, Andean Condor, *Cathartidae, genetic assessment, genetic monitoring, immunogenetics, major histocompatibility complex,* Vultur gryphus.

Estimación No Invasiva de Tamaños Mínimos Poblacionales y Variabilidad del Complejo Mayor de Histocompatibilidad en el Cóndor de los Andes

Resumen. La estimación de índices de abundancia de especies amenazadas es crucial para la preservación de la biodiversidad. Durante las últimas décadas, los muestreos no invasivos han permitido una aproximación directa y de bajo costo con relación a los métodos tradicionales de captura–marca–recaptura. En particular, el uso de plumas que han pasado por el proceso de muda han ha sido muy popular para el seguimiento de poblaciones de aves. No obstante, aún existe una carencia de marcadores moleculares con poder de diagnóstico, como los microsatélites, para un gran número de especies de interés en conservación. Recientemente, los genes altamente polimórficos del complejo mayor de histocompatibilidad (CMH) se han convertido en una alternativa razonable. En este estudio probamos la utilidad de los pérfiles de CMH como una huella genética en *Vultur gryphus*, una especie carroñera de difícil seguimiento mediante técnicas tradicionales. Para dicho fin, hemos aislado genes polimórficos y posiblemente funcionales de CMH de clase I (exón 3, 6 alelos) y de clase II B (exón 2, 11 alelos). Los análisis de polimorfismos conformacionales de cadena sencilla y de secuenciación directa, combinados con identificación del sexo con técnicas moleculares y estimaciones de la edad de los individuos, permitieron la identificación de 80 individuos distintos a partir de 110 plumas de muda colectadas en posaderos. Las proporciones entre sexos y entre las distintas clases de edad confirmaron estudios previos basados en observaciones directas de individuos. El número de machos adultos dobló el de hembras adultas, estando dicha proporción invertida en el caso de juveniles. Además de proporcionar patrones de variación genética relevantes en la respuesta inmune frente a patógenos, este estudio demuestra usos potenciales de los genes del CMH más allá de su bien conocido papel en ecología evolutiva.

Manuscript received 23 October 2009; accepted 5 April 2010.
[5]E-mail: malcaide@oeb.harvard.edu

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INTRODUCTION

The decimation of populations critically jeopardizes the preservation of biodiversity. Estimating population sizes is therefore one of the main challenges of conservation biologists. Molecular methods relying on polymorphic genetic markers have become increasingly popular for the monitoring of wildlife. Importantly, survey by means of samples taken noninvasively, such as hairs, molted feathers, or scat, has minimized many of the methodological difficulties with elusive, aggressive, or poorly accessible species (Schwartz et al. 2007). From all the battery of markers available, simple tandem repeats (SSRs) or microsatellites are the most popular because of their high polymorphism, codominancy, and cross-amplification between related species. The characterization of a relatively low number of microsatellite markers has been satisfactorily used for inferring minimum population sizes, individual identity, and relatedness in numerous species (Taberlet et al. 1997, Rudnik et al. 2005, Regnaut et al. 2006). Diagnostic genetic markers are lacking for the majority of species, however, given that microsatellites need to be isolated from at least closely related taxa or developed for each particular case. In spite of substantial technical advances, the generation of libraries of microsatellites still remains a time-consuming and expensive task.

The major histocompatibility complex (MHC) includes the most polymorphic genes described in vertebrates so far (Robinson et al. 2005). MHC genes encode cell-surface glycoproteins that play a crucial role during the activation of the adaptive immune system by recognizing small foreign peptides (antigens). The evolutionary significance of MHC polymorphism is linked to the ability of individuals and species to respond efficiently to a large spectrum of continuously evolving pathogens and parasites (Hughes and Nei 1992). Their recurrent consideration as "good genes" has also brought MHC genes into the focus of pathogen–host co-evolution, sexual selection, local adaptation, and conservation genetics (Sommer 2005). However, the isolation and characterization of MHC genes from other than a few model species is considered technically difficult and laborious. As a consequence of comparably small introns (Kaufman et al. 1999, Alcaide et al. 2007), long stretches of MHC sequences have been nonetheless isolated from various avian orders by means of the polymerase chain reaction (PCR) and primers sitting on conserved coding regions (Ekblom et al. 2003, Alcaide et al. 2007, 2009, Hughes et al. 2008). Recent studies have also revealed that boundaries between introns and exons might be quite well conserved within and even between different avian groups. As a result, our capabilities to cross-amplify highly polymorphic MHC loci across a huge number of avian species has been substantially enhanced (Alcaide et al. 2007, 2009). Moreover, high rates of gene conversion between multiple loci in the avian MHC (Wittzell et al. 1999, Alcaide et al. 2007, Miller and Lambert 2004) promote the co-amplification of gene duplicates through single PCR experiments.

In this study, we have characterized polymorphic MHC class I and class II loci in a species whose genome is little known, the Andean Condor (*Vultur gryphus*). This large scavenger, currently catalogued as "near threatened" by the International Union for the Conservation of Nature and included in Appendix I of the Convention on International Trade in Endangered Species (BirdLife International 2004), breeds and roosts at altitudes of up to 5000 m on inaccessible rocky outcrops throughout the Andes (del Hoyo et al. 1994, Lambertucci 2007, Lambertucci and Mastrantuoni 2008). This fact challenges traditional monitoring techniques relying on capture–mark–recapture, use of radio-transmitters, and even direct observation. Undoubtedly, the difficulty of access to the places where condors dwell, breed, and roost has hindered detailed monitoring of the species and is the most likely reason why many aspects of the condor's biology, such as accurate estimates of population size and trends, genetic diversity of populations, and the extent of foraging or dispersal movements, are poorly known (Lambertucci 2007). A recent proposal suggests a population around 6200 individuals, more than two-thirds probably in Argentina and Chile (Díaz et al. 2000). Since tens of individuals of both sexes and different age classes aggregate to roost at cliffs with shelves (Lambertucci et al. 2008), molted feathers are a suitable option for noninvasive genetic monitoring. As microsatellite sequences for any species of the family Cathartidae have been unavailable to the public until very recently (Romanov et al. 2009), we focused on individual MHC genotypes. Thus, we characterized and isolated polymorphic loci of MHC classes I and II and used their patterns of polymorphism, along with molecular sexing and age-class data inferred from feather color, to discriminate among individuals from a subset of roosts in Argentina.

METHODS

STUDY AREA

Feathers for this study were collected in Argentina during the summer of 2007, from 14 communal roosts in Rio Negro and Neuquén provinces ($n = 120$ feathers), northwestern Patagonia (36°–41° S, 70°–71° W), and one communal roost in Santa Cruz province ($n = 7$ feathers), approximately 1500 km farther south in southern Patagonia (50° S, 73° W, Fig. 1). The roosts selected for the study are a subset of the roosts in these areas but are the main known roosts (Lambertucci 2010; Lambertucci, unpubl. data). The feathers are generally found at the base of the cliffs on which the condors roost or breed. The number of feathers collected at each roost site ranged from 1 to 20, and each location was visited only once. Only fresh primary and secondary feathers from each cliff were collected. We also used differences in plumage colors to distinguish among age classes. Immature birds (juveniles and subadults, up to 5–6 years old) sport a matte brownish gray plumage, whereas adults (>6 years old) are black with pure white coverts on the upper wing and a white collar (Wallace and Temple

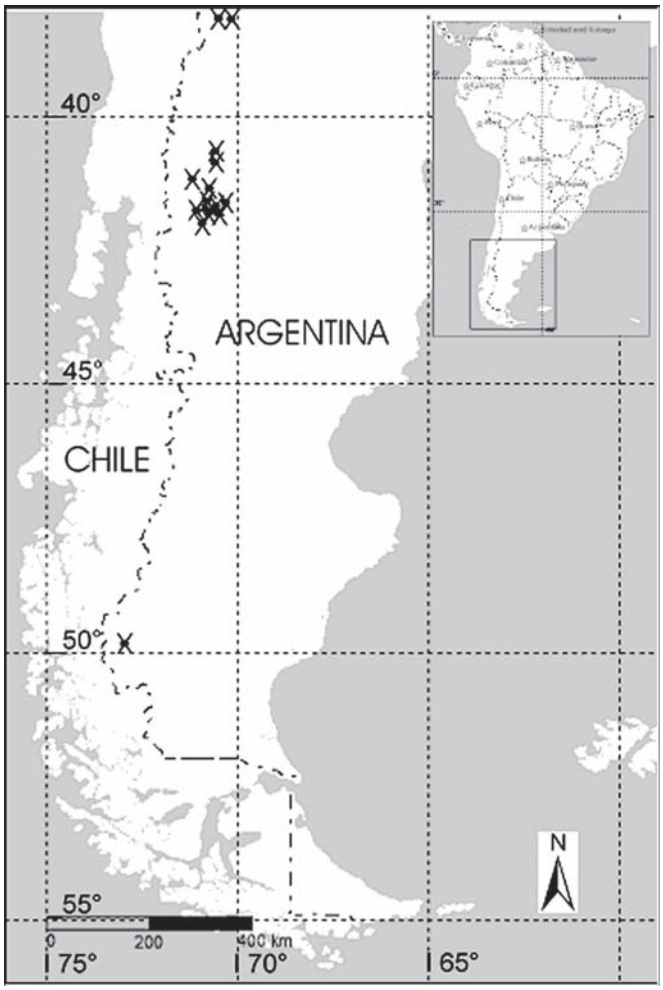

FIGURE 1. Study area in western Argentine Patagonia showing locations of the 15 communal roosts from which we collected Andean Condor feathers.

1987, del Hoyo et al. 1994). Fresh feathers are those whose physical appearance has not been dramatically affected by exposure to the sun and weather. We ensured the survey of recently molted feathers by gathering only nondegraded feathers, in which slight variations in color could be appreciated. The juvenile plumage of the Andean Condor is dark brown, then each successive plumage is paler, finally becoming gray with the molt preceding the adult plumage. This gradual transition of plumage coloration has been also reported in raptors (e.g., Herremans and Louette 2000). Therefore, we picked up different looking feathers in order to sample as many individuals as possible.

DNA EXTRACTION AND MOLECULAR SEXING OF MOLTED FEATHERS

The blood clot embedded in the superior umbilicus of each feather's shaft was treated according to a HotSHOT DNA-extraction protocol adapted from Truett et al. (2000). Blood clots in feathers have already proven to be a better source of DNA than samples from the feather's tip (Horváth et al. 2005). Feather shafts sliced at the level of the blood clot were suspended in 100–200 µL of a solution containing NaOH (25 mM) plus EDTA (0.2 mM) and incubated at 95 °C for 30 min. Reactions were neutralized by the addition of 100–200 µL of 40-mM Tris-HCl neutralization buffer and stored at −20 °C.

Using the universal primers P2 and P8 (Griffiths et al. 1998), we identified the sex of the bird that had shed the feather by PCR amplification of the CHD-W and CHD-Z genes. These primers amplify a single CHD-Z band from males and a CHD-Z plus a CHD-W band from females that can be distinguished after electrophoresis in 2.5% agarose gels (males, ZZ; females, ZW). We used feathers shed by a female condor kept at a zoo near Seville, Spain, as a control for our molecular sexing.

PCR AMPLIFICATION OF MHC GENES

We replicated the entire third exon of genes of MHC class I by using primers MHCIint2F (5′-CATTTCCCTGGTYGTGTTTCAGG-3′; Alcaide et al. 2009) and MHCI-ex3R (5′- CTCACCTTTCCTCTCCAG-3′). Primer MHCI-ex3R was designed de novo over a conserved 3′ distal region of exon 3 in the Falconiformes. Almost the whole coding sequence (255 out of 271 bp) of the second exon of genes of MHC class II B was amplified by means of primers MHCII-RapEx2F (5′-CAMACAGRGKTTTTTCCWGGAG-3′) and MHCII-CatInt2R (5′-CTCACCTTTCCTCTCCAG-3′). MHCII-RapEx2F targeted a conserved intron 1–exon 2 boundary in the Falconiformes, whereas MHCII-CatInt2R was specifically designed de novo for the Andean Condor. For that purpose, we partially sequenced the genomic fragment spanning exons 2 to 3 by using primers MHCII-RapEx2F and RapEx3CR (5′-CAGGCTGRCGTGCTCCAC-3′; Alcaide et al. 2007).

The PCR amplification profile consisted of 4 min at 94 °C, 35 cycles of 40 sec at 94 °C, 40 sec at 54 °C (for class I loci) or 56 °C (for class II loci), 40 sec at 72 °C, and finally 4 min at 72 °C. Each 25-µL reaction contained 1 unit of Taq polymerase (Bioline), 1× PCR manufacturer-supplied buffer, 1.5-mM $MgCl_2$, 0.02% gelatine, 0.2 mM of each dNTP, 10 pmol of each primer, and 5% DMSO.

AUTOMATED ANALYSIS OF MHC DIVERSITY BY SINGLE-STRAND CONFORMATIONAL POLYMORPHISM (SSCP)

Nondenaturing capillary electrophoresis enables separation of DNA sequences of the same length but different sequence. Migration times across a polymeric matrix are dependent on DNA folding, which, in turn, is affected by base composition (Kuhn et al. 2004, Alcaide et al. 2010). For SSCP electrophoresis with an ABI 3130xl Genetic Analyzer (Applied Biosystems), we labeled amplified MHC fragments from individual molted feathers with fluorescence by using primers marked with 6-FAM and VIC (MHC class II) and NED and

PET fluorochromes (MHC class I). PCR amplicons were diluted 1:10 and subsequently denatured with Hi-Di formamide (Applied Biosystems) and heat (95 °C for 3 min). Electrophoresis was carried out with a polymer composed of 5% conformational analysis polymer (Applied Biosystems), 10% glycerol, and 1x Genetic Analyzer running buffer (Applied Biosystems). Samples were run at optimized temperatures, 18 °C for class II loci and 33 °C for class I loci, with 0.7 µL of GeneScan 500-LIZ size standard. The temperature of the room holding the ABI 3130xl was maintained at or below the run temperature. We used GenMapper software version 3.7 (Applied Biosystems) to visualize and analyze the raw data. To test the feasibility and reproducibility of the technique, we processed several molted feathers of the female Andean Condor kept at the zoo in Seville.

MOLECULAR CLONING AND SEQUENCING ANALYSIS

In order to identify individual MHC sequences and the putative number of loci amplified, we purified eight selected PCR products (see below) for genes of MHC classes I and II in Microcon centrifuge tubes (Millipore) and, using the PGEM-T easy vector system II (Promega), subsequently cloned them into bacterial plasmids. We cloned 16 PCR amplicons (8 for class I genes and 8 for class II genes) displaying different electrophoretic chromatograms after the SSCP analysis of labeled MHC fragments. We assumed that different electropherograms and the presence of particular peaks might represent variations in allelic composition. We screened clones for the expected size of the insertion in 1.5% agarose gels by running a second PCR with M13 primers. We then selected between 12 and 16 positive clones per individual at random for sequencing analysis. All class I and class II PCR amplicons obtained from individual molted feathers were directly sequenced as well. We carried out sequencing reactions with the Big Dye 1.1 Terminator technology and resolved labeled fragments with the ABI 3130xl automated sequencer.

We used the software BioEdit 7.0.9 (Hall 1999) to align and edit MHC sequences of the Andean Condor. We considered cloned sequences to be true MHC alleles only when we found the same sequence in at least three different clones. We considered sequences differing by no more than two nucleotides from a redundant sequence to be artifacts of PCR or misincorportations of bases during bacterial replication and so discarded them. We used the software DNAsp (Rozas et al. 2003) to generate statistics on polymorphism at loci of both MHC classes I and II. Deviations from the neutral theory of molecular evolution were tested through Tajima's *D* test (Tajima 1989), also run in DNAsp. The frequencies of nonsynonymous (dN) and synonymous (dS) substitution ratios at those codons constituting positively selected sites in the well-studied MHC of the Lesser (*Falco naumanni*) and Common (*F. tinnunculus*) Kestrels (see Fig. 2) were calculated with MEGA 4.1 (Kumar et al. 2008).

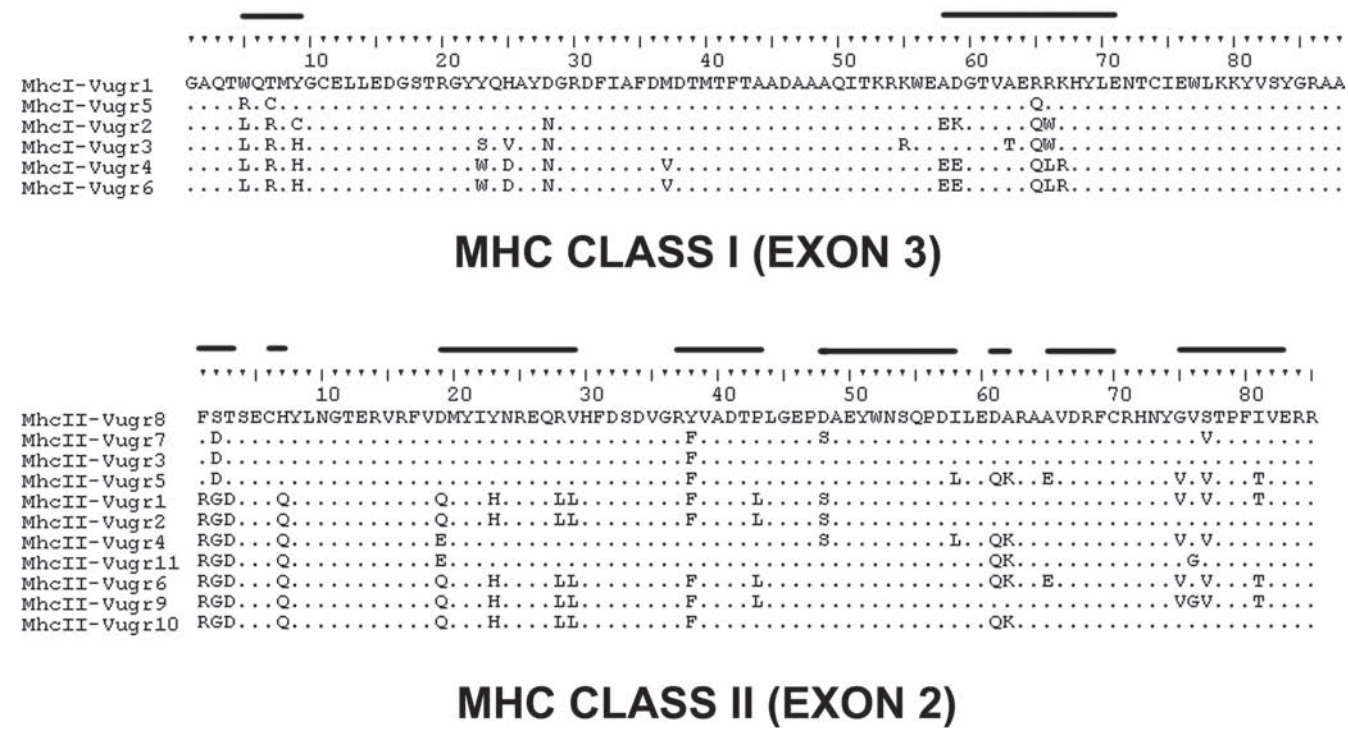

FIGURE 2. Putative amino acid sequences of loci of MHC classes I and II in the Andean Condor. MHC alleles have been named in accordance with the nomenclature recommended by Klein et al. (1990). The vast majority of polymorphic sites overlap with the coding regions encompassing positively selected amino acids in the well-studied MHC of the Lesser (*Falco naumanni*) and Eurasian (*F. tinnunculus*) Kestrels (putative antigen- binding regions indicated by black bars; see Alcaide et al. 2008, 2009 for details).

RESULTS

MHC DIVERSITY

We isolated six alleles of MHC class I and 11 of MHC class II from Andean Condor feathers (Gene Bank accession numbers EU120692-94, GU060474-78, and HM805049-5057; Fig. 2). The analysis of 264 bp of the third exon of genes of MHC class I revealed 27 segregating sites (S), a nucleotide diversity (π) of 0.049 (0.0018 per site); the average number of nucleotide differences between unique alleles (k) was 12.93. The analysis of 255 bp of the second exon of genes of MHC class IIB revealed 32 segregating sites (S), a nucleotide diversity (π) of 0.062 (0.0019 per site); the average number of nucleotide differences between unique alleles (k) was 15.84. Each individual yielded one to four alleles of MHC class II and two to four of MHC class I. These findings suggest the co-amplification of at least two genes of MHC class II and two of MHC class I. In some individuals MHC class I and/or MHC class II were represented by only one or two alleles. Our findings therefore suggest that gene duplicates might share identical alleles. This phenomenon, which has been already documented for other species of birds (Wittzell et al. 1999), can be attributed to high rates of gene conversion and concerted evolution between different loci (Miller and Lambert 2004, Alcaide et al. 2007, 2009).

Tajima's D test revealed an excess of high-frequency segregating (variable) sites ($D = 2.09$, $P < 0.05$) within alleles of MHC class II. Retention of high levels of nucleotide polymorphism can be attributed to balancing selection predominating over genetic drift. Although values of Tajima's D for genes of MHC class I were positive, evidence of balancing selection was not statistically significant ($D = 0.56$, $P > 0.10$). Rates of nonsynonymous substitution at codons presumably involved in antigen recognition were notably larger than those of synonymous substitution ($dN = 0.142 \pm 0.044$, $dS = 0.076 \pm 0.047$ for MHC class I genes; $dN = 0.603 \pm 0.109$, $dS = 0.106 \pm 0.055$ for MHC class II genes; see Fig. 2).

MHC GENOTYPES AS DNA FINGERPRINTS TO IDENTIFIY INDIVIDUALS

MHC profiles of individuals were compared through the analysis of SSCP and direct-sequencing electropherograms. Differences in times of migration across the CAP polymer of fluorescence-labeled alleles generated several types of SSCP electropherograms (Fig. 3). Direct-sequencing electropherograms, on the other hand, revealed variations in nucleotide compositions at several positions, according to the MHC alleles amplified in each particular case (Fig. 4). Independent

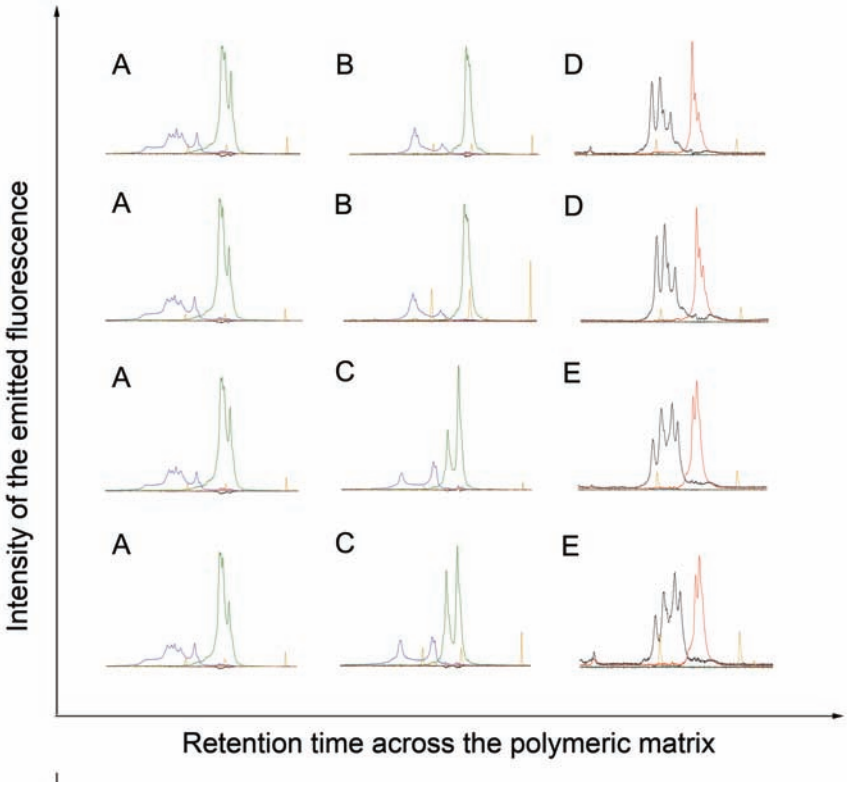

FIGURE 3. SSCP electropherograms of fluorescence-labeled PCR products for MHC genes of both class II (green and blue dyes) and class I (red and black dyes). Each color represents a different DNA strand of PCR amplicons. Orange peaks belong to the size standard and are aligned vertically to facilitate comparisons. Variations in nucleotide composition affect the topology of single-stranded DNA molecules and determine retention times during capillary electrophoresis. Reproducibility of five SSCP chromatograms (A, B, C, D, E) obtained from independent PCR of different molted feathers collected at the same or different roost sites. Differences in the y axis represent variations in the emitted fluorescence and may be related to small variations in the concentration of PCR products.

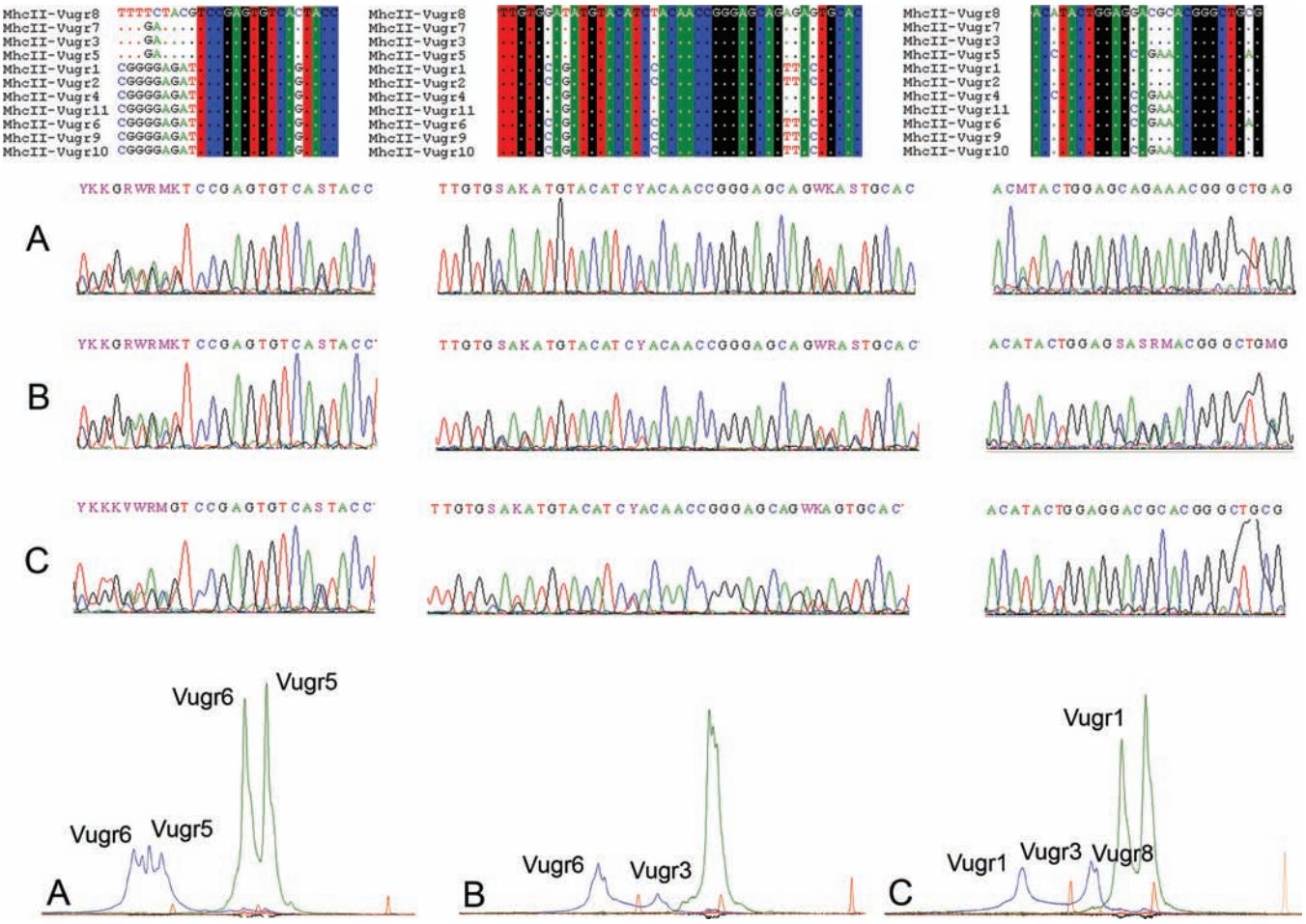

FIGURE 4. Correspondence between cloned MHC class II alleles (above), direct sequencing (middle) and SSCP electropherograms (below) in three Andean Condors (A, B, and C). Only the most polymorphic and informative coding regions of the second exon of MHC Class II B genes are displayed. Dots indicate identity with the top sequence. Degenerate nucleotide positions are indicated by IUPAC codes. The comparison of individuals with shared alleles allowed us to associate some characteristics SSCP peaks with particular alleles. For each single PCR amplicon, single-stranded DNA molecules were labelled with 6-FAM (blue) and VIC (green dyes). Orange peaks belong to the size standard. Only diagnostic peaks allowing the identification of particular alleles are labelled. For instance, alleles Vugr6 and Vugr3 generate a single peak in the green color but cannot be distinguished in the blue color.

PCR of the feathers from the captive Andean Condor yielded high reproducibility in the generation of these patterns, with only small variations in the *y* axis attributed to the concentration of each PCR amplicon and the fluorescence it subsequently emitted. Independent PCR of different feathers collected at the same or at different roosts yielded characteristic and informative electropherograms (see Figs. 3 and 4).

We successfully obtained sex and MHC profiles from 110 out of 127 molted feathers. Molecular sexing failed for some feathers probably because of degraded DNA preventing the PCR from amplifying the CDH-Z bands. Fourteen SSCP patterns for genes of MHC class I and 32 for those of MHC class II could be distinguished. Different MHC genotypes are derived from the combination of the six alleles of class I and the 11 of class II presumably shared by gene duplicates. This genetic information, combined with molecular sexing and determination of age class, enabled us to identify at least 80 genetically different individuals. Up to 19 individuals could be represented by two or even three feathers in our sample, which consisted of 33 adult males, 16 adult females, 9 juvenile males, and 22 juvenile females. We estimated the probability of finding two individuals of the most abundant sex and age class (adult males: 41.25%) with an identical MHC configuration at 1.4×10^{-2}. Our calculations considered the relative frequencies of each SSCP fingerprint, age class, and sex among the 80 genetically distinct individuals. This estimate yielded a frequency of 0.175 for the most abundant SSCP fingerprint of MHC class II and a frequency of 0.2 for the most abundant of MHC class I. This probability of identity does not take into account the possibility that closely related individuals were

sampled, so it should be interpreted with caution. Feathers that agreed in age, sex, and MHC profile were collected not only from the same but also from different locations. We estimate the probability that feathers from different roost sites are identical by chance as $<1 \times 10^{-3}$.

DISCUSSION

This is the first study reporting patterns of MHC variation in natural populations of a New World vulture. Taking advantage of previous sequence data on avian MHC genes, we successfully isolated polymorphic loci of MHC classes I and II from the Andean Condor. We did not find stop codons or disrupted reading frames in any coding region. Positive values of Tajima's *D* and an excess of nonsynonymous over synonymous substitutions at those codons presumably involved in antigen recognition also support the expression and functionality of the MHC genes we describe. Importantly, our molecular protocol might be useful for the investigation of MHC variation in other species of the family Cathartidae, including the highly endangered California Condor (*Gymnogyps californianus*). Inferences of individual MHC diversity via automated SSCP and direct-sequencing analyses, complemented with sex and age data, also permitted us to estimate minimum population sizes noninvasively in a species whose genome is little known.

MHC DIVERSITY IN THE ANDEAN CONDOR

Our survey of MHC variation in the Andean Condor (6 alleles of MHC class I, 11 of MHC class II) did not disclose the extraordinary high levels of genetic polymorphism (>100 alleles) found at other avian MHC loci (e.g., Alcaide et al. 2008, Pomerová et al. 2009). We estimated a minimum number of two genes each of MHC classes I and II in the Andean Condor, a finding that agrees with estimates reported for raptors (Alcaide et al. 2007, 2009). Nonetheless, the actual number of gene duplications can be confirmed only through Southern blots or a deeper characterization of the condor's MHC. The investigation of allele-segregation patterns from parents to offspring may also shed light on the number of loci amplified and the putative linkage of alleles into MHC haplotypes. The coordination of studbooks is challenging, however, because of the low productivity rates in captivity of this relatively scarce species (two nestlings at the most per breeding season) and our limitations in monitoring free-ranging breeding pairs.

Although we did not carry out molecular cloning for every single feather, the inspection of SSCP and direct-sequencing electropherograms did not suggest the amplification of alleles other than those depicted in Fig. 2. It is reasonable to expect that the amplification of novel alleles in some individuals should reveal new polymorphic sites. In this respect, direct-sequencing chromatograms always agreed with the overlapping of known alleles. Saying this, we cannot discount the underestimation of MHC diversity because we analyzed and cloned only a subset of individuals. Our findings nonetheless somewhat agree with the only genetic study of the Andean Condor published so far, which documented low variation across long stretches of mitochondrial DNA (Hendrickson et al. 2003). We isolated identical MHC profiles from distant sites (Fig. 1), a result that apparently negates the existence of strong patterns of MHC structuring in this species. Previous studies of other birds have reported remarkable patterns of MHC genetic structuring even at small geographical scales (e.g., Ekblom et al. 2007, Alcaide et al. 2008, Loiseau et al. 2009). Nevertheless, the small number of molted feathers we sampled from the most distant site suggests these preliminary results be interpreted with caution.

MHC GENOTYPES AS DNA FINGERPRINTS

Capillary electrophoresis of labeled PCR amplicons and direct sequencing of loci of MHC classes I and II generated reproducible and feasible DNA fingerprints that were suitable for discriminating individuals. It cannot be totally dismissed, however, that different sets of similar alleles may yield identical SSCP and direct-sequencing electropherograms. Thus we may conclude that some feathers come from the same individual when they in fact do not. This potential limitation nevertheless makes sense with an estimate of minimum population size. Genotyping errors represent, on the other hand, a potential source of population overestimation. Noninvasive genetic sampling is particularly prone to genotyping errors because it entails analysis of low concentrations of partially degraded DNA (Waits and Paetkau 2005). Allele dropout has been documented especially in the case of microsatellites, from which short alleles are amplified in preference to longer alleles. In the case of our target MHC loci, we obtained equally sized and reasonably small (~300 bp) amplicons by PCR. Thus, preferential amplification of some alleles over others is not expected in this case. It is also worth mentioning that the primers we used for sexing (P2/P8) have been associated with high rates of dropout of the female-specific W allele in molted feathers of two species (Gebhardt and Waits 2008; see also Casey et al. 2009). This potential pitfall has been attributed to mutations in the DNA sequences to which the primers anneal. In our sample, we did not assess rates of error in sexing, although repeated molecular sexing of the control female condor was always successful.

The minimum number of individuals that we estimated is around a third of the number of Andean Condors believed to occupy northwestern Patagonia. This population, origin of the vast majority of molted feathers we sampled, was estimated to host a minimum number of 246 birds by simultaneous censuses (Lambertucci 2010). We estimated only a part of the population because of the number of feathers we collected and because of the limitations linked to the resolving power of our genetic approach. Since we gathered feathers from each roost only once, it is unlikely that we sampled fresh juvenile and adult feathers from the same individual molting at different

times. In any case, we detected only three profiles identical by both the MHC and sex among differently colored feathers. The collection of primary and secondary feathers from the same individual is, on the other hand, more likely. In fact, we confirmed identical profiles in several pairs of feathers collected from the same or different roosts. Whether these feathers belong to the same individual cannot be completely assured because of the probabilistic limitations of our approach.

INFERRED SOCIAL STRUCTURE OF ANDEAN CONDOR ROOSTS

Features of the social structure of Andean Condor roosts have permitted us to test the accuracy of our noninvasive approach. One of our most interesting findings confirmed that sex ratios in the communal roosts were biased by age class. The number of adult males was double that of adult females (1:0.48), whereas the relationship was inverted in the case of juveniles (0.41:1). Our genetic inference of sex ratios in the Andean Condor for each age class is in agreement with data from direct observations of flying birds in Chile and digital images of birds feeding on carcasses in Bolivia (Sarno et al. 2000, Ríos-Uzeda and Wallace 2007). Differences between males and females in habitat use and mortality rate have been suggested to skew the sex ratios of adult birds in favor of males (Donázar et al. 1999, Sarno et al. 2000). In contrast to the majority of birds of prey, male Andean Condors are larger than females, and several studies have documented strong competition in the acquisition of resources, particularly for warmer and better-protected roost sites and for access to food (Donázar et al. 1999, Donázar and Feijoo 2002, Lambertucci et al. 2009). Although we cannot dismiss potential pitfalls in our molecular sexing protocol (see above), we believe that the concordance between our noninvasive approach and direct observations in the field adds reliability to our results. In addition, we found a ratio of age classes skewed in favor of adults (1:0.63), a ratio that has been related to the rate of reproduction and health of populations of long-lived species (Wallace and Temple 1988). Given that this estimate depends on the population investigated and the year, our ratio of adults to immatures differs from reported by previous studies (Sarno et al. 2000, Ríos-Uzeda and Wallace 2007), but it is in concordance with data for the same area (Lambertucci 2010).

CONCLUSION

Conservation efforts and initiatives may require as many suitable tools as possible. The survey of individual diversity in the MHC from molted feathers has proven successful for inferring variation in evolutionary relevant genes (Sommer 2005) as well as minimum population sizes for the Andean Condor. Outstanding advances in genomics predict an increase in our access to MHC markers in nonmodel organisms. Therefore, MHC profiles might augment and/or complement identification of individuals in vertebrates for which other molecular markers are lacking or polymorphism is low. Nevertheless, selectively neutral markers such as microsatellites should be, whenever possible, the first option for reaching these goals. In comparison to neutral markers, MHC loci have some important limitations that cannot be ignored. For instance, genes under selection are not adequate for inference of relatedness or gene flow, and, furthermore, the assignment of alleles to locus is challenging in the majority of cases (Alcaide et al. 2007, 2009).

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