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RESEARCH ARTICLE

Ancient DNA reveals substantial genetic diversity in the California Condor (*Gymnogyps californianus*) prior to a population bottleneck

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ABSTRACT

Critically endangered species that have undergone severe population bottlenecks often have little remaining genetic variation, making it difficult to reconstruct population histories to apply in reintroduction and recovery strategies. By using ancient DNA techniques, it is possible to combine genetic evidence from the historical population with contemporary samples to provide a more complete picture of a species' genetic variation across its historical range and through time. Applying this approach, we examined changes in the mitochondrial DNA (mtDNA) control region (526 base pairs) of the endangered California Condor (*Gymnogyps californianus*). Results showed a >80% reduction in unique haplotypes over the past 2 centuries. We found no spatial sorting of haplotypes in the historical population; the periphery of the range contained haplotypes that were common throughout the historical range. Direct examination of mtDNA from California Condor museum specimens provided a new window into historical population connectivity and genetic diversity showing: (1) a substantial loss of haplotypes, which is consistent with the hypothesis that condors were relatively abundant in the nineteenth century, but declined rapidly as a result of human-caused mortality; and (2) no evidence of historical population segregation, meaning that the available genetic data offer no cause to avoid releasing condors in unoccupied portions of their historical range.

Keywords: ancient DNA, California Condor, endangered species, genetic bottleneck, genetic variation, mitochondrial DNA, museum specimens, reintroduction

ADN antiguo revela considerable diversidad genética en *Gymnogyps californianus* antes de un cuello de botella poblacional

RESUMEN

Especies críticamente amenazadas que han sufrido disminuciones poblacionales severas por lo general tienen poca diversidad genética remanente, lo que dificulta la reconstrucción de las historias poblacionales para aplicarlas en estrategias de reintroducción y recuperación. Con el uso de técnicas de ADN antiguo es posible combinar la evidencia genética de la población histórica con muestras contemporáneas para presentar un panorama más completo de la variación genética de una especie a través de su área de distribución histórica y a lo largo del tiempo. Aplicamos esta aproximación para examinar los cambios en la región control (526 pb) del ADN mitocondrial (ADNmt) de la especie amenazada *Gymnogyps californianus*. Los resultados muestran una reducción mayor al 80% en el número de haplotipos únicos durante los dos siglos pasados. No encontramos estructura espacial en los haplotipos de la población histórica; la periferia del área de distribución contuvo haplotipos comunes en toda el área histórica de distribución. El examen directo del ADNmt de especímenes de museo de *G. californianus* presenta una nueva visión sobre la conectividad poblacional histórica y la diversidad genética demostrando (1) una pérdida considerable de haplotipos, lo que concuerda con la idea de que los cóndores eran relativamente abundantes en el siglo XIX pero sus poblaciones disminuyeron rápidamente como resultado de la mortalidad causada por humanos, y (2) ausencia de evidencia de segregación poblacional histórica, lo que significa que los datos genéticos disponibles no impiden la liberación de cóndores en las porciones no ocupadas de su área de distribución histórica.

Palabras clave: ADN antiguo, ADN mitocondrial, cóndor de California, cuello de botella poblacional, especies amenazadas, especímenes de museo, reintroducción, variación genética

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INTRODUCTION

The study of genetic variation in animal populations across space and through time is fundamental to our understanding of population and evolutionary biology (Mayr 1963, Harrison 1989) and for establishing effective conservation strategies (Frankham et al. 2006, Haig et al. 2011). For critically endangered species, with populations long confined to a small portion of their historical range, investigations of the contemporary population often provide little insight into the species' population history and structure prior to population decline (Matocq and Villablanca 2001, Johnson et al. 2007; but see Haig and Ballou 1995). Advances in DNA extraction from museum specimens now permit direct investigation of spatial and temporal changes in genetic variation that can provide crucial information for the development of effective conservation and reintroduction strategies (Murata et al. 2004, Draheim et al. 2012).

The observational record of California Condors (*Gymnogyps californianus*) suggests that they were widespread and locally abundant from southern British Columbia, Canada, to Baja California, Mexico, during Euro-American colonization (Snyder and Snyder 2000, D'Elia and Haig 2013). Population declines and range contractions were documented shortly thereafter, with condors disappearing from the Pacific Northwest, USA (north of San Francisco), in the early 1900s (D'Elia and Haig 2013), and from Baja California by the end of the 1930s (Wilbur and Kiff 1980). By the middle of the 20th century, the species was reduced to ~150 individuals limited to the mountains of southern California, USA (Koford 1953, Snyder and Snyder 2000). California Condor numbers continued to decline into the latter half of the century. The species was rescued from the brink of extinction in the late 1980s, when all of the remaining wild birds were caught for captive breeding (Snyder and Snyder 2000). Only 22 California Condors remained in 1982, and only 14 genetic founders (8 males, 6 females; Table 1) from 3 genetic clans were used to initiate the captive breeding program (Geyer et al. 1993, USFWS 1996, Ralls and Ballou 2004).

Through aggressive captive breeding efforts, followed by reintroductions beginning in 1992, population numbers have increased substantially. There are now >250 individuals in the wild, spread among 6 release sites in the southern half of their historical range (Hopper Mountain National Wildlife Refuge, southern California; Pinnacles National Park, central California; Big Sur, central California; San Simeon hills, central California; Vermillion Cliffs, Arizona, USA; and San Pedro Martir, Baja California, Mexico), and >150 individuals in captivity (Walters et al. 2010, Mace 2016, USFWS 2016). However, recovering population numbers belie significant remaining threats to the viability of the California Condor—primarily from continued contamination of their food supply by spent lead ammunition (Finkelstein et al. 2012). Furthermore, condors are still absent from the entire northern portion of their historical range, although reintroductions to this area are now being considered (D'Elia and Haig 2013, USFWS et al. 2014, D'Elia et al. 2015).

TABLE 1. Mitochondrial DNA control region haplotypes of the genetic founders of the California Condor captive breeding program.

Studbook #	Condor name	Sex	Haplotype
1	Topa-Topa	M	H1 [a]
4	AC-7	M	H1
6	AC-2	M	H1
11	Tama	F	H1
12	AC-8	F	H1
8 [b]	CVF	F	H1
5	AC-6	M	H4
7	AC-5	M	H4
13	UNI	F	H4
10	AC-3	F	H7
20	AC-4	M	H7
9 [c]	PPF	F	H7
2 [d]	CCM	M	unknown
3 [d]	SSM	M	unknown

[a] Heteroplasmic individual with somatic mutation at site 321.
[b] Progeny (studbook #23) used to identify haplotype.
[c] Progeny (studbook #33) used to identify haplotype.
[d] Genetic founders (wild birds whose eggs were brought into captivity) that died in the wild in 1984 and whose genotypes are unknown.

Studies of genetic variation in the California Condor have almost exclusively focused on the genetic founders of the captive population and their offspring. As expected, given their recent severe population bottleneck, these studies have revealed that the remaining population possesses low genetic diversity (Corbin and Nice 1988, Geyer et al. 1993, Chemnick et al. 2000, Adams and Villablanca 2007, Romanov et al. 2009). However, virtually nothing is known about the condor's genetic diversity or the spatial distribution of its genetic lineages prior to the population bottleneck. Technological advances in our ability to extract, amplify, and analyze ancient DNA from museum specimens now enable direct evaluation of genetic diversity through time, which can provide insights into a species' historical population structure and demographic history (Johnson et al. 2007, Wandeler et al. 2007, Draheim et al. 2012).

Studying variation in the historical California Condor population via genetic sampling of museum specimens was suggested as early as 1993 (Geyer et al. 1993), but was not feasible until recently (Clipperton 2005). Clipperton (2005) evaluated 324 base pairs (bp) from the mitochondrial DNA (mtDNA) control region of 41 individuals from the historical population (collected between 1886 and 1905

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from Baja California, Mexico, to Marin County, California, USA) and 12 of the 14 genetic founders. Results from Clipperton (2005) indicated that only a single mtDNA haplotype in the historical population was not represented in the contemporary population, suggesting that the species possessed low levels of mtDNA before the demographic bottleneck in the 20th century.

In this study, we further investigated historical California Condor mtDNA diversity using a larger sample of condor mtDNA from museum specimens. We tested the hypothesis that mtDNA diversity in the historical population of California Condors was not different from mtDNA diversity observed in the genetic founders of the captive population. We also investigated whether museum specimens from the Pacific Northwest, where reintroduction efforts are currently being considered, revealed any novel haplotypes which might suggest that the population there was once isolated from populations to the south.

METHODS

Samples

We obtained 93 California Condor tissue samples from museum specimens collected between 1825 and 1984 (Figure 1, Appendix Tables 5 and 6). These individuals represented a sample of the historical population (i.e. the population prior to the founding of the captive flock). We also obtained genomic DNA samples from the genetic founders of the captive population and their progeny from the San Diego Zoo's Institute for Conservation Research (Escondido, California, USA; Table 1). Samples of the genetic founders included those taken directly from 10 of the 14 founders, and 2 taken from progeny of female genetic founders that could not be sampled directly. Thus, we could directly or indirectly obtain the mtDNA haplotypes of 12 of the 14 genetic founders. The remaining 2 genetic founders were males (studbook #2 and #3; Table 1) that died in the wild in 1984, and were not available for sampling.

There are few specimens that were collected from the geographic periphery of the historical range (Wilbur 2012, D'Elia and Haig 2013). However, we sampled all available specimens with known provenance from the Pacific Northwest ($n = 5$; Figure 1, Appendix Table 5), and 2 specimens that were suspected to have been collected from this region (Appendix Table 6). Only 2 additional condor specimens that were definitively collected in the Pacific Northwest exist worldwide, both held at the St. Petersburg Zoological Museum in Russia (Specimens NHM1583 and NHM1584). However, our requests for samples from these individuals were denied due to museum policies. Another sample at the National Museum of the Czech Republic (Specimen P6V-41249) is suspected to have been collected in northern California (Wilbur 2012), but we were unable to secure a sample of this individual as it was housed in a sealed display case.

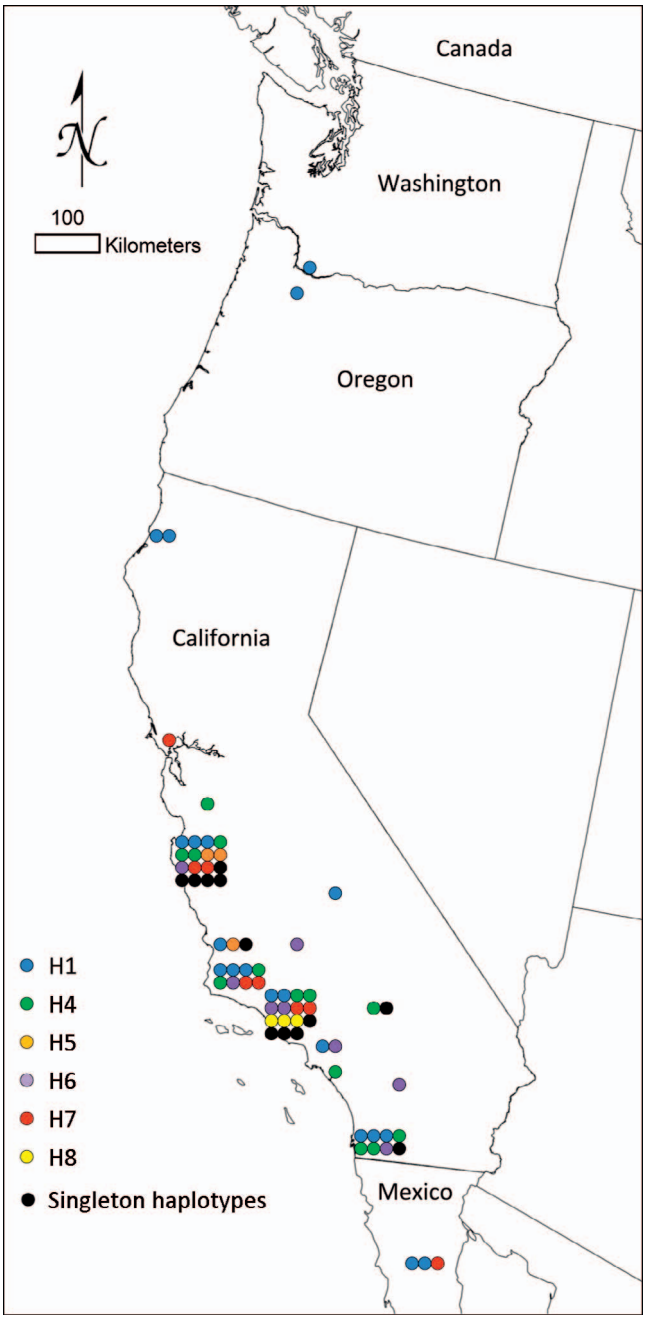


FIGURE 1. Spatial distribution of mtDNA control region haplotypes of California Condors collected from 1825 to 1984. Singleton haplotypes are haplotypes that were observed only once in our sample ($n = 67$) of the historical population.

Laboratory Analyses

Ancient DNA samples can be extremely susceptible to external contamination (Wandeler et al. 2007); therefore, we used negative controls for the extraction and amplifi-

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cation process throughout the procedure and followed appropriate ancient DNA techniques in a dedicated clean laboratory using a UV irradiated flow hood (see Draheim et al. 2012). Before extraction, we surface-sterilized museum tissues with sterile dH_20 and 80% ethanol washes, incubated them in a –80°C acetone bath, and then crushed them using a sterile mortar and pestle. We used a modified Qiagen DNeasy Kit (Qiagen, Valencia, California, USA) protocol for extractions, incorporating an additional 24-hr 55°C Protenase K digestion step.

Our analysis of genetic variation focused on the mtDNA control region, the major noncoding region of animal mtDNA that plays a role in replication and transcription of mtDNA molecules (Clayton 1992). We amplified the mtDNA of the genetic founders using the primer pair alt1 (Clipperton 2005) and CACO-R2 (Table 2), producing a 930 bp fragment containing domains I and II of the control region. Primer CACO-R2 was designed from an Andean Condor (*Vultur gryphus*) reference sequence (Genbank accession number AY129646.1; Hendrickson et al. 2003).

The degraded condition of some museum tissue samples required the use of multiple shorter PCR amplifications to generate a control region sequence. A combination of control region primers from Clipperton (2005) and California Condor primers designed from reference sequences generated from the genetic founders (CACO primers; see above) was used to produce 4 overlapping control region fragments ranging in size from 200 bp to 319 bp (primer combinations: alt1 and piel, Indel+ and CACO-R3, CACO-F5 and TDKD, and CACO-F3 and CACO-R4; Table 2). These fragments were aligned and assembled into a single 526 bp fragment that formed the basis of our analyses.

We performed PCR amplifications in 25 μL reactions containing 2.0 mM $MgCl_2$, 1 μM of primer, 100 μM of each dNTP, 1× PCR buffer, and 1 U AmpliTaq Gold DNA polymerase (Perkin Elmer, Waltham, Massachusetts, USA). PCR reaction conditions were: 5 min denaturation at 94°C, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 54°C for 30 s, and extension at 72°C for 30 s, followed by a 10-min 72°C extension. All PCR products were bidirectionally sequenced with BigDye 3.1 dye terminator sequencing chemistry (Applied Biosystems, Foster City, California, USA), and resolved on an ABI 3730 automated DNA sequencer (Applied Biosystems) at the University of Kentucky, Advanced Genetic Technologies Center (Lexington, Kentucky, USA). Resulting sequence chromatograms were aligned, edited, and trimmed using program BioEdit Sequence Alignment Editor (Hall 1999). Although studies of ancient DNA can be prone to miscoding substitutions, or deamination (Gilbert et al. 2003, Sefc et al. 2007), absence of double peaks, use of short fragment amplifications (rather than larger fragments that are more prone to false variation), and the fact that we generated sequences multiple times to verify variable positions meant that the variation in our dataset was likely real and not due to degradation of ancient DNA. All unique sequences were archived in GenBank (accession numbers KX379719–KX379736).

TABLE 2. Mitochondrial DNA control region primer sequences developed for California Condors.

Primer name	Primer sequence
CACO-R2	5′- CAC AAC ATC AGC ACT GAA ATT AC -3′
CACO-R3	5′- AAT GGT CCT GAA GCT GGT -3′
CACO-R4	5′- GGG AAC CAA AAG TGC TAA G -3′
CACO-F3	5′- ACC AGC TTC AGG ACC ATT C -3′
CACO-F5	5′- AAT GGT CTC AGG ACA TAA CAT G -3′

Data Analyses

We created input files for data analysis using program FaBox (Villesen 2007), which was also used to identify unique haplotypes and the number of variable sites. ARLEQUIN was used to calculate haplotype (h) and nucleotide diversity (π; Nei and Tajima 1981, Excoffier et al. 2005) for the historical population and the genetic founders. We constructed a median-joining network (Bandelt et al. 1999) using program NETWORK (http://www.fluxus-engineering.com/sharenet.htm) to illustrate relationships among haplotypes. We also calculated the percentage of haplotypes known to be lost (i.e. detected in our historical sample, but not present in the census of genetic founders), under the assumption of no new mutations from 1825 to 1984.

To compare our results with previous analyses, we trimmed our sequences to align with the sequence data of Clipperton (2005). Our dataset shared 274 bp with Clipperton's (2005) study that included 41 museum samples from the historical population. We excluded the first 50 bp analyzed by Clipperton (2005), as he showed no variation there among his 41 samples. Once aligned and trimmed, we evaluated similarities and differences in mtDNA sequences among the sampled individuals that were common to both studies ($n = 24$), and evaluated the number of haplotypes that we would have detected in the historical population had we restricted our analyses to the fragment of mtDNA analyzed by Clipperton (2005). We also analyzed the number of additional haplotypes that we detected among the shared samples as a consequence of sequencing a longer fragment of mtDNA. Although Clipperton (2005) identified 5 haplotypes among the historical samples in his study, 1 of those was based on a heteroplasmic male with a somatic mutation at a single bp that we identified as a member of a common haplotype (H1) for all of our analyses (Table 1).

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TABLE 3. Variable sites observed in a 526 base pair (bp) fragment of the mtDNA control region assayed from California Condors collected from 1825 to 1984 ($n = 67$).

	Position																		
Haplotype	33	62	79	113	120	187	195	217	250	258	268	298	333	339	430	442	464	522	545
1	C	A	C	C	A	T	C	C	A	C	C	C	G	C	C	T	C	G	C
2	T	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.
3	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.
4	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.
5	.	.	.	T	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.
6	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.
7	.	.	.	T	G	.	.	.	.	.	T	.	.	.	.	.	.	.	.
8	.	.	.	T	G	C	.	.	.	.	.	.	.	.	.	.	.	.	.
9	.	.	.	.	.	C	T	.	.	.	.	.	.	.	.	.	.	.	.
10	.	.	.	T	G	.	.	.	.	.	T	.	A	.	.	C	.	.	.
11	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T
12	.	G	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.
13	.	.	.	.	.	C	.	.	G	.	.	.	.	.	.	.	.	.	.
14	.	.	.	.	.	.	.	.	.	T	.	.	.	.	T	.	T	.	.
15	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
16	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.
17	.	.	.	T	G	.	.	T	.	.	T	.	.	.	.	.	.	.	.
18	.	.	.	.	.	C	.	.	.	.	.	T	.	.	.	.	.	.	.

Statistical inferences about the structure of the historical population were precluded by the small number of samples available at the periphery of the condor's historical range. Therefore, we simply report the haplotypes of all samples from the Pacific Northwest and how they relate to those in the central and southern portion of the species' historical range. We also mapped the distribution of these haplotypes, grouping samples by U.S. county or Mexican state (Figure 1).

RESULTS

We successfully amplified 526 bp of mtDNA control region sequences in portions of domains I and II from 67 museum samples (72% of museum specimens sampled; Appendix Tables 5 and 6) and all 12 genetic founders (or their progeny) for which samples were available (Table 1). The historical population contained a minimum of 18 haplotypes with 19 variable sites (Table 3), while our census of genetic founders of the captive population revealed only 3 haplotypes with 4 variable sites (Table 4).

TABLE 4. Comparison of California Condor mtDNA diversity in a sample of the historical population (1825–1984; $n = 67$) and in 12 of the 14 genetic founders of the captive breeding program. Numbers in parentheses represent ± 1 SD.

	Historical	Founders
Haplotypes	18	3
Polymorphic sites	19	4
Nucleotide diversity (π)	0.0036 (± 0.0022)	0.0029
Haplotype diversity (h)	0.851 (± 0.027)	0.682

This finding reflects a >80% decline in unique haplotypes from the historical population through the genetic bottleneck. The loss of most of these haplotypes occurred after 1900 (Appendix Table 5), although the specific timing of the decline cannot be determined with our dataset due to the small number of museum samples from the 20th century (Appendix Table 5).

The haplotype network was starlike, with low levels of sequence divergence and a high frequency of unique mutations (Figure 2). Haplotype diversity (h) declined 20%, from a mean value of 0.851 (± 0.027 SD) in the historical population to a value of 0.682 in the genetic founders. Nucleotide diversity (π) declined by 19%, from a mean value of 0.0036 (± 0.0022 SD) to 0.0029 (Table 4).

Twenty-four of our samples were from individuals also sequenced by Clipperton (2005). Our sequences matched those of Clipperton (2005), except for samples 41 and 70 (Appendix Table 5), where we saw variation at position 195 in sample 41 and at position 217 in sample 70 that Clipperton (2005) did not observe. We also found 2 additional haplotypes (samples 46 and 68) in the portion of domain II outside the region investigated by Clipperton (2005). Restricting all of our historical sequences to the fragment of mtDNA examined by Clipperton (2005) resulted in the identification of 13 haplotypes, as compared with only 4 haplotypes found by Clipperton (2005). Five additional haplotypes were detected in our study simply as a matter of sampling a larger fragment of mtDNA.

The haplotypes detected in the Pacific Northwest samples (H1 and H7) were common throughout the historical range, occurring from the Pacific Northwest to the southern terminus of the historical range in Baja

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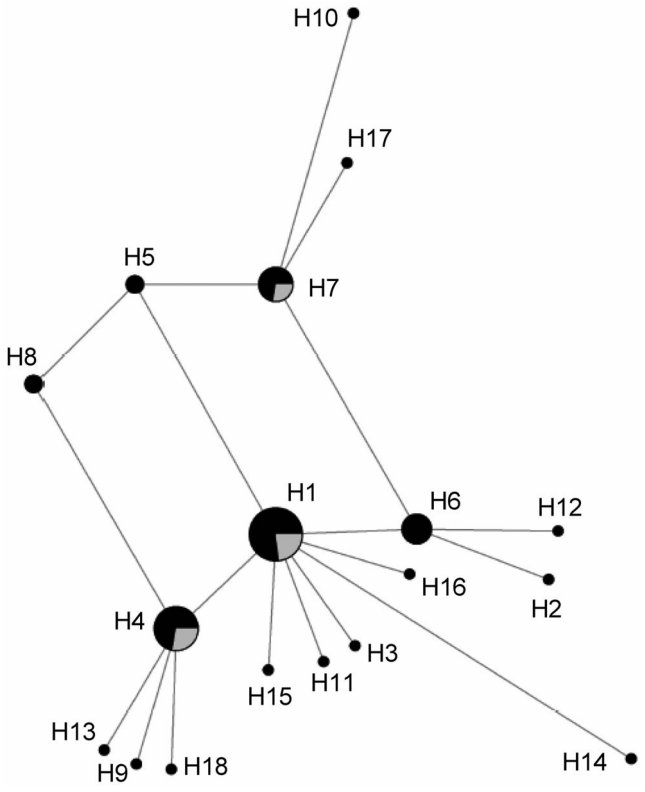


FIGURE 2. Median-joining network of a 526 bp (base pairs) fragment of California Condor control region mtDNA sequences. The proportions of individuals from the historical population (1825–1984) are shown in black and the proportions of genetic founders of the captive breeding program are shown in gray. Pie-chart diameter is proportional to the number of individuals with each haplotype.

California (Figure 1). These haplotypes survived the genetic bottleneck and are still represented in the contemporary population (Mace 2016; Table 1).

DISCUSSION

Our analysis revealed that California Condors have lost more than 80% of their mtDNA haplotypes over the past 2 centuries. As expected by theory (Allendorf 1986), haplotypes of low frequency were lost when California Condors experienced a major range contraction and reduction in population size. The small number of haplotypes remaining in the California Condor is similar to values reported for other bird species that have gone through a severe population bottleneck, including the Whooping Crane (*Grus americana*; Glenn et al. 1999), Crested Ibis (*Nipponia nippon*; Zhang et al. 2004), and Pink Pigeon (*Streptopelia mayeri*; Swinnerton et al. 2004).

Our results contrast with those of Clipperton (2005), who found little genetic diversity in the historical population of California Condors. This appears to be primarily a function of the larger sample size of our analysis and the larger fragment of mtDNA that we investigated. These contrasting results suggest that studies with small samples from the historical population, and those sampling small fragments of mtDNA, should be cautious about inferences that suggest inherently low genetic diversity in a species.

Numerous studies have reported low mtDNA haplotype diversity in large raptors, including the Andean Condor (*Vultur gryphus*; Hendrickson et al. 2003), Spanish Eagle (*Aquila adalberti*; Martínez-Cruz et al. 2004), White-bellied Sea-Eagle (*Haliaeetus leucogaster*; Shephard et al. 2005), Bonelli's Eagle (*Aquila fasciata*; Cadahía et al. 2007), and Cinereous Vulture (*Aegypius monachus*; Poulakakis et al. 2008). Reasons for this low diversity have been attributed to: past population bottlenecks followed by long recovery times after a bottleneck due to *K*-type life histories (Hendrickson et al. 2003, Romiguier et al. 2014); the slow spread of new mutations through a sparsely or linearly distributed population (Hendrickson et al. 2003); island colonization and subsequent isolation and genetic drift (Bollmer et al. 2006); and recent demographic crashes caused by humans (Krüger et al. 2015). However, low mtDNA diversity is not a universal trait of large raptors, especially those with extensive geographic ranges and large population sizes. For example, the Harpy Eagle (*Harpia harpyja*), the largest Neotropical bird of prey, with a geographic distribution from central Mexico to east-central Brazil, was found to possess 23 mtDNA haplotypes from a sample of 66 individuals, and had relatively high haplotype diversity ($h = 0.906$; Lerner et al. 2009). White-tailed Eagles (*Haliaeetus albicilla*), which have a disjunct distribution across the Palearctic and Greenland, also have retained a substantial amount of genetic diversity (38 mtDNA haplotypes, $n = 420$, $h = 0.797$; Langguth et al. 2013). The Golden Eagle (*Aquila chrysaetos*), one of the most widespread birds of prey, covering essentially the whole Palearctic, was found to have 30 haplotypes ($n = 252$) and total haplotype diversity consistent with many other large raptors that have sizeable populations and extensive geographic ranges ($h = 0.800$; Nebel et al. 2015). The Lammergeier (*Gypaetus barbatus*), whose range once extended across large regions of Asia, Europe, and Africa, had more than 50 mtDNA haplotypes represented in its historical population and had high haplotype diversity in central Asia ($h = 0.940$), where its range was most extensive (Godoy et al. 2004, Krüger et al. 2015). In addition, the White-rumped Vulture (*Gyps bengalensis*), which was formerly considered one of the most common raptors in the world, numbering in the millions, was shown to have a large number of mtDNA haplotypes (13 haplotypes detected in only 23 individuals) and considerable haplotype diversity ($h = 0.846$) prior to a recent bottleneck (Johnson et al. 2008). High levels of mtDNA

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diversity in these species has been attributed to: the longevity of large raptors, which may buffer against immediate losses of habitat or human persecution (Hailer et al. 2006, Lerner et al. 2009); conservation of multiple local populations distributed throughout the species' range (Hailer et al. 2006); and their large geographic ranges and, consequently, potentially high effective population sizes (Nebel et al. 2015). Comparably high mtDNA haplotype diversity in the historical population of California Condors (Table 4) is consistent with the 19th century observational record in the western United States, which suggests that condors were relatively widespread and numerous at the time of Euro-American contact (D'Elia and Haig 2013).

The appreciable genetic diversity revealed in the historical California Condor population, and reported for other wide-ranging raptors, contrasts with the results of studies of Andean Condors, which showed low amounts of mtDNA diversity (Hendrickson et al. 2003). This is unexpected, as Andean Condors are relatively widespread across the Andes Mountains and have an estimated population of at least 10,000 individuals (Birdlife International 2015). Reasons for this difference are not clear. It has been suggested that body size, metabolic rate, and generation time can influence rates of mtDNA evolution (Martin and Palumbi 1993), but these factors are likely similar for the 2 condors. There are several possible explanations for the observed differences in mtDNA diversity between the species. First, most of the control region sequences evaluated for Andean Condors were from domains II and III ($n = 38$), with only 5 sequences from domain I (Hendrickson et al. 2003). Given that considerable differences in diversity may exist between control region domains in avian species (Roques et al. 2004), and the more conserved nature of domain II (Brown et al. 1986, Marshall and Baker 1997), it is possible that the observed difference was the result of evaluating different portions of the mtDNA genome. Another possibility is that the small number of samples evaluated by Hendrickson et al. (2003; $n = 38$) was not sufficient to accurately estimate the amount of mtDNA diversity in the population. Finally, it is possible that Andean Condors truly have low levels of genetic diversity in their mitochondrial genome as a result of an evolutionary and demographic history that differs substantially from that of the California Condor. Future investigations using a larger number of Andean Condor sequences from domain I of the control region could provide additional insights into whether Andean Condor mtDNA diversity is truly much lower than in the historical population of California Condors.

We did not detect any unique haplotypes in our sample of California Condors from the Pacific Northwest, although we caution that sample sizes in this region were limited and mtDNA represents only a single locus. Despite our lack of statistical power to make definitive conclusions regarding the condor's historical population structure, the apparent lack of spatial structure is consistent with our understanding of condor movement ecology and the geography of its historical range. Condors are long-lived and known to move long distances while expending minimal energy due to their large wingspan and soaring mode of flight (Meretsky and Snyder 1992, Rivers et al. 2014). Mountainous areas are preferred by condors for soaring and looking for food because these areas provide upward-moving air currents that help large soaring birds to stay aloft (Rivers et al. 2014). Thus, the large north–south mountain chains of the Coast, Sierra, and Cascade ranges likely provided effective movement corridors for condors, facilitating genetic connectivity among local groups.

Various molecular studies of California Condors using an array of genetic markers have arrived at disparate groupings of the apparent genetic founders (e.g., Adams and Villablanca 2007; however, note that Adams and Villablanca 2007 incorrectly identified studbook #18 and #19 as genetic founders when neither individual produced any offspring; see Ralls and Ballou 2004). This is largely the result of using different genetic markers (i.e. nuclear vs. mitochondrial) or looking at different portions of the mitochondrial or nuclear genome. Nonetheless, Adams and Villablanca (2007) examined a portion of the mtDNA control region that largely overlapped with the region used in our study. Our results were identical to those of Adams and Villablanca (2007) with respect to the 12 genetic founders common to both studies, except that they identified an additional haplotype in studbook #1 ("Topa" in figure 4 of Adams and Villablanca 2007). This individual is known to be heteroplasmic (Clipperton 2005; Table 1), which explains the apparently different outcomes between our studies.

Examination of the current studbook for wild and captive California Condors (Mace 2016) suggests that all of the founder haplotypes are represented in the contemporary population, and thus that no net loss of haplotypes has occurred since the 1980s when the birds were brought into captivity. However, as of 2002, these mtDNA haplotypes were unequally distributed, with ~62% of the population assigned to haplotype H1, ~15% to H4, and ~23% to H7 (Adams and Villablanca 2007).

Current genetic management of the California Condor population is based on a kinship matrix of the complete nuclear genomes of all of the founders, followed by pedigree information on their descendants, rather than being based on mtDNA haplotypes (K. Ralls personal communication). Future investigations that apply these newly developed genomic tools to the historical population have the potential to provide further insights into the historical genetic diversity and demography of the California Condor.

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Conservation Implications

There has been a substantial loss of genetic diversity in California Condors over a relatively short period of time. As a result of this population bottleneck, inbreeding and decreased fitness have surfaced in the captive-bred population (Ralls et al. 2000, Ralls and Ballou 2004), as they have in other avian species that have undergone population bottlenecks (e.g., Jamieson 2010). California Condors now require, and will require for some time, intensive management to maximize retention of the remaining genetic diversity and to achieve demographic stability (Ralls and Ballou 2004). This situation with condors illustrates the importance of initiating captive breeding efforts when substantial genetic diversity still exists, as the loss of even one founder's genes can be significant (e.g., Haig et al. 1990). The efficacy of an early intervention strategy has recently been demonstrated in several species of *Gyps*, for which significant genetic variation has been retained in the captive flock despite populations collapsing in the wild due to diclofenac poisoning (Ishtiaq et al. 2015).

Currently, California Condors exist in a small number of disconnected and heavily managed populations and are absent from the northern portion of their historical range. Interest in reintroducing condors to the northern region has been growing, lead primarily by Native American tribes who view the condor as culturally important (D'Elia and Haig 2013, USFWS et al. 2014). Strategic placement of new reintroduction sites could facilitate range expansion, demographic connectivity, and gene flow between release sites (e.g., Álvarez et al. 2011). Expanding recovery efforts into unoccupied areas also offers the possibility of restoring condors into areas where habitat may be more extensive (D'Elia et al. 2015), where some threats may be less pronounced, or where management efforts needed to sustain the population may be less costly (USFWS et al. 2014). Discovery of shared haplotypes among specimens from the Pacific Northwest and elsewhere in the historical range suggests that there was historical gene flow among these populations. Thus, the available genetic data offer no reason to avoid releasing condors into unoccupied portions of their historical range. If the primary threat of lead exposure from spent ammunition can be sufficiently addressed (Epps 2014, Haig et al. 2014), such range expansion may offer the opportunity to rapidly increase the population of wild California Condors and aid in the retention of remaining genetic resources.

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APPENDIX TABLE 5. California Condor specimens collected from museums and sequenced (526 base pairs) for analysis of mtDNA.

Specimen number	Haplotype	County	State [a]	Collection date	Specimen location [b]	Museum catalog number
Northern specimens						
26	H1	Clark	WA	1825	RMNH	RMNH.AVES.162189
92	H1	Clackamas	OR	1835	USNM	USNM 78005
1	H1	Humboldt	CA	1892	EHS	Unnumbered mounted specimen
93	H1	Unknown N. CA	CA	1840–1841	MNd'HN	Unnumbered mounted specimen
50	H7	Marin	CA	1900–1905	FMNH	FMNH 39613
Southern specimens						
16	H1	San Diego	CA	1875	CAS	CAS-ORN 73275
87	H1	Monterey	CA	1885	USNM	USNM 103375
31	H1	San Diego	CA	1894	AMNH	AMNH 469946
71	H1	Los Angeles	CA	1898	LACM	LACM 5860
81	H1	Monterey	CA	1898	USNM	USNM 168797
39	H1	Santa Barbara	CA	1902	AMNH	AMNH 352007
40	H1	San Luis Obispo	CA	1902	AMNH	AMNH 90572
15	H1	Santa Barbara	CA	1903	CAS	CAS-ORN 25776
54	H1	Ventura	CA	1903	UMMZ	UMMZ 238685
89	H1	—	MX	1905	USNM	USNM 203218
90	H1	—	MX	1905	USNM	USNM 203217
47	H1	San Diego	CA	1906	DMNS	DMNS ZB.16354
44	H1	Ventura	CA	1916	AMNH	AMNH 352005
19	H1	Monterey	CA	1917	CAS	CAS-ORN 19010
5	H1	Santa Barbara	CA	1936	SBMNH	SBMNH AV196
67	H1	Tulare	CA	1984	LACM	LACM 101811
3	H2	Monterey	CA	1896	SBMNH	—
7	H3	Ventura	CA	1939	SDNHM	SDNHM 18117
64	H4	Monterey	CA	1855	BMNH	BMNH 1988.12.1
52	H4	Monterey	CA	1866	FMNH	FMNH 95160
10	H4	San Diego	CA	1893	CAS	CAS-ORN 44495
32	H4	San Diego	CA	1895	AMNH	AMNH 469944
34	H4	San Diego	CA	1896	AMNH	AMNH 469947
37	H4	Orange	CA	1897	AMNH	AMNH 469940
38	H4	Santa Clara	CA	1899	AMNH	AMNH 469942
51	H4	Monterey	CA	1899	FMNH	FMNH 130025
49	H4	Santa Barbara	CA	1900	FMNH	FMNH 11249
53	H4	San Bernardino	CA	1901	UMMZ	UMMZ 215051
77	H4	Ventura	CA	1901	USNM	USNM 389300
86	H4	Santa Barbara	CA	1902	USNM	USNM 188797
56	H4	Ventura	CA	1947	UMMZ	UMMZ 239901
12	H5	Monterey	CA	1877	CAS	CAS-ORN 73276
63	H5	San Luis Obispo	CA	1892	BMNH	BMNH 1915.7.12.1
76	H5	Monterey	CA	1901	USNM	USNM 192532
30	H6	San Diego	CA	1890	AMNH	AMNH 469945
17	H6	Riverside	CA	1893	CAS	CAS-ORN 73273
33	H6	Monterey	CA	1896	AMNH	AMNH 469943
13	H6	Santa Barbara	CA	1898	CAS	CAS-ORN 73274
74	H6	Ventura	CA	1900	USNM	USNM 201427
72	H6	Los Angeles	CA	1901	LACM	LACM 5859
55	H6	Ventura	CA	1940	UMMZ	UMMZ 238686
78	H6	Kern	CA	1950	USNM	USNM 418251
29	H7	Santa Barbara	CA	1889	AMNH	AMNH 469948
24	H7	Monterey	CA	1896	ANSP	ANSP 37094
35	H7	Santa Barbara	CA	1896	AMNH	AMNH 469949
91	H7	—	MX	1905	USNM	USNM 203219
43	H7	Ventura	CA	1908	AMNH	AMNH 750094
75	H7	Ventura	CA	1966	USNM	USNM 489359
58	H7	Monterey	CA	1852–1854	BMNH	BMNH 1955.6.N.20.135
36	H8	Ventura	CA	1897	AMNH	AMNH 469941
79	H8	Ventura	CA	1903	USNM	USNM 370997
73	H8	Ventura	CA	1923	LACM	LACM 4896
41	H9	Monterey	CA	1905	AMNH	AMNH 750092
42	H10	Ventura	CA	1906	AMNH	AMNH 750093

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APPENDIX TABLE 5. Continued.

Specimen number	Haplotype	County	State [a]	Collection date	Specimen location [b]	Museum catalog number
46	H11	San Bernardino	CA	1905	DMNS	DMNS ZB.26823
57	H12	Monterey	CA	1852–1854	BMNH	BMNH 1955.6.N.20.134
60	H13	Monterey	CA	1859	BMNH	BMNH 1955.6.N.20.137
61	H14	San Luis Obispo	CA	1897	BMNH	BMNH 1939.12.9.3411
62	H15	San Diego	CA	1900	BMNH	BMNH 1939.12.9.3414
68	H16	Ventura	CA	1916	LACM	LACM 20963
70	H17	Ventura	CA	1890	LACM	LACM 18877
84	H18	Monterey	CA	1898	USNM	USNM 168800

[a] WA = Washington, USA; OR = Oregon, USA; CA = California, USA; MX = Mexico.

[b] AMNH = American Museum of Natural History; ANSP = Academy of Natural Sciences of Philadelphia; BMNH = British Museum of Natural History; CAS = California Academy of Sciences; DMNS = Denver Museum of Nature & Science; EHS = Eureka High School, California; FMNH = Field Museum of Natural History; LACM = Natural History Museum of Los Angeles County; MNd'HN = Museum National d'Histoire Naturelle, Paris; RMNH = National Museum of Natural History Naturalis, The Netherlands; SBMNH = Santa Barbara Museum of Natural History; SDNHM = San Diego Natural History Museum; UMMZ = University of Michigan Museum of Zoology; USNM = U.S. National Museum of Natural History, Smithsonian Institution.

APPENDIX TABLE 6. California Condor specimens collected from museums that were not included in our genetic analyses due to: lack of amplification (A), insufficient number of base pairs (<526 bp; I), or uncertainty in provenance (P).

Specimen number	Reason	County	State [a]	Collection date	Specimen location [b]	Museum catalog number
2	I	Humboldt	CA	1889 or 1890	CHM	—
4	A	Santa Barbara	CA	1927	SBMNH	SBMNH OS545
6	A	San Diego	CA	1890	SDNHM	SDNHM 1
9	A	Fresno	CA	1900	CAS	CAS-ORN 34591
11	A	Monterey	CA	Prior to 1898	CAS	CAS-ORN 34590
14	A	Ventura	CA	1917	CAS	CAS-ORN 96024
18	A	Ventura	CA	1917	CAS	CAS-ORN 96025
20	A	Santa Barbara	CA	1903	CAS	CAS-ORN 25775
21	A	San Diego	CA	1894	CAS	CAS-ORN 19001
22	A	San Diego	CA	1894	CAS	CAS-ORN 19002
23	A, P	Unknown	CA	Prior to 1848	ANSP	ANSP 41
25	A, P	Unknown	Unknown	Prior to 1941	ANSP	ANSP 148147
27	A	Los Angeles	CA	1904	WFVZ	WFVZ 56020
28	A	Baja California	MX	1907	WFVZ	WFVZ 45293
45	A	Baja California	MX	1907	VT	VT 11374
48	A	Santa Barbara	CA	1900	FMNH	FMNH 11248
59	I	Monterey	CA	1853	BMNH	BMNH 1955.6.N.20.136
65	I	Monterey	CA	1855	BMNH	BMNH 1988.12.2
66	I	Kern	CA	1960	LACM	LACM 36096
69	A	Los Angeles	CA	1911	LACM	LACM 20962
80	I	Monterey	CA	1888	USNM	USNM 113663
82	A	Monterey	CA	1898	USNM	USNM 168798
85	A	Monterey	CA	1898	USNM	USNM 168801
95 [c]	I, P	Unknown	Unknown	Prior to 1858	MNd'HN	Unnumbered mounted specimen
94 [c, d]	P	Unknown	Unknown	Prior to 1858	MNd'HN	Unnumbered mounted specimen
83	I	Monterey	CA	1898	USNM	USNM 168799

[a] CA = California, USA; MX = Mexico.

[b] ANSP = Academy of Natural Sciences of Philadelphia; BMNH = British Museum of Natural History; CAS = California Academy of Sciences; CHM = Clarke Historical Museum, Eureka, California; FMNH = Field Museum of Natural History; LACM = Natural History Museum of Los Angeles County; MNd'HN = Museum National d'Histoire Naturelle, Paris; SBMNH = Santa Barbara Natural History Museum; SDNHM = San Diego Natural History Museum; USNM = U.S. National Museum of Natural History, Smithsonian Institution; VT = Virginia Polytechnic Institute and State University; WFVZ = Western Foundation of Vertebrate Zoology.

[c] These specimens were likely those collected by David Douglas near Fort Vancouver, Washington, from 1826 to 1827; however, their provenance is not definitive (Wilbur 2012).

[d] This specimen was genotyped as belonging to haplotype H1.

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