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Consequences of a Genetic Bottleneck in California Condors: A Mitochondrial DNA Perspective

Mary S. Adams and Francis X. Villablanca[1]

Abstract.—The California Condor (*Gymnogyps californianus*) has recently survived a severe population bottleneck. The entire population was reduced to 27 individuals in 1982. The number of genetic founders was even smaller. We obtained 482 base pairs of DNA sequence from the mitochondrial control region (CR) of all founder individuals that potentially represented unique maternal haplotypes. Four unique haplotypes were present in the genetic founders. One of these haplotypes is unique to Topatopa, a male brought into captivity in 1967, whose haplotype will not persist in the future population. Haplotype diversity (h) was reduced by 25% between the founder population and our census of the 2002 population. Nucleotide diversity (θ) did not vary significantly between the founders and the current population. Our results provide insights into condor genetics. First, where recessive deleterious alleles have been expressed in progeny (e.g., chondrodystrophy) the breeding pair shares the same mitochondrial haplotype. Second, we identified the presence of a nuclear copy of the mitochondrial control region and provide condor specific primer sequences to preferentially amplify DNA of mitochondrial origin. Third, we confirm low levels of genetic diversity in the captive population as suggested by previous research. Forth, we question whether the low level of diversity is a consequence of the 20th century bottleneck, or if diversity has been historically low over a much longer time scale.

California Condors (*Gymnogyps californianus*) are North America's largest soaring birds. Condors inhabited much of the continental United States prior to European settlement (Simons 1983, Steadman and Miller 1986, Wilbur 1978). However, the range of the species had contracted to

Biological Sciences Department, California Polytechnic State University, San Luis Obispo, California 93407, USA.
[1]*Address correspondence to this author. E-mail: fvillabl@calpoly.edu*

its final remnant distribution by at least the 1970s (Fig. 1). By 1987 the entire species was represented by only 27 individuals and that year the last wild Condor was brought into captivity (Snyder and Snyder 2000). All living California Condors are descended from that single population, which occupied southern California prior to its captivity.

The rapid decline of the California Condor population during the second half of the 20th century raised concerns about genetic inbreeding. Population genetics theory predicts that severe population bottlenecks result in a loss of genetic variation (Nei et al. 1975, Lacy 1997, Frankham 1995). This loss of genetic variation increases the likelihood of inbreeding, reducing individual fitness and overall population viability (Lande 1988). Inbreeding can reduce fitness through the production of homozygotes. Homozygotes result in reduced fitness when (1) heterozygotes for rare lethal or nearly lethal alleles interbreed (Lande 1988), or (2) when homozygotes

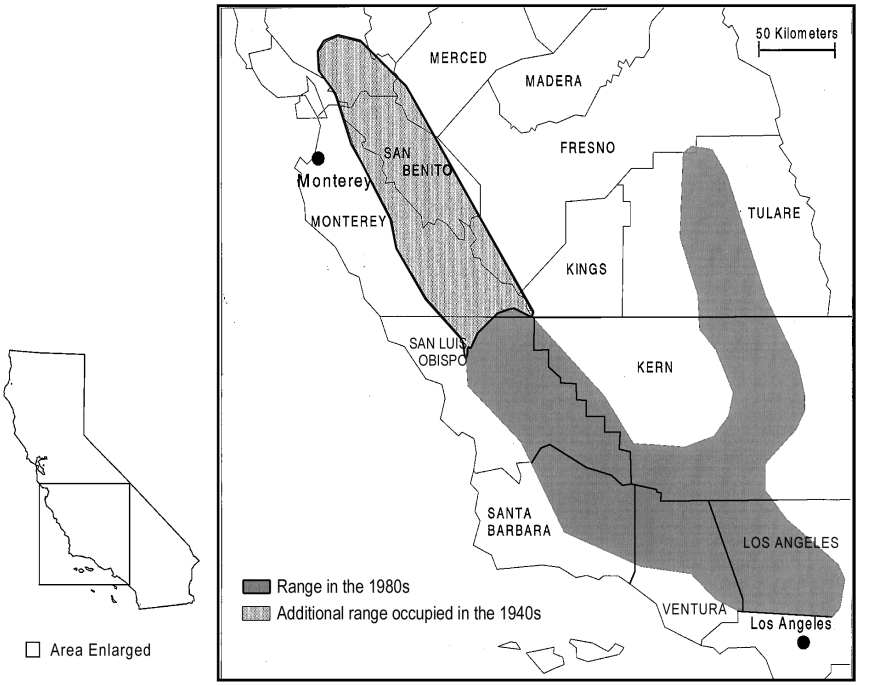

Fig. 1. Range of the recent historical California Condor population, as summarized by Snyder and Snyder (2000). The 1940s distribution (gray plus black) as proposed by Koford (1953) and Robinson (1940). The 1980 distribution (black) is based on intensive observations of marked and unmarked but individually identifiable birds (Snyder and Johnson 1985, Meretsky and Snyder 1992). County names and delineations are included.

are produced at loci where overdominace (heterozygote advantage) is acting. In addition to these well known inbreeding effects, theory also predicts that smaller populations are more likely to respond to genetic drift than to selection even when selection is acting (Barton and Charlesworth 1984, Ohta 1995). Overall, a loss of genetic diversity reduces individual fitness and mean population fitness, and results in less evolution through natural selection and more evolution via genetic drift.

The empirical effects of a genetic bottleneck include a loss of heterozygosity (Nei 1987, Frankham et al. 1999), a decrease in allele frequency, a loss of alleles (Bouzat et al. 1998, Glenn et al. 1999), and an increase in frequency or fixation of alleles that may be deleterious (Lacy 1997, Ralls et al. 2000). However since these measures are only meaningful if they can be used to demonstrate a loss or change, they are best interpreted through comparisons with a pre-bottleneck sample from the same population (Bouzat et al. 1998, Matocq and Villablanca 2000). Otherwise, we may erroneously attribute low genetic diversity to a demographic bottleneck (change) when in fact it reflects historically low levels of diversity (no change).

Review of condor genetics.—Of the 169 fertile California Condor eggs laid in captivity through 1998, five resulted in severely deformed embryos. These birth defects were diagnosed as chondrodystrophy, a lethal form of dwarfism (Ralls et al. 2000). On review of the expression of chondrodystrophy in condors, Ralls et al. (2000) concluded that chondrodystrophy is likely inherited as an autosomal recessive allele, which is the same mode of inheritance as in chickens, turkeys and quail. Ralls et al. estimated the frequency of the chondrodystrophy allele at 0.09 based on the observed expression of lethality and attributed this high frequency to a founder event. Since a founder event, or more specifically, the associated increase in inbreeding, is a requisite of the Ralls et al. hypothesis, their hypothesis is testable. One could determine if the demographic founders are more inbred relative to a pre-bottleneck population of California Condors. If there is no increase in inbreeding, then the chondrodystrophy allele would have been present at roughly the same frequency over the last 100+ years. In other words, there is an untested null hypothesis: the frequency of chondrodystrophy alleles may not have changed from the historic frequency.

Several studies have been conducted to evaluate relatedness and genetic diversity in California Condors. Corbin and Nice (1988) assessed genetic diversity using blood enzymes and found the population to be monomorphic or invariant at 24 of 31 loci surveyed. This is a relatively low level of polymorphism in comparison to other avian species (Corbin and Nice 1988). Geyer et al. (1993) employed minisatellite DNA fingerprints to characterize relatedness of 28 captive founders and 4 deceased founders for which tissues were available (Fig. 2). Fingerprint data were used to identify three distinct clans, where condors within a clan were more closely

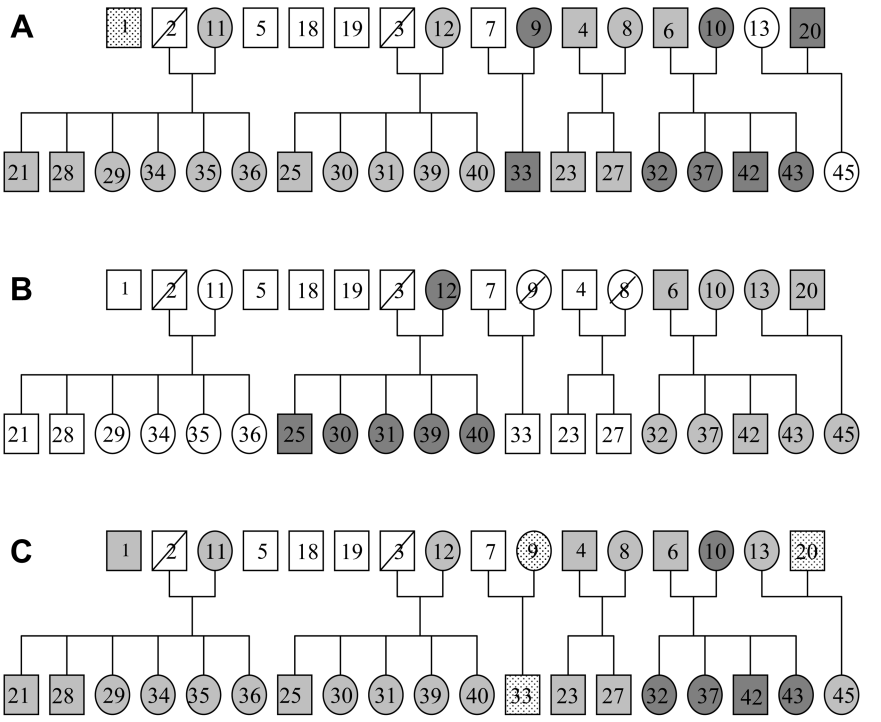

Fig. 2. Genealogical relationships between founding members of the captive flock and results from three different analyses of California Condor genetic variation. Numbers are the studbook (SB) numbers (Mace 2002). Circles represent females and squares represent males. Slashes indicate individuals for which data were not obtained, or were obtained indirectly. Individuals SB #2, and #3 represent two males that died in the wild and whose genotypes have not been recovered. Haplotypes for females SB #8 and #9 were obtained indirectly from offspring (A and C). Maternal genetic founders are the four individual females (SB #10, #11, #12, and #13) that produced female offspring (A and C). (A) Haplotypes identified using mtDNA sequence data. The un-shaded individuals represent haplotype 1, individuals shaded with light gray represent haplotype 2, spotted shading represents Topatopa (SB #3), an individual having a unique haplotype, and individuals shaded in dark gray represent haplotype 4. (B) Clans identified by Geyer et al. (1993) using nuclear DNA fingerprinting. No shading, light shading and dark shading identify individuals grouped into each of three clans. (C) Maternal haplotypes identified from mtDNA RFLP analyses (Chemnick et al. 1999). Unique haplotypes are identified by no shading, light shading, dark shading and spotted shading.

related to each other than to condors in any other clan. When compared to Andean Condors (*Vultur gryphus*), California Condors showed much less diversity and a higher degree of relatedness (Geyer et al. 1993). This is interesting given that Andean Condors themselves have very low genetic diversity (Hendrickson et al. 2003). Finally, Chemnick et al. (2000) used mitochondrial restriction fragment length polymorphism (RFLP) analysis to assess the genetic diversity of 14 potentially unrelated maternal lineages in the founding population (Fig. 2). This study identified four unique maternal lineages and showed that only two of these lineages persisted in the extant population.

The studies of Corbin and Nice (1988), Geyer et al. (1993), and Chemnick et al. (2000) have provided information on the relatedness of founder birds, and were instrumental in developing the initial captive breeding strategy. However, these studies do not provide information regarding the magnitude or rate of loss of genetic diversity in the extant population. Nor are the methods used applicable to the study of preserved (archived museum) specimens. Therefore, they cannot yield an assessment of change in genetic diversity. In other words, using these methods, we could never ask the larger question—has there been a reduction in genetic diversity over the last 100+ years?

Here, we consider the genetic effects of the California Condor population bottleneck. For several reasons, the California Condor offers an important opportunity to measure the genetic effects of a severe demographic bottleneck. First, the condor's decline has been well documented over the past 50 years, and the number of survivors is known (Wilbur 1978, Snyder and Snyder 2000). Second, the pedigree of over 90% of the surviving condors is known (Mace 2002). Third, there is no evidence of historic or recent population subdivision (N. Clipperton unpubl. data, N. Snyder pers. comm.) meaning that all individuals should represent a single breeding population (but *contra* Wilbur [1978] who contended that there were two breeding populations). We provide an assessment of changes in genetic diversity associated with the founding of a captive population. It is our intent that ultimately, data from archival museum specimens will be compared with our data, to determine patterns of genetic variation over a longer time scale.

Demographic and genetic founders.—The global population of wild California Condors was ultimately reduced to 27 individuals by 1987 (Snyder and Snyder 2000). We term these birds the demographic founders. Eleven of the demographic founders never bred in captivity, while sixteen of the demographic founders did breed or had offspring raised in captivity. We term these 16 the genetic founders (Fig. 2A, B, C). These 16 represent those individuals that are hypothetically unrelated and thus potentially represent unique lineages. Our objective was to determine the

level of genetic diversity in these 27 (demographic) and subsequently the 16 (genetic) founders. Four of the 16 genetic founders, two males and two females, died in the wild. Therefore, no genetic material was directly available from which to genotype these individuals. However, the mtDNA haplotype was obtained for the two deceased founder females (Table 1 and Fig. 2, studbook [SB] #8 and SB #9) by determining the haplotype of an offspring. Thus, DNA sources were available for 14 of the 16 genetic founders (lacking two males). Ralls and Ballou (2004) state that the genetic founders were 14 individuals. These authors do not consider the two males that were never captured, but which are known to have left progeny (Fig. 2, SB #2 and #3).

Importantly, the maternal inheritance of mitochondrial DNA reduces the genetic founders to only the *maternal* genetic founders. In the genetic founder population only four individual females, SB #10, #11, #12, and #13, produced female offspring who have since reproduced to maintain these maternal haplotype lineages (Fig. 2A, B, C).

In this study we explored mtDNA genetic diversity in three subpopulations of California Condors. Throughout we will refer to the 27 demographic founders (founders of the captive flock), the 16 genetic founders (of which 14 are potentially unrelated), and the 4 maternal genetic

Table 1. Identity of the 14 California Condors genotyped in this study (studbook numbers from Mace 2002), and respective sequence at all variable sites. Base 1 is at the 5' end of the Control Region's Region I and Base 482 is the base at the 5' end of the TDKD primer in CR Region II. Nucleotide positions that are not shown were not variable. Haplotypes are numbered sequentially in order of discovery within the founder California Condor population.

California Condor (Studbook #)	Haplo-type	Base 163	Base 170	Base 237	Base 318	Base 321
AC1 (19)	1	C	A	C	C	T
AC5 (7)	1	C	A	C	C	T
AC6 (5)	1	C	A	C	C	T
BFE (18)	1	C	A	C	C	T
UN1 (13)	1	C	A	C	C	T
AC2 (6)	2	C	A	**T**	C	T
AC7 (4)	2	C	A	T	C	T
AC8 (12)	2	C	A	T	C	T
Paxa (23)[a]	2	C	A	T	C	T
Tama (11)	2	C	A	T	C	T
Topa (1)	3	C	A	T	C	C
AC3 (10)	4	**T**	**G**	**T**	**T**	T
AC4 (20)	4	T	G	**T**	T	T
Sequoia (33)[a]	4	T	G	**T**	T	T

[a] Progeny used to identify the haplotype of a female genetic founder that was not captured from the wild.

founders (female founders that produce female offspring). Using data from the genetic founders and the pedigree, we extrapolate maternal haplotypes through the population bottleneck (as of 30 June 2002). The current population includes: all living demographic founders, and all living captive born birds (both captive and released).

Measures of genetic diversity.—The amount of genetic variation in a population is a function of the effective population size (N_e) (Wright 1938, Nei 1987, Frankham 1995, Crandall et al. 1999). When there is a rapid reduction in N_e, theory predicts that rare alleles are lost first (Allendorf 1986, Tajima 1989, Matocq and Villablanca 2000). Thus, the first detectable change should be a reduction in the *number* of haplotypes, due to a loss of rare or infrequent sequences, as measured by haplotype diversity. As more and more time accumulates, changes in haplotype *frequencies* can also be used to study loss of genetic diversity. Rare haplotypes, which are lost first, have a small effect on overall haplotype frequency. Significant changes in the frequency of the more common haplotypes are required before shifts in haplotype frequency are detectable. Nucleotide diversity (Nei 1987) reflects frequency differences, but is a measure that is slow to change compared to haplotype diversity.

Mitochondrial DNA.—Our study makes use of DNA sequences from the highly variable mitochondrial control region (CR). The avian CR is subdivided into three regions or domains (Fig. 3A) based on the relative rate of evolution within each region. Region II, the central conserved region (Clayton 1991), is flanked by two variable regions (Tarr 1995, Baker and Marshall 1997, Zink and Blackwell 1998, Saunders and Edwards 2000). Region I contains the most variation, and therefore is the most informative for studies of genetic diversity at the population level (Wenink et al. 1994, Baker and Marshall 1997, Glenn et al. 1999).

Mitochondrial DNA sequence data are a powerful tool for quantifying population level genetic variation (Wilson et al. 1985, Avise et al. 1987, Hillis and Moritz 1990, Geyer et al. 1993, Baker and Marshall 1997). The mitochondrial genome (haploid and maternally inherited) responds more quickly to drift than does the nuclear (diploid) genome (Birky et al. 1989, Palumbi et al. 2001), the mutation rate is up to 10 times faster than in nuclear loci, and there is no recombination (Hillis and Moritz 1990, Baker and Marshall 1997, Futuyma 1998, Palumbi et al. 2001).

Statistical power is greatest when numerous loci are used to make estimates of genetic diversity because of the inclusion of stochastic, or random, variance in genetic drift between loci (Lynch and Crease 1990). This is a compelling reason for using sequences from multiple nuclear loci. However, due to the rapid rate of evolution in the mitochondrial genome relative to the nuclear genome, and the rapid rate of lineage sorting (drift) relative to nuclear genes (Palumbi et al. 2001), our use of mtDNA maximizes

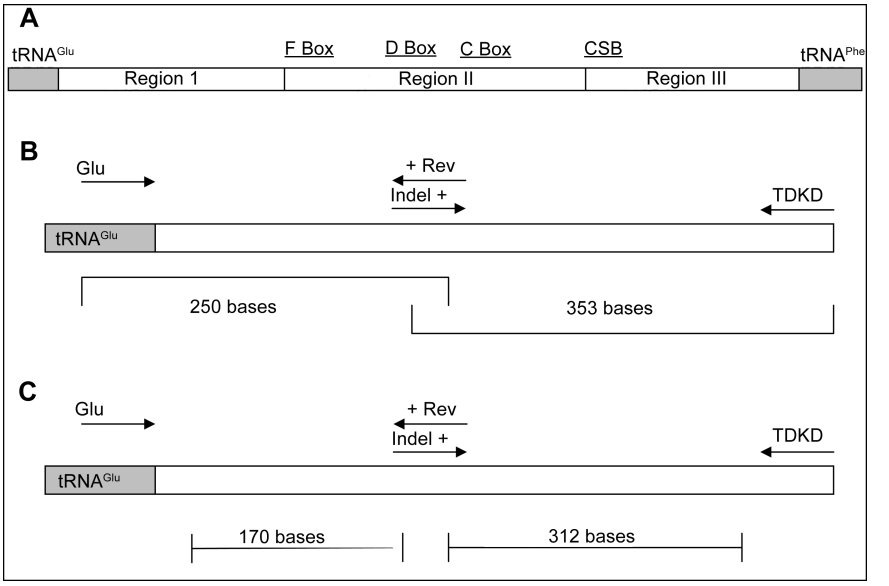

Fig. 3. Schematic representation of the mtDNA locus (Control Region [CR]) under investigation, and the primers used to amplify mitochondrial specific copies of the CR. (A) The three regions of the mitochondrial CR and the location of the conserved sequence blocks (F Box, D Box, C Box and CSB-1), shown on the L strand in 5'–3' orientation. Shaded portions flanking the control region represent tRNA's. (B) Primers shown amplify from $tRNA^{Glu}$ (Glu) to the D Box (TDKD) in two mitochondrial DNA specific fragments (250 and 353 nucleotides in length). (C) Data presented here are from the two fragments (170 and 312 nucleotides) without primers or overlap.

the power available from a single locus (Bouzat et al. 1998, Matocq and Villablanca 2000).

Nuclear copies of mtDNA.—Nuclear copies of mitochondrial sequences (numts) are known to exist. Nuclear paralogs (duplicated sequences) of avian mitochondrial sequences have been observed by several researchers (Quinn and White 1987, Quinn 1992, Kidd and Friesen 1998, Tiedemann and Kistowski 1998, Zhang and Hewitt 1996). Care must be taken to ensure that sequences used for analysis are orthologous (derived by mutation and not gene duplication). If undetected or misidentified, nuclear paralogs can confound phylogenetic and population genetic analysis as they are diploid, bi-parentally inherited (Bensasson et al. 2001), and would erroneously inflate estimates of genetic diversity.

During this study we identified a nuclear copy of our target mitochondrial DNA sequence (for details see Adams 2002). Thus, polymerase chain reaction (PCR) primers were designed to preferentially amplify each of the

two (mt and numt) sequences following the methods of Quinn and White (1987) and Sorenson and Fleischer (1996). Condor specific *and* mitochondria control region specific primers were used to preferentially amplify sequences presented herein.

In this study, we used DNA sequences from California Condors to quantify mitochondrial control region genetic diversity using 482 nucleotides of Region I and part of Region II. We evaluated the number of haplotypes, haplotype frequency, haplotype diversity (h), and nucleotide diversity (θ). These measures were then used to determine if there was a significant difference in genetic diversity between the genetic founders, the maternal genetic founders, and the current extant population (as of 30 June 2002), thereby directly assessing changes in genetic diversity over the last 25 years.

Methods

Samples.—DNA samples for all captive founders were provided by O. Ryder, Conservation and Research for Endangered Species (CRES). Whole genomic DNA was extracted from blood at CRES under sterile laboratory conditions. We obtained mtDNA sequence data from all genetic founders that were brought into captivity (Fig. 2 and Table 1). In addition, in order to confirm our methods, we obtained and compared data from several dam-offspring lineages (SB #12 [AC8] and offspring, n = 5; SB #11 [Tama] and offspring, n = 1; SB #10 [AC3] and offspring, n = 1). Finally, because we inferred the sequence of the CV female (SB #8) from her offspring, we sequenced both offspring (SB #23 and SB #27).

Amplification and sequencing.—Polymerase chain reactions were performed with the avian universal primer L16758 which is complementary to the $tRNA^{Glu}$ adjacent to the control region (Sorenson et al. 1999), and TDKD, a vertebrate universal primer, that binds the conserved D Box sequence (Quinn and Wilson 1993) (Fig. 3A). Each of these was paired with a Condor specific primer, Indel+ (5'-CAAGAACACTACCATCAGACC-3') or +Reverse (5'-GGTCTGATGGTAGTGTTCTTG-3') to amplify mitochondrial specific copied of California Condor control regions. A schematic of these amplicons is shown in Figure 3B, C.

Polymerase chain reactions were performed using ready-to-go PCR beads following the manufacturer's specifications (Amersham Pharmacia Biotech Inc. 2000). The 25 µl reactions included 2.2 µl of each 10 µM primer, 1.0 µl template, 1.5 µl ampliTaq DNA Polymerase, 10mM Tris HCl, 50mM KCL, 1.0mM $MgCl_2$, 200 µM each dNTP and BSA. Polymerase chain reactions were subjected to 30 cycles at 94°F for 30 s, 52–58°F for 30 s, and 72°F for 30 s in a PTC-100 Programmable Thermocycler (MJ Research, Inc. 1991). The PCR fragments (L16758/+reverse, and indel+ /TDKD) were

sequenced in both directions. Prior to cycle sequencing, the PCR products were cleaned using QIAquick PCR Purification Kit following the manufactures specifications (QIAGEN, 1999, QIAquick PCR purification kit, Valencia, California).

Cycle sequencing reactions were performed using cycle sequencing (Perkin-Elmer Corp., 1995, Big Dye Terminator Cycle Sequencing version 2.0., California) following manufacturer's specifications (PE Biosystems, 1999, ABI prism big dye terminator cycle sequencing ready reaction kit protocol, California). Cycle sequencing products were purified using ethanol precipitation methods modified from Promega Corp. (1996) and run on an ABI 377 DNA Sequencer.

Chromatograms were visualized, edited, and manually aligned using Sequencher 4.1 (Gene Codes Corporation, Inc. 1999). The composite fragment used in this study extended 482 bases, from Base 1 of the control region (Glenn et al. 1999) to the 5' end of the TDKD primer in the D box, and excluded the 21 base pair sequence of the Indel + and +Reverse primers (Fig. 3C).

Phylogenetics.—MODELTEST, version 3.0 was used to determine the most appropriate model of molecular evolution and to calculate the average nucleotide frequency among all sequences (Posada and Crandall 1998). MODELTEST sequentially compares nested models of DNA substitution using a hierarchical likelihood ratio test. The following parameters (null hypotheses) are tested: equal base frequencies, equal transition and transversion rate, equal rate among sites, and no invariant sites (Posada and Crandall 1998). The most likely model was the HKY model (Hasegawa et al. 1985). The parameters of this model are unequal nucleotide base frequencies (estimated at A = 0.3322, C = 0.2970, G = 0.1215 and T = 0.2493) and a transition bias (transition/transversion ratio) of 5:0. A weighted parsimony model (PAUP*) was used to generate a phylogeny of the haplotypes (Fig. 4). The HKY model was also used in analyses conducted using the FLUCTUATE program (LAMARC, Kuhner et al. 1997).

Haplotype and nucleotide diversity.—Pairwise sequence comparisons were conducted using PAUP (V 4.0b, Swofford 1998) to identify unique haplotypes, calculate number of polymorphic sites, and calculate haplotype frequencies in the genetic and maternal genetic founders. The haplotype frequency was tabulated as a function of the number of individuals in each group (genetic founders $n = 14$; maternal genetic founders $n = 4$; and the extant population as of June 2002, $n = 207$) having each of the possible haplotypes.

Haplotype diversity (h) was calculated for the demographic and maternal genetic founders, and for the current population (following Nei 1987, equation 8.1). Haplotype diversity ranges from 0 to 1 (Nei 1987). If all individuals in a large sample have a unique haplotype, the haplotype

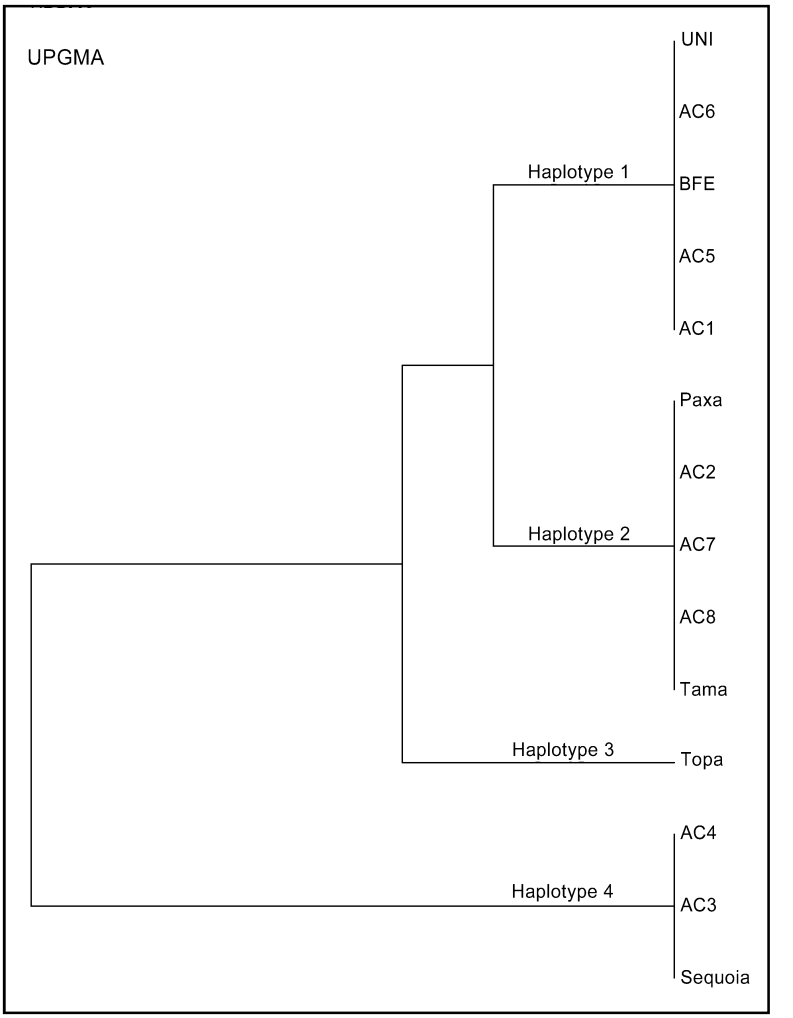

Fig. 4. Neighbor joining UPGMA phylogeny for all 14 genetic founders of the California Condor captive flock. These 14 founders processed 4 haplotypes.

diversity of the population will approach 1.0. If all individuals in a large sample have the same haplotype the haplotype diversity of the population is 0.

Nucleotide diversity, or the average sequence diversity at the nucleotide level (θ), was calculated for each of the three groups following Watterson (1975) and using the FLUCTUATE program, version 1.1 (Kuhner et al. 1997). Theta (θ) is a population parameter that is a function of effective population size (N_e) and the mutation rate (μ), ($\theta = N_e\mu$). Theta is

estimated from samples of sequences using nucleotide diversity (π) which is a function of the haplotype frequencies and the number of nucleotide differences (distance) between haplotypes (Nei 1987). The FLUCTUATE program uses Metropolis-Hastings Markov Chain Monte Carlo genealogy sampling to make a maximum likelihood estimate of the population parameters θ and g (growth rate) assuming the loci sampled are not affected by selection or recombination. This method for estimating θ is preferred since FLUCTUATE does not require an assumption of a stable population size. This is an assumption we were not willing to make since population size is a parameter we predict is changing. The accuracy of the θ estimate was determined using the likelihood curve or the graph of the log likelihood values associated with each estimate of theta. This curve is used to visualize an estimated 95% confidence interval of θ (Kuhner et al. 1998). We used a short chain length of 100 times the number of sequences and a long chain length of 1000 times the number of sequences, with 10 and 2 chains respectively per run. FLUCTUATE analyses for each of the three groups were replicated 500 times and the average θ was calculated for each set of replicates. Some replicates produced estimates of θ that were near infinity or negative infinity. These replicates were discarded and re-run. We used the data from the FLUCTUATE run that was nearest to the global average θ for further analysis.

Herein we have quantified the actual number of maternal lineages in the entire population and the fraction of those that are still extant in the breeding population. We have genotyped all female individuals that were brought into captivity or produced offspring that were brought into captivity. Consequently, we are not evaluating sample statistics, but are looking at the actual population parameter.

Results

Haplotype frequency and diversity.—The genetic founders revealed four haplotypes or unique sequences (Table 1 and Fig. 4). The four haplotypes are defined by five polymorphic sites (Table 1). The distribution of these haplotypes on the pedigree is shown in Figure 2A. One of these haplotypes is unique to Topatopa, the first male brought into captivity in 1967. Since males do not genetically transmit mtDNA to their offspring and Topatopa is the only living representative of this maternal lineage, only three of the four haplotypes will persist in the future California Condor population.

The frequencies of haplotypes have shifted over time (Table 2). Haplotype 1 has become progressively more rare and haplotype 2 progressively more common. Haplotype 4 was relatively uncommon in the genetic founders compared to haplotypes 1 and 2 but has increased its frequency relative to haplotype 1. As noted above, haplotype 3 will not survive into the

Table 2. Haplotype count (frequency) in genetic founders, maternal genetic founders, and the current California Condor population (as of 30 June 2002). Haplotype diversity (h) where $h = 1 - \sum x^2_i$, and x is the frequency of each haplotype.

Group name and population size	Haplotype 1	Haplotype 2	Haplotype 3	Haplotype 4	h
Genetic Founders					
$n = 14$	5 (0.357)	5 (0.357)	1 (0.071)	3 (0.214)	0.694
Maternal Founders					
$n = 4$	1 (0.25)	2 (0.50)	0 (0.00)	1 (0.25)	0.625
2002 Population					
$n = 207$	30 (0.145)	128 (0.618)	1 (0.005)	48 (0.232)	0.543

future. Assuming no mutation in the mitochondrial CR, and using the pedigree (Mace 2002) to extrapolate haplotypes, we predicted haplotypes for the entire extant California Condor population ($n = 207$). Haplotype diversity (h) for genetic founders was 0.694. In the extant population h was 0.524. The changes in h reflected a 25% decrease in haplotype diversity (Table 2).

Nucleotide diversity.—There was no shift in nucleotide diversity (Table 3). The mean nucleotide diversity (± standard error) in the maternal genetic founders overlaps with that of genetic founders in the 2002 population (Table 3).

Discussion

We examined mitochondrial control region sequences from genetic founders of the captive California Condor population. The nucleotide sequences of the entire Region I and part of Region II in the mitochondrial CR were determined by direct sequencing of 2 PCR products for 14 individuals (12 founders for which samples were available and 2 founder females for whom samples were only available through offspring; see Fig. 2). We detected a reduction in haplotype diversity and little or no change in nucleotide diversity. Thus, some diversity was lost in the population that was brought into captivity. Whether this population was genetically impoverished relative to pre-1900s California Condors remains unknown.

Table 3. Nucleotide diversity (θ) in Region I plus part of Region II of the mt Control Region in California Condors. None of the differences between founding groups are statistically significantly (standard errors [SE] overlap with mean values between comparisons).

Group name and population size (n)	θ	SE
Genetic founders (14)	0.00300	0.00027
Maternal genetic founders (4)	0.00470	0.00113
2002 population (207)	0.00333	0.00003

Inheritance patterns for haplotypes.—When tested, sequences of mitochondrial origin were identical between female parents and their offspring. This allowed us to verify that sequences within a maternal line were identical. In addition, because we inferred the sequence of the CV (SB #8) female from her offspring, we sequenced both Cuyama (SB #27) and Paxa (SB #23). As expected the two female offspring sequences were identical.

Haplotype frequencies.—Haplotype frequencies in the California Condor population have changed. Genetic drift theory predicts that in a randomly mating population haplotype frequencies would change due to chance alone, with some frequencies increasing, moving toward fixation, and some declining (Table 2). An alternative hypothesis is that natural selection on mtDNA haplotypes has shifted. With condors, this alternative hypothesis is unlikely. First, artificial selection has occurred since pairings were made at the captive breeding facilities according to the genetic desirability criteria (e.g., forced pairing with non-clan members following Geyer et al. 1993). Second, even if natural selection is acting on this small population, chance is more likely to effect genotype frequencies than selection simply because of the populations' small size (Barton and Charlesworth 1984, Ohta 1995).

Haplotye diversity.—We observed a 25% decrease in haplotype diversity (Table 2). This is a direct result of the recent increase in population size of condors in captivity, and the associated shifts in haplotype frequency. For example Topatopa's haplotype "fell" from a frequency of about 1 in 20 to about 1 in 200 simply as a result of population growth. Baker and Marshall (1997) compiled data from several avian species and assessed the haplotype diversity in Region I of the CR, finding that values of Nei's (1987) h ranged from 0.449 to 0.982. In their study, values for non-bottlenecked species ranged from 0.827 to 0.982. One species (the Knot, *Calidris canutus*) with known low genetic diversity for nuclear markers (allozymes) was the low value of 0.449. Indeed, when compared to Baker and Marshall's non-bottlenecked avian species, the haplotype diversity in the genetic founder ($h = 0.694$) and current California Condor population ($h = 0.524$) were both low enough to be outliers. Haplotype diversity in California Condors is lower than in the wide-ranging and apparently non-bottlenecked Andean Condor (Hendrickson et al. 2003). Thus, it is possible that the low values we observe are consistent with the low values expected for megafauna with a restricted range (Hendrickson et al. 2003), or it is possible that diversity has been lost during a bottleneck. A historic, pre-bottleneck population sample of the California Condor is necessary to correctly determine whether the starting value of haplotype diversity we observe ($h = 0.694$) represents a historical value or recent reduction.

Nucleotide diversity.—The nucleotide diversity estimate for the genetic founders reflects the remnant genetic diversity of the wild California Condor population prior to its captivity. The nucleotide diversity estimate for the 2002 population reflects the demographic history of the California Condor since its captivity and growth from the four maternal genetic founders. These two populations showed no significant difference in nucleotide diversity. Populations that have the same demographic history should have statistically indistinguishable values of theta (θ) (Slatkin 1987). Therefore, we expected that the log likelihood estimates of theta would not be significantly different under three possible scenarios: the population size reduction was not significant compared to past reductions; the population size reduction was too brief to have an evolutionary consequence; and/or the California Condor has already been through such demographic–genetic bottlenecks during its history. From the results of our study, it might be concluded that the population size reduction has not lasted long enough to have any effect on haplotype diversity. Most likely, if more time had passed, there would have been a greater (significant) reduction in nucleotide diversity. That no difference was observed implies that this measure of genetic diversity is insensitive to very large but temporary changes in population size. Or, alternatively, that this demographic crash (including its duration) is not too distinct from other naturally occurring crashes experienced by this species. Again, we would like to point out that a historic population sample of the California Condor is necessary to correctly discriminate between these alternative hypotheses.

Comparison with other genetic studies of California Condors.—The DNA fingerprint analysis of Geyer et al. (1993) was based on nuclear DNA digested with three restriction endonucleases. Their study revealed three clans. Their resulting groupings differed from the four haplotype groupings identified in this study. This difference might be expected when comparing mitochondrial sequence data to nuclear minisatellite data (see Fig. 2A, B, respectively). It is of note that in our study Topatopa was identified as having a unique mitochondrial haplotype, yet based on Geyer et al.'s nuclear analysis, Topatopa was placed into the largest clan.

The mitochondrial genome of California Condors has been studied previously through an RFLP analysis. Using five restriction enzymes, Chemnick et al. (2000) identified four haplotypes, but found only two persisting in the extant population. Our results were generally concordant with the results of the mtDNA RFLP analysis, with the exception that Topatopa has a unique mitochondrial haplotype in our analysis. It is important to note that none of the restriction enzymes used in the RFLP analysis would have detected the DNA sequence variation identified herein, because the recognition sequences of those enzymes are not contained in the fragment that we sequenced.

In summary, we corroborate the loss of genetic (mtDNA) diversity associated with the founding of the captive population. The number of haplotypes has been reduced as has haplotype diversity. Although our results do show a 25% decrease in the haplotype diversity between the genetic founders and the current population, our results did not show an apparent change in the average nucleotide diversity (θ). Haplotype diversity is more sensitive to changes in population size and the frequency of haplotypes within the population. In contrast nucleotide diversity is an estimate of heterozygosity at the nucleotide level. It is known that heterozygosity is slower to change than allele frequencies since rare alleles are the ones lost first and these contribute little to heterozygosity (Nei et al. 1975, Watterson 1975, Hartl and Clark 1989).

The genetic founders have only four unique mitochondrial CR haplotypes, defined by five polymorphic sites. Interestingly, haplotype 3 is unique to Topatopa, the oldest living California Condor brought into captivity as a chick in 1967. Due to the maternal inheritance of mitochondrial DNA this haplotype will not persist in the population, as Topatopa cannot pass it on to his offspring. Topatopa may be part of the largest nuclear clan, yet the unique mtDNA haplotype suggests that he also carries some additional nuclear genetic variance not found in other founders. Importantly, he has bred very successfully in the captive breeding program.

Inbreeding.—The entire captive flock was founded by six breeding pairs (Fig. 2). Males from two of these pairs were never genotyped. The dam and sire in three of the four genotyped pairs had different mitochondrial haplotypes from each other. Our DNA sequence result corroborates the same finding as the mitochondrial RFLP analysis (Chemnick et al. 2000; see our Fig. 2C). The CV pair (SB #4 and #8) is the only pair to share the same maternal haplotype. This is of interest because (1) it may indicate a higher level of relatedness within this pair; (2) these are the parents of the only known wild hatched chick that died with abnormal limb and skull development which are symptomatic of the lethal recessive chondrodystrophy gene (Snyder and Snyder 2000); and (3) they are also the parents of a male, Cuyama (SB #27), which fathered chicks expressing chondrodystrophy in captivity (Ralls et al. 2000).

Although a behavioral component has not yet been linked to chondrodystrophy or any another genetically based condition, behavior may affect fitness. Both Paxa and Cuyama exhibited abnormal behavior as chicks. Observed behaviors included excessive gaping and wing drooping, respectively (Snyder and Snyder 2000). Field observations in the early 1980s resulted in the documentation of several abnormal reproductive behaviors among wild pairs. These included poor coordination of egg incubation resulting in egg neglect and nest failure, aggressive interaction at the nest site and attempted homosexual copulation between two birds (Snyder and Snyder 2000).

Future implications.—In the captive California Condor population, the expression of deleterious alleles is concordant with the mating of individuals sharing the same haplotype (i.e., the CV pair [SB #4 and #8] and offspring). If we assume that individuals sharing a haplotype are more closely related than individuals with different haplotypes then we could use haplotypes to infer inbreeding. We suggest our results be used in exactly that manner.

Because mtDNA is passed along from a female to all her offspring without recombination, the techniques developed in this study can be used to identify maternal parents. For example, if maternity of a wild born condor is unknown and the potential female parents are from different maternal lineages (have different haplotypes) the maternal parent can be identified by sequencing a small fragment of mitochondrial DNA. A possible source of DNA that could be used includes feathers or egg shell fragments from the nest site.

Until recently, genetic diversity could only be assessed in extant populations. In addition, the amount of diversity was usually evaluated in comparison to some closely related species or population. However, this approach has the potential to lead to erroneous conclusions (see Bouzat et al. 1998, Matocq and Villablanca 2000). With current molecular methods and the primers developed in this study, it is possible to directly quantify the genetic variability of the California Condor both before and after the bottleneck of 1987. Such an analysis would allow us to fully understand the impact of this bottleneck and determine if and how much genetic diversity has been lost. Moreover, such an analysis would allow us to determine if the California Condor is one of a small number of species that show historically low levels of genetic diversity (Matocq and Villablanca 2000, Pertoldi et al. 2001).

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