

Population structure and genetic diversity in Swainson's Hawks (*Buteo swainsoni*): implications for conservation

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Received: 26 February 2007 / Accepted: 1 May 2007
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Abstract Swainson's Hawks (*Buteo swainsoni*) are large raptors with a breeding distribution extending across much of western North America where they were historically considered one of the most abundant raptors. Swainson's Hawks have declined precipitously in many parts of their range during the 20th century, and the historical range in California has been much reduced. In the Central Valley of California (CV), Swainson's Hawks exhibit behavioral and morphological characteristics apparently different from other regions. To describe the genetic diversity and population structure of Swainson's Hawks throughout their range, 19 microsatellite loci and 416 base pairs of the mitochondrial control region were analyzed. Microsatellite diversity appears high throughout the contemporary range. A Bayesian model-based analysis of microsatellite genotypes revealed clusters associated with the CV and the Great Basin/Great Plains region of North America (GBGP)

with overlap between regions. F_{ST} estimates suggest limited differentiation among Swainson's Hawks with isolation by distance. A heterozygote excess indicated a recent reduction in effective population size of Swainson's Hawks across all regions. Control region data revealed no population structure and provided evidence of historic population expansion in the GBGP. In the CV a weaker signature of population expansion was detected, possibly altered by recent declines. While genetic data suggests recent gene-flow across regions, apparent differences between the CV and GBGP in traits with potential fitness consequences (migratory behavior and morphology) along with marked decline in numbers in California call for careful conservation, management, and monitoring of Swainson's Hawks in the CV.

Keywords Swainson's Hawk · *Buteo swainsoni* · Population genetics · Mitochondrial DNA · Microsatellite

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Introduction

An on-going concern of conservation biology is the maintenance of biodiversity and identification of appropriate biological units for conservation. In order to best preserve existing biodiversity and evolutionary processes in human altered environments, genetic considerations such as population structure and genetic diversity must be incorporated into management strategies (Frankham et al. 2002). Birds of prey are often typified by large home ranges and low population densities. As human influences continue to alter natural environments raptor populations have become fragmented and have decreased often to the point of endangerment (Shepard et al. 2005; Newton 1991). As populations decline, genetic variability may be

lost as populations become subdivided, gene flow is reduced, and inbreeding increases. This pattern is often seen among naturally isolated populations of island endemics (Frankham 1997), as has been documented in the Galapagos Hawk (*Buteo galapagoensis*) (Bollmer et al. 2005) and has also been recorded among raptors in human fragmented landscapes (Martínez-Cruz et al. 2004; Roques and Negro 2005).

Maintenance of genetic diversity among currently widespread raptor species requires determination of population structure and the contemporary genetic diversity within populations. Low genetic differentiation tends to be characteristic of highly mobile species with broad distributions, such as raptors. Observed genetic variation may still be of consequence in conservation efforts, indicating, for example, incipient population divergence or differing selective regimes with ongoing gene flow.

Swainson's Hawks (*Buteo swainsoni*) are designated federally as a Species of Concern in some regions and listed as Threatened by the State of California. The Swainson's Hawk is a large, highly migratory raptor with a breeding distribution that extends across grasslands, open woodlands, and today incorporates many agricultural landscapes of western North America. Swainson's Hawks were historically considered to be one of the most abundant raptor species in western North America, but have declined precipitously in many parts of their range during the 20th century. Declines began during European settlement with many regions experiencing marked declines by 1900. The contemporary range of the Swainson's Hawk includes a contiguous portion of the Great Basin, Great Plains, and southwestern deserts (GBGP) and a geographically disjunct range in central California (CV; England et al. 1997; Fig. 1).

In California there was a precipitous decline in Swainson's Hawk numbers during the 20th century, with the population declining by as much as 90% (Bloom 1980). Along with a decline in census numbers, the historical range of Swainson's Hawks in California has been much reduced (England et al. 1997). Populations from coastal southern California and the coastal foothills and plains have been extirpated with populations still existing in portions of the Sacramento-San Joaquin Valleys in central California, the Modoc Plateau in northeastern California, and remnant population in Owen's Valley and the Mojave Desert (Bloom 1980; Grinnell 1944; Anderson pers. ob.). Recent threats to the persistence of Swainson's Hawks include pesticide poisonings in wintering ranges resulting in mass mortalities and loss of habitat within the breeding range (England et al. 1997; Risenbrough et al. 1989; Goldstein et al. 1999).

Dispersal between the CV and the GBGP may be limited. Study sites in California have documented high rates

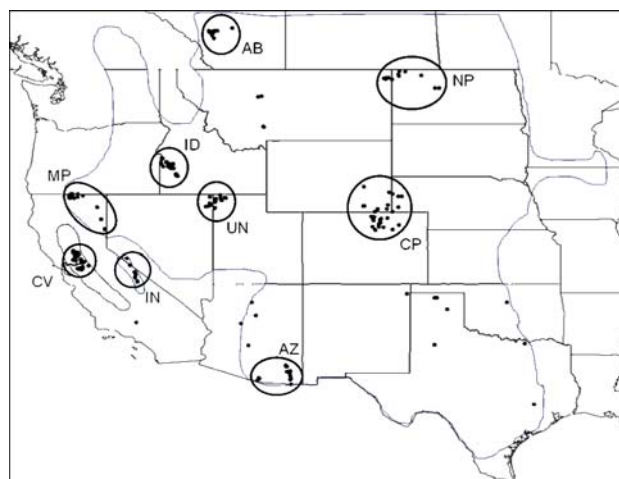


Fig. 1 Breeding range (shaded) of Swainson's Hawks (England et al. 1997) and distribution of Swainson's Hawk samples (dots). Nine sampling localities with greater than 10 breeding season samples are circled (AB = Alberta, $n = 13/13$; AZ = Arizona, $n = 23/23$; CP = Central Plains, $n = 39/33$; CV = Central Valley, $n = 79/71$; ID = Idaho, $n = 33/32$; IN = Inyo, $n = 13/12$; MP = Modoc Plateau, $n = 43/42$; NP = Northern Plains, $n = 19/18$; UN = Utah/Nevada, $n = 22/16$; number of multilocus microsatellite genotypes and mitochondrial sequences respectively)

of nest site fidelity with mean natal dispersal of less than 10 km (Estep 1989; Woodbridge et al. 1995) and mean adult dispersal of less than 5 km (Woodbridge et al. 1995) while natal dispersal in excess of 100 km has been observed in Alberta (Houston and Schmutz 1995). Behavioral and morphological studies suggest that Swainson's Hawks breeding in the CV might be genetically distinct from those breeding in the GBGP. Banding and telemetry data indicates that Swainson's Hawks from other regions winter in the pampas of Argentina, while the majority of CV Swainson's Hawks winter in western Mexico and Central America (Houston 1990; Wheeler 2003; Bradbury unpublished data). In addition to migratory differences, Swainson's Hawks occurring in California display a much greater frequency of dark, rufous, and intermediate plumaged individuals with local frequencies of approximately 85–90% within the CV compared with less than 40% outside of the state and less than 1% in the GBGP (Wheeler 2003). These dispersal, behavioral and morphological differences between regions along with documented population declines prompted an investigation to determine whether CV Swainson's Hawks are genetically distinct from those in other regions.

This investigation of Swainson's Hawk genetics had two goals: first, to use a panel of 19 Swainson's Hawk-specific microsatellite loci along with mitochondrial DNA sequence data to assess the genetic diversity of CV Swainson's Hawks and, second, to determine whether CV Swainson's Hawks are genetically distinct from those occurring in the

GBGP. Population genetic data describing contemporary genetic diversity and population structure among Swainson's Hawks provide an important compliment to previous ecological and behavioral studies.

Methods

Sample collection

Whole blood and contour feathers were collected from 357 Swainson's Hawks from California, east to North Dakota and Alberta, and south through Texas (Fig. 1) between 2003 and 2005, as well as five Ferruginous Hawks (*Buteo regalis*) for outgroup comparison. Samples were collected from CV, the nearest breeding populations to the north (Modoc Plateau), southeast (Arizona), and east (Inyo) as well as from five additional GBGP locations (Alberta, Central Plains, Idaho, Utah/Nevada, and Northern Plains). These sampling locations allowed comparisons between CV and the remainder of the Swainson's Hawk range in GBGP as well as for comparisons of equally distant locations within GBGP. Approximately 0.2 ml blood was drawn via medial metatarsal venipuncture and two feathers were plucked from the breast. Blood samples were stored in 1.2 ml of Longmire's lysis buffer (100 mM Tris pH 8.0, 100 mM EDTA, 10 mM NaCl, 0.5% SDS) at ambient temperature until delivered to laboratory facilities where they were preserved at -80°C . Feather samples were stored in paper envelopes and kept cool and dry. Samples were collected from adults, juveniles, and pre-fledge young in nests by licensed raptor biologists and from juveniles and adults treated at several wildlife rehabilitation facilities. Wild birds were leg-banded with U.S. Geological Survey tags and either released or returned to nests. One individual per presumed family group (nestlings and parents) was included in the sampling for this study. Total cell DNA was isolated from 25 μl of blood/buffer solution or feather calamus using QIAGEN DNeasy kits (QIAGEN Inc.). DNA was stored at 4°C while in use, and then transferred to -80°C upon completion of genetic work.

Data collection

Each individual was genotyped at 19 microsatellite loci (BswA110w, BswD122w, BswA204w, BswA317w, BswD210w, BswD220w, BswA303w, BswB111aw, BswD234w, BswD310w, BswD313w, BswB220w, BswB221w, BswD327w, BswA302w, BswA312w, BswD107w, BswD127w, and BswD324w; Hull et al. 2006) using DNA extracted from whole blood in six multiplex PCRs carried out following the conditions in Hull et al. 2006. PCR products were separated with a 3730 DNA Analyzer (Applied Biosystems Inc.). PCR

products were visualized and scored with STRand version 2.3.69 (Toonen and Hughes 2001).

A total of 416 base pairs of domain I of the mitochondrial control region were amplified via PCR using primers 16065F (Kimball et al. 1999) and H15414 (Bollmer et al. 2006). Both blood and feather samples were used from the same individuals to aid in detection of nuclear-mitochondrial insertions. PCR products were prepared for sequencing reactions using Ultra-clean purification kits (MoBio) and submitted to the UC Davis DNA Sequencing Facility for sequencing reactions and electrophoresis using primers 14965F and H15414. Primer 14965F, TTGTACATTAAC TATATTCCACATATCAT, was designed specifically for this project and is located 10 base pairs downstream from a poly-c repeat that inhibited the sequencing reaction. Sequences were examined and aligned using the software SEQUENCHER (Gene Codes Corporation).

Microsatellite data analysis

Samples from CV and GBGP regions were tested for deviations from Hardy-Weinberg equilibrium in the program ARLEQUIN version 3.01 (Excoffier et al. 2005) using Fisher's exact test with 10,000 dememorization and 100,000 Markov chain steps (Guo and Thompson 1992). Genotypic linkage equilibrium was tested using Fisher's exact test in GENEPOP with 1,000 dememorization steps, 100 batches, and 1,000 iterations per batch (Raymond and Rousset 1995). A Bonferroni correction for multiple tests was used for HWE and linkage equilibrium (Zar 1999). The presence of null alleles was tested in MICRO-CHECKER (van Oosterhout et al. 2004). Scoring error was evaluated by repeating amplification and scoring for 10% of samples at all loci and calculating the frequency of disagreement between runs. Heterozygosity and number of alleles per region, CV and GBGP, were tested for in MICROSATELLITE TOOLKIT (Park 2001). The number of private alleles in CV and GBGP was calculated in CONVERT (Glaubitz 2004) and allelic richness, which accounts for variation in sample sizes, was calculated in FSTAT (Goudet 1995).

The program STRUCTURE (Pritchard et al. 2000), a model-based Bayesian algorithm, was used to probabilistically cluster individuals from all sites, and separately for GBGP individuals, based upon their multilocus genotypes without using *a priori* information on sampling locality. A preliminary analysis using the admixture model with flat prior was run for 100,000 iterations with an initial burn-in of 10,000 iterations for $K = 1$ through $K = 10$. This parameter set was performed 10 times and the log Pr($X|K$) statistic averaged across runs. Based upon the preliminary results, a second parameter set, using the admixture model with flat prior, burn-in of 100,000 iterations, and run length of

1,000,000 iterations, was performed and $\log \Pr(X|K)$ statistic averaged across 10 runs. The most likely number of clusters, K , was chosen by determining the K value where the averaged $\log \Pr(X|K)$ was maximized and subsequently selecting the minimum value for K that did not sacrifice significant explanatory power (Pritchard and Wen 2002). Recently, this method has been shown to perform as well or better than the method presented by Evanno et al. 2005 (Waples and Gaggiotti 2006), especially in cases of moderate differentiation. We defined individual membership to a cluster based upon highest membership assignment probability.

Population genetic structure was further investigated by comparing the probability that each sampled individual could be genetically assigned to one of the nine sampling localities. Following a Bayesian approach (Rannala and Mountain 1997), as implemented in GENECLASS2 version 2.0.b (Piry et al. 2004), each Swainson's Hawk sampled during the breeding season at one of the nine sampling sites was removed from the total sample of individuals and treated as an unknown and the probability of assignment to each sampling sites was determined using the Monte-Carlo resampling method of Paetkau et al. 2004.

Population differentiation between regions (CV and GBGP) and between sampling sites was estimated in ARLEQUIN using pairwise F_{ST} , following the infinite alleles model (IAM), and R_{ST} , following the step-wise mutation model (SMM) of microsatellite evolution. F_{ST} estimations can be potentially depressed by high mutation rates such as those found in many microsatellite loci (Balloux and Lugon-Moulin 2002). In contrast, R_{ST} is less dependent upon mutation rates. However, R_{ST} has greater variance and estimations will be compromised by deviations from the SMM, such as imperfect repeats (Balloux et al. 2000). A Bonferroni correction for multiple tests was used to determine significance of F_{ST} and R_{ST} values among sampling locations and years (Zar 1999).

To estimate the time required for development of observed degree of population differentiation (between region F_{ST}), a hypothetical sample with two populations (simulating CV and GBGP regions) was modeled using EASYPOP (Balloux 2001). The model parameters were as follows: monogamy (England et al. 1997), equal sex ratio, no migration, step-wise mutation model, mutation rate of 0.001, and population estimates of 1400 pairs for CV and 38000 for GBGP (Anderson 2006; England et al. 1997). All parameters were set in such a way as to generate a minimum estimate of required time since divergence. For example, the setting of no migration, while not likely to be a reflection of biological reality, helps to establish a minimum divergence time. For the same reason, the estimates of population size used are low values of the range reported in the literature. The model was run for 200 generations and 50 replicates of the model were performed. The estimated F_{ST}

values were averaged across replicates and plotted against time in generations.

To investigate the potentially confounding effect of geographic distance, a Mantel test (Smouse et al. 1986) was used in ARLEQUIN to test for correlation between pairwise genetic and geographic distances. The dependent variable in this analysis was a matrix of genetic distances (expressed as $F_{ST}/(1-F_{ST})$, Slatkin 1995) while the independent matrix was composed of pairwise geographic distances between centroids of sampling localities. Because the Mantel test is potentially sensitive to small sample sizes, the relationship between genetic and geographic distance was investigated in two ways: first by comparing all pairs of the nine sampling sites and then by excluding those sites that were represented by fewer than 15 samples (Alberta and Inyo).

Tests for evidence of recent genetic bottlenecks were performed using Wilcoxon sign-rank tests in the program BOTTLENECK (Piry et al. 1999) using the strict IAM and a two-phase model (TPM) of microsatellite mutation which allows a user to specified proportion of the SMM into a multi-step model; we ran the TPM analysis with a setting of 70% SMM (12 of 19 loci appear to follow the SMM; Hull et al. 2006). A significant excess of heterozygotes is indicative of a recent population bottleneck.

Control region sequence analysis

Number of variable sites, number of haplotypes, haplotype diversity, and nucleotide diversity were calculated in DNAsp (Rozas et al. 2003). Pairwise Φ_{ST} values between each sampling site and between CV and GBGP regions were calculated using pair-wise Juke & Cantor distances and haplotype frequencies in ARLEQUIN and significance was assessed using 10,000 permutations. A Bonferroni correction for multiple tests was used to determine significance of Φ_{ST} values among sampling locations and years (Zar 1999). We tested for a correlation between genetic distance, as measured by Φ_{ST} , and geographic distance using a Mantel test (Smouse et al. 1986) with 100,000 permutations in ARLEQUIN. Again, comparisons were made both for all nine sample sites and those sites with 15 or greater samples (Alberta $n = 13$, Arizona $n = 23$, Central Plains $n = 33$, Northern Plains $n = 18$, Idaho $n = 32$, Inyo $n = 12$, Modoc Plateau $n = 42$, Utah/Nevada $n = 16$, and CV $n = 71$; Fig. 1).

Maximum likelihood and unweighted maximum parsimony trees were generated using PAUP* version 4.0b10 (Swofford 2002). Bayesian analyses were performed in MRBAYES version 3.0b4 (Huelsenbeck and Ronquist 2001). In all three analyses *B. regalis* was used as an outgroup. MRMODELTEST (Nylander 2004) was used to identify the model of sequence evolution and parameter

estimates that best fit the data. For heuristic maximum likelihood and Bayesian analyses, both hierarchical likelihood ratio and Akaike information criterion tests returned best scores for the fit of the data to the general time reversible plus invariable plus gamma model of nucleotide evolution. Maximum parsimony (100 replicate data sets) and maximum likelihood (500 replicate data sets) bootstrapping were performed in PAUP* using the same parameters as the original heuristic searches. For Bayesian analyses, the Markov Chain Monte Carlo simulation was started with a random tree. Four chains were run simultaneously for 10,000,000 generations with a tree sampled every 10,000 generations resulting in a total of 1,000 trees. Interpretation of the likelihood values indicated that the simulation achieved stability at approximately 80,000 generations. We conservatively estimated the first 250,000 generations to represent the initial burn-in; these were discarded. The relationship among haplotypes was further investigated through generation of minimum spanning networks in NETWORK version 3.1.1.1 (Bandelt et al. 1999).

Several metrics were used to investigate historical demography. The expansion coefficient (S/d), the ratio of variable sequence positions (S) relative to the mean number of pairwise differences between haplotypes (d), was calculated following Peck and Congdon (2004). Large values for S/d suggest recent population expansion while small values indicate constant population size. Mismatch distributions and raggedness statistics (rg) were calculated in DNAsp. Unimodal mismatch distributions are indicative of population expansion while a multimodal, ragged distribution of pairwise differences is more typical of a population at mutation-drift equilibrium (Rogers and Harpending 1992). We also calculated Fu's F_S statistic and used Tajima's D -test to detect past population expansions. Fu's F_S has greater statistical power to detect population expansion than other available tests (Fu 1997). Tajima's D -test compares the number of nucleotide differences between sequences and the number of differences between segregating sites; population expansion will result in a significant negative departure from zero (Tajima 1989). Fu and Li's D^* and F^* statistics were used to test for the confounding effect of background selection; significant values are associated with selection (Fu and Li 1993). Comparing F_S , D^* , and F^* can permit discrimination of population expansion from background selection. A significant F_S with non-significant D^* and F^* supports an interpretation of population expansion (Fu 1997).

Of 357 total Swainson's Hawk samples collected, 306 samples were collected during the breeding season prior to dispersal and migration and thus geographic origin could be confirmed. All 357 samples were used in determination of total number of alleles and haplotypes among Swainson's Hawks, initial STRUCTURE cluster identification,

construction of phylogenetic trees, and analysis of demographic history for the total Swainson's Hawk population. For all other analyses, only individuals collected during the breeding season were included in order to insure that sampled individuals were representative of local breeding populations and not migrants.

Results

Microsatellites

All 19 microsatellite loci amplified in Swainson's Hawks with no more than two alleles per individual. The 10% quality control analysis revealed a 0.3% rate of disagreement between initial and secondary scores. In CV mean observed heterozygosity was 0.81 ± 0.01 and the mean number of alleles per locus was 12.74 ± 6.90 . In GBGP mean observed heterozygosity was 0.83 ± 0.01 and the mean number of alleles per locus was 16.63 ± 9.65 . The number of private alleles was generally higher for GBGP than CV, however allelic richness at each locus was similar between populations (Table 1).

Preliminary STRUCTURE analyses indicated that $\log \text{Pr}(X|K)$ declined sharply beyond $K = 5$, consequently the second parameter set focused on K values of 1–5. For the second parameter set, $\log \text{Pr}(X|K)$, averaged across 10 runs, peaked at $K = 2$ and declined beyond $K = 3$ (Fig. 2). Geographic structure was apparent for the two clusters, with one cluster assigning primarily to GBGP and a second occurring mostly in CV with other individuals spread across GBGP (Fig. 3). For $K = 3$ a similar geographic pattern was evident for two of the clusters, while the third cluster appeared evenly in both regions and did not provide additional explanatory value (not shown). No geographic structure was evident when GBGP individuals were analyzed separately and a graph of $\log \text{Pr}(X|K)$ peaked at $K = 1$ (not shown).

Self-assignment of breeding Swainson's Hawks from nine sampling sites resulted in poor assignment to site of origin. In general, Swainson's Hawks sampled from GBGP sampling sites had a higher probability of assigning to a GBGP site than to CV. Similarly, CV individuals generally had a higher probability of assignment to CV than to any of the eight GBGP sampling sites. However, in both GBGP and CV a majority of individuals also had a low but non-trivial probability of assigning to the alternate region indicating that an origin from an alternate region could not be rejected (Table 2).

A pairwise comparison between CV and GBGP resulted in a significant, but small F_{ST} ($F_{ST} = 0.011$, $P < 0.001$) but non-significant R_{ST} ($R_{ST} = 0.001$, $P = 0.24$). When seven of the 19 loci (BswD122w, BswD210w, BswD310w,

Table 1 Polymorphism data for 19 Swainson’s Hawk-specific microsatellite loci tested across 306 individuals

Locus	A	A/locus CV	A/locus GBGP	AR _C CV	AR _C GBGP
BswA110w	13	10 (2)	11 (3)	9.92	10.00
BswD122w	24	16 (1)	23 (8)	15.88	19.78
BswA204w	18	12	18 (6)	11.92	15.49
BswA317w	11	9 (1)	10 (2)	8.92	9.01
BswD210w	26	22	26 (4)	21.90	23.90
BswD220w	15	9	15 (6)	9.00	13.50
BswA303w	12	7	12 (5)	6.99	9.57
BswB111aw	6	6 (1)	5	5.99	4.57
BswD234w	13	9	13 (4)	9.00	10.78
BswD310w	31	24	31 (7)	23.87	25.64
BswD313w	14	10	14 (4)	10.00	12.03
BswB220w	11	8	11 (3)	7.96	10.43
BswB221w	7	5	7 (2)	5.00	6.26
BswD327w	11	8 (1)	10 (3)	8.00	8.59
BswA302w	12	10 (1)	11 (2)	9.96	9.62
BswA312w	15	11	15 (4)	11.00	14.02
BswD107w	49	31 (3)	46 (18)	30.82	35.96
BswD127w	19	16 (2)	17 (3)	15.85	13.78
BswD324w	23	19 (2)	21 (3)	18.77	18.54

Total number of alleles (A), alleles per locus (A/locus) in the California Central Valley (CV) and Great Basin, Great Plains, and southwestern deserts (GBGP) number in parentheses indicates number of private alleles, and allelic richness in each region corrected for sample size (AR_C)

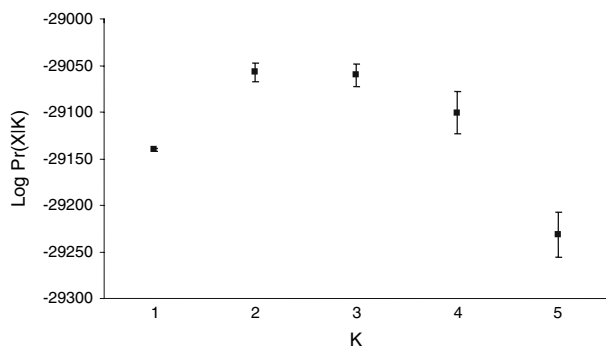


Fig. 2 Log Pr(X|K) for K = 1 through K = 5 ± 1 SE averaged across 10 STRUCTURE runs using the admixture model with a flat prior, a burn-in of 100,000 iterations, and a run length of 1,000,000 iterations

BswD313w, BswD107w, BswD127w, BswD324w, all pentanucleotide repeats) apparently deviating from the SMM (Hull et al. 2006), were removed from the analysis, both F_{ST} and R_{ST} were significant between CV and GBGP ($F_{ST} = 0.014$, $P < 0.001$; $R_{ST} = 0.012$, $P = 0.002$). Pairwise F_{ST} values were significant among a majority of sampling sites while R_{ST} values were entirely nonsignificant (Table 3) when all 19 loci were included. Upon removal of the seven loci not conforming to the SMM, eight pairwise R_{ST} values became significant.

The model results averaged across 50 EASYPOP simulations suggested that a minimum of between 68 and 75

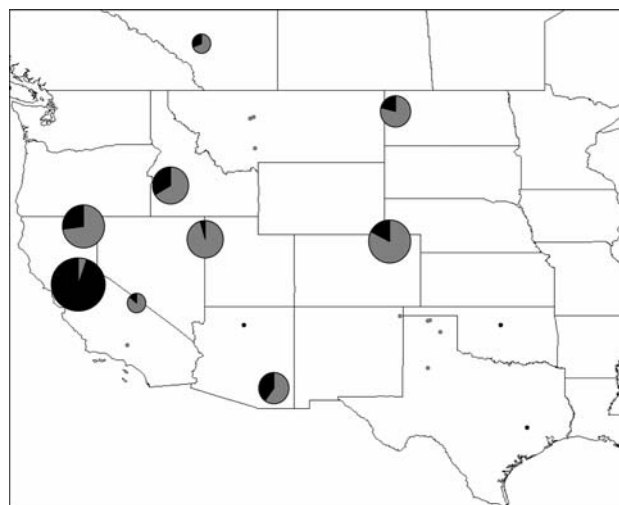


Fig. 3 Assignments using STRUCTURE of Swainson’s Hawks to two genetic clusters. Gray represents a cluster assigning to primarily the Great Basin, Great Plains and southwestern deserts (GBGP) and black shows a cluster found mostly in the California Central Valley (CV) with many individuals in the GBGP. Pie graphs depict the frequency of each cluster assignment at different sampling sites, the size of the graph reflects the number of samples collected at each site. Individual points represent single samples

generations were necessary to achieve the observed degree of population differentiation ($F_{ST} = 0.011$) between CV and GBGP. Using a generation time of three years for

Table 2 Average probability of individual assignment for Swainson’s Hawks sampled from nine sampling localities

Source	Average probability of assignment								
	AB	AZ	CP	NP	ID	IN	MP	UN	CV
AB	0.17	0.13	0.19	0.08	0.08	0.05	0.10	0.42*	0.09
AZ	0.33	0.28	0.13	0.03	0.12	0.06	0.09	0.36*	0.09
CP	0.48	0.18	0.53	0.08	0.37	0.10	0.33	0.64*	0.27
NP	0.56	0.21	0.38	0.29	0.27	0.05	0.31	0.60*	0.22
ID	0.48	0.16	0.32	0.08	0.52*	0.11	0.30	0.52*	0.30
IN	0.39	0.08	0.18	0.06	0.20	0.25	0.25	0.46*	0.13
MP	0.42	0.20	0.26	0.09	0.28	0.13	0.50*	0.50*	0.30
UN	0.32	0.07	0.16	0.03	0.19	0.06	0.11	0.38*	0.08
CV	0.43	0.28	0.44	0.13	0.47	0.12	0.48	0.75	0.87*

AB = Alberta, AZ = Arizona, CP = Central Plains, CV = Central Valley, ID = Idaho, IN = Inyo, MP = Modoc Plateau, NP = Northern Plains, UN = Utah/Nevada, *highest probability of assignment

Swainson’s Hawks (England et al. 1997), the time in generations required to attain the observed differentiation corresponds to roughly 200 years.

No significant relationship was found between genetic and geographic distance ($P = 0.43$, $r = 0.046$) among all nine sampling sites. However, when the two sampling sites with fewer than 15 samples (Alberta and Inyo) were removed from the analysis a significant effect of isolation-by-distance was found ($P = 0.04$, $r = 0.48$).

A significant excess of heterozygotes ($P < 0.001$ IAM, $P = 0.006$ TPM, Wilcoxon sign-rank test) was detected in the total population of Swainson’s Hawks indicating a relatively recent (within the past century) range-wide bottleneck. When considered separately, a significant excess of heterozygotes was detected in both CV and GBGP suggesting that both CV ($P < 0.001$ IAM, $P = 0.005$ TPM, Wilcoxon sign-rank test) and GBGP ($P < 0.001$ IAM, $P = 0.002$ TPM, Wilcoxon sign-rank test) regions have experienced recent bottlenecks in effective population size.

Control region sequences

A total of 279 control region sequences were generated following removal of individuals sampled outside the breeding season and cases of poor sequencing reactions. All sequences were archived in GenBank (accession numbers EF568738–EF568773). No evidence of nuclear copies of the control region was observed: replicate sequences from DNA extracted from feathers and blood yielded identical sequences, and no double peaks were observed in the electropherograms (see Sorenson and Quinn 1998). Additionally, sequences aligned with previously published *Buteo* control region sequences. Control region sequencing revealed 24 (5.7%) variable sites among Swainson’s Hawks, resulting in 33 haplotypes in 416 base pairs of control region sequence among 279 Swainson’s Hawks. Of these, 21 sites were parsimony informative. Among all samples haplotype diversity was 0.608 ± 0.035 with an associated nucleotide diversity of 0.00394. The

Table 3 Genetic differentiation at 19 microsatellite loci between Swainson’s Hawk sampling localities; F_{ST} below diagonal, R_{ST} above diagonal

	AB	AZ	CP	NP	ID	IN	MP	UN	CV
AB		0.0003	-0.0069	-0.0031	0.0184	0.0011	-0.0136	0.0001	-0.0139
AZ	0.0009		0.0330	0.0278 ^a	0.0162	0.0088	0.0123	0.0174 ^a	0.0117
CP	0.0002	0.0123*		0.0188	0.0141	0.0405	0.0113	0.0057	0.0016
NP	0.0052	0.0227*	0.0137*		0.0018 ^a	0.0393 ^a	0.0009	0.0388 ^a	0.0172 ^a
ID	0.0062	0.0147*	0.0055	0.0148*		0.0347 ^a	-0.0085	0.0307	0.0018
IN	0.0098	0.0250*	0.0167*	0.0358*	0.0236*		0.0294	0.0055	0.0260
MP	0.0037	0.0149*	0.0089*	0.0188*	0.0103*	0.0140*		0.0250	0.0056
UN	-0.001	0.0068	0.0036	0.0135*	0.0071	0.0211*	0.0114*		0.0140 ^a
CV	0.0148*	0.0149*	0.0162*	0.0193*	0.0156*	0.0300*	0.0161*	0.0152*	

* Significant pair-wise F_{ST} values following Bonferroni correction, ^a significant pair-wise R_{ST} values following removal of seven loci not conforming to the SMM and Bonferroni correction (AB = Alberta, AZ = Arizona, CP = Central Plains, CV = Central Valley, ID = Idaho, IN = Inyo, MP = Modoc Plateau, NP = Northern Plains, UN = Utah/Nevada)

average number of nucleotide differences among haplotypes was 1.638. Among 208 GBGP breeding samples, 28 haplotypes were identified (23 unique to GBGP) and 23 variable nucleotide positions, the average number of differences between GBGP haplotypes was 1.586. GBGP haplotype diversity was 0.573 ± 0.041 and nucleotide diversity was 0.00381. A total of 71 CV Swainson's Hawks yielded 8 haplotypes (3 unique to CV) among 14 variable sites with an average of 1.366 nucleotide differences between each haplotype. Haplotype and nucleotide diversity in CV were 0.590 ± 0.062 and 0.00328, respectively. Two singleton haplotypes were identified in individuals sampled outside of the breeding season.

Bootstrap analysis of maximum-likelihood and maximum parsimony trees and Bayesian posterior probabilities found 100% support for the distinction between the Swainson's Hawk clade and Ferruginous Hawks. Very limited support for differentiation among any of the Swainson's Hawk haplotypes was detected, and no geographic structure was evident. The nodes with weak bootstrap and Bayesian support do not correspond to particular regions and samples from the CV and GBGP are interspersed throughout the tree.

A minimum spanning network of all haplotypes revealed moderate structuring of haplotypes and suggested that haplotype A may have been the progenitor of the majority of Swainson's Hawk haplotypes (Fig. 4a). In contrast to GBGP, CV minimum spanning network displayed several missing intermediate haplotypes even though CV was the most extensively sampled site in the study (Fig. 4b and c). By combining samples from Modoc Plateau and Idaho a sample size ($n = 74$) similar to CV ($n = 71$) was achieved. The minimum spanning network of this partial GBGP data (Fig. 4d) set revealed twice as many haplotypes (16) and fewer missing steps than the CV group, suggesting the CV may have experienced a loss of haplotypes.

A pairwise comparison between CV and GBGP resulted in a non-significant Φ_{ST} using both haplotype frequencies and pairwise distances following Bonferroni correction ($\Phi_{ST} = 0.016$, $P = 0.026$ and $\Phi_{ST} = 0.025$, $P = 0.003$). Pairwise Φ_{ST} values were non-significant except in comparisons of Alberta with Arizona, Arizona with Northern Plains, Central Plains with Central Valley, and Alberta with Central Valley when using a pairwise distance estimate of Φ_{ST} (Table 4). All pairwise comparisons were non-significant when using haplotype frequencies. A Mantel test failed to reveal a significant correlation between genetic and geographic distance for control region sequences when comparing all sites ($P = 0.056$, $r = 0.333$) as well as in comparison of the seven sites with greater than 15 samples ($P = 0.123$, $r = 0.297$).

We evaluated the historical demography of pooled Swainson's Hawks from all sites as well as GBGP and CV

individually. In all three cases mismatch distributions closely fit the unimodal distribution expected for a recent population expansion (Total $\tau = 1.01$, range 0.00–11.61; GBGP $\tau = 0.00$, range 0.00–8.382; CV $\tau = 0.256$, range 0.00–6.166) and in each case the raggedness index was low (Total $rg = 0.047$, $P < 0.86$; GBGP $rg = 0.063$, $P < 0.79$; CV $rg = 0.049$, $P < 0.81$). Tajima's D was not significant in any of the groupings examined (Total $D = -1.57$, $P > 0.05$; GBGP $D = -1.61$, $P > 0.05$; CV $D = -1.52$, $P > 0.10$). Fu's F_S statistic was significant in the total population ($F_S = -28.437$, $P < 0.001$) and in the GBGP ($F_S = -21.824$, $P < 0.001$). In the CV, F_S was qualitatively much smaller and non-significant ($F_S = -2.120$, $P = 0.06$). Fu and Li's F^* and D^* were not significant in each case (Total $D^* = 0.379$, $P > 0.10$, $F^* = -0.521$, $P > 0.10$; GBGP $D^* = 0.427$, $P > 0.10$, $F^* = -0.475$, $P > 0.10$; CV $D^* = 0.495$, $P > 0.10$, $F^* = -0.250$, $P > 0.10$) suggesting selection was not responsible for the observed pattern. Expansion coefficients for the total sample set and GBGP were found to have large values of similar magnitudes (Total $S/d = 15.96$; GBGP $S/d = 14.50$), while the expansion coefficient was somewhat smaller in for CV ($S/d = 10.25$).

Discussion

Genetic diversity and population structure

Genetic data from multi-locus microsatellite genotypes suggests that Swainson's Hawks are subdivided into two clusters with one cluster primarily occurring in the Great Basin, Great Plains, and southwestern deserts (GBGP) and a second cluster found mostly in the California Central Valley (CV) with several individuals spread across the GBGP (Fig. 3). The observed heterozygosity was quite high and similar between the GBGP and CV as was allelic richness at each locus while a greater number of private alleles occurred in the GBGP than in the CV.

The overall genetic differentiation between GBGP and CV appears limited in absolute terms (as indicated by pairwise F_{ST} estimates) and is additionally accompanied by a pattern of isolation by distance. The self-assignment of individuals generally assigned samples to their region of origin (GBGP or CV) but not to individual sampling site. Many samples also had a low, but not negligible, probability of assigning to the alternate region, reflecting an overall low degree of differentiation between regions. Simulated populations help to provide a context for interpreting the low level of observed differentiation. The simulation conservatively suggests that this degree of differentiation would require approximately 200 years to develop; a timeframe corresponding roughly with European

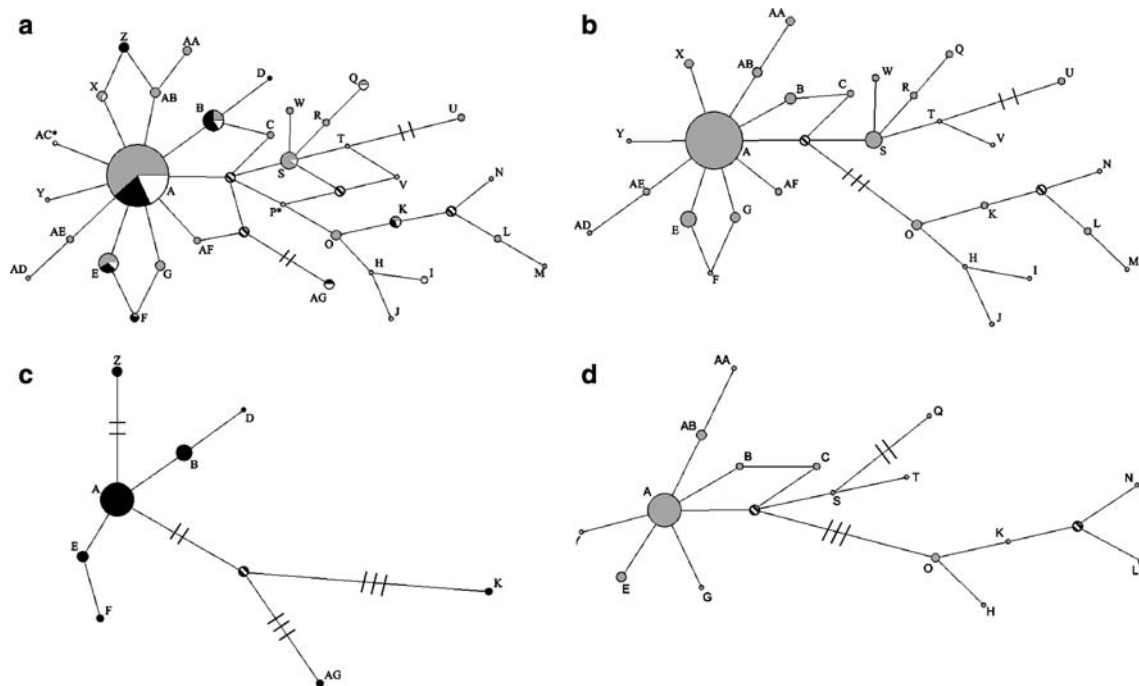


Fig. 4 Minimum spanning networks depicting absolute difference between haplotypes for **(A)** all sampling localities where the proportion of each haplotype occurring in the Great Basin, Great Plains and southwestern deserts (GBGP) is shaded in gray, the proportion occurring in the California Central Valley (CV) is shaded in black, and the proportion sampled outside of the breeding season is white, **(B)** GBGP sampling localities, **(C)** CV sampling sites, and **(D)** a partial GBGP sample composed of the Modoc Plateau and Idaho

sampling sites. Distinct haplotypes are labeled A through AF. The relative abundance of each haplotype is indicated by the size of the circles; hash marks indicate the number of mutated positions between haplotypes, where no mark occurs a single mutation is implied; * indicates haplotypes that were sampled exclusively outside of the breeding season; cross-hatched circles indicate missing haplotypes inferred by the analysis

Table 4 Genetic differentiation at 416 base pairs of control region sequence between nine Swainson’s Hawk sampling localities; pairwise Φ_{ST} values based upon haplotype frequencies below the diagonal and pairwise Φ_{ST} using the distance method above the diagonal

	AB	AZ	CP	NP	ID	IN	MP	UN	CV
AB		0.2073*	0.0432	-0.0266	0.0294	0.1080	0.0550	0.0429	0.1609*
AZ	0.0904		0.1134	0.0974*	0.0654	0.1202	0.0423	0.0991	0.0135
CP	0.0309	0.0206		0.0230	0.0430	0.0139	0.0478	-0.0054	0.0802*
NP	0.0071	0.0271	-0.0087		-0.0244	0.0574	0.0041	-0.0072	0.0722
ID	0.0421	0.0204	0.0107	0.0126		0.0547	0.0024	0.0153	0.0442
IN	0.0345	0.1032	0.0258	0.0641	0.0433		0.0529	0.0753	0.0490
MP	0.0868	0.0032	-0.0001	0.0137	0.0184	0.0765		0.0172	0.0134
UN	0.1111	0.0209	0.0151	0.0074	0.0466	0.1606	0.0019		0.0518
CV	0.0689	0.0112	0.0180	0.0323	0.0286	0.0610	0.0056	0.0435	

* Significant pairwise Φ_{ST} values following Bonferroni correction (AB = Alberta, AZ = Arizona, CP = Central Plains, CV = Central Valley, ID = Idaho, IN = Inyo, MP = Modoc Plateau, NP = Northern Plains, UN = Utah/Nevada)

settlement of western North America. Given the number of parameters that were estimated for the model, the precision of this estimate may be limited. However, the parameters were estimated purposefully in a way to minimize time since divergence (e.g., no migration). The resulting simulation data do indicate that the observed differentiation would not be achieved in a few generations, suggesting that

the degree of differentiation observed may not be due to short-term stochastic fluctuations in Swainson’s Hawk populations.

Interpretation of the significant, yet low, differentiation observed in the microsatellite data should be undertaken with caution. Considering the large sample sizes used here and the number of highly polymorphic markers employed, this study

had high statistical power to detect fine-scale population differentiation. Thus, very small differences between populations may be detected as statistically significant although differences may not be biologically meaningful (Hedrick 1999). Among Swainson's Hawks, differentiation between CV and GBGP is lower than reported in a previous investigation of clearly isolated island populations of Galapagos Hawks, phylogenetically the closest relative of Swainson's Hawks, using minisatellite data ($F_{ST} = 0.017\text{--}0.896$; Bollmer et al. 2005). While different types of molecular markers were employed, and the population size of the two species is drastically different, the difference in observed differentiation between Swainson's Hawks and Galapagos Hawks suggests that Swainson's Hawks have yet to reach the same level of isolation as found between island populations of Galapagos Hawks.

The absence of significant mitochondrial differentiation between CV and GBGP along with quite low values for Φ_{ST} between sampling sites indicates no population structure is present among mitochondrial control region lineages. Similar pairwise measures of Φ_{ST} were found between sampling sites in a continent-wide study of White-bellied Sea-Eagles in Australia ($\Phi_{ST} = 0\text{--}0.126$) where a high level of population connectivity was documented (Shepard et al. 2005). The absence of a correlation between genetic and geographic distance indicates that Swainson's Hawks are effectively panmictic with respect to the mitochondrial genome. As an effectively single non-recombining marker, the control region sequence data may not have enough statistical power to detect population structure at low levels.

Historical demography

For the total population and GBGP, control region sequences reveal a recent population expansion in Swainson's Hawks. Evidence of expansion is inferred from the close fit of mismatch distributions with theoretical expectations, low nucleotide diversities accompanied by high haplotype diversities, large expansion coefficients, significant F_S statistic, and star-like minimum spanning networks and control region phylogeny. The CV data are more complex with expansion suggested by the mismatch distribution and large expansion coefficient, but not by the F_S statistic which was non-significant and much smaller than in the total population and GBGP. The non-significant values obtained for Fu and Li's F^* and D^* statistics in all comparisons suggest that the observed patterns are not a consequence of background selection.

Considering the range of reported mutation rates for the avian control region domain I (Wenink 1996; Baker and Marshall 1997), the limited raptor fossil record available for estimating a Swainson's Hawk specific mutation rate, and error associated with expansion statistics, determining

timing of a possible population expansion with accuracy would be difficult. Previous studies of North American taxa have frequently detected signals of population expansion associated with the most recent glacial maxima approximately 18,000 years ago (Milot et al. 2000; Hull and Girman 2005). Retreat of glacial ice clearly provided Swainson's Hawks with the opportunity to occupy the northern plains and may have resulted in the signature of population expansion seen in control region data for the total population and GBGP.

The microsatellite data provides an interesting contrast to the evidence of population expansion seen in the mitochondria. For both CV and GBGP a significant excess of heterozygotes indicates that Swainson's Hawks have experienced recent population bottlenecks. The bottlenecks are likely associated with declines occurring during the 19th and 20th centuries as human settlement resulted in increased habitat loss and direct mortality (England et al. 1997). A severe population reduction among California Swainson's Hawks may have been responsible for the loss of control region haplotypes as indicated by the contrasting CV and partial GBGP minimum spanning networks, eight haplotypes versus 16 haplotypes. A loss of haplotypes may have consequently reduced the signal of previous population expansion.

Conservation implications

The highly polymorphic microsatellite loci employed here resulted in an observed heterozygosity of 0.81 for CV Swainson's Hawks. Using less polymorphic loci, previous studies have observed lower heterozygosity in several raptor species ranging from 0.099 in the Mauritius Kestrel (*Falco punctatus*) to 0.614 in the Lesser Kestrel (*F. naumanni*) (Nichols et al. 2001; Martínez-Cruz et al. 2004). The high level of observed heterozygosity at microsatellite loci among Swainson's Hawks occurring in CV belies the apparent loss of rare alleles which suggests that some evolutionary potential may have been lost. This assertion cannot be established definitively without a comparative sample collected prior to the bottleneck. In parallel with loss of rare microsatellite alleles, the minimum spanning network for CV suggests that several intermediate haplotypes may have been lost as a consequence of population decline.

The absence of both reciprocally monophyletic groups in control region data and significant allele frequency differences indicates that the Swainson's Hawks in CV do not meet the standards proposed by Moritz (1994) for an evolutionarily significant unit. The pattern suggested by the data is one of effective panmixia for control region and limited differentiation for the microsatellite loci. As discussed above, interpretation of weak differentiation between Swainson's Hawk populations can be challenging.

The observed divergence may reflect either an absence of population structure or incipient divergence, as seen in the analysis of simulated populations. Incipient divergence may be associated with recent changes observed in migratory behavior, a highly plastic trait (Berthold et al. 1992), among CV Swainson's Hawks. In contrast to the GBGP, where over-wintering Swainson's Hawks have not been documented, Swainson's Hawks in CV have recently established a winter population (Herzog 1996; Wheeler 2003). This change in behavior, along with the use of coastal western Mexico as wintering range by CV Swainson's Hawks (Wheeler 2003), may reflect an ecological response to changing environmental conditions, possibly local agriculture practices or climatic changes. Such changes in behavior could ultimately contribute to nascent divergence in Swainson's Hawks. As demonstrated by their migratory abilities, Swainson's Hawks are clearly capable of long distance dispersal which might preclude the formation of population structure. The limited dispersal observed among adults and juveniles in California (Estep 1989; Woodbridge et al. 1995) suggests that these birds may not mix as readily with individuals from other regions potentially allowing genetically distinct populations to emerge. The mechanism limiting dispersal within California Swainson's Hawks is not understood.

While the genetic data is somewhat ambiguous relative to distinct population segments, differences between CV and GBGP in traits with potential fitness consequences, such as migratory behavior and morphology, along with a precipitous decline in numbers in California call for careful conservation, management, and monitoring of Swainson's Hawks of the Central Valley. If populations are allowed to continue to decline and fragment, genetic diversity may be lost at an ever increasing rate as has been observed previously in raptors (Red Kite, Roques and Negro 2005; Spanish Imperial Eagle, Martínez-Cruz et al. 2004; California Condor, Geyer et al. 1993), and ultimately require an expensive and time consuming recovery effort. Continued efforts should be directed towards monitoring of census population size in the Central Valley, determination of population trends, periodic evaluation of genetic diversity and population structure as census numbers change, and habitat conservation.

Acknowledgments We would like to thank the Arizona Game and Fish Department, Calgary Wildlife Rehabilitation Society, California Department of Fish and Game, Cascades Raptor Center, Golden Gate Raptor Observatory, HawkWatch International, Laramie Raptor Refuge, Last Chance Forever, Lindsay Wildlife Hospital, Montana Raptor Center, Rocky Mountain Raptor Project, South Plains Refuge, Swainson's Hawk Technical Advisory Committee, UC Davis Raptor Center, UC Davis Veterinary Teaching Hospital, C Boal, P Bloom, J Papp, B Mattox, J McKinley, J Mulholland, R Murphy, T Bollinger, P Parker, S Blackman, A Disbrow, D Racine, C Briggs, and M Dorin for assistance with sample collection. S Brown, J Eadie, A Hull, T

Hull, J Keane, J Kurushima, B May, B Sacks, W Savage, B Stedman, L Tell, J Well, and many others for their technical assistance and advice. Financial support for this project was provided by the Swainson's Hawk Technical Advisory Committee (via the California Department of Water Resources and the California Department of Fish and Game), the University of California Genetic Resources Conservation Program, the Veterinary Genetics Laboratory at UC Davis, and graduate student funding support through the UC Davis Graduate Group in Ecology. We thank associate editor Stuart Piertney and two anonymous reviewers for their comments on previous versions of this manuscript.

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