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Landscape characteristics influence morphological and genetic differentiation in a widespread raptor (*Buteo jamaicensis*)

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Abstract

Landscape-scale population genetic structure in vagile vertebrates was commonly considered to be a contradiction in terms whereas recent studies have demonstrated behaviour and habitat associated structure in several such species. We investigate whether landscape features influence morphological and genetic differentiation in a widespread, mobile raptor. To accurately describe genetic differentiation associated with regional landscape factors, we first investigated subspecies relationships at a continental scale. We used 17 microsatellite loci and five morphological measurements to investigate differentiation between eastern and western subspecies of red-tailed hawks (*Buteo jamaicensis*) and to identify patterns between differentiation and habitat within western North America. Bayesian and frequency-based analyses of microsatellite data revealed clear distinctions between *B. j. borealis* (eastern) and *B. j. calurus* (western) samples. Furthermore, hawks sampled in Texas were stouter than those collected from the Rocky Mountains and farther west. Among western samples, birds from the Great Basin, Rocky Mountains, and Washington were significantly different in morphology than those from Oregon and California. We identified a pattern of isolation by distance among western breeding sites around the Sierra Nevada. Given the long-range dispersal capabilities of raptors, this pattern suggests that population-specific habitat preferences, corresponding with habitat breaks between eastern and western slopes of the Sierra Nevada, and/or regionally variable population densities limit migration between the Mediterranean habitat of central California and the xeric habitats of southern California and interior west. We suggest habitat preferences and regionally disparate population densities may play a role in shaping genetic structure in vagile avian taxa.

Keywords: *Buteo jamaicensis*, habitat, microsatellite, mitochondrial, morphology, red-tailed hawk

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Introduction

Until recently, there has been little interest in the landscape-scale population genetic structure of highly vagile organisms because of the assumption that the high levels of gene flow would prevent population genetic structure at smaller spatial scales (Roy *et al*. 1994; Paetkau *et al*. 1998). This assumption would be valid if gene flow were spatially random. However, a host of recent studies on wide-ranging terrestrial vertebrates indicate that this often is not the case and that populations can exhibit high levels of structure on the landscape scale (Geffen *et al*. 2004; Manel *et al.* 2003; Sacks *et al*. 2004; McRae *et al*. 2005; Pilot *et al*. 2006; Boulet *et al*. 2007; Carmichael *et al*. 2007). In particular, habitat has been identified as an important determinant of population genetic structure in wide-ranging habitat generalists even where no physical barriers exist.

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Although there is evidence that birds may have dispersal biases with respect to habitat (Davis & Stamps 2004), little is known of the population genetic consequences of such behaviour, especially in wider-ranging species. Habitat-biased dispersal has been found to strongly influence population genetic structure among island populations of birds, overriding patterns driven by geographical distance (Tonnis *et al*. 2005). However, the influence of habitat on population genetic structure in birds has primarily been observed among species with limited distributions (Cicero & Johnson 1998; Cicero 2004). Studies of more widespread species have tended to focus on geographical distance and Pleistocene vicariance events, which manifest themselves on larger scales (Merliä *et al*. 1997; Irwin 2002; Godoy *et al*. 2004; Spellman *et al*. 2007). For example, broadly distributed avian species often display genetically distinct clades, associated with isolated Pleistocene glacial refugia, but rarely exhibit population structure within clades (Milot *et al*. 2000; Kimura *et al*. 2002; Peters *et al*. 2005). The absence of population substructure has been attributed to high levels of contemporary gene exchange and post-Pleistocene population expansion (Gibbs *et al*. 2000; Hull & Girman 2005). However, use of mtDNA markers might fail to show population substructure occurring because of contemporary patterns of gene flow as shaped by habitats over the landscape scale.

A contributing factor to absence of habitat-associated population structure in widespread species of birds may be that many of the species investigated to date have distributions restricted to particular habitats (Milot *et al*. 2000; Kimura *et al*. 2002; Peters *et al*. 2005). A promising avenue of investigation is the study of widespread, habitat generalists which might display genetic structure concordant with habitat breaks. Such research provides an opportunity to gain further insights about the relative importance of Pleistocene refugia and landscape features in shaping genetic structure in broadly distributed, mobile organisms. Here, we investigate these questions in a widespread North American raptor, the red-tailed hawk (*Buteo jamaicensis*).

Red-tailed hawks are a polytypic raptor distributed throughout Central and North America and breed in all available habitats except tundra, including scrub desert, plains, broken coniferous forests, deciduous woodlands, and tropical rainforests (Preston & Beane 1993). They display a high degree of territory fidelity suggesting that fine-scale population structure may exist within regional populations (Janes 1984). Here, we investigate the population genetic structure of three subspecies of red-tailed hawks, *Buteo jamaicensis calurus*, *B. j. borealis*, and *B. j. fuertesi*. *Buteo jamaicensis calurus* is found throughout western North America from Alberta to southern Arizona and New Mexico, *B. j. borealis* in eastern North America from northeastern Canada south to the Gulf Coast and west through the Great Plains, and *B. j. fuertesi* in south-central North America (Fig. 1). Comparison of *B. j. calurus*, *B. j. borealis*, and *B. j. fuertesi* provides an opportunity to compare observed continental-scale genetic differentiation to previous investigations of the effects of Pleistocene refugia on avian population differentiation.

Additional examination of *B. j. calurus* within the spatially heterogeneous landscape of western North America permits investigation of whether habitat breaks contribute to observed patterns of genetic differentiation. Regional ecogeographical morphological variation has been described within breeding and wintering *B. j. calurus*, with the largest hawks occurring in southwestern deserts and California grasslands and the smallest found in forests of the Pacific Northwest (Fitzpatrick & Dunk 1999). A similar pattern has been observed in Northern goshawks (*Accipiter gentilis*; Squires & Reynolds 1997) while the opposite is seen in great horned owls (*Bubo virginianus*; McGillivray 1989). These habitat-associated morphological differences suggest that genetic partitions may also be associated with habitat breaks.

The primary objective of this study was to use highly polymorphic microsatellite loci to investigate relationships between population genetic structure and landscape features among red-tailed hawks in western North America. In order to effectively elucidate patterns within western North America and to separate contemporary from historical influences, three topics were addressed:

1 *Subspecies distinctions*: determine whether three described subspecies (*B. j. borealis*, *B. j. calurus*, and *B. j. fuertesi*) displayed genetic differentiation from one another.
2 *Continental-scale influences*: describe the possible landscape and historical factors responsible for shaping population genetic structure at the continental scale.
3 *Regional landscape factors*: investigate whether genetic differentiation could be detected at a finer scale among western red-tailed hawks (*B. j. calurus*). *Buteo jamaicensis calurus* is a promising choice for investigations of landscape effects on population genetic structure because of the heterogeneity of environment and topography found throughout their western North American range. Since size variation within *B. j. calurus* has been previously documented (Fitzpatrick & Dunk 1999), we sought to determine whether morphological variation was correlated with observed population genetic variation among western red-tailed hawk populations.

Together, these study objectives provide an opportunity to understand the roles of isolation, geographical distance, and habitat features on differentiation in a mobile, widespread species.

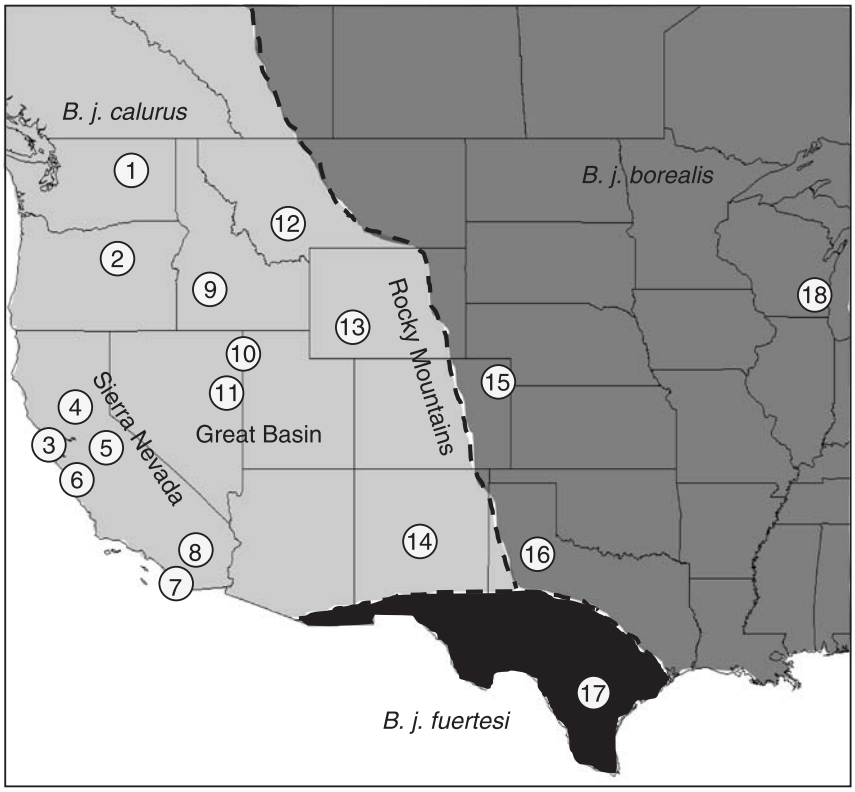

Fig. 1 Red-tailed Hawk sampling locations. 1, Washington migration, $n = 27$; 2, Oregon migration, $n = 80$; 3, coastal California migration, $n = 265$; 4, Central Valley migration, $n = 220$; 5, Central Valley breeding, $n = 9$; 6, coastal California breeding, $n = 58$; 7, southern California migration, $n = 21$; 8, southern California breeding, $n = 36$; 9, Idaho breeding, $n = 29$; 10, Nevada/Utah breeding, $n = 41$; 11, Nevada migration, $n = 93$; 12, Montana breeding, $n = 6$; 13, Wyoming migration, $n = 14$; 14, New Mexico migration, $n = 92$; 15, central plains breeding, $n = 6$; 16, Texas breeding, $n = 5$; 17, Texas migration, $n = 47$; 18, Wisconsin breeding, $n = 34$. Breeding samples were collected during the months of May through mid-July. Migration samples include migration and wintering hawks collected between mid-August and mid-March. The dashed north–south line reflects the subspecies boundary between *Buteo jamaicensis calurus* (west; light grey shading) and *Buteo jamaicensis borealis* (east; dark grey shading). South of the dashed east–west line reflects the range of *Buteo jamaicensis fuertesi* (black shading).

Materials and methods

Sample collection

Whole blood and contour feathers were collected from a total of 1083 red-tailed hawks at 18 sites (nine breeding and nine migration/winter) from California, east to Wisconsin, north through Montana, and south through Texas (Table 1, Fig. 1) between 2003 and 2006. Samples from 357 Swainson's hawks (*Buteo swainsoni*) were included for outgroup comparison. Approximately 0.2 mL of blood was drawn from each individual 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 dry in paper envelopes at 22 °C. Samples were collected from free-flying adults and juveniles during migration and on wintering grounds using bownets, mistnets, dho-ghazzas, and bal-chatris (Berger & Meuller 1959; Clark 1970; Bloom 1987; Bub 1991), and from prefledge young in nests by licensed raptor biologists as well as from juveniles and adults treated at several wildlife rehabilitation facilities. Wild birds were leg-banded with US Geological Survey bands and either released or returned to nests. Only one individual per presumed family group (nestlings and parents) was included in sampling for this study, consequently sample size and number of sampling points are equivalent for breeding sites. 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 transferred to –80 °C upon completion of work.

Microsatellite data collection

Each individual was genotyped at 17 microsatellite loci (BswA110w, BswD122w, BswA204w, BswA317w, BswD210w, BswD220w, BswA303w, BswB111aw, BswD234w, BswD310w,

Table 1 Sample location, season, and sample size for 18 red-tailed hawk sampling localities. Subscripts $_B$ and $_M$ indicate breeding season and migration samples, respectively

Site	Location	Subspecies	Genetic	Morphology
CC_B	Coastal California	*Buteo jamaicensis calurus*	58	0
CV_B	Central Valley	*B. j. calurus*	9	0
ID_B	Idaho	*B. j. calurus*	29	0
MT_B	Montana	*B. j. calurus*	6	0
NV_B	Nevada	*B. j. calurus*	41	0
SC_B	Southern California	*B. j. calurus*	36	0
CP_B	Central Plains	*Buteo jamaicensis borealis*	6	0
TX_B	Texas	*B. j. borealis*	5	0
WI_B	Wisconsin	*B. j. borealis*	34	0
CC_M	Coastal California	*B. j. calurus*	265	228
CV_M	Central Valley	*B. j. calurus*	220	121
NM_M	New Mexico	*B. j. calurus*	92	108
NV_M	Nevada	*B. j. calurus*	93	104
OR_M	Oregon	*B. j. calurus*	80	68
SC_M	Southern California	*B. j. calurus*	21	0
WA_M	Washington	*B. j. calurus*	27	12
WY_M	Wyoming	*B. j. calurus*	14	12
TX_M	Texas	*Buteo jamaicensis fuertesi*	47	16
Total	—	—	1083	669

BswD313w, BswB220w, BswB221w, BswD327w, BswA302w, BswA312w, and BswD127w) in six multiplex polymerase chain reactions (PCR) following the conditions described in Hull *et al*. 2007a. PCR products were separated with a 3730 DNA Analyser (Applied Biosystems Inc.) and PCR products visualized and scored with STRAND version 2.3.69 (Toonen & Hughes 2001).

Analysis of genetic diversity

Samples from all 18 sampling sites were tested for deviations from Hardy–Weinberg equilibrium (HWE) 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 & Thompson 1992). Genotypic linkage equilibrium was tested using Fisher's exact test in the program GENEPOP with 1000 dememorization steps, 100 batches, and 1000 iterations per batch (Raymond & Rousset 1995). A sequential Bonferroni correction for multiple tests was used in HWE and linkage equilibrium analyses (Rice 1989). We tested for presence of null alleles using MICRO-CHECKER (van Oosterhout *et al*. 2004). Scoring error was evaluated by re-amplifying and analysing 10% of the samples at all loci and calculating the frequency of disagreement between runs. Heterozygosity and number of alleles per region were quantified using MICROSATELLITE TOOLKIT (Park 2001). The number of private alleles was calculated in CONVERT (Glaubitz 2004), and allelic richness, which accounts for variation in sample sizes, was calculated in FSTAT (Goudet 1995).

Previous research indicated that occasional hybridization occurs among *Buteo* species (Clark & Witt 2006; Hull *et al*. 2007b). We performed assignment tests in the program STRUCTURE to test for the presence of hybrid individuals among our samples (Pritchard *et al*. 2000). Samples were assigned to either a red-tailed or Swainson's hawk population using the 'pop info' option. The relationship of potential hybrids to red-tailed and Swainson's hawk samples was then visualized using a factorial correspondence analysis (FCA) in the program GENETIX 4.05.2 (Belkhir *et al*. 2000). Presumed hybrid samples were removed from further analyses.

Differentiation among populations

To visualize the relationships between sampling localities with greater than 10 individuals, average allele frequency differences among sampling sites was computed (Nei 1987) using 10 000 bootstrap replicates. The resulting divergence matrices were used, with both neighbour-joining and UPGMA algorithms, to make a clustering tree using the program PHYLIP (Felsenstein 1989).

STRUCTURE (Pritchard *et al*. 2000) was subsequently used to probabilistically cluster individuals from all regions, and separately for western samples, based upon their multilocus genotypes without using a priori information on sampling locality of origin. A preliminary analysis using the admixture model with a flat prior, correlated allele frequencies (Falush *et al*. 2003), a burn-in of 10 000 iterations, and run length of 100 000 iterations was conducted for $K = 1$ through 10. This parameter set was performed 10 times and the log $\Pr(X|K)$ statistic averaged across runs. Based upon preliminary results, burn-in and run length were increased to 500 000 and 1 000 000, respectively, for $K = 1$

through 5 and the log Pr(X | *K*) statistic was averaged across 10 runs. The most likely number of clusters, *K*, was chosen by determining the *K* value where averaged log Pr(X | *K*) was maximized and subsequently selecting the minimum value for *K* that did not sacrifice significant explanatory power (Pritchard & Wen 2002). Recently, this method, although partly subjective, has been shown to perform as well or better than the method presented by Evanno *et al*. (2005; Waples & Gaggiotti 2006), especially in cases of moderate differentiation. We defined individual membership to a cluster based upon the highest membership assignment probability.

Differentiation between sampling sites was estimated in ARLEQUIN using pairwise F_{ST} comparisons. Analysis of molecular variance (AMOVA) was used to test for differentiation among four a priori groups: (i) breeding samples from presumed subspecies [*Buteo jamaicensisborealis*: sites WI_B, CP_B, TX_B (see Table 1 for explanation of site codes); *Buteo jamaicensis calurus*: ID_B, MT_B, NV_B, SC_B, CV_B, CC_B; see Fig. 1]; (ii) breeding samples within *B. j. calurus* grouped by STRUCTURE results; (iii) migration and wintering samples grouped by band-recovery patterns (Pacific: WA_M, OR_M, CC_B, CV_B, CV_M, CM_M, SC_M; Great Basin/Rocky Mountain: ID_B, MT_B, NV_B, NV_M, NM_M, WY_M, SC_B); and (iv) observed morphological differences in migratory and wintering samples discerned from principal components analysis. A sequential Bonferroni correction for multiple tests was used to determine significance of F_{ST} values among sampling locations and regions (Rice 1989).

To provide a coarse estimate of the time since eastern and western populations diverged, we modelled two hypothetical populations using the simulation software EASYPOP (Balloux 2001). Population parameters were monogamy (Preston & Beane 1993), equal sex ratios, no migration, infinite alleles model, a mutation rate of 0.0001, and effective population estimates of 30 000 individuals per population (census population size in excess of 500 000). These parameters are not likely to precisely reflect biological reality; however, they were selected in order to provide a minimum estimate of time since divergence (e.g. no migration between populations). The model was allowed to run for 10 000 generations and divergence estimates were averaged across 10 runs. Time in generations was converted to absolute time using a generation time of 3 years (Preston & Beane 1993).

Landscape and genetic relationships

We used Monmonier's maximum distance algorithm, as implemented in BARRIER version 2.2 (Manni *et al*. 2004), to help interpret where barriers to gene flow may occur. We computed two barriers to test for breaks between eastern and western subspecies and to test for presence of a barrier within western populations. Barrier confidence was assessed using 1000 replicate matrices of genetic distance based on bootstrapped data sets. To further investigate the relationship between geography and genetic distance, a Mantel test (Smouse *et al*. 1986) was used in ARLEQUIN to test for a correlation between pairwise genetic and geographical distances among four western breeding populations for which we had sufficient sample size. The dependent variable in this analysis was a matrix of pairwise genetic distances expressed as $F_{ST}/(1 - F_{ST})$ (Slatkin 1995) while the independent matrix was composed of pairwise geographical distances between centroids of sampling localities. We investigated these relationships using both straight-line distances between sampling sites and the circumferential distance between sites around the Sierra Nevada (CC_B to SC_B to NV_B to ID_B).

Morphological data collection

Wing-chord, tail length, hallux length, exposed culmen length, and mass (Golden Gate Raptor Observatory 1998) were recorded from 669 juvenile migrating and wintering red-tailed hawks sampled in California (coastal, and Central Valley regions), Oregon, Washington, Nevada, Texas, New Mexico, and Wyoming (Table 1). Individuals were identified as male or female based upon the methods of Donohue & Dufty (2006) and Pitzer *et al*. (in press).

Morphological data analysis

We calculated means and standard deviations for each metric and then standardized the data according to variance. We used principal components analysis (PCA) to describe morphological differences between juvenile red-tailed hawks sampled at eight migration and winter sites. Eigenvectors were rotated using varimax rotation (Krzanowski 2000). Because of sexual size dimorphism (Preston & Beane 1993), PCA was conducted separately for males and females. We used analysis of variance to test for effects of sample site and interaction between sample site and sex on mean principal component 1 (PC1) and 2 (PC2) values. We used Tukey tests to test for pairwise differences in PCA scores between sites. Analyses were performed using SYSTAT 11.0 (SYSTAT Software Inc. 2004).

We tested for a correlation between pairwise genetic and morphological distances among birds sampled at seven western migration and wintering sampling sites for which morphological measurements were taken. As before, we used a Mantel test where the dependent variable was a matrix of genetic distances while the independent matrix was composed of pairwise differences of mean morphological differences between sites as represented by mean PC2 scores obtained from the between site morphometric comparison.

Table 2 Sample size, mean, and standard deviation for female and male red-tailed hawks measured at eight migration sites

Location	Sex	*n*	Wing	Tail	Weight	Culmen	Hallux
CC_M	F	106	403 ± 15	240 ± 9	1094 ± 114	27.1 ± 2.0	30.8 ± 1.4
	M	122	379 ± 14	226 ± 10	875 ± 168	24.8 ± 1.2	28.2 ± 1.3
CV_M	F	42	401 ± 12	231 ± 37	1199 ± 227	27.2 ± 1.2	31.1 ± 1.4
	M	79	372 ± 17	224 ± 9	915 ± 172	25.0 ± 1.1	28.0 ± 1.1
NM_M	F	57	412 ± 10	243 ± 11	1068 1 ± 13	26.0 ± 1.4	30.5 ± 1.7
	M	51	374 ± 33	231 ± 9	846 ± 93	24.1 ± 0.8	27.8 ± 1.1
NV_M	F	55	410 ± 11	242 ± 8	1113 ± 111	26.7 ± 2.0	30.4 ± 1.0
	M	49	381 ± 17	228 ± 7	880 ± 84	24.4 ± 0.9	27.7 ± 1.5
OR_M	F	27	398 ± 12	235 ± 9	1096 1 ± 27	27.1 ± 1.0	31.0 ± 2.0
	M	41	374 ± 11	221 ± 10	880 ± 92	24.6 ± 1.0	28.3 ± 1.3
TX_M	F	11	393 ± 11	229 ± 10	1246 ± 122	28.4 ± 1.1	32.1 ± 1.5
	M	5	366 ± 7	219 ± 5	968 ± 136	24.9 ± 1.3	28.4 ± 0.8
WA_M	F	6	412 ± 17	244 ± 14	992 ± 112	25.7 ± 1.1	31.2 ± 1.1
	M	6	375 ± 13	220 ± 10	850 ± 60	24.2 ± 1.2	26.8 ± 2.7
WY_M	F	7	410 ± 9	241 ± 6	974 ± 86	25.8 ± 0.6	29.4 ± 1.6
	M	5	390 ± 6	224 ± 17	909 ± 104	23.2 ± 1.5	28.0 ± 1.5

Table 3 Principal component loadings for principal component 1 (PC1) and 2 (PC2) in male and female red-tailed hawks

	Juvenile males		Juvenile females	
	PC1	PC2	PC1	PC2
Wing	0.583	–0.584	0.482	–0.695
Tail	0.616	–0.542	0.323	–0.722
Mass	0.523	0.427	0.697	0.190
Culmen	0.654	0.292	0.606	0.408
Hallux	0.482	0.540	0.718	0.286
Variance explained	33.04%	23.88%	34.12%	27.25%

Results

Morphological data analysis

Morphological analysis of juvenile red-tailed hawks suggested that hawks from Texas (TX_M) were generally largest, those from California (CC_M, CV_M) and Oregon (OR_M) intermediate, and Washington (WA_M), Nevada (NV_M), Wyoming (WY_M), and New Mexico (NM_M) generally smallest (Table 2). Principal components analysis resulted in two useful components for both males and females (Table 3). Principal component 1 (PC1) was interpreted as representing body size, correlated positively with all measurements, and accounted for 33% of the variance in the male data set and 34% of the variance in the female data set. Mass, culmen, and hallux loaded positively onto the second principal component (PC2), which was interpreted as a shape descriptor, and accounted for 24% of the variance in the male data set and 27% of the variance in the female data set. Hawks with relatively larger mass, culmen, and hallux for similar wing and tail lengths were described as stouter individuals.

Analysis of variance (ANOVA) indicated no significant effect of sex ($F_{1,653} = 1.07$, $P = 0.30$; $F_{1,653} < 0.001$, $P = 0.99$) nor interaction between sex and sampling site ($F_{7,653} = 0.98$, $P = 0.44$; $F_{7,653} = 1.05$, $P = 0.40$) for either PC1 or PC2. Consequently, we grouped males and females for further analyses. The ANOVA indicated that both PC1 and PC2 were significantly different among sites (PC1: $F_{7,653} = 2.78$, $P = 0.007$; PC2: $F_{7,653} = 21.28$, $P < 0.001$). However, Tukey tests on PC1 indicated no significant pairwise comparisons between sites, whereas Tukey tests performed on PC2 revealed several significant comparisons (Fig. 2, Table 4). Birds from Texas (*Buteo jamaicensis borealis* and *B. j. fuertesi*) were significantly stouter than all birds from all other sites (*Buteo jamaicensis calurus*). Within *B. j. calurus*, birds from Pacific migration sites in California and Oregon were generally stouter than birds from Washington and interior migration sites from Great Basin migration sites (Fig. 2, Table 4).

Analysis of genetic diversity

All 17 microsatellite loci amplified in red-tailed hawks with no more than two alleles per individual, indicating that cross–species application of Swainson's hawk microsatellites in red-tailed hawks did not result in nontarget amplification. Five deviations from Hardy–Weinberg equilibrium (out of 136 comparisons; three in CC_B, one in NV_B, and one in SC_B) and no indication of linkage disequilibrium were detected. None of the 17 loci displayed evidence of null alleles. The 10% quality control analysis revealed a 0.8% rate of disagreement between initial and secondary scores. Overall and site-specific heterozygosity, number of alleles, allelic richness, and private alleles are summarized in Table 5. Similar levels of heterozygosity and allelic richness

	OR_M	CV_M	CC_M	NV_M	WA_M	NM_M	WY_M	TX_M
OR_M	—	0.525	0.349	0.937	0.962	1.000	0.871	0.255
CV_M	1.000	—	1.000	0.992	0.427	0.328	0.248	0.868
CC_M	0.030	0.015	—	0.973	0.374	0.145	0.205	0.871
NV_M	< 0.001	< 0.001	0.001	—	0.681	0.874	0.473	0.643
WA_M	0.002	0.002	0.030	0.998	—	0.958	1.000	0.153
NM_M	< 0.001	< 0.001	< 0.001	0.631	1.000	—	0.857	0.208
WY_M	0.001	0.001	0.051	0.868	0.999	0.999	—	0.083
TX_M	0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	—

Table 4 ***P*** values of morphologic pairwise comparisons of principal components 1 (above diagonal) and 2 (below diagonal) between eight red-tailed hawk migration localities. Shaded boxes indicate significant pairwise comparisons following sequential Bonferroni correction

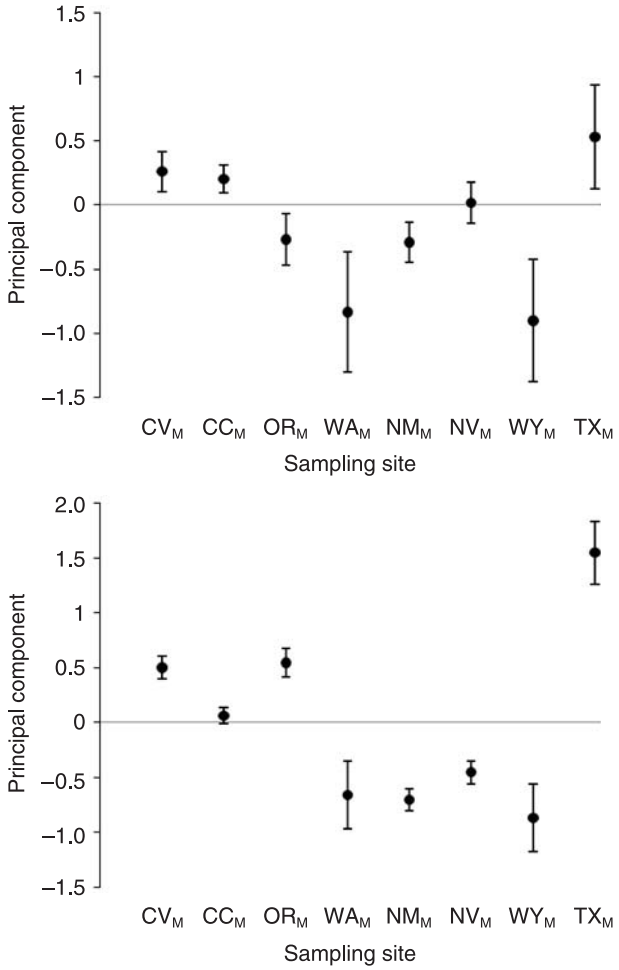

Fig. 2 Mean+-one SE among eight migration sites for (a) Principal component 1, and (b) Principal component 2. Generally, the stoutest hawks appear to occur in Texas while the least stout occur in Washington (WA_M) and the Great Basin/Rocky Mountains (NM_M, NV_M, WY_M).

were observed at all sampling sites suggesting that relative to the population as a whole, none of the regions examined has experienced a recent decline in genetic diversity.

Table 5 Polymorphism data for 1057 red-tailed hawks from sampling locations with greater than 10 individuals. Number of samples (*N*), heterozygosity corrected for sample size (H_C), average number of alleles per locus (*A*/locus), allelic richness corrected for sample size (AR_C), and number of private alleles in each region (A_P)

Site	*N*	H_C	*A*/locus	AR_C	A_P
CC_B	58	0.72 ± 0.05	10.94 ± 7.43	6.97 ± 3.62	1
NV_B	41	0.74 ± 0.05	11.18 ± 7.20	7.64 ± 3.84	3
ID_B	29	0.72 ± 0.04	9.41 ± 5.77	7.21 ± 3.51	1
SC_B	36	0.73 ± 0.05	9.59 ± 5.11	7.24 ± 3.28	0
WI_B	34	0.76 ± 0.04	9.76 ± 5.26	7.30 ± 3.11	3
WA_M	27	0.73 ± 0.04	9.06 ± 4.92	7.10 ± 3.27	0
OR_M	80	0.73 ± 0.04	11.82 ± 8.20	7.26 ± 3.57	0
CC_M	265	0.72 ± 0.05	15.12 ± 11.74	7.25 ± 3.71	5
NV_M	92	0.74 ± 0.04	12.88 ± 9.14	7.39 ± 3.71	1
NM_M	93	0.74 ± 0.04	12.76 ± 9.28	7.46 ± 3.82	1
WY_M	14	0.75 ± 0.04	7.47 ± 3.68	7.20 ± 3.37	2
CV_M	220	0.73 ± 0.04	14.18 ± 10.65	7.31 ± 3.65	5
SC_M	21	0.71 ± 0.05	8.24 ± 4.38	6.94 ± 3.27	2
TX_M	47	0.75 ± 0.05	11.06 ± 7.42	7.61 ± 3.63	3
Total	1057	0.74 ± 0.04	18.17 ± 14.14	7.49 ± 3.73	—

Differentiation among populations

Assignment tests in STRUCTURE revealed one individual that did not assign well to either Swainson's or red-tailed hawks. Factorial correspondence analysis of 1083 red-tailed hawks and 357 Swainson's hawks revealed two distinct clusters corresponding to species designation. The probable hybrid sample, collected during migration at the Goshute Mountains, was located between the two species clusters in the analysis. This result is similar to previously described red-tailed hawk × Swainson's hawk hybrids (Hull *et al.* 2007b). This individual was removed from all other analyses.

Analysis of mean allele frequency differences among sampling sites was used to create a UPGMA tree (Fig. 3). Strong bootstrap support (90%) indicated a distinction between eastern and western subspecies of red-tailed hawks. Several nodes were supported among western sampling sites, strong support was found for the node grouping Central Valley and central coastal California (100%).

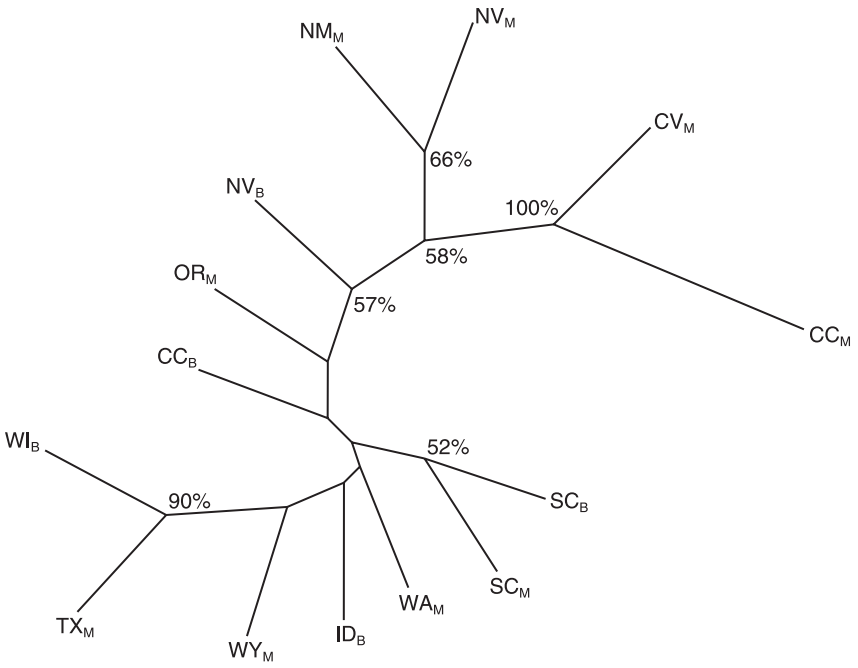

Fig. 3 UPGMA clustering of sampling localities with greater than 10 samples based on allele frequency differences using 10 000 bootstrapped data sets. Nodes with greater than 50% bootstrap support are indicated. CC_B, coastal California breeding; CC_M, coastal California migration; CV_M, Central Valley migration; ID_B, Idaho breeding; NM_M, New Mexico migration; NV_B, Nevada breeding; NV_M, Nevada migration; OR_M, Oregon migration; SC_B, southern California breeding; SC_M, southern California migration; TX_M, Texas migration; WA_M, Washington migration; WI_B, Wisconsin breeding; WY_M, Wyoming migration.

Neighbour-joining tree topology was similar, but bootstrap support was only found for the distinction between eastern and western sites (79%) and for Central Valley and central coastal California (52%) distinctions.

Analysis in STRUCTURE indicated that three genetic clusters were most likely for the total red-tailed hawk data set. Samples collected from east of the Rocky Mountains were predominantly a single cluster, while samples collected from the Rocky Mountains and farther west were composed of large portions of each cluster (Fig. 4). A second STRUCTURE analysis of western red-tailed hawks (sampled west of the Rocky Mountains) suggested the presence of two clusters. The most common genetic clusters differed between central California and Great Basin/Rocky Mountain migration and breeding sites (Fig. 5).

Pairwise F_{ST} comparisons between breeding sites with greater than 10 samples were significant except between NV_B and ID_B and between NV_B and SC_B (Table 6). Pairwise F_{ST} comparisons between migration sites indicated a mixture of significant and nonsignificant comparisons within the range of *B. j. calurus*; however, comparisons between TX_M and all other migration sites except WY_M were significant (Table 6). Very low pairwise differences were detected between breeding and migration samples collected in the same area (F_{ST} from 0.000 to 0.013). An AMOVA comparing *B. j. borealis* and *B. j. calurus* breeding sites indicated significant differentiation between subspecies ($F_{ST} = 0.031$, $P < 0.001$; $F_{CT} = 0.019$ $P < 0.001$). Further AMOVAS detected significant, but low differentiation between western breeding sites as indicated in the STRUCTURE analysis (Central California: CC_B, CV_B, and Great Basin: SC_B, ID_B, NV_B, MT_B; $F_{ST} = 0.012$, $P < 0.001$; $F_{CT} = 0.002$, $P = 0.07$). Very low levels of differentiation were identified between migration sites grouped by band-recovery patterns ($F_{ST} = 0.009$, $P < 0.001$; $F_{CT} = 0.006$, $P = 0.03$) as well as between groups of morphologically similar migrants (large: OR_M, CC_M, CV_M and small: WA_M, NV_M, NM_M, WY_M; $F_{ST} = 0.009$, $P < 0.001$; $F_{CT} = 0.006$, $P = 0.02$). These values are comparable with differentiation observed between Swainson's hawk populations ($F_{ST} = 0.011$; Hull *et al*. 2007).

The model results averaged across 10 EASYPOP simulations suggested that a minimum of 5588 generations were necessary to achieve the observed degree of population differentiation ($F_{ST} = 0.031$) between *B. j. borealis* and *B. j. calurus*. Using a generation time of 3 years for the time in generations required to attain the observed differentiation corresponds to roughly 17 000 years. This is likely to underestimate time since divergence since the model was run under the assumption of no migration and Bayesian clustering analyses suggest migration between regions. However, this estimate provides a useful minimum estimate.

Landscape and genetic relationships

Results from Monmonier's maximum distance algorithm strongly supported a barrier between *B. j. calurus* and *B. j. borealis* corresponding roughly with the Rocky Mountains (Fig. 6). Within western North America, a barrier was

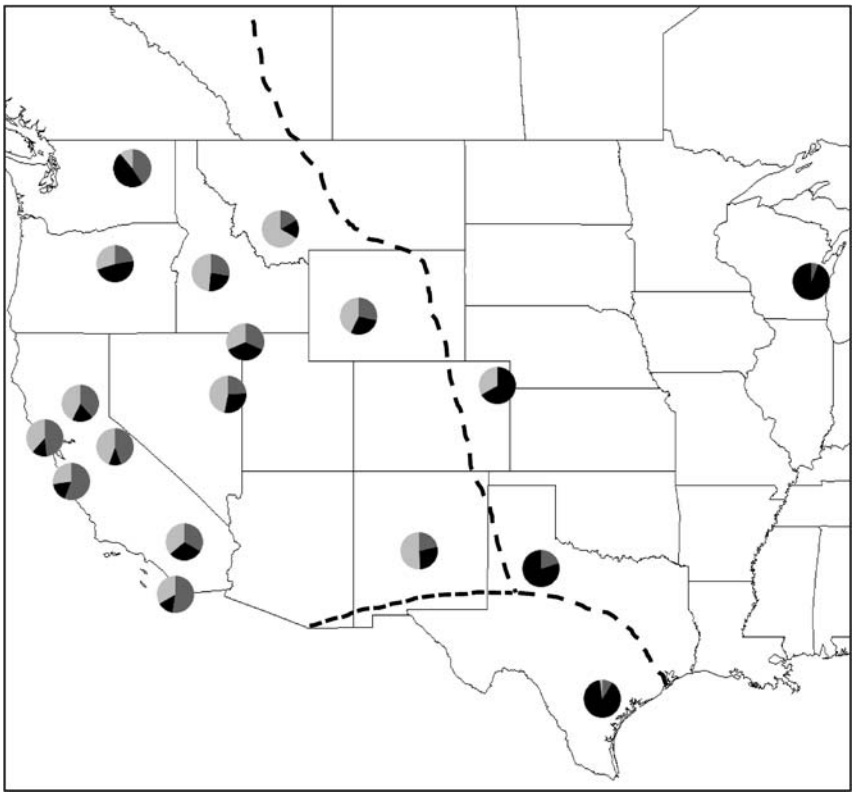

Fig. 4 Delineation using STRUCTURE of North American red-tailed hawks into three genetic clusters. Black represents a cluster assigning to primarily the eastern North America (*Buteo jamaicensis borealis*) with some individuals west of the Rocky Mountains and the two grey shades represent clusters found mostly in western North America (*Buteo jamaicensis calurus*) with few individuals in eastern North America. Pie graphs depict the frequency of each cluster assignment at different sampling sites. The dashed north–south line reflects the subspecies boundary between *B. j. calurus* (west) and *B. j. borealis* (east). South of the dashed east–west line reflects the range of *Buteo jamaicensis fuertesi*.

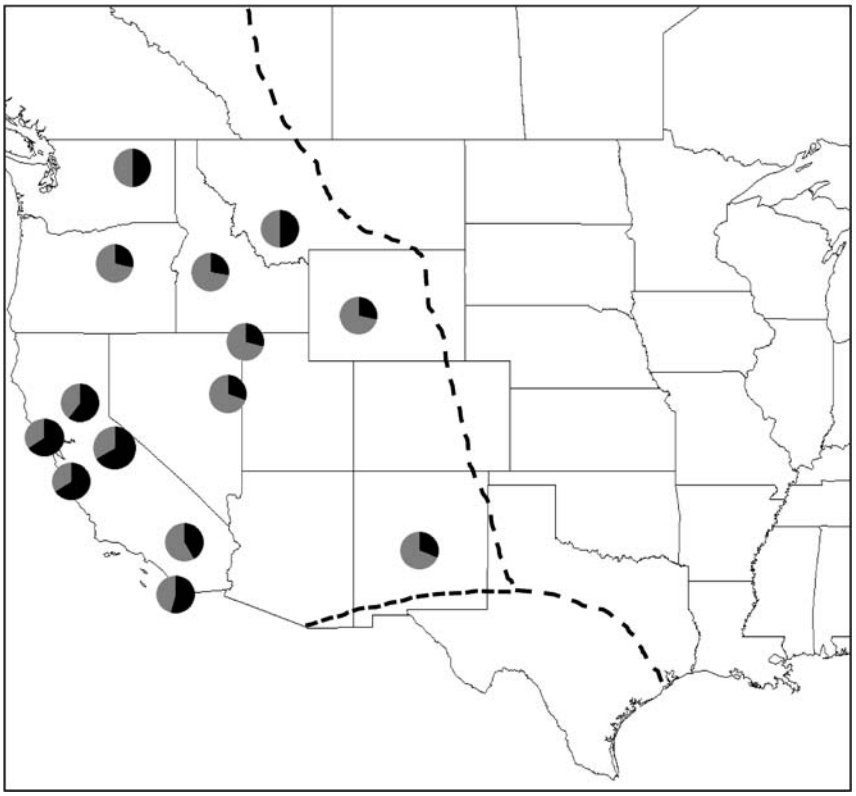

Fig. 5 Delineation using STRUCTURE of western North American red-tailed hawks into two genetic clusters. Black represents a cluster with greatest frequency in central California and decreases counterclockwise around the Sierra Nevada. The opposite pattern is observed in the grey cluster. Pie graphs depict the frequency of each cluster assignment at different sampling sites. The dashed north–south line reflects the subspecies boundary between *Buteo jamaicensis calurus* (west) and *Buteo jamaicensis borealis* (east). South of the dashed east–west line reflects the range of *Buteo jamaicensis fuertesi*.

Table 6 Genetic differentiation (F_{ST}) between red-tailed hawk sampling localities at 17 microsatellite loci. Dashed line separates *Buteo jamaicensis calurus* from *B. j. borealis* and *B. j. fuertesi*. Shaded boxes indicate significant pairwise F_{ST} values following sequential Bonferroni correction

	B. j. calurus														*B. j. borealis/fuertesi*			
	CC_M	CC_B	CV_M	CV_B	SC_M	SC_B	OR_M	WA_M	NV_M	NV_B	ID_B	MT_B	NM_M	WY_M	CP_B	TX_B	TX_M	WI_B
CC_M	—																	
CC_B	0.005	—																
CV_M	0.001	0.006	—															
CV_B	0.015	0.029	0.013	—														
SC_M	0.006	0.018	0.007	0.032	—													
SC_B	0.008	0.016	0.009	0.023	0.001	—												
OR_M	0.009	0.020	0.007	0.022	0.008	0.012	—											
WA_M	0.009	0.023	0.008	0.020	0.005	0.013	0.001	—										
NV_M	0.004	0.013	0.003	0.013	0.008	0.004	0.005	0.006	—									
NV_B	0.005	0.011	0.003	0.016	0.012	0.009	0.006	0.005	0.000	—								
ID_B	0.008	0.018	0.008	0.020	0.016	0.016	0.014	0.011	0.005	0.006	—							
MT_B	0.007	0.010	0.005	0.042	0.000	0.008	0.012	0.005	0.007	0.010	0.005	—						
NM_M	0.007	0.012	0.006	0.016	0.008	0.006	0.007	0.004	0.000	0.001	0.005	0.004	—					
WY_M	0.001	0.012	0.001	0.004	0.000	0.005	0.003	0.000	0.000	0.000	0.005	0.010	0.000	—				
CP_B	0.000	0.006	0.000	0.006	0.006	0.000	0.000	0.000	0.000	0.000	0.000	0.007	0.000	0.000	—			
TX_B	0.028	0.041	0.032	0.045	0.032	0.036	0.024	0.023	0.025	0.022	0.040	0.032	0.024	0.017	0.000	—		
TX_M	0.022	0.030	0.021	0.030	0.027	0.020	0.017	0.013	0.017	0.019	0.025	0.028	0.017	0.019	0.000	0.015	—	
WI_B	0.032	0.038	0.030	0.039	0.041	0.036	0.031	0.024	0.025	0.026	0.036	0.039	0.028	0.020	0.000	0.019	0.005	—

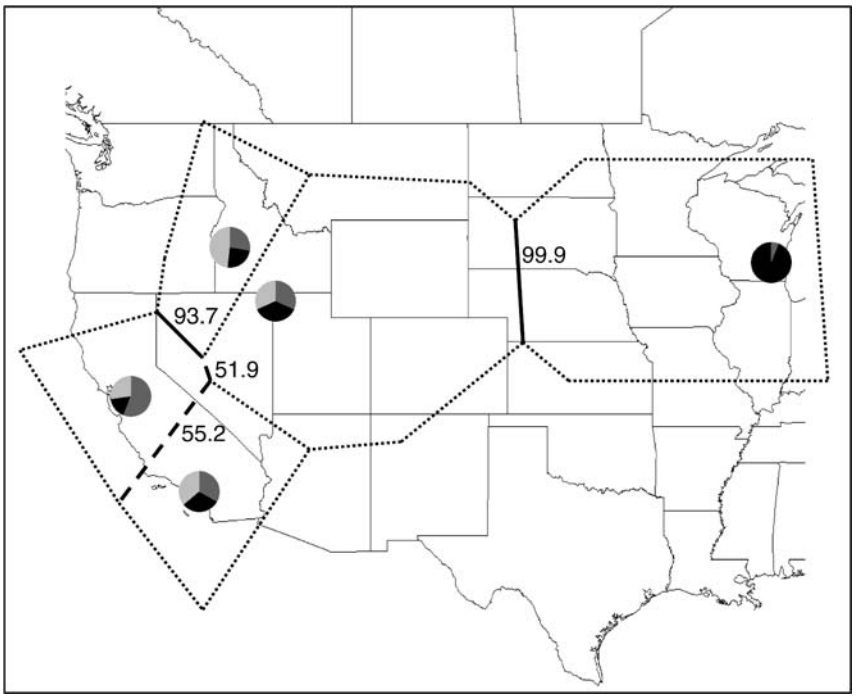

Fig. 6 Barriers to gene flow as detected by Monmonier's algorithm roughly corresponding to the Rocky and Sierra Nevada mountain ranges. Solid lines represent well-supported barriers, heavy dashed lines indicate barriers with low support, and light dotted lines indicate edges of Voronoï tessellation polygons. Numbers next to barriers indicate the proportion of bootstrap replicates supporting a break in that location.

detected along the Sierra Nevada, supported with high confidence in the northern part of the mountain range but with declining support between central and southern California (Fig. 6). No significant relationship was found between genetic and geographical distance among four western breeding sites when straight-line distances were used ($P = 0.17$, $r = 0.42$). When the relationship was tested using distance around the Sierra Nevada, a significant correlation between genetic and geographical distance was observed ($P = 0.04$, $r = 0.58$). Whether the higher r estimate associated with the indirect relative to the direct path reflected a better fit (as opposed to chance) is unclear but, in any case, supported the results from Monmonier's maximum distance algorithm.

A Mantel test of the correlation between morphological and genetic differences between seven western migration and wintering sites did not detect a significant correlation ($P = 0.09$, $r = 0.35$).

Discussion

Although red-tailed hawks have a high degree of mobility and a continuous distribution across North America, this study identified population structure among North American red-tailed hawks at both the continental and regional scale. At the continental scale, limited admixture between populations suggested either recent divergence and incomplete lineage sorting or, more likely, secondary contact following a period of divergence in isolation. Within western North America, population differentiation appears to coincide with the crest of the Sierra Nevada Mountains, suggesting population structure of wide-ranging and vagile taxa may be associated with regional variations in habitat.

Continental-scale influences

At the continental scale, an east–west division in red-tailed hawk microsatellite genotypes was detected with population boundaries roughly coinciding with the Rocky Mountains. This result parallels patterns of differentiation documented in other widespread North American avian taxa (e.g. Kimura *et al*. 2002; Peters *et al*. 2005) and conforms to previously described red-tailed hawk mitochondrial restriction fragment length polymorphism (RFLP) data (Pearlstine 2004). While our microsatellite data allow only a coarse calculation of time since the eastern and western populations diverged, observed population structure and divergence simulations are consistent with a pattern of isolation during Pleistocene refugia with subsequent range expansion and secondary contact. This pattern has been documented in many species of North American birds (Scribner *et al*. 2003; Hull & Girman 2005; Jones *et al*. 2005) and should be investigated more thoroughly in future analysis of red-tailed hawk DNA sequence data.

Bayesian clustering analysis suggests that the population occurring east of the Rocky Mountains appears to be composed of primarily a single genetic cluster, with a small proportion of individuals that appear to have western ancestry. In contrast, the western population includes three genetic groups, including the predominant eastern type. The rare occurrence of western ancestry in the eastern population coupled with the more frequent occurrence of eastern ancestry west of the Rocky Mountains suggests that asymmetrical dispersal may occur from eastern areas into western North America. While additional data describing individual movements are required to more thoroughly address this possibility, biased migration from eastern into western North America may be a consequence of geographical features. The wintering grounds of *Buteo jamaicensis borealis* are predominantly defined by open plains, the Mississippi River Valley, and coastal plains where prominent geographical features to aid in navigation during migration are largely absent. Consequently, the movements of these birds may be subject to greater variation due to weather and winds. In contrast, migrants of *Buteo jamaicensis calurus* in western North America migrate along many prominent landscape features (e.g. the Rocky Mountains and Sierra Nevada) that may be used to assist individuals in staying on course during migration, reducing the possibility of straying to the east. Additionally, the Great Basin also may act as a population sink, drawing immigrants from easterly populations but not contributing emigrants east of the Rocky Mountains. Detailed satellite telemetry studies may help to resolve this issue.

Subspecies distinctions

The observed east–west distinction among breeding sites corresponds with previously described range limits for the subspecies *B. j. borealis* (eastern) and *B. j. calurus* (western), with the Rocky Mountains serving as a primary geographical delineation (Preston & Beane 1993). Divergence as measured by microsatellites was low between eastern and western subspecies ($F_{ST} = 0.031$). In comparison, using mitochondrial sequence data, Hull & Girman (2005) found a Φ_{CT} (an F_{CT} analogue used in the analysis of haplotypic data) of 0.17 between eastern and western populations of sharp-shinned Hawks. The low F_{ST} estimates found here may have been influenced by relatively large effective population sizes of red-tailed hawks, the high degree of heterozygosity observed among microsatellite loci, and the use of nuclear rather than mitochondrial data.

Among migration sampling sites, all western sites (except WYM; $n = 14$) were distinct from samples collected in southern Texas. This pattern is consistent with the pattern observed between eastern and western sites during the breeding season. Samples collected in southern Texas during winter clustered predominantly with the eastern *B. j. borealis* breeding population, although many of the individuals had plumage characteristics associated with *Buteo jamaicensis fuertesi*. Sampling within the strict breeding range of *B. j. fuertesi*, as well as additional sampling of *B. j. borealis* populations from the central plains and the remaining North American subspecies (*Buteo jamaicensis alascensis*, *B. j. umbrinus*, and *B. j. harlani*) during the breeding season is required to resolve their relationships.

Our morphological analysis found red-tailed hawks from southern Texas that were genetically similar to *B. j. borealis* to be significantly stouter (thicker culmen and hallux for a given overall size) than western individuals. This finding supported previous descriptions of subspecific differentiation

between *B. j. borealis* and *B. j. calurus* collected in New Jersey and Nevada, respectively (Pearlstine & Thompson 1994). The size distinction between Texas and western samples provides further support for limited mixing of *B. j. borealis* and *B. j. calurus* during migration and winter.

Regional landscape factors

Among *B. j. calurus* breeding sites in western North America, we found support for genetic isolation by distance around the Sierra Nevada. Idaho and coastal central California represented end points, with central Great Basin and southern California as intermediate points. This suggests that admixture of red-tailed hawk populations may be limited to some extent by environmental or behavioural factors correlated with the Sierra Nevada. Given the extensive dispersal capabilities of red-tailed hawks, it is unlikely that the Sierra Nevada themselves pose a significant barrier to dispersal. Nest records extend into western foothills of the Sierra Nevada (Fitch *et al*. 1946) and into areas of the high Sierra (Grinnell & Miller 1944; Orr & Moffitt 1971; Gaines 1988). This suggests that environmental or behavioural factors associated with the Sierra Nevada may be responsible for structuring red-tailed hawk populations.

A similar pattern of divergence is seen in the *Vireo solitarius* species complex between Cassin's and Plumbeous vireos (*V. cassinii* and *V. plumbeous*), as well as between oak and juniper titmice (*Baeolophus inoratus* and *B. ridgwayi*; Cicero & Johnson 1998; Cicero 2004). In both species groups, limited dispersal and the crest of the Sierra Nevada appear to pose a barrier to migration, with limited areas of secondary contact occurring in northeastern California. Differing habitat preferences also appear to play a critical role in preventing these sister taxa from interbreeding, as Plumbeous vireos and juniper titmice prefer more xeric pinion-juniper habitats east of the Sierra Nevada, while Cassin's vireos and oak titmice occur west of the Sierra Nevada throughout California's mixed coniferous and oak woodlands (Curson & Goguen 1998; Cicero 2000; Goguen & Curson 2002).

Habitat preferences also may play a role in population structure observed among western red-tailed hawks. Within the Great Basin, red-tailed hawks nest at relatively low densities, primarily in junipers, cottonwoods, and firs, while red-tailed hawks in central California nest extensively in native oak and pine trees, as well as introduced eucalyptus, and achieve some of the highest recorded densities of nesting red-tailed hawks (Fitch *et al*. 1946; Preston & Beane 1993; Janes 2003). These habitat differences may result in natal imprinting for particular environmental characteristics resulting in habitat-biased dispersal and population structure (Davis & Stamps 2004; Sacks *et al*. 2004). Habitat-associated population differentiation is supported by available band-recovery and satellite-tracking data. Tracking results for red-tailed hawks from the Goshute Mountains in the Great Basin indicate fidelity to arid habitats within the Great Basin and southwestern deserts (HawkWatch International 2007), with two individuals apparently immigrating into southern California deserts from the central Great Basin. In contrast, no immigration has been observed among migrants from the northern Great Basin that pass through coastal California. Additionally, band recoveries of juvenile red-tailed hawks from southern California deserts show both local dispersal as well as long distance dispersal to similarly arid habitats of extreme northeastern California and the Great Basin but not into intervening mesic habitats of central California (Bloom 1985). A similar pattern of habitat-biased dispersal without regard to intervening geographical distance has been documented among island populations of warbler finches in the Galápagos archipelago (*Certhidea olivacea* and *C. fusca*; Tonnis *et al*. 2005).

If habitat-biased dispersal is a contributing factor in structuring red-tailed hawk populations, we may expect to find a nonsignificant relationship between genetic and geographical distance (Tonnis *et al*. 2005). This is indeed the case when we compared populations directly to each other instead of considering the distance around the Sierra Nevada. The spatial arrangement of habitats around the Sierra Nevada results in an association between genetic and geographical distance around the Sierra Nevada. The relatively mesic habitats of central California give way to more arid habitats further south and the arid southern California habitats are contiguous with arid habitats of the Great Basin in the eastern rain shadow of the Sierra Nevada.

Habitat-specific population structure in red-tailed hawks may be reinforced by associated differences in population densities. Within central California, nonmigratory red-tailed hawks achieve high population densities compared to the primarily migratory Great Basin individuals that remain at a comparatively low density (Fitch *et al*. 1946). These differences in behaviour and breeding density may serve to increase population distinctions due to habitat imprinting by reducing the likelihood of successful immigration of migratory Great Basin hawks into central California.

Our morphological findings within western North America are somewhat comparable to those of Fitzpatrick & Dunk (1999). The results of Fitzpatrick & Dunk (1999) suggested that the largest breeding and wintering red-tailed hawks occurred in grasslands of California and the smallest in forests of the Pacific Northwest. In the present study, we found the stoutest, migrant hawks in central California, but also in Oregon. Additionally, we found that hawks sampled in Washington were similar to those sampled throughout the Great Basin and Rocky Mountains. The differences we observed in morphology are similar to the pattern of differentiation in the microsatellite data of breeding populations, with a general distinction between California and Great Basin/Rocky Mountain populations. Although not

statistically significant, the weak correlation between morphological and genetic differentiation is suggestive of a positive relationship. A positive correlation could represent either further evidence of isolation by distance and habitat segregation across the west or differing selection on body shape between regions.

The observed differences in stoutness may be associated with adaptive variation associated with regional environmental or behavioural differences. Stout birds have relatively larger culmen and hallux measurements for a given size. Larger culmens and halluxes have been associated with taking larger ground-dwelling prey in *Accipiter* species (Wattel 1973; Whaley & White 1994), however, previous studies suggest that red-tailed hawks in the eastern Great Basin take larger prey than individuals breeding in the foothills of California (Fitch *et al*. 1946; Smith & Murphy 1973). Seasonality may also be a contributing factor to regional variation in body size, as in Bergmann's rule which predicts larger-bodied individuals in colder climates. This pattern is not supported by the previous morphometric analysis (Fitzpatrick & Dunk 1999) or the results presented here.

Differences in body shape may be related to a behavioural correlate of environment. Stouter birds are relatively more massive for a given flight surface resulting in higher wing-loading and less efficient long-distance flight. Among our morphological sampling sites, individuals from Great Basin and Rocky Mountain locations experience seasonably variable food resource and weather and show a greater tendency to be migratory than individuals from both central California and Oregon. The lighter wing-loading of interior red-tailed hawks may reflect an adaptation to a more migratory life history. Alternatively, the differences in size might be the result of phenotypic plasticity due to differing environmental conditions. For example, abundant prey resources in central California may allow red-tailed hawks to grow to a larger size than those in the Great Basin. Sampling adults during the breeding season may allow a better understanding of relationships between morphology and genetic differentiation.

Conclusions

Together, regional genetic and morphological data for red-tailed hawks in western North America provide insights into landscape factors that may shape population differentiation in widespread, mobile species. Rather than panmixia or isolation by distance, the population structure of western red-tailed hawks appears to be affected by environmental factors, including habitat breaks, resulting in habitat-biased dispersal and population structure. This finding suggests that additional study of continuously distributed, mobile, habitat generalists may reveal previously unidentified behavioural and landscape characteristics responsible for shaping population genetic structure.

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