Effects of Holocene climate change on the historical demography of migrating sharp-shinned hawks (*Accipiter striatus velox*) in North America

JOSHUA M. HULL*† and DEREK J. GIRMAN*
*Department of Biology, Sonoma State University, 1801 East Cotati Avenue, Rohnert Park, CA 94928-3609, USA; †Golden Gate Raptor Observatory, Building 201 Fort Mason, San Francisco, CA 94123, USA

Abstract

DNA sequences of the mitochondrial control region were analysed from 298 individual sharp-shinned hawks (*Accipiter striatus velox*) sampled at 12 different migration study sites across North America. The control region proved to be an appropriate genetic marker for identification of continental-scale population genetic structure and for determining the historical demography of population units. These data suggest that sharp-shinned hawks sampled at migration sites in North America are divided into distinct eastern and western groups. The eastern group appears to have recently expanded in response to the retreat of glacial ice at the end of the last glacial maximum. The western group appears to have been strongly effected by the Holocene Hypsithermal dry period, with molecular evidence indicating the most recent expansion following this mid-Holocene climatic event 7000–5000 years before present.

Introduction

Changes in the distribution of species through recent geological time have long been of interest to ecologists. For example, Davis (1969) examined variation in a New England forest community through the Holocene and found a strong effect of climatic change during this period. The abundance of fossil pollen has made examination of changes in terrestrial plant distributions through time easier to address than similar studies of animals, which have often left a scant fossil record. With advances in molecular genetic methods, techniques have become available that allow ecologists to investigate how the distributions of animal species may have changed in response to climatic events in recent geological history (Avise & Walker 1998; Douglas *et al*. 2003). Many studies have focused on speciation events related to the Pleistocene Refugia Hypothesis, wherein a series of glaciations followed by glacial retreat and habitat recovery was proposed to potentially account for patterns of avian species diversity across North America (Klicka & Zink 1997).

Pleistocene refugia have been suggested to also explain much of the current population structure within species. For example, molecular studies of North American migratory bird species have revealed genetic differentiation between eastern and western subspecies or populations (divided by the Great Plains and Rocky Mountains) (Milot *et al*. 2000; Kimura *et al*. 2002). Analyses of historical demography in avian species have suggested that populations were isolated during glacial maxima and subsequently expanded into their present ranges in response to retreat of glacial ice, approx. 16 000 years before present (ybp), and the concurrent recovery of suitable habitat during interglacial periods (Merilä *et al*. 1997; Mila *et al*. 2000; Peck & Congdon 2004).

Climatic factors associated with the Hypsithermal period (7000–5000 bp) may also have had a profound effect on the distribution of some North American species. The Hypsithermal period appears to have been a time of rapid climate change. Stable isotope ratios of deuterium, analysis of atmospheric CO_2 taken from Antarctic ice core samples, and fossil pollen records indicate that the global Hypsithermal climate was more arid, causing a prolonged drought throughout western North America (Steig 1999). This period has been implicated in historic changes observed in the

distribution of a variety of North American plant taxa (Davis 1969) and nonavian vertebrates (Webb 1984; Douglas *et al*. 2003).

In this paper, we examined population structure and historical demography of sharp-shinned hawks (*Accipiter striatus*) to test hypotheses about the impacts of climatic events associated with Pleistocene and Hypsithermal periods. First, we tested whether sharp-shinned hawk populations were partitioned in an east–west manner similar to other avian species. Sharp-shinned hawks banded during migration through western North America have been subsequently recovered only within western regions during both migration and the breeding season (Hoffman *et al*. 2002; Hull 2003). A similar pattern has been observed in sharp-shinned hawks migrating in eastern North America (Clark 1985). These patterns, along with strong fidelity to breeding ground observed in the closely related European sparrowhawk (*Accipiter nisus*) (Newton 1979), provided a priori support for the hypothesis that North American sharp-shinned hawks likely display an east–west genetic subdivision.

Second, we examined historical demography to determine whether populations had recently expanded, as has been found in other North American species, and, if so, how long ago such expansions occurred. Established paradigms regarding the continental scale genetic structure and historical demography in birds have been built largely on studies of songbird or seabird species which, to date, have not been found to be affected by the Hypsithermal (Klicka & Zink 1997; Kidd & Friesen 1998; Congdon *et al*. 2000; Mila *et al*. 2000). In contrast, because of their reliance on dense conifer stands with younger trees (Rosenfield *et al*. 1991), sharp-shinned hawks provide a model for species whose populations may have been particularly sensitive to arid conditions prevalent during the Hypsithermal. Consequently, we might expect to find a demographic effect associated with the Hypsithermal in this species. We analysed mitochondrial DNA sequence data from the control region to investigate these questions.

Materials and methods

Sampling

Feathers were analysed from 12 sampling sites associated with migratory routes from across North America: Bonney Butte, Oregon, USA (OR, $n = 29$); Sausalito, California, USA (CA, $n = 31$); Boise, Idaho, USA (ID, $n = 35$); Goshutes, Nevada, USA (NV, $n = 27$); Manzano Mountains, New Mexico, USA (NM, $n = 24$); Hawk Cliff, Ontario, Canada (ON, $n = 23$); Hawk Ridge, Minnesota, USA (MN, $n = 21$); Cedar Grove, Wisconsin, USA (WI, $n = 20$); Cape May Point, New Jersey, USA (NJ, $n = 21$); Little Gap, Pennsylvania, USA (PA, $n = 24$); Wise Point, Virginia, USA (VA, $n = 20$); La Mancha, Veracruz, Mexico (VC, $n = 20$). Mist nets, bow nets, and dho ghazzas (a square 2 m × 2 m net) were used to capture individuals at each site. Two contour feathers were plucked from the breast of each individual, approximately 2 cm below the crop. Samples were collected between August 15, 1999, and October 15, 1999, during peak sharp-shinned hawk migration. Ten or fewer individuals were sampled per site in any 1-week period to avoid over-representing certain latitudes.

Data generation

DNA extraction was performed following the Qiagen DNeasy protocol for animal tissues (Qiagen). Polymerase chain reaction (PCR) was used to isolate and amplify portions of the mitochondrial genome (Saiki *et al*. 1980). Owing a novel rearrangement in the position of the control region within the mitochondrial genome of Accipitriidae, we used an atypical combination of primers to amplify a portion of the control region (Haring *et al*. 2001). Primer Thr16064, which flanks the control region on the 5′ end, and primer CR537, which is approximately 600 bp downstream from Thr16064, were used for all samples (Sorenson *et al*. 1999). Double-stranded PCR products were generated in 50-μL reactions consisting of buffer (100 mM Tris-HCl, pH 9.0, 500 mM KCl, 1% Triton X-100), 2.5 mM $MgCl_2$, 0.8 mM dNTPs, 0.1 mM of each primer, 1.25 units of *Taq* DNA polymerase (Promega), 20–60 ng of genomic DNA in an MJ Research thermocycler. The PCR parameters were 1 cycle of 95 °C for 2 min, 34 cycles of 94 °C for 30 s, 53 °C for 45 s and 72 °C for 1 min, and 1 cycle of 72 °C for 10 min.

We also conducted a preliminary cytochrome *b* analysis on a subset of samples. We used universal primers (LCBOB and HCBETY) developed in previous studies (Outlaw *et al*. 2003). The PCR parameters for cytochrome *b* amplification were 1 cycle of 95 °C for 2 min, 34 cycles of 94 °C for 30 s, 48 °C for 30 s, and 72 °C for 1 min, and 1 cycle of 72 °C for 10 min.

The PCR products were run in low-melt agarose gels, excised, and subsequently isolated using an UltraClean DNA Purification Kit (MoBio Laboratories). They were used in a cycle sequencing reaction using a Thermo Sequenase fluorescent labelled cycle sequencing kit with 7-deaza-dGTP from Amersham Pharmacia and run in a 5% acrylamide gel in a Li-Cor Gene ReadIR 4200 automated sequencer. Sequences were examined and aligned by eye using the program SEQUENCHER (Gene Codes Corporation).

Data analysis

Relationships among sharp-shinned hawk mtDNA control region sequences were analysed using three approaches. The relationships of the genotypes were first estimated by unweighted maximum parsimony analysis using PAUP* version 4.0 (Swofford 2003) with *Accipiter nisus* as an

outgroup. *Accipiter nisus* was used as an outgroup based on previous morphological studies, studies of DNA-DNA hybridization, and unpublished analyses of the cytochrome *b* gene (Wattel 1973; Sibley & Ahlquist 1991; J. M. Hull unpublished). The strength of each node was assessed using 1000 bootstrap replicates. Second, the genetic distances among control region nucleotide sequences were estimated using the Tamura–Nei model, which estimates the differential rates of transitional substitution between purines and between pyramidines and the proportion of transversional differences (Tamura & Nei 1993). Genetic distances were used to construct neighbour-joining trees in PAUP* 4.0 (Swofford 2003). Finally, we generated minimum-spanning networks, in which haplotypes are the nodes of a network rather than the terminal tips of a tree. All possible minimum spanning networks were determined using pairwise absolute distances generated in ARLEQUIN (Schneider *et al*. 1997).

To examine differences among localities, average sequence divergence among sampling sites was computed (Nei 1987). Estimates of mean sequence divergence between populations (p_A) were corrected for polymorphisms within each population (Nei 1987). The resulting divergence matrix was used, with a neighbour-joining algorithm, to make a clustering tree using the program PHYLIP (Felsenstein 1989).

The significance of geographical divisions among sample sites was evaluated by estimating Φ_{ST}, the proportion of total genetic variation attributable to among-site variation vs. within-site variation using the program ARLEQUIN version 2.0 (Schneider *et al*. 1997). Pairwise estimates of Φ_{ST} were used to estimate subdivision among all sampling locations.

Population groupings were analysed using an analysis of molecular variance (AMOVA) (Excoffier *et al*. 1992). This method was used because, unlike other F_{ST} analogues, it is not sensitive to deviations from a normal distribution of genotypes (Takahata & Palumbi 1985; Hudson *et al*. 1992). Groupings tested were identified on the basis of the pattern of geographical occurrence of haplotypes, the pattern of average sequence divergence among populations, and biogeographical proximity of sampling sites. Those groupings that maximized values of Φ_{CT} in an analysis of molecular variance (AMOVA) and were significantly different from distributions of individuals generated from 1000 random permutations of the DNA sequences should reflect the most probable geographical subdivisions (Excoffier *et al*. 1992).

Historical demography was inferred through a variety of methods. First, nucleotide diversity (π) and haplotype diversity (h) (Nei 1987) within all groups along with pairwise differences were calculated using the program ARLEQUIN (Schneider *et al*. 1997). Comparing haplotype diversity and nucleotide diversity can reveal information about patterns of historical demography. High haplotype diversity in conjunction with low nucleotide diversity can suggest recent population growth while high haplotype diversity with high nucleotide diversity is indicative of a long-standing population (Mila *et al*. 2000). We also calculated an expansion coefficient (S/d) or the ratio of variable sequence positions (S) relative to the mean number of pairwise nucleotide differences (d) between haplotypes (Peck & Congdon 2004). Recent population expansions are indicated by large values and constant long-term population size is indicated by small values (von Haeseler *et al*. 1996).

We also investigated historical patterns of population structure using the mismatch distribution of pairwise nucleotide differences in pooled genetically distinct groups with the programs ARLEQUIN version 2.0 and DNASP version 4.00 (Rogers & Harpending 1992; Schneider *et al*. 1997; Rozas *et al*. 2003). If a population has undergone rapid expansion, a unimodal mismatch distribution approximating a Poisson curve is expected (Rogers & Harpending 1992), whereas populations approaching mutation drift equilibrium are expected to produce a multimodal or 'ragged' mismatch distribution. We examined the 'raggedness' statistic (rg) and assessed the statistical significance of this value from the distribution of the statistic determined by simulations (Harpending *et al*. 1993).

Statistics based on the mismatch distribution, such as rg, are thought to be less robust at detecting expansion, therefore, we also employed a range of neutrality statistics to detect traces of past population growth or stability based on DNA sequences (Ramos-Onsins & Rozas 2002). Fu's (1997) F_S test statistic uses information from the haplotype distribution to test specifically for population growth and has been shown to be among the best statistics for detecting population growth in comparisons of statistical power (Fu 1997; Ramos-Onsins & Rozas 2002). We used Tajima's (1989) D-test, which contrasts the number of nucleotide differences between sequences (π) and the number of differences between segregating sites (θ). Population expansions can cause significant negative departures of Tajima's D from 0 (Tajima 1989). Fu & Li's (1993) D^* and F^* statistics were calculated for comparison with Fu's F_S. The effects of background selection can be distinguished from population growth or range expansion by examining the pattern of significance between F_S, F^*, and D^* (Fu 1997). If F_S is significant and F^* and D^* are not, then population growth or range expansion is indicated, whereas the reverse suggests selection (Fu 1997). All of these calculations were completed using DNASP 4.0 (Rozas *et al*. 2003).

To estimate time since expansion, we used the mismatch distributions and the nonlinear least-square approach in ARLEQUIN version 2.0 (Schneider *et al*. 1997). The relationship of $\tau = 2ut$ was used where t is the number of generations elapsed between initial population and current population and $u = 2\mu k$, where μ is the mutation rate per million years and k is the length of the sequence (Rogers

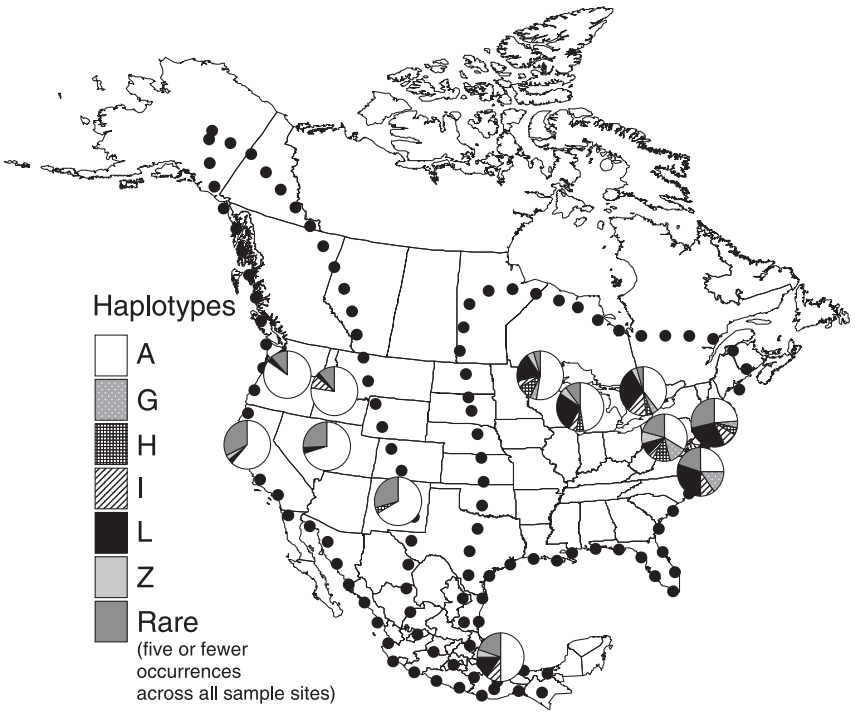

Fig. 1 Map of North American sampling localities (samples collected between August 15, 1999, and October 15, 1999) indicating east–west division (Clark 1985; Hoffman *et al.* 2002) and frequency of haplotype occurrence at each site. Identification of localities is provided in the text.

1995). This algorithm assumes rather than tests for population expansion, therefore, the time estimate is only valid insofar as the assumption holds.

Results

Patterns of genetic variation

We found no variation in cytochrome *b* sequences among 12 sharp-shinned hawk samples examined from 12 geographically disparate localities, each containing distinct and widely varying control region sequences.

Domain I of the control region displayed considerably more variation within sharp-shinned hawks, with 26 (4.90%) variable sites of the 531 examined (GenBank accession numbers AY791851–AY791883). Of these 26 variable sites, 23 had transitions and two had transversions. Empirical nucleotide frequencies among all samples were found to be: $A = 28.75\%$, $C = 30.05\%$, $G = 13.43\%$ and $T = 27.77\%$. A total of 33 control region haplotypes were identified (Fig. 1, Table 1). Haplotype *A* was the only one found at all sample localities. Of the remaining 32 haplotypes, 10 occurred among both eastern and western sites, 13 among western sites only (five at multiple western sites), and nine among eastern sites only (four at multiple eastern sites).

Bootstrap analysis of the maximum parsimony tree found no support for differentiation among any ingroup haplotypes (Fig. 2). There was 100% bootstrap support for *A. s. velox* haplotypes as distinct from *A. nisus*.

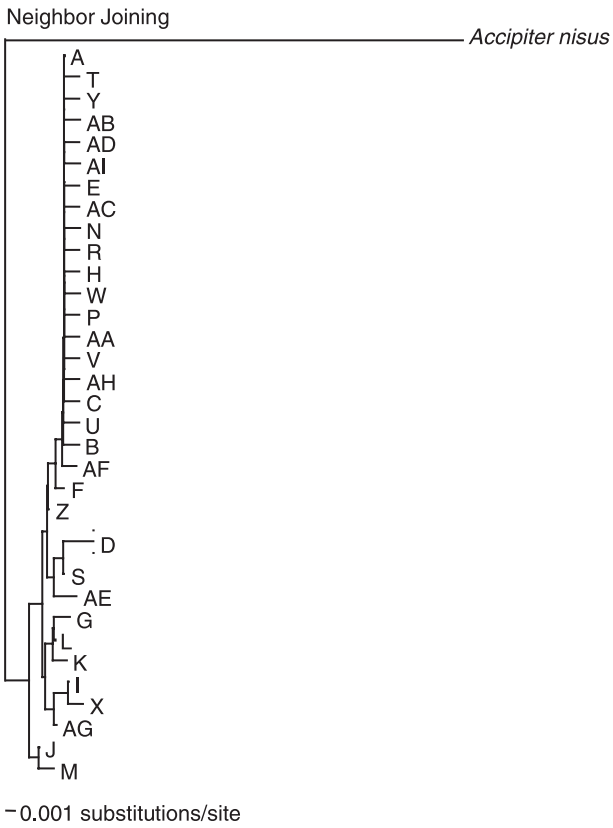

Fig. 2 Neighbour-joining tree from Tamura-Nei distances of control region haplotypes for *Accipiter velox* sampled in North America with *Accipiter nisus* as the outgroup (Tamura & Nei 1993).

Table 1 Haplotype distribution among 12 sampling localities

	Migration sample sites											
Haplotype	OR	CA	ID	NV	NM	ON	MN	WI	NJ	PA	VA	VC
A	22	19	27	19	16	9	11	9	5	8	5	10
B		1	1	1								
C		1	1		1							
D		1										1
E		1		1					2			
F		1										
G						1	1		1	3	3	
H					1	1	2	1	1	3		
I			3			3	1	1	2	1	2	2
J								1	1	1		
K						1			1	1		
L		1		1		7	4	5	6	2	6	3
M									1			
N									1			
P		1										1
R	1											
S	1		1					1				1
T	1			1	1							
U		1		1								
V				1								
W				1				1		1	1	1
X							1					
Y		1			3							
Z	1	1	1			1	1	1		2		1
AA		1										
AB		1										
AC					1							
AD				1								
AE			1									
AF					1					1		
AG										1	1	
AH											1	
AI											1	

Sampling sites: OR, Bonney Butte, Oregon, USA (n = 29); CA, Sausalito, California, USA (n = 31); ID, Boise, Idaho, USA (n = 35); NV, Goshutes, Nevada, USA (n = 27); NM, Manzano Mountains, New Mexico, USA (n = 24); ON, Hawk Cliff, Ontario, Canada (n = 23); MN, Hawk Ridge, Minnesota, USA (n = 21); WI, Cedar Grove, Wisconsin, USA (n = 20); NJ, Cape May Point, New Jersey, USA (n = 21); PA, Little Gap, Pennsylvania, USA (n = 24); VA, Wise Point, Virginia, USA (n = 20); VC, La Mancha, Veracruz, Mexico (n = 20).

Analysis of mean sequence divergence among sampling sites was used to create an average sequence divergence tree (Fig. 3). This tree supports an east–west division among migration sites. Eastern sites were distinct from western sites and were separated from each other by larger genetic distances than sites within the western region. Western sites were more tightly grouped and separated from each other by lower levels of genetic distance. The VC site is near the centre of the network, but appears to have a smaller genetic distance from eastern sites than from western sites.

A minimum spanning network of haplotypes also suggested a difference in haplotype history between East and the West (Fig. 4a). Consideration of minimum spanning networks for eastern and western populations separately (Fig. 4b,c) can yield insight into evolution of the control region in these two geographical areas. In both regions, the strong star-like tree topology suggests a recent population expansion. In the West (Fig. 4b), high frequency and ubiquity of haplotype A suggested that it was the progenitor of most western haplotypes, except for D, I, L, S and AE. The minimum spanning network for the eastern sites (Fig. 4c) displayed more structure, with both haplotype A and haplotype L acting as important progenitors for eastern haplotypes.

Pairwise Φ_{ST} comparisons among sites revealed no significant genetic differentiation among eastern sampling sites including the Veracruz sampling locality ($P > 0.05$,

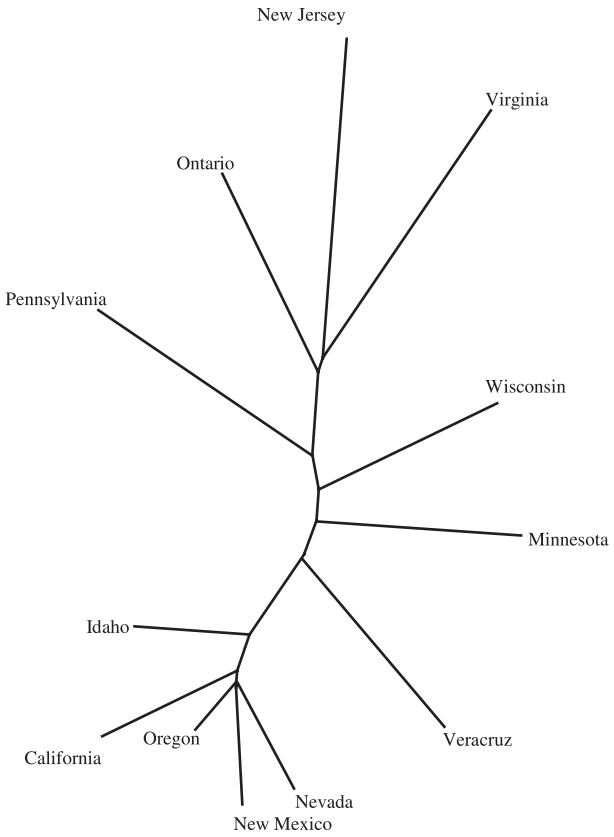

Fig. 3 Neighbour-joining clustering network of sampling localities based on average sequence divergence among sites.

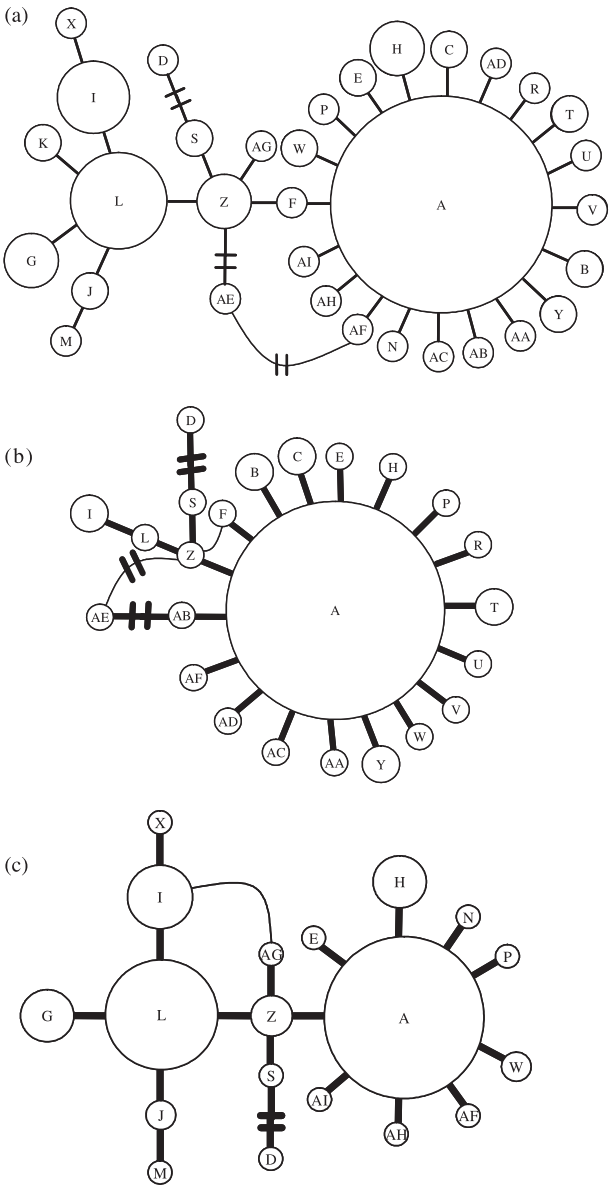

Fig. 4 Minimum spanning networks depicting absolute difference between haplotypes for (a) all sampling localities, (b) western sampling localities only and (c) eastern sampling localities only. The relative abundance of each haplotype is indicated by the sizes of the circles, hash marks indicate the number of base pair changes between haplotypes (a single base pair change is indicated by absence of a hash mark).

Table 2). In the West, the only significant distinction indicated by pairwise Φ_{ST} comparisons was between ID and NM. Except for ID, which was not significantly differentiated from VC or MN, western locations were significantly distinct from all eastern locations. Several groupings of sites were compared with test hypotheses about groupings that might maximize partitioning of genetic variance and, in particular, to sort out the placement of ID, VC and MN. The grouping of sites that displayed highest Φ_{CT} value was the West, including OR, CA, ID, NV and NM, and the East, including ON, MN, WI, NJ, PA and VA along with VC ($\Phi_{CT} = 0.16895$; $P < 0.01$) (Fig. 1). The groupings with next highest support were: a western group containing OR, CA, NV, ID, NM, MN, and VC, and an eastern group containing ON, NJ, PA, VA, WI ($\Phi_{CT} = 0.10850$; $P < 0.01$). All other logically hypothesized groupings based on biogeographical proximity, haplotype distribution among sampling sites, and/or results of pairwise Φ_{ST} analyses gave nonsignificant results, as exemplified by a western group consisting of OR, CA, NV and NM, and an eastern group composed of ON, MN, WI, NJ, PA, VA, VC and ID ($\Phi_{CT} = 0.05095$; $P = 0.062$).

Analysis of genetic diversity among eastern sites revealed 17 transitions and no transversions in 17 polymorphic sites, a haplotype diversity of 0.793, a nucleotide diversity of 0.00324 and an average pairwise distance value of 1.72. In the West, 19 transitions and two transversions were found among 21 polymorphic sites with a haplotype diversity value of 0.473, a nucleotide diversity of 0.00140 and an average pairwise distance value of 0.741. Both eastern and western groups demonstrate a classic pattern of high haplotype diversity along with low nucleotide diversity often associated with a recent population expansion.

Table 2 Pairwise ϕ_{ST} values between sampling sites

	OR	CA	ID	NV	NM	ON	MN	WI	NJ	PA	VA	VC
OR	0.000											
CA	−0.013											
	–	0.000										
ID	0.013	−0.007										
	–	–	0.000									
NV	−0.011	−0.011	0.016									
	–	–	–	0.000								
NM	0.022	0.006	0.051	0.114								
	–	–	+	–	0.000							
ON	0.353	0.259	0.215	0.323	0.353							
	+	+	+	+	+	0.000						
MN	0.179	0.110	0.068	0.157	0.193	0.005						
	+	+	–	+	+	–	0.000					
WI	0.215	0.132	0.095	0.191	0.233	−0.006	−0.036					
	+	+	+	+	+	–	–	0.000				
NJ	0.333	0.250	0.220	0.304	0.333	−0.032	0.020	0.002				
	+	+	+	+	+	–	–	–	0.000			
PA	0.191	0.130	0.096	0.176	0.202	−0.004	−0.031	−0.028	0.006			
	+	+	+	+	+	–	–	–	–	0.000		
VA	0.365	0.277	0.238	0.335	0.365	−0.033	0.026	0.014	−0.024	0.007		
	+	+	+	+	+	–	–	–	–	–	0.000	
VC	0.139	0.076	0.041	0.133	0.168	0.030	−0.023	−0.032	0.044	−0.013	0.048	
	+	+	–	+	+	–	–	–	–	–	–	0.000

Sampling sites: OR, Bonney Butte, Oregon, USA (n = 29); CA, Sausalito, California, USA (n = 31); ID, Boise, Idaho, USA (n = 35); NV, Goshutes, Nevada, USA (n = 27); NM, Manzano Mountains, New Mexico, USA (n = 24); ON, Hawk Cliff, Ontario, Canada (n = 23); MN, Hawk Ridge, Minnesota, USA (n = 21); WI, Cedar Grove, Wisconsin, USA (n = 20); NJ, Cape May Point, New Jersey, USA (n = 21); PA, Little Gap, Pennsylvania, USA (n = 24); VA, Wise Point, Virginia, USA (n = 20); VC, La Mancha, Veracruz, Mexico (n = 20). +, Significant values ($P < 0.05$); –, nonsignificant values.

Historical demography

Significant genetic differentiation between eastern and western groups led us to evaluate historical demography of the eastern and western regions separately. Mismatch distributions closely fit unimodal curves typically associated with recent population expansion (Fig. 5). These populations did not show a pattern associated with expectations of stationarity and their raggedness indices were low, as expected for populations under expansion in both the East (rg = 0.031, $P < 0.05$) and the West (rg = 0.093, $P < 0.05$).

The results for the various neutrality tests are summarized in Table 3. Fu's F_S statistic was significant for both populations showing greater negative deviation in the West ($F_S = -29.73$, $P < 0.001$) than in the East ($F_S = -7.96$, $P = 0.006$). Results for Fu's F^* and D^* were not statistically significant for either the western ($F^* = -2.27$, $P > 0.05$; $D^* = -2.26$, $P > 0.05$) or the eastern population ($F^* = -2.10$, $P > 0.05$; $D^* = -2.12$, $P > 0.05$). Tajima's D-values were significantly negative in the West ($D = -2.16$, $P = 0.005$), however, in the East Tajima's D, although negative, was not significantly different from zero ($D = -0.97$, $P = 0.173$). Finally, the expansion coefficients (S/d) for both regions were found to have large values, with the value in the West (S/d = 28.95) being larger than that in the East (S/d = 9.87).

Estimates of time since expansion for each population were generated from the mismatch distributions resulting in a τ of 2.160 for eastern sites and 0.855 for western sites. Using a mutation rate of 14.8% per million years for domain I of the control region (Wenink *et al*. 1996; Merilä *et al*. 1997) and a generation time of two years for sharp-shinned hawks (Bildstein & Meyer 2000), time since expansion was estimated to be approximately 14 000 BP for the eastern group and 5000 BP for the western group.

Discussion

The genetic data support a recent history of expansion of sharp-shinned hawks in North America. However, this study and others suggest that these migratory raptors appear to have an East–West continental distinction. Our analyses suggest that sharp-shinned hawks associated with these two regions were differentially affected by the climatic events during the Holocene.

Population structure

As expected based upon mark–recapture data, control region data for migratory sharp-shinned hawks support an east–west division among birds migrating through North

	East	West	Expectation Selection	Expansion
Number of haplotypes	20	24		
Nucleotide diversity	0.00324	0.00140	Low	Low
Haplotype diversity	0.79	0.47	Low	High
Expansion coefficient (S/d)	9.87	28.95		High
Tajima's (1989) D	−0.968	−2.164*	Significant	Significant
Fu & Li's (1993) F^*	−2.10	−2.27	Significant	Not significant
Fu & Li's (1993) D^*	−2.12	−2.26	Significant	Not significant
Fu's (1997) F_S	−7.96**	−29.73**	Not significant	Significant
Raggedness (rg)	0.031*	0.093*		
τ	2.160	0.855		
Time since expansion (years)	*c*. 14 000	*c*. 5000		

Table 3 Diversity, neutrality and expansion time estimates for migratory sharp-shinned hawks for eastern and western regions of North America from mitochondrial control region sequences

Expectations under mechanisms of selection or population expansion are given (Peck & Congdon 2004). Significant values in the data are indicated with asterisks: *$P < 0.05$; **$P < 0.01$.

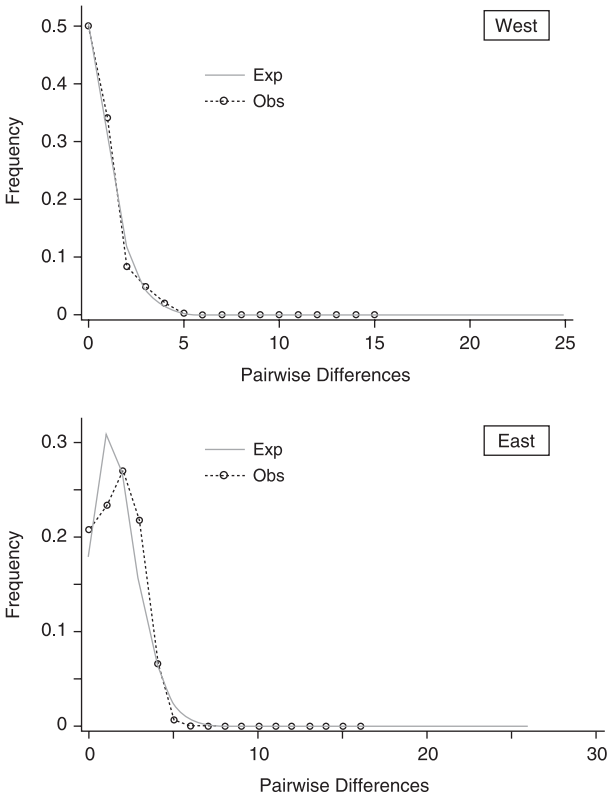

Fig. 5 Mismatch distributions for (a) western sampling localities and (b) eastern sampling localities: grey line, expected; dashed line with circles, observed.

America corresponding to areas separated by the Rocky Mountains and Great Plains. The western group was described by sampling sites along the Pacific coast and in the Great Basin (OR, CA, ID, NV and NM). The eastern group was composed of the Midwestern and Atlantic sampling sites (MN, WI, ON, NJ, VA, PN and VC). This grouping of samples appears to be most appropriate based upon the AMOVA results and the neighbour-joining tree of sampling sites, and correlates well with known band recovery data. Banding studies have found that individuals migrating through western migratory sites were strictly associated with putative western breeding populations and those caught travelling through eastern migratory sites were associated with eastern breeding sites (Clark 1985; Hoffman *et al*. 2002; Idaho Bird Observatory 2004). The east–west division of genetic variation found in this study is consistent with previous studies of continental scale population structure in North American migratory birds (Milot *et al*. 2000; Kimura *et al*. 2002). Identification of eastern and western populations, along with previous band recovery data, may be of use to researchers at North American banding stations, and suggests that, although some straying may occur, the East and the West are generally behaving as two separate demographic units. Thus, perhaps population trends for eastern and western North America should be monitored and considered separately. Further examination of samples collected in breeding and wintering grounds will be important in the process of connecting the events being monitored at migration stations to endpoint locations.

Population expansion

The results from this study are consistent with those predicted under a scenario of population expansion of sharp-shinned hawks in both the East and the West. However, the analyses show differences between the East and West indicating disparate demographic histories.

Statistical tests of neutrality provide strong support for the differential patterns of expansion in the eastern and western regions. Although various factors such as

background selection, population growth and selective sweeps can account for deviations from neutrality, they can be distinguished from one another by evaluating the significance of different analyses. Fu's (1997) F_S is particularly sensitive to population growth and selective sweeps relative to Fu & Li's (1993) D^* and F^*, whereas D^* and F^* are more sensitive to background selection than F_S (Fu 1997). Therefore, a pattern of significant F_S with nonsignificant F^* and D^* indicates population expansion or selective sweep and the opposite pattern would indicate effects of background selection (Peck & Congdon 2004).

Our data support a pattern of population expansion showing significant negative values for Fu's F_S and nonsignificant values for Fu and Li's D^* and F^* (Table 3). In all cases the values associated with the western population were more negative, perhaps indicating a stronger expansion signal than that for the East. This association with population expansion was also supported by the mismatch distributions, which were not significantly different from the expectation of expansion, and had low levels of raggedness. A difference in the mismatch distributions in the West and the East is noticeable and may correspond to the differences in the pattern of expansion suggested by the neutrality tests described above. This difference is more keenly highlighted by the results of Tajima's D, which has been used to test for population expansion, but is known to be intermediate (between Fu's F_S and Fu & Li's F^* and D^*) in its sensitivity to population expansion (Tajima 1989; Congdon *et al*. 2000; Ramos-Onsins & Rozas 2002). Tajima's D was significantly negative in the West and was negative but nonsignificant in the East, suggesting a stronger signal for expansion in the West, which may suggest a more recent expansion event in the West relative to the East (Peck & Congdon 2004). Although the results from the neutrality tests employed here can suggest either population expansion or selective sweeps, it would require additional data from independent nuclear loci to definitively distinguish between the two mechanisms. However, we know of no empirical evidence of selective sweeps in vertebrate mtDNA using comparisons of independent nuclear loci. Rather, we find an increasing prevalence of studies that report results indicating a signature of rapid expansion (Donnelly *et al*. 2001; Hahn *et al*. 2002).

Other indications of population expansion come from the pattern of a low level of nucleotide diversity and a high level of haplotype diversity. This was supported by large values of the expansion coefficient, which were greater than those found in other studies reporting population expansion (von Haeseler *et al*. 1996; Peck & Congdon 2004). In addition, the parsimony tree, the neighbour-joining tree and the minimum spanning trees generated in this study display star-like patterns, which suggest a recent, rapid population expansion (Rogers 1995; Mila *et al*. 2000). This pattern is seen for sharp-shinned hawks as a whole, as well as for both the eastern and western populations individually, indicating each population has experienced rapid population growth.

Finally, we found a lower level of genetic variation within North American sharp-shinned hawk populations than has been previously found in North American Passerines. The cytochrome *b* data for the Sharp-shinned hawks analysed revealed no sequence variation across the continent. This lack of variability was unexpected given the large geographical range sampled for *A.* s. *velox* and an estimated census population size of over one million (Ferguson-Lees & Christie 2001).

Haplotype A was the most prevalent haplotype examined, forming an internal node in the minimum spanning tree that led to 19 terminal haplotypes, suggesting that this may be a progenitor haplotype in this species. Within *A. s. velox* in the West, haplotype A was found at all sites, comprised the majority of haplotypes sampled, and occupied an interior node of the minimum spanning tree. Haplotype A also appeared to be the immediate progenitor of the majority of haplotypes in the West. In the east, haplotype A was still prominent but haplotype L was also common and occupied another central node of the minimum spanning tree for the East. From these patterns it appears that haplotypes A (East and West) and L (East only) were widely distributed during the time of population expansions and may be progenitors of many of the other haplotypes seen with more restricted distributions among sites. Moreover, it suggests that the West and the East have different demographic histories.

Climatic impacts on the history of sharp-shinned hawks in North America

The timing of the recent population expansions in the east and west differed, with estimations of 14 000 ybp and 5000 ybp, respectively. While the accuracy of these estimates depends on the mutation rate, they suggest that the western group is considerably younger than the eastern group. The estimate associated with the eastern group is consistent with a response to glacial retreat from the last glacial maximum approximately (22 000–16 000 years ago (Webb & Bartlein 1992; Eyles 1993). This pattern of population expansion is similar to postglacial population growth experienced by other bird species (Avise & Walker 1998; Mila *et al*. 2000; Peck & Congdon 2004).

There are two possible explanations for such a recent expansion of sharp-shinned hawks in the West. It is possible that the hawks were confined to eastern North America prior to about 5000 years ago and exhibited late growth from a single expanding population (see Zink *et al*. 2000) or that they were present in the West and then experienced a critical population decline. The fossil record for sharp-shinned hawks indicates they were present in larger numbers in the

west 12 000 years ago, following the last glacial cycle, and prior to 5000 years ago (Howard & Miller 1933; Brodkorb 1964). Consequently, it appears that a significant event occurred roughly 5000 years ago resulting in either a severe reduction of the western population or total elimination of breeding sharp-shinned hawks in western North America.

Such a population reduction could conceivably have resulted from climatic events of the Hypsithermal, which reduced Sharp-shinned Hawk habitat along their migration route and within their breeding range. During this period, desert flora expanded in the North American south-west, replacing woodlands while seasonal droughts and fire frequency increased (Delcourt & Delcourt 1993; Buck & Curtis 1999; Carcaillet *et al*. 2001). Concurrent with western aridity, drought stress in the Pacific North-west breeding range resulted in development of forest communities with more open canopies such as Douglas fir and a decrease in prevalence of mesophytic conifers (Delcourt & Delcourt 1993; MacDonald *et al*. 1993; Foley 1994; Davis 1999; Anderson *et al*. 2001). This shift in community structure could have greatly reduced both the foraging and nesting habitat of sharp-shinned hawks (Rosenfield *et al*. 1991).

Current paradigms of avian genetic structure and historical demography have been largely shaped by studies of North American songbird population structure and colonization patterns. However, Hypsithermal effects have not been reported in studies of North American songbird taxa examined to date. Patterns in these studies have typically been found to be concordant with interglacial expansions during the Pleistocene but have not demonstrated an influence of the Hypsithermal in the West (Mila *et al*. 2000; Milot *et al*. 2000; Kimura *et al*. 2002). In contrast to sharp-shinned hawks, songbird species studied previously, such as Wilson's warblers (*Wilsonia pusilla*), MacGillivray's warblers (*Dendroica petechia*), and yellow warblers (*Oporornis tolmiei*), have broader habitat tolerances (Bent 1953). Thus, loss of dense stands of conifer may not have resulted in a severe mid-Holocene population decline in these types of species.

The Hypsithermal has been implicated in range contraction in other organisms. Among avian species, low genetic variation in the Japanese rock ptarmigan (*Lagopus mutus japonicas*) appears to have been the result of a bottleneck during the Hypsithermal dry period caused by a decrease in Japanese stone pine (*Pinus pumila*) zone that is the required habitat of the Japanese rock ptarmigan (Yoshiyuki *et al*. 2001).

Climatic events during the Hypsithermal have also been implicated in altering population distribution of a variety of nonavian organisms. For example, Douglas *et al*. (2003) used molecular genetic evidence to suggest that population divergence in flannelmouth suckers (*Catostomus latipinnis*) may have resulted from drought conditions that peaked in the American west between 7000 ybp and 4000 ybp. Similarly, a study of the ringed salamander (*Ambystoma annulatum*) revealed low levels of genetic variation that may be the result of a recent range expansion following the climatic events and habitat changes during the Hypsithermal (Phillips *et al*. 2000). Finally, anthropological studies of Native American human populations suggest that humans abandoned dwellings in the Atacama desert during the Hypsithermal period (Nunez *et al*. 2002).

This study suggests that, species tied to dense forest habitats in western North America may have been differentially impacted by the Hypsithermal relative to their conspecifics in the East. However, it remains to be seen whether the Hypsithermal had a broad effect on North American raptor species in particular and/or whether the effect will be increasingly discovered across North American taxa requiring dense forest habitat.

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