IBIS
BOU international journal of avian science
Ibis (2023) doi: 10.1111/ibi.13182

Population genetics and phylogeography of north American Merlins (*Falco columbarius*) in the post-DDT era

BREANNA L. MARTINICO,*[1,2] GEORGE K. SAGE,[3] MEGAN C. GRAVLEY,[3] SANDRA L. TALBOT,[3] RYAN P. BOURBOUR,[1,2] ANGUS C. HULL,[2] BRUCE A. HAAK,[4] ALLEN M. FISH[2] & JOSHUA M. HULL[1,2]
[1]*Department of Animal Science and Graduate Group in Ecology, University of California, Davis, California, USA*
[2]*Golden Gate Raptor Observatory, Golden Gate National Parks Conservancy, San Francisco, California, USA*
[3]*Far Northwestern Institute of Art and Science, Anchorage, Alaska, USA*
[4]*Eagle, Eagle, Idaho, USA*

In North America, the population genetic structure of many raptor species has been shaped by patterns of post-glacial population expansion and anthropogenic forces, such as the widespread use of the organochlorine pesticide dichlorodiphenyltrichloroethane (DDT) during the mid-20th century. While common themes of post-glacial avian population expansion have emerged, little is known about the genetic impacts of DDT on raptor species that experienced a population bottleneck but were not the focus of conservation efforts. We investigated how the combination of post-Pleistocene environmental change and the DDT-era population bottleneck have influenced the contemporary population structure of Merlins *Falco columbarius* in North America. We genotyped migrating Merlins across North America (n = 272) at 23 polymorphic microsatellite loci and generated sequence data for a 569-base-pair segment of the mitochondrial control region. We used hierarchical analysis of molecular variance, pairwise F_{ST}/ϕ_{ST} comparisons and Bayesian clustering analyses to assess genetic differentiation between individuals from eastern and western North America, distinct migratory flyways, and three recognized North American subspecies. Across all analyses, we found low or no population differentiation, suggesting that North American Merlins largely comprise one panmictic population showing evidence of a post-glacial population expansion with little genetic differentiation detected between regions. Furthermore, we did not detect a contemporary signal of a genetic bottleneck that could have resulted from the DDT-era population decline with the markers used in this study. Consistent with other avian species, we found a correlation between allele length variation at a microsatellite isolated from the 3′ untranslated region of the *ADCYAP1* gene and migratory versus sedentary characteristics in Merlin subspecies. We detected two common mitochondrial control region haplotypes in the geographical regions sampled, a unique pattern among other widespread North American raptor species. This study furthers our understanding of the genetic and demographic history of Merlins in North America and can inform future genomic studies of this species.

Keywords: *ADCYAP1*, Falconiformes, microsatellite, migration monitoring, mitochondrial control region, population structure, raptor..

Extant patterns of phylogeographical structure of avian taxa have been shaped by large-scale Pleistocene and post-Pleistocene climatic and environmental changes (Klicka & Zink 1997). Intense anthropogenic impacts on natural systems over recent centuries may also alter patterns of population structure and genetic diversity of avian species

*Corresponding author.
Email: bmartinico@ucdavis.edu

(Pimm *et al.* 2006, Jetz *et al.* 2007, Brook *et al.* 2008, Spooner *et al.* 2018). The Merlin *Falco columbarius*, a small falcon with three defined subspecies in North America (*F. c. columbarius*, *F. c. richardsonii* and *F. c. suckleyi*), experienced range-wide population declines due to pesticide exposure (Newton *et al.* 1982, Warkentin *et al.* 2020) and has an unknown phylogeographical history despite being a cosmopolitan species. Understanding the relative importance of these two drivers in shaping population genetic structure can provide insights into the roles of natural and anthropogenic forces on the population structure of species in North America (Petit *et al.* 1998).

In the mid-20th century, many raptor species, including the Merlin, experienced significant population declines due to environmental contamination from the widespread use of the organochlorine pesticide dichlorodiphenyltrichloroethane (DDT; Schick *et al.* 1987, Fry 1995). Raptor population declines were attributed to biomagnification of contaminants in the food web combined with a high physiological sensitivity to DDT, which led to failed reproduction from eggshell thinning that caused egg breakage during incubation (Ratcliffe 1967, Hickey & Anderson 1968, Fox 1971, Cooke 1973, Meek 1988). In addition to direct demographic effects, severe population declines have the potential to negatively affect the genetic health of populations through reduction of overall genomic variation, decreased heterozygosity and loss of alleles, in addition to increased vulnerability to inbreeding depression, genetic drift and extinction associated with small population sizes (O'Brien 1994). Recovering raptor populations may still be vulnerable after these bottleneck events because surviving populations may be less genetically diverse with a lower capacity for future adaptive response to environmental change (Bürger & Lynch 1995).

The declines, subsequent conservation actions and recovery for select raptor species affected by DDT have been well documented (Cade 1974, Simons *et al.* 1988, Bowerman *et al.* 1995, Cade *et al.* 1988, U.S. Fish and Wildlife Service 2003, Henny *et al.* 2010). However, not all raptor species severely affected by DDT have been well studied (Schick *et al.* 1987). Despite receiving no focused conservation efforts or special status designations, North American Merlin populations appear to have rebounded after DDT was banned, as documented through migration counts (Hoffman & Smith 2003), Breeding Bird Surveys (Sauer *et al.* 2013) and Christmas Bird Counts (James *et al.* 1987, Niven *et al.* 2004). Merlins in North America have even expanded their range to include novel urban areas in some regions (Sodhi *et al.* 1992, Houston & Hodson 1997, Warkentin *et al.* 2020). Comprehensively inferring the status and population trends with traditional monitoring methods for North American Merlins has proven to be difficult because the majority of breeding occurs in remote areas and non-breeding populations are dispersed over a vast geographical area. North American Merlins have yet to be the focus of range-wide genetic research, so we have little understanding of current population structure, levels of genetic diversity, adaptive potential or how the DDT-era population bottleneck may have affected the genetics of the species.

In addition to anthropogenic impacts, post-Pleistocene processes can influence range-wide patterns of population structure and genetic diversity within a species through bottleneck and expansion events that happened over geological time (Hewitt 1996). Due to advancing and retreating glaciers (Holocene climate change), populations of many North American avian species were reduced to isolated patches of non-frozen refugial habitat, a process that played an important role in creating the patterns of North American avian species diversity observed today (Klicka & Zink 1997, Petit *et al.* 2003). Once isolated, populations differentiated and developed distinct genetic lineages via genetic drift and natural selection (Hewitt 2000). Upon glacial retreat following the last glacial maxima, population expansion and secondary contact contributed to creating patterns of regional differentiation. Because extant lineages carry genetic signatures reflecting their individual histories of isolation and expansion, it is possible to investigate historical impacts through molecular analyses (Zink 1996). As with many other avian taxa with distinct eastern and western North American populations or subspecies, climatic events during the Pleistocene and Holocene eras have been implicated in contributing to the observed genetic differentiation and population structure (Hull & Girman 2005, Hull *et al.* 2008, 2010). For wide-ranging species, population structure and diversity resulting from both Pleistocene glaciation and Holocene climatic events may reflect differing histories of drift and selection across their range and can be an important factor

in delineating evolutionarily significant units and management units for conservation.

In this study, we investigated how the combination of anthropogenic factors and post-Pleistocene historical demography have influenced the population structure of North American Merlins by using neutral genetic markers. We tested for genetic differentiation between eastern and western regions, among distinct migratory flyways, and among described subspecies. Our specific objectives were to: (1) quantify contemporary population structure and investigate potential genetic impacts from the DDT era using 23 polymorphic nuclear microsatellite loci; (2) investigate genetic differences between the three described North American subspecies using microsatellite loci to determine whether neutral genetic differences corresponded to subspecies designations assigned based on plumage characteristics; and (3) determine how Pleistocene glaciation and Holocene climate change impacted the population structure of Merlins in North America using a 569-base-pair sequence of the control region of the mitochondrial DNA.

METHODS

Sample collection

We collected DNA samples via two plucked breast feathers from Merlins (n = 272) in 2009 at 13 migration monitoring sites across North America. In addition to Merlins sampled at migration monitoring sites, samples from Alaska were also augmented with samples (blood, tissue, feather or epithelial swab) provided by a wildlife rehabilitation centre or from dead Merlins salvaged by colleagues and made into study specimens. We tested for differences among a priori groups delineated by four distinct North American raptor migration corridors (flyways): the Pacific, Intermountain (a distinct raptor migration corridor within the Central Flyway), Mississippi and Atlantic flyways (Fig. 1a). These samples are of unknown breeding origin, although these groupings are biologically relevant because past genetic analyses have revealed that migration sites correspond to distinct breeding populations of many North American raptors (Hull & Girman 2005, Goodrich & Smith 2008, Hull *et al.* 2010). Merlins sampled in Alaska were considered a fifth group because of the unknown migratory routes used (Warkentin *et al.* 2020). Hereafter this grouping is referred to as 'Alaska/flyways'. Within the Pacific Flyway, samples were collected from Marin, California, USA (n = 33). Within the Intermountain Flyway, samples were collected from Chelan Ridge, Washington (n = 19), Bonney Butte, Oregon (n = 9), Goshutes Mountain, Nevada (n = 6), Manzano Mountain, New Mexico (n = 1), Commissary Ridge, Wyoming (n = 4), and Boise, Idaho (n = 36). Within the Mississippi Flyway, samples were collected from Cedar Grove, Wisconsin (n = 36), and Hawk Ridge, Minnesota (n = 30). Within the Atlantic Flyway, samples were collected from Cape May, New Jersey (n = 31), Eastern Shore of Virginia National Wildlife Refuge, Virginia (n = 30), and Florida Keys, Florida (n = 7). For the Alaska group, samples were collected during autumn migration from Palmer, Alaska (n = 21); these samples were augmented by samples collected from dead Merlins found by colleagues during the autumn months (n = 2) and from 11 Merlins treated in a wildlife rehabilitation centre, nine of which were admitted during the nesting season and two that may have been migratory (see Sage *et al.* 2020). Additionally, we assigned individuals to east and west groups for comparisons, hereafter referred to as 'east–west'. Individuals were delineated as eastern if they were sampled in the eastern flyways, Mississippi and Atlantic, and western if they were sampled in Alaska or the western flyways, Pacific and Intermountain, following the biogeographical pattern observed in multiple North American avian taxa (Zink 1996).

As currently described, named subspecies of North American Merlins have relatively distinct breeding ranges and exhibit distinct plumage coloration from dark (*F. c. suckleyi*, Fig. 1b) in the Pacific Northwest, to moderate (*F. c. columbarius*, Fig. 1c) across the North American taiga, to pale (*F. c. richardsonii*, Fig. 1d) in the northern prairie region (Haak 2012, Warkentin *et al.* 2020). Eastern Idaho is one of the few locations where all three subspecies are known to occur in large numbers during the non-breeding season and where subspecies determination based on an individual's plumage was standard procedure at the sampling location (Haak 2012): *F. c. columbarius* (n = 16), *F. c. richardsonii* (n = 6) and *F. c. suckleyi* (n = 14). Individuals were not systematically identified to subspecies at other sampling locations and determination cannot be done post-sample collection.

Samples were collected and donated under authorization of the US Geological Survey's Bird

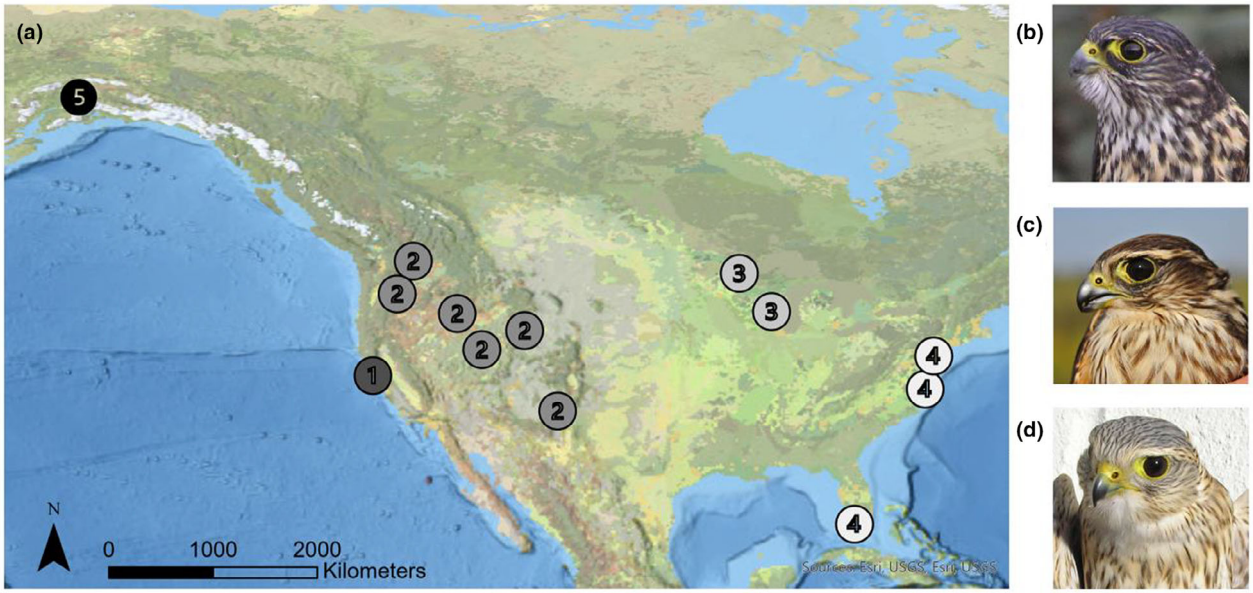

Figure 1. (a) Map of North America showing sampling locations labelled by flyway (1: Pacific, 2: Intermountain, 3: Mississippi and 4: Atlantic; 5 corresponds to the Alaska sampling location and it is not known to which flyway Merlins originating in this region belong). Flyway delineations for North American raptors are described in Goodrich and Smith (2008). Plumage variation of North American Merlin subspecies (b) *Falco columbarius suckleyi*, (c) *Falco columbarius columbarius* and (d) *Falco columbarius richardsonii* (Photos: B. Haak).

Banding Laboratory through site-specific permits issued to individual research stations. Funding for work on this project was provided by USGS Alaska Science Center and the Golden Gate National Parks Conservancy/Golden Gate Raptor Observatory.

Data collection

DNA was extracted from muscle, blood, feather or epithelial swab samples at the USGS Alaska Science Center using procedures outlined in Medrano *et al.* (1990) and modified as outlined in Sonsthagen *et al.* (2004). For feather and epithelial swab samples, extraction procedures were further modified as described in Talbot *et al.* (2011). Genomic DNA extractions were quantified using fluorometry and diluted to 50 ng/μL working solutions.

We genotyped each individual at seven microsatellite loci known to be polymorphic in other falcon species (Peregrine Falcon *Falco peregrinus*: NVHfp31, 46–1, 79–4, 89, 92–1, 107, Nesje *et al.* 2000; Gyrfalcon *Falco rusticolus*: NVHfr34, Nesje & Røed 2000) and 16 microsatellite loci isolated and characterized in Merlin (Fco001, 002, 003, 005, 006, 006–1, 008, 011, 012, 013, 014–1, 014–2, 015, 016, 020, 022, and in the 3′ untranslated region (UTR) of the adenylate cyclase-activating polypeptide 1 (*ADCYAP1*) gene; Hull *et al.* 2020), and sequenced a 569-base-pair segment of domain 1 of the mitochondrial control region, using primer sequences and following simultaneous bidirectional sequence procedures similar to those described in detail elsewhere for Peregrine Falcon (Talbot *et al.* 2011, 2017, White *et al.* 2013). Some samples did not produce a product using the mitochondrial DNA primers listed in Talbot *et al.* (2011), so we used a set of internal primers (H299 5′-GCAGTAGTCCGAACCTCGTG-3′ and L319 5′-CACGAGGTTCGGACTACTGC-3′) that amplified two smaller, overlapping portions of the target sequence. For quality control purposes, DNA from two to five individuals representing each designated population was extracted, amplified and sequenced in duplicate.

Microsatellite data analysis

We included individuals that had fewer than three missing loci in the microsatellite analyses and calculated genetic diversity statistics at three levels: (1) Alaska/flyways: Alaska (n = 30), Pacific (n = 31),

Intermountain (n = 70), Mississippi (n = 66) and Atlantic (n = 66); (2) east–west: east (Mississippi and Atlantic; n = 132) and west (Alaska, Pacific and Intermountain groups; n = 131); and (3) overall (n = 263). Genetic diversity metrics included observed (H_O) and expected (H_E) heterozygosities, Hardy–Weinberg equilibrium, and number of alleles using Arlequin ver 3.5.2.2 (Excoffier *et al.* 1992, Excoffier & Lischer 2010), and the number of private alleles using GenAlEx ver 6.5 (Peakall & Smouse 2006, 2012). We performed an analysis of molecular variance (AMOVA) in Arlequin to test for population differentiation between the east–west groups. We calculated pairwise F_{ST} using Arlequin to evaluate significance and measure genetic differentiation among Alaska/flyways, east–west and subspecies groups: *F. c. columbarius* (n = 16), *F. c. richardsonii* (n = 6) and *F. c. suckleyi* (n = 14). We tested for a recent genetic signature of population fluctuation using a Wilcoxon signed-rank test with the two-phase model of microsatellite evolution (90% stepwise mutation model and 10% variation) in the program BOTTLENECK ver 1.2.02 (Piry *et al.* 1999).

Additionally, we used a Bayesian clustering algorithm, STRUCTURE ver 2.3.4 (Pritchard *et al.* 2000, 2003), following an admixture model and no prior to estimate number of clusters over all samples and for individuals identified to subspecies. For all samples, we considered k = 1 to k = 10 with 10 iterations of each k and with an initial burn in of 10 000 Markov chain Monte Carlo (MCMC) and 100 000 subsequent iterations. We performed a second STRUCTURE analysis including only individuals with subspecies identification (without categorizing them for the analysis). We ran k = 1 to k = 5 with 10 iterations of each k and with an initial burn in of 10 000 MCMC and 100 000 subsequent iterations. We visualized plots of mean likelihood L(k) and Δk with STRUCTURE HARVESTER (Earl & vonHoldt 2012) and used the Δk method to infer the most likely number of populations.

Lastly, we investigated variation in the average allele length of the microsatellite found in the 3′ UTR of the *ADCYAP1* gene among individuals of known subspecies that exhibit variation in migratory behaviour. The *ADCYAP1* gene codes for a neuropeptide with a broad functional spectrum, where longer alleles in the microsatellite found at the 3′ UTR of the *ADCYAP1* gene are correlated with early dispersal and migration and greater migratory tendencies in populations of certain avian species, including a raptor species (Mueller *et al.* 2011, Chakarov *et al.* 2013). We compared average allele length between subspecies with a greater migratory tendency (*F. c. columbarius* and *F. c. richardsonii*; n = 22) with the subspecies that is described as largely sedentary (*F. c. suckleyi*; n = 14; Warkentin *et al.* 2020) using a Welch two-sample t test in R ver 3.5.1 (R Core Team 2018).

Mitochondrial data analysis

We included individuals with completely homologous sequences in mitochondrial control region analyses for (1) Alaska/flyways: Alaska (n = 31), Pacific (n = 15), Intermountain (n = 58), Mississippi (n = 41) and Atlantic (n = 47); (2) east–west: east (Mississippi and Atlantic; n = 88) and west (Alaska, Pacific and Intermountain groups; n = 104); and (3) overall (n = 192). We calculated nucleotide diversity (π) and haplotype diversity (H) with DnaSP ver 5 (Librado & Rozas 2009) and created a minimum spanning network of haplotypes from all individuals using a median-joining method in the program NETWORK ver 10.0.0.0 (Bandelt *et al.* 1999). We tested for differences among the east–west groupings using an AMOVA framework (Excoffier *et al.* 1992) and determined significance by calculating ϕ_{ST} between both sets of groupings using Arlequin. We calculated statistics in Arlequin and DnaSP to look for genetic evidence of a population expansion, including Tajima's D (Tajima 1989), Fu's F_S (Fu 1997), and Fu and Li's D^* and F^* statistics (Fu & Li 1993).

RESULTS

Microsatellite

We assessed Hardy–Weinberg equilibrium for all microsatellite loci and found no significant deviation following Bonferroni correction for multiple tests ($\alpha < 0.002$; Table S1). The average number of alleles across the five groups (flyways/Alaska) was 6.6 and the number of private alleles ranged from four to 11 (Table 1). We performed an AMOVA to test for population differentiation among the east–west groups and found that 99.3% of the variation was attributed to differences within sampling sites rather than among sampling sites (Table 2). Pairwise F_{ST} between east–west

Table 1. Characterization of microsatellite loci based on location. The table includes sample sizes (n), expected (H_E) and observed (H_O) heterozygosities, average number of alleles (N_A), and number of private alleles (N_P) for east–west, flyways, Alaska and all individuals.

Group	n	H_E	H_O	N_A	N_P
West	131	0.55	0.53	8.1	22
Alaska	30	0.56	0.57	6.0	8
Pacific	31	0.55	0.54	6.0	4
Intermountain	70	0.54	0.51	7.0	10
East	132	0.55	0.54	8.2	20
Mississippi	66	0.57	0.57	7.6	11
Atlantic	66	0.54	0.54	6.7	9
All	263	0.55	0.54	6.6	0

was low but significant (F_{ST} = 0.006; $P < 0.05$). Between flyways/Alaska, F_{ST} values support little to no genetic differentiation and ranged from 0 to 0.013. Although F_{ST} values were low, significant values of P ($P < 0.002$) were found in three pairwise comparisons (Alaska–Midwest, Alaska–Atlantic, Intermountain–Atlantic pairwise comparisons; Table 3). Subspecies pairwise F_{ST} comparisons indicated little to no genetic differentiation among recognized subspecies at neutral genetic markers with small non-significant values; *F. c. columbarius–F. c. richardsonii*: $F_{ST} = 0$, $P = 0.81$; *F. c. columbarius–F. c. suckleyi*: F_{ST} = 0.003, P = 0.31; *F. c. richardsonii–F. c. suckleyi*: F_{ST} = 0.004, P = 0.44 (Table S2). We could not perform a Mantel test to test for isolation-by-distance based on known geographical origins because samples were obtained from migrating birds with an unknown location of breeding origin (with the possible exception of some of the Alaska samples). It is possible that different individuals from the same breeding population may be sampled at various points throughout the north–south flyways (e.g. New Jersey and Florida), which confounds relationships between genetic and geographical distance.

We tested for recent genetic signature of population fluctuation using BOTTLENECK with a Wilcoxon signed-rank test and did not detect an excess of heterozygosity (P = 0.99); populations that have recently undergone a genetic bottleneck typically show a signature of excess heterozygosity. The test showed that these data skew towards a deficit of heterozygosity (P = 0.02), which is indicative of a recent population expansion, although this result was not significant after a Bonferroni correction ($\alpha < 0.002$). STRUCTURE analyses with no a priori sampling information found support that North American Merlins and associated subspecies generally represent one panmictic population (Fig. S1). For both analyses, all samples and those of known subspecies, the mean likelihood values and the Δk method indicated that k = 1 is the most supported number of populations. Evaluation of the average allele length of the *ADCYAP1* microsatellite showed significant differences in average allele lengths between migratory and less migratory subspecies (T = 2.4, df = 19.5, P = 0.03), with *F. c. suckleyi* having shorter average allele lengths than *F. c. columbarius* and *F. c. richardsonii*.

Mitochondrial control region

For the mitochondrial control region in North American Merlins, haplotype diversity ranged from 0.701 to 0.860 among flyways/Alaska (Table 4), and we identified 27 unique haplotypes in these groups (Table S3). The pattern of haplotype distribution of the minimum spanning network of all samples (Fig. 2) showed two common haplotypes, A and E, appearing in high frequencies in all

Table 2. AMOVA results among *a priori* Alaska and western flyways – Pacific and Intermountain – compared with eastern flyways – Mississippi and Atlantic. Results are based on (1) 23 microsatellite loci with significant values shown in bold type following a Bonferroni correction ($P < 0.002$), and (2) the mitochondrial control region with no values significant at the $\alpha = 0.05$ level.

Source of variation		df	Sum of squares	Variance components	Percentage of variation
(1)	Among groups	1	13.2	0.028	0.52
	Among populations within groups	11	62.6	0.010	0.19
	Within populations	513	2725.7	**5.313**	99.29
(2)	Among groups	1	0.6	−0.003	−0.41
	Among populations within groups	11	8.9	0.000	1.54
	Within populations	179	119.9	0.670	98.88

Table 3. Pairwise F_{ST} values for 23 microsatellite loci shown below the diagonal between Alaska and the four flyways – Pacific, Intermountain, Mississippi and Atlantic, and bold type indicates significance following Bonferroni correction ($P < 0.002$). Pairwise ϕ_{ST} values for the mitochondrial control region for the same groups are shown above the diagonal, and bold values indicate significance ($P < 0.05$).

	Alaska	Pacific	Intermountain	Midwest	Atlantic
Alaska	–	0.000	**0.057**	0.017	**0.058**
Pacific	0.000	–	0.003	0.000	0.000
Intermountain	0.008	0.000	–	0.000	0.000
Midwest	**0.011**	0.003	0.003	–	0.000
Atlantic	**0.013**	0.004	**0.008**	0.001	–

Table 4. Mitochondrial control region diversity statistics, including number of individuals sampled (n), number of haplotypes (N_H), haplotype diversity (H), number of polymorphic sites (S), number of private substitution sites, and nucleotide diversity (π) for east–west, and number of private substitution sites (P_{HS}) for Alaska and all North American flyways.

Group	n	N_H	H	S	P_{HS}	π
West	104	18	0.77	17	8	0.003
Alaska	31	12	0.86	11	1	0.003
Pacific	15	4	0.71	13	4	0.004
Intermountain	58	10	0.73	8	1	0.002
East	88	16	0.74	11	2	0.002
Midwest	41	13	0.78	8	0	0.002
Atlantic	47	8	0.70	7	2	0.002
All	192	27	0.76	19	0	0.002

groups (see also Fig. S2). The third most common haplotype, F, was present in lower frequencies in all groups while the fourth most common haplotype, B, was absent in the Pacific and Mississippi flyways; all other haplotypes were present in fewer than five individuals. Additionally, the Alaska group had six private haplotypes, the highest among all groups.

We found a single instance of a highly divergent haplotype in the Pacific Flyway group, haplotype K, that diverged from the next closest haplotype by eight mutations. Results of a BLAST search showed that haplotype K shared 98.77% identity with the homologous sequence of the control region for a Merlin that was sampled in Inner Mongolia, China (Accession number: KM264304; Dou *et al.* 2016). In contrast, similarity in the homologous sequences between KM264304 and the most common haplotypes in our study, A and E, were a 97.89% and 97.72% match, respectively.

We performed an AMOVA and found that nearly all variation was due to within-sampling-site variation rather than variation between sampling sites or groups. For the east–west comparison, the variation within sampling sites was 98.9% (Table 2). Pairwise ϕ_{ST} comparisons showed little to no genetic differentiation between east–west ($\phi_{ST} = 0$; $P = 0.46$). ϕ_{ST} comparisons among flyways/Alaska groups in the Alaska–Intermountain ($\phi_{ST} = 0.057$) and Alaska–Atlantic ($\phi_{ST} = 0.058$) showed low to moderate genetic differentiation that was found to be significant ($P < 0.05$; Table 3). As a result of the overall absence of population differentiation, we interpreted the expansion statistics at the continent-wide level as the most relevant biological unit (Table 5).

DISCUSSION

We investigated the potential impacts of DDT use and post-Pleistocene climatic events on the population structure and historical demography of Merlins in North America using microsatellite and mitochondrial control region sequences. We did not find evidence of a recent genetic bottleneck, strong genetic differentiation or population structure across their North American range using the genetic markers in this study. We found several small but significant pairwise comparisons, which may indicate weak genetic differentiation among some regions. There were no significant pairwise comparisons between subspecies, but a correlation was detected in allele length at a microsatellite isolated from the 3′ UTR of the *ADCYAP1* gene known to be associated with migratory behaviour in avian species. Additionally, our study revealed that North American Merlins display a pattern of mitochondrial genetic structure with two common haplotypes present in all flyways, a unique pattern compared with other North American species.

The general absence of strong genetic structure across the continent suggests that North American Merlins comprise largely a single panmictic

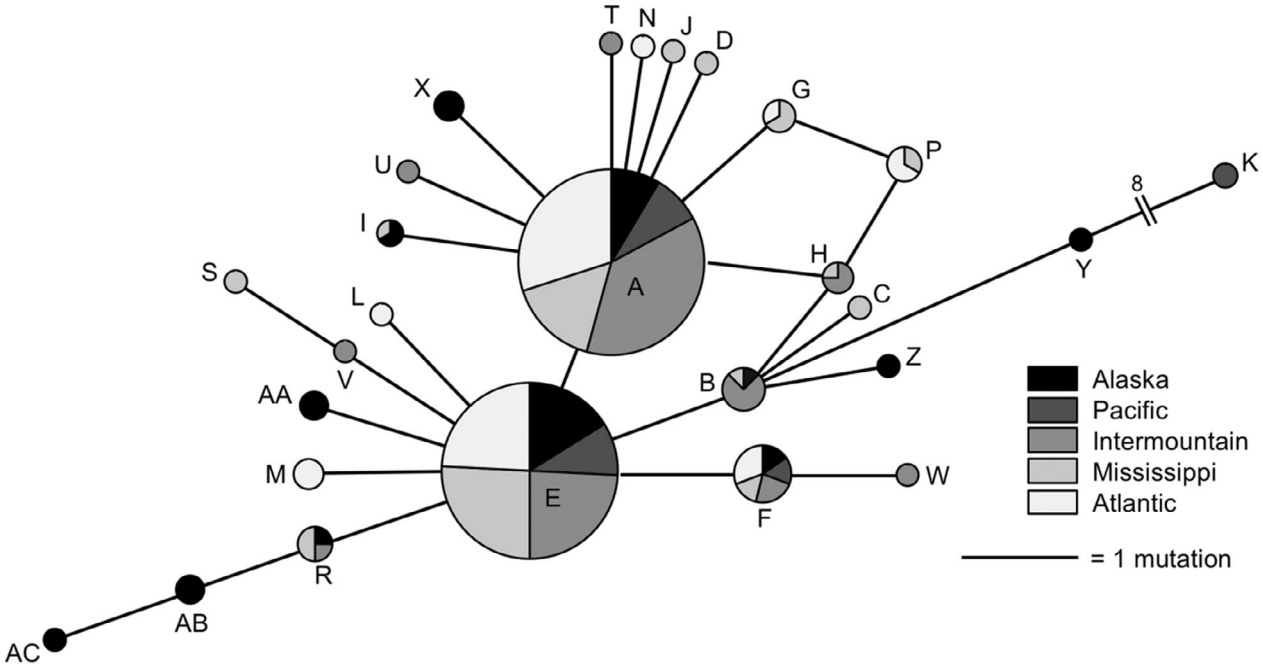

Figure 2. Mitochondrial control region haplotype minimum spanning network for all samples. Circle size is proportional to number of individuals with a particular haplotype. Branch length indicates number of mutations.

Table 5. Expansion statistics based on the mitochondrial control region for four flyways – Pacific, Intermountain, Mississippi and Atlantic – and Alaska. Values in bold type are significant ($P < 0.05$).

Group	Tajima's *D*	Fu's F_S	Fu & Li's *D**	Fu & Li's *F**	*S*/*d*	*k*
West	−1.50	**−28.27**	−1.70	−1.98	11.17	1.52
Alaska	−0.95	**−27.09**	−0.08	−0.41	5.68	1.94
Pacific	**−1.72**	**−19.10**	**−2.32**	**−2.47**	5.78	2.25
Intermountain	−1.00	**−29.51**	−2.38	−2.27	7.43	1.08
East	−1.24	**−29.36**	−0.52	−0.91	9.49	1.16
Mississippi	−0.88	**−28.32**	0.63	0.18	6.19	1.29
Atlantic	−0.92	**−29.92**	−0.29	−0.58	6.72	1.04
All	−1.37	**−28.81**	−2.30	**−2.45**	6.72	1.36

population, with weak differentiation among certain locales, in particular Alaska. We found small but significant differences in pairwise F_{ST} between Alaska–Intermountain, Alaska–Mississippi and Alaska–Atlantic flyways, in addition to the Atlantic–Pacific and Atlantic–Intermountain flyways, using 23 polymorphic microsatellite loci. These results suggest weak levels of isolation-by-distance, genetic drift or both; however, we could not test for isolation-by-distance because of the unknown origin of the migrating individuals sampled and the confounding effects of long-linear flyways on estimating distance among sites. The high degree of similarity between Merlins sampled in Alaska and those sampled in the Pacific Flyway may be a result of a high degree of genetic connectivity and indicates that the Pacific Flyway is an important migratory corridor for Merlins breeding in Alaska, as it is for other migratory avian species in North America (Wilson *et al.* 2018). Genetic studies describing potential source populations of migrants are important in identifying patterns of geographical connectivity, especially for species that have not been large enough for the use of satellite transmitters (Hull *et al.* 2009, Warkentin *et al.* 2020).

After a notable population decline in the mid-20th century associated with the widespread use of DDT, census numbers of North American Merlins have increased, along with an expansion of their geographical range into novel urban habitats (Warkentin & James 1988, Sodhi *et al.* 1992). We did not find evidence that the decrease in census numbers and the subsequent recovery left a detectable genetic bottleneck in our contemporary samples. Similarly, Peregrine Falcons in North America do not show a signature of a genetic bottleneck from the DDT era and rather show increased genetic diversity post-recovery, probably due to the prompt conservation efforts and effects of extensive captive propagation and release programmes, which involved multiple distinct genetic lineages (Brown *et al.* 2007, Talbot *et al.* 2017). Detection of a bottleneck in genetic data may not occur unless the population bottleneck was intensive – i.e. a drop in numbers to 25 or fewer breeding individuals (Luikart *et al.* 1999) – or prolonged enough to cause the loss of rare alleles and declines in genetic diversity (Nei *et al.* 1975, England *et al.* 2003). Additionally, as the number of generations increases post-bottleneck, so does the difficulty in detecting a signature of a demographic bottleneck in genetic analyses, even if the population decline was strong (Mátics *et al.* 2017). The lack of lingering genetic effects from the DDT-era population decline in Merlins may be attributed to the relatively quick cessation of the widespread use of DDT following the discovery of the environmental consequences of its use or the inability to detect it with BOTTLENECK (Talbot *et al.* 2017). Augmentation of this dataset with additional microsatellite data from populations using pre-DDT-era museum samples would allow us to make comparisons between contemporary and historical Merlin population structure and genetic diversity (Brown *et al.* 2007). Such analyses could provide insights into the potential loss of adaptive alleles and the overall impact of a widespread population decline on this species.

For North American Merlin subspecies, differences in regional plumage variation associated with named subspecies are not consistent with differentiation at the neutral genetic markers used in this study. This could indicate that plumage variation is persisting with ongoing interbreeding among subspecies despite their distinct geographical breeding locales. The environmental and/or genetic mechanisms responsible for maintenance of this plumage variation have yet to be discovered and may be relevant to other raptor species that exhibit geographical plumage polymorphism and lack genetic structure (Hull *et al.* 2010). Alternative causes that may be responsible for maintaining plumage variation in Merlins include regional environmental influences or strong selection pressure at a few loci rather than geographical isolation. For Peregrine Falcons, microsatellite analyses separated the darker northwest subspecies (*Falco peregrinus pealei*) from other subspecies (Brown *et al.* 2007, Talbot *et al.* 2017). Interestingly, we found that the subspecies with the highest sedentary tendency, *F. c. suckleyi*, had shorter average allele lengths in the 3′ UTR *ADCYAP1* microsatellite, where longer lengths are associated with increased migratory activity (Mueller *et al.* 2011, Chakarov *et al.* 2013). Although we found an absence of genetic structure among subspecies and flyways with our analyses using neutral markers, broad geographical regions may have different selection pressures acting on specific regions of the genome not analysed in this study.

We found little genetic differentiation in the mitochondrial control region between Merlins in eastern and western North America and among distinct migration flyways. Notably, we did not find the east–west pattern of post-Pleistocene genetic structure as found in other raptor species with continent-wide distributions (Hull & Girman 2005, Hull *et al.* 2008, 2010, Machado *et al.* 2018). The pattern of low nucleotide diversity and high haplotype diversity in combination with the expansion statistics and the minimum spanning network support a pattern of post-Pleistocene population expansion. However, we found two common haplotypes present in each flyway across the North American range of the species, a pattern that has not been previously reported in raptor species with continent-wide distributions. The exact processes and demographic history that led to the presence of two common haplotypes in all groups is unclear and could not be determined through our analyses. It may be possible that historical populations separated by isolated glacial refugia may have experienced a high degree of post-Pleistocene population mixing and/or dispersal compared with other North American raptors. Morphological evidence, habitat affinities and migration tendencies of North American Merlins have previously been thought to indicate a double invasion of Merlins into North

America from the Palaearctic during the Pleistocene (Temple 1972).

Additional genomic data are needed to inform the continuing management of Merlins in North America. Current management prescriptions are based on a model of population stability or range-wide increase (Warkentin *et al.* 2020), although subspecies may have regional differences in selection pressures and the potential for functional evolutionary differences associated with migratory behaviour not captured in this study. Additionally, impacts from anthropogenic threats – for example, habitat loss, pesticides and heavy metal contaminants – vary across North America (Chandler *et al.* 2004, Bourbour *et al.* 2019, Konrad *et al.* 2020). Future studies should prioritize collection of high-quality genetic samples, as further investigation using a whole genome approach was not possible with the quantity of DNA obtained in this study. Studies of migrating and wintering individuals from known breeding origins and pre-bottleneck museum specimens may provide additional insights into fine-scale population differentiation, historical demography and patterns of selection unable to be characterized in this study.

We would like to thank the community scientist volunteers at the Golden Gate Raptor Observatory, and those at HawkWatch International, Hawk Ridge Bird Observatory, Cedar Grove Hawk Research Station and Cape May Raptor Banding Project for sample collection efforts. We thank Ted Swem and Chris Dau (USFWS), Clayton White, Bob Dittrick and the Bird Treatment and Learning Center for providing samples from Alaskan Merlins. We thank Bobbi Pierson for data curation activities. We would also like to thank Tom Hahn, Michelle Hawkins, Megan Crane, Shannon Skalos, Autumn Iverson and the anonymous peer-reviewers for valuable feedback on the manuscript. Any use of trade, product or firm names is for descriptive purposes only and does not imply endorsement by the US Government. This is contribution number 204 of the Golden Gate Raptor Observatory.

CONFLICTS OF INTEREST

The authors declare there were no conflicts of interest.

ETHICAL NOTE

Samples were collected and donated under authorization of the United States Geological Survey's Bird Banding Lab through site-specific permits issued to individual research stations.

FUNDING

None.

AUTHOR CONTRIBUTIONS

Joshua M. Hull: Conceptualization and sample collection; writing – review and editing. **Bruce A. Haak:** Coordination of sample collection. **Angus C. Hull:** Coordination of sample collection. **Allen M. Fish:** Coordination of sample collection; writing – review and editing. **George K. Sage:** Laboratory work; writing – review and editing. **Megan C. Gravely:** Laboratory work; writing – review and editing. **Sandra L. Talbot:** Conceptualization, coordination of sample collection, laboratory work; writing – review and editing. **Breanna L. Martinico:** Conceptualization; data analysis; writing – original draft; writing – review and editing. **Ryan P. Bourbour:** Data analysis; writing – original draft; writing – review and editing.

Data Availability Statement

The data that support the findings of this study are openly available from Sage *et al.* (2020) at https://doi.org/10.5066/P9BOU6CP. For sequence data, GenBank accession numbers are OQ104417–OQ104608.

REFERENCES

Bandelt, H.J., Forster, P. & Röhl, A. 1999. Median-joining networks for inferring intraspecific phylogenies. *Mol. Biol. Evol.* **16**: 37–48.

Bourbour, R.P., Martinico, B.L., Ackerman, J.T., Herzog, M.P., Hull, A.C., Fish, A.M. & Hull, J.M. 2019. Feather mercury concentrations in North American raptors sampled at migration monitoring stations. *Ecotoxicology* **28**: 379–391.

Bowerman, W.W., Giesy, J.P., Best, D.A. & Kramer, V.J. 1995. A review of factors affecting productivity of bald eagles in the Great Lakes region: Implications for recovery. *Environ. Health Perspect.* **103**(Suppl 4): 51–59.

Brook, B.W., Sodhi, N.S. & Bradshaw, C.J. 2008. Synergies among extinction drivers under global change. *Trends Ecol. Evol.* **23**: 453–460.

Brown, J.W., Van Coeverden De Groot, P.J., Birt, T.P., Seutin, G., Boag, P.T. & Friesen, V.L. 2007. Appraisal of the consequences of the DDT-induced bottleneck on the level and geographic distribution of neutral genetic variation in Canadian peregrine falcons, *Falco peregrinus*. *Mol. Ecol.* **16**: 327–343.

Bürger, R. & Lynch, M. 1995. Evolution and extinction in a changing environment: Quantitative-genetic analysis. *Evolution* **49**: 151–163.

Cade, T.J. 1974. Plans for managing the survival of the Peregrine falcon. Management of Raptors. *Raptor Res. Rep.* **89**: 104.

Cade, T.J., Enderson, J.H., Thelander, C.G. & White, C.M. (eds) 1988. *Peregrine Falcon Populations: Their Management and Recovery*. Proceedings of the 1985 International Peregrine Conference. Boise, ID: Sacramento The Peregrine Fund, Inc.

Chakarov, N., Jonker, R.M., Boerner, M., Hoffman, J.I. & Krüger, O. 2013. Variation at phenological candidate genes correlates with timing of dispersal and plumage morph in a sedentary bird of prey. *Mol. Ecol.* **22**: 5430–5440.

Chandler, R.B., Strong, A.M. & Kaufman, C.C. 2004. Elevated lead levels in urban house sparrows: A threat to sharp-shinned hawks and merlins? *J. Raptor Res.* **38**: 62–68.

Cooke, A.S. 1973. Shell thinning in avian eggs by environmental pollutants. *Environ. Pollut.* **4**: 85–152.

Dou, H., Zhao, C., Yang, X., Liu, G., Zhang, J. & Zhang, H. 2016. Complete mitochondrial genome of the Merlin (*Falco columbarius*). *Mitochondrial DNA A DNA Mapp. Seq. Anal.* **27**: 1547–1548.

Earl, D.A. & vonHoldt, B.M. 2012. STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conserv. Genet. Resour.* **4**: 359–361.

England, P.R., Osler, G.H., Woodworth, L.M., Montgomery, M.E., Briscoe, D.A. & Frankham, R. 2003. Effects of intense versus diffuse population bottlenecks on microsatellite genetic diversity and evolutionary potential. *Conserv. Genet.* **4**: 595–604.

Excoffier, L. & Lischer, H.E. 2010. Arlequin suite version 3.5: A new series of programs to perform population genetics analyses under Linux and windows. *Mol. Ecol. Resour.* **10**: 564–567.

Excoffier, L., Smouse, P.E. & Quattro, J.M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. *Genetics* **131**: 479–494.

Fox, G.A. 1971. Recent changes in reproductive success of the pigeon hawk. *J. Wildl. Manage.* **35**: 122–128.

Fry, D.M. 1995. Reproductive effects in birds exposed to pesticides and industrial chemicals. *Environ. Health Perspect.* **103**: 165.

Fu, Y. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking, and background selection. *Genetics* **147**: 915–925.

Fu, Y. & Li, W.H. 1993. Statistical tests of neutrality of mutations. *Genetics* **133**: 693–709.

Goodrich, L.J. & Smith, J.P. 2008. Raptor migration in North America. State of North America's birds of prey. *Ser. Ornithol.* **3**: 37–149.

Haak, B.A. 2012. Winter occurrence of three Merlin subspecies in southwestern Idaho, USA. *J. Raptor Res.* **46**: 220–223.

Henny, C.J., Grove, R.A., Kaiser, J.L. & Johnson, B.L. 2010. North American osprey populations and contaminants: Historic and contemporary perspectives. *J. Toxicol. Environ. Health Part B* **13**: 579–603.

Hewitt, G.M. 1996. Some genetic consequences of ice ages, and their role in divergence and speciation. *Biol. J. Linn. Soc.* **58**: 247–276.

Hewitt, G. 2000. The genetic legacy of the Quaternary ice ages. *Nature* **405**: 907–913.

Hickey, J.J. & Anderson, D.W. 1968. Chlorinated hydrocarbons and eggshell changes in raptorial and fish-eating birds. *Science* **162**: 271–273.

Hoffman, S.W. & Smith, J.P. 2003. Population trends of migratory raptors in western North America, 1977-2001. *Condor* **105**: 397–419.

Houston, C.S. & Hodson, K.A. 1997. Resurgence of breeding Merlins, *Falco columbarius richardsonii*, in Saskatchewan grasslands. *Can. Field-Nat.* **111**: 243–248.

Hull, J.M. & Girman, D.J. 2005. Effects of Holocene climate change on the historical demography of migrating sharp-shinned hawks (*Accipiter striatus velox*) in North America. *Mol. Ecol.* **14**: 159–170.

Hull, J.M., Strobel, B.N., Boal, C.W., Hull, A.C., Dykstra, C.R., Irish, A.M., Fish, A.M. & Ernest, H.B. 2008. Comparative phylogeography and population genetics within *Buteo lineatus* reveals evidence of distinct evolutionary lineages. *Mol. Phylogenet. Evol.* **49**: 988–996.

Hull, J.M., Ernest, H.B., Harley, J.A., Fish, A.M. & Hull, A.C. 2009. Differential migration between discrete populations of juvenile red-tailed hawks (*Buteo jamaicensis*). *Auk* **126**: 389–396.

Hull, J.M., Mindell, D.P., Talbot, S.L., Kay, E.H., Hoekstra, H.E. & Ernest, H.B. 2010. Population structure and plumage polymorphism: The intraspecific evolutionary relationships of a polymorphic raptor, *Buteo jamaicensis harlani*. *BMC Evol. Biol.* **10**: 224.

Hull, J.H., Sage, G.K., Sonsthagen, S.A., Gravley, M.C., Martinico, B.L., Booms, T.L., Swem, T. & Talbot, S.L. 2020. Isolation and characterization of microsatellite loci in merlins (*Falco columbarius*) and cross-species amplification in gyrfalcons (*F. rusticolus*) and peregrine falcons (*F. peregrinus*). *Mol. Biol. Rep.* **47**: 8377–8383.

James, P.C., Smith, A.R., Oliphant, L.W. & Warkentin, I.G. 1987. Northward expansion of the wintering range of Richardson's Merlin (*Falco columbarius richardsonii*). *J. Field Ornithol.* **50**: 112–117.

Jetz, W., Wilcove, D.S. & Dobson, A.P. 2007. Projected impacts of climate and land-use change on the global diversity of birds. *PLoS Biol.* **5**: e157.

Klicka, J. & Zink, R.M. 1997. The importance of recent ice ages in speciation: A failed paradigm. *Science* **277**: 1666–1669.

Konrad, P.M., Shaffer, J.A. & Igl, L.D. 2020. The effects of management practices on grassland birds—Merlin (*Falco columbarius*). In Johnson, D.H., Igl, L.D., Shaffer, J.A. & DeLong, J.P. (eds) *The Effects of Management Practices on Grassland Birds*: 12. Jamestown, ND: U.S. Geological Survey Professional Paper 1842. https://doi.org/10.3133/pp1842R

Librado, P. & Rozas, J. 2009. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **25**: 1451–1452.

Luikart, G., Cornuet, J.M. & Allendorf, F.W. 1999. Temporal changes in allele frequencies provide estimates of population bottleneck size. *Conserv. Biol.* **13**: 523–530.

Machado, A.P., Clément, L., Uva, V., Goudet, J. & Roulin, A. 2018. The Rocky Mountains as a dispersal barrier

between barn owl (*Tyto alba*) populations in North America. *J. Biogeogr.* **45**: 1288–1300.

Mátics, R., Hoffmann, G., Farkas, S., Dawson, D., Frantz, A., Varga, D., Mátics, E. & Klein, Á. 2017. Demographic decline and detection of genetic bottleneck in a population of barn owl *Tyto alba* in Hungary. *J. Ornithol.* **158**: 811–821.

Medrano, J.F., Aasen, E. & Sharrow, L. 1990. DNA extraction from nucleated red blood cells. *Biotechniques* **8**: 43.

Meek, E.R. 1988. The breeding ecology and decline of the Merlin *Falco columbarius* in Orkney. *Bird Study* **35**: 209–218.

Mueller, J.C., Pulido, F. & Kempenaers, B. 2011. Identification of a gene associated with avian migratory behaviour. *Proc. R. Soc. B Biol. Sci.* **278**: 2848–2856.

Nei, M., Maruyama, T. & Chakraborty, R. 1975. The bottleneck effect and genetic variability in populations. *Evolution* **29**: 1–10.

Nesje, M. & Røed, K.H. 2000. Microsatellite DNA markers from the gyrfalcon (*Falco rusticolus*) and their use in other raptor species. *Mol. Ecol.* **9**: 1438–1440.

Nesje, M., Røed, K.H., Lifjeld, J.T., Linberg, P. & Steen, O. 2000. Genetic relationships in the peregrine falcon (*Falco peregrinus*) analysed by microsatellite DNA markers. *Mol. Ecol.* **9**: 53–60.

Newton, I., Bogan, J., Meek, E. & Little, B. 1982. Organochlorine compounds and shell-thinning in British Merlins *Falco columbarius*. *Ibis* **124**: 328–335.

Niven, D.K., Sauer, J.R., Butcher, G.S. & Link, W.A. 2004. Christmas bird count provides insights into population change in land birds that breed in the boreal forest. *Am. Birds* **58**: 10–20.

O'Brien, S.J. 1994. A role for molecular genetics in biological conservation. *Proc. Natl Acad. Sci. U.S.A.* **91**: 5748–5755.

Peakall, R. & Smouse, P.E. 2006. GENALEX 6: Genetic analysis in excel. Population genetic software for teaching and research. *Mol. Ecol. Notes* **6**: 288–295.

Peakall, R. & Smouse, P.E. 2012. GenAlEx 6.5: Genetic analysis in excel. Population genetic software for teaching and research – An update. *Bioinformatics* **28**: 2537–2539.

Petit, R.J., El Mousadik, A. & Pons, O. 1998. Identifying populations for conservation on the basis of genetic markers. *Conserv. Biol.* **12**: 844–855.

Petit, R.J., Aguinagalde, I., de Beaulieu, J.L., Bittkau, C., Brewer, S., Cheddadi, R., Ennos, R., Fineschi, S., Grivet, D., Lascoux, M., Mohanty, A., Müller-Starck G, Demesure-Musch B, Palmé A, Martín JP, Rendell S, Vendramin GG. 2003. Glacial refugia: Hotspots but not melting pots of genetic diversity. *Science* **300**: 1563–1565.

Pimm, S., Raven, P., Peterson, A., Şekercioğlu, Ç.H. & Ehrlich, P.R. 2006. Human impacts on the rates of recent, present, and future bird extinctions. *Proc. Natl Acad. Sci. U.S.A.* **103**: 10941–10946.

Piry, S., Luikart, G. & Cornuet, J.M. 1999. BOTTLENECK: A computer program for detecting recent reductions in the effective population size using allele frequency data. *J. Hered.* **90**: 502–503.

Pritchard, J.K., Stephens, M. & Donnelly, P. 2000. Inference of population structure using multilocus genotype data. *Genetics* **155**: 945–959.

Pritchard, J.K., Wen, W. & Falush, D. 2003. Documentation for structure software: Version 2. Available at: http://web.stanford.edu/group/pritchardlab/software/structure_v.2.3.1/documentation.pdf

R Core Team 2018. *R: A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing Available at: https://www.R-project.org/

Ratcliffe, D.A. 1967. Decrease in eggshell weight in certain birds of prey. *Nature* **215**: 208–210.

Sage, G.K., Pierson, B.J., Sonsthagen, S.A., Gravley, M.C. & Talbot, S.L. 2020. Genetic data for Merlin (*Falco columbarius*) and cross-species microsatellite amplification in select Falco species, North America. U.S. Geological Survey data release, Version 2. https://doi.org/10.5066/P9BOU6CP

Sauer, J.R., Link, W.A., Fallon, J.E., Pardieck, K.L. & Ziolkowski, D.J. Jr. 2013. The North American breeding bird survey 1966–2011: Summary analysis and species accounts. *N. Am. Fauna* **79**: 1–32.

Schick, C.T., Brennan, L.A., Buchanan, J.B., Finger, M.A., Johnson, T.M. & Herman, S.G. 1987. Organochlorine contamination in shorebirds from Washington state and the significance for their falcon predators. *Environ. Monit. Assess.* **9**: 115–131.

Simons, T., Sherrod, S.K., Collopy, M.W. & Jenkins, M.A. 1988. Restoring the bald eagle. *Am. Sci.* **76**: 253–260.

Sodhi, N.S., James, P.C., Warkentin, I.G. & Oliphant, L.W. 1992. Breeding ecology of urban Merlins (*Falco columbarius*). *Can. J. Zool.* **70**: 1477–1483.

Sonsthagen, S.A., Talbot, S.L. & White, C.M. 2004. Gene flow and genetic characterization of northern goshawks breeding in Utah. *Condor* **106**: 826–836.

Spooner, F.E.B., Pearson, R.G. & Freeman, R. 2018. Rapid warming is associated with population decline among terrestrial birds and mammals globally. *Glob. Chang. Biol.* **24**: 4521–4531.

Tajima, J. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* **123**: 585–595.

Talbot, S.L., Palmer, A.G., Sage, G.K., Sonsthagen, S.A., Swem, T., Brimm, D.J. & White, C.M. 2011. Lack of genetic polymorphism among peregrine falcons *Falco peregrinus* of Fiji. *J. Avian Biol.* **42**: 415–428.

Talbot, S.L., Sage, G.K., Sonsthagen, S.A., Gravley, M.C., Swem, T., Williams, J.C., Longmire, J.L., Ambrose, S., Flamme, M.J., Lewis, S.B., Anderson, C., White, C.M. & Phillips, L. 2017. Intraspecific evolutionary relationships among peregrine falcons in western north American high latitudes. *PLoS One* **12**: e0188185.

Temple, S.A. 1972. Systematics and evolution of the North American Merlins. *Auk* **89**: 325–338.

U.S. Fish and Wildlife Service. 2003. Monitoring Plan for the American Peregrine Falcon, A Species Recovered Under the Endangered Species Act: 53. U.S. Fish and Wildlife Service, Divisions of Endangered Species and Migratory Birds and State Programs, Pacific Region, Portland, OR.

Warkentin, I.G. & James, P.C. 1988. Nest-site selection by urban Merlins. *Condor* **90**: 734–738.

Warkentin, I.G., Sodhi, N.S., Espie, R.H.M., Poole, A.F., Oliphant, L.W. & James, P.C. 2020. Merlin (*Falco columbarius*), version 1.0. In Billerman, S.M. (ed) *Birds of*

the World. Ithaca, NY: Cornell Lab of Ornithology. https://doi.org/10.2173/bow.merlin.01

White, C.M., Sonsthagen, S.A., Sage, G.K., Anderson, C. & Talbot, S.L. 2013. Genetic relationships among some subspecies of the Peregrine falcon (*Falco peregrinus L.*), inferred from mitochondrial DNA control-region sequences. *Auk* **130**: 78–87.

Wilson, R.E., Ely, C.R. & Talbot, S.L. 2018. Flyway structure in the circumpolar greater white-fronted goose. *Ecol. Evol.* **8**: 8490–8507.

Zink, R.M. 1996. Comparative phylogeography in north American birds. *Evolution* **50**: 308–317.

Received 1 April 2022;
Revision 3 December 2022;
revision accepted 9 January 2023.
Associate Editor: Jose Antonio Sanchez-Zapata.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Hardy–Weinberg equilibrium for all microsatellite loci by locus and group (Alaska and flyways – Pacific, Intermountain, Mississippi, Atlantic).

Table S2. (A) Pairwise F_{ST} and (B) P values for 23 microsatellite loci for comparison among North American Merlin subspecies.

Table S3. Mitochondrial control region haplotype frequencies observed in Alaska and four North American flyways – Pacific, Intermountain, Mississippi and Atlantic.

Figure S1. Structure plot for all samples shown at $k = 2$ clusters, indicating lack of genetic structure and similar likelihood of each individual belonging to a particular population.

Figure S2. Distribution of proportion of haplotypes in each Flyways/Alaska group of the four most common haplotypes (A, B, E, F) and all others (Rare) in Alaska (top left) and flyways – Pacific, Intermountain, Mississippi and Atlantic (shown from left to right).