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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*

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Abstract

Peregrine falcon populations underwent devastating declines in the mid-20th century due to the bioaccumulation of organochlorine contaminants, becoming essentially extirpated east of the Great Plains and significantly reduced elsewhere in North America. Extensive re-introduction programs and restrictions on pesticide use in Canada and the United States have returned many populations to predecline sizes. A proper population genetic appraisal of the consequences of this decline requires an appropriate context defined by (i) meaningful demographic entities; and (ii) suitable reference populations. Here we explore the validity of currently recognized subspecies designations using data from the mitochondrial control region and 11 polymorphic microsatellite loci taken from 184 contemporary individuals from across the breeding range, and compare patterns of population genetic structure with historical patterns inferred from 95 museum specimens. Of the three North American subspecies, the west coast marine subspecies *Falco peregrinus pealei* is well differentiated genetically in both time periods using nuclear loci. In contrast, the partitioning of continental *Falco peregrinus anatum* and arctic *Falco peregrinus tundrius* subspecies is not substantiated, as individuals from these subspecies are historically indistinguishable genetically. Bayesian clustering analyses demonstrate that contemporary genetic differentiation between these two subspecies is mainly due to changes within *F. p. anatum* (specifically the southern *F. p. anatum* populations). Despite expectations and a variety of tests, no genetic bottleneck signature is found in the identified populations; in fact, many contemporary indices of diversity are higher than historical values. These results are rationalized by the promptness of the recovery and the possible introduction of new genetic material.

Keywords: genetic bottleneck, microsatellite DNA, mitochondrial DNA, museum specimens, peregrine falcons, systematics

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Introduction

Maintaining genetic diversity is important to a population in terms of responding to present evolutionary and environmental forces as well as maintaining the potential to adapt to future conditions (Reed & Frankham 2003). Populations that experience a large reduction in census size (a population bottleneck) are expected to exhibit a reduced level of genetic variation (Nei *et al*. 1975; Maruyama & Fuerst 1984, 1985; Cornuet & Luikart 1996). In contrast with large populations, small populations have a fewer total number of allele copies for a given locus, and are more susceptible to genetic drift and inbreeding depression. As an exacerbating effect, the latter has been

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linked to increased extinction risk (Mills & Smouse 1994; Frankham 1995, 1998; Saccheri *et al*. 1998).

The observed genetic consequences of a bottleneck depend on several factors. First, separate genomes can respond differently to a population decline. Nuclear loci are expected to be less sensitive to bottleneck effects than are cytoplasmic genomes because of differences in effective population size (N_e); however, this relationship can be reversed under skewed mating systems (e.g. Johnson *et al*. 2003). Second, changes in the genetic constitution of a population depend on both the strength and duration of a bottleneck. Of these, the latter is more important in regards to the retention of genetic diversity; theory predicts that the majority of diversity can be retained in populations suffering a severe decline if the bottleneck is not prolonged (Nei *et al*. 1975; Maruyama & Fuerst 1984, 1985; England *et al*. 2003). Third, the time since a bottleneck occurred is the most important variable determining whether genetic signatures will be detected. When a population recovers after a bottleneck, the distribution of genetic diversity returns to equilibrium conditions (Cornuet & Luikart 1996). This consequently puts an upper limit on the age of bottleneck events that can be detected with molecular markers.

The expectation of low levels of genetic variability in reduced populations is borne out empirically. In a recent review, Spielman *et al*. (2004) report that 77% of threatened taxa (74% of threatened birds) exhibit lower heterozygosity than nonthreatened sister taxa, and that on average heterozygosity is 35% lower in these threatened populations. A large reduction in population size may therefore decrease the long-term (evolutionary) potential of a population through erosion of standing genetic variation (Newman & Pilson 1997; Westemeier *et al*. 1998; England *et al*. 2003).

Peregrine falcons (*Falco peregrinus*) possess several characteristics associated with elevated extinction risk (Purvis *et al*. 2000): they occur at low population densities, they have a relatively low reproductive rate, and they are high trophic-level predators. This risk was made manifest during the mid-20th century with the introduction of dichlorodiphenyl-trichloroethane (DDT)-containing pesticides (and to a lesser extent other organochlorines; Risebrough & Peakall 1988). Ratcliffe (1967) first noted that a reduction in eggshell thickness correlated with chlorinated hydrocarbon levels was responsible for reduced reproductive success. As a result of the bioaccumulation of these contaminates, North American peregrine populations underwent unprecedented declines, becoming essentially extirpated east of the Great Plains and significantly reduced elsewhere (Hickey 1969; Fyfe *et al*. 1976; Cade *et al*. 1988). Extensive re-introduction programs in Canada and the United States, together with the establishment of severe restrictions on pesticide use in both countries, have helped populations return to predecline sizes in much of the historical range (Millsap *et al*. 1998; Mesta 1999; Rowell *et al*. 2003).

Three North American subspecies are currently recognized based on morphological, geographical, and ecological characteristics (White & Boyce Jr. 1988; White *et al*. 2002), and each subspecies was affected by DDT to different degrees. *Falco peregrinus pealei* Ridgway 1873, the largest of the world's 19 peregrine subspecies (White & Boyce Jr. 1988), is a Pacific marine subspecies distributed from northwestern Washington state to the Aleutian Islands of Alaska. It is characterized by its diet (predominantly alcids and procellariids), dark plumage, and year-round residency (Beebe 1960). DDT affected this subspecies the least, presumably because of its distinctive prey that are more removed from areas of pesticide use. During the population nadir Fyfe *et al*. (1976) proclaimed *F. p. pealei* populations off the coast of British Columbia as the only stable peregrine populations in North America.

The arctic subspecies *Falco peregrinus tundrius* White (1968) is generally smaller and paler than other North American subspecies, and is highly migratory. Its distribution extends north of the treeline from Alaska to Greenland. Their decline in abundance due to DDT contamination was estimated at 50–60%, with initial abundance estimates at several thousand individuals (Fyfe *et al*. 1976). Much of its historical range has since been naturally re-populated. *Falco p. tundrius* was downlisted on the US Endangered Species List to Threatened in 1984, and de-listed altogether in 1994 (Swem 1994). Canadian populations of *F. p. pealei* and *F. p. tundrius* currently have Special Concern status under the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) and the Species at Risk Act (SARA).

Falco peregrinus anatum Bonaparte 1838 has a continental distribution south of the treeline and east of the Pacific coast. This subspecies is generally intermediate between the other two in size, colour, and migratory behaviour (White *et al*. 2002). *Falco p. anatum* was most severely affected by DDT, being extirpated from the eastern half of the continent by the mid-1970s (Fyfe *et al*. 1976). Historically, some biologists recognized separate western and eastern forms of *F. p. anatum*, the latter being referred to as the 'rock' peregrine (White 1968). If this distinction had evolutionary significance then the rock peregrine is now extinct.

Falco p. anatum was the focus of extensive recovery efforts, with breeding programs initiated in both the United States and Canada in 1974 and 1975, respectively. In all, approximately 7000 individual birds were released (White *et al*. 2002). An important distinction between the American and Canadian recovery plans is that, while in the former a minimum of seven subspecies from around the world were used as breeding stock (Tordoff & Redig 2001), only pure *F. p. anatum* individuals were bred and released in Canada (G. Holroyd, personal communication). *Falco p. anatum* has generally recovered throughout Canada; although eastern Canadian populations are still somewhat small and fragmented, most are stable and many are

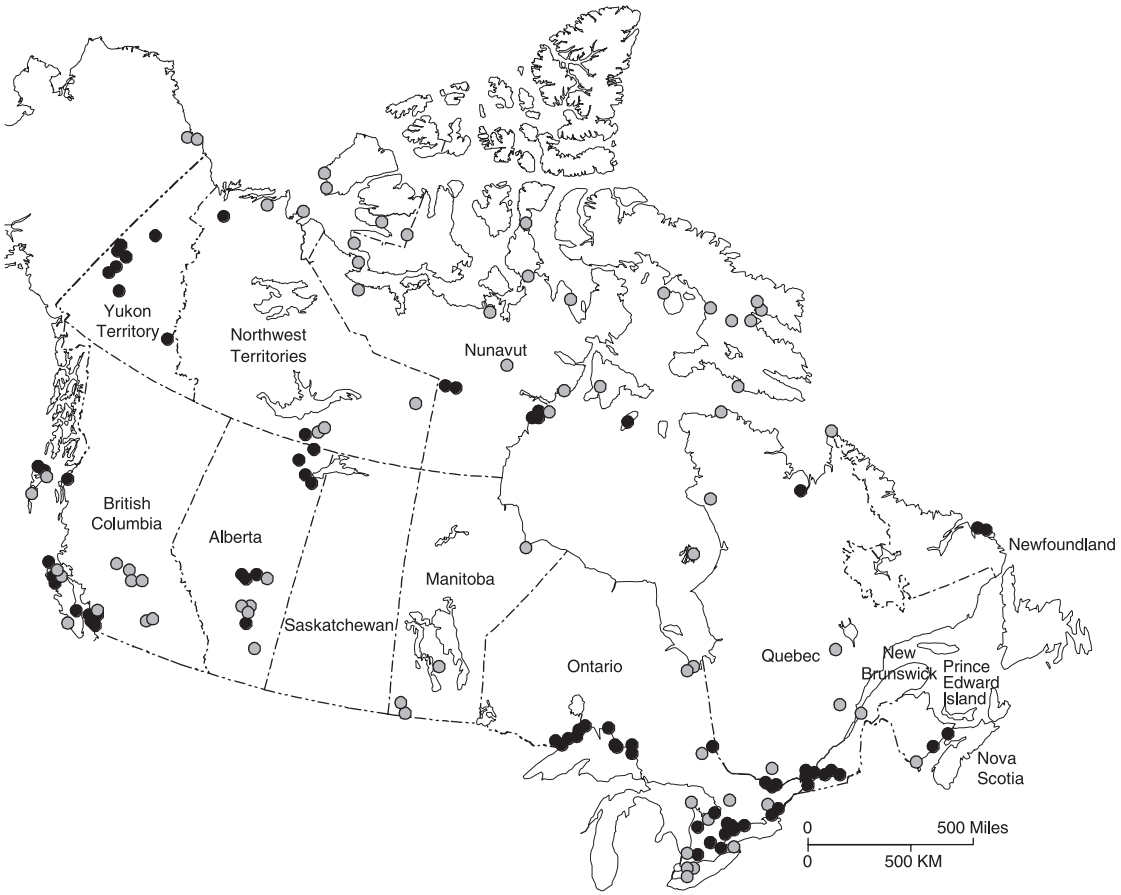

Fig. 1 Sampling locations for historical (grey; $n = 94$) and contemporary (black; $n = 184$) Canadian peregrine falcons. Circles may represent multiple individuals.

experiencing high rates of growth (Rowell *et al*. 2003). The recent increase in census numbers prompted the removal of *F. p. anatum* from the US Endangered Species List in 1999 without first being downlisted to threatened status (Mesta 1999), although this decision was not without debate (Pagel *et al*. 1996; Cade *et al*. 1997; Pagel & Bell 1997; Millsap *et al*. 1998). In Canada, this subspecies currently has threatened status under COSEWIC and SARA.

Despite the success of the North American peregrine recovery, little is known about the genetics of contemporary populations. We utilize data from the mitochondrial (mtDNA) control region and 11 polymorphic microsatellite loci taken from both historic ($n = 95$) and contemporary individuals ($n = 184$) to directly examine the level and distribution of neutral genetic variation through both space and time. Although peregrine falcons can be found throughout North America, we examine only Canadian populations for two reasons: first, all three subspecies are well represented in Canada; second, only native birds were released in the Canadian re-introduction programs, enabling a more direct appraisal of the bottleneck. We focus on three central questions: (i) In terms of genetic diagnosability, what is the validity of current taxonomy that recognizes three North American subspecies? (ii) Did the decline and subsequent recovery alter levels of genetic differentiation among subspecies? (iii) In terms of levels of genetic diversity, what were the genetic consequences of the DDT-induced bottleneck of the mid-20th century? Conclusions from analyses using only contemporary data are compared to those that make use of data from both time periods, thereby evaluating the efficiency of the former in reconstructing the demographic past.

Materials and methods

Tissue collection and DNA extraction

Blood ($n = 143$), feather ($n = 31$), or muscle ($n = 10$) tissue samples were collected from contemporary birds from across the Canadian breeding range between 1991 and 2004 (Fig. 1, Table 1; Table S1, Supplementary material). Historical samples consist of toe pads sampled from skins stored at the Royal Ontario Museum in Toronto, Ontario ($n = 49$) and Canadian Museum of Nature in Ottawa, Ontario

Table 1 Sample sizes and tissue types of peregrine falcon specimens

Population	Sample size	Tissue type
Historical samples		
F. p. anatum	24	Toe pad
F. p. tundrius	49	Toe pad
F. p. pealei	15	Toe pad
Hybrids[1]	7	Toe pad
Total	95	
Contemporary samples		
F. p. anatum	109	Blood, feather
F. p. tundrius	46	Blood
F. p. pealei	24	Blood
Unknown B.C.[2]	5	Blood
Total	184	

[1]Six putative *F. p. anatum* × *F. p. tundrius*, one putative *F. p. anatum* × *F. p. pealei*.
[2]Samples of undetermined subspecies status.

(n = 46); these birds were originally collected between 1885 and 1966. Although population allele frequencies may have changed over the course of the individual temporal samples, it is assumed that any such changes would pale in comparison to the major changes brought about by the DDT-induced bottleneck. Because historical samples were all collected before the description of *F. p. tundrius* (White 1968), subspecies status for historical non-*F. p. pealei* individuals was determined from a comparison of original sampling location with currently recognized subspecies boundaries. Individuals were collected during the breeding season and obvious close relatives were excluded.

DNA from contemporary samples was extracted using a standard phenol–chloroform extraction (Sambrook *et al*. 1989). Purification of historical DNA from museum skins was accomplished using the DNeasy tissue extraction kit (QIAGEN) with the following modifications to the suggested protocol: to maximize the amount of DNA recovered, the elution buffer was diluted 1:3 in ddH_2O and heated to 37 °C prior to elution. DNA was eluted with 150 μL of the diluted buffer and then concentrated to 50 μL using an evacuated centrifuge to return salt concentrations to the original buffer.

Genotyping and sequencing

A 405-bp fragment of the mtDNA control region was amplified using existing primers (S. Berlin, personal communication): PEFA-3F 5′-CCCCATTACATGATAACTCAATG-3′, and PEFA-4R 5′-CTGACCGAGGAACCAGAGG-3′. Samples were screened initially using single-stranded conformational polymorphisms (SSCP) following Friesen *et al*. (1997). Optimized amplifications were conducted in 15-μL reaction volumes containing 1× buffer, 0.4 mM each dNTP, 0.4 μM each primer, 0.5 U *Taq* DNA polymerase (Fisher Scientific), 1 μCi α-^{33}P-dATP (Amersham Life Science Inc.), and 2 μL template DNA. Approximately 50% of contemporary samples and 75% of historic samples were re-amplified, which served to confirm haplotype scoring. For samples representing distinct haplotypes, 10 μL of polymerase chain reaction (PCR) product was sequenced with direct incorporation of α-^{33}P-dATP using the ThermoSequenase Terminator Cycle Sequencing Kit (Amersham Life Science Inc.). To corroborate the SSCP results, 10 or more random individuals were sequenced for each observed haplotype (when available).

PCR primers for 12 microsatellite DNA loci developed for peregrine falcons were utilized (Nesje *et al*. 2000b). One primer for each locus was radioactively end-labelled with (γ^{33}P)-dATP following Ibarguchi *et al*. (2000). Microsatellite amplifications were conducted in 11-μL reaction volumes containing 0.27 mM each dNTP, 0.27 μM each primer, 0.045 μM end-labelled primer, 0.05 U *Taq* DNA polymerase (Fisher Scientific), and 1.25 μL template DNA. PCR products were electrophoresed in 0.6× TBE (Tris-borate-EDTA) buffer through 6% polyacrylamide gels. Eight to 10 standard samples were run on every gel. Approximately 10% of individuals within each temporal sample were re-amplified for each locus to confirm genotype scoring. To check for potential allelic dropout, amplifications were repeated under less stringent PCR conditions (lower annealing temperature and higher $MgCl_2$ concentration).

Data analysis

Meaningful estimates of genetic diversity and tests for population bottleneck signatures require that populations are correctly defined. Therefore, the degree of genetic distinctiveness of recognized subspecies was first considered to help define populations for these subsequent tests.

Analysis of population genetic structure

Pairwise Φ_{ST} and F_{ST} (Weir & Cockerham 1984) estimates were computed in ARLEQUIN 2.0 (Schneider *et al*. 2000) using an analysis of molecular variance (AMOVA; Excoffier *et al*. 1992) for mtDNA and microsatellite data, respectively. Statistics were calculated both within and between time periods. Although the latter is not strictly valid, it indicates whether large genetic changes have occurred within a population across time. Significance was determined through 10 000 random Monte Carlo permutations.

As a complementary assessment of population genetic structure, assignment tests were performed using GENECLASS 2 (Cornuet *et al*. 1999; Piry *et al*. 2004) using the Bayesian

procedure of Rannala & Mountain (1997). This approach is superior to other assignment methods for all combinations of sample size, number of loci, and levels of differentiation (Cornuet *et al*. 1999). Tests were performed both within each time period and between them; in the latter case, historic samples were used as reference populations into which contemporary individuals were assigned. Assignment tests were conducted with and without mtDNA data; in the former case the 'second mitochondrial allele' was coded as missing.

To summarize population relationships, dendrograms were constructed from pairwise microsatellite distance matrices of both Nei *et al*.'s (1983) D_A distance and the chord distance D_C of Cavalli-Sforza & Edwards (1967) using the neighbour-joining algorithm (Saitou & Nei 1987) in POPULATIONS 1.1 (Langella 2002). To gauge confidence, bootstrapping was performed across both loci and across individuals (100 pseudoreplications each); it is not clear which bootstrapping method is more appropriate (J. Felsenstein, personal communication). These metrics of interpopulation distance are preferred for summary comparisons because with fixation indices such as F_{ST} each pairwise comparison has a different theoretical maximum (Hedrick 1999), and are therefore not directly comparable.

A major assumption in population genetics studies is that our perceived population designations reflect biological reality. To examine whether currently recognized subspecies boundaries correspond to genetic entities, we used the Bayesian clustering method implemented in STRUCTURE 2.0 (Pritchard *et al*. 2000; Falush *et al*. 2003). This method assumes populations are in Hardy–Weinberg equilibrium (HWE), and assigns individuals to a prespecified number of clusters, *K*, using allele frequencies from multilocus genotype data and Markov chain Monte Carlo (MCMC) sampling. An enormous advantage of this method over those listed above is that populations are not defined by the investigator, but are instead identified by the data. The approach can be used to determine the value of *K* that best fits the data by comparing overall likelihood scores for different values of *K*. Analyses were performed separately to both periods and also for the combined data set. Preliminary analyses determined that a chain length of 10^6 iterations following a burn-in period of 10^5 iterations gave consistent results. Analyses were run assuming an ancestry model that incorporates admixture and correlated allele frequencies across loci. A separate α was used for each cluster to account for unequal representation of the clusters in the dataset (Falush *et al*. 2003). No prior information regarding sampling origin was used. Analyses at each value of *K* (from one to 10) were run three times to investigate convergence of the Markov chain. Analyses were run with and without mtDNA, as described above. Plots of the results were generated using DISTRUCT (Rosenberg 2004).

Estimates of genetic diversity

Indices of mitochondrial DNA diversity, including nucleotide diversity (π), and haplotype diversity (h), as well as Tajima's D (Tajima 1989), were compared across all spatial and temporal populations using DNASP 3.52 (Rozas & Rozas 1999). For microsatellite loci, GDA 1.1 (Lewis & Zaykin 2000) was used to test each population for linkage disequilibrium and departures from HWE using a standard Bonferroni correction (Rice 1989). A genic differentiation test (Goudet *et al*. 1995), which determines whether allelic complements differ between samples, was performed using GENEPOP 3.4 (Raymond & Rousset 1995). Mean observed heterozygosity (H_O) was calculated using GDA. To accommodate differences in sample sizes we calculated allelic richness (the standardized mean number of alleles/locus), and private allelic richness (the standardized mean number of private alleles/locus) using rarefaction as implemented in HP-RARE 1.0 (Kalinowski 2005). Values were standardized to 15 individuals for the subspecies comparisons (size of the historical *F. p. pealei* sample) and 95 individuals for the temporal comparisons (size of the combined historical sample). Statistics for each population were compared across time using a Wilcoxon matched-pairs signed-ranks test.

Bottleneck tests

In addition to straight temporal comparisons of genetic diversity, a variety of approaches were used to test specifically for bottleneck signatures. First, we used the *M*-ratio method of Garza & Williamson (2001). *M* is the ratio of the total number of distinct allelic states observed at a locus to the overall allelic size range at that locus. Populations that experience a bottleneck are expected to lose rare alleles (Nei *et al*. 1975), creating allelic vacancies and therefore decreasing *M*. Significance of the *M* was determined from 10 000 populations simulated under mutation–drift equilibrium with a user-defined initial level of diversity ($\theta = 4N_e\mu$). We set the proportion of single-step mutations (p_s) to 90% and the average size of multistep mutations (Δ_g) to 3.5, as prescribed by the authors. A mean mutation rate $\mu = 5 \times 10^{-4}$ is assumed by the program. MIGRATE 1.7.6 (Beerli & Felsenstein 1999, 2001) was used to estimate predecline values of θ from historical genotype data. Microsatellite mutation was modelled as a continuous Brownian process and gamma-distributed variation across loci. Four simultaneous Markov chains were run for a given analysis and each analysis was repeated three times to ensure convergence of the Markov chain.

An alternative test for bottleneck signatures was performed using BOTTLENECK 1.2 (Piry *et al*. 1999). Populations that have experienced a recent ($0.2N_e$–$4N_e$ generations) bottleneck exhibit transient elevated levels of heterozygosity (*sensu* Nei *et al*. 1975) compared to levels expected

under mutation–drift equilibrium (Cornuet & Luikart 1996). The expected equilibrium level of heterozygosity (H_{eq}) was calculated from the sample size (n) and the observed number of alleles (k) through a coalescent process under mutation–drift equilibrium. A two-phase model (TPM) assuming 95% of mutations were single-step and a variance among multiple steps of 12 was used, as prescribed by Piry *et al*. (1999).

We calculated various estimates of effective population size N_e as a complement to the bottleneck tests above. First, MLNE 1.0 (Wang & Whitlock 2003) was used to estimate harmonic mean effective population sizes across time. This maximum-likelihood method makes inferences based on observed changes in allele frequencies using temporally separated samples from a population, and does not require any of the temporal samples to be in migration-drift equilibrium. Employing a generation time of 4 years, the time between the historical and contemporary samples is ~15 generations. However, because both temporal samples were each collected over several years, the program was run multiple times assuming the mean separation of temporal samples to be 5, 10, 15, and 20 generations.

Second, BAYALLELE (Nichols & Freeman 2004) was used to estimate relative effective population sizes before and after the population decline. Because the same genetic markers were used across populations it was possible to estimate relative values of N_e by assuming that mutation rates were equivalent across populations (but not necessarily across loci). BAYALLELE uses MCMC sampling to approximate posterior probability distributions of migration rates and relative N_e while treating locus-specific mutation rates as nuisance parameters. Effective population size of each population i is given by $\log(\bar{N}_e i) = N + \Delta N_i$ (Nichols *et al*. 2001). Vague uniform priors were put on N_e (mean = 5.5, variance = 15) and migration (mean = –6.5, variance = 20) values, and mutation rate priors were set as prescribed by the author (mean = –6.5, variance = 20; R. Nichols, personal communication). Migration was prohibited in comparisons that involved temporal samples. This method requires that the markers be in mutation–drift–migration equilibrium. Because loci with a high mutation rate can reach equilibrium more quickly (Nichols & Freeman 2004), analyses were limited to the seven most quickly evolving microsatellite loci as determined through preliminary runs of the program. Preliminary analyses determined that a chain length of 2.56×10^6 iterations following a burn-in period of 2.56×10^6 iterations gave consistent results. Multiple runs were performed to ensure convergence.

Results

No discrepancies were found between the mtDNA control region results obtained through SSCP analysis and direct sequencing. All contemporary samples were successfully amplified and scored, but 13 historical samples (13.7%) were apparently too degraded to amplify the 405-bp fragment, giving a final historical mtDNA sample size of 82. Most samples from both time periods were successfully amplified and genotyped for the 12 microsatellite loci. One microsatellite locus (NVH fp79–1) was monomorphic for all populations and hence was dropped from subsequent analyses.

The mtDNA control region fragment was 405 bp for every haplotype observed. The most frequent haplotype was identical to the published sequence in Mindell *et al*. (1999). No insertions or deletions were observed. A concern with the SSCP method is that variation may be missed (Sunnucks *et al*. 2000). In none of the more than 10 randomly sequenced individuals for each observed haplotype (when available) was a novel haplotype found.

Analysis of population genetic structure

Despite extremely low levels of mitochondrial control region polymorphism (Table 2), significant population genetic structure was detected (Table 3). Most notable was a change in structure between the two time periods. Historically, a significant genetic difference existed only between *F. p. tundrius* and *F. p. pealei*. In contemporary samples, the pairwise Φ_{ST} between the *F. p. anatum* and

Table 2 Mitochondrial control region variable sites and frequency distribution among geographic and temporal samples. Numbers for variable sites refer to sites within the published mitochondrial genome of *Falco peregrinus* (Mindell *et al*., 1999). 'Cont.' and 'Hist.' refer to contemporary and historical populations, respectively

	Variable site				*F. p. anatum*		*F. p. tundrius*		*F. p. pealei*	
Haplotype	15331	15332	15338	15350	Cont.	Hist.	Cont.	Hist.	Cont.	Hist.
HapA	T	T	A	A	0.817	0.842	0.435	0.667	1.000	1.000
HapB	C	—	—	—	0.073	0.158	0.457	0.311	—	—
HapC	—	C	—	—	0.009	—	0.087	0.022	—	—
HapD	—	—	G	—	0.092	—	0.022	—	—	—
HapE	—	—	—	G	0.009	—	—	—	—	—

Table 3 Pairwise estimates of population differentiation derived from mtDNA (Φ_{ST}; above diagonal) and microsatellite (F_{ST}; below diagonal) data. Numbers in bold are significantly greater than zero at $\alpha = 0.05$

	Historical populations			Contemporary populations		
Population	*F. p. anatum* ($n = 24$)	*F. p. tundrius* ($n = 49$)	*F. p. pealei* ($n = 15$)	*F. p. anatum* ($n = 109$)	*F. p. tundrius* ($n = 46$)	*F. p. pealei* ($n = 24$)
Historical						
F. p. anatum	—	0.019	0.083	0.016	**0.113**	0.134
F. p. tundrius	0.003	—	**0.176**	**0.134**	0.021	**0.216**
F. p. pealei	**0.077**	**0.080**	—	0.005	**0.256**	0
Contemporary						
F. p. anatum	**0.012**	**0.015**	**0.048**	—	**0.251**	0.023
F. p. tundrius	0.007	0.001	**0.076**	**0.013**	—	**0.299**
F. p. pealei	**0.087**	**0.091**	0.020	**0.047**	**0.081**	—

F. p. tundrius subspecies was also significant and was an order of magnitude larger than the historical value. This significant Φ_{ST} value can be explained in terms of large haplotype frequency differences between contemporary *F. p. anatum* and *F. p. tundrius* samples (Table 2). Most notably, HapB was found in contemporary *F. p. tundrius* populations at a frequency of 45.7%, but had a frequency of only 7.3% in contemporary *F. p. anatum* populations. Haplotypes HapC and HapD showed similar differences between subspecies. Φ_{ST} values between temporal samples of individual subspecies revealed no significant change through time for any subspecies, although the power to detect this and other potential differences was limited by the paucity of variation at the control region. However, there was a change in the range of Φ_{ST} values across time similar to changes observed in other studies (e.g. Johnson *et al*. 2004); contemporary populations had a larger range of values ($\Phi_{ST} = 0.023–0.281$) than historical populations ($\Phi_{ST} = 0.015–0.170$), indicating higher levels of differentiation in contemporary populations.

None of the subspecies from either time period exhibited significant linkage disequilibrium or departures from HWE for any of the microsatellite loci after a standard Bonferroni correction. Population structure in microsatellites exhibited a similar pattern to the mtDNA data (Table 3). Historically *F. p. pealei* was well differentiated from the other two subspecies with significant F_{ST} values (0.078 with *F. p. tundrius* and 0.076 with *F. p. anatum*). In contemporary samples, however, all populations were significantly differentiated. The lone difference between the two time periods, as with the mtDNA data above, was significant structure between *F. p. anatum* and *F. p. tundrius*.

Because of the higher power of the microsatellites the source of this change could be determined by estimating temporal F_{ST} values analogous to those for the mtDNA data above (Table 3). Both *F. p. tundrius* and *F. p. pealei* showed no significant difference between temporal datasets, however, *F. p. anatum* exhibited weak but significant differentiation between historical and contemporary samples. In addition, historical *F. p. anatum* and contemporary *F. p. tundrius* showed no sign of genetic difference, which is expected given the lack of differentiation between the two subspecies historically. Thus the change in structure appears to be due entirely to changes within *F. p. anatum* alone, and is supported by the genic differentiation test which indicates significant different allelic complements between temporal *F. p. anatum* samples ($P = 0.002$, d.f. = 22).

The neighbour-joining population dendrograms constructed using D_A and D_C had identical topologies (Fig. 2; D_A tree not shown) and supported the results above. Both *F. p. pealei* and *F. p. tundrius* exhibited clustering of temporal samples, the historical *F. p. anatum* sample clustered with the *F. p. tundrius* populations, and the contemporary *F. p. anatum* sample is an apparent outlier.

Results from assignment tests with and without mtDNA information were equivalent; only the former are reported. Two patterns were apparent (Table 4). First, *F. p. pealei* had high assignment success in both time periods (historical self-assignment = 73%, contemporary = 92%). Second, there was a change in the cross-assignment between *F. p. anatum* and *F. p. tundrius*. In the historical sample this cross-assignment was not significantly different from random — individuals from *F. p. anatum* and *F. p. tundrius* were equally likely to be assigned to the other population as they were to their own (Fisher exact test, two-tailed $P = 0.308$). In contrast, the cross-assignment in the contemporary sample was significantly nonrandom (Fisher exact test, two-tailed $P < 0.001$); self-assignment success increased for both *F. p. anatum* (68%) and *F. p. tundrius* (80%). Few *F. p. anatum*/*F. p. tundrius* individuals were incorrectly assigned to *F. p. pealei* in either time period.

Results from the temporal assignment (Table 4) mirrored the results of the temporal *F*-statistics above. Contemporary *F. p. pealei* individuals were assigned with high

Table 4 Population level Bayesian assignment test results. Numbers in bold represent proportions of individuals successfully assigned to their recognized population

	Assigned population					
	Historical populations			Contemporary populations		
Sampled population	(*n* = 24) *F. p. anatum*	(*n* = 49) *F. p. tundrius*	(*n* = 15) *F. p. pealei*	(*n* = 109) *F. p. anatum*	(*n* = 46) *F. p. tundrius*	(*n* = 24) *F. p. pealei*
Historical						
F. p. anatum	**0.50**	0.42	0.08			
F. p. tundrius	0.37	**0.57**	0.06			
F. p. pealei	0.07	0.20	**0.73**			
Contemporary						
F. p. anatum	**0.40**	0.34	0.26	**0.68**	0.24	0.08
F. p. tundrius	0.25	**0.71**	0.04	0.20	**0.80**	0.00
F. p. pealei	0.04	0.04	**0.92**	0.04	0.04	**0.92**

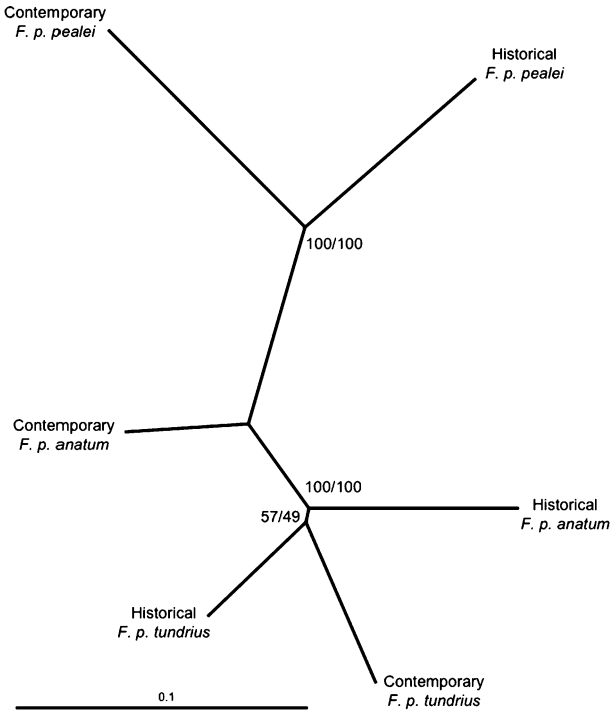

Fig. 2 Neighbour-joining population dendrograms constructed using Cavalli-Sforza & Edwards's (1967) D_C distance. Numbers at the nodes represent nonparametric bootstrap proportions (across individuals/loci).

success to the historical *F. p. pealei* population. The contemporary *F. p. anatum* samples were nearly equally assigned to each of the historical samples. Interestingly, contemporary *F. p. tundrius* were assigned to the historical *F. p. tundrius* population with higher success than would be predicted from temporal F_{ST} values (Table 3).

Using the Bayesian clustering method implemented in STRUCTURE the highest posterior probability for either of the individual time periods or the complete data set was found for $K = 2$ (Table 5; analyses with and without mtDNA data delivered similar results and those including mtDNA data are reported). Analyses assuming a model with admixture gave results similar to those assuming no admixture. Assignment by subspecies to inferred clusters for $K = 2$ was roughly similar in both time periods (Fig. 3). Historical samples showed a very strong signature of differentiation with nearly all *F. p. anatum* and *F. p. tundrius* individuals assigned to cluster 1 and almost all *F. p. pealei* individuals grouped into cluster 2. The lack of differentiation between *F. p. anatum* and *F. p. tundrius* indicates that historically these two subspecies were genetically indistinguishable. Contemporary samples gave a less distinctive picture. Samples from *F. p. pealei* and *F. p. tundrius* had high assignment to clusters 2 and 1, respectively, but *F. p. anatum* samples were no longer limited to a single cluster. Three contemporary *F. p. anatum* populations contributed substantially to this difference: Québec, northern Ontario, and British Columbia (Fig. 3). Historical *F. p. tundrius* individuals assigned to cluster 2 with posterior probability of 0.25–0.5 exhibit no geographical pattern.

Given the results from the analyses above, for the remainder of this paper the lumped *F. p. anatum* and *F. p. tundrius* samples will be referred to as '*F. p. anatum/tundrius*'. Analyses will focus on the *F. p. anatum/tundrius* and *F. p. pealei* populations, while separate *F. p. anatum* and *F. p. tundrius* statistics are provided for comparison purposes.

Estimates of genetic diversity

No population demonstrated significant departures from neutral expectations for the control region data as indicated by Tajima's D (Table 6). Contemporary samples exhibited extremely low levels of haplotype diversity, with only five haplotypes found range-wide (Table 2). However,

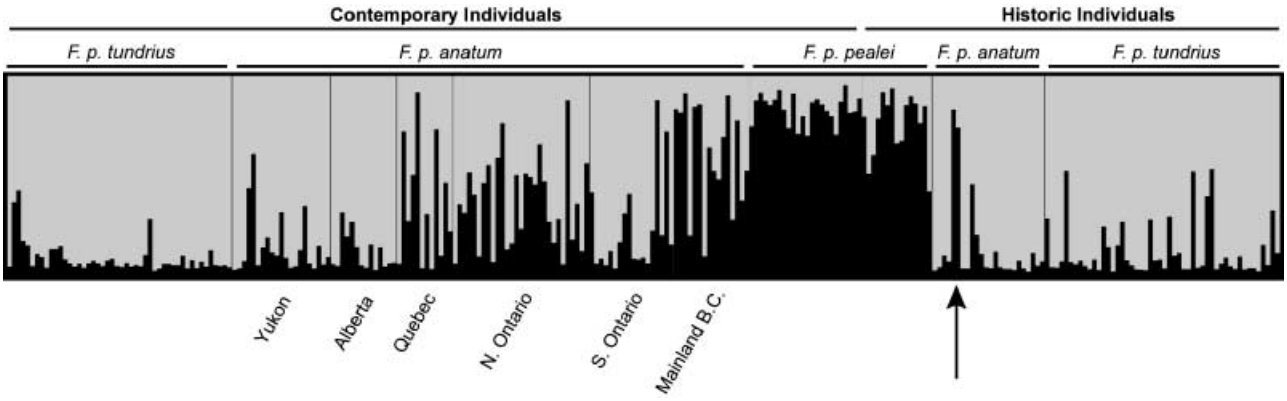

Fig. 3 Assignment of individuals to $K = 2$ inferred clusters from the STRUCTURE analysis using data from both mtDNA control region and microsatellite loci (results from microsatellite data alone were equivalent). Colours (grey and black) indicate different inferred clusters. For each individual, colours within a column represent the inferred posterior probability that the individual belongs to a particular cluster. Results are for one run; each analysis yielded nearly identical results. Contemporary *F. p. anatum* samples are sorted by geographic location. The arrow identifies two historical *F. p. anatum* individuals from British Columbia that were assigned to the *F. p. pealei* cluster.

	Historical		Contemporary		Both time periods	
K	Ln P(D)	Var[Ln P(D)]	Ln P(D)	Var[Ln P(D)]	Ln P(D)	Var[Ln P(D)]
1	–2411.7	29.3	–4817.8	26.7	–7262.5	30.6
2	–2358.1	120.3	–4737.0	164.3	–7094.5	200.3
3	–2439.0	307.8	–5017.3	766.1	–7405.7	949.9
4	–2502.3	444.8	–5186.3	1136.8	–7738.0	1676.1

Table 5 Posterior probabilities and variances for models assuming various numbers of clusters (*K*) both for individual time periods and for the entire data set. Both microsatellite and mtDNA data were used; results using microsatellite data alone were equivalent. Statistics for each value of *K* represent average values over three simulations

historical samples exhibited even lower diversity with only three haplotypes range-wide, all of which were found in contemporary populations. Although 13 historical samples would not amplify, there is no obvious reason to believe that these individuals possessed haplotypes not already present in the data set. One haplotype (HapA; Table 2) was present in all populations at very high frequency. All *F. p. pealei* and west coast *F. p. anatum* individuals exhibited this haplotype. Only one haplotype, HapE, was endemic to a particular subspecies (*F. p. anatum*), and it was found in a single individual. Haplotype diversity was significantly higher in the contemporary *F. p. anatum/tundrius* population than the historical population, and this was due to an increase in the diversity of the *F. p. tundrius* sample (Table 6). Low overall mtDNA diversity results are corroborated with preliminary SSCP screenings of 12S rRNA (397 bp) and two cytochrome *b* fragments (239 and 485 bp) for 32 individuals from disparate locations across Canada, which failed to detect any polymorphism (data not shown).

A total of 69 microsatellite alleles were detected across all populations and loci (Table 6). Thirteen alleles were unique to a particular time period (historic, five; contemporary, eight), and 10 of these were unique to a particular population (historic *F. p. anatum*, one; historic *F. p. tundrius*, three; contemporary *F. p. anatum*, four; contemporary *F. p. tundrius*, two). All of these alleles were present in low frequencies (≤ 2%) except one that was present in contemporary *F. p. anatum* populations at a frequency of 6%. The historical private alleles may represent 'ghost alleles' (*sensu* Groombridge *et al*. 2000), or may simply be a sampling artefact. When standardized for sample size, the number of private alleles/locus did not differ significantly across time, although most contemporary values were higher (Table 6). Contemporary private alleles may reflect a sampling artefact as historical sample sizes are much smaller, although they may also represent allelic introgression from re-introduced birds of non-native stock (Tordoff & Redig 2001).

The microsatellite data exhibited a similar pattern to the control region data with no significant reduction in diversity across the population decline (Table 6). In fact, most metrics of nuclear variation showed higher diversity after the decline, and this was statistically significant for observed heterozygosity in the *F. p. anatum/tundrius* sample. The only exception to this increase in diversity was the temporal *F. p. pealei* sample, which showed a slight decrease in diversity, although this was not statistically significant.

Table 6 Indices of mitochondrial and microsatellite DNA diversity for historical and contemporary peregrine falcon samples. Standard errors are given in parentheses. Numbers in bold represent significantly higher contemporary values when compared to historical values

		mtDNA control region				Microsatellites (11 loci)				
Population	Sample size (mtDNA/micro)	Number of haplotypes	Nucleotide diversity	Haplotype diversity	Tajima's D[6]	Mean alleles/locus	Allelic richness[1]	Private alleles[2]	Private richness[1]	H_O[3]
Historical populations										
F. p. anatum/tundrius[4]	65/73	3	0.0010 (0.0001)	0.415 (0.007)	0.006	5.36 (0.94)	4.41 (0.72)	16	0.84 (0.32)	0.50 (0.09)
F. p. anatum	19/24	2	0.0007 (0.0002)	0.281 (0.027)	–0.035	4.73 (0.81)	4.45 (0.75)	1	0.24 (0.11)	0.50 (0.09)
F. p. tundrius	45/49	3	0.0012 (0.0002)	0.469 (0.008)	0.102	5.27 (0.94)	4.41 (0.71)	5	0.23 (0.12)	0.50 (0.08)
F. p. pealei	14/15	1	0	0	N/A	4.00 (0.56)	4.00 (0.56)	1	0.21 (0.11)	0.52 (0.08)
Pooled Historical[5]	82/95	3	0.0010 (0.0001)	0.379 (0.006)	–0.067	5.45 (0.91)	5.44 (0.91)	5	0.46 (0.21)	0.50 (0.08)
Contemporary populations										
F. p. anatum/tundrius	155	5	0.0013 (0.0001)	**0.461** (0.003)	–0.528	5.82 (1.01)	4.59 (0.71)	25	**1.11** (0.34)	**0.54** (0.09)
F. p. anatum	109	5	0.0008 (0.0002)	0.322 (0.005)	–1.074	5.64 (0.95)	4.53 (0.73)	4	0.30 (0.10)	0.55 (0.09)
F. p. tundrius	46	4	0.0018 (0.0003)	**0.608** (0.006)	0.094	5.27 (0.84)	4.49 (0.64)	2	0.42 (0.13)	0.53 (0.08)
F. p. pealei	24	1	0	0	N/A	4.00 (0.56)	3.79 (0.54)	0	0.11 (0.06)	0.51 (0.09)
Pooled Contemporary	184	5	0.0011 (0.0001)	**0.411** (0.003)	–0.643	5.82 (1.01)	5.61 (0.95)	9	0.63 (0.18)	**0.54** (0.09)

[1]Standardized to $n = 15$ individuals except pooled populations which are standardized to $n = 95$ individuals.
[2]Compared within a time period (either 3 subspecies, or *F. p. anatum/tundrius* vs. *F. p. pealei*), except for pooled populations which were compared across time.
[3]H_O, observed heterozygosity.
[4]The pooled *F. p. anatum* and *F. p. tundrius* samples (see text for rationalization).
[5]Pooled samples include six putative hybrid individuals.
[6]Not significant for any sample.

Bottleneck tests

None of the tests were able to detect significant bottleneck signatures for any population or either time period. First, the Wilcoxon matched-pairs signed-ranks test failed to show a decrease in genetic diversity across the population decline for any of the subspecies. In fact, most of the diversity metrics were higher after the decline for most contemporary populations, and several of these increases were statistically significant (Table 6). Likewise, the *M*-ratio method did not detect a significant proportion of vacant potential allelic states in any population ($P > 0.07$; data not shown). Finally, using the Wilcoxon test as implemented in BOTTLENECK, no signature of elevated heterozygosity was detected for any population, historical or contemporary ($P > 0.45$; data not shown).

As expected, estimated values for N_e from MLNE varied considerably depending on the assumed number of generations separating temporal samples (Table 7). Given the results from STRUCTURE above, estimates for *F. p. pealei* and *F. p. anatum/tundrius* were made without including migration in the model. N_e estimates were small for the *F. p. pealei* sample, reflecting the restricted range size of this subspecies. Estimates for *F. p. anatum/tundrius* were quite high given the demographic history of this population.

Analysis of relative effective population size, ΔN_e, revealed interesting patterns between populations (Fig. 4; note log scale). Populations that differ in mean ΔN_e by one log unit are inferred to be an order of magnitude different in size, although the absolute sizes are unknown. Results were insensitive to choice of prior distributions, and analyses using randomized subsets of the contemporary *F. p. anatum* sample ($n = 50$) returned identical results (data not shown). Posterior densities spanned a range of two log units (two orders of magnitude), although most of the volume lies within one log unit. *Falco p. pealei* populations had the smallest values of ΔN_e, again likely representing its limited range. No detectable change in ΔN_e was found in

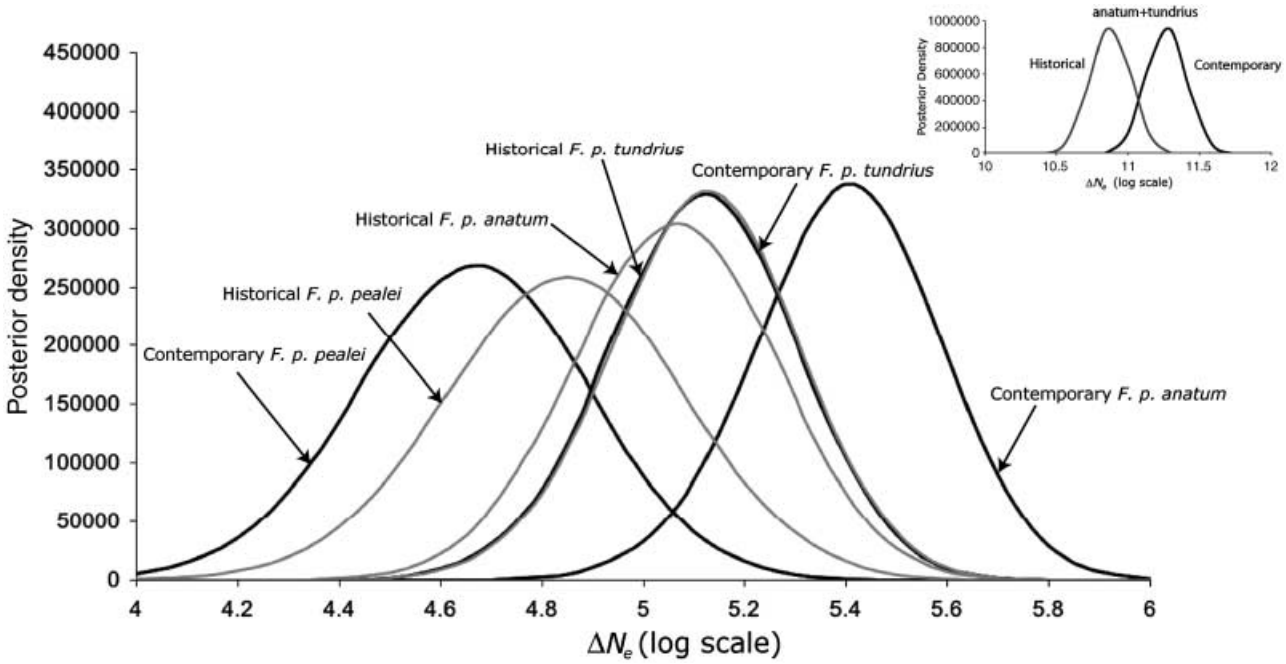

Fig. 4 Posterior probability densities of relative N_e estimates for all samples (note log scale). Grey curves represent historical populations and black curves represent contemporary populations. Inset: posterior probability densities of relative N_e estimates for temporal *anatum* + *tundrius* samples (see text for rationalization).

Table 7 Estimates of N_e derived by MLNE under various assumed numbers of generations separating temporal samples. Brackets indicate 95% confidence intervals (lower, upper)

No. generations[1]	*F. p. pealei*		*F. p. anatum/tundrius*	
	N_e	95% CI	N_e	95% CI
5	151	(67–787)	591	(319–1395)
10	224	(98–1133)	873	(471–2071)
15[2]	293	(130–1485)	1160	(624–2750)
20	366	(161–1837)	1442	(778–3429)

[1]Assumed time separating temporal samples.
[2]The mean number of generations between temporal samples assuming a generation time of 4 years.

F. p. tundrius when comparing the two temporal samples, but a large increase was found in *F. p. anatum*, and consequently for the *F. p. anatum/tundrius* sample as a whole (Fig. 4, inset).

Discussion

Mitochondrial DNA sequences and nuclear microsatellites are found to be useful in interpreting the consequences of the DDT-induced bottleneck. We discern that recognized subspecies designations are unsubstantiated from a genetic viewpoint, as *F. p. anatum* and *F. p. tundrius* cannot be differentiated. Using a revised taxonomy recognizing gene pools of *F. p. pealei* and *F. p. anatum/tundrius*, we detect no signature of a genetic bottleneck for either population. The inclusion of the historical samples is found to be indispensable for both evaluating the validity of subspecies designations and providing a direct basis of comparison for interpreting contemporary levels of genetic variation.

Population genetic structure

Markers from the two genomes analysed give patterns that are consistent with increased genetic differentiation between recognized subspecies following the bottleneck (Table 3). Both mtDNA and microsatellite data show that *F. p. anatum* and *F. p. tundrius* were genetically indistinguishable historically and that contemporary samples are weakly but significantly differentiated. For the mtDNA data, there is an indication that haplotype frequencies fluctuated in both subspecies. For example, the frequency of HapB increases from 31.1% (historical) to 45.7% (contemporary) in *F. p. tundrius* populations, while the frequency of the same haplotype decreases from 15.8% to 7.3% in *F. p. anatum* populations (Table 2). These shifts are likely a bottleneck consequence, however, low polymorphism limits the detection of significant differences between temporal samples. An increase in the range of pairwise Φ_{ST} values among contemporary populations vs. values among

historical populations is also observed, indicating that populations have become increasingly differentiated through time (Johnson *et al*. 2004).

Nuclear microsatellite analyses employing population dendrograms (Fig. 2), F_{ST} (Table 3), Bayesian assignment (Table 4) and Bayesian clustering (Table 5; Fig. 3) approaches are all in agreement. First, all four approaches indicate that *F. p. pealei* is well differentiated from all other peregrine falcon populations in Canada. This corroborates earlier morphological assessments of peregrine falcon subspecies (White & Boyce Jr. 1988). Second, similar to the mtDNA data, the latter three methods find that historical *F. p. anatum* and *F. p. tundrius* subspecies were genetically indistinguishable, yet, among contemporary populations, the two subspecies are differentiated. Because of the increased number of loci and level of polymorphism it appears that this change in relationship is principally due to frequency changes within *F. p. anatum* alone. Since historical *F. p. anatum* and *F. p. tundrius* samples are indistinguishable from contemporary *F. p. tundrius* samples, the contemporary arctic populations appear to be representative of the ancestral *F. p. anatum/tundrius* population.

The STRUCTURE analyses identify two clusters: one with most *F. p. pealei* birds, and another with all other individuals (Fig. 3). Three geographic regions are responsible for the apparent recent differentiation of *F. p. anatum*: Québec, northern Ontario and British Columbia. Peregrine falcons were extirpated from Québec and northern Ontario, so birds in these regions are all of re-introduced ancestry. Because the Canadian re-introduction program involved only pure *F. p. anatum* individuals, the skewed allele frequencies of these regions likely represent the limited gene pool from the breeding program. However, the possibility remains that this differentiation is due to the introgression of alleles from non-native stock, as the breeding program of the eastern United States involved a minimum of seven subspecies from around the world, five of which are known to have successfully contributed genes to breeding populations (Tordoff & Redig 2001). In fact, banded American-released birds have been observed breeding in Ontario (G. Holroyd, personal communication). However, strong support for this hypothesis will require a comprehensive population genetic survey of American peregrine falcon populations.

The third group of individuals to contribute to the apparent contemporary differentiation of *F. p. anatum* and *F. p. tundrius* are those from southwestern British Columbia. Most of these are assigned with high probability to the *F. p. pealei* cluster in the STRUCTURE analyses. For instance, individuals from the northern part of Vancouver Island are considered *F. p. pealei*, while individuals in the south are considered *F. p. anatum*. From the analyses presented here all of these individuals belong genetically to *F. p. pealei*. Furthermore, the two historic *F. p. anatum* individuals that were assigned to the *F. p. pealei* cluster both came from British Columbia (Fig. 3). Thus, while the recognition of *F. p. pealei* as a subspecies appears valid, the currently recognized geographic boundaries of this subspecies are not.

A plausible hypothesis for the origin of *F. p. pealei* invokes glacial refugia. Using radiocarbon dating and plant macrofossil data, Warner *et al*. (1982) demonstrate that parts of Haida Gwaii were ice-free during the last glacial maximum, approximately 16 000 years ago. Today this area shows a high degree of both floral and faunal endemism (Byun *et al*. 1999). Taken together, these data have been used to hypothesize a glacial refugium for several taxa (humans: Rogers *et al*. 1991; song sparrow *Melospiza melodia*: Zink & Dittmann 1993; black bear *Ursus americanus*: Byun *et al*. 1997; rainbow trout *Oncorhynchus mykiss*: McCusker *et al*. 2000; coho salmon *Oncorhynchus kisutch*: Smith *et al*. 2001; ermine *Mustela erminea*: Fleming & Cook 2002; Steller's jays *Cyanocitta stelleri*: Burg *et al*. 2005; but see Demboski *et al*. 1999). The present-day distribution of *F. p. pealei* is similar to many of these taxa. Although a lack of mtDNA monophyly precludes dating the origination of *F. p. pealei*, low levels of genetic divergence (*F. p. pealei* does not possess any novel microsatellite alleles or mtDNA haplotypes) suggest that this subspecies differentiated from *F. p. anatum/tundrius* relatively recently.

Genetic diversity and bottleneck signatures

Contemporary samples show relatively low levels of microsatellite diversity and extremely low levels of mtDNA diversity (Table 2). The levels of overall genetic variation observed here are similar to those in the endangered Spanish imperial eagle (*Aquila adalberti*; Martínez-Cruz *et al*. 2004), the declining red kite (*Milvus milvus*; Roques & Negro 2005), and the endemic Galápagos hawk (*Buteo galapagoensis*; Bollmer *et al*. 2006). Mitochondrial DNA nucleotide diversity is actually lower than that found in whooping cranes (*Grus americana*; Glenn *et al*. 1999), which declined to 14 individuals. However, the inclusion of museum specimens here shows that observed low contemporary levels of genetic variation are *not* a result of the DDT-induced bottleneck, as no decline in diversity is observed. Rather, peregrine falcons seem to naturally exhibit a paucity of genetic variation in both genomes. The veracity of these results is supported by direct sequencing of Greenland peregrine falcons, which also failed to find extensive variation at the mtDNA control region (J. Johnson, personal communication). Although Lifjeld *et al*. (2002) report reduced minisatellite variation in postbottleneck Norwegian peregrine falcon populations, their conclusions are based on cross-species comparisons which may be confounded by life history differences. Our results, based on direct temporal comparisons, re-affirm the importance of using appropriate reference populations when

interpreting population genetic data (Bouzat 2000; Matocq & Villablanca 2001).

Given that *F. p. anatum* was pronounced extirpated from eastern North America and *F. p. tundrius* declined an estimated 50–60% (Fyfe *et al*. 1976), strong signatures of a genetic bottleneck were expected. For example, Bellinger *et al*. (2003) used historical samples to quantify a loss of genetic variation in greater prairie chickens (*Tympanuchus cupido*) following a comparatively severe population bottleneck. However, despite extensive empirical evidence (Spielman *et al*. 2004) and theoretical expectations, loss of genetic diversity across a bottleneck is not ubiquitous. Retention of significant genetic variation has been observed in diverse taxa, including fish (Schönhuth *et al*. 2003), amphibians (Burns *et al*. 2004), mammals (Dinerstein & McCracken 1990; Spong & Hellborg 2002), and birds (Mundy *et al*. 1997; Paxinos *et al*. 2002; Hailer *et al*. 2006). Therefore, the results from the present study, although rare, are not unprecedented. Hailer *et al*. (2006) recently observe high levels of genetic variation in populations of white-tailed eagles (*Haliaeetus albicilla*), which also declined precipitously due to DDT-contamination. These authors argue that long lifespans can act as an intrinsic buffer to stave off genetic erosion. If true, this may be operating in peregrine falcons as well, where longevity can reach 16–20 years (White *et al*. 2002). While the interpopulation movement of breeding individuals will also diminish losses in genetic diversity, we currently have no estimates on the extent of this movement among Canadian peregrine falcon populations.

Estimates of harmonic average N_e across the bottleneck are 1160 for *F. p. anatum/tundrius* and 293 for *F. p. pealei*, assuming 15 generations separating the temporal samples and negligible migration between the two groups (Table 7). The value for *F. p. pealei* is reconcilable with actual census size estimates for this subspecies. White *et al*. (2002) estimate 378 occupied eyries in Pacific coastal North America; assuming two birds are present at each eyrie, an N_e/N ratio estimate of 0.39 is obtained. We recently calculated a similar ratio of 0.32 for a stable population of arctic peregrine falcons (Brown *et al*. in review), and Reed *et al*. (1986) calculate an N_e/N ratio of 0.41 for the northern goshawk (*Accipiter gentilis*), the only other raptor estimate currently available. The agreement of these numbers supports the notion that *F. p. pealei* populations were reasonably stable across the DDT bottleneck.

The calculated harmonic average N_e estimate of 1160 for *F. p. anatum/tundrius* is much higher than expected given its well-documented demographic history. The obtained value can be rationalized in three ways. Firstly, although *F. p. anatum* was nearly exterminated, *F. p. tundrius* historically comprised several thousand individuals (Fyfe 1969), and so a significant number of individuals are believed to have survived the decline. Secondly, the promptness of recovery programmes in both Canada and the United States likely prevented the erosion of genetic diversity, as theory predicts that the majority of genetic diversity can be retained in populations suffering a severe decline if the bottleneck is not prolonged (Nei *et al*. 1975; Maruyama & Fuerst 1984, 1985; England *et al*. 2003). Finally, the estimate of N_e is undoubtedly upwardly biased by the re-introduction of individuals into the wild (i.e. the population did not simply decline and recover). Introgression of alleles from these re-introduced individuals, some of which were possibly of extra-continental origin (Tordoff & Redig 2001), would have replenished depleted natural genetic stocks.

A complementary interpretation is based on relative N_e estimates (Fig. 4). Unlike the values in Table 7, these figures do not involve average bottleneck population size, but rather compare 'snapshots' of N_e before and after the bottleneck. Contemporary N_e values are not lower than predecline estimates (Fig. 4). In fact, postrecovery estimates for *F. p. anatum* and *F. p. anatum/tundrius* are higher than historical values. Similar to the maximum-likelihood estimates in Table 7, these estimates are also likely biased by the genetic influence of re-introduced individuals. Contemporary N_e estimates may therefore be temporarily inflated (i.e. are higher than would be supported naturally), and may decline as populations approach mutation–drift equilibrium.

Taxonomy and conservation of North American peregrine falcons

Our results raise the question of whether current taxonomy of peregrine falcons needs to be refined. This question necessarily involves the definition of 'subspecies', a concept that has been controversial, especially in ornithology (Wilson & Brown. 1953; Barrowclough 1982; Mayr 1982; Zink 2004; Phillimore & Owens 2006). Importantly, evolutionary and conservation research requires a genetic basis for taxonomy. The required degree of genetic uniqueness depends on the investigator. Zink (2004) uses mtDNA monophyly as a firm criterion for designating subspecies status. Using this approach, all peregrine falcons in Canada (including *F. p. pealei*) would be lumped into the same subspecies because none exhibit monophyly. This is not surprising, given that 97% of recognized subspecies surveyed by Zink (2004) do not meet these strict requirements (but see Phillimore & Owens 2006). Barrowclough (1982) proposes the more general criterion of 'predictiveness', that subspecies designations should facilitate confident predictions of multiple character states. Taken from a genetics perspective, *F. p. pealei* qualifies as a subspecies given the high success rate of assignment from multilocus genotypic data, although *F. p. anatum* and *F. p. tundrius* would not qualify as distinct subspecies because little genetic differentiation exists between them.

Crandall *et al*. (2000) propose a more comprehensive approach based on ecological and genetic exchangeability, with each being testable. Additionally, this approach involves a temporal axis of comparison. Following this method, *F. p. pealei* qualifies as a valid subspecies, as it is genetically distinguishable in both time periods and exhibits unique ecological characteristics. At face value, both *F. p. tundrius* and *F. p. anatum* appear to qualify because, despite the lack of historical genetic differentiation, they are now genetically differentiated and show ecological differences (migratory behaviour, prey, size, plumage). However, contemporary genetic differentiation as shown here is likely due to anthropogenic causes. Furthermore, it is unclear whether perceived ecological differences have a genetic foundation. James (1983), for example, shows that morphological differentiation in birds can have little genetic basis. This is consistent with the results of Nesje *et al*. (2000a), who find a higher degree of genetic differentiation within the European subspecies *F. p. peregrinus* than is found here between North American subspecies *F. p. anatum* and *F. p. tundrius*, lending further support to the notion that the original description of *F. p. tundrius* was artificial. Given the results of the present study, we recommend that *F. p. tundrius* be subsumed into *F. p. anatum*.

Our findings have implications in the continued management and conservation of North American peregrine falcons. First, we show that contemporary levels of genetic diversity are on par with historical levels. This indicates that the DDT-induced bottleneck did not likely reduce the evolutionary potential of this species in Canada. Second, if it is accepted that *F. p. tundrius* be subsumed into *F. p. anatum*, then the controversial de-listing of *F. p. anatum* (Pagel *et al*. 1996; Cade *et al*. 1997; Pagel & Bell 1997; Millsap *et al*. 1998) from the endangered species list (Mesta 1999) is vindicated because substantial numbers of *F. p. anatum* individuals exist in the wild. However, census sizes should not imply that management is no longer required. More than 30 years after banning DDT and DDE, these pesticides are still a major concern for the welfare of peregrine falcon populations. Significant levels of DDE are still observed in some areas of North America where pesticide use was especially heavy (Harris *et al*. 2000; Mora *et al*. 2002; Elliott *et al*. 2005). Additionally, Fyfe *et al*. (1990) show that migratory peregrines may ingest and accumulate organochlorine contaminants when wintering in Latin America, where restrictions on pesticide use are less strict. Finally, we have shown here and elsewhere (Brown *et al*. in review) that the ratio N_e/N for North American peregrine falcons is relatively small (~0.32–0.39); this ratio defines the context for interpreting direct census counts, and suggests that these counts be treated conservatively. Given these reasons, monitoring of peregrine falcon populations should continue.

Conclusions

Genetic surveys can provide insights into the status of natural or re-introduced populations and can be used to provide recommendations for future management decisions. However, we show here that the interpretation of such data should be within the framework of a sound taxonomy and utilize suitable reference populations. To this end, we strongly advocate the temporal approach employed here, as inferences are more accurate and direct than standard cross-species approaches. Employment of this approach is clearly limited by the provenance of archived genetic samples; however, this difficulty can be overcome through carefully planned monitoring schemes. Serial genetic samples are inexpensive, nondestructive and easily stored, and provide a wealth of direct information that would otherwise be lost forever.

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Supplementary material

The supplementary material is available from
http://www.blackwellpublishing.com/products/journals/suppmat/MEC/MEC3151/MEC3151sm.htm

Table S1 Sample provenance

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This work represents part of Joseph's MSc research conducted in the Friesen/Birt laboratory. Joseph is currently pursuing a PhD at the University of Michigan investigating the influences of lineage-specific rate heterogeneity on phylogenetic inference and molecular dating. V. L. Friesen and T. P. Birt use molecular markers to study mechanisms of population differentiation in vertebrates, primarily seabirds. P. V. C. de Groot and P. Boag use molecular markers to address applied conservation genetic problems in birds, fish and mammals. G. Seutin manages the endangered species program at the Parks Canada Agency.
