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Short Communication

The DDT-induced decline influenced genetic diversity in naturally recovered Peregrine falcons (*Falco peregrinus*) nesting within the Alaska Arctic and eastern interior

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We assessed the influence of the severe mid-20th century population decline on genetic diversity in non-augmented Peregrine Falcon *Falco peregrinus* populations nesting within the Alaska Arctic and eastern Interior. Microsatellite and mitochondrial DNA (mtDNA) data were analysed for Peregrine Falcons sampled from three periods: pre-decline, decline and post-decline. The influence of the decline on genetic diversity differed between the two locales. The Alaska Arctic was characterized by shifts in mtDNA haplotype frequencies, increased inbreeding coefficient, reduction in effective population size and increase in private haplotypes, and a signature of post-decline population growth was detected; by contrast, the eastern Interior showed a reduction in haplotype diversity and no differences in allelic or haplotypic frequencies between pre- and post-decline periods, though pre-decline birds clustered away from the other two periods and allelic frequency differences were observed between decline and post-decline periods. Patterns in genetic diversity suggest populations recovered through recruitment from within and immigration.

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By altering allelic and haplotypic frequencies through the stochastic process of genetic drift, population declines can dramatically influence genetic diversity and may ultimately change the evolutionary trajectory of populations. The impacts of declines on genetic diversity depend on several factors: pre-decline effective population size, severity and duration of the decline, and aspects of species biology (longevity, reproduction, philopatry, etc.) (Brown *et al.* 2007). Connectivity among populations can mitigate genetic consequences of reductions in population size (Jangjoo *et al.* 2016), especially where declines are localized events. However, if declines are widespread, connectivity may be reduced, further isolating declining populations and potentially increasing the influence of genetic drift.

The marked mid-20th century crash of Peregrine Falcon *Falco peregrinus* populations associated with bioaccumulation of dichlorodiphenyltrichloroethane (DDT) extirpated the species from parts of North America and Europe and reduced populations by over 75% in some regions (Enderson *et al.* 1995). Resource managers augmented natural recovery by introducing captive-bred Falcons to some extirpated or remnant populations. In Canada and Scandinavia, analyses of such post-decline populations uncovered genetic shifts between time periods (Brown *et al.* 2007, Jacobsen *et al.* 2008). However, inferences drawn from those studies are limited. Locale-specific pre- and post-decline samples were not assayed (Canada), and augmentation of populations conducted in Scandinavia used birds of non-Scandinavian ancestry.

Here, we used a temporal approach to assay genetic diversity in two Peregrine Falcon populations – Arctic and eastern Interior Alaska – that were not augmented with captive-bred birds (Enderson *et al.* 1995). We utilized archived samples collected during three general time periods (pre-decline, decline and post-decline) to compare genetic signatures using microsatellite fragment and mitochondrial DNA (mtDNA) sequence data. Both populations were targeted in long-term studies: based on current abundance estimates, Alaska Arctic declined by ~75% (Colville River; Swem & Matz 2018) and eastern Interior by ~80% (upper Yukon River; Ambrose *et al.* 2016; Fig. 1). Natural recovery of these high-latitude Peregrine Falcon populations provides an

opportunity to evaluate interactions among microevolutionary and demographic processes unaffected by reintroduction efforts.

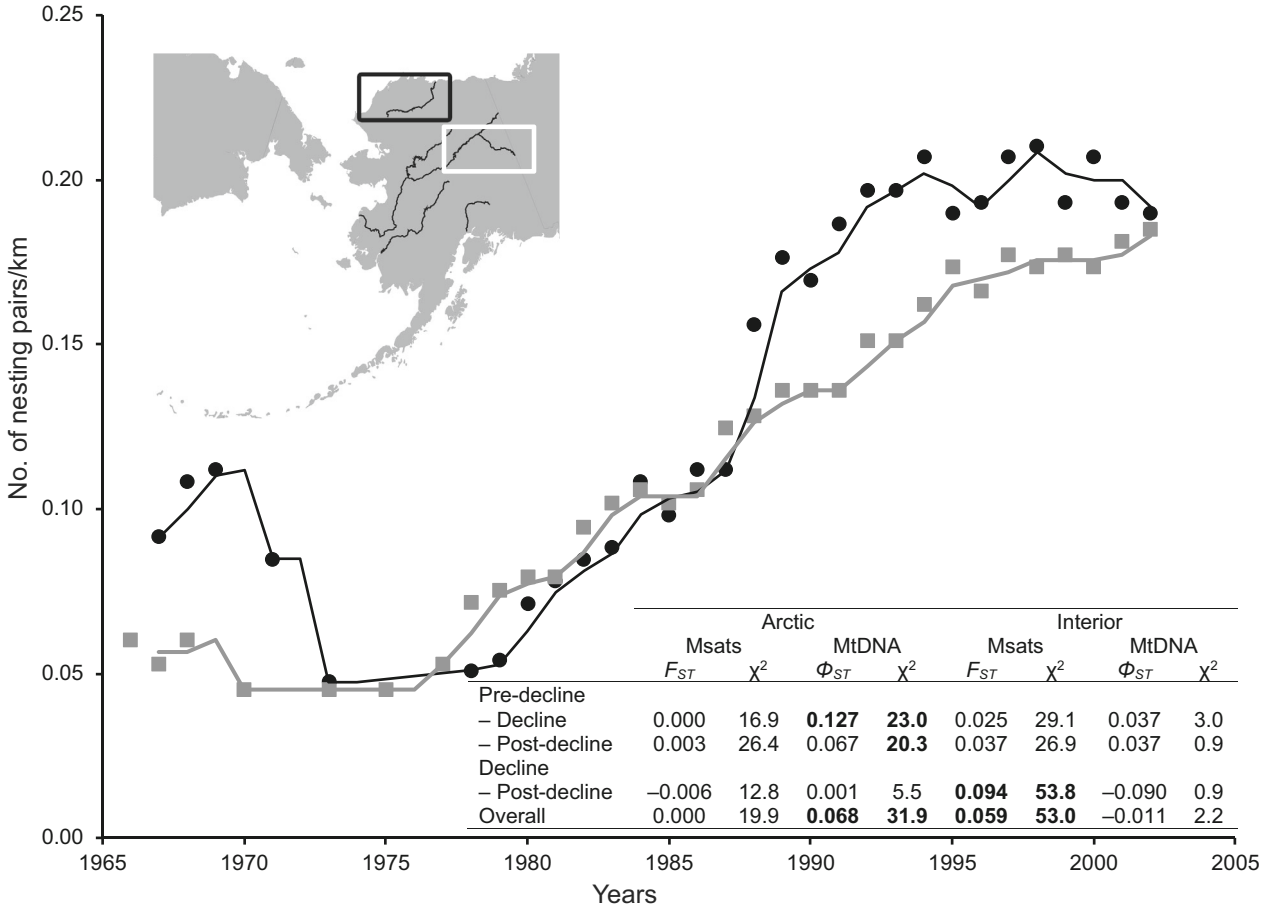
METHODS

Samples

Samples representing Arctic and eastern Interior populations prior to the decline (hereafter 'pre-decline') were taken from toe-pads of museum specimens collected from 1899–1968 (Appendix S1). Blood, feather or buccal samples were collected at nest-sites in 1985 and 2001–2009 along the Colville River (hereafter 'Arctic') and upper Yukon River (hereafter 'Interior') (Table 1). Only a single representative per nest was included in the study. We assume the 1985 samples carry signatures of the decline (hereafter 'decline'), although we acknowledge that recovery started in the early to mid-1970s. The 2001–2009 samples represent 'post-decline'. The decline samples represent 17.2% (Arctic; Swem & Matz 2018) and 18.5% (Interior; Ambrose *et al.* 2016) of the breeding pairs observed.

Laboratory techniques

The DNA extraction, microsatellite genotyping at 11 loci (NVHfp5, 13-1, 31, 46-1, 54, 79-4, 82-2, 86-2, 89-2, 92 and 107; Nesje & Røed 2000), mtDNA control region sequencing (559 base pairs) and data processing followed Talbot *et al.* (2011), with three exceptions for pre-decline specimens. DNA extractions were performed in a low-copy laboratory using a phenol–chloroform protocol (Sambrook *et al.* 1989), followed by use of a Microcon centrifugal filter (Millipore Sigma, Burlington, MA, USA), and included one water blank per five samples (no



	Arctic				Interior			
	Msats		MtDNA		Msats		MtDNA	
	F_{ST}	χ^2	Φ_{ST}	χ^2	F_{ST}	χ^2	Φ_{ST}	χ^2
Pre-decline								
– Decline	0.000	16.9	**0.127**	**23.0**	0.025	29.1	0.037	3.0
– Post-decline	0.003	26.4	0.067	**20.3**	0.037	26.9	0.037	0.9
Decline								
– Post-decline	–0.006	12.8	0.001	5.5	**0.094**	**53.8**	–0.090	0.9
Overall	0.000	19.9	**0.068**	**31.9**	**0.059**	**53.0**	–0.011	2.2

Figure 1. Location of rivers (upper left panel: Arctic bounded in black, Interior bounded in light grey), plot of density of Peregrine Falcons from 1966 to 2002 (Arctic denoted by black line, Interior denoted by light grey line), estimates of genetic structure (F_{ST} and Φ_{ST}), and genic and haplotypic distributions (χ^2) at three time periods (lower right panel) of Peregrine Falcons nesting in the Alaska Arctic and Interior. Significant comparisons ($\alpha = 0.05$) are in bold type. Nest-site occupancy data are from Ambrose *et al.* (2016) and Swem and Matz (2018).

Table 1. Indices of genetic diversity: mean number of alleles and haplotypes, allelic and haplotypic richness, number of private alleles and haplotypes, observed (H_o) and expected heterozygosity (H_e), inbreeding coefficient (F_{IS}), effective population size (*Ne*), haplotype (*h*) and nucleotide (π) diversity, percentage change between pre-decline and post-decline metrics, and sample size (*n*) based on microsatellite and mtDNA control region loci for Peregrine Falcons nesting in Alaska Arctic and Interior sampled at three periods. Single standard deviation is in parentheses. Significant metrics are in bold type.

	Arctic				Interior			
	Pre-decline	Decline	Post-decline	% Change	Pre-decline	Decline	Post-decline	% Change
Microsatellites								
n	28	10	23	–	6	10	8	–
No. of alleles	4.8 (2.6)	4.0 (1.9)	5.2 (2.5)	7.7	3.0 (1.3)	3.6 (1.9)	3.6 (2.2)	9.4
Allelic richness[a]	4.1 (1.8)	4.0 (1.9)	4.3 (1.8)	3.7	3.1 (1.7)	3.1 (1.3)	2.9 (1.2)	0.0
Private alleles	3	1	9	66.7	2	6	7	66.7
H_o (%)	48.3 (2.9)	44.5 (4.7)	46.5 (3.1)	−3.9	38.8 (6.1)	52.7 (4.8)	46.3 (5.5)	7.8
H_e (%)	51.5 (8.3)	52.6 (7.4)	53.8 (7.5)	–	47.7 (8.8)	51.6 (8.5)	50.4 (7.5)	–
F_{IS}	0.064	0.161	**0.138**	–	0.204	−0.024	0.087	–
Ne	**107.1 (40.1–∞)**	∞ (26.0–∞)	**13.3 (7.7–25.9)**	–	∞ (19.6–∞)	∞ (24.2–∞)	26.4 (5.4–∞)	–
mtDNA								
n	26	10	19	–	5	9	7	–
No. of haplotypes	4	3	6	33.3	2	3	2	0.0
Haplotypic richness[b]	3.5	3.0	5.3	34.0	2.0	2.6	1.9	−5.3
Private haplotypes	1	0	3	66.7	0	1	0	0.0
h	0.588 (0.075)	0.511 (0.164)	0.602 (0.124)	2.3	0.600 (0.175)	0.417 (0.191)	0.250 (0.180)	−50.0
π	0.0012 (0.0011)	0.0001 (0.0010)	0.0017 (0.0014)	29.4	0.0011 (0.0012)	0.0008 (0.0009)	0.0004 (0.0006)	−57.1

[a]Allelic richness is based on a minimum sample size of 10 individuals for Arctic populations and six for Interior time periods. [b]Haplotypic richness is based on a minimum sample size of 10 individuals for Arctic populations and five for Interior time periods.

product was amplified). Samples were genotyped in duplicate for all loci; no inconsistencies were observed. The mtDNA control region was amplified in two fragments: primer pairs L15206 and H299 (5'-GCAGTAGT CCGAACCTCGTG-3'), and L257 (5'-ACCCACATTA GTTCACGTAG-3') and H15856.

Data analysis

We calculated the mean number of alleles (A), allelic richness (AR), inbreeding coefficient (F_{IS}), observed and expected heterozygosities, Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) at microsatellite loci in FSTAT 2.9.3 (Goudet 1995). The mtDNA haplotype (h) and nucleotide (π) diversity were calculated in Arlequin 3.1 (Excoffier *et al.* 2005). An unrooted mtDNA haplotype network was constructed in Network 5.0.1.1 (Fluxus Technology, Clare, UK 2015).

We used POWSIM 4.1 (Ryman and Palm 2006) to assess the statistical power of detecting population genetic structure. We ran simulations at 10 generations (1000 dememorizations, 100 batches, 1000 iterations per batch, 1000 runs) with observed allele frequencies and sample sizes in the present study and varied effective population sizes (Ne = 50–400, increments of 50) to test statistical power.

Genetic structure was assessed among time periods within Arctic and Interior for microsatellite (F_{ST}) and mtDNA (Φ_{ST}) data in Arlequin. We used chi-square analysis to test for differences in the temporal distribution of alleles and haplotypes (Raymond & Rousset 1995). Multiple comparisons were adjusted using Bonferroni correction ($\alpha = 0.05$). A Tamura and Nei (1993) nucleotide substitution model was used to calculate Φ_{ST} (see Talbot *et al.* 2011). We visualized between-individual relationships using a principal coordinate analysis (PCoA) in GenAlEx version 6.5 (Peakall & Smouse 2012). We used Structure 2.3.2 (Pritchard *et al.* 2000; Hubisz *et al.* 2009) to assign individuals to clusters based on microsatellite data (search strategy: admixture model assuming correlated frequencies, time-period as a prior, K = 1–5, 50 000 burn-in, 500 000 Markov chain Monte Carlo iterations, 10 replicates).

Contemporary Ne was estimated with NeESTIMATOR v2 (Do *et al.* 2014). Microsatellite data were analysed using the LD-based method and evaluated effects of low-frequency alleles on point estimates by excluding rare alleles (Pcrit = 0.0–0.2). Variance in Ne estimates across a range of Pcrit values is suggestive of gene flow, whereas stable estimates are indicative of isolated populations (Waples & England 2011).

Wilcoxon sign-rank tests tested for fluctuations in population size under three mutation models (infinite allele model, stepwise mutation model (SMM), two-phased model; parameters: 79% SMM, variance 9, 5000 permutations) based on microsatellite data and were implemented in Bottleneck 1.2.02 (Piry *et al.* 1999).

RESULTS

Genetic diversity

Microsatellite loci and populations were in linkage equilibrium and HWE. Indices of genetic diversity based on microsatellite loci were similar across time periods (Table 1). Increases in diversity metrics were observed, though confidence intervals (CI) overlapped for most metrics. Within Arctic, the post-decline period had the highest number of private alleles (PA) and significant increase in F_{IS}. Similarly, post-decline Interior had the highest number of PA.

Nine mtDNA haplotypes defined by seven variable sites were observed, with time periods represented by 2–6 haplotypes (Table 1; Appendix S2). Within Arctic, h was similar across time periods, while π was lowest in the decline period and highest post-decline. The post-decline period was represented by the highest number of haplotypes (H) and private haplotypes (PH). Within Interior, h and π were highest pre-decline, with the lowest values observed in the decline period.

Genetic structure

Simulations in POWSIM indicated sufficient power (> 90%) to detect genetic differences based on allele frequencies and sample sizes. Patterns of temporal variance varied between marker types and sites (Fig. 1). For Arctic, neither genetic structure nor differences in genic distribution were detected across periods at microsatellite loci. Conversely, genetic structure and differences in haplotypic distribution were observed in pre-decline comparisons and overall based on mtDNA. Within Interior, genetic structure and differences in gene distribution were detected between decline and post-decline periods and overall at microsatellite data, but not mtDNA. The first three coordinates explained 59.3% (Arctic) and 63.0% (Interior) of the total variation of the PCoA (Appendix S3). No patterns were present in the PCoA for Arctic. PCoA indicated two main clusters, albeit weakly separated, in Interior, with pre-decline individuals clustering away from the other two time periods. Structure failed to uncover genetic structure in Arctic (LnP|K(1) = −1478.3; LnP|K(2) = −1489.0, $r > 3.4$) or Interior (LnP|K(1) = −556.0; LnP|K(2) = −569.4, $r > 8.2$).

Population demography

Reduction in Ne was observed for Arctic, as the CI did not overlap between pre-decline and post-decline

periods (Table 1). No signature of a reduction in *Ne* for Interior was observed based on overlapping CI. Variation in *Ne* estimates across Pcrit values was observed for Arctic pre-decline and post-decline and Interior post-decline individuals, indicative of past gene flow (Fig. 2; Appendix S4).

Fluctuations in population size were detected for Arctic; heterozygote deficiency was observed under the SMM ($P = 0.008$) for the post-decline period, suggesting population growth. Remaining time periods were in mutation-drift equilibrium across all tests.

DISCUSSION

Although the population decline was of short duration, it influenced the levels and distribution of genetic variation within naturally recovered Peregrine Falcon populations. Differences among time periods were observed at various metrics. Sample sizes were low for the decline; however, samples represent 17.2% (Arctic) and 18.5% (Interior) of the pairs detected in monitored breeding populations and therefore probably captured the genetic diversity present at that time. Furthermore, power analyses indicated our microsatellite dataset has sufficient power to uncover genetic structure. Indeed, we observed differences in haplotypic (Arctic) and allelic (Interior) frequencies across time periods. Although sample sizes for Arctic are robust across time frames, fewer samples for Interior pre- and post-decline may reduce the power for some analyses to detect differences.

Patterns in diversity differed between Alaska Arctic and Interior. Biases in diversity metrics (PA, H, PH and π) increased post-decline (although CI overlapped) and structure (Φ_{ST} and χ^2) at mtDNA was observed within Arctic. Conversely, biases in diversity metrics (h and π) decreased post-decline (albeit CI overlapped) and structure (F_{ST} and χ^2) was uncovered at microsatellite loci within Interior. Differences in genetic signatures between Arctic and Interior may be attributable to contrasts in aspects of population demography and history. Reproductive performance metrics were similar between rivers throughout the recovery. However, interannual variation in the proportion of pairs with young was ~2× greater in the Interior (Appendix S5; Ambrose *et al.* 2016, Swem & Matz 2018). Abundance stabilized in the mid-1990s within Arctic (Swem and Matz 2018) but not until the early-2010s in Interior (Ambrose *et al.* 2016). Indeed, productivity (young per pair) appeared to stabilize in Arctic after 1992, whereas interannual productivity estimates varied considerably in Interior (Appendix S5; Ambrose *et al.* 2016, Swem & Matz 2018). As metrics of genetic diversity and structure are influenced by demographic processes (i.e. growth, effective size and census size), and populations experience different time-lag rates prior to reaching equilibrium following change (Epps & Keyghobadi 2015), the Arctic population may be closer to achieving equilibrium than Interior. The Arctic reached population stability recently (10–15 years after the pre-decline samples), following only a few generations; thus, genetic variation can change rapidly, especially as species respond and recover via the continual action of microevolutionary and demographic process (e.g. Sonsthagen *et al.* 2017, 2019).

Although genetic signatures for Arctic and Interior differed, patterns in genetic diversity suggest that recovery in both populations resulted from recruitment from within and from immigration. First, significant Φ_{ST} and χ^2 among periods within Arctic are suggestive of variance in reproductive output and philopatry and the increase in F_{IS} and reduction in *Ne* is indicative of inbreeding. Although the signature is not as marked, possibly due to sample size limitations, differences in the biases of diversity metrics pre- and post-decline, and significant F_{ST} and χ^2 among periods within Interior are also consistent with recovery involving only a few Falcons. Secondly, increases in the biases of genetic diversity metrics, influx of new alleles/haplotypes, population growth inferred in Bottleneck (Arctic), and variance of *Ne* across Pcrit values in post-decline periods suggest the recovery was also augmented by natural immigration. Prior research found that gene flow estimates among regions (post-decline) are asymmetric between Arctic Canada and Arctic Alaska (microsatellite data) and between Interior Alaska and Arctic Alaska (mtDNA data), with no genetic structure detected between Interior and Arctic Alaska (Talbot *et al.* 2017). Thus Peregrine Falcons were probably dispersing across the landscape as populations recovered. The marked change in the distribution of haplotypes, relative to genic distributions, within Arctic suggests female dispersal may be driving this pattern. Furthermore, gene flow among regions would dampen effects of genetic drift by increasing *Ne* of remnant populations, thus retaining genetic diversity during the decline (Jangjoo *et al.* 2016), as seen here. As patterns of genetic structure have not changed pre- and post-decline between Arctic and Interior (pre-decline: microsatellite $F_{ST} = 0.011$, $P = 0.207$; mtDNA $\Phi_{ST} = -0.105$, $P = 0.991$), the decline does not appear to have influenced dispersal rates in Peregrine Falcons, and populations were probably consistently connected through gene flow. Indeed, variance in *Ne* across Pcrit values for pre-decline Arctic support our inference of connectivity among historical Peregrine Falcon populations.

Patterns of diversity in Alaska's non-augmented populations differ from patterns observed in studies involving augmented populations (Brown *et al.* 2007, Jacobsen *et al.* 2008), suggesting that reintroduction efforts probably altered the spatial and temporal distribution of allelic and haplotypic frequencies within Peregrine Falcons in

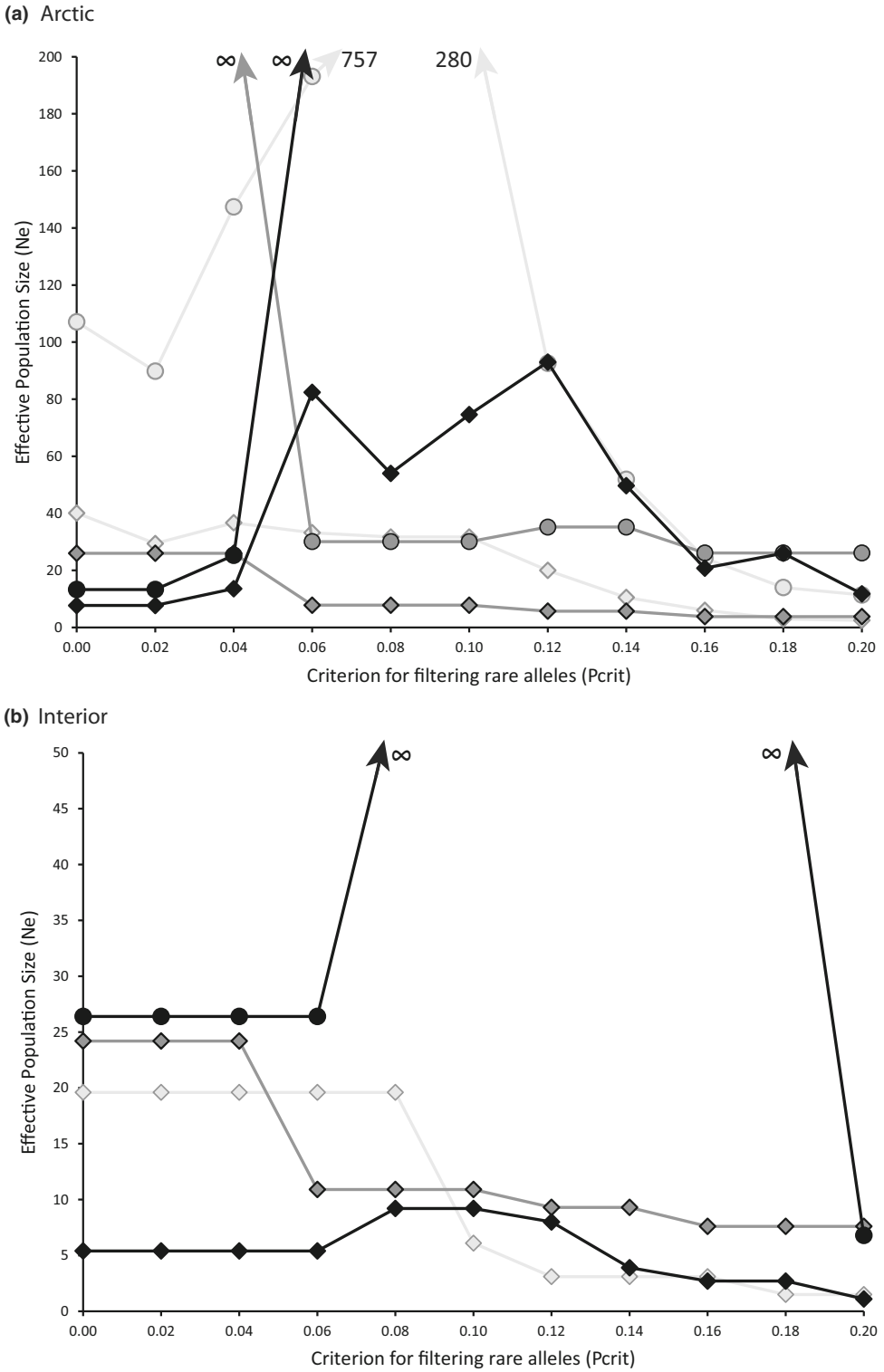


Figure 2. Effective population size (*Ne*) as a function of excluding rare alleles (Pcrit) in Peregrine Falcons nesting in Alaska (a) A tic and (b) Interior sampled at pre-decline (shown in light grey), decline (shown in dark grey) and post-decline (shown in black) ti periods. Circles represent point estimates of *Ne*, and diamonds are lower 5% confidence limits. *Ne* for Interior decline was ∞ or v large. Point estimates and 95% confidence limits are reported in Appendix S4.

Canada and Scandinavia. Additionally, unlike another non-augmented populations studied in England (Weaving *et al.* 2021), we observed increases in levels and distribution of genetic diversity following the decline and recovery. Our results, coupled with recent results from Weaving *et al.* (2021), provide valuable inference for assessing results of unassisted recovery of Peregrine populations but also highlight the importance of evaluating the impacts of declines on a case-by-case basis. It is the interplay of ecological, demographic and microevolutionary processes that shapes the trajectory of genetic diversity through time.

The manuscript was improved by comments by Rebecca Kimball and two anonymous reviewers. Any use of trade, firm or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

AUTHOR CONTRIBUTIONS

Sarah A. Sonsthagen: Conceptualization (equal); data curation (equal); formal analysis (lead); investigation (equal); methodology (lead); writing – original draft (lead). **Ted Swem:** Conceptualization (equal); funding acquisition (equal); methodology (equal); resources (equal); writing – review and editing (equal). **Skip Ambrose:** Conceptualization (equal); funding acquisition (equal); resources (equal); writing – review and editing (equal). **Melanie J. Flamme:** Conceptualization (equal); funding acquisition (equal); resources (equal); writing – review and editing (equal). **Clayton M. White:** Conceptualization (equal); methodology (equal); resources (equal); writing – review and editing (equal). **George K. Sage:** Conceptualization (equal); data curation (equal); investigation (equal); writing – review and editing (equal). **Sandra L. Talbot:** Conceptualization (equal); data curation (equal); formal analysis (supporting); funding acquisition (lead); investigation (equal); methodology (equal); writing – review and editing (equal).

ETHICAL NOTE

Peregrine Falcons were handled following protocols outlined by the Ornithological Council (Fair *et al.* 2010).

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

Data Availability Statement

Mitochondrial sequence data are available from NCBI GenBank (KY981769, ON5643722–ON564402). Microsatellite genotype data and detailed sample information are available from the U.S. Geological Survey data repository (Sonsthagen & Pierson 2017; https://doi.org/10.5066/F7F18WV0).

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SUPPORTING INFORMATION

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

Appendix S1. List of museum catalogue numbers* of Peregrine Falcons *Falco peregrinus* assayed for the pre-decline Alaska Arctic and eastern Interior populations.

Appendix S2. Unrooted network illustrating relationships of mtDNA control region haplotypes assayed from Peregrine Falcons residing in the Alaska (A) Arctic and (B) Interior sampled at three periods: pre-decline (1899–1968), decline (1985) and post-decline (2001–2009, respectively). Numbers denote haplotype names. The size of the circle is proportional to the number of individuals representing that haplotype.

Appendix S3. Plots of inter-individual relationships visualized through principal components analyses based on microsatellite genotype data collected from Peregrine Falcons nesting in Alaska (A) Arctic and (B) Interior at three time periods: pre-decline (1899–1968), decline (1985) and post-decline (2001–2009). Pre-decline individuals are shown in light grey, decline in dark grey and post-decline in black. Percentages of variation explained by each axis are shown.

Appendix S4. Effective population size (*Ne*) estimates and 95% confidence limits as a function of excluding rare alleles (Pcrit) in Peregrine Falcons sampled at three time periods in Alaska Arctic and Interior. Non-overlapping *Ne* estimates between pre-decline and post-decline are in bold type.

Appendix S5. Reproductive performance of Peregrine Falcons nesting in Alaska Arctic (black) and Interior (grey) between 1987–2002 plotted as (A) proportion of pairs with young, (B) number of young per pair and (C) number of young per successful pairs. Plots were restricted to years in which surveys were conducted annually. Data are from Ambrose *et al.* (2016) and Swem and Matz (2018).