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Temporal increase in mtDNA diversity in a declining population

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Abstract

In small and declining populations levels of genetic variability are expected to be reduced due to effects of inbreeding and random genetic drift. As a result, both individual fitness and populations' adaptability can be compromised, and the probability of extinction increased. Therefore, maintenance of genetic variability is a crucial goal in conservation biology. Here we show that although the level of genetic variability in mtDNA of the endangered Fennoscandian lesser white-fronted goose *Anser erythropus* population is currently lower than in the neigbouring populations, it has increased six-fold during the past 140 years despite the precipitously declining population. The explanation for increased genetic diversity in Fennoscandia appears to be recent spontaneous increase in male immigration rate equalling 0.56 per generation. This inference is supported by data on nuclear microsatellite markers, the latter of which show that the current and the historical Fennoscandian populations are significantly differentiated (F_{ST} = 0.046, P = 0) due to changes in allele frequencies. The effect of male-mediated gene flow is potentially dichotomous. On the one hand it may rescue the Fennoscandian lesser white-fronted goose from loss of genetic variability, but on the other hand, it eradicates the original genetic characteristics of this population.

Keywords: anser erythropus, effective population size, female philopatry, migration, sex-biased dispersal, temporal genetic variation

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Introduction

Maintenance of genetic diversity is considered to be one of the main goals of conservation biology, because genetic variability increases the population's probability of persistence due to its positive effects on reproductive fitness and adaptability (Frankham *et al.* 2002; Spielman *et al.* 2004). Because measurement of quantitative genetic variation is difficult, evolutionary potential of populations is commonly approximated using neutral genetic variation. A fundamental relationship exists between levels of genetic variability and effective size (N_e) of a population. For selectively neutral regions of genome, the rate of introduction of genetic variation is expected to be a function of the mutation rate and N_e (Kimura 1983). Whereas mutations are the source of novel genetic variation, random genetic drift—the effect of which is inversely proportional to the N_e—affects the chances of the alleles to persist in a population. Thus, loss of genetic diversity is expected in small populations (Wright 1931) and experimental studies (e.g. Montgomery *et al.* 2000) support this theoretical prediction. Furthermore, comparisons of threatened and closely related non-endangered species (O'Brien 1994), as well as time-series analyses of declining populations (Glenn *et al.* 1999) testify that lower levels of genetic variability are evident in populations experiencing size reductions.

The design and implementation of management programs are particularly challenging in situations where management of ecological connectivity or population

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supplementation need to be considered. The lesser white-fronted goose *Anser erythropus* is one of the most threatened bird species in the Palearctic, especially in the western part of its distribution (Jones *et al.* 2008). The recent global estimate of the mid-winter population is 28 000–33 000 individuals (Wetlands International 2006). The Fennoscandian population of this species has declined from several thousands to less than 100 individuals during the last century (Fig. 1a). With only 25 breeding pairs in Fennoscandia, concerns about possible inbreeding and inbreeding depression have emerged. Here we report an assessment of levels of mitochondrial and nuclear genetic variation in the prebottleneck population using museum material, as well as compare it to the current levels of genetic diversity in Fennoscandia and Russia.

Material and methods

DNA isolation and PCR

DNA was isolated from 48 museum feathers (hereafter, historical sample; Table 1) using proteinase K digestion and ethanol precipitation (Sambrook & Russell 2001). DNA isolation and pre-PCR work were done in a facility where no other DNA work was done and UV light was used to eliminate possible contamination. All chemicals were kept separate from other DNA work and filtered tips were used in all pre-PCR stages. Negative controls were included both in DNA isolation and PCR reactions.

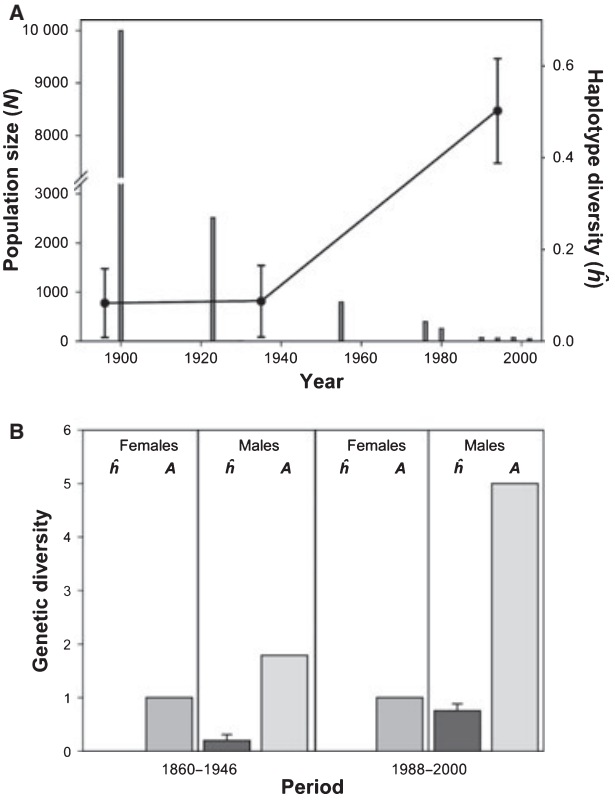

Fig. 1 (a) Estimated population size and mtDNA haplotype diversity ($\hat{h}$ ± SD) as a function of time in the Fennoscandian lesser white-fronted goose population. (b) Haplotype diversity ($\hat{h}$ ± SD) and haplotypic richness (*A*) in historical and current samples for males and females separately.

Nuclear microsatellites were analysed for 47 museum individuals (Table 1, Fig. 2). Data on microsatellite variability in the current Fennoscandian (Norway, Finland, $N = 14$) and West Russian (Bolshezemelskaya Tundra, Yamal Peninsula, the closest known breeding areas east of Fennoscandia, $N = 16$) populations using the same loci have been published before (Ruokonen *et al.* 2007), but the allele sizes had to be synchronized between laboratories. We used ten loci: Hhiμ1, Hhiμ3, Bcaμ2, Bcaμ6, Bcaμ7, Bcaμ8, Bcaμ9 (Buchholz *et al.* 1998), Aalμ1 (Fields & Scribner 1997), APH11 (Maak *et al.* 2000), and TTUCG5 (Cathey *et al.* 1998). PCR conditions were: 2 pmol of TET or HEX-labelled primers, or 3 pmol of FAM-labelled primers, 1 × Multiplex Mastermix (Qiagen) and 0.5 × Q-Solution (Qiagen) together with 1:10 diluted DNA (new samples) or undiluted 3 μl of DNA (historical sample). Total volume of PCR reaction was 10 μl. PCR cycling profile was 15 min at 95 °C, followed by 32 (new samples) or 40 (historical sample) cycles 30 s at 95 °C, 90 s at 54 °C and 60 s at 72 °C, and a final extension at 72 °C for 10 min. The PCR products were diluted 1:750 with water, mixed with Et-ROX 400 standard (GE Healthcare, Life Sciences) and resolved in a MegaBace 1000 capillary sequencer (GE Healthcare, Life Sciences). Genotypes were scored with the program Fragment Profiler 1.2 (GE Healthcare, Life Sciences). All PCR reactions were repeated at least once. Three of the loci were rejected at this stage: locus Bcaμ6 was not polymorphic (frequency of the most common allele >0.98), and loci TTUCG5 and Bcaμ8 showed inconsistent results between replicate PCR reactions. For the remaining seven loci, the average amplification success was 99.1% for West Russian, 96.9% for Fennoscandian and 90.0% for the historical sample.

A 276 bp fragment of mitochondrial control region was PCR-amplified using primers L180 and H466 as described in Ruokonen *et al.* (2000). Double-stranded sequencing of PCR products was carried out by using BigDye 3.1 and ABI PRISM 3730 Sequencer (Applied Biosystems) according to manufacturer's instructions. PCR primers were used for sequencing. Sequences have been submitted to GenBank under accession numbers EF125747–EF125794. Additionally, mtDNA data from Fennoscandia and West Russia published by Ruokonen *et al.* (2004) were used (Table 2, Fig. 2).

Table 1 Historical lesser white-fronted geese sampled from museums (collected in 1860–1946)

Museum*)	Museum code	Sampling locality	Sampling date
NRM	569278	Torne Lappmark, Sweden	17 July 1860
NRM	569271	Terebirki, Kola, Russia	17 July 1877
NRM	569270	Tuloma, Kola, Russia	18 May 1879
TMU	569	Hammerfest, Norway	June 1888
TMU	567	Balsfjord, Norway	27 August 1892
TMU	568	Nordkjosen, Norway	28 August 1892
ZMUO	3672	Sandnäs, Sör-Varanger, Norway	17 June 1893
NHMM	4.944	Lapland, Sweden	17 July 1899
NHMG	2898	Torne Lappmark, Sweden	27 May 1906
NHMG	3185	Lule Lappmark, Sweden	4 July 1907
NHMG	3400	Torne Lappmark, Sweden	22 June 1908
NHMG	3792	Lule Lappmark, Sweden	28 May 1911
NHMM	2.942	Scania, Sweden	21 October 1915
NHMG	4481	Lule Lappmark, Sweden	27 May 1916
NHMG	4478	Lule Lappmark, Sweden	27 May 1916
NHMG	4479	Lule Lappmark, Sweden	27 May 1916
NHMG	4480	Lule Lappmark, Sweden	27 May 1916
NRM	569275	Torne Lappmark, Sweden	14 April 1921
NRM	569274	Torne Lappmark, Sweden	14 April 1921
NHMG	5315	Torne Lappmark, Sweden	25 June 1922
NHMG	5319	Torne Lappmark, Sweden	25 July 1922
NHMG	5318	Torne Lappmark, Sweden	25 June 1922
NHMM	1.941	Scania, Sweden	January 1924
NRM	569272	Finland	26 September 1924
NHMF	FOR303	Valkeajärvi, Keuruu, Finland	Autumn 1925
NRM	620004	Sorsele, Lapland, Sweden	7 June 1927
NRM	569277	Norrbotten, Sweden	8 September 1928
ZMUO	3673	Sör-Varanger, Norway	25 May 1928
NHMM	7.1177	Lapland, Sweden	20 June 1931
OA	538	Österhankmo, Finland	9 September 1932
FMNH	11292	Hailuoto, Finland	20 August 1934
ZMOU	1342	Liminka, Finland	8 May 1934
FMNH	11566	Inari, Finland	12 May 1937
ZMUB	10037	Nord-Varanger, Finnmark, Norway	4 June 1938
ZMUB	9803	Vadsö, Finnmark, Norway	25 May 1939
ZMUB	10077	Krampenes, Finnmark, Norway	19 May 1940
ZMUB	10076	Kiberg, Finnmark, Norway	21 May 1940
NHMG	8167	Torne Lappmark, Sweden	14 June 1945
ZMOU	1343	Hailuoto, Finland	15 September 1945
NHMG	8364	Lycksele Lappmark, Sweden	27 June 1946
NHMG	8363	Lycksele Lappmark, Sweden	27 June 1946
NHMG	8366	Lycksele Lappmark, Sweden	13 June 1946
NHMG	8367	Lycksele Lappmark, Sweden	11 June 1946
NHMG	8365	Lycksele Lappmark, Sweden	13 June 1946
NHMG	8368	Lycksele Lappmark, Sweden	15 June 1946
NHMG	8362	Lycksele Lappmark, Sweden	27 June 1946
NHMM	9.2494	Lapland, Sweden	10 September 1946
Oulun Lyseo, Oulu	NA	Finland	Prior to 1950†

*FMNH, Finnish Museum of Natural History, Helsinki; NHMF, Natural History Museum, Forssa; NHMG, Natural History Museum, Gothenburg; NHMM, Natural History Museum, Malmö; NRM, Swedish Museum of Natural History, Stockholm; OA, Ostrobothnia Australis, Vaasa; TMU, Tromsø Museum; ZMOU, Zoological Museum of Oulu University; ZMUB, Bergen Museum; ZMUO, Natural History Museum, Oslo. †No exact sampling date available, individual excluded from the two historical periods.

The Fennoscandian population has declined and disappeared from some of the historical breeding areas (most notably, Sweden), and therefore the historical and current sampling areas differ (Fig. 2). Comparison of the Swedish (N = 29) and other individuals (N = 19) in the historical sample showed that they do not differ

Fig. 2 Breeding area and sampling localities of the lesser white-fronted goose in Fennoscandia and Russia. Dark grey colour indicates the historical breeding area (1950s) and the lined patches show the current breeding area (in 1990s, von Essen *et al.* 1996) in Fennoscandia. The distributional area in Kola Peninsula is not known and is therefore left unmarked. Grey circles show sampling localities for the historical material from years 1860–1924, grey/black circles for historical material 1925–1946 and black crosses for the current samples (Fennoscandia 1988–2000, West Russia 1996–1999). Two individuals in the historical sample collected from Scania, Southern Sweden, are not shown on the map.

Table 2 Mitochondrial haplotypes found in the temporal samples in Fennoscandia and in West Russia. The sex of the individuals is given in parenthesis after the number of individuals carrying each haplotype (males/females/sex unknown)

mtDNA haplotype	Fennoscandia, historical (1860–1946)	Fennoscandia, current (1988–2000)	West Russia, current (1996–1999)
W1	46 (17/21/8)	16 (5/8/3)	11 (4/1/6)
W2		1 (1/0/0)	4 (2/2/0)
W3			3 (2/1/0)
W4		1 (1/0/0)	1 (0/0/1)
W7			1 (0/0/1)
W8		1 (1/0/0)	3 (0/0/3)
E1	2 (2/0/0)	4 (2/0/2)	14 (2/1/11)
E5			2 (0/0/2)
N	48 (19/21/8)	23 (10/8/5)	39 (10/5/24)

either in terms of mitochondrial DNA (mtDNA, Φ_{ST} = 0.01, P = 0.50) or nuclear microsatellites (F_{ST} = 0.002, P = 0.39). Hence, any difference between the current and the historical Fennoscandian sample is unlikely to be due to bias introduced by sampling different areas in different times.

For some analyses, the historical sample was divided in two based on sampling years (historical samples 1860–1924 and 1925–1946). There was no genetic differentiation between these two historical periods in either marker type employed (mtDNA; Φ_{ST} = 0, P = 0.99, microsatellites: F_{ST} = 0.007, P = 0.18).

Because allele drop-out could explain high F_{IS} estimates and temporal differences in allele frequencies (see Results and Discussion), we examined the putative problem in detail and found no indications of this (see Supporting Information).

Estimation of genetic diversity

Microsatellites. Expected (H_e) and observed heterozygosity (H_o) and allelic richness (A_R) for the historical and current samples of Fennoscandian and West Russian lesser white-fronted geese were estimated with program FSTAT (Goudet 1995). Population level inbreeding was estimated by $F_{IS} = 1 - (H_o/H_e)$.

To test if H_e and H_o of the current sample differ from the historical sample, we randomly resampled with replacement the same number of individuals as in the current sample (N = 14) from the historical sample (N = 48) using PopTools (Hood 2009). Proportion of samples giving H_e or H_o higher than or equal to what was observed in the current sample were used as the probabilities of current H_e and H_o being similar to the historical sample.

mtDNA. A 221 bp mitochondrial DNA (mtDNA) control region sequence was used to estimate genetic diversity. Haplotype diversity ($\hat{h}$), nucleotide diversity (π) and mutation parameter (Watterson's theta, $\theta_w = 2N_e\mu$) were estimated using program DnaSP 4.10 (Rozas *et al.* 2003). To test if diversity estimates $\hat{h}$, π and θ_w differ among the samples, one-tailed t-tests were applied. To make the test more conservative, the variance for females was assumed to be equal to that of the males when comparing their diversity estimates. Haplotypic

richness (A) was estimated in HP-Rare (Kalinowski 2005) using rarefaction.

Simulations

Simulations of nuclear data were run in program BottleSim (Kuo & Janzen 2003). The mean sampling years of the Fennoscandian historical and current sample were 1922 and 1997, respectively, and during this period the population census size dropped from 2500 individuals to 82 individuals (Siivonen 1949; Aarvak *et al.* 2009). Hence, we simulated the effect of population decline on the levels of genetic variability during a 75-year period assuming annual geometric growth rate of –4.5%, starting with the allele frequencies in the microsatellite data of the historical sample. Ratio of males to females was set to 1:1, age at maturation to 2 years, longevity to 7 years, and generations were assumed to be overlapping. Longevity was estimated by life table analysis based on current reproduction and mortality rates (Aarvak & Øien 2009). Because no estimates of N_e/N_c were available, we run the simulations under three scenarios regarding the ratio of effective (N_e) and census (N_c) population sizes: $N_e = N_c$, $N_e = 1/2N_c$ and $N_e = 1/10N_c$.

The expected loss of mtDNA diversity was estimated using $H_t = H_0 \prod_{i=1}^{t} \left(1 - \frac{1}{N_{ef,i}}\right)$ (Hedrick 1995). The current haplotype diversity of 0.76 in the West Russian lesser white-fronted goose population was used as H_0, the original mtDNA diversity in Fennoscandia. This is probably a conservative estimate, because also Russian populations are currently declining (Jones *et al.* 2008). Fennoscandian females showed no genetic variation in mtDNA ($H_t = 0$). In order to get conservative estimates for size and duration of the bottleneck, H_t was set to <0.096 ($\hat{h} = 0.096$ if one out of 21 females in the historical sample had an mtDNA haplotype other than the rest of the females). Population growth decreases loss of genetic variation after a bottleneck (Hedrick 1995). Because the timing of the historical bottleneck is not known, population demography after the bottleneck was not taken into consideration. Thus, the estimates of bottleneck sizes and durations are conservative in this sense too. For calculating the population census estimates from the female effective population sizes and duration of the bottlenecks we assumed a sex ratio of 1:1, 20% juveniles and generation length of 7 years. Because it has been shown that the effective size of mtDNA in isolated populations with random mating can approach the female population size (Chesser & Baker 1996), we assumed $N_e/N_c = 1$.

Estimation of migration and N_e

The proportion of male geese migrating from the West Russian population to Fennoscandia and N_e were estimated using a model for spatial and temporal dynamics of biparentally and uniparentally inherited genes (equation 16 in Chesser & Baker (1996); also see Chesser (1998)). The parameters used for divergence were θ_{ST} = 0.100 (95% CI 0.072–0.119) between current populations of Fennoscandia and West Russia for mtDNA (based on data in Ruokonen *et al.* 2004), and F_{ST} = 0.011 (95% CI –0.013 to 0.052) for microsatellite divergence. Because males import the unique variation and do not pass it to their progeny, and because the θ_{ST} was determined using males and females sampled from the Fennoscandian population, mtDNA suffices for estimating male immigration. Mean progeny per female was estimated at $k = 1.44$ and variance in progeny per female, σ^2_k = 0.77 based on field data (T. Aarvak, unpublished). We assumed monogamy with permanent pair-bonds and the frequency of multiple paternity equal to zero. Population sizes were estimated as 25 breeding pairs for Fennoscandia and 700 for West Russia.

Ecological data

The sex was known for 40 geese in the historical sample, and was determined in the field based on morphology and behaviour for 18 and 15 geese from the current Fennoscandian and West Russian populations, respectively. Out of ten males from the current population, eight were known breeders (seen with *pulli* or moulting at the breeding areas) and two were seen paired at the staging area in Finnmark, Norway, from where the birds move to the nearby breeding grounds. Population size estimates for the Fennoscandian lesser white-fronted goose were collected from the literature (Siivonen 1949; Soikkeli 1973; Ulfstrand & Høgstedt 1976; Norderhaug & Norderhaug 1984; Aarvak & Timonen 2004).

Results

Despite the fact that the Fennoscandian population of the lesser white-fronted goose has declined in between the historical (1860–1924 and 1925–1946) and current samples (1988–2000), measures of nuclear genetic diversity, H_e and A_R, show only minor differences among the temporal samples (Table 3, Fig. 3). We used simulations to obtain an estimate of expected change in the level of nuclear genetic variability in the Fennoscandian population. During the sampling period the population declined from 2500 to 82 individuals (census estimates from the mean sampling years of the historical and current samples, 1922 and 1997). Because the ratio of effective population size N_e to census size of the population N_c is not known we used N_e/N_c of 1, 0.5 and 0.1

Table 3 Genetic diversity estimates for the microsatellite data, including simulation results (see text for details). Number of individuals (N), allelic richness (A_R) and expected and observed heterozygosities (H_e, H_o) with standard deviations (SD). Simulation results show the effect of population decline on H_e in the absence of male immigration

	N	A_R	H_e	SD¤	H_o	SD#
Fennoscandia, historical 1860–1924	24	3.6	0.513	0.209	0.437	0.196
Fennoscandia, historical 1925–1946	23	3.5	0.496	0.257	0.344	0.188
Fennoscandia, current simulated, $N_e = N_c$	–	–	0.467	0.036*	–	–
Fennoscandia, current simulated, $N_e = 0.5N_c$	–	–	0.443	0.050*	-	–
Fennoscandia, current simulated, $N_e = 0.1N_c$	–	–	0.289	0.083*	–	–
Fennoscandia, current 1988–2000	14	3.8	0.534	0.180	0.517	0.155
West Russia, current 1996–1999	16	3.4	0.491	0.250	0.478	0.145

¤ SDs were estimated over loci, except SD# over individuals and SD* over 1000 simulations.

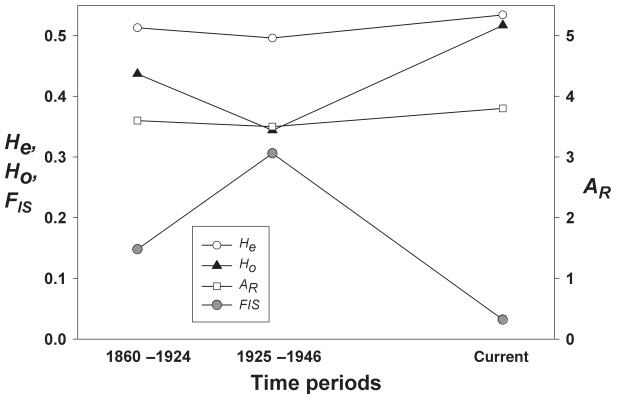

Fig. 3 Temporal change in expected (H_e) and observed heterozygosity (H_o), allelic richness (A_R) and inbreeding (F_{IS}).

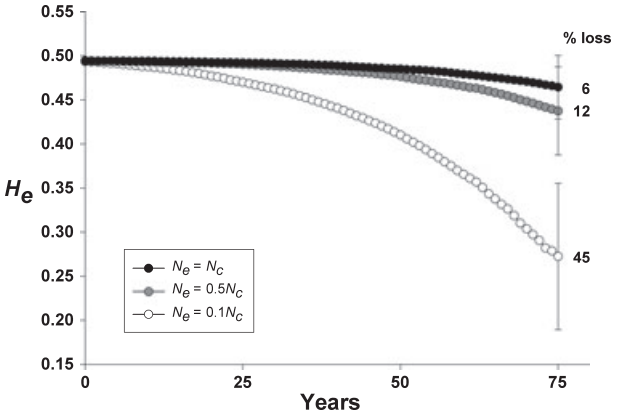

Fig. 4 Temporal change in expected heterozygosity (H_e) in simulations assuming different ratios of effective (N_e) and census (N_c) population sizes. Bars show SDs over 1000 simulations. See text for details.

in the simulations, which yielded an expected loss of 6, 12 or 45% of the genetic variability (Table 3, Fig. 4).

Temporal changes in the observed heterozygosity, H_o, and F_{IS} were more dramatic (Table 3, Fig. 3). In both historical samples H_e exceeded H_o and F_{IS} estimates are positive ($F_{IS(1860–1924)} = 0.148$ and $F_{IS(1925–1946)} = 0.306$). In agreement with the population decline, H_o decreased and F_{IS} increased between the two historical periods. In the current sample H_o approaches H_e and hence F_{IS} approaches zero (Fig. 3). This is an unexpected result, considering that the population has been constantly declining. Based on resampling (see Materials and methods), H_o of the current sample differs from the H_o of the historical sample ($P = 0.001$ of being from the same population), whereas no difference was observed in the H_e values ($P = 0.1$).

We analysed sequence variation in the mtDNA control region for 48 lesser white-fronted geese from the historical periods (years 1860–1924 and 1925–1946), and compared levels of genetic variability to a sample of 23 geese from the current population (1988–2000). A sixfold temporal increase in haplotype diversity ($\hat{h}$) and a fourfold increase in nucleotide diversity (π) were detected (Fig. 1a, Table 4). At the same time, haplotypic richness (A) increased from 1.96 to 5.00 and mutation parameter (θ_w) from 1.35 to 1.90 (Table 4). Increase in mtDNA variability is highly unexpected considering the population history.

Female geese are known to return to their natal areas to breed, whereas males sometimes pair with females from non-natal populations and move to new breeding areas (Greenwood 1980; Anderson *et al.* 1992; Lindberg *et al.* 1998). This behavioural pattern prompted us to examine mtDNA haplotype frequencies separately for the two sexes, and indeed, a sex-bias was found. All sampled females carried the same mtDNA haplotype, and haplotype diversity for females was zero (Fig. 1b). Males were much more variable (Fig. 1b): in the historical sample 11% and in the current population 50% of the males carried mtDNA haplotypes that each exists with low frequencies. These rare haplotypes are found in West Russian lesser white-fronted geese (Table 2, Ruokonen *et al.* 2004) suggesting that the males

Table 4 Genetic diversity estimates based on mtDNA in the lesser white-fronted goose samples. Number of individuals (N), haplotypic richness (A), haplotype diversity ($\hat{h}$: the probability that two randomly chosen individuals have different haplotypes), nucleotide diversity (π; the average number of pairwise nucleotide differences among haplotypes per bp), and mutation parameter (θ_w; Watterson's estimate of theta $\theta=2Ne\mu$ per gene)

Sample	N	A	$\hat{h}$ ± SD	π ± SD	θ_w ± SD
Both sexes, 1860–1924	24	1.96	0.083 ± 0.075	0.0019 ± 0.0017	1.35 ± 0.71
Both sexes, 1925–1946	23	2.00	0.087 ± 0.078	0.0020 ± 0.0018	1.35 ± 0.73
Both sexes, 1988–2000	23	5.00	0.502 ± 0.114*	0.0081 ± 0.0023¶	1.90 ± 0.91§§
Females, 1860–1946	21	1.00	0	0	0
Females, 1988–2000	8	1.00	0	0	0
Males, 1860–1946	19	1.79	0.199 ± 0.112 †	0.0045 ± 0.0025‡	1.44 ± 0.77¶¶
Males, 1988–2000	10	5.00	0.756 ± 0.130†§	0.0109 ± 0.0031‡††	2.48 ± 1.31¶¶‡‡

*$\hat{h}$: 1988–2000 > 1860–1924 $t(37) = 14.8$, $P < 0.001$; 1988–2000 > 1925–1946 $t(38) = 14.4$, $P < 0.001$.
†$\hat{h}$ males > females: 1860–1946 $t(37) = 5.6$, $P < 0.001$; 1988–2000 $t(15) = 12.3$, $P < 0.001$.
§$\hat{h}$ males: 1988–2000 > 1860–1946 $t(16) = 11.5$, $P < 0.001$.
¶π 1988–2000 > 1860–1924 $t(40) = 10.5$, $P < 0.001$; 1988–2000 > 1925–1946 $t(41) = 10.0$, $P < 0.001$.
‡π males > females: 1860–1946 $t(37) = 5.7$, $P < 0.001$; 1988–2000 $t(15) = 7.4$, $P < 0.001$.
††π males: 1988–2000 > 1860–1946 $t(15) = 5.6$, $P < 0.001$.
§§θ_w 1988–2000 > 1860–1924 $t(41) = 2.3$, $P = 0.013$; 1988–2000 > 1925–1946 $t(42) = 2.3$, $P = 0.015$.
¶¶θ_w males > females: 1860–1946 $t(37) = 5.9$, $P < 0.001$; 1988–2000 $t(15) = 4.0$, $P < 0.001$.
‡‡θ_w males: 1988–2000 > 1860–1946 $t(12) = 2.3$, $P = 0.042$.

carrying them in Fennoscandia are first-generation immigrants.

Discussion

Considering that the Fennoscandian population of the lesser white-fronted goose has declined 95% in size during the last century, the most salient findings of this study are the increased mtDNA diversity, moderate changes in nuclear diversity and decrease in the level of inbreeding. These results suggest that factors other than the recent reduction in population size have affected genetic diversity. Immediately after a bottleneck, genetic variability is expected to decline due to the loss of rare haplotypes/alleles, whereas e.g. nucleotide diversity is affected later on (Nei *et al.* 1975; Tajima 1989). Historically low genetic diversities have been observed earlier in some currently threatened populations (Matocq & Villablanca 2001; Hoffman & Blouin 2004), but the present study is the first to report increase in mtDNA diversity in a declining population.

Based on the observed sex-bias increased immigration of male geese from Russia to Fennoscandia appears to be the most plausible explanation of the recent temporal increase in genetic diversity. Several possible explanations for the increase in male recruitment to Fennoscandia can be given. Because pair-formation takes place during the non-breeding season, it is possible that the non-breeding areas for Fennoscandian and West Russian breeding populations currently overlap (Aarvak & Øien 2003). Also, because the Fennoscandian population is presently extremely small, females may have difficulties finding a mate from the natal population (Stephens & Sutherland 1999) or they may attempt to avoid inbreeding (Pusey 1987) by pairing with a male from a non-natal group.

The unisexual and effectively unidirectional migration of males from Russia into the Fennoscandian population creates a scenario wherein the genetic divergences between populations will differ for mitochondrial and nuclear DNA. Immigrant males carrying the mtDNA of their mothers (in Russia) will not transmit those genes to their progeny. Thus, the Russian mtDNA will be only represented in the male dispersers. Nevertheless, male dispersal rate can be estimated directly because mtDNA differentiation (θ_{ST} = 0.100; 95%CI 0.072–0.119) was calculated on the basis of both sexes. We estimated the proportion of male geese migrating from Russia to Fennoscandia to be 0.02 (0.016–0.026). Because there are approximately 700 breeding male geese in the West Russian population, it is estimated that 14 (11.2–18.2) males from Russia pair with females from the Fennoscandia population (estimated at 25 pairs) each generation. Thus, the rate of male migration into the Fennoscandia population is about 0.56 (0.45–0.73). Despite this high rate of migration the differentiation of mtDNA for progeny born within Fennoscandia and within Russia will change relatively little over time. Nuclear genes in Fennoscandia, on the other hand, are expected to undergo rapid frequency changes associated with the high rate of introgression from the Russian population. Nuclear genes characteristic of the Fennoscandian population will be eliminated from the segregating gene pool within 10–15 generations (i.e.

70–105 years) if introgression by males from Russia proceeds at the estimated rate. Although the level of mtDNA variability is currently lower in Fennoscandia than in Russia (Ruokonen *et al.* 2004), the level of variability in nuclear markers in the Fennoscandian population exceeds that of the much larger West Russian breeding population (Table 3).

During 1980–1990s captive lesser white-fronted geese were released in Fennoscandia (von Essen 1996) and this could be an alternative explanation for the increase in genetic variability. However, half of the rare haplotypes in the Fennoscandian population have not been found in captive stocks, whereas all of them occur in wild Russian lesser white-fronted geese (Ruokonen *et al.* 2004, 2007). Also, if these rare haplotypes were of captive origin, they should have been found in both sexes, not only in males.

Simulations showed that rather subtle decline in genetic variability in the Fennoscandian population would be expected in the absence of male immigration, unless if the effective size of the population is much smaller than the census size. A closer look on the nuclear allele frequencies in the temporal samples shows that eleven alleles were lost, seven of them with a frequency of >5% in the historical sample, and five alleles increased and three decreased considerably in frequency in between the historical and current sample. At the same time, five new alleles appeared into the Fennoscandian population, most likely as a consequence of male immigration from Russia. Therefore it is not surprising that no temporal changes in H_e or A_R—which do not consider allele identities—were observed. However, these changes are shown by significant genetic differentiation between the historical and current Fennoscandian population (F_{ST} = 0.046, P = 0). This degree of temporal differentiation within a population is remarkable, considering that the current Fennoscandian and West Russian populations differ only by F_{ST} = 0.011 (P = 0.063).

The low level of mtDNA variation in the historical females indicates that the Fennoscandian lesser white-fronted goose population has bottlenecked already before the current decline. Based on the expected loss of mtDNA diversity, a bottleneck of, e.g. six females for 12 generations or ten females for 20 generations is needed to inflate mtDNA variation close to the level currently observed in the Fennoscandian lesser white-fronted goose females. In census numbers these estimates translate into 15 and 25 individuals, and the durations of the bottlenecks are 84 and 140 years, respectively. The main reason behind the current population decline is thought to be hunting (Jones *et al.* 2008). It seems highly unlikely that historical hunters (Itkonen 1948) could have caused such a severe population bottleneck. Thus, it seems plausible that the Fennoscandian population has been also historically relatively small and isolated or that the westernmost breeding areas were colonized by a few individuals after the last ice age, less than 10 000 years ago.

According to the genetic rescue hypothesis, population persistence is improved by immigrants through their genetic contribution alone (e.g. Ingvarsson 2001). When viewed solely from the perspective of the mtDNA, the introgression could be regarded as genetic rescue, but the effect is ephemeral due to maternal inheritance of the mtDNA. Loss of unique nuclear alleles characteristic of the Fennoscandian population, including potential locally adaptive genetic variation, is also a possibility. With exception of the decrease in the level of inbreeding reported here, no positive or negative fitness effects have been reported for the population thus far.

Because of female natal philopatry, the immigrant males merely replace Fennoscandian males and no demographic rescue takes place. In the absence of female migration and because of the female natal philopatry, the size of the population is primarily determined by its production of female offspring. In isolation, the N_e of the Fennoscandian population is likely to be lower than the currently estimated census number of 60–80 individuals. Due to the introduction of genes from the Russian population N_e is dramatically elevated to 748–768 individuals for biparentally inherited markers. Consequently, the level of genetic diversity in the Fennoscandian population is largely determined by the genetic variability of the neighbouring Russian population.

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Supporting information

Additional supporting information may be found in the online version of this article.

Fig. S1 Individual observed heterozygosities (*Ho*) of the historical lesser white-fronted geese with respect of the age of the sample as indicated by the sampling year. *Ho* and sampling year are not correlated (Pearson's $r^2 = 0.026$, $P = 0.288$).

Fig. S2 Allele frequencies in the three temporal samples of the lesser white-fronted goose from Fennoscandia.

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