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Conservation genetics of the threatened horned grebe (*Podiceps auritus* L.) population of the Magdalen Islands, Québec

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Abstract

The horned grebe (*Podiceps auritus*) population of the Magdalen Islands in the St. Lawrence Gulf (Québec, Canada) has declined sharply over the last decades. It is the only breeding population of this species in eastern North America with nearest breeding populations being >2500 km apart in western North America and Europe, We used three types of genetic markers: mitochondrial (mt) DNA ND2 sequence, α-enolase intron sequence, and 25 amplified fragment length polymorphism loci (AFLPs) to quantify the genetic diversity within the Magdalen Island population and to assess its genetic distinctiveness relative to populations from western Canada (five sites) and Iceland (one site). The Magdalen Island population retained a comparable amount of genetic diversity to the average diversity observed across all populations in all three markers. Horned grebe mtDNA sequences formed a monophyletic group and nearly all haplotypes present in Québec were found elsewhere. In the ND2 fragment, populations partitioned into two groups corresponding to subspecies (Iceland versus North American sites) and more strongly in three groups according to geographic disjunctions (Iceland versus Québec versus western Canada). In contrast, there was no evidence of structure between sites in the α-enolase intron. In the AFLPs, Iceland showed the greatest level of differentiation, followed by the Québec and British Columbia populations. For conservation purposes, we suggest that the Magdalen Islands population should be recognized as a separate unit.

Introduction

Several case studies have showed that populations with wide disjunct distributions may have been isolated in distinct refugia during the Pleistocene glaciation events or other large-scale geological events (Pielou 1991; Bernatchez and Wilson 1998; Flagstad and Røed 2003; Holder et al. 2004, but see Avise 2000). As a result, these disjunct populations may now be distinct based on genetic and ecological characters and this distinctiveness must be accounted for in conservation plans (Moritz et al. 1995; Crandall et al. 2000; Fraser and Bernatchez 2001). The horned grebe (*Podiceps auritus*) shows such a disjunct distribution. The breeding distribution of this medium-distance

migratory bird has two components: it includes a continuous range over continental Eurasia and western North America, as well as a suite of islands: Magdalen Islands in the Gulf of St. Lawrence (Canada), Greenland, Iceland, and Scotland (Fjeldså 1973; Stedman 2000). Horned grebes from Eurasia, including Iceland, belong to the subspecies *P. a. auritus* and have a darker and blacker plumage, whereas North American individuals belong to the subspecies *P. a. cornitus* and have a paler and greyer plumage with contrasting light-grey feather edges on back (Stedman 2001).

The Magdalen population is thus the only breeding population in eastern North America and is strongly isolated geographically. Indeed, nearest well established populations include the extended population in western Canada and Northwest U.S.A. (about 100,000 individuals, located >2500 km west) and the Icelandic population (about 400–750 individuals, located >3000 km apart east) (O'Donnel and Fjeldså 1997; Stedman 2000). Since the first report of horned grebes nesting on the Magdalen Islands in 1897 (Young 1897), the population has never been very abundant. Historically, the population included about 40 individuals in 1956 (Hagar 1956) and 41 individuals in 1989 (Fradette 1992). Since then, the population has shrunk to an average of 18 observed adults/year and 7 breeding pairs/year between 1991 and 2001 (Shaffer and Laporte 2003). Data relative to the reproductive success are scarce but quite alarming, The hatching success of eggs was 54% (36/67) in 1994–1995. Of these 36 nestlings that successfully hatched, only ten reached the age of 36 days (Shaffer and Laporte 2003). The sharp decline in homed grebes prompted the designation of the Magdalen Island population as threatened by the Government of Québec in 2000, according to the provincial law "*Loi sur les espèces vulnérables et menacées*" (Act respecting threatened or vulnerable species) (Gazette officielle du Québec 2000). At the national level, however, the species does not have any particular conservation status due to its wide abundance in western Canada (Canadian Endangered Species Conservation Council 2001).

The horned grebe population nesting on the Magdalen Islands may not be self sustainable via local recruitment in the long term, and reduced genetic variation may lead to inbreeding depression (Hedrick 2001; Frankham et al. 2002). We therefore conducted a study on the conservation genetics of this population relative to nearest regular breeding populations. Specifically, our objectives were to quantify the level of genetic diversity within the Magdalen Island population, compare it to the genetic diversity found in populations from western Canada and Iceland, and determine the genetic distinctiveness of the Magdalen Island population relative to the other populations under study. We hypothesized that the Magdalen Island population would be genetically depauperate due to its small census size and genetically distinct compared to populations from western Canada and Iceland due to the large geographical distances among these regions, Three types of genetic markers were used to test our hypotheses: (1) mitochondrial (mt) DNA ND2 sequence, (2) nuclear sequence in the α-enolase intron, and (3) amplified fragment length polymorphism (AFLPs) (Vos et al. 1995).

Methods

Data collection and DNA extraction

We collected 128 horned grebe samples from six breeding sites located in Canada (Alberta, British Columbia, Manitoba, Northwest Territories, Yukon, and Québec) and one breeding site in Iceland during the breeding seasons of 1995, 1996, 2002, and 2003 (Table 1). For adults, we punctured the brachial vein and we preserved 70 μl of blood in 600 μl lysis buffer (Seutin et al. 1991). For embryos, we preserved tissues in a salt solution containing DMSO (20% DMSO, 0.25 Na-EDTA, saturated with NaCl). We extracted DNA using QIAamp DNA Mini kit (blood samples) or DNeasy Tissue kit (tissue samples) (QIAGEN Inc., Valencia, CA) following the manufacturer protocol but we resuspended the DNA in 35–70 μl of double distilled water.

Mitochondrial and nuclear DNA sequence

We initially amplified a ND2 mtDNA fragment (1094 bp) using universal primers L5219-met-F and H6313-trp-R (Sorenson et al. 1999). We subsequently designed internal species-specific ND2 primers (PoAu2F 5′-AGCAATCACTGGATCATAGC-3′ and PoAu2R 5′-TGGGGATAGAGG-

Table 1. Summary of genetic polymorphism in each sampling site for (a) mtDNA; (b) enolase intron; and (c) AFLP

Groups	Western Canada					QC	IC
Site	AB	BC	MA	NWT	YU		
(a) mt-DNA							
n	16	20	18	20	18	15	21
# Haplotypes	5	9	7	8	4	3	2
p (times 100)	0.071	0.120	0.098	0.089	0.038	0.073	0.059
H	0.450	0.705	0.634	0.590	0.314	0.562	0.524
(b) Intron							
n	13	13	12	14	11	15	20
# Alleles	3	4	4	4	3	4	4
H_e	0.557	0.483	0.649	0.564	0.437	0.582	0.476
H_o	0.769	0.539	0.583	0.643	0.455	0.733	0.400
(c) AFLP							
n	13	11	10	14	12	15	15
H	0.333	0.337	0.392	0.347	0.322	0.370	0.290

For mtDNA, values are given for the number of birds genotyped (n), number of haplotypes observed (# haplotypes), nucleotide diversity (p), and haplotype diversity (H). For enolase intron, values are number of birds genotyped (n), number of different alleles observed (# alleles), gene diversity (H_e) and observed heterozygosity (H_o). For AFLP, values reported are number of birds genotyped (n), and Nei's gene diversity (H). Abbreviations are: AB: Alberta, BC: British Columbia, IC: Iceland, MA: Manitoba, NWT: Northwest Territories, QC: Québec, and YU: Yukon.

TAGTAGTAGG-3′) that amplified a 885 bp fragment and trimmed all sequences to that size. Using these two sets of primers, we obtained mtDNA ND2 sequences for 128 horned grebes, as well as for two outgroup species: one eared grebe (*Podiceps nigricollis*) and one red-necked grebe (*Podiceps grisegena*). Depending on the primer set, we amplified the ND2 region using the polymerase chain reaction (PCR) in: (1) 50 μl reactions with 1× PCR buffer (10 mM Tris–HCL [pH 9.O], 1.5 mM $MgCl_2$, 0.1% Triton X-100, 50 mM KCL), 320 μM of dNTPs, 1 U *Taq* polymerase, 20 ng of DNA and 1 μmol each of L5219-met-F and H6313-trp-R; 2) 30 μl reactions with 1× PCR buffer, 270 μM of dNTPs, 0.5 U *Taq* polymerase, 50 ng of DNA and 230 pmol each of PoAu2-F and PoAu2-R. Fragments were amplified as follows: initial denaturation at 95 °C (5 min); 35 cycles of 95 °C for 1 min, 62 °C (L5219-met-F and H6313-trp-R) or 56 °C (PoAu2F and PoAu2R) for 1 min, 72 °C for 1 min; terminal extension of 72 °C for 10 min. For the intron α-enolase, we directly amplified a 312 bp sequence of using the primers EnolaseL731 and EnolaseH912 in a subset of horned grebe samples (n = 98, see Table 1) following Friesen et al. (1997).

Both mitochondrial and nuclear products were resolved on 1.2% agarose gels and purified using the QIAquick Gel Extraction kit (QIAGEN Inc., Valencia, CA). Fragments were then sequenced with a BigDye terminator cycle sequencing kit on an ABI 3100 automated analyzer (Applied Biosystems Inc., Foster City, CA). We aligned sequences manually with the BIOEDIT 5.0.9 software (Hall 1999) and collapsed the sequences into unique haplotypes using the TCS 1.13 software (Clement et al. 2000). Aligned sequences are available on GenBank, accession numbers DQ157674 to DQ157692.

Amplified fragment length polymorphism (AFLP)

We amplified AFLP loci in 90 horned grebes following the protocol developed by Vos et al. (1995) with slight modifications. Essentially, we digested 15 ng of high molecular weight DNA with restriction enzymes *Eco*RI and *Mse*I and we ligated the resulting fragments with adaptors. We then amplified fragments via PCR reaction using the preselective primers *Eco*RI-A and *Mse*I-C that annealed to the ligated fragments. We re-amplified a subset of the fragments obtained from the first PCR using selective primers (*Eco*RI-ACA and *Mse*I-CCG; *Eco*RI-ACT and *Mse*I-CCC; *Eco*RI-AAG and *Mse*I-CCG, which provided clear and variable profiles in preliminary tests, M. Boulet, unpubl. data). We separated the AFLP fragments on a BaseStation analyzer and analyzed them using

Cartographer 1.2.6 software (previously MJ Research Inc., South San Francisco, CA, now Bio-Rad, Hercules, CA). We retained 25 clear polymorphic loci for the genetic analyses and scored fragments by eye as being present or absent on the gels.

Genetic diversity

For sequence data, we calculated nucleotide diversity (p) and haplotype diversity (H) in mtDNA, gene diversity (H_e) and observed heterozygozity (H_o) in α-enolase intron using the program Arlequin 2.000 (Schneider et al. 2000). The program AFLP-SURV 1.0 provided estimates of Nei's gene diversity (H) for the AFLP data (Vekemans 2002). Using Arlequin 2.000, we also verified departures from Hardy–Weinberg equilibrium in the α-enolase sequences and tested for population expansion employing Tajima's D statistic (Tajima 1989).

Phylogenetic network

We used two techniques to assess phylogenetic relationships among mitochondrial haplotypes and detect monophyletic groups within the species. First, we identified the appropriate model of evolution of the sequence (HKY85 + gamma) using the program MODELTEST 3.06 (Posada and Crandall 1998) and then conducted a Bayesian phylogenetic analysis with the selected model in Mr.Bayes 3.0 (Huelsenbeck 2000). We included two grebe species as outgroups (see mitochondrial and nuclear DNA sequence). Second, we restricted our analysis to horned grebe sequences and generated a minimum spanning network presenting the intraspecific relationships among sequences using TCS 1.13 (Clement et al. 2000).

Population structure

For sequence data, we performed global and hierarchical analyses of molecular variance to test for the significance of two factors in delineating populations: (1) subspecies designations by comparing Iceland versus all other sites; (2) geographic disjunctions by comparing Iceland versus Québec versus all western Canada sites using the program Arlequin 2.000 (Schneider et al. 2000). We computed pairwise comparisons of population differentiation using sequential Bonferroni adjustments (Rice 1989). We also calculated and estimator of population divergence, $G_{ST}(k)$, for each population based on frequency data in CONTRIB (Petit et al. 1998). Each $G_{ST}(k)$ value is a relative measure of differentiation of a given population k versus all other populations.

For AFLP data, we performed a global analysis of molecular variance using the program AFLP-SURV 1.0 (Vekemans 2002). We estimated genotype frequencies using a Bayesian method because AFLPs are dominant markers and heterozygote individuals cannot be distinguished from dominant individuals at a given locus (Zhivotovsky 1999). Population pairwise F_{ST} estimates between sites were calculated in the same software using 1000 random permutations to assess significance with $\alpha = 0.05$ (Vekemans 2002).

Population assignment and demographic connectivity

We evaluated the level of demographic connectivity between groups (4, see Results) that emerged from population structure analyses using AFLPOP 1.1 (Duchesne and Bernatchez 2000). This program estimates the likelihood that particular individuals belong to a given group. The procedure was composed of three steps: (1) simulation of 500 genotypes to quantify the assignment power based on the allelic frequencies; (2) re-allocation of real individuals to geographical groups to identify individuals that were more likely coming from other locales than the locales where they were caught, (3) exclusion of individuals from populations at $P = 0.01$ to provide a measure of confidence associated with individual assignments (Duchesne and Bernatchez 2002; Manel et al. 2005). We used a log-likelihood threshold value of 1.3 for assignments, meaning individuals were assigned if they had at least 20 times more chances of coming from one group than another. This value approximately corresponds to an $\alpha = 0.05$. We used the 25 AFLP loci plus the mtDNA marker coded as a single binary mitochondria locus, where individuals having the most frequent haplotype observed in Québec (HOGR01) were scored with "1s" and all other individuals were scored with "0s". The α-enolase locus was not included in this analysis because it did not provide sufficient assignment information in preliminary analyses (but see Results).

Results

Genetic diversity and demography

We identified 19 haplotypes in the 885 bp mtDNA fragment distinguished by 16 polymorphic sites. Haplotype diversity showed a wide range of values, from 0.314 (Yukon) to 0.705 (British Columbia) and nucleotide diversity was very low overall, ranging from 0.000377 (Yukon) to 0.001195 (British Columbia, Table 1). Iceland had the fewest haplotypes (n = 2) while British Columbia harboured the highest number of haplotypes (n = 9, Table 1). In this marker, all western populations showed evidence of past population expansion (Tajima D statistics all < -1.6, *P*s all < 0.05) whereas the Québec and Iceland populations showed no evidence for such expansion (Québec: Tajima D statistics = 0.13, $P = 0.64$; Iceland: Tajima D statistics = 1.6, $P = 0.77$).

We found seven alleles in the α-enolase intron and four polymorphic sites. Global gene diversity was 0.535, very similar to the mean haplotype diversity observed in mtDNA (0.540). However, gene diversity showed less variation among populations than in mtDNA (min: 0.437 in Yukon, max: 0.649 in Manitoba) and the number of alleles per population did not differ among sites (n = 3 or in all sites, see Table 1). Observed mean heterozygosity was 0.589 and ranged from 0.400 in Iceland to 0.769 in Alberta. Only the Icelandic population showed a significant deviation from the Hardy–Weinberg equilibrium ($P = 0.01$). For AFLPs, estimates of gene diversity of each population did not depart very much from the mean gene diversity within population of 0.342 (minimum value = 0.290 in Iceland, maximum value = 0.392 in Manitoba, Table 1).

Phylogenetic analyses

The 19 horned grebe haplotypes were weakly differentiated from each other and there was no evidence of reciprocal monophyly among regional groups (tree not showed). Two small clades had high support (81 and 86 %) and connected haplotypes mostly confined in Western populations: HOGR07 (found in Northwest Territories, Yukon and British Columbia) with HOGRO5 (Northwest Territories) and HOGR09 (Alberta, British Columbia and Manitoba) with HOGR13 (Iceland, Manitoba, Northwest Territories and Yukon). The minimum spanning-network exhibited a typical star-like shape, without clear regional subgroups of haplotypes (Figure 1).The central haplotype HOGR02 was present in every population sampled. Overall, distances among haplotypes were very small as the most differentiated haplotypes were only two mutations away from HOGR02 (Figure 1).

Population structure

For mtDNA, three haplotypes were particularly abundant: HOGR02 (n = 78/128), HOGR01 (n = 15/128), and HOGR13 (n = 13/128, Figure 2). Three more haplotypes were present in at least two populations (HOGRO4, HOGRO7, HOGRO9, n = 3 each), while 13 haplotypes were private (Figure 2). The G_{ST} estimates obtained from haplotype frequencies identified Québec as the most divergent population (G_{ST}QC = 0.268), followed by Iceland (G_{ST}IC = 0.194), This pattern was mainly due to the differential abundance of HOGR02 between Western Canada and Iceland, of HOGRO1 between Québec and the other groups, and finally of HOGR13 between Iceland and the other groups.

We found a moderate but significant level of genetic differentiation in mtDNA that was well partitioned between subspecies (among group variance: 15.7%, see Tables 2 and 3) but even more so between geographic disjunctions (among group variance: 25.6%, see Tables 2 and 3). Pairwise F_{ST} values involving QC or IC were highest and generally significant after Bonferroni adjustments (range of F_{ST} values = 0.15–0.49, Table 3). In contrast, there was no significant genetic differentiation between western sites (F_{ST} values all close to 0.0, *Ps* all > 0.05, Table 3).

For the α-enolase intron, two alleles were predominant and present in every population: A01 (n = 117/196) and A02 (n = 63/196). Alleles A04, A05 and A06 occurred in three populations each, while A03 and A07 were private alleles (Figure 3). The G_{ST} estimates of differentiation of each population versus all other populations revealed the same pattern as recovered by mtDNA: Québec and Iceland had the highest G_{ST} values (G_{ST}QC = 0.028 and G_{ST}IC = 0.027). There was no evidence of genetic differentiation based on subspecies or geographic disjunctions (*P*s < 0.05,

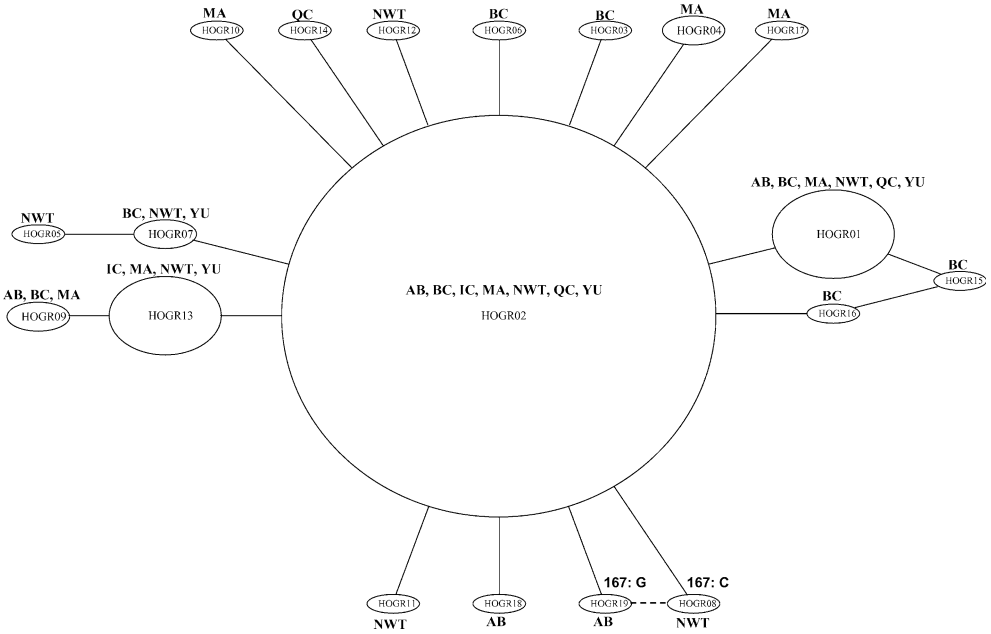

Figure 1. Minimum spanning-network showing the relationships among the 19 horned grebe haplotypes in mtDNA ND2 fragment. Each line connecting haplotypes (ovals) represent a single mutation. Ovals are proportional to haplotype frequency. Two different mutations occurred at locus 167 (HOGR08: A–C; HOGR19: A–G).

Tables 2 and 3). Thus, none of the pairwise comparisons between western sites were significant (all $F_{ST} < 0.0$, Ps > 0.05, Table 3).

In AFLPs, global among sites was weak but significant ($F_{ST} = 0.040$, $P < 0.0001$). This was mainly due to a significant differentiation between Iceland and every other sites including Québec ($F_{ST} = 0.11$) and British Columbia ($F_{ST} = 0.10$, Table 3). In addition, Québec and British Columbia were significantly distinct from three

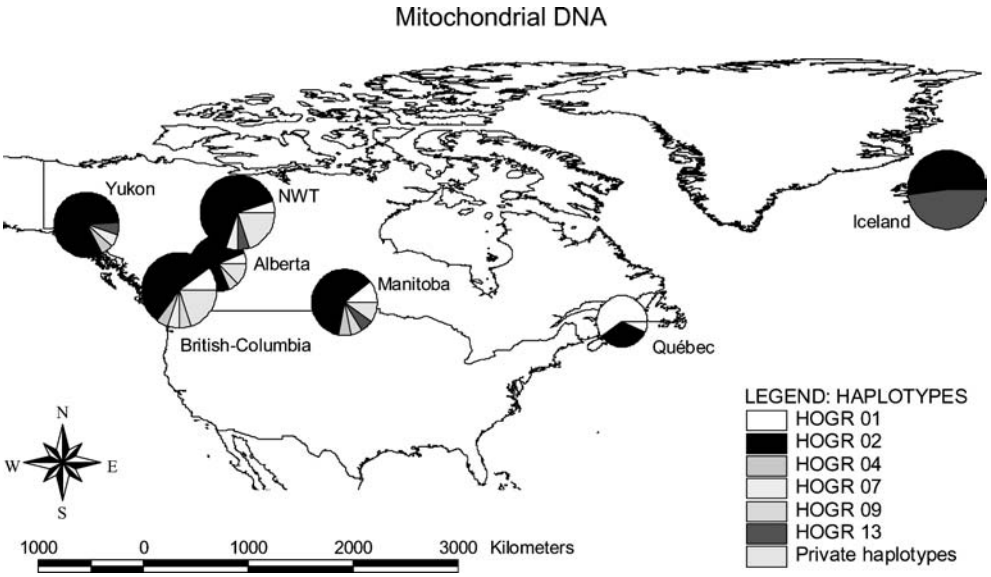

Figure 2. Geographic distribution of horned grebe haplotypes (HOGR). Circles are proportional to the number of samples in a given site. Private haplotypes (n = 13) refer to haplotypes observed in a single population. Refer to electronic version for coloured map.

Table 2. Hierarchical analyses of molecular variance in ND2 mtDNA and a-enolase intron 2 fragments

Inferences	Groups	Variance	% Total	Fixation index, *P* values
ND2 mtDNA				
Global	[IC, QC, MB, AB,	AP	11.9%	$F=0.14$, $P<0.001$
	NWT, YU, BC]	WP	88.1%	
Subspecies	[IC] [QC, MB, AB,	AG	15.7%	$F=0.157$, $P=0.15$
	NWT, YU, BC]	AP/WG	7.7%	$F=0.091$, $P<0.001$
		WP	76.6%	$F=0.230$, $P<0.001$
Geographic disjunction	[IC] [QC] [MB, AB,	AG	25.6%	$F=0.256$, $P<0.001$
	NWT, YU, BC]	AP/WG	−0.8%	$F=-0.010$, $P=0.86$
		WP	75.2%	$F=0.248$, $P<0.001$
α-enolase				
Global	[IC, QC, MB, AB,	AP	0.6%	$F=0.01$, $P=0.30$
	NWT, YU, BC]	WP	99.4%	
Subspecies	[IC, QC, MB, AB,	AG	0.8%	$F=0.008$, $P=0.29$
	NWT, YU, BC]	AP/WG	0.3%	$F=0.003$, $P=0.62$
		WP	99.0%	$F=0.010$, $P=0.32$
Geographic disjunction	[IC] [QC] [MB, AB,	AG	0.9%	$F=0.009$, $P=0.30$
	NWT, YU, BC]	AP/WG	0.1%	$F=0.000$, $P=0.28$
		WP	99.1%	$F=0.009$, $P=0.31$

AP = among population; WP = within populations; AG = among groups; AP/AG = among populations within groups.

North American sites each (Table 3). In summary, the AFLP data suggested four groups: Iceland, Québec, British Columbia, and the west central sites (Manitoba, Yukon, Northwest Territories, Alberta).

Population assignment and demographic connectivity

Based on evidence of population structure at mtDNA and AFLP markers, we assessed demographic

Table 3. Pairwise F_{ST} estimates between grebe populations at three markers (mtDNA, enolase intron, and 25 AFLP loci)

	AB	BC	MB	NWT	YU	QC
BC (mt)	−0.02					
(enol)	0.00	–				
(AFLP)	0.03					
MB (mt)	−0.03	−0.02				
(enol)	−0.03	0.01	–			
(AFLP)	−0.02	0.04*				
NWT (mt)	−0.02	0.00	0.00			
(enol)	−0.03	−0.02	−0.02			
(AFLP)	0.02	0.05*	0.03			
YU (mt)	−0.01	0.00	−0.01	−0.02		
(enol)	0.06	−0.02	0.06	0.01−	−	
(AFLP)	0.00	0.03	0.01	−0.01		
QC (mt)	0.30*	0.17**	0.22**	0.28*	0.42*	
(enol)	−0.03	0.03	−0.03	−0.01	0.09	–
(AFLP)	0.04*	0.04*	0.02	0.05*	0.01	
IC (mt)	0.22*	0.19*	0.15*	0.21*	0.27*	0.49*
(enol)	0.03	−0.02	0.02	−0.01	−0.02	0.05
(AFLP)	0.07*	0.10*	0.08*	0.08*	0.04*	0.11*

The symbol *refers to significant differences after Bonferoni adjustments (mtDNA) or after 1000 random randomisations (AFLP) and **refers to *P* values <0.05 but not significant after adjustments (mtDNA).

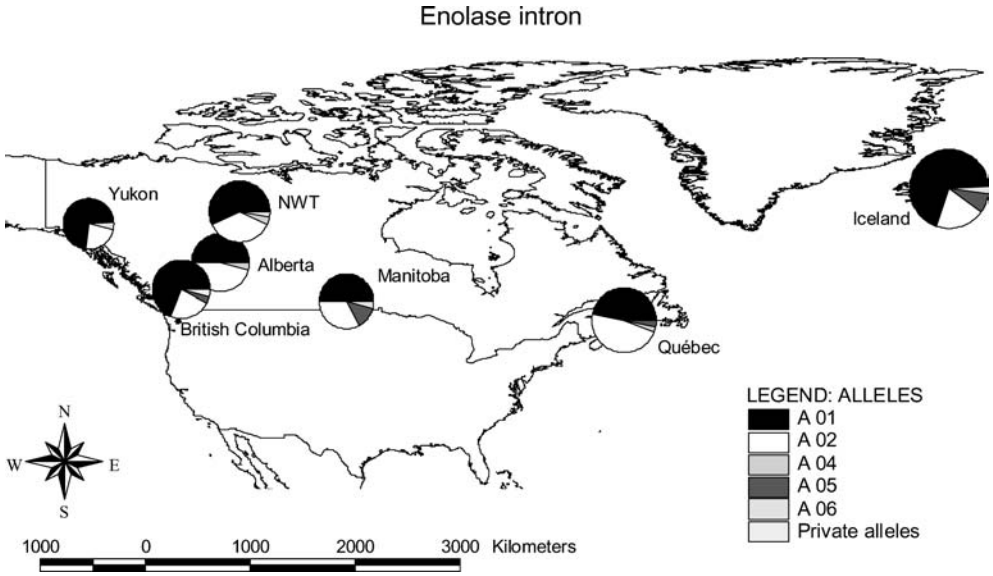

Figure 3. Geographic distribution of horned grebe enolase alleles (A). Circles are proportional to the number of samples in a given site. Private alleles (n = 2) refer to alleles that were observed in a single population. Refer to electronic version for coloured map.

connectivity between four main regions listed above to determine whether the Magdalen Island population was exchanging individuals with North American or Icelandic populations. Overall, 56.4% of the simulated genotypes could be assigned to Québec, 64.6% to Iceland, 32.2% to the west central group and 31.4% to British Columbia, which indicates a limited power of assignation, and particularly so for the westernmost sites. For the assignment of real individuals, 53.3% (8/15) of Québec, 60.0% (9/15) of Iceland, 33.3% (15/45) of the west central, and 30.0% (3/10) of British Columbia individuals were assigned to one of the four groups. There was no evidence of strong immigration into the Magdalen Island population: of the eight Magdalen Island grebes that could be assigned, all but one (QC22) were allocated to this population (Table 4). This assignment was probably the result of the haplotype coding rule, because individual QC22 had a private haplotype from Québec (HOGR14) that was coded as "0" in the AFLPOP analysis.

There was no evidence of demographic connectivity between Québec and Iceland: all assignations involving Iceland birds confirmed their Icelandic origin and all Quebec birds were excluded from Iceland (Table 4). In contrast, we recorded possible demographic connectivity between Québec and west North America. In west central sites, while 13 birds were apparently truly from one of the west central sites, two were more likely from Québec (Table 4). In addition, four of the Québec could not be excluded from British Columbia and ten could be not excluded from west central Canada (Table 4). However, with the limited power of assignation in the west we cannot rule out the presence of wrong assignments between these areas. In brief, our results suggest that if immigration does occur within the Magdalen Islands, it is more likely with the west central populations than with Iceland.

Discussion

The Magdalen Islands population does not show signs of extreme reduction in genetic diversity at any of the markers and still retains a comparable amount of genetic diversity to the average diversity observed across all populations at mtDNA and nuclear markers. Second, while the Magdalen Islands population is not phylogeographically distinct from other populations, it definitely shows population differentiation at mtDNA and AFLP markers when compared to western North American populations. Below, we discuss the implications of these results for the conservation of the Magdalen Islands horned grebe population.

Table 4. Population assignment of Québec individuals and mis-assigned western individuals based on a threshold log-likelihood difference ≥1.3 and corresponding exclusion from groups

Individuals	Group assignment	Groups excluded ($P = 0.01$)
QC01	None (0.75)	Iceland
QC02	None (0.10)	British Columbia, Iceland
QC03	None (0.90)	Iceland
QC04	None (0.04)	Iceland
QC05	Québec (4.76)	All except Québec
QC07	None (0.37)	Iceland
QC10	Québec (2.29)	All except Québec
QC11	Québec (1.71)	British Columbia, Iceland
QC12	Québec (2.29)	British Columbia, Iceland
QC14	None (0.10)	British Columbia, Iceland
QC15	Québec (5.22)	All except Québec
QC21	Québec (1.71)	All except Québec
QC22	West central (2.50)	British Columbia, Iceland
QC29	None (1.13)	British Columbia, Iceland
QC37	Québec (1.42)	All except Québec
AB01	British Columbia (2.87)	Iceland, Québec
BC09	Iceland (1.84)	Québec
MA11	Québec (2.12)	British Columbia, Iceland
NW87	Québec (1.36)	British Columbia, Iceland

Values of the log-likelihood difference are indicated in parenthesis.

Genetic diversity

On the Magdalen Islands, the low hatching success of eggs, the high mortality in young, and the reduced number of adults raised concerns about level of genetic diversity still present in the population, in the event that a lack of reduced genetic variation leads to inbreeding depression (Shaffer et al. 1994). Yet, the genetic diversity in the Québec population was fairly comparable to the average diversity across all populations in all three markers. We therefore conclude that the recent decline in the population has not reduced its genetic diversity very much at least at neutral markers and at one enzymatic gene. However, the population is currently so small that it may in fact suffer from inbreeding depression, which may not have been detected with the markers used due to limited genome coverage and possible retention of ancestral polymorphism (Bulgin et al. 2003). Alternatively, this diversity may be restored or maintained through the immigration of a few individuals wintering in the Maritimes or the East Coast but breeding in central/western North America. This is suggested by the non exclusion of all Québec birds from British Columbia and the west central populations in particular.

Population structure and origin

We found that the Icelandic and North American horned grebes, including those from the Magdalen Islands, form a single phylogenetic group. Yet the horned grebe showed significant global population differentiation at the mitochondrial ($F_{ST} = 0.14$) and AFLPs ($F_{ST} = 0.04$) markers. These results contrast with the absence of structure observed in the intron where $F_{ST} = 0.01$. In ducks and other vertebrates, the gene α-enolase encodes for important structural proteins: the enzyme α-enolase which is involved in skeletal myogenesis, and tau-crystallin, a structural lens protein (Wistow et al. 1988, http://www.ncbi.nlm.nih.gov/gquery/gquery.fcgi). Because introns are strongly linked to exons, the α-enolase intron may be under stabilising selection and represents a single locus, whereas the AFLPs include several loci throughout the genome that may be neutral or under different types of selection.

The star-shaped phylogenetic network suggested that an expansion had occurred in the North American range of the species (Slatkin and Hudson 1991), and this was conformed by highly negative values in Tajima D statistics in the west. The historical records refer to an expansion of the breeding

range into new areas at the beginning of the 20th century that included the Magdalen Islands around 1900's as well as Scotland around 1908 (Fjeldså 1973). Thus the Magdalen Island population may be of relatively recent origin, but the lack of resolution of the network and possible contemporary immigration does not provide us the power to determine the exact source population. Alternatively, this population could represent a relict of a population that used to breed on the exposed continental shelf during Pleistocene glaciations, as observed in the Newfoundland rock ptarmigan population (Holder et al. 1999, 2004), with possibly relatively recent gene flow from other areas.

The present-day winter distribution of the species provides a hypothetical ecological explanation about the possible origin of the Magdalen population. About 50% of horned grebes from western North America migrate along the Mississippi and Atlantic flyways to reach the Atlantic coasts for the wintering season (Fjeldså and 1973; Root 1988; Sauer et al. 1996; Stedman 2000). For these individuals, an establishment on the Magdalen islands for the breeding season would represent a major advantage: it would shorten the migratory route and reduce the risks associated to migration such as predation, starvation and competition on stopovers. Grebe species in general are not particularly efficient at flying (Palmer 1962). Some horned grebes winter as north as the Atlantic coasts of Nova Scotia, New Brunswick, and Maine (Root 1988; Sauer et al. 1996). If the Magdalen Islands grebes use these wintering grounds, this would translate into a migration distance of about 400–800 km, as opposed to a migration distance of at least 2500 km for western birds migrating to the Atlantic coast (i.e., from Manitoba to the Atlantic coast).

Conservations implications

Presently, the conservation status of the Magdalen horned grebe population in Canada is "secure" due to the abundant populations in the western part of the country (Canadian Endangered Species Conservation Council 2001). In a management plan, it would thus be grouped with the western populations. Below, we discuss the conservation implications of our results in respect with the criteria for population enlisting under the newly adopted Canadian *Species at Risk Act* (*SARA*).

The SARA legislation allows status designation below the species level, i.e., it permits the listing of subspecies, varieties and "geographically or genetically distinct" populations in its definition of wildlife species (Committee on the status of endangered wildlife in Canada 2003). To qualify for enlisting, a designatable unit below the species level must conform to any of the following criteria, although the first criteria have more weight: (1) named subspecies or varieties based on faunal checklist such as the American Ornithologist Union checklist for birds; (2) units identified as genetically distinct based on appropriate inherited traits (morphological, life history, behaviour) and/or genetic markers; (3) units separated by major range disjunction such that dispersal of individuals between separated regions has been limited for an extended period of time and will remain restricted in the near future; (4) units identified as biogeographically distinct based on occupation of different eco-geographic regions that are relevant to the species and reflect historical or genetic distinction.

We showed that there were significant allele frequency differences in mtDNA and AFLP markers between the Magdalen Island population and Iceland as well as with most western populations. This type of population distinctiveness corresponds to the definition of a management unit (*sensu* Moritz et al. 1995). Specifically, a management unit (MU) is a demographically independent set of population identified to help short-term management of larger entities. Thus the Magdalen Island population qualifies for *SARA* enlisting under genetic criteria. On the ecological side, we currently do not know whether the Magdalen Island population is distinct from the western populations based on criteria such as life history traits and heritable morphological traits. However, the extent of migratory behaviour and the migratory direction are partly controlled by a few genes (Berthold 2001). We suspect the Magdalen Island population winters along the Atlantic coast (for example, in Nova Scotia, New Brunswick or New England) and if so, it would be distinct based on a heritable behaviour (different migration pathways, abbreviated migration). The same conclusion would apply if this population winters along the Pacific coast (which is unlikely) or along the

Greenland coasts: in any cases, the overall migratory pathway would be distinct.

Finally, the Magdalen Island breeding population clearly appears as being geographically isolated from any other populations by thousands of kilometres. In addition, it is not demographically connected with Iceland. None of the Québec birds with the exception of QC22 with the private Québec haplotype were assigned to western populations. The nonexclusion of some Québec birds from the western sites and the assignation of two west birds to Québec suggest possible genetic exchanges between these disjunct areas that may be essential to demographically sustain the population on a short-term basis. Because the level of differentiation recorded by mtDNA was higher than the predicted ''four times rule of thumb'' based on mutation-drift equilibrium assumptions (Mills and Allendorf 1996), it is possible that the immigration events are mostly mediated via western males, as observed in some other birds species with male-biased dispersal (Greenwood 1980; Clarke et al. 1997). The number of female immigrants per generation Nm_f derived from $F_{ST} = 1/(4Nm + 1)$ (Wright 1931) for every pairwise Québec comparisons approached the general rule of thumb of one immigrant per generation to prevent and minimize the loss of polymorphism and heterozygosity within subpopulations ($Nm = 0.3$–1.2).

In conclusion, we suggest that the Magdalen Island population warrants the status of a conservation unit below the species level under the Canadian *SARA* based on criteria 2 and 3. The few occurrences of breeding pairs in other areas of eastern North America suggest that successful long-term establishments are extremely rare and the population is at high risk of extinction. Indeed, why there are no horned grebes elsewhere than on the Madgdalen Islands in eastern North America (ex.: pounds in mainland Québec and Ontario, where presumably suitable habitats are available) is still a mystery. At this time, the population is at high risk of extinction and immediate efforts should be targeted to increase the productivity of adults, which are particularly low: 0.1–1.1 fledging/pair depending on chick age and year (see Shaffer and Laporte 2003). The exact causes of the horned grebe decline on the Magdalen Islands are unknown but we suspect that predation on nests and adults, destruction of nests after water floods, intoxication of individuals by lead, and competition with the aggressive and newly established pied-billed grebe (*Podilymbus podiceps*) play a role (Shaffer and Laporte 2003).

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References

Avise JC (2000) *Phylogeography*, Harvard University Press, Cambridge, Massachusetts.

Bernatchez L, Wilson CC (1998) Comparative phylogeography of nearctic and palearctic freshwater fishes. *Mol. Ecol.*, **7**, 431–452.

Berthold P (2001) *Bird Migration: A General Survey*, 2nd edn. Oxford University Press, London, UK.

Bulgin NL, Gibbs HL, Vickery P, Baker AJ (2003) Ancestral polymorphisms in genetic markers obscure detection of evolutionary distinct populations in the endangered Florida grasshopper sparrow (*Ammodramus savannarum floridanus*). *Mol. Ecol.*, **12**, 831–844.

Canadian Endangered Species Conservation Council (2001) *Wild Species 2000: The General Status of Species in Canada. Version 1.*, Minister of Public Works and Government Services Canada, Ottawa, Ontario.

Clarke AL, Saether B-E, Roskaft E (1997) Sex biases in avian dispersal: A reappraisal. *Oikos*, **79**, 429438.

Clement M, Posada D, Crandall KA (2000) TCS: A computer program to estimate gene genealogies. *Mol. Ecol.*, **9**, 1657–1659.

Committee on the status of endangered wildlife in Canada (2003) *Cosewic's Assessment Process and Criteria*, COSEWIC, Ottawa, Ontario.

Crandall KA, Bininda-Emonds O, Mace G, Wayne RK (2000) Considering evolutionary processes in conservation biology. *Trends Ecol. Evol.*, **15**, 290–295.

Duchesne P, Bematchez L (2002) AFLPOP: A computer program for simulated and real population allocation based on AFLP data. *Mol. Ecol. Notes*, **3**, 380–383.

Fjeldså J (1973) Distribution and geographical variation of the horned grebe *Podiceps auritus* (Linnaeus, 1758) *Ornis Scandinavica*, **4**, 55–86.

Flagstad Ø, Røed KH (2003) Refugial origins of reindeer (*Rangifer tarandus* L.) inferred from mitochondrial DNA sequences. *Evolution*, **57**, 658–670.

Fradette P (1992) *Les oiseaux des Îles-de-la-Madeleine: Population et sites d'observation*. Attention Frag' Îles.

Frankham R, Ballou JD, Briscoe DA (2002) *Introduction to Conservation Genetics*, Cambridge University Press, Cambridge, UK.

Fraser DJ, Bernatchez L (2001) Adaptative evolutionary conservation: Towards a unified concept for defining conservation units. *Mol. Ecol.*, **10**, 2741–2752.

Friesen VL, Congdon BC, Walsh HE, Birt TP (1997) Intron variation in marbled murrelets detected using analyses of single-stranded conformational polymorphisms. *Mol. Ecol.*, **6**, 1047–1058.

Gazette officielle du Québec (2000) Règlement sur les espèces fauniques menacées ou vulnérables *Lois et Règlements Partie 2,132 année, 12 avril 2000*, **44**, 2404–2405.

Greenwood PJ (1980) Mating systems, philopatry, and dispersal among birds and mammals *Anim. Behav.*, **28**, 1140–1162.

Hagar JA (1956) Birds seen at Magdalens in 1956. In: *Manuscript*, p. 15.

Hall TA (1999) BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT *Nucleic Acids Symp. Ser.*, **41**, 95–98.

Hedrick PW (2001) Conservation genetics: Where are we now? *Trends Ecol. Evol.*, **16**, 629–636.

Holder KR, Mongomerie R, Friesen VL (1999) A test of the glacial refugium hypothesis using patterns of mitochondrial and nuclear DNA sequence variation in Rock ptarmigan (Lagopus mutus). *Evolution*, **53**, 1936–1950.

Holder KR, Montgomerie RA, Friesen VL (2004) Genetic diversity and management of Nearctic rock ptarmigan (*Lagopus mutus*). *Can. J. Zool.*, **82**, 564–575.

Huelsenbeck JP (2000) *MrBayes 3.0: Bayesian Inferences of Phylogeny (Software)*, University of Rochester, Rochester, NY.

Manel S, Gaggiotti OE, Waples RS (2005) Assignment methods: matching biological questions with appropriate techniques. *Trends Ecol. Evol.*, **20**, 136–142.

Mills LS, Allendorf FW (1996) The one-migrant-per-generation rule in conservation and management. *Conserv. Biol.*, **10**, 1509–1518.

Moritz C, Lavery S, Slade R (1995) Using allele frequency and phylogeny to define units for conservation management. *Amer. Fisheries Soc. Symp.*, **17**, 249–262.

O'Donnel C, Fjeldså J (1997) *Grebes – Status Survey and Conservation Action Plan*, IUCN/SSC Grebe Specialist Group, Gland, Switzerland.

Palmer RS (1962) *Handbook of North American Bird Vol. 1 (Loons through Flamingos)*, Yale University Press, New Haven, CT.

Petit RJ, El Mousalik A, Pons O (1998) Identifying populations for conservation on the basis of genetic markers. *Conserv. Biol.*, **12**, 844–855.

Pielou EC (1991) *After the Ice Ages: The Return of Life to Glaciated North America*, The University of Chicago Press, Chicago, Illinois.

Posada D, Crandall KA (1998) Modeltest: Testing model of DNA substitution. *BioInformatics*, **14**, 817–818.

Rice WR (1989) Analysing tables of statistical tests *Evolution*, **43**, 223–225.

Root T (1988) *Atlas of Wintering North American Birds. An Analysis of Christmas Bird Count Data*, The University of Chicago Press, Chicago, Illinois.

Sauer JR, Schwartz S, Hoover B (1996) *The Christmas Bird Count Home Page. Version 95.1*, Patuxent Wildlife Research Center, Laurel, MD.

Schneider S, Roessli D, Excoffier L (2000) *Arlequin 2.000: A Software Package for Population Genetics*, Genetics and Biometry Laboratory, Department of Anthropology, University of Geneva, Geneva, Switzerland.

Seutin G, White BW, Boag PT (1991) Preservation of avian blood and tissue samples for DNA analyses. *Can. J. Zool.*, **69**, 82–90.

Shaffer F, Laporte P (2003) *Le Grèbe esclavon (Podiceps auritus) aux Îles-de-la-Madeleine: Population, nidification et habitat*, Service canadien de la faune, Direction de la conservation de l'environnement, Environnement Canada., Québec, Québec.

Shaffer F, Laporte P, Robert M (1994) *Plan d'action pour le rètablissement du Grébe cornu (Podiceps auritus) au Québec*, Service canadien de la faune, Direction de la conservation de l'environnement, Environnement Canada, Québec, Québec.

Slatkin M, Hudson RR (1991) Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations. *Genetics*, **129**, 555–562.

Sorenson MD, Ast JC, Dimcheff DE, Yuri T, Mindell DP (1999) Primers for a PCR-based approach to mitochondrial genome sequencing in birds and other vertebrates. *Mol. Phylogenet. Evol.*, **12**, 105–114.

Stedman SJ (2000) Horned grebe (*Podiceps auritus*) In: The Birds of North America, No. 5 (eds. Poole A, Gill F), pp. 1–28. The Birds of North America Inc., Philadelphia, PE.

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

Vekemans X, Beauwens T, Lemaire M, Roldan-Ruiz I (2002) Data from amplified fragment length polymorphism (AFLP) markers show indication of size homoplasy and of a relationship between degree of homoplasy and fragment size. *Mol. Ecol.*, **11**, 139–151.

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: A new technique for DNA fingerprinting. *Nucleic Acids Res.*, **23**, 4407–4414.

Wistow GJ, Lietman T, Williams LA, Stapel SO, de Jong WW, Horwitz J, Piatigorsky J (1988) Tau-crystallin/alpha-enolase: One gene encodes both an enzyme and a lens structural protein. *J. Cell Biol.*, **107**, 2729–2736.

Wright S (1931) Evolution in Mendelian populations *Genetics*, **16**, 97–259.

Young CJ (1897) Notes on the birds of the Magdalen Islands, PQ. *Ottawa Nat.*, **11**, 145–158.

Zhivotovsky LA (1999) Estimating population structure in diploids with multilocus DNA markers *Mol. Ecol.*, **8**, 907–913.