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SHORT COMMUNICATION

Conservation genetics of high-arctic Gull species at risk: I. Diversity in the mtDNA control region of circumpolar populations of the Endangered Ivory Gull (*Pagophila eburnea*)

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Abstract

The high-arctic Ivory Gull (*Pagophila eburnea*) has recently undergone a sharp decline in numbers, and in Canada it is listed as "Endangered" under the Species-At-Risk Act. To test for circumpolar genetic distinctiveness, we examined 264 bp of the mtDNA Control Region Domain I from 127 museum specimens collected during the breeding season from northern Canada, Greenland, and Norway, and during the non-breeding season from adjacent overwintering grounds in Canada, Greenland, and a disjunct area in Alaska adjacent to the Bering Sea. Partition of genetic variance according to various phylogeographic and breeding ground models indicates no strong population structure, except that Alaska birds are consistently differentiated from other locations, and there are significant temporal shifts in haplotype frequencies. The evidence suggests that Ivory Gulls in Canada, Greenland, and Norway are a single genetic entity, in contrast to Alaska birds, which may represent a distinctive Siberian population.

Keywords

Conservation genetics, COSEWIC, endangered species, ivory gull, mtDNA

History

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The cold coast of Greenland is barren and bare
No seedtime, no harvest are ever known there.
And the birds here sing sweetly in mountain and dale
But there's no bird in Greenland to sing to the whale
-Farewell to Tarwathie, trad

Introduction

The Canadian breeding population of Ivory Gulls, *Pagophila eburnea* (Phipps, 1774), underwent a precipitous decline from an estimated 1200 pairs in the 1980s to as few as 250 pairs in 2005 (Gilchrist & Mallory, 2005; Haney & McDonald, 1995; Renaud & McLaren, 1982), and they now breed only in northern Nunavut (Figure 1). Based on a projected decline to 200 birds by 2015, the species has been assessed as ''Endangered'' under the Species-At-Risk Act (SARA) by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC, 2006). Another high arctic gull species, Ross's Gull (*Rhodostethia rosea*), has been assessed as ''Threatened'', recognizing it as a rare species in Canada with small numbers of birds breeding at a few newly established colonies (Royston & Carr, 2015).

The global population of this circumpolar and high-arctic species appears also to be in decline from the previous estimate of 10,000 breeding birds by Vuilleumier (1995). The best estimate for northern and eastern Greenland showed 1800 birds, with decline in the south (Gilg et al., 2009). It is classified as Declining at Svalbard in Norway (Anker-Nilssen et al., 2000), and rare in the islands of the Kara Sea of Siberia (Zubakin, 1984). Colonies documented in the late nineteenth and early twentieth centuries at Franz Josef Land and Spitsbergen have either disappeared or been severely reduced (Bateson & Plowright, 1959; Birkenmajer, 1969; de Korte & Volkov, 1993; Haney & McDonald, 1995; Krajick, 2003; MacDonald, 1976; Volkov & de Korte, 1996).

Declines in Ivory Gull abundance have been attributed to several factors (Gilchrist & Mallory, 2005). Although now protected under SARA, they were formerly shot for food in Nunavut and off the northeast coast of Newfoundland, and bands recovered from shot birds show that they remain vulnerable to hunting pressure during migration to and from breeding grounds (Stenhouse et al., 2004). Intense and unregulated hunting still occurs in Greenland, and on the coast of eastern Siberia adjacent to the Bering Sea wintering grounds (Greg Robertson, personal communication). The species is strongly dependent on the sea ice: reduced ice on the breeding grounds is associated with lower reproductive success (Dalgety, 1932), whereas increased sea ice at the wintering grounds appears to have a negative impact on food availability (Krajick, 2003). Gilg et al. (2009) suggest that it may well become the first bird species to become extinct as a result of anthropogenic global warming.

We consider here the circumpolar population genetic structure of Ivory gulls, in particular those on their summer breeding grounds in high-arctic Canada and northern and eastern Greenland and adjacent wintering areas, as well as the Norway breeding grounds and the disjunct Alaska wintering ground. The species' geographic remoteness and conservation status preclude

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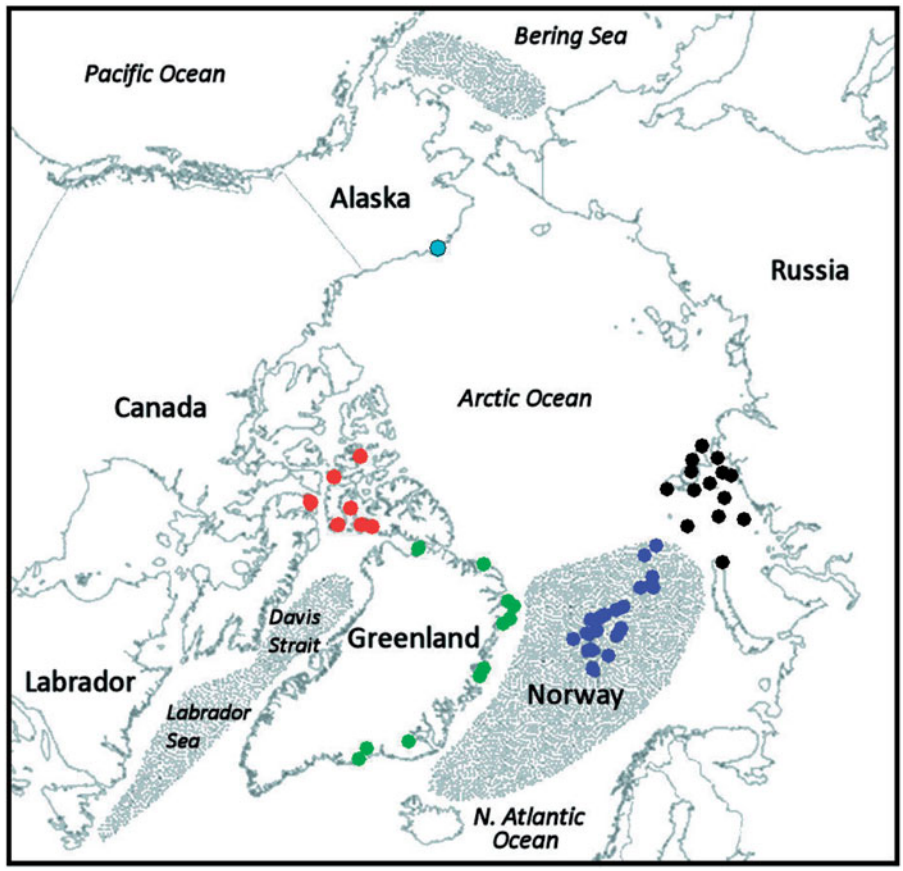

Figure 1. Circumpolar distribution of Ivory Gulls (redrawn from COSEWIC, 2006). Known breeding colonies in Canada, Greenland, Norway, and Siberia are shown as red, green, blue, and black dots, respectively; non-breeding areas in the Bering Sea, Greenland Sea, and Davis Strait/Labrador Sea are stippled. The majority of the Alaska non-breeding sample comes from Pt Barrow, AK (light blue dot).

live sample collection: we used tissue from museum material, an approach that is invaluable in avian conservation genetic studies (Cooper, 1994; Rocha et al., 2014; Royston & Carr, 2015; Vallianatos et al., 2002).

Methods

Samples

Details of location and date of collection for 127 museum specimens of Ivory Gulls are given in the Supplementary materials. Individual birds are designated as ''breeding'' if the specimen was collected at a known breeding colony during the summer breeding season (June–early September: Haney & McDonald, 1995), and as ''non-breeding'' if taken elsewhere, or at other times of the year.

DNA extraction, amplification, and sequencing

A small ($\sim$1 mm^2) piece of tissue was cut with a sterile razor blade from either a toe pad or skin sample. DNA was extracted from the tissue with the QIAamp DNA Mini Kit Tissue Protocol (Qiagen Inc., Clifton Hill, Cananda).

PCR was done with species-specific primers that amplified a region that included 264 bp from the Control Region (CR) Domain I of the mtDNA genome:

GullCR1-F 5′-CCT ACA CCC CTA GCC CAT CTT GCT CTT TTG-3′

GullCR1-R 5′-CCA GTT GTT TGG CAA AGT GCA TCA GTG AGG-3′

The PCR cycle comprised an initial denaturation at 90 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 2 min, annealing at 50 °C for 45 s, and extension at 72 °C for 5 min, and a further 10 min extension after the last cycle. PCR products were sequenced with the BigDye chemistry on an ABI 377 sequencer.

Population genetic analysis

Sequences were aligned and single nucleotide polymorphisms (SNPs) identified with Sequencher (GeneCodes, Ann Arbor, MI). Haplotype numbers, haplotype diversity (h), nucleotide diversity (p) (Nei, 1987), and the mean number of pairwise differences (k) (Tajima, 1983) were calculated following Nei (1987) as implemented in DnaSP version 4.0 (Omega Bio-Tek, Norcross, GA) (Rozas & Rozas, 1999).

Pairwise Φ_{ST} values among geographic locations were calculated with Arlequin 3.0c (Excoffier et al., 2005). To evaluate phylogeographic structure, five AMOVA partitions were assessed. For partitions III, IV, and V, the Alaskan non-breeding birds were included as a proxy representative of Siberian breeding birds.

(I) Six separate locations
(II) Two breeding populations: Western [Canada + Greenland] *versus* Eastern [Norway]
(III) Two breeding populations: as in II, with Eastern = [Norway + Alaska]
(IV) Three breeding populations: Western *versus* Eastern *versus* Alaska
(V) Two non-breeding locations: [Canada + Greenland] *versus* [Alaska]

Differences in haplotype frequencies were assessed by a modification of Fisher's Exact Test for a 2×3 contingency table (Freeman & Halton, 1951).

Results

CR sequence variation

The 264 bp region of CR Domain I included six polymorphic positions, including a single-base deletion in a run of 10 T bases, which was recoded as a SNP (cf. Pearce, 2006). The polymorphic sites defined seven haplotypes, Pe1–Pe7 (Figure 2 and Tables 1 and 2). Sequences for these haplotypes were submitted to

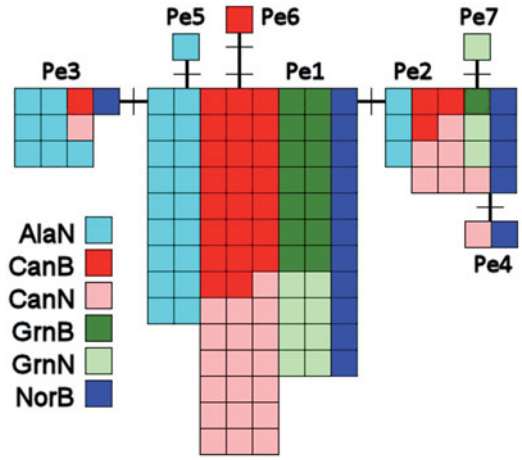

Figure 2. Minimum Spanning Network and geographic distribution of CR haplotypes in Ivory Gull. AlaN = Light Blue = Alaska non-breeding; CanB = Dark Red = Canada breeding; CanN = Light Red = Canada non-breeding; GrnB = Dark Green = Greenland breeding; GrnN = Light Green = Greenland non-breeding; NorB = Dark Blue = Norway breeding.

Table 1. Geographic distribution of Ivory Gull CR haplotypes.

Haplotype distribution	Pe1	Pe2	Pe3	Pe4	Pe5	Pe6	Pe7	Total
Alaska non-breeding	18	3	7	0	1	0	0	29
Canada breeding	23	3	1	0	0	1	0	28
Canada non-breeding	19	6	1	1	0	0	0	27
Greenland breeding	14	1	0	0	0	0	0	15
Greenland non-breeding	8	2	0	0	0	0	1	11
Norway breeding	11	4	1	1	0	0	0	17
Total	93	19	10	2	1	1	1	127

GenBank and assigned the accession numbers KP120957–KP120963, inclusive.

Geographic structure of CR variation

None of the samples from breeding areas are significantly differentiated by pairwise Φ_{ST} nor were breeding birds differentiated from non-breeding birds, either in Canada or in Greenland (Table 3). Alaska birds were differentiated from all other locations except Norway. More than 0.9 of the variance occurs among individuals in all models (Table 4). Among the AMOVA partitions, the highest Φ_{ST} value contrasts non-breeding Canada + Greenland versus Alaska birds ($\Phi_{ST} = 0.136$).

Temporal trends

Collectively, birds taken after 1934 had marginally greater diversity than those collected earlier ($\Phi_{ST} = 0.0218$, $p = 0.088$, ns) (Table 3). Proportions of the three major haplotypes were significantly different between pools of pre- and post-1934 birds (exact test, $p = 0.0046$), apparently in consequence of haplotype Pe3, which occurs almost exclusively in Alaska <1934 (exact test, $p = 0.00023$) (Table 5a and b). Analyzed separately, haplotype proportions in earlier and later Alaska samples do not differ significantly (Table 5c).

Discussion and conclusions

Ivory Gulls from the three breeding areas in Canada, Greenland, and Norway are not genetically differentiated, which suggests that they can be considered a single population for threat assessment. Greenland and Canadian birds can therefore provide a mutual ''rescue effect'' (COSEWIC, 2006). There are no significant differences between breeding and non-breeding samples either from Canada or Greenland, which suggests that the Labrador Sea/Davis Strait and Greenland Sea overwintering aggregations can be considered a part of the same gene pool.

Ivory Gulls from the Alaska wintering ground are consistently differentiated from other locations. Their ecology is poorly understood. Banding recoveries indicate that they are capable of long-distance movement to and from the Siberian breeding grounds in the Kara Sea (Salomonsen, 1967, 1979; Tomkovich, 1990). If Alaska non-breeding birds do in fact represent the Siberian population, the predominance of haplotype Pe3 in pre-1934 Alaska samples might indicate either a distinctive phylogeographic pattern or a species-wide temporal shift in haplotype frequencies.

Ivory Gull CR genotypes form a star-like phylogeny around a central, common Pe1 haplotype. This is typically taken as an indication of recent origin from a single bottlenecked population (Avise et al., 2000), and many arctic species including other larids seem to be characterized by weak structure due to establishment of colonies since the last glacial period, and/or to long-distance dispersal events (Moum & Arnason, 2001; Patirana et al., 2002). However, central ''stars'' in such phylogenies based on short mtDNA sequences have, upon dissection with complete mtDNA genomes, been shown to be paraphyletic assemblages of quite ancient lineages (Carr & Marshall, 2008; Carr et al., in review). For further investigation of their genetic structure, it would be useful to have a larger sample of the mtDNA genomes of these birds.

Table 2. Genetic diversity among Ivory Gull CR sequence by sampling location.

Origin and status	Individuals	Haplotypes	h	π	k
(a)					
Alaska non-breeding	29	3	0.589	0.00256	0.675
Canada breeding	28	4	0.345	0.00168	0.443
Canada non-breeding	27	4	0.470	0.00207	0.547
Greenland breeding	15	2	0.133	0.00051	0.133
Greenland non-breeding	11	3	0.472	0.00234	0.618
Norway breeding	17	4	0.551	0.00256	0.676
(b)					
<1934	40		0.413	0.00192	0.531
>1934	87		0.524	0.00233	0.614

Table 3. Pair-wise Φ_{ST} values for Ivory Gull CR sequence (above); p values (below).

	CanB	GrnB	NorB	CanN	GrnN	AlaN
CanB	–	0.0331	0.0234	0.0126	0.0111	0.0661
GrnB	ns	–	0.0706	0.0451	0.0676	0.0978
NorB	ns	ns	–	0.0464	0.0537	0.0722
CanN	ns	ns	ns	–	0.0463	0.0883
GrnN	ns	ns	ns	ns	–	0.0994
AlaN	*	*	ns	*	*	–

*$p < 0.05$. Abbreviations as in Figure 2.

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Table 4. Analysis of molecular variance (AMOVA) of Ivory Gull CR sequence data for five models of phylogeographic structure.

AMOVA model	Groups	Variance component	Variance fraction
I – Geographic groups	CanB, CanN, GrnB, GrnN, NorB, AlaN	Φ_{SC}: 0.0118 Φ_{ST}: 0.265	Φ_{SC}: 0.0427 Φ_{ST}: 0.957*
II – Western versus Eastern breeding	(1) CanB + GrnB (2) NorB	Φ_{CT}: 0.0194 Φ_{SC}: −0.00795 Φ_{ST}: 0.216	Φ_{CT}: 0.0851 Φ_{SC}: −0.0350 Φ_{ST}: 0.950
III – Western versus Eastern (inc Alaska)	(1) CanB + GrnB (2) NorB + AlaN	Φ_{CT}: 0.00722 Φ_{SC}: 0.0112 Φ_{ST}: 0.257	Φ_{CT}: 0.0262 Φ_{SC}: 0.0405 Φ_{ST}: 0.933*
IV – Western versus Eastern versus Alaska	(1) CanB + GrnB (2) NorB (3) AlaN	Φ_{CT}: 0.0305 Φ_{SC}: −0.0101 Φ_{ST}: 0.257	Φ_{CT}: 0.110 Φ_{SC}: −0.0364 Φ_{ST}: 0.927*
V – Western versus Eastern non-breeding	(1) CanN, GrnN (2) AlaN	Φ_{CT}: 0.0461 Φ_{SC}: −0.0141 Φ_{ST}: 0.307	Φ_{CT}: 0.136 Φ_{SC}: −0.0414 Φ_{ST}: 0.906*

*$p < 0.05$. Abbreviations: CanB, Canada breeding; CanN, Canada non-breeding; GrnB, Greenland breeding; GrnN, Greenland non-breeding; NorB, Norway breeding; AlaN, Alaska non-breeding; AMOVA partitions: CT, among groups; SC, among locations within groups; ST, within locations.

Table 5. 2×3 contingency tests for haplotype distribution between pre- and post-1934 birds (taken together, or Alaska only), and Alaska *versus* non-Alaska birds.

	Haplotypes		
	Pe1	Pe2	Pe3
(a) Total			
<1934	26	5	8
>1934	67	14	2
			$p = 0.0046$
(b) Total			
AlaN	17	3	8
not AlaN	76	16	2
			$p = 0.00023$
(c) AlaN			
<1934	13	3	8
>1934	4	0	0
			$p = 0.39$

Declaration of interest

The authors declare there are no competing relationships that could influence the authors' work. Data collection was supported by a Canadian Wildlife Service contract to SMC and IL Jones, and an NSERC Discovery Grant to S. M. C.

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Supplementary material available online

Supplementary material