

SHORT COMMUNICATION

**Conservation genetics of high-arctic Gull species at risk: II.
Diversity in the mtDNA control region of Threatened Ross's Gull
(*Rhodostethia rosea*)**

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Abstract

Ross's Gull (*Rhodostethia rosea*) is the rarest of Canadian high-arctic gulls, and is listed as Threatened under Canada's Species-At-Risk Act. The large majority of birds breed in Siberia: the origins and affinities of four extremely small breeding colonies observed since 1978 in the Canadian high arctic are unknown. We compared a 515-bp region of the mtDNA Control Region amplified from material in museum collections taken from non-breeding birds in Canada ($n=8$) and Alaska ($n=6$), the latter passage migrants from the Siberian populations. The Alaskan birds all have distinct haplotypes that differ by as many as six SNPs: Canadian birds taken in the vicinity of the breeding colonies show only two of these. We hypothesize the origins of the Canadian breeding colonies as recent founder events by small numbers of passage migrants from Siberia via Alaska. Ross's Gull maintains a very tenuous breeding presence in the Canadian high Arctic.

KeywordsConservation genetics, COSEWIC, mtDNA,
Ross's Gull, Threatened species**History**Received 7 August 2014
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Ross's Gull (*Rhodostethia rosea* (MacGillivray, 1842)) is the rarest species of gull breeding in North America, and in Canada is classified as Threatened by COSEWIC (Committee on the Status of Endangered Wildlife in Canada) due to its low population numbers and low productivity (Alvo et al., 1996). The species' holotype, a Canadian bird, was collected in June 1823 on the east coast of the Melville Peninsula in what is now Nunavut (Blomqvist & Elander, 1981). Breeding populations were first observed in 1978: only four Canadian breeding locations have been confirmed, at Churchill in Manitoba (Chartier & Cooke, 1980), and three locations in Nunavut, at the Cheyne Islands (MacDonald, 1978), Prince Charles Island (Bechet et al., 2000), and a small island east of Bathurst Island (Mallory et al., 2006) (Figure 1). It is possible that some sites remain undiscovered (Mallory et al., 2006). None of these locations includes more than five breeding pairs. Ross's Gull appears to have nested almost every year from 1980 to 1994 in Churchill and the Cheyne Islands; it is unknown if this involves return migrants (Alvo et al., 1996). Breeding success has historically been low, due to bad weather and predation by foxes, wolves, and other gulls (Densley, 1999). Disturbance of nest sites is an increasing problem, especially in Churchill (Alvo et al., 1996). The species is believed

never to have existed in large numbers in Canada (Alvo et al., 1996; Buturlin, 1906), and for this reason was assessed by COSEWIC as a rare Threatened species rather than as a formerly more abundant Endangered one, as is the case with the more numerous but now Endangered high-arctic Ivory Gull (Royston & Carr, 2015).

The main distribution of Ross's Gull is in northeastern Siberia, between the Chukotka and Taymyr Peninsulas (Zubakin & Avdanin, 1983; Zubakin et al., 1990). Estimates place numbers as high as 50–100,000 individuals (Alvo et al., 1996), and a survey of northern Yakutia indicates that the species is more widespread than previously assumed (CAFF, 2004). The highly productive polar ice that borders the Barents and Greenland Seas serves as an important summer feeding and molting area for non-breeding birds (Meltofte et al., 1981). Ross's Gulls seem to be the most common bird in the Central Arctic Ocean north of 85°N (Hjort et al., 1997).

Ross's gulls in Siberia breed in late summer. They undertake an annual post-breeding migration, first from the Chukchi Sea to the vicinity of Point Barrow, Alaska, and then to the Beaufort Sea. Population aggregations in the near-shore zone of Chukchi and Beaufort Seas are thought to include as many as 20–40,000 birds. A major conservation concern is the potential for devastation of this aggregation by pollution from nearby oil drilling (Alvo et al., 1996). After freeze-up in October, there is a return movement to the Chukchi Sea (Degtyarev et al., 1987; Divoky et al., 1988).

The origin and affinities of the Canadian breeding colonies of Ross's Gulls are unknown. Extremely low breeding numbers in Canada, and the conservation threats faced by the breeding

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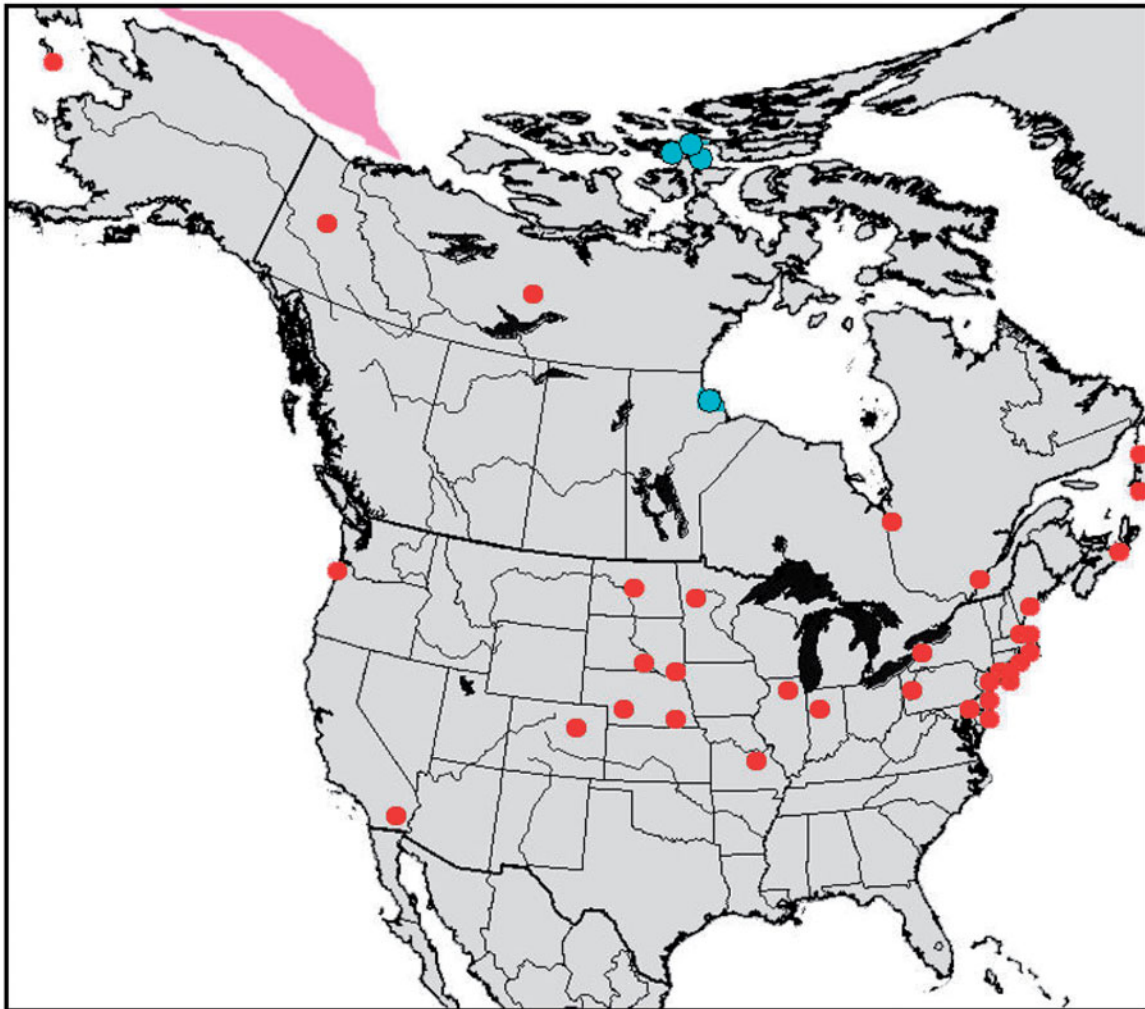


Figure 1. Distribution of Ross's Gull (*Rhodostethia rosea*) (after T. Sohl, based on Ridgely et al., 2003). Canadian breeding colonies are in light blue, Alaskan passage migration areas in pink, and vagrant sightings in red.

colonies, preclude collection or analysis of live birds. We instead made an analysis of material available in North American museum collections, which have been an effective resource for other avian conservation studies (Cooper, 1994; Freeland et al., 2006; Royston & Carr, 2015; cf. Rocha et al., 2014).

Methods

Samples and DNA extraction

We compared eight Canadian museum specimens collected 1974–1985, from Churchill, Manitoba, and Seymour I., Nunavut, with five Alaskan specimens collected at Point Barrow on the Bering Sea 1928–1931 and one in 1961 (Table 1). Based on the date of collection, none of the birds was taken during the species' breeding season: the Alaskan birds likely represent passage migrants from Siberian breeding populations. Specimens were obtained under CITES permit to SMC.

DNA was extracted from a $\sim 1\text{ mm}^2$ piece of the footpad from each sample with a QIAamp DNA Mini Kit Tissue Protocol (Qiagen Inc., Valencia, CA) according to the protocol of the manufacturer.

PCR amplification

Gull-specific oligonucleotide primers were designed for the Control Region (CR) based on the homologous region from the

Kelp Gull (*Larus dominicanus*) mtDNA genome sequence (Slack et al., 2007; GenBank accession NC_007006). The primer sequences are the followings:

GullCR#1F: 5'-TCAGCAACCCGGTGTAGG AAAGATCCTA CG-3'

GullCR#1R: 5'-ATCACGGTTAATCTTTTCAGTTAAACTTC C-3'

DNA was amplified in a total volume of 25 μL volume, including 10 μL of dH_2O , 2.5 μL of $10\times$ PCR buffer, 0.5 μL of dNTPs (20 mM), 0.5 μL of each primer (10 mM), and 0.2 μL (1 U) of Hot Start Taq polymerase (ProMega, Madison, WI), along with 10 μL of the DNA extract. Negative controls were done with 10 μL dH_2O . Amplifications were performed in an Eppendorf Mastercycler. After an initial 15 min incubation at 95 $^\circ\text{C}$ to activate the Taq polymerase activity, the PCR amplification cycle was denaturation for 45 s at 93 $^\circ\text{C}$, primer annealing for 35 s at 52 $^\circ\text{C}$, and primer extension for 60 s at 72 $^\circ\text{C}$, repeated 45 times, followed by a final extension at 72 $^\circ\text{C}$ for 5 min, after which the samples were held at 5 $^\circ\text{C}$. PCR products were purified with the Qiagen PCR cleanup protocol according to the protocol of the manufacturer. PCR products were sequenced with the same primers used for amplification, on both strands. DNA sequences were generated on an ABI 377 Automated DNA Sequencer (Applied Biosystems, Foster City, CA), and analyzed with Sequencher 4.2 (Gene Codes).

Table 1. Location, museum information, SNP sites, and haplotype assignments in the mtDNA CR of 14 specimens of Ross's Gull.

Location	Museum sample	Origin	Year	Base position												Haplotype
				18	30	42	106	115	273	284	302	304	345	370	424	
CA	CMN60081	Seymour I., Nunavut	1974	C	A	C	G	C	C	T	A	G	C	C	C	H1
CA	CMN60082	Seymour I., Nunavut	1974	C	A	C	G	C	C	T	A	G	C	C	C	H1
CA	MM3791	Churchill, Manitoba	1982	C	A	C	G	C	C	T	A	G	C	C	C	H1
CA	MM3792	Churchill, Manitoba	1982	C	A	C	G	C	C	T	A	G	C	C	C	H1
CA	CMN70031	Fogo I., Newfoundland	1976	C	A	C	G	C	C	T	A	G	C	C	C	H1
AK	FM158717	Pt Barrow, Alaska	1931	C	A	C	G	C	C	T	A	G	C	C	C	H1
CA	CMN86167	Baffin I., Nunavut	1985	C	A	C	G	C	C	C	A	G	C	C	C	H2
CA	MM4260	Churchill, Manitoba	1983	C	A	C	G	C	C	C	A	G	C	C	C	H2
CA	CMN60083	Seymour I., Nunavut	1974	C	A	C	G	C	C	C	A	G	C	C	C	H2
AK	FM160703	Pt Barrow, Alaska	1928	C	A	C	G	C	C	C	A	G	C	C	C	H2
AK	FM160702	Pt Barrow, Alaska	1928	T	G	C	G	C	C	C	A	G	C	C	T	H3
AK	FM160710	Pt Barrow, Alaska	1929	C	A	C	G	C	C	C	A	G	A	T	C	H4
AK	FM160709	Pt Barrow, Alaska	1928	C	A	T	G	T	T	C	A	A	C	C	C	H5
AK	MVZ158296	Singoalik R., Alaska	1961	C	A	C	A	C	C	C	G	A	C	C	C	H6

Haplotype 2 is taken as the reference; SNP differences in other haplotypes are shown in bold.

CMN, Canadian Museum of Nature; MM, Manitoba Museum; FM, Field Museum of Natural History; MVZ, Museum of Vertebrate Zoology, University of California.

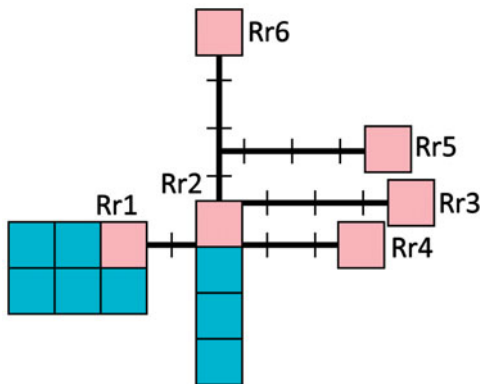


Figure 2. Minimum spanning network of six mtDNA control region haplotypes among 14 museum specimens of Ross's Gull. Haplotypes found in Canadian birds are shown in light blue, and those in Alaskan birds in pink.

Population genetic analysis

Haplotype diversity (H_d) and nucleotide diversity (π) were obtained with DnaSP, v. 4.0 (Qiagen, Valencia, CA) (Rozas & Rozas, 1999). An analysis of molecular variance (AMOVA) was performed with Arlequin, version 3.0 (Qiagen, Valencia, CA) (Excoffier et al., 2005); here, F_{ST} measures the degree of population structure between the Canadian and Alaskan samples. The long-term effective population size of females was estimated as $N_e = \pi \times 10^6 / (s)(g)$, where π is the nucleotide diversity, s is the rate of sequence divergence, and g is the average generation time (Wilson et al., 1985). We used $g = 10$ (Alvo et al., 1996), $s = 0.085$ subs/MY as estimated for the Lesser Black-backed Gull (*Larus fuscus*) (Liebers & Helbig, 2002; Liebers et al., 2001).

Results

In a 515-bp segment of the CR, there are 12 SNP sites that define six haplotypes (Rr1–Rr6) separated by up to five SNPs (Table 1 and Figure 2). All sequences were submitted to GenBank and assigned the accession numbers KP120964–KP120969, inclusive.

All six of the Alaskan birds have distinct haplotypes ($H_d = 1.000$, $\pi = 8.2 \times 10^{-3}$). Among the eight Canadian birds,

only two haplotypes (Rr1 and Rr2) are present, which differ by one SNP ($H_d = 0.535$, $\pi = 1.0 \times 10^{-3}$); both haplotypes are present at the Seymour I. and Churchill sites. A significant proportion of the AMOVA variance occurs between the two geographic locations ($F_{ST} = 0.126$; $p \leq 0.04$). For the combined samples, the estimated female $N_e = 5058$, which suggests a total $N_e \sim 10,000$. For the Alaskan sample alone, the estimated female $N_e = 9650$ suggests a total $N_e \sim 20,000$.

Discussion and conclusions

Genetic structure and population history

There is significant genetic differentiation between the Alaskan and Canadian Ross's Gulls, as captured in an $F_{ST} = 0.126$, which reflects their different haplotypic diversity levels. The two haplotypes observed in Canadian birds are a subset of those in the Alaskan birds, and both are present in both (non-breeding) Churchill and Nunavut samples. Based on these observations, and with appropriate caveats for small samples size, a simple scenario is recent origin of the present Canadian breeding colonies as founder events by small numbers of passage migrants from Siberia via Alaska. Breeding colonies have been reported in Greenland since the 1800s (Kampp & Kristensen, 1980): the absence of records from Canada prior to 1978 may be evidence of absence and recent establishment, or simply failure to detect a rare phenomenon in a remote location.

Implications for conservation

Ross's Gull maintains a tenuous presence as a breeding species in the Canadian High Arctic. It is an intrinsically rare bird in Canada: in any given year, more vagrants undoubtedly pass through than breed in Canada. Its COSEWIC listing as "Threatened" reflects this. Estimation of threat level also allows for "rescue effect", whereby the degree of risk of extinction in Canada is mitigated if extirpated breeding populations may be replaced from elsewhere. If Canadian breeding populations at Churchill and in Nunavut are indeed recent, it remains an open question whether they are also ephemeral. An appropriate conservation strategy should verify the inter-annual stability of breeding colony composition, which could include non-invasive genotyping during the nesting season.

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 S. M. C. and I. L. Jones, and an NSERC Discovery Grant to S. M. C. The paper is based on data from an MSC thesis by Stephanie Royston who was supported by a fellowship from the School of Graduate Studies at Memorial University during the study.

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