

Population genetic structure in migratory sandhill cranes and the role of Pleistocene glaciations

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Abstract

Previous studies of migratory sandhill cranes (*Grus canadensis*) have made significant progress explaining evolution of this group at the species scale, but have been unsuccessful in explaining the geographically partitioned variation in morphology seen on the population scale. The objectives of this study were to assess the population structure and gene flow patterns among migratory sandhill cranes using microsatellite DNA genotypes and mitochondrial DNA haplotypes of a large sample of individuals across three populations. In particular, we were interested in evaluating the roles of Pleistocene glaciation events and postglaciation gene flow in shaping the present-day population structure. Our results indicate substantial gene flow across regions of the Midcontinental population that are geographically adjacent, suggesting that gene flow for most of the region follows an isolation-by-distance model. Male-mediated gene flow and strong female philopatry may explain the differing patterns of nuclear and mitochondrial variation. Taken in context with precise geographical information on breeding locations, the morphologic and microsatellite DNA variation shows a gradation from the Arctic-nesting subspecies *G. c. canadensis* to the non-Arctic subspecies *G. c. tabida*. Analogous to other Arctic-nesting birds, it is probable that the population structure seen in Midcontinental sandhill cranes reflects the result of post-glacial secondary contact. Our data suggest that subspecies of migratory sandhills experience significant gene flow and therefore do not represent distinct and independent genetic entities.

Keywords: Gruidae, isolation by distance, microsatellite DNA, Pleistocene glaciation, population genetics, satellite telemetry

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Introduction

Pleistocene glacial influences on Arctic bird populations

There is a growing body of knowledge regarding the migration and population genetic patterns of Arctic-nesting birds. First described for waterfowl, glaciations during the Pleistocene are thought to have been major factor in the differentiation of many Arctic-nesting birds (Ploeger 1968). Modern DNA methodologies allow researchers to assess current and historical patterns of gene flow in an effort to find explanations for the phylogeographical distributions seen today (Rogers 1995; Webster *et al.* 2002). Using

mitochondrial DNA (mtDNA) sequencing, significant divergences have been discovered in many taxa previously thought to be closely related (Klicka & Zink 1997, 1999; Avise & Walker 1998; Avise *et al.* 1998; citations therein). Although there is much debate on the accuracy of using DNA to estimate divergence time (Klicka & Zink 1997), recent research indicates that Pleistocene glaciations have been a major influence on the divergences seen in many bird species (Avise & Walker 1998; Avise *et al.* 1998; Klicka & Zink 1999).

Current theories of speciation by glaciation contend that widespread ancestral populations were split by Pleistocene glaciations and subsequently evolved significant genetic differences among their allopatric populations (Avise & Walker 1998). However, not all populations ancestrally split by glaciation progressed to speciation. The results of postglaciation secondary contact of previously allopatric

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populations may provide insight into the progression of the speciation process (Barton & Hewitt 1985; Hewitt 1996). During glacial isolation, associated demographic processes (such as reduced population sizes) may result in uneven partitioning of existing genetic variation or the occurrence of new mutations in some populations. If these processes result in differentiated mitochondrial haplotype distributions and allele frequencies at nuclear loci, the outcome of postglaciation contact can be assessed through population genetic analysis (Ball & Avise 1992; Rogers 1995; Hewitt 1996). In many Arctic birds, researchers have used genetic markers to measure the outcomes of such secondary contact events. In some bird species [e.g. rock ptarmigan, *Lagopus mutus* (Holder *et al.* 1999), dunlins, *Calidris alpina* (Wenink *et al.* 1996), Brewer's sparrow, *Spizella breweri* (Klicka & Zink 1997)] genetic and morphometric characteristics remain geographically localized, suggesting that two taxa were formed. Alternatively, with species like the snow geese (*Chen caerulescens*), genetic analysis shows that high gene flow has caused the distinct mtDNA haplotypes produced by Pleistocene glaciations to be shared across many populations (Avise *et al.* 1992). In this case, the morphological and nuclear diversity shows a gradient of variation across their range. Intermediate to these examples are species such as Canada geese (*Branta canadensis*) in which a combination of natal philopatry and male-mediated gene flow produce a population where mtDNA and morphological variation may be localized, but nuclear variation has a more clinal distribution (Van Wagner & Baker 1990). Although sandhill cranes (*Grus canadensis*) are reported to have strong natal philopatry (Walkinshaw 1949; Littlefield 1968; Drewien 1974; Littlefield & Ivey 1995; Nesbitt *et al.* 2002), it is not known whether the geographical partitioning of two major mtDNA clades, reported to be a result of Pleistocene glaciation (Rhymer *et al.* 2001), has been maintained by female philopatry, and whether nuclear variation also reflects the effects of glaciation. Studies of nuclear variation will help assess reinforcement or the progression of hybridization in post-Pleistocene sandhill populations, and provide evidence for gender-biased gene flow should it exist.

Sandhill crane population history

The sandhill crane is the most abundant of the 15 described species of cranes (Meine & Archibald 1996). The world's population of more than 500 000 birds is distributed widely across North America and extends to northeastern Siberia and as far south as Cuba (Fig. 1, Walkinshaw 1973; Johnsgard 1983; Meine & Archibald 1996). The taxonomy of sandhill cranes, originally derived through morphometric and geographical distinctions, has been the subject of many studies. Within this species, a detailed history chronicles the designation of as many as eight populations and six subspecies (Fig. 1, Table 1). In contrast to the type specimen

collected from the Arctic region of North America (lesser sandhill, *Grus canadensis canadensis*), a nonmigratory population of sandhill cranes was discovered in Florida and designated by Meyer (1794) as *Grus pratensis*. The Florida population was later recognized as a subspecies of sandhill crane (Florida sandhill, *Grus canadensis pratensis*) (Peters 1925). Subsequently the Cuban sandhill (*Grus canadensis nesiotus*) was identified in the mid-19th century by Poey (1854) and then named as a subspecies by Bangs & Zappey (1905). Although subspecies to this point were primarily distinguished on the basis of geography and migratory habit, subsequent changes to sandhill taxonomy used morphology to differentiate subspecies. An additional migratory subspecies, greater sandhill (*Grus canadensis tabida*) from central North America was named by Peters (1925) due to its larger body size. Walkinshaw (1949) defined a subset of birds geographically and morphologically intermediate to the northern *G. c. canadensis* and southern *G. c. tabida* breeding in central Canada. These birds were subsequently named the Canadian sandhill (*Grus canadensis rowani*) (Walkinshaw 1965; Aldrich 1979). A final nonmigratory subspecies (Mississippi sandhill, *Grus canadensis pulla*) was named by Aldrich (1972) based on recent population restrictions and minor plumage differences.

There are currently five major populations of migratory sandhill cranes (Fig. 1, Table 1). The largest of these populations, the Midcontinental population (MCP), breeds in the northern regions of North America and Siberia and contains all three migratory subspecies (Johnson & Stewart 1973). The birds of the MCP migrate great distances annually to overwinter in areas of the Texas Plains, Texas Gulf Coast, New Mexico, Arizona, and northern Mexico (Fig. 1) (Tacha *et al.* 1984; Drewien *et al.* 1996; Krapu & Brandt 2002). Like the MCP, cranes using the flyway in states and provinces along the Pacific coast are represented by all three migratory subspecies. The Pacific Flyway population (PFP) breeds in Alaska primarily from the Alaskan Peninsula east to the Kenai Peninsula and upper Cook Inlet (Petrula & Rothe 2002, unpublished); however a segment of this population has recently been identified on islands off British Columbia and far into the southeastern Alaskan coasts (Ivey *et al.* in press). An adjacent population, the Central Valley population (CVP), contains only *G. c. tabida* and breeds in portions of British Columbia, Washington, Oregon, Nevada, and California (Pacific Flyway Council 1983; Pogson & Lindstedt 1991). Collectively, the sandhill cranes from the CVP and PFP converge on a common wintering ground in the Central Valley of northern California (Pogson & Lindstedt 1991; Petrula & Rothe 2002, unpublished). In addition to these northern populations, the Eastern Flyway population (EFP) breeds in the Great Lakes region of the USA and in southern Canada east of Hudson Bay (Meine & Archibald 1996) and winters in areas of southern Georgia and central Florida (Walkinshaw 1973;

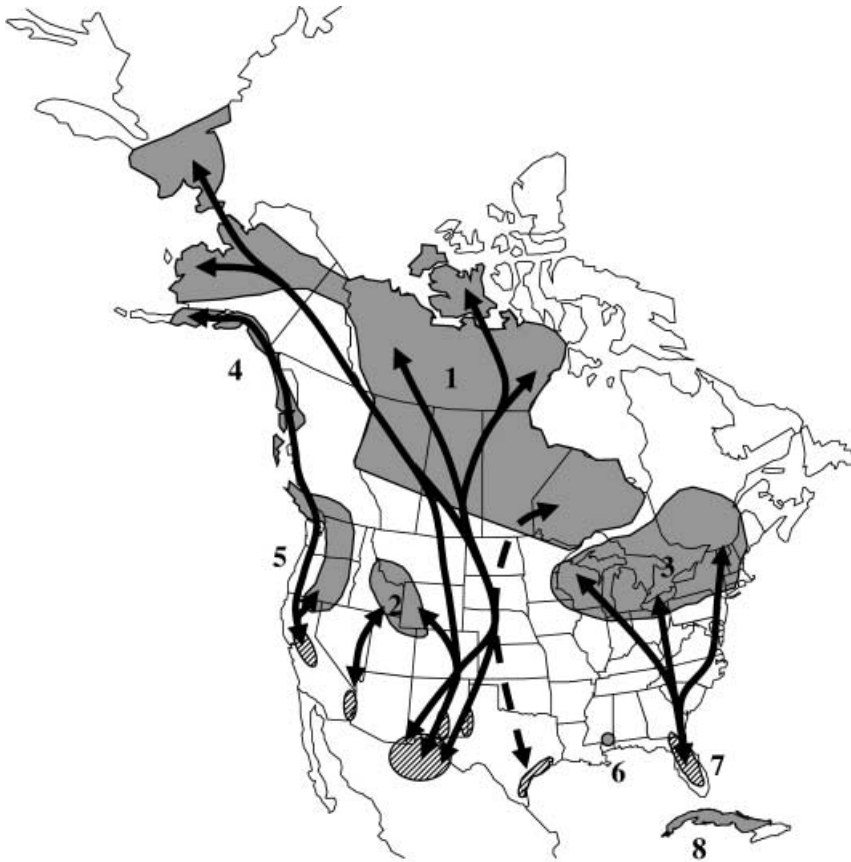


Fig. 1 Migration and range map for the sandhill crane. Breeding (grey) and overwintering (hashed) ranges, as well as migratory paths are shown (adapted from Johnsgard 1983; Meine & Archibald 1996; Krapu & Brandt 2002). A key to population designation is given in Table 1.

Table 1 Key to sandhill crane populations

Population	Subspecies	Breeding location	Wintering location
1. Midcontinental population (MCP)	<i>G. c. canadensis</i> , <i>G. c. rowani</i> , <i>G. c. tabida</i>	Eastern Siberia, Alaska, central Canada from the Canadian Rockies to Hudson Bay, and Minnesota	Texas Plains, New Mexico, Arizona, Mexico, and Gulf Coast of Texas
2. Rocky Mountain population (RMP)	<i>G. c. tabida</i>	Colorado, Idaho, Montana, Utah, and Wyoming	New Mexico, Arizona, and Mexico
3. Eastern Flyway population (EFP)	<i>G. c. tabida</i>	Great Lakes Region of the USA and southeastern Canada	Southern Georgia, and central Florida
4. Pacific Flyway population (PFP)	<i>G. c. canadensis</i> , <i>G. c. rowani</i>	Coastal regions of southern Alaska and northern British Columbia	Central Valley of California
5. Central Valley population (CVP)	<i>G. c. tabida</i>	British Columbia, Washington, Oregon, Nevada, and California	Central Valley of California
6. Mississippi Sandhill population	<i>G. c. pulla</i>	Mississippi Sandhill Crane National Wildlife Refuge	Nonmigratory
7. Florida Sandhill population	<i>G. c. pratensis</i>	South-central Georgia and central Florida	Nonmigratory
8. Cuban Sandhill population	<i>G. c. nesiotus</i>	Mainland Cuba and the Isla de la Juventud	Nonmigratory

Lewis 1977). The last of the migratory populations, the Rocky Mountain population (RMP), contains only *G. c. tabida* and breeds in the Rocky Mountain region of the USA from southern Idaho and Montana to northern Utah and

Colorado (Drewien *et al.* 1999). The eastern portion of RMP cranes migrate through Colorado where they encounter and subsequently overwinter with a portion of the migratory birds of the MCP (Drewien *et al.* 1999).

Genetic studies of migratory sandhill cranes

There has been much debate in the literature on the validity of the taxonomic designations given to sandhill cranes (see Johnson & Stewart 1973; Tacha 1981, 1988; Tacha *et al.* 1985; Rhymer *et al.* 2001; Glenn *et al.* 2002). Given that subspecific designations are solely derived through differences in morphology, plumage, and geography, subspecific designations may not reflect genetically distinct taxa. For example, decades after Oberholser (1921) first noted the geographical integration of *G. c. canadensis* and *G. c. tabida* within the MCP, Tacha *et al.* (1985) observed mixed pairs of small- and medium-sized birds, as well as pairs of small and large sized birds, providing the first evidence that mating potentially occurs between migratory subspecies. As assignments of sandhill cranes to subspecies is difficult due to overlapping morphological ranges (Johnson & Stewart 1973; Gaines & Warren 1984; Tacha *et al.* 1985), there have been five studies that attempt to describe the three migratory subspecies through genetic analysis. Gaines & Warren (1984) presented evidence for a fixed allozyme allele difference between birds of the *G. c. canadensis* subspecies and those from southern Canada (i.e. *G. c. rowani* and *G. c. tabida* subspecies). In a follow-up study, Tacha *et al.* (1986) showed that this 'fixed' allozyme allele was present in 88% of *G. c. tabida*, 64% of *G. c. rowani*, and 18% of *G. c. canadensis*, thus indicating the possibility of greater gene flow between the subspecies than was previously thought. Recently, three studies were completed using mtDNA sequencing. Glenn *et al.* (2002) and Petersen *et al.* (2003) used the highly variable portion of the control region of the mitochondrial genome to assess variation in the MCP only, whereas Rhymer *et al.* (2001) used the entire control region to quantify variation among all but the Cuban subspecies. All three studies produced similar results. Within all the named subspecies, there seems to be only two evolutionary lineages: lineage I, which is comprised primarily of *G. c. canadensis*; and lineage II, which contains the remaining *G. c. rowani*, *G. c. tabida*, *G. c. pratensis*, *G. c. pulla*, and *G. c. nesiotis* subspecies (Rhymer *et al.* 2001). As lineages I and II are 5.3% divergent, Rhymer *et al.* (2001), Glenn *et al.* (2002), and Petersen *et al.* (2003) concluded that only two of the three migratory subspecies were phylogenetically valid. A call to consolidate *G. c. rowani* and *G. c. tabida* was made. Although these three studies clarified the mtDNA variation of this species, sampling of nuclear variation has been limited. The specific objectives of this study were to quantify the population structure and/or gene flow between three of the major populations of migratory sandhill cranes using microsatellite DNA and to determine if nuclear variation in combination with mtDNA provides a more complete picture of the genetics of the MCP. The more general objectives were to compare patterns of mitochondrial and nuclear

differentiation among migratory sandhill crane populations, and to use these patterns to infer population history and the possible role of Pleistocene glaciations in determining the present genetic structure of the species.

Methods*DNA sampling*

A total of 191 blood samples were obtained from the following populations: Midcontinental population (91), obtained as part of USGS Northern Prairie Wildlife Research Center's studies to delineate the breeding grounds, migration routes, and wintering grounds of subspecies and subpopulations that form the Midcontinental population (Krapu & Brandt 2002); the Rocky Mountain population (26), obtained from a study by the Colorado Division of Wildlife and USGS to examine the ecology of cranes that migrate through the San Luis Valley in Colorado (Gammonley & Laubhan 2002); and the Eastern Flyway population (74), obtained from the International Crane Foundation's studies of cranes that breed in Wisconsin (Hayes *et al.* 2003). For all samples, whole blood was drawn and placed into a storage/lysis buffer [0.1 M Tris, 0.1 M EDTA, 5% SDS, 0.01 M NaCl; (Longmire *et al.* 1991)] for later extraction. DNA isolation from the lysate followed protocols for the Promega Wizard Genomic Purification Kit (Promega Corp.).

Molecular genetic analysis

Samples were genotyped at 13 microsatellite loci [Gam μ 1, Gam μ 2, Gam μ 3, Gam μ 4, Gam μ 5, Gam μ 6, Gam μ 7, Gam μ 9, Gam μ 12, Gam μ 15, Gam μ 21, Gam μ 101, Gam μ 102 (Glenn *et al.* 1997; Jones *et al.* 2002)] originally designed for the whooping crane (*Grus americana*) (Glenn 1997). Microsatellite amplification followed procedures previously published for other cranes (Jones *et al.* 2002; Jones 2003). Fluorescently labelled PCR products were pooled together with an internal size standard (Promega CXR (red) 60–400 bp ladder) and run on an MJ BaseStation automated DNA sequencer (MJ Research). Genotypes were scored using CARTOGRAPHER software (MJ Research).

Subspecies, evolutionary lineage, and breeding location

To accompany the microsatellite DNA genotypes, data were collected on morphotype (i.e. subspecies) and mtDNA lineage (lineages I and II, Rhymer *et al.* 2001). For all individuals sampled, birds were categorized by morphology as *G. c. canadensis*, *G. c. rowani*, or *G. c. tabida* using the discriminant methods of Johnson & Stewart (1973), and mtDNA lineage diagnosed using the PCR-RFLP methods of Glenn *et al.* (2002). For the MCP, Krapu &

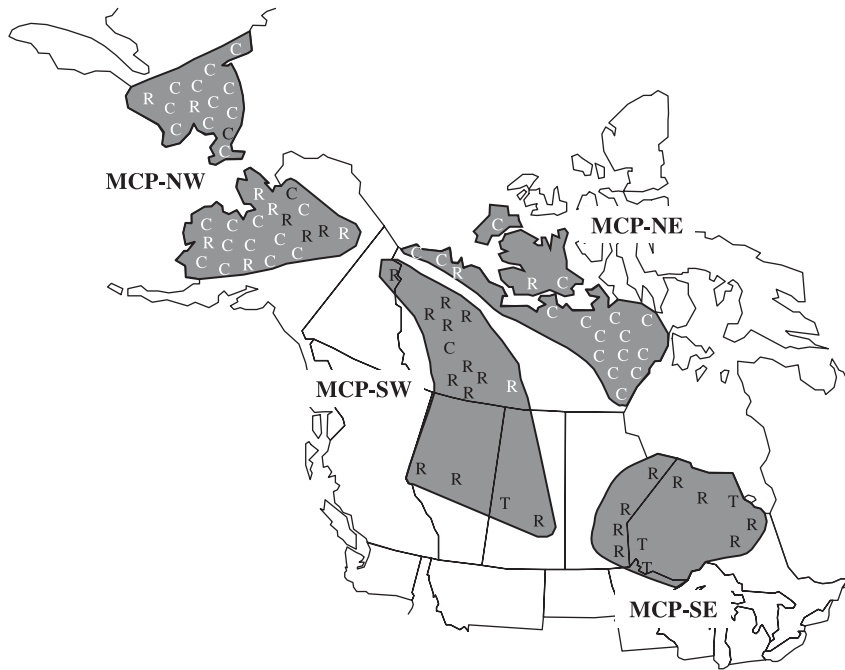


Fig. 2 Distribution of the sandhill crane morphotypes and mtDNA lineages within the Midcontinental population. Approximate geographical placement of the subspecies *Grus canadensis canadensis* (C), *Grus canadensis rowani* (R), and *Grus canadensis tabida* (T), as well as mtDNA lineages I (white) and II (black) based on Krapu & Brandt's (2002) satellite telemetry are shown. Due to geographical redundancy of some samples, not all birds are represented.

Brandt (2002), captured and marked birds with platform transmitting terminals (PTTs) while at their primary spring staging area in the Central Platte and North Platte river valleys of Nebraska. PTTs were then used to follow bird movements via satellite telemetry. Using GIS coordinates of birds during the breeding season, we defined four geographical regions within the MCP: northwest (MCP-NW), northeast (MCP-NE), southwest (MCP-SW), and southeast (MCP-SE) (Fig. 2). The population was subdivided using the following geographical boundaries: a 300-km span of mountain range within the Yukon Territory, consisting of the Mackenzie and Richardson ranges, separate the MCP-NW birds from the others; the MCP-NE birds were differentiated from those in southern Canada due to their tundra nesting habit; and the MCP-SE region was defined as all birds known to use the southeasternmost migratory route (dashed migratory route, Fig. 1). The subdivisions used here are analogous to the subpopulations delineated by Krapu & Brandt (2002) except that we have included all birds within Alaska into the MCP-NW group.

The EFP, from the Great Lakes region, is known through banding and radio-tracking to winter in Florida (Walkinshaw 1973; Nesbitt 1975; Lewis 1977), isolated from the MCP. As their breeding and wintering range are not known to overlap with the MCP, the EFP is considered to be a distinct breeding population (Meine & Archibald 1996). To test this distinction, we used birds sampled from Wisconsin breeding areas as representatives of the EFP in close proximity to the MCP. We know from previous studies that the ranges of MCP and EFP are the closest in the western Great Lakes region (Tacha *et al.* 1994; Krapu & Brandt 2002).

Although the breeding range of the RMP is isolated from the MCP, these two populations converge during winter. Birds from the Arctic regions of Canada, Alaska, and Siberia are known to frequent the migratory pathway of the RMP (Krapu & Brandt 2002, in press). As samples were obtained from the San Luis Valley in Colorado, a known stopover site for this migratory path, morphometric and mitochondrial analyses were used to differentiate the RMP *G. c. tabida* from MCP *G. c. canadensis* and *G. c. rowani*. Birds collected from the San Luis Valley found to be *G. c. canadensis* or *G. c. rowani* from the MCP were removed before analysis. We acknowledge this sampling method as a potential source of error. However, from the satellite telemetry to date (Krapu & Brandt 2002), we have no evidence that the larger birds (i.e. *G. c. tabida*) of the MCP use the San Luis Valley migration route. *Grus canadensis tabida* from the MCP overwinter in the Texas Plains and along the Texas Gulf Coast and migrate annually through Nebraska's Central Platte Valley (Krapu & Brandt 2002).

Microsatellite DNA analyses

Genetic diversity within breeding locations was explored by calculating the proportion of polymorphic loci, mean number of alleles per locus, mean number of alleles per polymorphic locus, observed heterozygosity (H_O), and expected heterozygosity (H_E) using the computer program GENETIC DATA ANALYSIS (GDA, Lewis & Zaykin 2000). Allelic richness, an estimate of allelic diversity that compensates for unequal sample size, was calculated using FSTAT (Goudet 2001) and averaged across loci. GENEPOP (Raymond

& Rousset 1995) was used to determine if populations had significant deviations from Hardy–Weinberg expectations and whether loci exhibit linkage disequilibrium. For both Hardy–Weinberg and disequilibrium analyses, Bonferroni correction (Rice 1989) was used to compensate for multiple comparisons.

Population differentiation was examined using several methods. Using Weir & Cockerham's (1984) theta (θ) as an estimator of population co-ancestry coefficient F_{ST} , overall and pairwise analyses of F_{ST} were conducted using GDA and tested for significance by bootstrapping. Overall and pairwise values for the number of migrants per generation between populations (Nm) were calculated using Slatkin's private allele method (Slatkin 1985; Barton & Slatkin 1986). An analysis of molecular variance (AMOVA, Excoffier *et al.* 1992) was carried out and tested for significance by permutation using ARLEQUIN (Schneider *et al.* 2000). Correlations of allele frequencies and subsequent genotypes between all populations were also investigated with a principal components analysis (PCA) using PCA-GEN, a software program written by Jérôme Goudet, Institute of Ecology, University of Lausanne, Lausanne, Switzerland. From this analysis, a two-dimensional canonical plot was created that represents the first two principal components.

To analyse isolation by distance in the overall study, a Mantel test was carried out on the matrices of genetic distance (in this case the F_{ST} estimator θ) and geographical distance and tested for significance by permutation using GENEPOP. Geographical distance between populations was calculated using the latitude and longitude of the centre point of each group. Additionally, isolation by distance was assessed within the MCP itself. To accomplish this, the two largest subpopulations were divided into two smaller constituent units. Samples within the MCP-NW were divided at the Bering Strait providing Siberian and Alaskan samples; and MCP-SW population was split at the 60th parallel making north and south divisions.

In contrast to the preceding analyses based on user-defined populations, we used the Bayesian-clustering method of Pritchard *et al.* (2000) to identify cryptic genetic

structure using only genotypic data. Pritchard *et al.*'s (2000) program STRUCTURE, which employs this method, can detect genetic structure without imposing any preconceived ideas of population substructure. This iterative method takes the information from given genotypes and statistically assigns individuals, based on Hardy–Weinberg expectations, to a user-defined number of anonymous genetic clusters (K), thus elucidating genetic structure that may not be apparent otherwise. To analyse genetic structure within and between the sandhill populations, STRUCTURE was used to perform six independent analyses using simulations of 50 000 burn-in iterations and 100 000 data iterations to assign individuals into $K = 1–6$ populations, respectively. The posterior probability of K ($P(K|X)$) was determined using the methods of Pritchard *et al.* (2000). After the STRUCTURE analysis was carried out, individual admixture proportions (i.e. the proportion of an individual's genetics assigned to each genetic cluster) were sorted and displayed by population.

Results

Among the samples used in this study, we identified the three size morphs from Johnson & Stewart (1973) that correspond with the subspecies definitions of *Grus canadensis canadensis*, *Grus canadensis rowani*, and *Grus canadensis tabida*. Although the small morph (*G. c. canadensis*) was found only in the northern reaches of the MCP and the larger morph (*G. c. tabida*) found only in the lower portions, the intermediate morph (*G. c. rowani*) has considerable overlap with both northern and southern forms (Table 2). The approximate placement of each sample based on satellite telemetry (Fig. 2), provides insight to subspecies placement within the MCP and the extent of subspecies mixing. The mixing of the subspecies within the central regions of the MCP was better illustrated when the information from mtDNA sequence analysis was overlaid (Fig. 2). As expected for the EFP, and defined for the RMP, the southern populations contained only large-bodied (*G. c. tabida*) birds with type II mtDNA (Table 2).

Table 2 Subspecific composition of sampled populations

Population	Number sampled	Breeding location assumptions	Proportions of each morphotypic subspecies			Proportions of each mtDNA lineage	
			<i>G. c. canadensis</i>	<i>G. c. rowani</i>	<i>G. c. tabida</i>	I	II
MCP-NW	40	Known (PTT)	0.75	0.25	0.00	0.87	0.13
MCP-NE	21	Known (PTT)	0.91	0.09	0.00	1.00	0.00
MCP-SW	16	Known (PTT)	0.06	0.88	0.06	0.06	0.94
MCP-SE	14	Known (PTT)	0.00	0.79	0.21	0.00	1.00
RMP	26	Inferred	0.00	0.00	1.00	0.00	1.00
EFP	74	Known (radio telemetry)	0.00	0.00	1.00	0.00	1.00

Table 3 Descriptive statistics for each population derived from multilocus microsatellite DNA genotypes

Population	Number of samples genotyped	Proportion of polymorphic loci	Alleles per locus	Alleles per polymorphic locus	Allelic richness	Expected heterozygosity (H_E)	Observed heterozygosity (H_O)	Inbreeding within subpopulations (F_{IS})
MCP-NW	40	0.92	5.08	5.42	4.31	0.52	0.42	0.20*
MCP-NE	21	0.92	5.31	5.67	4.81	0.55	0.48	0.14*
MCP-SW	16	0.92	4.38	4.67	4.25	0.54	0.43	0.22*
MCP-SE	14	0.92	3.92	4.17	3.92	0.54	0.48	0.10
RMP	26	0.85	3.92	4.46	3.54	0.49	0.44	0.11
EFP	74	0.92	3.92	4.17	3.35	0.49	0.43	0.13*

*Significant heterozygote deficiency ($P < 0.05$).

Table 4 Locus-by-locus tests for Hardy–Weinberg equilibrium. For each locus, F_{IS} values indicate lack (positive) or excess (negative) of heterozygosity. Dashes indicate monomorphic loci

Locus	MCP-NW	MCP-NE	MCP-SW	MCP-SE	RMP	EFP
Gam μ 1	0.08	0.15	0.10	0.09	0.21	0.30*
Gam μ 2	—	—	—	—	—	—
Gam μ 3	0.21	0.08	0.07	-0.18	0.07	0.13
Gam μ 4	0.46	0.47	0.46	-0.04	0.66	0.31
Gam μ 5	0.17	0.16	0.24	0.32	0.16	0.31*
Gam μ 6	0.13	0.03	0.12	0.14	0.04	0.06
Gam μ 7	0.56*	0.76*	0.66*	0.27	0.24	0.14
Gam μ 9	0.36	0.02	0.28	0.32	0.13	0.10
Gam μ 12	0.19	-0.25	-0.05	-0.08	—	-0.02
Gam μ 15	0.14	-0.08	0.06	0.32	0.30	0.02
Gam μ 21	0.55*	0.34	0.34	0.11	0.01	0.07
Gam μ 101	-0.01	-0.19	0.10	-0.13	-0.10	0.14
Gam μ 102	-0.11	0.01	0.08	-0.07	0.13	-0.09

*Significant heterozygote deficiency after Bonferroni correction ($P < 0.05$).

The microsatellite DNA genotypes (Jones 2003; available online from <http://www.savingcranes.org>) exhibited no evidence of linkage disequilibrium; however there were deviations from Hardy–Weinberg expectations. All populations showed heterozygote deficiencies, with MCP-NW, MCP-NE, MCP-SE, and EFP significantly so ($P < 0.05$) after Bonferroni correction (Table 3). To explore this deficiency further, a locus-by-locus presentation of F_{IS} (Table 4) illustrates that the reduction in observed heterozygosity involved multiple loci that exhibit equilibrium in other populations and other species (Jones *et al.* 2002; Jones 2003), thus the deficiency is likely due to population history or structure, not genotyping problems (e.g. null alleles). Although we did not see evidence of null genotypes (e.g. no individuals failed to produce PCR products), PCR competition can cause one of the two alleles at a locus to amplify at a lower rate thus providing an erroneous homozygote genotype (allelic dropout). To detect and reduce the

Table 5 Alleles found in only one population (i.e. private alleles)

Locus	Allele	Frequency	Found in
Gam μ 12	119	0.01	MCP-NW
Gam μ 5	196	0.01	MCP-NW
Gam μ 102	139	0.04	MCP-NW
Gam μ 1	150	0.02	MCP-NE
Gam μ 1	146	0.02	MCP-NE
Gam μ 102	145	0.02	MCP-NE
Gam μ 7	143	0.02	MCP-NE
Gam μ 3	131	0.02	MCP-NE
Gam μ 6	143	0.10	MCP-NE
Gam μ 7	149	0.10	MCP-NE
Gam μ 12	125	0.03	MCP-SW
Gam μ 5	176	0.03	MCP-SW
Gam μ 21	128	0.04	MCP-SE
Gam μ 102	148	0.04	MCP-SE
Gam μ 6	136	0.02	RMP
Gam μ 9	183	0.02	RMP
Gam μ 6	138	0.06	RMP
Gam μ 1	134	0.01	EFP
Gam μ 21	140	0.01	EFP
Gam μ 21	142	0.01	EFP
Gam μ 101	237	0.03	EFP
Gam μ 102	124	0.05	EFP

possibility of allelic dropout, we genotyped homozygote individuals multiple times. With respect to gene diversity (H_E), allelic diversity, allelic richness, as well as private alleles, only nonsignificant differences were seen between populations (Tables 3 and 5).

Significant F_{ST} values ($P < 0.05$) were found overall and for most pairwise combinations of populations (Table 6), with all regions of the MCP significantly differentiated from the RMP and EFP samples. The MCP appears to follow an isolation-by-distance model of population divergence, where adjacent regions (MCP-NW, MCP-SW, and MCP-NE; and MCP-SW and MCP-SE, respectively) are not significantly different with respect to their allele frequencies and regions farthest apart (MCP-NW and MCP-SE; and

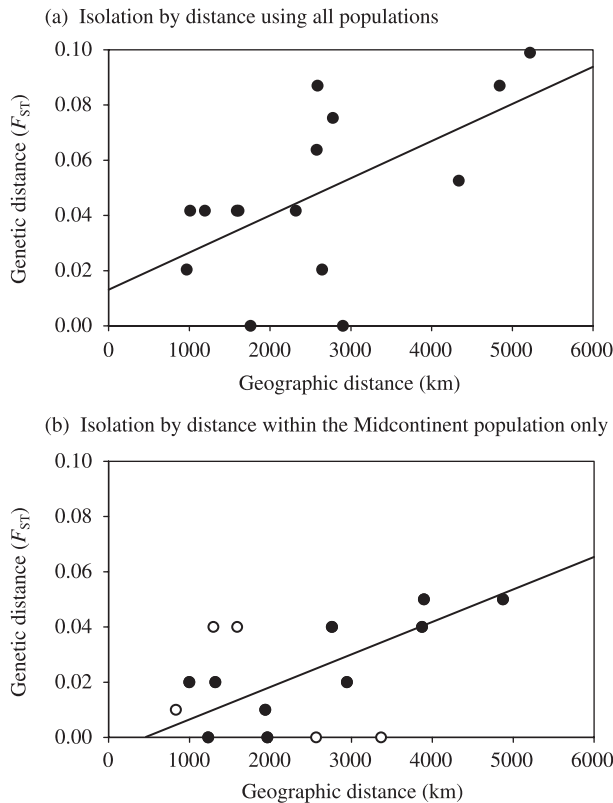


Fig. 3 Pairwise estimates of F_{ST} plotted against geographical distance over all populations (a) and for the Midcontinental population only (b). Regression lines are shown. Open circles (b) represent comparisons with the MCP-NE subpopulation.

Table 6 Overall and pairwise estimates of F_{ST} (lower) and Nm (upper) based on microsatellite DNA genotypes. Asterisks indicate significant deviations ($P < 0.05$) of allele frequencies across all loci

	MCP-NW	MCP-NE	MCP-SW	MCP-SE	RMP	EFP
MCP-NW	—	3.65	2.26	1.41	1.42	1.01
MCP-NE	0.00 NS	—	3.75	2.05	1.84	2.02
MCP-SW	0.02 NS	0.02 NS	—	2.68	2.62	1.21
MCP-SE	0.05*	0.04*	0.00 NS	—	2.42	1.36
RMP	0.08*	0.07*	0.04*	0.04*	—	1.89
EFP	0.09*	0.08*	0.06*	0.04*	0.04*	—
Overall F_{ST}	0.06*					
Overall Nm	3.66					

MCP-NE and MCP-SE, respectively) have significantly different allele frequencies ($P < 0.05$) (Table 6). Analysis for geographical similarity of allele frequencies (Fig. 3) indicates that F_{ST} is positively correlated to geographical distance between all populations (Fig. 3a; $r = 0.59, P = 0.03$), yet shows that within the MCP isolation by distance is more constrained. Within the conglomerate of MCP as a whole, geographical distance is weakly correlated to genetic distance ($r = 0.45,$

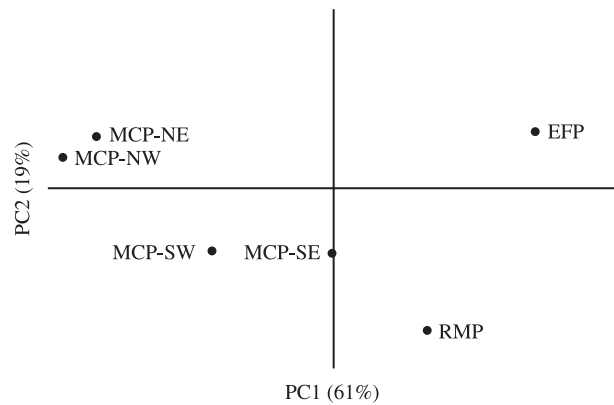


Fig. 4 Canonical plot of the first two principal components illustrate the partitioning of variance of overall F_{ST} across all populations. Graphical proximity conveys information on correlations of allele frequencies between populations presumably due to coancestry and/or gene flow.

$P = 0.09$). However, if comparisons involving MCP-NE (open circles) are removed from the analysis, the association of genetic to geographical distance becomes much stronger ($r = 0.82, P = 0.01$).

Like the estimates of F_{ST} , gene flow estimates (Nm), based on the number and frequency of private alleles indicates a high rate of gene flow within the MCP (Table 6). Although Nm estimates are lower between the MCP, EFP, and RMP, Nm rates greater than one migrant per generation indicate that a low level of gene flow may exist between all migratory populations (Mills & Allendorf 1996 and citations therein). Similarly, the analysis of molecular variance shows that most of the genetic variability lies within populations (93.3%, $P < 0.001$), and only a small amount of the variation (4.2%, $P = 0.068$) is due to the differences among the three migratory populations and among the subpopulations of the MCP (2.5%, $P < 0.001$). Additional hierarchical analyses for samples within the MCP reveal that little variation is explained by morphological subspecies (1.3%, $P = 0.025$) and mtDNA haplotype differences approximate that of the geographically derived subpopulations (2.5%, $P < 0.001$). In a visual representation of F_{ST} , the PCA (Fig. 4) illustrates the pattern of gene flow within the MCP, as well as between the MCP, EFP and RMP.

Although individuals within six geographical groups were tested, the Bayesian STRUCTURE analysis revealed the highest posterior probability for four genetic clusters ($P(K|X) = 0.95$, Table 7). To explore the geographical placement of these genetic clusters, we sorted and graphed the individual admixture proportions by population (Fig. 5). Where two genetic clusters were specified ($K = 2$), the genetic variation clustered such that it illustrated a basic difference between far northern birds (e.g. Arctic nesting) and all other birds. A new grouping, consistent with admixture between the two initial genetic clusters, was delineated

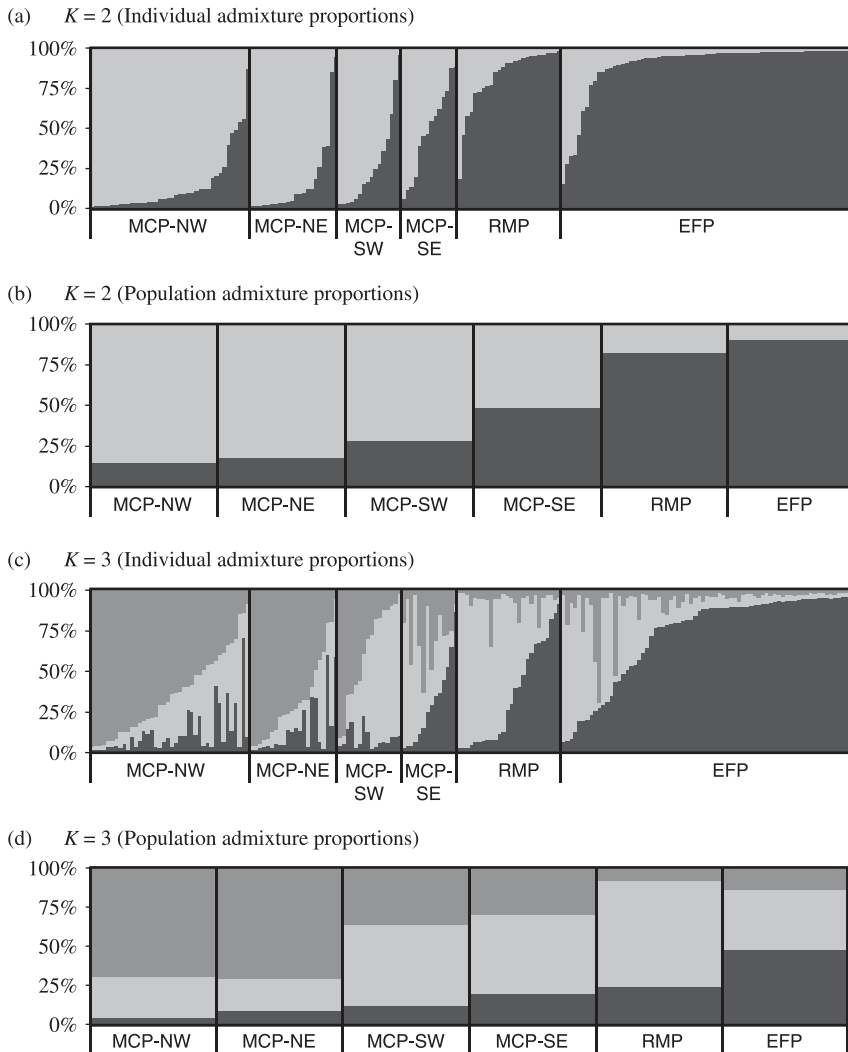


Fig. 5 Bayesian analysis of the microsatellite DNA genetic structure. The proportion of ancestry assigned to each of the K subgroups was plotted for each population by individuals (a, c), and by population mean (b, d).

Table 7 Inferred number of genetic clusters (K)

K	$\log P(X K)$	$P(K X)$
1	-5637.3	-0
2	-5414.2	-0
3	-5384.1	-0
4	-5361.1	0.95
5	-5364.2	0.05
6	-5404.5	-0

when the variance was partitioned further ($K = 3$). The primary geographical placement of this admixture again suggests isolation by distance for the microsatellite DNA variation. When $K = 4$ was used (the value with the highest posterior probability), the analysis only served to subdivide the admixed variation further. As the admixture proportions for $K = 4-6$ did not elucidate any new genetic structure, they are not presented here.

Discussion

The sandhill cranes are the most numerous, and one of the most genetically diverse, species of crane. Our data show that although the estimates of allelic richness and gene diversity (H_E) are low compared to many bird species (Neff & Gross 2001), of the five crane species sampled to date, the sandhill cranes are second only to red-crowned cranes (*Grus japonensis*) in overall diversity (Hasegawa *et al.* 2000; Jones *et al.* 2002; Jones 2003). One notable detail in the data is significant heterozygote deficiencies in the majority of groups tested. While genotyping errors or null alleles cannot be completely eliminated as an explanation, we feel that demographic history is a more likely explanation. As the boundaries of the MCP regions were inferred through geographical or migratory differences, they may not represent true genetic boundaries. Given that this species has a high degree of natal philopatry (Walkinshaw 1949; Littlefield 1968; Drewien 1974; Littlefield & Ivey 1995; Nesbitt *et al.*

2002), the geographical boundaries used in this study likely encompass many localized demes. Population genetic theory suggests that if a population contains multiple demes, an excess of homozygosity is likely to be found (i.e. the Wahlund effect, Wahlund 1928). Although the EFP also shows a significant lack of heterozygotes (Table 4), the root cause may be different. It is known that the midwest sector of the EFP went through a severe population reduction within the last 100 years and was almost completely extirpated from Wisconsin by 1930 (Henika 1936; Leopold 1949; Walkinshaw 1949, 1973). The sandhill crane sample from Wisconsin shows both reduced allelic richness and heterozygote deficiency that likely reflects this population bottleneck.

With regard to allelic differentiation among populations, there were few microsatellite alleles unique to any one population. No private alleles were observed at a frequency higher than 10% (Table 5). The population-level analyses uncovered only slight differences between any two populations (i.e. $F_{ST} < 0.05$ is considered little differentiation, Wright 1978; Hartl & Clark 1997). The results of this study show that the nuclear diversity, within the MCP and overall, is clinal throughout the 6000-kilometre-wide range. As evidenced by a nonsignificant F_{ST} , no discernable differences in microsatellite DNA allele frequencies were seen between the two regions where lineage I mtDNA predominated (MCP-NW and MCP-NE, Table 6). Gene flow between these two regions is sufficient to sustain similarity of allele frequencies (Table 6). Although the subspecific composition of these two northern regions is different from the southern regions (Table 2), neither MCP-NW or MCP-NE has a significant difference in allele frequencies compared to samples from the MCP-SW region (Table 6). These results in tandem with nonsignificant F_{ST} estimates between MCP-SW and MCP-SE regions and significant F_{ST} estimates between the MCP-SE and MCP-NW/MCP-NE (Table 6) indicate isolation by distance. This north/south cline is echoed throughout the results of this study [e.g. morphologic and mtDNA subspecific composition (Table 2), Nm (Table 6), PCA (Fig. 4), and the STRUCTURE analysis (Fig. 5)]. To further test for isolation by distance of these populations, a Mantel test of geographical and genetic distance between migratory populations showed a significant ($P < 0.03$) positive association between genetic differentiation and interpopulation distance (Fig. 3a). Although the majority of the comparisons within the MCP (Fig. 3b) showed a similar tendency for isolation by distance (filled circles), comparisons involving MCP-NE (open circles) indicate that gene flow to and from this subpopulation do not follow a simple isolation-by-distance model. Comparisons of MCP-NE and the constituent samples of the MCP-NW (Siberia and Alaska) (lower right open circles) show that the distance between the Arctic regions is not a significant barrier to gene flow. Conversely, comparisons

among MCP-NE, southern MCP-SW, and MCP-SE (upper left open circles) suggest that the Canadian Shield, a sterile rocky landscape produced by glaciation that separates MCP-NE from the central and southern sectors of the population, is producing a barrier to gene flow.

The analyses in total show only slight allele frequency differences between the MCP and the other migratory populations. It has long been known that the MCP cranes intermingled with those of the RMP in their wintering grounds (Lewis 1977), thus it is not surprising that these two populations are genetically similar. In relation to the genetic connectivity of the EFP and the MCP, it is plausible that the MCP and the EFP have occasional genetic interchange. Tacha *et al.* (1994) suggested that transition from MCP to the EFP was near the Ontario/Manitoba border in south central Canada. However, via satellite telemetry, we now know that the birds in western Ontario are in fact a part of the MCP (Krapu & Brandt 2002). Thus, the transition from MCP to EFP runs from central Minnesota across to southeastern Ontario (Fig. 1). Although it is known that the MCP and EFP over-winter in different locations, their breeding populations are nearly contiguous. Due to fluctuations in range, it is likely that gene flow could occasionally occur between these populations.

Subspecies discrimination

The microsatellite and mtDNA data in combination with information on morphology and geography provide new insights into the history of the MCP. Given that the *G. c. canadensis* morph and mtDNA lineage I remain clustered mainly in the north and that the larger body morphs of *G. c. rowani* and *G. c. tabida* and mtDNA lineage II remain mainly south (Fig. 2, Table 2), it is easy to see how previous studies have come to the conclusion that the population is heavily subdivided with only occasional hybridizations (Walkinshaw 1949, 1965; Johnson & Stewart 1973; Lewis 1977; Aldrich 1979; Meine & Archibald 1996; Rhymer *et al.* 2001; Glenn *et al.* 2002; Petersen *et al.* 2003). However, taken in context with the precise geographical information, the morphologic and microsatellite DNA variation shows that there is a gradation from the Arctic-nesting *G. c. canadensis* to the non-Arctic *G. c. tabida* (Figs 2, 4 and 5; Tables 2 and 6). Although the results of this study, like previous studies, nullify *G. c. rowani* as a genetically distinct subspecies, these results also nullify the possibility of these birds being simply an alternate body form of the *G. c. tabida*. Data to date indicate that this morphotype is intermediate in morphology, geography, and genetics. Given the new data on nuclear similarity to both northern and southern birds, the *G. c. rowani* subspecies should be considered a transitional form, as suggested previously (Stephen 1967; Tacha *et al.* 1985; Rhymer *et al.* 2001; Petersen *et al.* 2003).

Pleistocene glaciation and sandhill cranes

By providing a nuclear data set matched with mtDNA, morphology and geographical data, this study provides a great opportunity to advance the knowledge of this species. We can now examine the data and look for clues to historical and ongoing processes that have shaped the populations we see today. Given the considerable sequence divergence in mtDNA lineages shown by Rhymer *et al.* (2001), it is not likely that this divergence could have evolved in the face of current levels of gene flow. Similarly to other North American species (Klicka & Zink 1997, 1999; Avise & Walker 1998), Rhymer *et al.* (2001) suggests the degree of mtDNA divergence seen in sandhills presumably dates to the mid-Pleistocene. Similar to other arctic populations [e.g. marbled murrelets, *Brachyramphus marmoratus* (Congdon *et al.* 2000), common guillemot, *Uria aalge* (Friesen *et al.* 1996), and Canada geese (Van Wagner & Baker 1990)], it is likely that what we see in the MCP today reflects secondary contact of a previously subdivided population.

The results of this study, showing the sandhill mtDNA localized in a north/south pattern and the morphometric and nuclear variation clinal, suggest that the current MCP is, in part, the result of postglaciation secondary contact. The Beringian glacial refugium, an area of eastern Siberia and western Alaska that remained unglaciated throughout the Pleistocene, was an ecological refuge where many species survived the Pleistocene glaciation (Hopkins *et al.* 1982). This unglaciated area coincides with westernmost limit of this species and represents the area currently occupied by the birds of the MCP-NW region, a stronghold of the birds with lineage I mtDNA. It is likely that the ancestral population of sandhill cranes, that resided in North America for millions of years prior to the Pleistocene (Brodkorb 1967), were bisected by the progression of early Pleistocene glaciation, producing two refugia, one in Beringia or west of the glaciers extent on the Siberian steppe and one in the southern portions of North America. These refugia remained isolated a sufficient amount of time to evolve significant differences in mtDNA sequence (Rhymer *et al.* 2001). At the end of the last glaciation event (c. 11 000 BP), the Laurentide and Cordilleran ice sheets retreated and produced an ice-free migratory corridor from Beringia to the southern portions of North America (Arnold 2002). Upon expansion of the Siberian/Beringian population of *G. c. canadensis* southeast and the southern population of *G. c. tabida* northwest, this corridor which parallels the east slope of the Canadian Rockies, was a region of secondary contact and introgression.

With the high degree of nuclear gene flow seen in this study, coalescence theory would have predicted a population structure more similar to that of snow geese (Avise *et al.* 1992). As mtDNA has a much faster coalescence time (one-fourth that of nuclear genes), the mtDNA lineages

would have spread throughout the population well before that of the nuclear microsatellite DNA if random mating existed. However, given that mtDNA variation in this population is still highly structured geographically and that the nuclear DNA variation is clinally distributed, strong female natal philopatry and male mediated gene flow is suggested. It is not known whether the MCP is currently in genetic equilibrium or whether the integration of these two refugia populations is still in progress. Continued monitoring of the genetic composition of the MCP may provide insight into rates of introgression. If the intermediate morphology of the *G. c. rowani* does in fact result from the hybridization between *G. c. canadensis* and *G. c. tabida*, this morph will become more common in areas of the range where it is advantageous, or remain localized if it is disadvantageous (Rhymer & Simberloff 1996). Conversely, if the population is currently in equilibrium, the *G. c. rowani* morph may represent a physiological intermediate adapted to more median environments. In any case, the MCP should be considered a single genetic population whose origin stems from the introgression of two formerly divided populations. As with the snow goose (Avise *et al.* 1992), given the high rates of gene flow that exist today, any genetic differentiation remaining in the MCP today will likely decline over time.

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