SHORT COMMUNICATIONS

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HYBRIDIZATION AND POPULATION SUBDIVISION WITHIN AND BETWEEN ROSS'S GEESE AND LESSER SNOW GEESE: A MOLECULAR PERSPECTIVE

JASON D. WECKSTEIN[1,5], ALAN D. AFTON[2], ROBERT M. ZINK[3] AND RAY T. ALISAUSKAS[4]
[1]*Department of Biological Sciences and Museum of Natural Science, Louisiana State University, Baton Rouge, LA 70803*
[2]*U.S. Geological Survey, Louisiana Cooperative Fish and Wildlife Research Unit, Louisiana State University, Baton Rouge, LA 70803*
[3]*J. F. Bell Museum and Department of Ecology, Evolution and Behavior, 100 Ecology Building, University of Minnesota, St. Paul, MN 55108-6097*
[4]*Canadian Wildlife Service, 115 Perimeter Rd., Saskatoon, SK 57N 0X4, Canada*

Abstract. We reanalyzed Quinn's (1992) mtDNA control region data set including new sequences from nine Lesser Snow Geese (*Chen caerulescens caerulescens*) and 10 Ross's Geese (*Chen rossi*) and found the same divergent lineages that Quinn (1992) attributed to vicariant separation of Lesser Snow Goose populations during the Pleistocene. However, peculiar patterns of mtDNA control region sequence variation, including a multimodal mismatch distribution of mtDNA sequences with two levels of population structuring and the sharing of two divergent haplotype lineages, are consistent with two hybridization episodes in *Chen* geese. Comparisons of mtDNA variation with historical and allozyme data sets compiled by Cooke et al. (1988) are consistent with the hypothesis that sharing of two mtDNA haplotype lineages between Ross's Goose and Lesser Snow Goose resulted from hybridization (Avise et al. 1992). Furthermore, population structure found within one haplotype cluster is consistent with Cooke et al.'s (1988) hypothesis of past allopatry between blue and white Lesser Snow Geese.

Key words: Chen caerulescens caerulescens, Chen rossii, *control region, hybridization, mtDNA sequencing, population genetics.*

Resumen. Reanalizamos los datos de la región de control del ADN mitocondrial (ADNmt) de Quinn (1992), junto con nuevas secuencias de nueve individuos de la especie *Chen caerulescens caerulescens* y 10 de *Chen rossi*. Encontramos los mismos linajes divergentes que Quinn (1992) atribuyó a la separación vicariante de las poblaciones de *C. c. caerulescens* durante el Pleistoceno. Sin embargo, encontramos que las dos especies comparten dos linajes de haplotipos divergentes, y la distribución de ''mismatch'' en secuencias del ADNmt mostró multimodalidad con dos niveles de estructuración de la población. Estos patrones peculiares están de acuerdo con la hipótesis de que hubo dos episodios de hibridización en gansos del género *Chen*. Los datos históricos y de aloenzimas compilados por Cooke et al. (1988) también apoyan esta hipótesis (Avise et al. 1992). Además, la estructura de la población dentro de un grupo de haplotipos es consistente con la hipótesis de Cooke et al. (1988) acerca de la pasada alopatría entre los morfos azul y blanco de *C. c. caerulescens*.

Lesser Snow Goose (*Chen caerulescens caerulescens*) and Ross's Goose (*Chen rossi*) appear to be valid species; however, previous molecular analyses revealed a peculiar sorting of mitochondrial lineages, suggesting an unusual evolutionary history. Avise et al. (1992) examined mitochondrial Restriction Fragment Length Polymorphisms (RFLP) in Ross's Geese and Lesser Snow Geese and noted the existence of two divergent haplotype lineages, which are shared between the two species. Avise et al. (1992) classified these RFLP haplotypes into Clade I and Clade II types and attributed this pattern to either hybridization or lack of lineage sorting, but did not go on to evaluate which hypothesis was correct. Quinn (1992) sequenced a small section

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Manuscript received 26 November 2001; accepted 22 January 2002.
[5] E-mail: jweckst@lsu.edu

of the mitochondrial DNA control region to build a genealogy of Lesser Snow Goose populations. Quinn (1992) found a deep mtDNA split within Lesser Snow Geese and attributed it to a historical vicariant event. However, he did not include sequences from Ross's Goose. We reanalyzed Quinn's (1992) data set and added new Lesser Snow and Ross's Goose sequences. Our reanalysis, taken in light of historical information compiled by Cooke et al. (1988), allows us to make new inferences about hybridization, population structure, and the species identities of the ancestors that originally carried each sequence type.

METHODS

SAMPLE LOCATIONS

We used previously published sequences ($n = 82$; Quinn 1992) from three localities (from west to east): Wrangel Island, northeastern Russia (71°N, 180°E), Anderson River, Northwest Territories, Canada (69°N, 129°W), and La Pérouse Bay, Manitoba, Canada (58°N, 90°W). In addition, we analyzed new Ross's Goose ($n = 10$) and Lesser Snow Goose ($n = 9$) samples from Karrak Lake (Nunavut, Canada 67°14′N, 100°16′W), which is located approximately midway between Anderson River and La Pérouse Bay (Dunn et al. 1999).

PCR AMPLIFICATION AND SEQUENCING

We analyzed 178 bp of the mitochondrial control region from 10 Ross's Geese and 91 Lesser Snow Geese. We amplified the new samples from Karrak Lake, using Quinn's (1992) primers (16775L and 287H-M) and the PCR protocol of Kocher et al. (1989) with the following thermal cycling profile: 40 cycles of 94°C for 40 sec, 57°C for 40 sec, 72°C for 40 sec, and one final extension of 72°C for 5 min. We examined PCR products on a 1% agarose gel to verify the presence of desired products. We purified PCR products using a QIAquick PCR Purification Kit (Qiagen, Valencia, California) and used approximately 75 ng of this double-stranded PCR product for cycle sequencing with fluorescent dye terminators and AmpliTaq FS (Applied Biosystems, Foster City, California). We sequenced PCR products in both directions using Quinn's (1992) heavy strand primer 287H-M and our own light strand primer LCHENCRI (5′-TTGGTTATGCATATTCG TGC-3′). We removed unincorporated dyes from sequencing reaction products using Centri-sep columns (Princeton Separations, Adelphia, New Jersey) filled with Sephadex G-50. Sequenced products were electrophoresed on an ABI377 (Applied Biosystems) automated DNA sequencer. Automated sequences were examined and reconciled from both forward and reverse strands using Sequencher (ver. 3.1, Genecodes Co., Ann Arbor, Michigan). These sequences are deposited in GenBank (AF467108–AF467126) and an alignment of all 26 haplotypes found is available from the authors.

SEQUENCE ANALYSIS

We calculated pairwise Kimura (1980) 2-parameter distances between all sequences using MEGA (Kumar et al. 1993). We used the computer program PAUP* (Swofford 1998) to construct a neighbor-joining tree of haplotypes from Kimura (1980) 2-parameter distances. The neighbor-joining tree was rooted using control region sequences from Tundra Bean Goose (*Anser fabalis rossicus*, AF159951), Pink-footed Goose (*A. brachyrhynchus*, AF159954), Lesser White-fronted Goose (*A. erythropus*, AF159956), European White-fronted Goose (*A. albifrons albifrons*, AF159958), and Western Greylag Goose (*A. anser anser*, AF159962), which were deposited in GenBank by Ruokonen et al. (2000). We used Arlequin (ver. 1.1; Schneider et al. 1997) to calculate mismatch distributions (distribution of pairwise genetic differences) for Ross's and Lesser Snow Geese, χ^2 values to test the goodness of fit between observed and expected mismatch distributions (Rogers and Harpending 1992, Harpending et al. 1993), and population pairwise F_{st} values (a measure of population subdivision). Using Arlequin, we input different population structures to test for genetic structure within all sequences, between clades (sequence types A and B), between Ross's Geese and Lesser Snow Geese, and between localities. We calculated among-colony pairwise F_{st} values for all sequences together and for each of the sequence types (A and B) separately.

TABLE 1. Observed numbers of individual Ross's Geese and Lesser Snow Geese per haplotype found at the Karrak Lake Colony, Nunavut, Canada.

Haplotype number	Sequence type	Lesser Snow Goose	Ross's Goose
1	B	6	7
2	A	2	3
3	B	1	0

RESULTS

We found 3 haplotypes among geese from the Karrak Lake Colony (Table 1) and 26 haplotypes among all 101 individuals included in the analysis (10 Ross's Geese and 91 Lesser Snow Geese). A neighbor-joining tree of these haplotypes (Fig. 1) showed two divergent sequence types equivalent to Quinn's (1992) type A and type B sequences and Avise et al.'s (1992) clade I and clade II lineages, respectively. Representatives of both sequence types were distributed throughout populations of both goose species (Fig.1). Ross's Goose and Lesser Snow Goose shared two haplotypes. Haplotype 1 (type B) was shared by 7 Ross's Geese and 28 Lesser Snow Geese, and haplotype 2 (type A) was shared by 3 Ross's Geese and 24 Lesser Snow Geese.

The mismatch distribution for all 101 mtDNA sequences was multimodal, with one large peak and two moderately sized peaks (Fig. 2). This distribution was significantly different ($\chi^2_{27} = 22\,783.1$, $P < 0.01$) from the expected distribution given rapid expansion from a recent population bottleneck. The mismatch distribution for type B sequences was bimodal, whereas that for type A sequences was monomodal (Fig. 2).

Average Kimura (1980) 2-parameter sequence divergence between type A and B sequences was 7.4%. Sequence divergences averaged 0.9% and 1.8% within

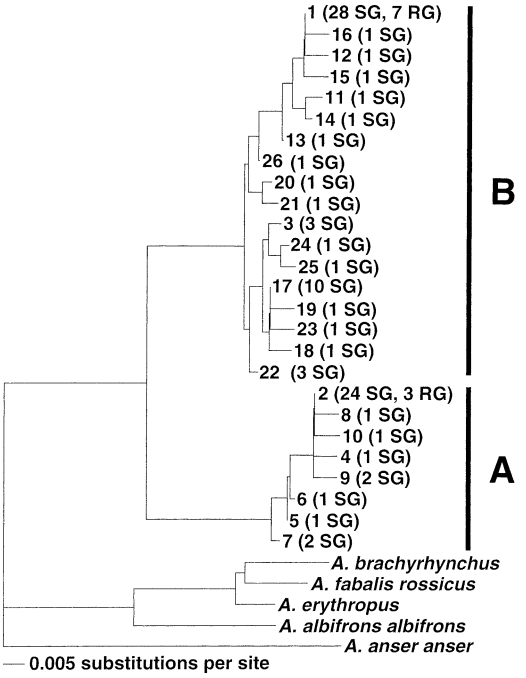

FIGURE 1. Neighbor-joining tree showing genealogical relationships between 26 haplotypes found in Ross's Geese and Lesser Snow Geese. Numbers at the tips of branches are haplotype numbers we assigned. Numbers of Ross's Geese (RG) and Lesser Snow Geese (SG) carrying each haplotype are in parentheses. Haplotype 1 is identical to Quinn's (1992) reference sequence 9, while haplotype 2 corresponds to Quinn's (1992) reference sequence 1. Average divergence between sequence types A and B is 7.4%. Branch lengths are proportional to Kimura (1980) 2-parameter sequence divergence.

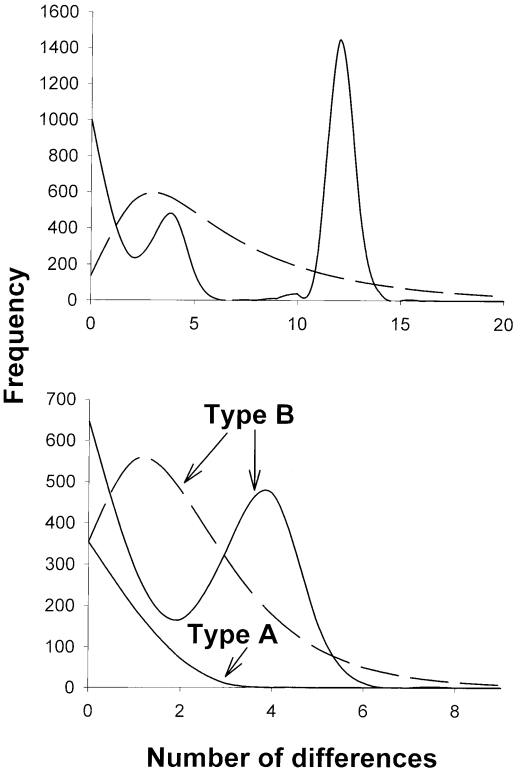

FIGURE 2. Mismatch distributions for Ross's Geese and Lesser Snow Geese mtDNA sequences. The solid curves indicate the observed distribution of pairwise genetic differences for (upper plot) all pairs of *Chen* mtDNA sequences and (lower plot) type A and type B sequences separately. The dashed curves are expected mismatches calculated using Rogers' (1995) model of sudden demographic expansion. Frequency refers to the number of pairwise comparisons that exhibit a given number of nucleotide differences. In the upper plot, the observed distribution differs from the expected distribution ($\chi^2_{27} = 22\,783.1$, $P < 0.01$), and therefore is not consistent with a history of recent bottleneck and rapid expansion in the *Chen* geese. The expected mismatch in the lower plot is for type B sequences only and differs from the observed mismatch ($\chi^2_9 = 1165.7$, $P < 0.01$). For type A sequences, we could not estimate the expected mismatch because the observed mismatch mean was greater than the observed variance.

types A and B, respectively. The estimate of F_{st} between sequence types A and B was 0.87 ($P < 0.05$). F_{st} calculations among the four sampled colonies (including sequences from both Ross's and Lesser Snow Geese) yielded two significant ($P < 0.05$) pairwise values, 0.12 (Wrangel Island vs. La Pérouse Bay) and 0.16 (Wrangel Island vs. Karrak Lake). When analyzed separately (by sequence type), population structuring among colonies differed between sequence types A and B. Pairwise F_{st} calculations among colonies for type A sequences yielded little structuring with one significant ($P < 0.05$) but relatively low value, 0.11 (Wrangel Island vs. La Pérouse Bay). However, pairwise F_{st} calculations among colonies for type B sequences yielded five significant ($P < 0.05$) pairwise values, 0.76 (Wrangel Island vs. La Pérouse Bay), 0.70 (Wrangel Island vs. Karrak Lake), 0.27 (Wrangel Island vs. Anderson River), 0.41 (Anderson River vs. La Pérouse Bay), and 0.29 (Anderson River vs. Karrak Lake).

DISCUSSION

HISTORICAL DISTRIBUTIONS, ALLOZYMES, AND mtDNA

Prior to the 1970s, most Ross's Geese wintered in the lower San Joaquin Valley, California (Bellrose 1976, Ryder and Alisauskas 1995), and consequently were rare in the central and eastern United States (Alisauskas 1998). Since 1949, the continental population of Ross's Geese has increased, and migration routes have shifted eastward (Trauger et al. 1971, Ryder and Ali-

sauskas 1995). This eastward shift in wintering distribution and migratory routes brought Ross's Geese into contact with Lesser Snow Geese during winter and more importantly during spring migration when most pair formation is thought to occur (Robertson and Cooke 1999; ADA, RTA, pers. obs.). This shift in wintering areas led to opportunities for interspecific hybridization. Indeed, intermediate geese of both color phases frequently are observed, and mixed-species pairs have successfully nested (Trauger et al. 1971, McLandress and McLandress 1979, Cooke et al. 1995). Some authors have suggested that the rare blue phase Ross's Goose originated from backcrossing of hybrids between white Ross's Geese and blue phase Lesser Snow Geese (McLandress and McLandress 1979).

Cooke et al. (1988) summarized historical distributional records and gathered allozymic evidence indicating that the two color phases of Lesser Snow Goose were allopatric as recently as the 1920s (see also Cooke et al. 1995). As agriculture opened up the region between allopatric wintering Lesser Snow Goose populations, winter distributions and migratory routes eventually overlapped leading to pairings between blue and white phase individuals (Cooke et al. 1988). Presently, plumage coloration is distributed clinally across both wintering and breeding populations (Cooke et al. 1988). This clinal distribution is similar to the distribution of formerly allopatric taxa merging in a hybrid zone. White Lesser Snow Geese predominate in western North America, whereas blue phase Lesser Snow Geese are more common in eastern North America (Cooke et al. 1988). In addition to their historical evidence, Cooke et al. (1988) found that color phases of Lesser Snow Geese exhibit marginally significant differences in allozyme variants at 6 loci, supporting the conclusion that the two goose color morphs were separate taxa until recently. Finally, color phases of Lesser Snow Geese mate assortatively (Cooch and Beardmore 1959), which may represent a preference evolved in allopatry.

Significant mtDNA F_{st} values between Wrangel Island, the westernmost colony in this study, and La Pérouse Bay and Karrak Lake, two eastern colonies, are consistent with historical and allozyme data (Cooke et al. 1988) indicating that eastern and western populations of Lesser Snow Geese (presumably blue and white forms, respectively) came into sympatry only recently. Although dispersal to and from Wrangel Island from other colonies has been documented (Johnson 1995), mtDNA gene flow might be limited because dispersal of males is much more frequent than that of females (Cooke and Sulzbach 1978, Johnson 1995).

The neighbor-joining tree, mismatch distributions, and F_{st} values document population structuring among mtDNA haplotypes found in Ross's Geese and Lesser Snow Geese. The presence of two divergent lineages (sequence types A and B) in the neighbor-joining tree indicates that mitochondrial DNA sequences historically have undergone some sort of population isolation, because this level of polymorphism would be unlikely to persist for a long period of time in a single population (Moore 1995). Furthermore, the significant F_{st} between type A and type B sequences indicates this pattern of population subdivision. However, both Ross's Geese and Lesser Snow Geese carry both sequence types. One explanation is that this sharing of sequence types is a result of retention of an ancestral polymorphism (Avise et al. 1992). However, in light of the depth of the haplotype tree, the historical data on range shifts, and the existence of phenotypically intermediate individuals, we argue that hybridization is a more likely cause of the sharing of two divergent lineages between Ross's Goose and Lesser Snow Goose.

Three peaks in the mismatch distribution highlight three groups of pairwise comparisons: (1) those showing large numbers of differences, (2) those showing moderate to low numbers of differences, and (3) those showing almost no differences. This pattern reflects that found in average divergences within and between sequence types. One interpretation of this pattern is a relatively old isolation between groups carrying the A and B types, with a more recent isolation of populations typified by the B type. The type A lineage does not appear to have segregated into isolated populations. Individual mismatch distributions of each of the two sequence types (Fig. 2, lower plot) helps us to better elucidate patterns of population structure. Pairwise comparisons between type A sequences produce a monomodal curve with a mean of 0.58, which is a signature of little or no structure, and relatively recent population expansion. The curve for type B sequences is bimodal (or ragged), indicative of a stable population, or one with structure (which the F_{st} confirmed).

By combining historical distributional, observational, and genetic data, we can speculate which species (Ross's or Lesser Snow) originally carried each of the sequence types. Ross's Goose populations historically were limited in distribution and population size (Bellrose 1976, Alisauskas 1998, Ryder and Alisauskas 1995), which would yield a low F_{st} value, such as that found for type A sequences among colonies. The mismatch distribution for type A sequences also suggests recent population expansion, consistent with the evidence on recent changes in distribution. Therefore, the monomodal distribution of sequence type A suggests that type A sequences originally were carried by a Ross's Goose mother because historical Ross's Goose populations, limited in wintering distribution to western North America, likely were not subdivided into multiple populations. The mismatch distribution of type B sequences produced a bimodal distribution, which indicates (1) population structure nested within the type B sequences or (2) a history with two bottlenecks in population size (Zink 1997). Both historical data and allozymes indicate that until recently (~1920) Lesser Snow Goose color phases were allopatric both in winter and during breeding. Such a pattern of allopatry over time could produce the bimodal pattern exhibited by the mismatch distribution of type B sequences. Moreover, this pattern of allopatry would yield high F_{st} values, such as those from the among-colony calculations for type B sequences. Therefore, the bimodal pattern of subdivision in the type B sequence mismatch distribution and significant F_{st} values suggest that type B sequences originally were carried by Lesser Snow Goose mothers. Further analyses, us-

ing both nuclear and mitochondrial markers for the same individuals across the range of Ross's Goose and Lesser Snow Goose are needed to test these hypotheses. Moreover, using nuclear and mitochondrial markers would allow tracking and confirmation of the patterns of hybridization between Lesser Snow Goose color phases and between Ross's Geese and Lesser Snow Geese.

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