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MOLECULAR GENETIC STATUS OF ALEUTIAN CANADA GEESE FROM BULDIR AND THE SEMIDI ISLANDS, ALASKA[1]

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Abstract. We conducted genetic analyses of Aleutian Canada Geese (*Branta canadensis leucopareia*) from Buldir Island in the western Aleutians and the Semidi Islands in the eastern portion of their breeding range. We compared data from seven microsatellite DNA loci and 143 base pairs of the control region of mitochondrial DNA from the two populations of Aleutian Canada Geese and another small-bodied subspecies, the Cackling Canada Goose (*B. c. minima*) which nests in western Alaska. The widely separated island-nesting Aleutian geese were genetically more closely related to each other than to mainland-nesting small-bodied geese. The populations of Aleutian geese were genetically differentiated from one another in terms of mitochondrial DNA haplotype and microsatellite allele frequencies, suggesting limited contemporary gene flow and/or major shifts in gene frequency through genetic drift. The degree of population genetic differentiation suggests that Aleutian Canada Goose populations could be considered separate management units. There was some evidence of population bottlenecks, although we found no significant genetic evidence of non-random mating or inbreeding.

Key words: Aleutian Canada Geese, bottlenecks, Branta canadensis leucopareia, *genetics, microsatellites, mtDNA.*

INTRODUCTION

Three small-bodied subspecies of Canada Geese (*Branta canadensis*) breed in Alaska: Taverner's Canada Geese (*B. c. taverneri*) nest predominantly in the interior portion of the state and north of the Brooks Mountain range in northern Alaska; Cackling Canada Geese (*B. c. minima*) nest predominantly in coastal regions of western and southwestern Alaska; and Aleutian Canada Geese (*B. c. leucopareia*) nest only on a few small islands (Buldir, Nizki, Alaid, Agattu, Little Kiska, Chagulak, Amukta) in the Aleutian chain and on the Semidi Islands (Kiliktagik and Anowik) southwest of Kodiak Island.

Aleutian Canada Geese were extirpated from most of their historical range (Fig. 1) after the introduction of non-native predators for fur production between the 1830s and 1930s (USFWS 1991). The only population known to survive was on Buldir Island where predators had not been released due to the island's remoteness and difficult terrain (Byrd and Woolington 1983). Restricted distribution and low remnant population numbers prompted the United States Fish and Wildlife Service to list Aleutian Canada Geese as endangered in 1967.

Hatch and Hatch (1983) subsequently described ''Aleutian-like'' Canada Geese from Kiliktagik of the Semidi Islands, and Bailey and Trapp (1984) discovered a population of ''Aleutian-like'' Canada Geese on Chagulak Island (Fig. 1). Conventional morphometric studies were inconclusive regarding the taxonomic and reproductive status of these populations in relation to those on Buldir (S. Hatch, pers. comm.). However, Shields and Wilson (1987) determined that newly discovered ''Aleutian-like'' Canada Geese from Chagulak and Kiliktagik could not be genetically differentiated from *B. c. leucopareia* from Buldir based on 21 restriction enzymes and examination of nearly 200 restriction fragments from mitochondrial DNA (mtDNA). MtDNAs of the two other small-bodied Canada geese in Alaska (Cackling Canada Geese and Taverner's Canada Geese) were clearly distin-

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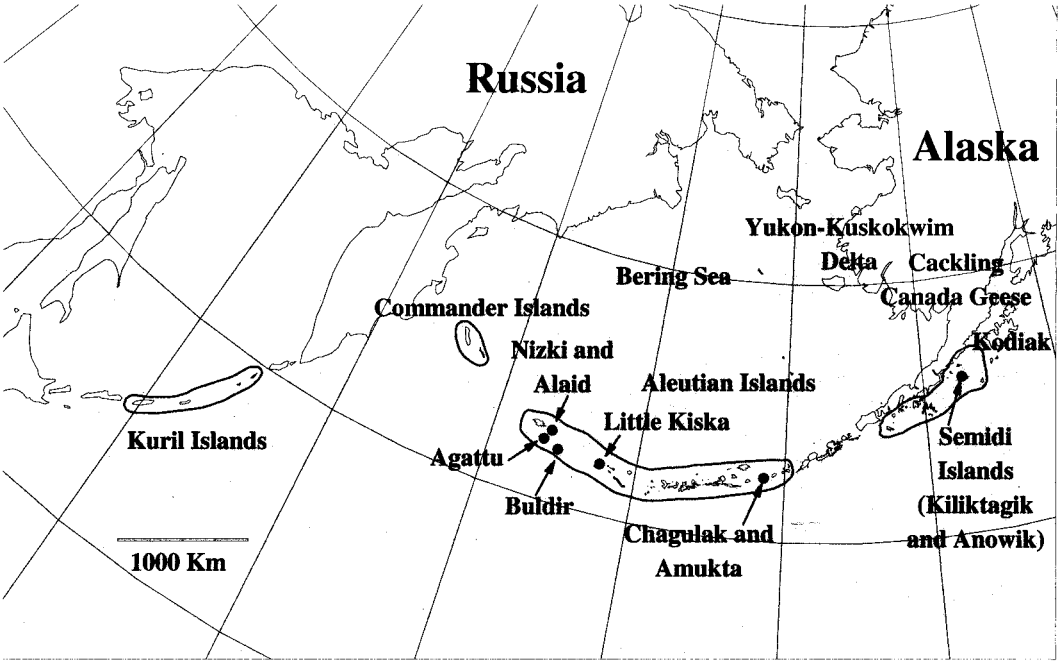

FIGURE 1. Historical distribution (polygons) and current U.S. (black circles) breeding populations of Aleutian Canada Geese (USFWS 1991). Locations sampled in this study were Aleutian Canada Geese from Buldir Island and the Semidi Islands and Cackling Canada Geese from the Yukon-Kuskokwim Delta.

guishable from those of the three island populations of Aleutian Canada Geese. The discovery of the two previously unknown breeding populations of Aleutian Canada Geese on Chagulak and Kiliktagik and an increase from approximately 800 birds in the mid-1970s to 32,000 birds in 1999, led the U.S. Fish and Wildlife Service to propose delisting Aleutian Canada Geese as threatened (DeGange 1999).

Despite the lack of genetic evidence of distinctions, banding data suggest there are at least two demographically distinct sub-populations of Aleutian Canada Geese. Buldir birds stage and winter in northern and central California, respectively, whereas Semidi birds winter in Oregon (USFWS 1991). The large distance between breeding sites on Buldir and those on the Semidi Islands, the time interval since the breeding distribution was more continuous (ca. 1750, DeGange 1999), and the two populations' current use of different wintering areas suggest the two populations may be reproductively isolated. Migration patterns and wintering locations for geese of Amukta and Chagulak of the central portion of the Aleutian Island chain are not well defined, but some banding data indicate these geese winter in the same areas as the Buldir geese (USFWS 1991).

Previous genetic studies of Aleutian Canada Geese (allozymes, Morgan et al. 1977; mtDNA, Shields and Wilson 1987) were based on small sample sizes and were not sufficiently polymorphic to provide data with which to establish population relationships and comparative population levels of variation. Recently, techniques including analysis of biparentally-inherited nuclear microsatellite loci have proven to be highly informative regarding questions about dispersal, gene flow, and relationships of isolated populations (McDonald and Potts 1997). In this study we used seven recently developed biparentally-inherited microsatellite loci (Buchholz et al. 1998, Cathey et al. 1998) and DNA sequence analysis of 143 base pairs of domain I of the maternally-inherited avian mtDNA control region to study Aleutian Canada Geese from Buldir and the Semidi Islands. These two non-coding genetic systems are subject to higher rates of mutation than coding regions of the genome (Brown et al. 1982, Goldstein and Pollock 1997) and consequently are thought to be more informative at the population level. Our objective was to use

molecular genetic markers to assess the extent of gene flow among populations, the potential genetic effects of population bottlenecks, and genetic relationships of Aleutian Canada Geese from Buldir and the Semidi Islands. For comparison, we used Cackling Canada Geese from the Yukon-Kuskokwim (Y-K) Delta, which also have experienced reductions in population numbers, although not as drastic as Aleutian Canada Geese have.

METHODS

SAMPLE COLLECTION AND PREPARATION

During 1991, whole blood samples were collected from geese at Buldir Island ($n = 29$), the Semidi Islands ($n = 8$), and from captive geese raised at the University of California, Davis from eggs taken at Buldir ($n = 5$). In addition, tissue samples ($n = 12$) identified as being from Semidi Islands birds based on morphology and their discrete wintering location were collected from carcasses found on wintering grounds in 1993 and 1994 in Tillamook County, Oregon. Samples were placed in tissue preservation buffer (4 M Urea, 0.2 M NaCl, 100 mM Tris-HCl pH 8.0, 0.5% n-Lauryl sarkosine, 10 mM EDTA) and shipped at ambient temperatures. Blood quills from molting Cackling Canada Geese ($n = 41$) were collected in 1995 on the Y-K Delta and were placed in the tissue preservation buffer described above. All samples were stored at −20°C in the laboratory prior to analysis. Genomic DNA was extracted from blood and tissue using standard phenol-chloroform extraction procedures and quantified using a fluorometer.

MICROSATELLITE ANALYSES

Each population was characterized using six dinucleotide repeat microsatellite loci (TTUCG-4 and TTUCG-1, Cathey et al. 1998; Bcaμ 1, Bcaμ 3, Bcaμ 9, and Bcaμ 11, Buchholz et al. 1998) and one 5-bp repeat microsatellite locus (TTUCG-5, Cathey et al. 1998). Each locus was amplified in 25 μl reaction volumes using 100–150 ng DNA, PCR buffer (10 mM Tris-HCl pH 8.5, 1.5 mM $MgCl_2$, 50 mM KCl, 10 μg ml^{-1} nuclease-free BSA, 0.0025% Tween-20), 1.5 U of AmpliTaq DNA Polymerase (Perkin Elmer, Foster City, California), dNTPs at 200 μM (TTUCG-4, TTUCG-5, TTUCG-1), 120 μM (Bcaμ 3), or 80 μM (Bcaμ 1, Bcaμ 9, and Bcaμ 11), 0.04 μM of γ-^{32}P-ATP-labeled forward primer, and non-labeled primers at locus-specific concentrations [0.8 μM (TTUCG-4) or 0.36 μM (TTUCG-5, TTUCG-1, Bcaμ 1, Bcaμ 3) non-labeled forward primer, 1.2 μM (TTUCG-4) or 0.4 μM (TTUCG-5, TTUCG-1, Bcaμ 1, Bcaμ 3) or 0.12 μM (Bcaμ 9) or 0.04 μM (Bcaμ 11) reverse primer]. Thermocycler conditions included initial denaturation at 94°C for 2 min, followed by 25–35 cycles of denaturing at 94°C for 1 min, annealing at locus-specific temperatures 56°C (TTUCG-4, Bcaμ 1, Bcaμ 3, Bcaμ 9, Bcaμ 11) or 60°C (TTUCG-5, TTUCG-1) for 1 min, and extending at 72°C for 1 min.

Genotypes at each microsatellite locus were assigned relative to an M13 sequence standard and internal controls (individuals of known genotype run on each gel) after standard polyacrylamide gel electrophoresis and autoradiography. Microsatellite allele frequencies, estimates of observed and expected heterozygosity, and genetic distance (chord distance, Cavalli-Sforza and Edwards 1967) were derived using BIOSYS (version 1.7, Swofford and Selander 1981). The chord distance has been shown to be robust with respect to tree topology for microsatellite loci surveyed for intra-specific populations (Takezaki and Nei 1996). Genetic distances were used to generate a multi-locus neighbor-joining tree in MEGA (Kumar et al. 1993). Bootstrap values could not be generated for the tree due to an insufficient number of populations. Estimates of Wright's (1969) inbreeding coefficient (F) were derived and tested for statistical significance as described by Li and Horvitz (1953). Inbreeding coefficients range from −1.0 to 1.0, with 1.0 indicating complete homozygosity (and thus severe inbreeding or invariance) among the individuals sampled.

Analyses of variation in allele frequency within and among Aleutian Canada Goose populations were conducted with both *F*-statistics (Cockerham 1969) using the program FSTAT (F_{IT}, F_{ST}, and F_{IS}, Goudet 1994) and an analog of Nei's (1973) *G*-statistics (R_{ST}) using the program RstCalc (Goodman 1997). F_{IT} is the proportion of variance among all samples regardless of population; F_{ST}/R_{ST} is the proportion of variance between populations; and F_{IS} is the proportion of variance within populations. The analog, R_{ST}, and *F*-statistics differ in that R_{ST} is derived from variances in mean allele size and frequency in relation to number sampled, while

F-statistics are derived from variances in frequency alone.

Tests to assess the possibility of recent bottlenecks in each population were conducted using the program BOTTLENECK which identifies an excess of heterozygosity over all polymorphic loci by performing randomizations of data under two microsatellite models of mutation theory assuming mutation-drift equilibrium (Cornuet and Luikart 1996). Outputs of these randomizations were tested for heterozygosity excess and normal distributions (Cornuet and Luikart 1996). Computer simulations indicate that the Stepwise Mutation Model (SMM) output of the Wilcoxon one-tail test for heterozygosity excess is more appropriate for this data set because our data have fewer than 30 individuals per population and fewer than 10 microsatellite loci (G. Luikart, pers. comm.).

MTDNA ANALYSES

Primers C1 and C1R (Sorenson and Fleischer 1996) were tested using cesium chloride-purified Canada Goose mtDNA and nuclear DNA to confirm amplification of mtDNA and absence of nuclear sequences of mitochondrial origin (Numt) product as described by Sorenson and Quinn (1998). A hypervariable portion of the mtDNA control region (3′ end of domain I, Baker and Marshall 1997) was sequenced from a sample of geese in each population (Buldir, $n = 9$; Semidi, $n = 13$; Cackling Canada Geese, $n = 17$). An ≈400 bp fragment of mtDNA was amplified in 50 μl reaction volumes using 50 ng of total genomic DNA, PCR buffer (67 mM Tris-HCl, pH 8.0, 6.7 mM $MgCl_2$, 0.01% Tween-20), 1 mM of each dNTP, 1 μM of C1 primer, 1 μM of C1R primer, and 1 U of AmpliTaq DNA Polymerase (Perkin Elmer, Foster City, California). Thermocycler parameters included a hot start at 80°C for 5 min before adding the AmpliTaq DNA Polymerase and cycling 40 times at 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min.

PCR products were cleaned with Ultrafree-MC, 30,000 NMWL Filter Unit spin columns (Millipore Corporation, Bedford, Massachusetts) and then sequenced using the internal labeling α^{32}P-dATP protocol in the SequiTherm EXCEL DNA Sequencing Kit (Epicentre Technologies, Madison, Wisconsin) and 1.5 μM of the C1 primer. Thermocycler parameters were 95°C for 5 min followed by 30 cycles of 95°C for 30 sec, 56°C for 30 sec, and 70°C for 1 min. To eliminate stops in variable regions noted in initial sequence optimization experiments, each dideoxy termination reaction was incubated for 30 min at 37°C with 1 μl of a Terminal Deoxynucleotidyl Transferase (TdT) mix containing 3.33 mM dNTPs, 1X sequencing reaction buffer, 2.5 U TdT (Life Technologies, Inc., Frederick, Maryland), before adding 3 μl of stop solution.

MtDNA sequences were aligned manually after standard polyacrylamide gel electrophoresis and autoradiography. Haplotype designations were based on 143 bp of sequence and were submitted to GenBank under accession numbers AF175474, AF175483-AF175490, and AF175492. Estimates of population haplotype frequency were used in subsequent analyses. Estimates of haplotype heterogeneity [specifically, the nucleon (or haplotype, designated by *h*) and nucleotide diversity (π) indices for nonselfing populations (equations 8.1 and 10.4, respectively, of Nei 1987)] were estimated for each population using the DA function in the program REAP (McElroy et al. 1991) from pairwise gamma distances between haplotypes (Kimura 2-parameter model, $\alpha = 0.5$, Kimura 1980) generated in MEGA (Kumar et al. 1993). The nucleon diversity index (*h*) is approximately equivalent to the probability that two randomly chosen individuals will have different haplotypes. The nucleotide diversity index (π, Nei and Tajima 1981) measures the average pairwise nucleotide difference between individuals within samples. The measurement π corrects *h* for the size of the nucleon examined (Nei 1987).

Heterogeneity of haplotype distribution among populations was tested for significance using the MONTE function in the program REAP (McElroy et al. 1991) using 1,000 replicates based on Roff and Bentzen's (1989) Monte Carlo chi-square test. This test has been shown to be appropriate for data sets in which many elements (in our case, haplotypes) occur fewer than five times (Roff and Bentzen 1989).

Significance of population differences in haplotype frequency (Phi_{ST}) were estimated using the program AMOVA (Excoffier et al. 1992), an mtDNA-analog of standard *F*-statistics (Cockerham 1969) which are commonly employed for biparentally inherited loci. Phi_{ST} was calculated both with and without weighting the haplotype frequencies with pairwise Euclidean distances of nucleotide differences between haplotypes. Maximum parsimony analyses of mtDNA hap-

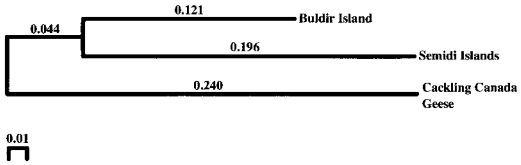

FIGURE 2. Neighbor-joining tree based on Cavalli-Sforza and Edwards (1967) chord distances from seven microsatellite loci of Canada Geese.

lotype sequences could not be completed because there were fewer informative sites than haplotypes (i.e., identical site changes found in more than one haplotype used to infer evolutionary relationships). As an alternative to a maximum parsimony analysis, pairwise gamma distances between haplotypes (Kimura 2-parameter model, $\alpha = 0.5$, Kimura 1980) were used to construct a minimum spanning tree network.

An alpha level ≤ 0.007 (i.e., 0.05 divided by 7 [the number of tests]) was considered significant for all tests or sets of multiple comparisons. Means ± SE are presented in the text.

RESULTS

MICROSATELLITE DNA

All seven microsatellite loci were polymorphic, with the number of alleles observed at individual loci ranging from 2 to 21. Cackling Canada Geese and Semidi Islands geese were monomorphic for one allele at locus TTUCG-4, but all three populations were polymorphic at each of the other loci. Mean heterozygosities over all loci were similar across all three populations (range: 0.57–0.59), although heterozygosities at individual loci varied greatly between populations. A neighbor-joining tree, based on Cavalli-Sforza and Edwards (1967) chord distances, revealed that the Buldir and Semidi populations were more genetically similar (0.32) than either Aleutian Canada Goose population was to Cackling Canada Geese (Buldir x Cackling Canada Geese = 0.40; Semidi × Cackling Canada Geese = 0.48; Fig. 2). Inbreeding coefficients (F, Wright 1969) were not significant at any locus or in any population except locus TTUCG-4 for Buldir Island ($\chi^2_1 = 7.0$, $P < 0.01$).

All loci appeared to be in Hardy-Weinberg equilibrium given the small and nonsignificant values of F_{IS} (variance within populations, Table 1). When either F_{ST} or R_{ST} (variance between populations) values were calculated, two to three of the seven loci showed significant spatial heterogeneity in allele frequency as did the overall values when all loci were considered for F_{ST} (Table 1). Individually significant loci differed between F_{ST} (Bcaμ 1, Bcaμ 9) and R_{ST} (TTUCG-1, TTUCG-4, Bcaμ 3). This may be because R_{ST} is corrected for the variance from the mean allele size in each population. Although the overall R_{ST} value was not significant after a correction for multiple tests ($P = 0.02$), the value is approximately the same as the overall F_{ST} value. These results suggest that these populations experience little genetic exchange with each other and/or that population bottlenecks have resulted in large shifts in allele frequency over recent time periods. However, we failed to detect recent bottlenecks using the program BOTTLENECK (Cornuet and Luikart 1996) for each population (SMM Wilcoxon one-tail test for heterozygosity excess Buldir $P = 0.22$; Semidi $P = 0.42$; Cackling Canada Geese $P = 0.42$).

TABLE 1. Summary of *F*-statistics after 500 permutations (Goudet 1994) and *R*-statistics after 1,000 permutations (Goodman 1997) at individual loci and across all microsatellite loci, and mtDNA Phi_{ST} values after 500 bootstrap iterations (Excoffier et al. 1992).

Locus	F_{IT}	F_{ST}	F_{IS}	R_{ST}
TTUCG-1	−0.144	0.080	−0.243	0.078[a]
TTUCG-4	0.560	0.075	0.525	0.113[a]
TTUCG-5	0.078	0.016	0.062	−0.012
Bcaμ 1	0.107	0.064[a]	0.047	0.009
Bcaμ 3	0.096	0.031	0.067	0.124[a]
Bcaμ 9	0.219	0.124[a]	0.108	−0.016
Bcaμ 11	−0.042	−0.005	−0.038	0.065
Total over all microsatellites	0.081	0.050[a]	0.033	0.052
mtDNA		Phi_{ST}		
Without pairwise distance information		0.345[a]		
Including pairwise distance information		0.280[a]		

[a] Value is significantly different from expectation.

MTDNA

Cackling Canada Geese and Aleutian Canada Geese did not have distinct mtDNA in the 143 base pairs of the mtDNA genome surveyed, although substantial differences in haplotype frequency were observed between the two subspecies and between the Buldir and Semidi populations of Aleutian Canada Geese. Ten mtDNA haplotypes with 10 variable sites were detected (seven tran-

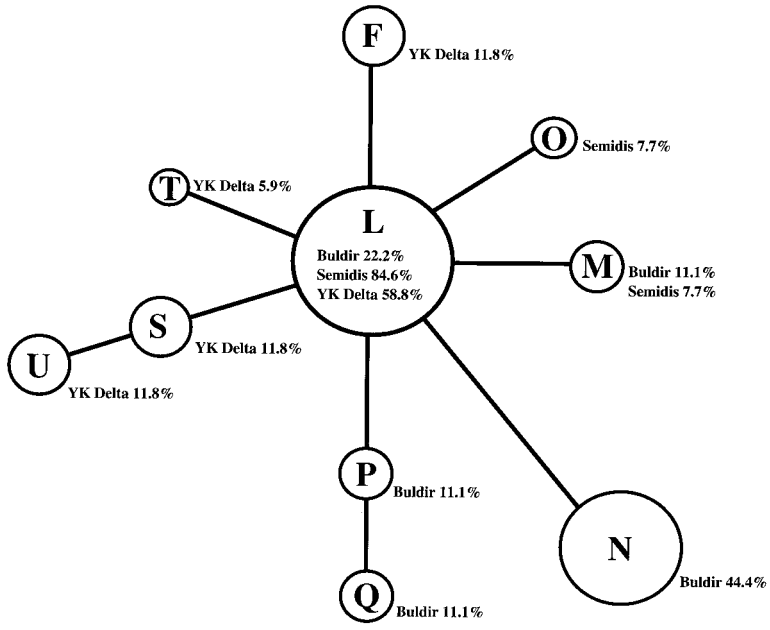

FIGURE 3. Minimum spanning tree indicating relationships among 10 mtDNA haplotypes identified in this study and haplotype frequencies in each population of Canada Geese. Each branch represents a single nucleotide difference with the exception of haplotype N which differed from haplotype L by two base pairs. The tree is drawn to scale based on pairwise gamma distances between haplotypes (Kimura 2-parameter model, $\alpha = 0.5$, Kimura 1980).

sitions, two transversions, and one insertion/deletion; GenBank accession numbers AF175474, AF175483-AF175490, and AF175492) and aligned with published mtDNA sequences from Snow Geese (*Chen caerulescens*, Quinn 1992). Haplotype L was the most common haplotype overall, and geographically ubiquitous. Many haplotypes were found in only one subspecies or one population (e.g., haplotypes F, S, T, and U were observed only in Cackling Canada Geese, haplotypes N, P, and Q were observed only in Buldir Island geese, whereas haplotype O was observed only in Semidi Islands geese). Haplotype diversities (h) ranged from 0.28 ± 0.11 (Semidi Islands geese) to 0.76 ± 0.08 (Buldir Island) with the haplotype diversity of Cackling Canada Geese ($h = 0.63 \pm 0.08$) being intermediate. Nucleotide diversity (π) values were much lower because they are corrected for the number of base pairs examined, but followed the same trends as haplotype diversity (data not shown). Monte Carlo simulations testing for haplotype heterogeneity across populations were significant whether or not Cackling Canada Geese were included ($P = 0.001 \pm 0.001$).

The analysis of mtDNA haplotype variance and resulting F-statistic, Phi_{ST}, were significant whether or not the Euclidean squared distances between haplotypes were used (Table 1). The minimum spanning tree network central position is occupied by haplotype L which is geographically ubiquitous. All other haplotypes are no more than two mutations from haplotype L (Fig. 3).

DISCUSSION

GENETIC INFERENCES

The seven microsatellite loci and mtDNA sequence data indicate significant genetic differentiation between the two Aleutian Canada Goose populations, but a closer relationship to each other than either population is to Cackling Canada Geese. A neighbor-joining tree based on microsatellite data (Fig. 2) and an analysis of molecular variance from microsatellite data (F_{ST} and R_{ST}), as well as the mtDNA analog (Phi_{ST}, Table 1) supports this relationship. The Phi_{ST} value is approximately four times the value of the nuclear loci, as expected, because mtDNA is

maternally inherited and has an effective size 1/4 of nuclear markers (Avise 1994). Significant differences in haplotype heterogeneity ($P = 0.001 \pm 0.001$) also indicate a high degree of reproductive isolation between the two Aleutian Canada Goose populations. The statistically significant inter-population variance analyses of both mtDNA and microsatellite allele frequencies argue for consideration of these populations as separate management units (Moritz 1994).

Historically, there is evidence these populations were much smaller than they are today (USFWS 1991). Thus, an alternative explanation for the degree of differentiation between these two populations of Aleutian Canada Geese could be that population bottlenecks have caused large shifts in allele and haplotype frequency between the two populations. Indeed, the data show that Semidi Islands geese have fewer alleles overall and more heterozygotes than expected for five of seven microsatellite loci, as well as the lowest haplotype and nucleotide diversity for mtDNA, three potential indicators of a recent bottleneck (Nei et al. 1975). However, statistical tests of these data fail to indicate inbreeding or a recent bottleneck, indicating that if a bottleneck occurred, it was not of long duration. Our results suggest the differentiation observed is more likely due to limited contemporary gene flow than due to recent bottlenecks.

Although low sample size in the Semidi Islands population ($n = 18$) may explain the low number of alleles in comparison with our other two populations, it does not explain the increased levels of heterozygosity. Furthermore, a comparison of statistics for a Canada Goose population with identical sample size revealed greater allele abundance and lower than expected levels of heterozygosity (unpubl. data for Canada Geese from Washington state). Therefore, although microsatellite allele and mtDNA haplotype frequencies would likely change with increased sample sizes from the Semidi Islands, we do not believe observed locus characteristics are an artifact of low sample size.

A locus-by-locus evaluation of Wright's (1969) inbreeding coefficient (F) and statistical significance as described by Li and Horvitz (1953) shows inbreeding is insignificant for all populations at all loci with the exception of the Buldir Island population at locus TTUCG-4. The statistical significance of this locus is most likely an artifact of locus characteristics (i.e., only two alleles with the second allele occurring in only a few individuals from Buldir) and not an indication of inbreeding. F_{IS} values are not significant. The absence of additional significant inbreeding coefficients or F_{IS} values suggests that all populations surveyed are in Hardy-Weinberg equilibrium for all loci and do not appear to be inbred.

Both microsatellite and mtDNA frequency distributions are consistent with the current taxonomic separation of Aleutian Canada Geese from Cackling Canada Geese (Bellrose 1978). Shields and Wilson (1987) were able to differentiate Aleutian Canada Geese from Cackling Canada Geese based on restriction enzyme digests of the entire mtDNA for two individuals per population (Buldir, Chagulak, Kiliktagik, and Y-K Delta). However, these small sample sizes may not have been sufficient to establish taxonomic divergence (Shields and Wilson 1987). With our larger sample size, we were unable to designate individuals to a particular subspecies based on this short portion of mtDNA sequence alone. Shared microsatellite alleles and mtDNA haplotypes between both subspecies, and between the Aleutian Canada Goose populations, indicate either a common ancestry among all three populations, or recent, but limited gene flow, or both. The minimum spanning tree network (Fig. 3) provides a graphical summary of haplotype relationships with some geographic relevance. The lack of phylogenetically informative sites may be due to the small number of nucleotides sequenced (too few nucleotides sequenced for phylogenetic analysis) or because the subspecies have only recently diverged.

CONSERVATION IMPLICATIONS

Our objective was to assess the extent of gene flow and effects of population bottlenecks on Aleutian Canada Geese from Buldir and the Semidi Islands. The occurrence of shared alleles and haplotypes indicates common ancestry, but may suggest some level of historical gene flow as well. Statistical analyses of our data indicate there is little, if any, current gene flow between these populations; this is based on significant variances between populations for both microsatellites and mtDNA (Table 1). Although the populations on Buldir Island and the Semidi Islands have experienced different rates of population growth since listing, we found no statis-

tically significant genetic evidence that either has gone through a recent population bottleneck, although increased sample size and number of loci would increase the power of our tests. Aleutian Canada Geese from the central Aleutian Islands (Chagulak) were not available for analysis in this study. Because little is known about this population's migration corridors, wintering areas, current population numbers, or genetic status, it is possible that genetic analyses of this geographically intermediate population would reveal gene flow not detected in our study. We distinguish populations of Aleutian Canada Geese as genetically differentiated from each other. Furthermore, the lower number of alleles observed in the Semidi Islands population are consistent with the lower population numbers and slower growth rate relative to the Buldir Island population. Continued poor recruitment in the Semidi Islands population may jeopardize an irreplaceable component of the overall diversity of the subspecies. We recommend that future conservation measures for Aleutian Canada Goose populations include increasing genetic sample size in the Buldir and Semidi Islands, genetic sampling and analyses of geese from Chagulak Island in the central Aleutian Island chain to ascertain the role of that population in Aleutian Canada Goose genetic structure, as well as careful monitoring of the Semidi Islands population.

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