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Source: Waterbirds, 36(2) : 166-178

Published By: The Waterbird Society

URL: https://doi.org/10.1675/063.036.0206

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Population Genetics and Evaluation of Genetic Evidence for Subspecies in the Semipalmated Sandpiper (*Calidris pusilla*)

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Abstract.—Semipalmated Sandpipers (*Calidris pusilla*) are among the most common North American shorebirds. Breeding in Arctic North America, this species displays regional differences in migratory pathways and possesses longitudinal bill length variation. Previous investigations suggested that genetic structure may occur within Semipalmated Sandpipers and that three subspecies corresponding to western, central, and eastern breeding groups exist. In this study, mitochondrial control region sequences and nuclear microsatellite loci were used to analyze DNA of birds (microsatellites: $n = 120$; mtDNA: $n = 114$) sampled from seven North American locations. Analyses designed to quantify genetic structure and diversity patterns, evaluate genetic evidence for population size changes, and determine if genetic data support the existence of Semipalmated Sandpiper subspecies were performed. Genetic structure based only on the mtDNA data was observed, whereas the microsatellite loci provided no evidence of genetic differentiation. Differentiation among locations and regions reflected allele frequency differences rather than separate phylogenetic groups, and similar levels of genetic diversity were noted. Combined, the two data sets provided no evidence to support the existence of subspecies and were not useful for determining migratory connectivity between breeding sites and wintering grounds. Birds from western and central groups displayed signatures of population expansions, whereas the eastern group was more consistent with a stable overall population. Results of this analysis suggest that the eastern group was the source of individuals that colonized the central and western regions currently utilized by Semipalmated Sandpipers. *Received 6 August 2012, accepted 29 January 2013.*

Key words.—*Calidris pusilla*, genetic structure, microsatellite DNA, migratory connectivity, mitochondrial DNA, Semipalmated Sandpiper, subspecies.

Waterbirds 36(2): 166-178, 2013

Semipalmated Sandpipers (*Calidris pusilla*) are one of the most commonly observed North American shorebirds (Hicklin and Gratto-Trevor 2010). Using flyways along the U.S. Atlantic coast and the North American midwest, the species breeds in Arctic Canada and Alaska, and winters primarily in South America (Hicklin and Gratto-Trevor 2010; Gratto-Trevor *et al.* 2012). Estimates of the total number of Semipalmated Sandpipers are high for shorebirds (2,000,000-3,500,000; Morrison *et al.* 2000, 2006), making the species of "least concern" on the IUCN Red List (BirdLife International 2009). However, temporal trends indicate that population declines are likely occurring, just as they are with most shorebird species that breed in North America (Bart *et al.* 2007; Jehl 2007; Andres *et al.* 2012; Morrison *et al.* 2012).

No named subspecific forms are currently recognized for Semipalmated Sandpipers (American Ornithologists' Union 1957). However, Gratto-Trevor *et al.* (2012) recently postulated that up to three subspecies may exist based primarily on variation in average bill lengths of breeding individuals, with shorter bills in the west and longer bills in the east (Harrington and Morrison 1979). Discontinuities in the distributions of bill lengths suggest the presence of distinct western, central, and eastern breeding groups (Gratto-Trevor *et al.* 2012). Geographic variation in migratory patterns also exists. In spring, western and central Arctic breeders migrate north through the interior of North America, while eastern breeders use more easterly, often coastal, areas. In fall, western breeders migrate south through the interior

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of North America, while eastern and central breeders stage primarily on the Atlantic coast, especially in areas such as the Bay of Fundy, Canada (reviewed in Gratto-Trevor *et al.* 2012). Therefore, the central Arctic breeders in particular show an elliptical migration, migrating southwards considerably farther east than their northward spring migration route (Gratto-Trevor and Dickson 1994). In winter, considerable mixing occurs, although birds in Brazil appear to be almost entirely eastern breeders and virtually all on the Pacific coast of South America are from western breeding areas (Gratto-Trevor *et al.* 2012).

The geographic variation in morphology and migratory patterns suggests that there may be genetic structure within Semipalmated Sandpipers as a result of limited movement of individuals between eastern, central, and western Arctic breeding populations. Birds that demonstrate long distance migratory capabilities often display reduced genetic structure relative to their non-migratory counterparts (Miller *et al.* 2012b). However, genetic analyses in some cases nonetheless reveal substantial genetic structure that corresponds to the presence of distinct subspecies, even among species that engage in long-distance seasonal migration (Miller *et al.* 2010). In many cases, genetic structure may even be of sufficient magnitude such that genetic data can be used to help identify the breeding population of migrating or wintering individuals (Wenink *et al.* 1993; Webster *et al.* 2002; Haig *et al.* 2011).

Previously, genetic analyses of a small number of Semipalmated Sandpipers were performed as part of a multispecies study designed to assess the feasibility of using randomly amplified polymorphic DNA (RAPD) markers to track migratory shorebirds throughout the annual cycle (Haig *et al.* 1997). Inferences for Semipalmated Sandpipers were limited in scope due to the small number of individuals analyzed. Analyses based on DNA sequences of the nuclear β-fibrinogin intron seven locus have also revealed no genetic differentiation of mixed wintering populations from northern Brazil (Gonçalves *et al.* 2007). To date, however, there has not been a complete genetic analysis of breeding populations within the species. Thus, in this investigation, we used microsatellite loci and mitochondrial DNA sequences to: 1) quantify genetic differentiation, diversity, and population history patterns in Semipalmated Sandpipers, and 2) evaluate evidence to either support or refute the existence of different Semipalmated Sandpiper subspecies.

Methods

Sample Collection

Our samples included birds collected from seven locations across northern North America (Table 1; Fig. 1). With the exception of birds from Delaware Bay (New Jersey, USA), blood or tissue samples were obtained from adults captured on the nesting grounds during breeding season. Delaware Bay samples comprised the set of individuals with the longest bills captured during spring migration rather than nesting individuals (D. S. Mizrahi, pers. commun.). Given that these individuals possessed very long bills and were collected along the Atlantic Coast migratory flyway during spring, we assumed that all were eastern breeders for the purposes of our analyses. The assumption was later justified by our analyses, where we observed similar haplotype frequencies and no genetic differentiation between the Delaware Bay samples and known breeding samples from La Perouse Bay within the eastern group. Geographic groupings of sampling locations based on bird bill length variation (Gratto-Trevor *et al.* 2012) are shown in Fig. 1.

Molecular Methods

DNA extractions from blood or tissue samples were performed using standard phenol/chloroform extraction protocols (Sambrook *et al.* 1989). Microsatellite analyses were based on genotypes from 120 birds (Table 1), with each individual genotyped at nine microsatellite loci (Cme1, Cme2, Cme3, Cme4, Cme5, Cme6, Cme7, Cme9, Cme10) originally designed for *C. melanotos* as described by Carter and Kempenaers (2007). Primers for each locus were labeled with either 5' 6-FAM or HEX labels, and PCR products from each individual were run on an ABI 3730 automated DNA sequencer. Microsatellite allele sizes for each individual were determined using program GENEMAPPER (Applied Biosystems 2009).

Mitochondrial DNA (mtDNA) sequence data were obtained for a 470 bp region of the mitochondrial control region in 114 birds (Table 1). PCR amplification of the mtDNA region was performed using 10 mL reactions containing 1X PCR buffer (PerkinElmer, Inc.), 1.5 mM $MgCl_2$, 10 mM of each dNTP, 0.15 mM of each PCR primer TS96L and TS778H (Wenink *et al.* 1994),

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Table 1. Sample sizes (*n*) and genetic diversity estimates for seven Semipalmated Sandpiper collection locations grouped into three geographic regions. Numbers in parenthesis adjacent to each location name correspond to numbered locations in Fig. 1. H_e: expected heterozygosity; *A*: allelic richness; A_r: rarefied allelic richness; π: nucleotide diversity; *H*: haplotype diversity.

		Microsatellites				mtDNA				
Region	Location	*n*	H_e	*A*	A_r	*n*	π	*H*	*A*	A_r
Western	Nome (1)	6	0.697	5.22	4.73	4	0.0011	0.500	2	2.00
	Prudhoe (2)	18	0.663	7.33	4.43	18	0.0020	0.601	5	2.33
	Group	24	0.668	7.78	7.69	22	0.0018	0.580	6	6.00
Central	Mackenzie Delta (3)	20	0.689	8.11	4.69	19	0.0014	0.573	4	2.24
	Banks Island (4)	20	0.724	7.56	4.77	18	0.0010	0.471	2	1.83
	Rasmussen (5)	13	0.677	6.67	4.56	12	0.0014	0.455	4	2.00
	Group	53	0.698	9.33	8.06	49	0.0013	0.503	6	4.05
Eastern	La Perouse Bay (6)	21	0.667	7.44	4.50	21	0.0028	0.471	4	1.98
	Delaware Bay (7)	22	0.727	7.78	4.72	22	0.0029	0.455	2	1.81
	Group	43	0.702	8.67	7.84	43	0.0028	0.457	4	3.02
Total		120				114				

and 0.2 U of AmpliTaq Gold (PerkinElmer, Inc.). Thermal-cycling parameters included 30 cycles of denaturing at 94 °C (30 sec), annealing at 50 °C (30 sec), and extension at 72 °C (60 sec). PCR products were visualized via agarose gel electrophoresis with ethidium bromide staining to confirm successful amplification. PCR products were bi-directionally sequenced with BigDye version 3 dye terminator sequencing chemistry using primers TS96L and TS778H. Sequencing reactions were resolved on an ABI 3730 automated DNA sequencer, with resulting chromatograms aligned, edited, and trimmed to the final 470 bp alignment length using program BIOEDIT (Hall 1999).



Figure 1. Map highlighting seven geographic locations where individual Semipalmated Sandpipers (*Calidris pusilla*) were collected for genetic analyses. Circles, triangles, and squares represent locations that were grouped into western, central, and eastern breeding groups based on Gratto-Trevor *et al.* (2012). Location names associated with each number are provided in Table 1. With the exception of the Delaware Bay samples (location 7), all locations corresponded to Semipalmated Sandpiper breeding sites. Samples from location 7 were from very long billed spring migrants and assumed to be part of the eastern geographic group.

Genetic Differentiation, Diversity, and Population History

Genetic diversity was calculated for each sampling location and geographic region using several different measures. For the mtDNA data, we used Arlequin (Excoffier *et al.* 2005) to calculate haplotype diversity (*H*) and nucleotide diversity (π). Program HP-Rare (Kalinowski 2005) was used to calculate allelic richness (*A*: the observed number of alleles) within each sampling location as well as a rarefaction-based estimate of allelic richness (A_r) that accounted for sample size differences among groups of individuals. For the microsatellite data, program GDA version 1.1 (Lewis and Zaykin 2001) was used to calculate expected heterozygosity (H_e) and HP-Rare was used to calculate *A* and A_r for the microsatellite data. GDA was further used to test for deviations from Hardy-Weinberg genotypic proportions at each locus and to test for linkage disequilibrium between pairs of loci. Hardy-Weinberg disequilibrium test results were combined over loci by using the *Z*-transform test based on locus-specific *P*-values (Whitlock 2005).

We used two approaches to evaluate genetic structure patterns. First, the Bayesian clustering algorithm encapsulated in program STRUCTURE (Pritchard *et al.* 2000) was used to identify the number of clusters suggested by the microsatellite loci and to perform anonymous individual cluster assignments without reference to the collection location of each sample. We used 10 replicates of each assumed number of clusters (*K*), where *K* ranged from one through six. Individual analysis replicates were based on $2*10^5$ burn-in steps followed by $3*10^6$ analysis steps. Analysis options included

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the correlated allele frequency model and the admixture model as suggested by Falush *et al.* (2003). Analysis results from values of *K* that resulted in the highest average likelihood score were summarized using the computer program CLUMPP (Jakobsson and Rosenberg 2007). Second, we used Arlequin to obtain global and pairwise measures of genetic structure using the Analysis of Molecular Variance (AMOVA; Excoffier *et al.* 1992) framework. Estimates of differentiation based on Φ_{ST} were calculated for the mtDNA data set, whereas in Weir and Cockerham's (1984) θ was calculated for the microsatellites. Analysis variants were performed where individuals were placed in separate western, central, or eastern groups and where the seven individual collections were treated as separate "populations" for the purposes of the analysis. The significance of global and pairwise values of Φ_{ST} and θ were obtained based on a randomization procedure using 10,000 randomization replicates.

We explored population histories by evaluating evidence for prior population bottlenecks or expansions. We used program BOTTLENECK (Piry *et al.* 1999) to test the microsatellite data for signatures of recent genetic bottlenecks within the western, central, and eastern groups of individuals. We performed separate analyses that assumed either a strict stepwise mutational model, the infinite alleles model, or the more realistic two-phase model that incorporates aspects of both the stepwise and infinite alleles models (Di Rienzo *et al.* 1994). In two-phase model analyses, we assumed that either 60% or 80% of mutations were pure stepwise mutations. These values bracket the range of values observed in studies of microsatellite mutational dynamics in avian pedigrees (Miller *et al.* 2012a). We likewise specified two-phase model variances to be 2, 4, 9, 16, or 25. Each analysis was performed using 10,000 replicate simulation realizations, with results over loci derived from the Wilcoxon Signed-Rank test (Cornuet and Luikart 1996). The mtDNA data were used to evaluate evidence for population expansions within each region. Analyses were performed using DnaSP (Librado and Rozas 2009) and included calculation of Tajima's *D* (Tajima 1989), Fu's (1997) F_S statistic, and the R2 statistic of Ramos-Onsins and Rozas (2002). These statistics are among the most useful for detecting past population expansions (Ramos-Onsins and Rozas 2002). DnaSP was used to implement coalescent-based simulations to determine the significance of each statistic based on 10,000 replicates. We further considered evidence for population expansions using mismatch distributions (Rogers and Harpending 1992). In this case, DnaSP was used to compare observed mismatch distributions to expected mismatch distributions from constant-sized and growing populations.

Genetic Evidence for the Presence of Subspecies

We used our data to determine if the microsatellite loci and mtDNA sequences could be used to diagnose different subspecific forms (*sensu* Patten and Unitt 2002). We considered the degree of differentiation of the three geographic regions by evaluating the extent of mtDNA differentiation and haplotype sharing between putative subspecies. We used program TCS (Clement *et al.* 2000) to generate a haplotype network and infer the genealogy of mtDNA sequences using the statistical parsimony approach (Templeton *et al.* 1992) with 95% connection confidence. The haplotype network allowed us to determine if haplotype groups could be identified that corresponded to different geographic groupings of individuals. We also considered results of our STRUCTURE analyses (Pritchard *et al.* 2000). If STRUCTURE identified more than one cluster, then patterns of individual assignments to different clusters may help provide evidence for the presence and diagnosability of distinct subspecies (Miller *et al.* 2010).

Results

Genetic Differentiation, Diversity, and Population History

We observed no evidence for deviations from Hardy-Weinberg genotypic proportions at any individual sampling location after Bonferroni corrections for multiple tests. However, while no single locus produced a significant outcome, a significant combined probability test over loci was observed for the central group when all individuals from the region were analyzed as a single unit ($z = -2.40$, $P = 0.008$). No evidence for linkage disequilibrium between pairs of loci was detected. Five percent ($n = 108$) of tests performed across the three geographic regions (3 regions × 36 tests of locus pairs per region = 108 tests) produced significant results at the $\alpha = 0.05$ level, consistent with random expectations. Likewise, only five of the 252 linkage disequilibrium tests (~2%) performed across all seven sampling locations produced a significant outcome at the 0.05 level. Genetic diversity levels within the microsatellite data were similar among individual sampling locations and geographic regions (Table 1).

We observed 11 unique mtDNA haplotypes among the 114 individuals sequenced (Table 2; Genbank accession numbers JX437167-JX437177). One haplotype (H1) was shared by 77 (68%) individuals across all regions. Two additional haplotypes (H3 and H4) were also detected in more than one geographic region, but at much lower frequencies (Table 2). With the exception of

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Table 2. Observed frequencies of 11 mtDNA haplotypes within geographic regions and collection sites for Semipalmated Sandpipers.

	Haplotype											
Area	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	Total
Region												
Western	14	1	1	4	1	1						22
Central	33			2			1	11	1		1	49
Eastern	30		11	1						1		43
Total	77	1	12	7	1	1	1	11	1	1	1	114
Location												
Nome	3					1						4
Prudoe	11	1	1	4	1							18
Mackenzie Delta	12			2				4			1	19
Banks Island	12							6				18
Rasmussen	9						1	1	1			12
La Perouse Bay	15		4	1						1		21
Delaware Bay	15		7									22
Total	77	1	12	7	1	1	1	11	1	1	1	114

H8, which was detected at moderate levels within the central region locations, the remaining haplotypes were detected in single individuals. The La Perouse Bay and Delaware Bay samples from the eastern region possessed similar haplotype frequencies (Table 2), which further justified treatment of the Delaware Bay samples as part of the eastern breeding group. As with the microsatellite data, genetic diversity levels were largely similar among sampling locations and regions (Table 1). The possible sole exception was the rarefied estimate of allelic richness (A_r) obtained for the mtDNA data from the western group. In this case, the rarefied number of alleles (haplotypes) was nearly twice the comparable number observed within the eastern group.

Genetic structure patterns varied depending on the data set analyzed (microsatellites vs. mtDNA). The microsatellite data provided no evidence of genetic structure within the species. STRUCTURE (Pritchard *et al.* 2000) analyses indicated that the $K = 1$ solution was most likely, suggesting the presence of a single Semipalmated Sandpiper genetic cluster (Fig. 2). Likewise, global estimates of θ from the microsatellites were low and non-significant (Tables 3 and 4). A single pairwise contrast between the Prudhoe and Banks Island sampling locations was significant ($\theta = 0.173$; $P = 0.001$), but we considered this result to be a likely Type I error associated with performing large numbers of individual analyses.

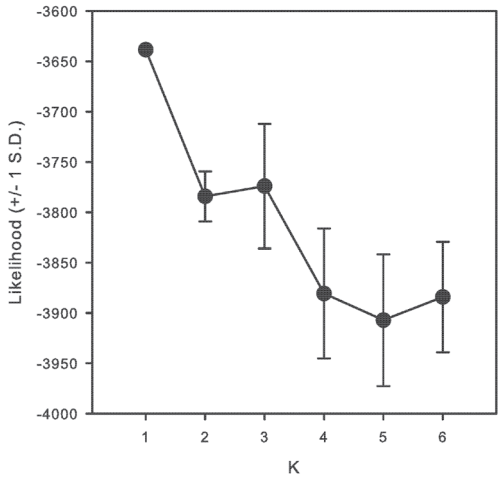


Figure 2. Results of analyses based on program STRUCTURE (Pritchard *et al.* 2000) for Semipalmated Sandpipers (*Calidris pusilla*). The greatest overall likelihood was associated with the K = 1 case, indicating the presence of a single genetic cluster.

Unlike the microsatellites, the mtDNA data revealed moderate levels of differentiation among regions ($\Phi_{ST} = 0.166$; $P < 0.001$) and individual sampling locations ($\Phi_{ST} = 0.124$; $P < 0.001$). Values of Φ_{ST} for pairwise combinations of regions ranged from 0.089 to 0.221, and all were significant at the 0.05

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Table 3. Global and pairwise estimates of Φ_{ST} (mtDNA) and θ (microsatellites) obtained for three regional groupings of Semipalmated Sandpiper samples. Values of Φ_{ST} and θ are below matrix diagonals, and associated P-values are above the diagonals. Significant pairwise values at the α = 0.05 level are highlighted in bold.

Area		Western	Central	Eastern
Microsatellites				
Overall θ = 0.003, *P* = 0.960	Western	—	0.186	0.225
	Central	0.003	—	0.066
	Eastern	0.004	0.004	—
mtDNA				
Overall Φ_{ST} = 0.166 *P* < 0.001	Western	—	**0.003**	**0.037**
	Central	**0.089**	—	**0.001**
	Eastern	**0.103**	**0.221**	—

level (Table 3). Likewise, Φ_{ST} from pairwise combinations of sampling locations ranged from negative values up to 0.275 (Table 4). In general, significant tests were associated with contrasts involving sampling location pairs from different geographic regions (Table 4). No significant differentiation was detected between location pairs from the same region.

We found little evidence for the occurrence of recent past bottlenecks in any regional group. Of the 36 individual tests performed across different mutational models and mutational model parameter sets, we identified only one significant test result (analysis of the eastern region under the infinite alleles model; $P = 0.014$). *P*-values from the infinite alleles-based tests were > 0.10 for the other two regions, and *P*-values from tests based on the more realistic two-phase model were > 0.5 in all three regions. In contrast, results of the mtDNA data analyses were suggestive of past population expansions within the western and central regions (Table 5). In this case, significant (or approximately significant) analysis results were associated with all three test statistics, whereas the eastern region produced clear non-significant analysis outcomes. Mismatch distributions were less informative for the western and central groups, where the observed distributions and expected distributions under the constant-sized and expanding population models were very similar (Fig. 3). However, a bimodal mismatch distribution was detected in the eastern region that was conspicuously different from both theoretical models (Fig. 3).

Genetic Evidence for the Presence of Subspecies

The statistical parsimony network produced by our mtDNA data revealed a shallow, star-like genealogical relationship among haplotypes (Fig. 4) as opposed to evidence for the presence of deep phylogenetic structure, regional haplotype groups, or different subspecies. Because results of STRUCTURE analyses (Fig. 2) indicated that the $K = 1$ solution was most likely, the microsatellite data also provided limited evidence to support the presence of distinct genetic groups in Semipalmated Sandpipers.

Discussion

Results of our genetic analyses are likely consistent with those expected for a bird species that possesses long distance migration capabilities and large population sizes. Evidence for subtle genetic structure exists among breeding populations of Semipalmated Sandpipers across North America. The structure is not apparent in the nuclear microsatellite data (Tables 3 and 4; Fig. 2) and is only reflected by small differences in mtDNA haplotype frequencies among regions (Table 2). Population genetic theory predicts that genetic structure is usually stronger in the mitochondrial versus nuclear genome due to its haploid structure and uniparental (maternal) inheritance (Barrowclough and Zink 2009), which leads to faster genetic drift and shorter coalescence times. Sex-biased dispersal could also nominally account for such patterns if

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Table 4. Global and pairwise estimates of Φ_{ST} (mtDNA) and θ (microsatellites) obtained for Semipalmated Sandpipers collected at seven different locations. Values of Φ_{ST} and θ are below matrix diagonals and associated *P*-values are above the diagonals. Significant pairwise values at the α = 0.05 level are highlighted in bold. Individual location numbers correspond to those listed in Fig. 1.

		1	2	3	4	5	6	7
Microsatellites								
Overall θ = 0.001, *P* = 0.999	Nome (1)	—	0.855	0.242	0.448	0.170	0.223	0.938
	Prudhoe (2)	-0.013	—	0.844	0.706	0.610	0.089	0.531
	Mackenzie Delta (3)	0.009	-0.005	—	0.902	0.866	0.108	0.596
	Banks Island (4)	0.003	-0.003	-0.006	—	0.513	**0.012**	0.721
	Rasmussen (5)	0.015	-0.002	-0.007	0.000	—	0.303	0.315
	La Perouse (6)	0.012	0.010	0.008	**0.017**	0.004	—	0.136
	Delaware Bay (7)	-0.014	0.001	-0.001	-0.002	0.005	0.008	—
mtDNA								
Overall Φ_{ST} = 0.124, *P* < 0.001	Nome (1)	—	0.446	0.326	0.103	0.727	0.312	0.103
	Prudhoe (2)	0.005	—	0.064	**0.001**	0.067	0.154	**0.035**
	Mackenzie Delta (3)	0.038	0.049	—	0.372	0.283	**0.014**	**0.002**
	Banks Island (4)	0.175	**0.173**	-0.003	—	0.107	**0.002**	**0.003**
	Rasmussen (5)	-0.024	0.049	0.009	0.076	—	0.059	**0.028**
	La Perouse (6)	0.043	0.050	**0.145**	**0.202**	0.106	—	0.560
	Delaware Bay (7)	0.126	**0.131**	**0.231**	**0.275**	**0.187**	-0.030	—

Table 5. Results of tests for past population expansions within the western, central, and eastern Semipalmated Sandpiper breeding regions using analyses of the mtDNA data generated in this study. Test statistics and *P*-values are reported for Tajima's *D* (Tajima 1989), Fu's (1997) F_S, and the R2 statistic of Ramos-Onsins and Rozas (2002).

Regions	*D*	*P*-value	Fs	*P*-value	R2	*P*-value
Western	-1.50	0.050	-2.62	0.020	0.09	0.073
Central	-1.42	0.051	-2.67	0.045	0.07	0.119
Eastern	0.38	0.675	1.56	0.813	0.13	0.670

female Semipalmated Sandpipers demonstrate markedly greater natal and breeding site fidelity than males. However, most bird species demonstrate female-biased dispersal rather than site fidelity (Greenwood 1980; Clarke *et al.* 1997), and furthermore, no evidence for any sex-biased dispersal exists in Semipalmated Sandpipers (Gratto *et al.* 1985; Clarke *et al.* 1997; Jehl 2007). Our results, therefore, likely indicate that mtDNA provides a better understanding of genetic structure patterns in Semipalmated Sandpipers than microsatellites.

The level of mitochondrial differentiation detected in Semipalmated Sandpipers is lower than that observed among Dunlin (*C. alpina*), a wader that has numerous described subspecies and an even broader breeding distribution (Wenink *et al.* 1996). In this case, as opposed to our detection of a single common haplotype among the majority of individuals in our analysis (Table 2), Dunlin breeding in Alaska, central Canada, and Greenland possess different haplotype groups (Wenink *et al.* 1996). Likewise, the proportion of individuals possessing the same control region mtDNA haplotype in Semipalmated Sandpipers (68%; Table 2) is roughly comparable to results from analyses of *C. temminckii*, where 81% of individuals sampled from Fennoscandia and Siberia harbored the same mtDNA haplotype (Rönkä *et al.* 2008). The value of Φ_{ST} reported for *C. fuscicollis* in Arctic Canada (global Φ_{ST} = 0.066; Wennerberg *et al.* 2002) is nonsignificant and numerically lower than comparable values obtained for Semipalmated Sandpipers (Tables 3 and 4), but a direct comparison of data sets is likely confounded by differences in the spatial scales of each investigation and the low power of the *C. fuscicollis* analysis due to very small sample sizes.

Low levels of genetic structure and differentiation among populations may be detected in a species for two main reasons. First, individual dispersal among breeding sites can promote gene flow and lead to the maintenance of similar allele frequencies among populations. Assuming an idealized island model of population structure at drift-

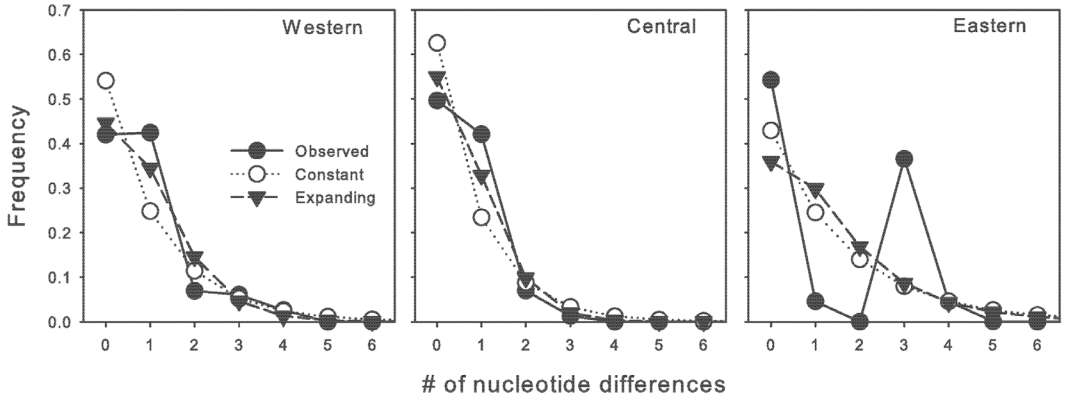


Figure 3. Observed mismatch distributions generated from mitochondrial DNA sequences among western, central, and eastern Semipalmated Sandpiper groups. Expected mismatch distributions from a constant-sized population and expanding population are provided for comparison purpose.

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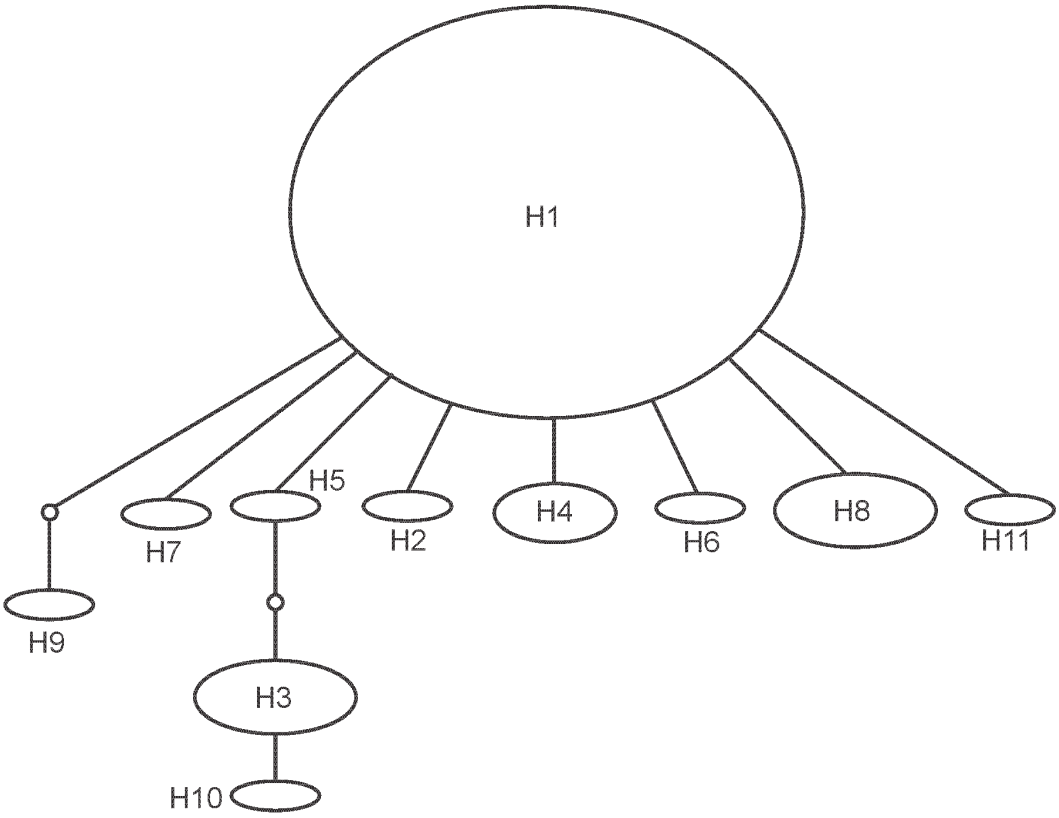


Figure 4. Haplotype network illustrating relationships among 11 unique control region haplotypes detected in Semipalmated Sandpipers (*Calidris pusilla*). Frequencies of each haplotype are listed in Table 2. The size of each symbol in the network reflects the overall frequency of each haplotype. Lines reflect the number of mutational differences between haplotypes.

migration equilibrium, the well-known expressions $F_{ST} \approx 1/(4Nm + 1)$ or $F_{ST} \approx 1/(2Nm + 1)$ can be solved for diploid or haploid genomes, respectively, to obtain numerical values for *Nm*: the effective number of migrants per generation required to account for the observed level of genetic structure (Slatkin 1985). Acknowledging the caveats and criticisms associated with literally interpreting such migration rates (Whitlock and McCauley 1999), the global values of θ and Φ_{ST} listed in Table 3 provide estimates of ~83 and ~2.5 migrants per generation for the nuclear and mitochondrial data sets, respectively. Semipalmated Sandpipers are considered to have high natal and breeding site fidelity, or low levels of dispersal, among breeding populations. Of the thousands of individual birds banded at a natal or breeding site and resighted on the breeding grounds, none has ever been detected at a different breeding location to provide direct evidence of gene flow (C. Gratto-Trevor, unpubl. data). In all likelihood, if dispersal is occurring, it is probably prevalent at a sufficiently low level such that it goes undetected within the Semipalmated Sandpiper breeding populations that are routinely monitored. Given the large global population size of the species, a substantial number of individuals would need to be banded on the breeding ground for detection of infrequent dispersal events.

Low genetic structure may also be observed if Semipalmated Sandpipers underwent a large-scale population expansion that carried representative genetic diversity from an initial source population to new locales. If a comparatively recent occurrence

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relative to the age of the source population, then insufficient time may have elapsed for genetic drift to promote differentiation of groups, especially if said groups are large. Long distance seasonal migration to latitudinal extremes is considered to be a mechanism that allows species to minimize intra- and interspecific competition for resources in more temperate or tropical regions (Cox 1968). Migration is generally considered to be a recently-evolved behavior that arose in response to glacial retreat at the end of the Pleistocene (Cox 1985; Milá *et al.* 2000; Milá *et al.* 2006). Given a release from competitive constraints on population size, populations may plausibly increase dramatically. Population size increases may also be facilitated by the capacity for long distance movement that allows species such as the Semipalmated Sandpiper to occupy the full spatial extent of seasonally favorable habitats across the North American Arctic. In our analyses, evidence for population expansions was evident, particularly in the central and western groups of samples (Table 5). Likewise, the overall haplotype network (Fig. 4) generated from our mtDNA sequences was starlike and consistent with the genealogical relationships that occur during population establishment and growth (Slatkin and Hudson 1991). In contrast, while the western and central groups showed no clear patterns in their mismatch distributions (Fig. 3), the eastern group demonstrated a multimodal distribution. While this distribution also differed from the theoretical distribution produced by DnaSP under expectations for a constant-sized population, multimodal distributions are much more consistent with the signature of a long-term, stable population than they are with a population experiencing rapid growth (Slatkin and Hudson 1991). Combined, these results may indicate that the eastern group represents the ancestral population that served as the sources of individuals that ultimately occupied the central and western regions.

Genetic data are among the varied types of information that may be useful in confirming (Miller *et al.* 2010) or refuting (Draheim *et al.* 2010) the validity of different subspecies (Haig *et al.* 2006; 2011). Fourteen out of the 52 North American shorebird species possess named subspecies, with four of these poly-subspecific taxa found among the 13 North American species of *Calidris* (American Ornithologists' Union 1957; Morrison *et al.* 2006). In our analyses of Semipalmated Sandpipers, the microsatellite data provided no evidence for population structure across the species' range (Tables 3 and 4; Fig. 2). While mtDNA illustrated the evidence for allele frequency differences and some genetic structure among groups (Tables 3 and 4), most individuals shared the same common haplotype (Table 2). The genetic data, therefore, provide no evidence to support the existence of different subspecies within the Semipalmated Sandpiper and, furthermore, do not provide a reliable means for diagnosing among putative groupings of the birds included in this investigation. By extension, the genetic data have limited utility for identifying the respective breeding populations associated with individuals captured on wintering grounds or during migration.

Information on morphology or behavior is also possibly useful in determining if valid subspecies exist (Haig *et al.* 2006). Variation in bill length is currently considered to be useful for identifying Semipalmated Sandpiper breeding groups in North America, and has also been discussed as a possible indicator for the presence of different subspecies (Gratto-Trevor *et al.* 2012). Neutral genetic loci such as the mtDNA and microsatellites included in our analyses may not reflect patterns seen at functionally-relevant characters under natural selection (Greenberg *et al.* 1998; Crandall *et al.* 2000). However, it is currently unknown if longitudinal bill length variation in Semipalmated Sandpipers has a functional genetic basis, let alone if it is influenced by selective processes. Harrington (1982) reported that long-billed individuals forage in muddier habitats than short-billed individuals, which may imply advantages and disadvantages of shorter or longer bills in slightly different habitats. However, this study possessed small sample sizes and was restricted to a few geographic locations in the eastern USA during migra-

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tion. Gratto *et al.* (1984) noted that female Semipalmated Sandpipers, which possess longer bills than males, tended to consume longer *Corophium* prey items during migration through the Bay of Fundy, Canada. While such patterns suggest functional differences in bill lengths, it is unclear if this sexually dimorphic pattern also holds for the longitudinal patterns observed within both sexes across northern North America. Baker (1979) illustrated that variation in culmen lengths among six shorebird species (including Semipalmated Sandpipers) was associated with vegetation height and diversity within individual feeding areas. As with the other studies noted above, it is unclear if these findings also reflect the continent-wide longitudinal variation in feeding habits within Semipalmated Sandpipers alone. More detailed information on regional variation in feeding habitats and prey species may be required to better understand the source of continent-wide bill length variation in this species. Further, the degree with which bill length can reliably diagnose (*sensu* Patten and Unitt 2002) breeding groups is currently unknown. Rather than defining distinct groups, bill length may instead represent a simple cline, with the appearance of discontinuities in the cline stemming from sampling of individuals at discrete locations (Patten and Unitt 2002). Therefore, a formal evaluation of the true diagnostic utility of this character is likely needed to provide final resolution to the issue.

ACKNOWLEDGMENTS

We acknowledge the U.S. Geological Survey, Forest and Rangeland Ecosystem Science Center, and the Science and Technology Branch, Environment Canada, for financial support. We would like to thank the Canadian Wildlife Service (Environment Canada), Queen's University, Manomet Centre for Conservation Sciences, and Cumulative Impacts Monitoring Program (Aboriginal Affairs and Northern Development Canada) for logistical support while collecting samples. Assistance in collecting samples was provided by M. Colwell, V. Johnston, S. Pepper, J. Rausch, and L. Pirie. Work from this study was covered under the following permits: USGS-Bird Banding Laboratory permit #21241; Environment Canada scientific permit WSS-03-NWT/91; NWT Wildlife Research permits 1700, WL000240, and WL001078; Environment Canada Animal Care permits 1991-1995; Endangered/Threatened Species Wildlife Permit # MA10833A-0; APHIS Permit for Importation and Transportation of Controlled Materials and Organisms and Vectors (outside of U.S.) #101946; and Migratory Bird Import/Export Permit #MB175920-0. Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

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