

**BIOLOGICAL IMPACTS OF CLIMATIC CHANGE ON A BERINGIAN
ENDEMIC: CRYPTIC REFUGIA IN THE ESTABLISHMENT AND
DIFFERENTIATION OF THE ROCK SANDPIPER
(*CALIDRIS PTILOCNEMIS*)**

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Abstract. The importance of climatic change on the establishment and differentiation of high-latitude species is largely unknown. Biological effects of historic climate change can be determined from historic signals in genetic data. The rock sandpiper (*Calidris ptilocnemis*) is a good test case for examining these biological effects because it has a known sister species, the purple sandpiper (*Calidris maritima*), and it is the only endemic Beringian bird with multiple described subspecies, suggesting a process of initial establishment and subsequent differentiation in the high-latitude regions of Beringia. We sequenced 2,074 bp of mitochondrial DNA from 40 rock sandpipers from nine breeding locations and four purple sandpipers. We used phylogenetic and coalescence methods to reconstruct trees and to evaluate the population demography, migration rates, and relative times of population divergence. Phylogenetic trees show that purple and rock sandpipers are monophyletic sister species. Within the rock sandpiper, clade branching patterns and coalescence estimates suggest that there were multiple refugial populations in Beringia, which correspond loosely to different glacial cycles. Rock sandpipers show the establishment, persistence, and accumulation of partitioned genetic diversity across several glacial cycles, implicating the presence of multiple cryptic biological refugia in this region through repeated cycles of climate change.

1. Introduction

The importance of climatic change and biological refugia in the establishment and differentiation of high-latitude species is largely unknown. The biological effects of historic climate change can be determined from historic signals in genetic data. This is accomplished by examining the genetic markers that are assumed to be acquiring mutations in a nearly neutral manner (markers not under strong selection pressures), and so the fate of these mutations within isolated populations (a population that does not interbreed with other populations) are primarily determined through genetic drift (Kimura, 1983; Hartl and Clark, 1997). As time elapses, some new mutations become fixed, while ancestral mutations (those shared with the parental population) are randomly lost (Hartl and Clark, 1997). By examining these changes, inferences about the history and the effects of climate change on these populations can be determined.

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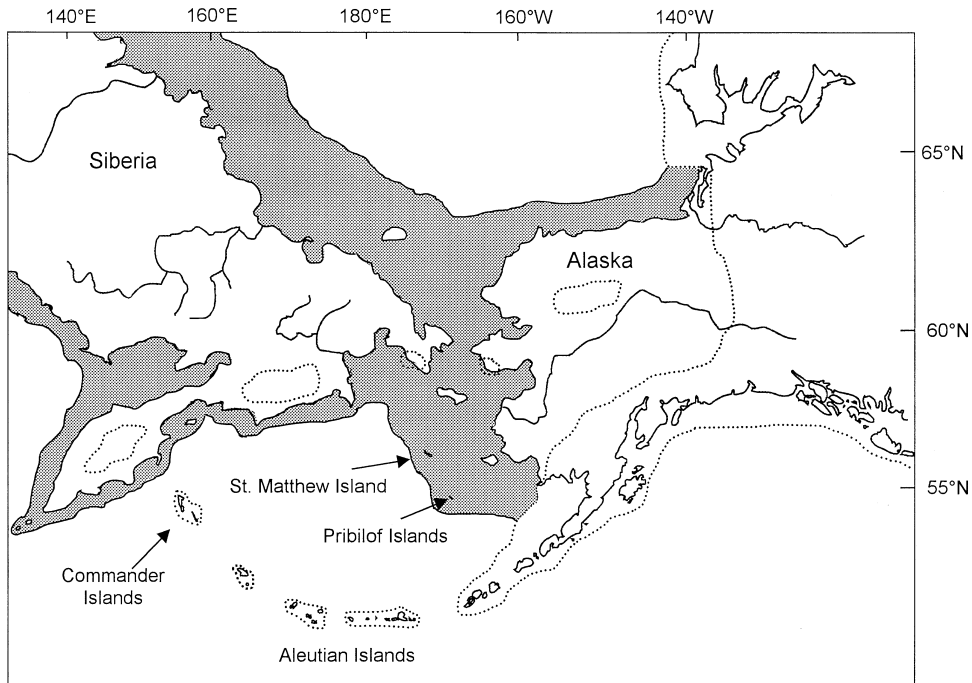


Figure 1. Map of Beringia including the extent of the Bering land bridge (shaded in gray), present sea levels or coastlines and areas glaciated during the last glacial maximum (areas within dotted lines).

Beringia, which includes most of Alaska, northeastern Siberia, and the Bering Strait, was strongly affected by climate change during the Wisconsin glaciation (10,000–117,000 ybp; Hamilton et al., 1986; Williams et al., 1998). Geologic evidence suggests that during this hundred-thousand year glacial period, there were multiple cycles of warming and cooling (interstadials), which likely caused the expansion and contraction of ice-free habitats (Hopkins et al., 1982; Hamilton et al., 1986). These glacial cycles also caused sea levels to fluctuate, which intermittently exposed and submerged the Bering land bridge that connected Asia and North America (Hopkins, 1967; Hopkins et al., 1982; Pielou, 1991; Figure 1).

By the time of the last glacial maximum (LGM: 10,000–30,000 ybp), ice covered substantial portions of the current distributions of birds breeding in Beringia. However, ice-free areas probably served as refugia (a biotic refuge from surrounding inhospitable conditions) for many species (Hamilton et al., 1986; Hewitt, 2000; Stewart and Lister, 2001; Figure 1). There is evidence that at least one large glacial refugium was present in Beringia in the areas north of the ice in the Bering land bridge area and in present-day Alaska and Siberia (Pielou, 1991; Figure 1). Researchers studying the phylogeography (geographic distributions of genetic lineages; Avise, 2000) of two bird species, the rock ptarmigan (*Lagopus mutus*; Holder et al., 1999) and the marbled murrelet (*Brachyramphus marmoratus*; Congdon et al.,

2000), have postulated that one or two previously unknown refugia were present in the Bering Sea region, likely in the Aleutian Islands. However, there is presently no geological or fossil evidence to corroborate these cryptic Bering Sea refugia.

Species that have all or most of their breeding distributions in Beringia would have been strongly affected by these climate changes, and thus could serve as useful models for examining the biological effects of climate change and the importance of refugia for high-latitude taxa during the Wisconsin period. To gain a better understanding of the biological effects of historic climate change in high latitudes, we examined the phylogeographic and demographic history of the rock sandpiper (*Calidris ptilocnemis*), a species endemic to Beringia.

Several factors make rock sandpipers ideal for determining the biological effects of climate change in Beringia. First, because both morphological (Gill et al., 2003) and molecular (Borowik, 1996; Borowik and McLennan, 1999) evidence has shown that purple sandpipers (*Calidris maritima*) are probably the sister taxon or closest extant relative of rock sandpipers, it is possible to examine whether the Wisconsin glacial cycles caused recent speciation between these two taxa. These two species are very similar morphologically, and at one time were considered to be the same species ('*Arquatella*' *maritima*; American Ornithologists' Union, 1910). Later they were separated because of differences in breeding plumage and their disjunctive breeding distributions (American Ornithologists' Union, 1931). Although current gene flow (exchange of genes between populations due to interbreeding) is unlikely, at least one purple sandpiper has occurred in Alaska in the last 20 years (Gibson and Kessel, 1992). It is possible that rock sandpipers diverged genetically from purple sandpipers during the Wisconsin glaciation (<117,000 ybp) due to isolation.

Secondly, rock sandpipers are the only endemic Beringian bird with recognized subspecies (American Ornithologists' Union, 1957), or formally recognized intra-specific groupings based on morphological distinctiveness. The rock sandpiper has four subspecies: *ptilocnemis*, *tschuktschorum*, *couesi* and *quarta* (Peters, 1934; American Ornithologists' Union, 1957; Gibson and Kessel, 1997; Figure 2). Other birds endemic to Beringia are thought to be monotypic (lacking described subspecies; American Ornithologists' Union, 1957; Fay and Cade, 1959). The lack of spatially structured variation within other endemic Beringian birds would seem to fit a model of a single Beringian biological refugium. But sub-specific differentiation in rock sandpipers, which implies historic patterns of population isolation, suggests the possibility that historic climatic effects in the region caused there to be multiple glacial refugia in this region. In rock sandpipers, intergrades between subspecies have been documented (Murie, 1959), and the distributional limits of some subspecies are uncertain. It is unclear whether these sub-specific differences are due to protracted isolation in several glacial refugia, to variable ecological factors (e.g., rainfall, temperature, or food sources) affecting different populations, or to both. By examining each subspecies genetically, it is possible to determine whether a single refugium or multiple refugia harbored this species in the Wisconsin period

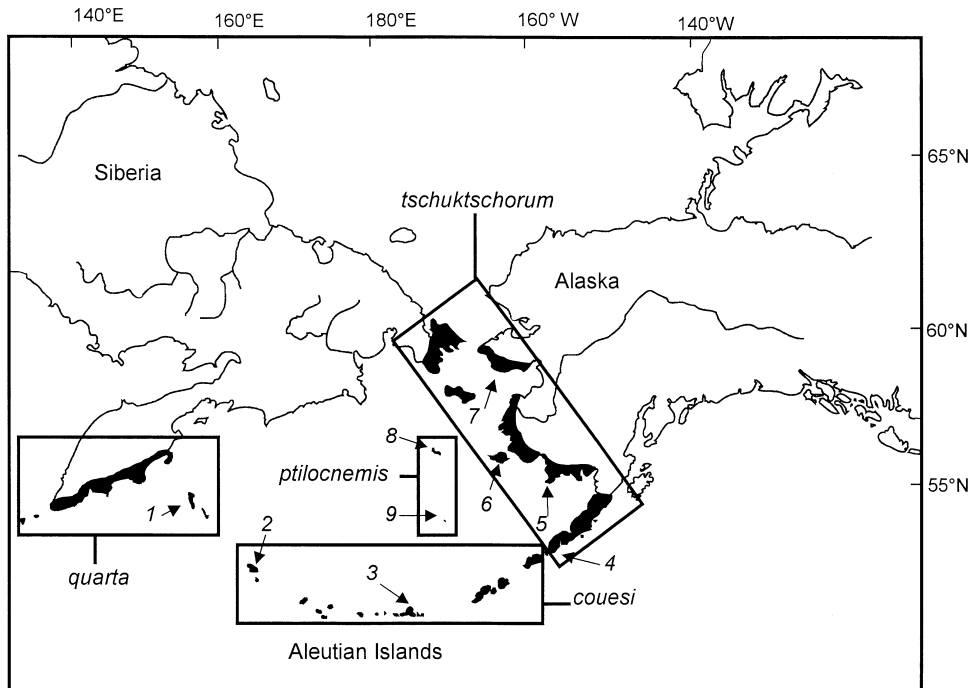


Figure 2. Map of current rock sandpiper breeding range (shaded) outlined by subspecies. Numbers correspond to collection locations of birds used in this study: 1) Commander Islands, 2) Attu Island, 3) Adak Island, 4) Izembek Lagoon, 5) Cape Peirce, 6) Nunivak Island, 7) Nome, 8) St. Matthew Island, and 9) Pribilof Islands.

and to determine how past episodes of climate change have affected a Beringian endemic.

Thirdly, a portion of the current breeding distribution of rock sandpipers was glaciated during the LGM (Figures 1 and 2), and thus a scenario of postglacial population expansion out of glacial refugia and into newly available habitat is likely. Several unglaciated areas might have served as sources for postglacial population expansions, including a large Beringian refugium or a refugium among islands in the Bering Sea. Most subspecies' distributions are near to at least one of these possible refugia (Figures 1 and 2). Thus, the phylogeography of rock sandpipers throughout much of their breeding distribution should provide insight into whether the currently recognized sub-specific differences are due to historic isolation in different glacial refugia.

Using genetic data (mtDNA) for individuals from all of the subspecies of rock sandpipers and from several purple sandpipers, we examine the phylogeographic patterns of these taxa to answer three questions about the biological effects of climate change in Beringia: 1) did rock and purple sandpipers differentiate as a result of recent glacial cycles; 2) were rock sandpipers isolated in a single glacial

refugium or multiple refugia during the LGM, with recent population expansion into the current breeding range; and 3) is there a sub-specific correspondence between refugial source(s) and expansion areas?

2. Materials and Methods

2.1. DNA SEQUENCING

Whole genomic DNA was extracted (Glenn, 1997) from the tissues of 40 rock sandpipers collected during the breeding season from nine locations (Figure 2, Table 1) and from four purple sandpipers from two locations. Most of the mitochondrial cytochrome *b* gene (1,033 bp) and the entire NADH dehydrogenase subunit 2 gene (ND2; 1,041bp) were amplified and cycle-sequenced using four primer pairs per gene per individual. Primers used for cytochrome *b* included: L14851 (Kornegay et al., 1993), H16064 (Harshman, 1996), L15350 (Klicka and Zink, 1997), and H15424 (Hackett, 1996). Primers used for ND2 were L5216, H6313, H5758, and H5766 (Sorenson et al., 1999). Amplified products were sequenced in both directions using an ABI 373A or 3100 automated sequencer (Applied Biosystems Inc., Foster City, CA). All sequences were deposited in GenBank (Table 1).

TABLE I

Species, subspecies, sampling locations, and GenBank accessions for rock and purple sandpipers used in this study. Museum voucher numbers are provided on GenBank.

Species	Location	<i>n</i>	GenBank accession	
			Cytochrome <i>b</i>	ND2
<i>Calidris ptilocnemis quarta</i>	Commander Is., Russia	2	AY156153–154	AY156129–130
<i>C. p. couesi</i>	Attu Is., Aleutian Is., Alaska	5	AY156101–105	AY156223–227
<i>C. p. couesi</i>	Adak Is., Aleutian Is., Alaska	5	AY156233–237	AY156091–095
<i>C. p. tschuktschorum</i>	Izembek Lagoon, Alaska Pen., Alaska	5	AY156247–251	AY156109–113
<i>C. p. tschuktschorum</i>	Togiak NWR, Cape Peirce, Alaska	5	AY156254–258	AY156124–128
<i>C. p. tschuktschorum</i>	Nunivak Is., Alaska	5	AY156137–141	AY156096–100
<i>C. p. tschuktschorum</i>	Nome, Seward Pen., Alaska	3	AY156142, 252–253	AY156106–108
<i>C. p. ptilocnemis</i>	St. Matthew Is., Alaska	5	AY156143–147	AY156114–118
<i>C. p. ptilocnemis</i>	Pribilof Is., Alaska	5	AY156148–152	AY156119–123
<i>C. maritima</i>	Reykjavik, Iceland	3	AY156155–157	AY15131–133
<i>C. maritima</i>	Cameron Parrish, Louisiana	1	AY156158	AY156134

2.2. NEUTRALITY TESTS, MISMATCH DISTRIBUTIONS, AND PHYLOGENETIC ANALYSES

To examine whether sequences were evolving in a neutral manner, tests for neutrality were performed using Tajima's test of selective neutrality (Tajima, 1989) in Arlequin 1.1 (Schneider et al., 1997). Arlequin was also used to determine mismatch distributions, in which pairwise combinations of sequences are examined to determine the distribution of differences (Hartl and Clark, 1997). A unimodal distribution often indicates that a population has undergone a population expansion (Rogers and Harpending, 1992). All rock sandpiper sequences were grouped together for both analyses to determine whether they had expanded out of a single refugium after the LGM.

A partition homogeneity test was used to verify that the two molecular data sets (cytochrome *b* and ND2) could be combined for phylogenetic analyses (Farris et al., 1995a, b). The two data sets were not significantly different ($P = 0.24$), so they were combined in all analyses. Genetic distance, maximum parsimony, and maximum likelihood analyses were used to construct phylogenetic trees (PAUP 4.0b10, Swofford, 2001). The most appropriate model and parameter estimates for likelihood analyses were determined using Modeltest 3.06 (Posada and Crandall, 1998). The most appropriate was the Hasegawa, Kishino, and Yano model (Hasegawa et al., 1985) with among-site variation approximated using the proportion of invariable sites (HKY+I). Bootstrap support for distance, parsimony, and likelihood trees were evaluated by resampling the data matrix 100 times (Felsenstein, 1985).

Bayesian inference was also used to develop phylogenetic trees using the same model of evolution as above (MrBayes 2.01; Huelsenbeck and Ronquist, 2001). This technique provides the most probable tree and the posterior probabilities for each clade under a Bayesian framework using a Markov chain Monte Carlo algorithm (Larget and Simon, 1999). These posterior probabilities can be used as an alternative statistic to bootstrapping for determining the accuracy of the estimated phylogeny. Also, Bayesian methods might provide a more reliable estimate of the true phylogeny because, unlike bootstrapping, the posterior probabilities generated are the true probabilities of each clade under the evolutionary models used (Rannala and Yang, 1996).

Multiple runs with a random starting tree were used to ensure that the Markov chain converged at optimal likelihood values. Trees sampled before the Markov chain reached a plateau were discarded, and the remaining trees were used to approximate the posterior probability of the phylogeny (Huelsenbeck and Ronquist, 2001). Trees were then imported into PAUP 4.0b10 (Swofford, 2001), and a majority rule consensus tree was created. The posterior probabilities of clades were determined as the percentage of occurrence of each clade among all sampled trees (Huelsenbeck and Ronquist, 2001).

Dunlin (*Calidris alpina*; Genbank AY156135, 156159) was used as an out-group in both of the phylogenetic analyses, because morphological and molecular

evidences have historically suggested that it is the closest living relative of rock and purple sandpipers (American Ornithologists' Union, 1910; Borowik, 1996; Borowik and McLennan, 1999). Also, curlew sandpiper (*Calidris ferruginea*; Genbank AY156136, 156160) was used as a more distant outgroup.

2.3. COALESCENT ESTIMATES

A coalescent approach was chosen because this technique has been shown to provide accurate results for population parameters (e.g. degrees of differentiation and divergence times) when small sample sizes are used (Harding, 1996; Miura and Edwards, 2001). Assuming a neutral model of evolution, this technique traces backward in time the most likely pathway to the coalescence point of two lineages. Based on differences in coalescence times among different lineages, inferences about historic population divergence and demography (e.g., population contractions or expansions) can be determined (Harding, 1996).

Non-equilibrium based estimates of θ ($2\mu N_e$), divergence times ($T = t/2N_e$), and gene flow ($M = 2N_e m$) for population pairs were determined using the program Mdiv (Nielsen and Wakeley, 2001), where N_e is the effective population size, t is the time to population divergence in generations, m is the migration rate in number of individuals per generation, and μ is mutation rate per sequence per generation. Divergence times for the combined cytochrome *b* and ND2 datasets were estimated by assuming a mutation rate of 4.14×10^{-5} mutations per sequence per generation based on molecular clock rates ($\sim 2\%$ per MY) found for a variety of bird species for total mitochondrial DNA (Shields and Wilson, 1987; Tarr and Fleischer, 1993), the cytochrome *b* gene (Krajewski and King, 1996; Nunn et al., 1996; Randi, 1996; Fleischer et al., 1998), and the cytochrome *b* and ND2 genes (Johnson and Sorenson, 1998; Zink and Blackwell, 1998; Johnson and Clayton, 2000). A generation time of 1 year was used as a conservative estimate, given that rock sandpipers molt into full adult breeding plumage in their first spring (Hayman et al., 1986), and the evidence strongly suggests that they breed in their first year.

Non-equilibrium estimators were used because of the probability that rock sandpipers experienced recent population expansions, bottlenecks, or incomplete lineage sorting and thus would not be in equilibrium between drift and migration. In addition, Griswold and Baker (2002) found that in expanding chaffinch (*Fringilla coelebs*) populations, non-equilibrium based estimators more accurately approximated migration rates than equilibrium-based methods. The HKY model (Hasegawa et al., 1985) of molecular evolution, which takes into account multiple nucleotide substitutions at the same position, nucleotide frequency differences, and any transition/transversion bias, was used to generate Bayesian posterior distributions of T , M , and θ . Parameter estimates were derived from the modes of each distribution, and Bayesian credibility intervals were determined by taking the shortest intervals containing 95% of the posterior distributions. Mdiv also provides an estimate of

time to most recent common ancestor (TMRCA), which is not necessarily the same as T ($t/2N_e$; Edwards and Beerli, 2000). These two parameters might differ due to gene flow between recently isolated populations or to renewed gene flow after populations have been isolated for extended periods. TMRCA estimates were also used to date the coalescences of major clades within the phylogenetic tree using the methods described above.

3. Results

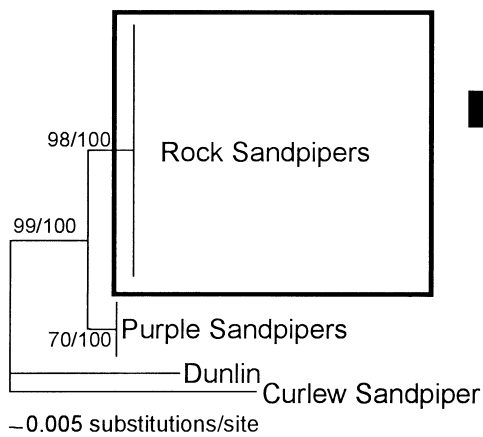
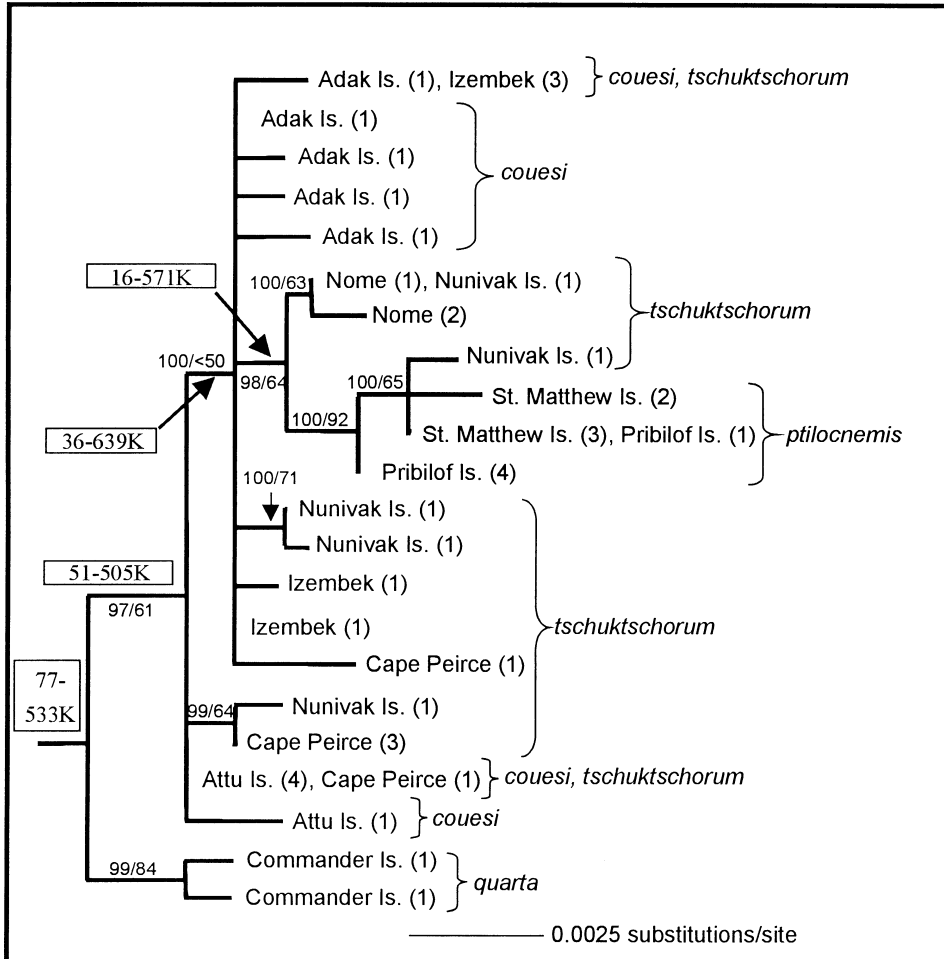
3.1. NEUTRALITY TESTS AND MISMATCH DISTRIBUTIONS

The neutrality test was negative ($D = -1.1656$) and not significantly different from zero ($P = 0.126$), supporting the assumption of neutrality and consistent with a species or group of populations that have expanded or experienced a recent bottleneck (Tajima, 1989; Rand, 1996). However, it is unlikely that this negative value is due to a recent expansion from a single refugium, because the mismatch distribution (not shown) was not unimodal, suggesting that populations are subdivided. This result is also found when the Commander Island sequences are removed and the distributions reanalyzed.

3.2. PHYLOGENETIC ANALYSES

The 2,074 bp of mtDNA sequence were combined for phylogenetic analysis to produce a single maximum likelihood tree (Figure 3, see methods). This tree was identical in topology to the trees derived using distance and maximum parsimony methods. Rock and purple sandpipers are clearly sister species, but divergence estimates predate the Wisconsin glaciation (approximately 3% sequence divergence, or 1.5 Mya). Although purple sandpipers from only two locations were examined (Table 1), each species is clearly monophyletic (all grouping together in a single clade; Figure 3). Bootstrap support for these relationships is high, but support for structure within the rock sandpiper clade was generally low, probably because few base pair differences (2–4 bp) supported individual clades (Figure 3). Bootstrap support, based on maximum parsimony and distance trees, are similar to those

Figure 3. Maximum likelihood trees of the phylogenetic relationships of rock sandpipers based on mitochondrial cytochrome *b* and ND2 sequences (2,074 bp) and the subspecies associated with each population. Smaller tree (below) shows relative branch lengths for each species, while larger tree shows relationships within the rock sandpiper clade, with location of haplotype and number of individuals with that haplotype in parentheses. Bayesian posterior probability (first number) and bootstrap (second number) values are provided at each branch. Time to most recent common ancestor (TMRCA) estimates are provided in boxes for each major clade.



reported on the likelihood tree with >50% support for all nodes shown in the tree (Figure 3).

Four separate Bayesian analyses with random starting parameters were examined, and all analyses converged at similar log likelihood scores (−4,595.12 to −4,638.68). These likelihood values were similar to those produced during the maximum likelihood analysis (−4,621.88). The initial 165,700 generations of a total of 400,000 generations were discarded (Huelsenbeck and Ronquist, 2001). A majority rule consensus tree was constructed from 40,001 trees produced during the analysis. This consensus tree had a topology identical to that of the maximum likelihood tree, but provided better resolution within the rock sandpiper clade (Figure 3). This might indicate that Bayesian probability methods are more useful than the traditional bootstrap analyses in resolving recently diverged populations, possibly due to the conservative nature of bootstrap analyses (Murphy et al., 2001; Zhaxybayeva and Gogarten, 2002). The tree (Figure 3) suggests that rock sandpipers and purple sandpipers descended from a common ancestor that colonized the north Pacific and the north Atlantic oceans.

There was substantial structure within the rock sandpiper clade, but branch lengths were generally short, indicating relatively recent population divergences (Figure 3). The tree (Figure 3) suggests that the Commander Island population is sister to all other rock sandpiper populations to the east in North America. Several clades were supported by >95% posterior probabilities and >60% bootstrap values, including a clade containing all individuals from St. Matthew Island, a clade that had all rock sandpipers sampled from Nome, and the monophyletic Commander Island clade (Figure 3). However, there was also evidence for incomplete lineage sorting (the maintenance of ancestral haplotypes in a population that has not been isolated for a sufficient period of time for new mutations to become fixed in that population; Avise, 2000) or recent gene flow within many of these clades. The Nunivak Island sample, for example, had individuals placed throughout the tree, with individuals grouping with birds from Nome, St. Matthew/Pribilof Islands, and Cape Peirce. An unusual instance of shared haplotypes was that of a rock sandpiper from Cape Peirce that had the same haplotype as birds from Attu Island. There was also a strong relationship between Adak Island and Izembek Lagoon, with many individuals sharing the same or similar haplotypes (Figure 3).

We found some genetic basis for the currently described morphologically based subspecies. The subspecies *ptilocnemis* and *quarta* were each found in a single clade, but both *tschuktschorum* and *couesi* did not group independently and were difficult to differentiate using mtDNA sequences alone (Figure 3). Because the rate of neutral genetic change is likely to be decoupled from rates of phenotypic change due to natural selection, these data do not address the question of whether these are 'valid' subspecies. Instead, the apparent genetic mixing could be due to a recent instance of gene flow between these subspecies or retention of ancestral polymorphisms through incomplete lineage sorting during historic isolation.

3.3. COALESCENT ESTIMATES

Values for θ , including the maxima and minima for the respective 95% credibility intervals, ranged from 0.1 (St. Matthew and Pribilof Islands) to 17.0 (Commander Islands and Nunivak Island) for rock sandpiper populations (Table 2). These credibility intervals were very broad, so differences in effective population sizes among populations are difficult to assess. However, the largest values for θ were found for pairwise comparisons between rock sandpipers and purple sandpipers. These values ranged from 2.7 (Attu Island and purple sandpiper) to 19.8 (Nunivak Island and purple sandpiper). These higher values may reflect the differences in breeding range and effective population sizes between these two species.

Divergence times and TMRCA were standardized for differences in effective population size by multiplying these pairwise estimates by θ (Griswold and Baker, 2002). All TMRCA estimates are almost twice as large as divergence time estimates (Table 3); this is likely due to the maintenance of ancestral polymorphisms (incomplete lineage sorting) or historically high levels of gene flow between now-isolated populations (Edwards and Beerli, 2000). Pairwise estimates between purple and rock sandpipers had divergence dates that predate the Wisconsin glaciation and correspond fairly well with estimates based on percent sequence divergence (approximately 1.5 Mya, Table 3). All TMRCA estimates for rock sandpipers predate the LGM and range from 36,460 ybp (St. Matthew and Pribilof Islands) to 192,574 ybp (Nunivak and Commander Islands; Table 3). Divergence estimates (T ; Table 3) are probably more conservative indicators of population divergence (Nielsen and Wakeley, 2001) and suggest that some population pairs diverged at the end of the LGM (ca. 10,000 to 30,000 ybp). These recent estimates include the Adak Island–Izembek Lagoon pair (13,771 ybp) and the Attu Island–Cape Peirce pair (14,545 ybp). Most of the population pairs had divergence times that occurred between 30,000 and 60,000 ybp (Table 3). However, two locations consistently had the oldest divergence estimates: Commander Islands (67,702–241,731 ybp) and St. Matthew Island (27,936–241,731 ybp; Table 3). In addition, these two populations were the most divergent from one another (241,731 ybp; Table 3). However, all pairwise estimates with the Commander Island population must be viewed with caution. Because of the small sample size from this location ($n = 2$), these estimates might be skewed upward.

To minimize problems with limited sample sizes from individual populations, major splits within the phylogenetic tree were dated using an estimate of TMRCA for all sequences within each clade (e.g., to determine the coalescence date of the split between the Commander Islands and all other rock sandpipers, the TMRCA for all rock sandpiper sequences was estimated; Figure 3). This measure should provide a robust estimate, because the coalescence point of all sequences within a clade is examined regardless of population affiliation. Thus, problems with contemporary gene flow and incomplete lineage sorting should be minimal. These estimates likely predate the actual population divergence (Edwards and Beerli, 2000); thus, ranges

TABLE II
 Mdiv estimates of θ ($2 N_e \mu$) above diagonal and $N_e m$ below diagonal of population pairs for rock and purple sandpipers. Values in parentheses are 95% credibility intervals. See Figure 1 for population locations

	Commander	Attu	Adak	Izembek	C. Peirce	Nunivak	Nome	St. Matt.	Pribilofs	<i>maritima</i>
Commander Is.	-	1.54 (0.4-8.3)	3.38 (1.2-12.0)	2.41 (0.5-9.5)	2.57 (0.7-12.2)	5.49 (1.9-17.0)	2.16 (0.6-9.9)	1.73 (0.6-8.5)	1.75 (1.0-12.2)	8.04 (1.73-14.45)
Attu Is.	0.01 (0.01-5)	-	2.41 (0.6-9.1)	1.07 (0.2-5.5)	1.16 (0.2-7.0)	3.29 (0.9-11.3)	1.02 (0.3-5.5)	1.23 (0.3-6.1)	1.08 (0.2-5.3)	4.61 (2.7-12.1)
Adak Is.	0.03 (0.01-5)	0.05 (0.01-5)	-	1.78 (0.5-8.3)	2.96 (0.8-11.1)	5.06 (1.9-15.7)	2.50 (0.7-10.2)	2.18 (0.7-10.4)	1.99 (0.6-9.5)	7.25 (4.0-16.5)
Izembek NWR	0.01 (0.01-5)	0.01 (0.01-5)	3.32 (0.45-5)	-	1.63 (0.4-8.1)	3.41 (1.2-12.5)	1.46 (0.3-6.7)	1.60 (0.4-7.1)	1.07 (0.3-6.3)	5.01 (3.0-13.2)
Cape Peirce	0.01 (0.01-5)	0.16 (0.01-5)	0.09 (0.01-5)	0.03 (0.01-5)	-	3.36 (1.0-12.2)	1.61 (0.3-8.7)	1.58 (0.4-9.4)	1.43 (1.9-15.7)	5.17 (2.9-15.4)
Nunivak Is.	0.08 (0.01-5)	0.35 (0.01-5)	0.52 (0.01-5)	0.30 (0.01-5)	0.63 (0.15-5)	-	2.68 (0.9-11.1)	3.00 (0.7-12.3)	2.33 (0.6-11.2)	7.31 (4.1-19.8)
Nome	0.03 (0.01-5)	0.01 (0.01-5)	0.01 (0.01-5)	0.01 (0.01-5)	0.01 (0.01-5)	1.09 (0.27-5)	-	1.28 (0.3-6.7)	0.99 (0.2-4.4)	5.29 (2.8-12.2)
St. Matthew Is.	0.01 (0.01-5)	0.01 (0.01-5)	0.02 (0.01-5)	0.01 (0.01-5)	0.01 (0.01-5)	0.17 (0.01-5)	0.01 (0.01-5)	-	0.46 (0.1-3.0)	5.15 (3.0-12.1)
Pribilof Is.	0.01 (0.01-5)	0.01 (0.01-5)	0.01 (0.01-5)	0.01 (0.01-5)	0.01 (0.01-5)	0.26 (0.03-5)	0.01 (0.01-5)	0.14 (0.01-5)	-	4.75 (2.8-11.0)
<i>maritima</i>	0.01 (0.01-5)	0.01 (0.01-5)	0.01 (0.01-5)	0.01 (0.01-5)	0.01 (0.01-5)	0.01 (0.01-5)	0.01 (0.01-5)	0.01 (0.01-5.8)	0.01 (0.01-5)	-

TABLE III

Mdiv estimates (corrected for unequal N_e by multiplying by θ) of T ($t/2N_e$) above diagonal and time to most recent common ancestor (TMRCAs) below diagonal of population pairs for rock and purple sandpipers. Values in parentheses are 95% credibility intervals of T . Bottom numbers are years before present assuming a generation time of 1 year. Mdiv does not provide credibility intervals for TMRCAs estimates. See Figure 1 for population locations

	Commander	Attu	Adak	Izembek	C. Peirce	Nunivak	Nome	St. Matt.	Pribilofs	<i>maritima</i>
Commander Is.	-	3.64 (0.7-7.7)	3.51 (1.3-16.9)	4.72 (1.4-24.1)	2.80 (1.0-12.9)	4.55 (0.7-27.4)	8.11 (0.9-21.6)	10.01 (1.5-17.3)	8.96 (0.1-8.7)	75.25 (14.2-69.2)
Attu Is.	4.25	-	2.33 (0.9-12.0)	1.31 (0.52-5.3)	0.60 (0.12-5.4)	1.51 (0.6-16.4)	1.85 (0.5-5.1)	5.90 (0.6-6.2)	3.81 (0.5-5.4)	68.93 (5.6-69.2)
Adak Is.	6.64	4.08	-	0.57 (0.04-8.9)	1.78 (0.5-14.8)	2.48 (0.7-25.3)	1.65 (0.6-12.5)	4.09 (1.1-10.9)	2.70 (1.0-9.9)	54.97 (5.7-72.5)
Izembek NWR	6.72	2.52	2.86	-	1.27 (0.5-8.1)	1.43 (0.4-17.1)	3.11 (0.6-7.3)	3.64 (0.7-8.0)	2.41 (0.5-5.3)	70.40 (6.0-75.1)
Cape Peirce	5.93	3.56	4.73	3.63	-	2.72 (0.1-16.8)	2.06 (0.7-8.1)	6.46 (0.8-7.9)	2.02 (0.2-7.1)	58.38 (5.4-77.4)
		143,174	85,897	114,354	87,796	65,730	49,796	156,136	48,886	1,420,040

(Continued on next page)

TABLE III
Continued

	Commander	Attu	Adak	Izembek	C. Peirce	Nunivak	Nome	St. Matt.	Pribilofs	<i>maritima</i>
Nunivak Is.	7.93	5.93	6.06	5.30	5.86	-	6.07	3.90	3.48	66.02
	192,574	143,201	146,442	128,048	141,451		(0.03-13.4)	(0.5-15.0)	(0.3-11.7)	(9.4-109.7)
Nome	6.88	3.24	4.11	3.50	4.28	5.35	-	4.19	2.65	66.02
	166,300	78,280	99,251	84,447	103,363	129,248		(0.4-6.4)	(0.4-5.0)	(5.6-79.3)
St. Matthew Is.	7.53	5.01	5.48	5.48	5.36	6.25	4.67	-	1.16	73.21
	181,806	121,073	132,387	132,259	129,374	150,946	112,723		(0.1-2.32)	(6.2-77.2)
Pribilof Is.	7.35	4.25	4.41	3.41	4.57	4.88	3.48	1.51	-	70.63
	177,650	102,573	106,541	82,396	110,460	117,793	84,123	36,460		(4.8-71.2)
<i>maritima</i>	69.86	51.19	56.45	52.38	51.03	52.57	51.44	59.49	55.14	-
	1,687,623	1,236,556	1,363,544	1,265,147	1,232,607	1,269,902	1,242,445	1,436,874	1,331,804	

of possible divergence dates were determined based on 95% credibility intervals of θ . We feel that these estimates provide a very conservative dating scheme for rock sandpiper divergences. TMRCA dates of coalescence within the phylogenetic tree reveal at least four major historic events in the genetic signal of rock sandpipers: 1) divergence of the Commander Islands population from all North American populations (77,030–533,096 ybp); 2) divergence of the Attu Island and Cape Peirce populations from all other North American populations (50,708–504,554 ybp); 3) divergence of the Adak Island and Izembek Lagoon populations from northern Bering Sea populations (35,040–639,316 ybp); and 4) the split of the Nome population from those of St. Matthew Island and the Pribilof Islands (15,940–571,189 ybp; Figure 3).

3.4. GENE FLOW

Estimates of gene flow showed very little movement between most population pairs (Table 2). Only two pairs had N_{em} values larger than 1.0 (Izembek Lagoon–Adak Island and Nunivak Island–Nome), which suggests that levels of gene flow between these two population pairs have been sufficient to overcome genetic drift (Hartl and Clark, 1997). Once again, credibility intervals are large, possibly due to small sample sizes; however, large credibility intervals are not necessarily a sole consequence of small sample size. A recent study found equally large credibility intervals when larger sample sizes were used ($n = 7–13$ per location, Griswold and Baker, 2002). Many of the pairwise estimates had very low values (0.01), suggesting a lack of gene flow between these locations. As expected, this low value was also found for all purple sandpiper and rock sandpiper pairs. These species have likely not shared individuals for approximately 1.5 MY, but pairwise comparisons between rock sandpiper populations and purple sandpipers also have large credibility intervals (0.01–5.80 migrants per generation). This suggests that these intervals are skewed upward, possibly due to the maintenance of ancestral polymorphisms, incomplete lineage sorting, or relatively small sample sizes.

4. Discussion

4.1. ROCK AND PURPLE SANDPIPER PHYLOGEOGRAPHY

Rock and purple sandpipers are sister taxa that diverged well before the beginning of the Wisconsin glaciation. Based on both percent sequence divergence and coalescence estimates (approximately 1.5 Mya; Table 3) these species diverged around the beginning of the Pleistocene (1.8 MYa), which corresponds with the onset of many major glaciations (Williams et al., 1998). It is difficult to attribute this divergence to any specific event, but by examining the current distributions of these

species a pattern emerges. Both species are coastally distributed, but rock sandpipers are only found in the north Pacific and purple sandpipers occur in the north Atlantic. Large expanses of land (North America and Asia) appear to be the primary barrier between these species. Although land might seem to be an unusual barrier to gene flow for terrestrial birds, both rock and purple sandpipers have limited migratory movements, and their entire lives are spent on or near saltwater beaches (Murie, 1959; del Hoyo et al., 1996). The most northern breeding populations of both species are seasonal migrants, and these migratory populations correspond to areas where pack ice reaches shore in winter (locations in the Bering Sea, the Bering Strait, Siberia, and northern Europe). Thus, both ice and land appear to constrain the present distributions of both species. A common ancestor likely colonized the north Pacific and north Atlantic, and speciation between the two populations likely developed during subsequent (and continued) isolation.

4.2. POPULATION EXPANSIONS AND CONTRACTIONS

There appear to have been several range expansions and intervening contractions among rock sandpiper populations during the late Pleistocene. Coalescence estimates (Table 3) and the phylogenetic tree (Figure 3) indicate that an initial expansion occurred with colonization of the Pacific before the Wisconsin glaciation. It is likely that this Pacific population expanded broadly, given that the oldest pairwise estimates of divergence (181,806–241,731 ybp, Table 3) are between two geographically distant populations (Commander Islands and St. Matthew Island). However, more recent pairwise coalescent dates (<117,000 ybp; Table 3) suggest that with the advent of the Wisconsin glaciation, populations probably contracted into several refugial areas.

Other expansions probably occurred in one or both of the Wisconsin interstadials (approximately 25,000 ybp and 75,000 ybp, Williams et al., 1998). Associations between populations that show historic gene flow (shared haplotypes) but current isolation (incomplete lineage sorting with older coalescent dates) provide evidence for expansions and subsequent isolation events. This kind of relationship is found between the Attu Island and Cape Peirce populations and the St. Matthew Island and Pribilof Island populations, in which there are low pairwise estimates of gene flow (Table 2) but shared haplotypes (identical mtDNA sequences) between locations (Figure 3).

A final series of range expansions probably occurred within the last 12,000 years as the glacial ice receded at the end of the Pleistocene. Populations that have a post-glacial signature of expansion include those that have high gene flow estimates, younger coalescent dates, and shared haplotypes. These include Adak Island and Izembek Lagoon and Nome and Nunivak Island (Tables 2 and 3; Figure 3).

4.3. REFUGIA

If a single refugium had harbored rock sandpipers through the LGM and subsequent post-glacial colonization had occurred, then the mismatch distributions would likely have been unimodal (Rogers, 1995). However, the distributions were bimodal. In addition, most pairwise coalescence estimates of divergence time predate the LGM, and some even predate the beginning of the Wisconsin glaciation (Table 3). These results suggest that rock sandpipers were isolated in several refugia, and that these populations experienced different histories of persistence and expansion.

Phylogenetic reconstructions and TMRCA estimates indicate that at least five areas served as refugia for rock sandpiper populations that persisted throughout the Wisconsin glaciations (117,000–10,000 ybp; Figure 3). These refugia correspond loosely to the Commander Islands (a western Pacific refugium), two locations in the Aleutian Islands (Attu and Adak Islands), and Nome and St. Matthew/Pribilof Islands (two Bering land bridge refugia). If we use the most conservative dating scheme possible (based on TMRCA 95% credibility intervals; Figure 3), then these locations all have coalescence dates that precede the end of the LGM and might correspond with several of the interstadial or interglacial events that occurred during the Quaternary (Williams et al., 1998).

Two of these “genetic” refugia consistently have the oldest pairwise coalescence estimates (Table 3), indicating that rock sandpipers persisted and were isolated for longer periods of time in these areas. These refugia correspond to the Commander Islands (the western Pacific refugium) and St. Matthew Island and Pribilof Islands (a Bering land bridge refugium). Other possible refugial populations have intermediate pairwise divergence estimates (30,000–90,000 ybp), including Nome and Attu Island and Adak Island in the Aleutian Islands (Table 3; Figure 2). Genetic support for the Nome refugium includes the grouping of the three individuals from Nome in a single well supported clade (Figure 3) and pairwise divergence estimates that all predate the LGM (Table 3). However, an individual from Nunivak Island is also found in the Nome clade, which suggests that the Nunivak Island population might have colonized Nome. This scenario seems unlikely, because individuals from Nunivak Island are scattered throughout the tree (Figure 3), and the gene flow estimates between Nome and Nunivak Island are greater than one individual per generation, which indicates that post-glacial Nunivak was probably colonized by several refugial populations (Figure 3).

The Aleutian locations also have refugial signatures (Attu and Adak islands; Table 3, Figure 4C). These two locations have likely not had gene flow for 56,000 years (Table 3). However, both locations share haplotypes with different populations on the mainland. Cape Peirce and Attu Island are grouped together in the phylogenetic tree and share one haplotype (Figure 3). However, estimates of gene flow are less than one per generation (Table 2), which might indicate that there has been incomplete lineage sorting between these locations. Adak Island shares a haplotype with Izembek Lagoon (Figure 3) and has gene flow estimates of 3

to 4 individuals per generation (Table 2). Contemporary gene flow is suggested. Additional support for these two locations as refugia comes from other studies that have shown that one or both of these locations were refugia for other bird species (Holder et al., 1999; Congdon et al., 2000).

4.4. ROCK SANDPIPER DIFFERENTIATION

Climate change and the concordant isolation of populations in refugia appears to have caused the pattern of morphological and sub-specific differentiation evident in rock sandpipers today. There is strong evidence that the subspecies *quarta* and *ptilocnemis* represent distinctly different evolutionary units (as evidenced by diagnosable plumage differences and molecular distinctiveness) that have been isolated for much of the Wisconsin period (Figure 2). These two morphologically based entities were isolated in two refugia that permitted the differentiation of these subspecies: the western Pacific (*quarta*) and Bering land bridge (*ptilocnemis*) refugia. However, *couesi* and *tschuktschorum*, although morphologically separable from the other two subspecies, are impossible to differentiate based on the genetic data presented here. Although isolation in multiple refugia probably caused the morphological variation found in these two subspecies, contemporary gene flow might be relatively high or these units have not been isolated from one another long enough for complete lineage sorting. An examination of population structure using more rapidly evolving molecular markers might indicate whether the populations on Attu Island and Nome, for example (the former being sedentary and the latter migratory), are currently isolated from other populations.

4.5. HIGH LATITUDE BIODIVERSITY AND CRYPTIC REFUGIA

Refugia are important for the long-term retention of biodiversity, particularly for high-latitude and temperate species (e.g. Taberlet and Cheddadi, 2002). Equally important, however, is the role of refugia in generating biodiversity (Stewart and Lister, 2001; Tzedakis et al., 2002). This role may be particularly important at high latitudes, where the relative effects of refugia on biodiversity generation could be quite large in the absence of non-refugial contributions to these processes: ice- and sea-covered landscapes leave little other than refugia for terrestrial species to occupy.

Rock sandpipers show the establishment, persistence, and accumulation of partitioned genetic diversity across several glacial cycles in the north Pacific. An unreconstructed (and perhaps not reconstructable) mosaic of multiple, intra-Beringian refugia seem to have played a major role in differentiation within this species following its initial colonization and speciation. The combined physical effects of glacial ice and fluctuating sea levels have made geological reconstruction of these

refugia difficult. For example, there is not yet any physical evidence for the existence of refugia in the Aleutian Islands. But the genetic echoes of these refugia are unmistakable. Rock sandpipers therefore reveal multiple cryptic refugia through repeated cycles of climate change, evident only through examination of the genetic records of the lineages affected. As more Beringian lineages are examined, we expect that more of these high-latitude cryptic refugia will be found (e.g. Fedorov and Stenseth, 2002) and that further genetic corroboration will be obtained for those already revealed.

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