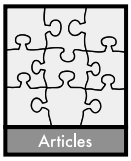

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Mitochondrial DNA data imply a stepping-stone colonization of Beringia by arctic warbler *Phylloscopus borealis*

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Arctic warbler *Phylloscopus borealis* is one of several high-latitude Passerines which are widely distributed across one northern continent but restricted to the Beringian part of the other. Most species with such asymmetric intercontinental ranges are monomorphic across Beringia, suggesting either recent colonization of the second continent or considerable gene flow across the Bering Strait. Arctic warbler is the only migratory species in this group that has three different subspecies in Beringia: *Ph. b. borealis* (Scandinavia to western Beringia, south to Mongolia), *Ph. b. xanthodryas* (Japan, Sakhalin, Kamchatka, western Beringia), and *Ph. b. kennicotti* (Alaska). This polymorphism may indicate that Arctic warbler has a unique and complex phylogeographic history that differs significantly from other species with similar ranges. Our analyses of complete mtDNA ND2 sequences of 88 Arctic warblers collected across the species range showed that the clade comprised of birds breeding on Sakhalin Island and Kamchatka Peninsula diverged from the Palearctic/Beringian clade by 3.8% in ND2 sequence. Beringian birds formed a recently derived clade embedded within the Palearctic clade. Nucleotide diversity declined sharply eastward from Palearctic to western Beringia and then to eastern Beringia. Our data provided no support for currently recognized subspecies. They suggested that the barrier at the western edge of Beringia was crossed by Arctic warbler earlier than the Bering Strait resulting in a stepping-stone colonization of Beringia by this species. Gene flow appears to be restricted across the western border of Beringia but not the Bering Strait.

Arctic warbler *Phylloscopus borealis* is the only *Phylloscopus* (Aves: Sylviidae) warbler that breeds in North America (AOU 2007). Although this species breeds across the Palearctic, its Nearctic range is restricted to Alaska (Lowther 2001). Such an asymmetric intercontinental range, when a species is widely distributed across one of the two northern continents but found only in the Beringian part of the other, is a characteristic of several other passerines. Among them are gray-headed chickadee *Poecile cincta* (Hailman and Haftorn 1995), bluethroat *Luscinia svecica* (Guzy and Mccaffery 2002), gray-cheeked thrush *Catharus minimus* (Stepanyan 2003), eastern yellow wagtail *Motacilla tschutschensis* (Badyaev et al. 1998), white wagtail *Motacilla alba* (Badyaev et al. 1996), and red-throated pipit *Anthus cervinus* (Stepanyan 2003). Furthermore, northern wheatear *Oenanthe oenanthe* breeds in two isolated areas in the Nearctic, in eastern Beringia and in eastern Canada and Greenland (Kren and Zoerb 1997). The migratory route of the east Beringian northern wheatear population across the Bering Strait suggests considerable similarity between its biogeographic history and that of the species with asymmetric intercontinental ranges listed above.

With the exception of polymorphism in Arctic warbler, the asymmetry of Palearctic and Nearctic ranges, migratory routes, and lack of phenotypic differentiation between populations on opposite sides of the Bering Strait argue that colonization events across the Bering Strait are recent, perhaps more recent than the Last Glacial Maximum (LGM). The eastern yellow wagtail may be an exception, as *M.t. tschutschensis*, breeding on both sides of Beringia, is phenotypically distinct from *M.t. plexa* breeding across the northern Palearctic. This phenotypic difference has been interpreted as evidence for *M.t. tschutschensis* surviving the LGM in Beringia in isolation from *M.t. plexa* (Badyaev et al. 1998). This hypothesis received some support in a recent mtDNA study (Pavlova et al. 2003). Although localities sampled in the northern Palearctic and Beringia were not genetically differentiated, nucleotide diversity declined from west to east across northern Siberia. However, it was equal on both sides of the Bering Strait and was 4.5 times greater than at the western border of Beringia (Pavlova et al. 2003: Table 2). A pattern such as this, where decreasing nucleotide diversity across a species range is abruptly met with a population of much greater nucleotide diversity, is consistent with the existence of two refugia

during the LGM – one in western and the other in eastern Palearctic.

The phenotypic differentiation between west and east Beringian populations of gray-headed chickadee and Arctic warbler indicate that unlike other species, these populations may have survived the LGM in isolation and evolved distinct phenotypes. In general, subspecific differences are poor predictors of evolutionary independence of avian populations breeding in different geographic areas (Zink 2004). It is especially true when subspecies are described on the bases of slight, often clinal, variation in plumage color and other phenotypic characters. This appears to be the case in both Arctic warbler and gray-headed chickadee, in which differences between subspecies are characterized as minimal (Hailman and Haftorn 1995, Lowther 2001). However, rapid evolution of phenotypic differences is unlikely if species breed and winter in similar habitats and are not affected by sexual selection. Both species are sexually monomorphic suggesting that their phenotypes are not under strong sexual selection. Yet, evolution of reproductive isolation and genetic divergence are possible in populations separated by geographic barriers, such as the Bering Strait, without significant divergence of phenotypes.

In this paper, we use complete sequences of the mitochondrial ND2 gene to test two related sets of alternative hypotheses. First, we test whether the three currently recognized subspecies of Arctic warbler *Ph. b. borealis* (Europe to Kolyma and Russian Far East), *Ph. b. xanthodryas* (Chukotka, Kamchatka, Sakhalin, Japan), and *Ph. b. kennicotti* (Alaska) represent evolutionary independent units. Second, we test whether the historic biogeographic barriers at the western edge of Beringia (Lena-Kolyma barrier; Hewitt 2004; Fig. 1) and Bering Strait are reflected in the pattern of genetic variation in Arctic warbler.

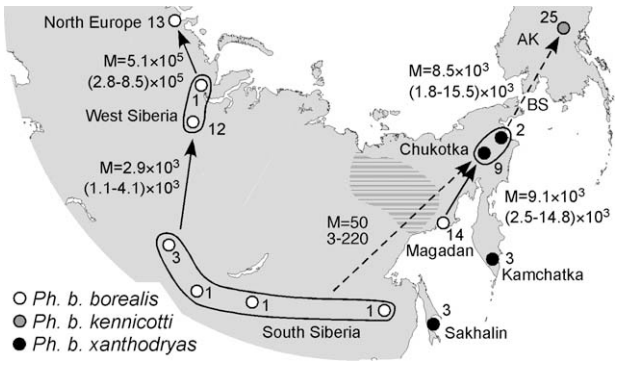

Figure 1. Sampling localities and gene flow estimates. Sampled localities (identified by the circles on the map). Numbers next to the circles represent sample sizes. Arrows represent direction of gene flow. Dashed arrows represent significant F_{st}-values, solid arrows – non-significant F_{st}-values. M-values (m/μ) next to the arrows represent the levels of gene flow estimated in Migrate. The 95% confidence intervals are indicated under each M-value. The area shaded with horizontal lines of uneven spacing represent approximate location of the Lena-Kolyma barrier formed by mountains (2000–3100 m) of Verkhoyanskiy, Suntar-Khayata, and Cherskogo Ranges, and Yano-Oymyakonskoye and Okhotsko-Kolymskoye highlands between Lena and Kolyma rivers.

Material and methods

A total of 88 tissue samples of locally breeding or fledged Arctic warblers from 13 localities across the Palearctic and Alaska was obtained from avian collections (Fig. 1, Appendix 1). Adults were considered local breeders if males had enlarged seminal vesicles, and if females had either one or a combination of a brood patch, enlarged ova, or oviduct. Juvenile birds were considered locally fledged if they had growing feathers and no fat reserves. These data were provided by the collections housing voucher specimens. We used additional samples of two chiffchaffs *Phylloscopus collybita*, two willow warblers *Ph. trochilus*, two greenish warblers *Ph. trochiloides*, and one Sakhalin leaf-warbler *Ph. borealoides* as outgroups.

We use mtDNA sequences for this study because they are more sensitive to population demographic processes, e.g. bottlenecks or founder effects, than nuclear sequences (Berggren et al. 2005). Nuclear alleles have four times the effective population size of mtDNA, so they require four times the number of generations for equal changes in allele frequency (lineage sorting) than does mtDNA. The high demographic sensitivity of mtDNA is essential for distinguishing between an isolation of Asian and Alaskan Arctic warblers during the LGM with recent reestablishment of gene exchange from post LGM colonization of Alaska from Asia with continuing gene exchange.

We selected ND2 gene over other mtDNA loci because its evolution is rapid and consistent with the molecular clock (Drovetski et al. 2005). Total genomic DNA was extracted using DNeasy Blood and Tissue Kit (QIAGEN, Valencia, CA). For each individual, the complete mitochondrial ND2 gene (1041 bp) was amplified via PCR using GoTaq Green Master Mix (Promega, Madison, WI) and primers L5219Met (5′CCCATACCCCGAAAATGATG 3′) and H6313Trp (5′ CTCTTATTTAAGGCTTTGAAGGC 3′; Sorenson et al., 1999) in the quantities suggested by the GoTaq user's guide. The PCR profile consisted of an initial DNA denaturation for 3 min at 95°C, followed by 35 cycles of 45 s denaturation at 95°C, 45 s annealing at 57°C, and 1 min extension at 72°C. The final extension step was carried out at 72°C for 10 min Sequencing was performed on ABI 3730 48-Capillary Genetic Analyzer (Applied Biosystems, Foster City, CA,) at the DNA analysis facility on Science Hill at Yale University.

Sequences were aligned automatically in Sequencher 4.2 (Gene Codes Corporation, Ann Arbor, MI). The alignment did not require editing because there were no indels in the ND2 sequences. Translation of the sequences revealed no stop codons except at the end. Substitution rates, nucleotide and codon composition were similar across haplotypes, suggesting that we did indeed sequence mtDNA ND2 gene for all individuals.

Unique haplotypes were identified from a data set of individual sequences in DnaSP 4.10.9 (Rozas et al. 2003). We used the McDonald-Kreitman test (McDonald and Kreitman 1991) implemented in DnaSP to test whether Arctic warbler ND2 sequences are affected by selection. A maximum likelihood (ML) analysis implemented in PAUP* 4.0 (Swofford 1998) was used to reconstruct the phylogeny of warbler haplotypes. ML model and parameters were

determined in Modeltest 3.06 (Posada and Crandall 1998) using AIC (Akaike 1974). Haplotypes were added randomly for both ML (10 replicates) and bootstrap analyses (500 replicates).

Pairwise F_{st} values, nucleotide diversity (π_n), Tajima's D (Tajima 1989, 1996), and Fu's Fs (Fu 1997) were calculated in Arlequin 2.0 (Schneider et al. 2000). Arlequin also was used to conduct Mantel test and an AMOVA to test for presence of geographic structuring in our dataset and to determine whether our data support currently recognized subspecies. Levels and directions of gene flow among sampled localities were identified using Migrate 2.3 (Beerli and Felsenstein 2001). We used the settings suggested in the user manual for the initial run of Migrate. This initial run was used to estimate starting parameters for the final run of the program which used the following settings: ttratio = 2.0; freqs-from-data = YES; seqerror-rate = 0.0; categories = 1; rates = 1: 1.0; prob-rates = 1: 1.0; autocorrelation = NO; weights = NO; fast-likelihood = NO; usertree = RANDOMTREE; random-seed = AUTO; use-M = YES; aic-modeltest = YES; theta = Own:{0.004 0.002 0.2 1.0 1.0 0.001}; migration = Own:{-75.0 0.0001 0.0001 0.0001 0.0001 0.0001-0.0001 0.0001 0.0001 0.0001 0.0001 1523.0-0.0001 0.0001 0.0001 5527.0 0.0001 0.0001-0.0001 0.0001 0.003 0.003 35727.0 0.003-0.003 3279.0 0.0003 0.0003 0.0003 0.0003-}; mutation = CONSTANT; fst-type = MIGRATION; custom-migration = {**}; geo = NO; bayes-update = NO; short-chains = 10; short-inc = 100; short-sample = 5000; long-chains = 5; long-inc = 20; long-sample = 50000; burn-in = 100000; heating = ADAPTIVE:1:{1.0,1.333333,2.0,4.0,1000000.0}; moving-steps = NO; long-chain-epsilon = INFINITY; gelman-convergence = Yes:Sum; replicate = YES:10; resistance = 0.000001. We report summary values and 95% confidence intervals for ten runs with different random starting trees.

Results

Phylogenetic analysis of Arctic warbler haplotypes

The initial phylogenetic analysis (results not shown) that included seven individuals of four other species – chiffchaff, Sakhalin leaf-warbler, willow and greenish warblers – identified two divergent clades within Arctic warbler. One of these clades consisted of three haplotypes identified among six individuals from Kamchatka Peninsula and Sakhalin Island that represent subspecies *Ph. b. xanthodryas*. The other clade included all haplotypes identified among continental Palearctic and Alaskan individuals representing all three subspecies *Ph. b. borealis, Ph. b. xanthodryas,* and *Ph. b. kennicotti*, respectively. Therefore, we used Sakhalin and Kamchatka haplotypes as a composite outgroup for the final phylogenetic analysis.

DnaSP identified 44 unique haplotypes among 88 individuals of Arctic warbler. The AIC selected the TrN + I model (Posada and Crandall 1998) for the ND2 sequences. The TrN + I is a submodel of the general time reversible (GTR) model (Rodríguez et al. 1990) in which transversions are weighted equally and sites are divided into two categories – variable with equal probability of change and invariable. Guanine was under-represented and cytosine was over-represented in ND2 sequences (A = 29.37%, C = 36.01%, G = 11.45%, T = 23.17%; df = 3, G-test $P < 0.001$). All taxa shared this nucleotide bias and there was no evidence of heterogeneity of base composition among taxa. NCBI accession numbers for our ND2 sequences are EU481411-EU481504.

The ML analysis of Arctic warbler haplotypes resulted in a single tree consistent with the molecular clock (non-clock -ln L = 1967.99341, -ln L with molecular clock enforced = 1988.54946; $-2\Delta\ln L = 41.1121$, df = 42, P = 0.51). The distance between Sakhalin/Kamchatka and the continental Palearctic/Alaska clades was 3.8% (Fig. 2) and this division was supported by 100% bootstrap value. The McDonald-Kreitman neutrality test conducted using these two clades revealed that ND2 sequence evolution in Arctic warbler is consistent with neutrality (NI = 1.286; Fisher's exact P = 0.80). These data indicate a long-term isolation and independent evolutionary history of these two clades.

Within the continental Palearctic/Beringian clade, all haplotypes from Beringia (Magadan, Chukotka, and Alaska) formed a recently derived clade. This clade was supported by bootstrap value of 63%, and distance between this clade and its sister haplotype from West Siberia was 0.2% (Fig. 2). Beringian haplotypes formed a star phylogeny, characteristic of recent population expansion from a small number of founders (Avise 2000). The most common (31 of 50 individuals, 62%), widespread haplotype was in the center of the star and a few derived haplotypes differed from it by one or two mutational steps (Fig. 3). Only one derived haplotype was shared by two individuals – one from Magadan and one from Chukotka. All other derived haplotypes were found in single individuals.

In contrast to Beringia, Palearctic haplotypes formed a complex phylogeny with many divergent haplotypes of low frequency (Fig. 3). Although the ancestral haplotype was found in all three Palearctic localities, only 5 individuals of 32 (15.6%) shared this haplotype, and another haplotype shared by birds from North Europe and West Siberia was more frequent (6 of 32 birds, 18.8%). These phylogenetic patterns suggest a relatively long-term stable population size and gene flow between localities in Palearctic and a recent colonization of Beringia.

Population structure and gene flow

AMOVA did not support division of continental Palearctic and Beringian Arctic warblers into the three subspecies, *Ph. b. borealis, Ph. b. xanthodryas,* and *Ph. b. kennicotti*. Differences in ND2 sequences between these subspecies failed to explain any proportion of variance observed in our dataset. However, it revealed significant differentiation among our sampled geographic areas that accounted for 55.6% ($P < 0.001$) of the observed variance. Differences among individuals within areas accounted for the remaining 44.4% ($P < 0.001$) of variance. This differentiation among geographic areas could be related to significant Isolation-by-Distance (IBD) identified by Mantel test (r = 0.88, P = 0.001).

Pairwise F_{st} values were significant in comparisons of populations divided by geographic barriers (Lena-Kolyma

Figure 2. ML phylogenetic relationships, geographic origin, and frequency of Arctic warbler ND2 haplotypes. Numbers next to the branches indicated bootstrap values based on 500 replicates.

barrier and Bering Strait) or large distances, and were not significant in comparisons of neighboring populations on the same side of either barrier (north Europe – west Siberia, west Siberia – south Siberia, and Magadan – Chukotka; Table 1). These results indicate that both IBD and geographic barriers affected the patterns of genetic variation and gene flow across the species' range.

Tajima's D values were significantly negative only for Beringian localities (Magadan, Chukotka, Alaska; Table 1) suggesting that Arctic warbler experienced recent population expansion in Beringia, but had stable populations in Palearctic. Nucleotide diversity declined sharply from west to east (Fig. 4) indicating possible direction of recent colonization of Beringia from Palearctic and Alaska from northeast Asia. However, the decline of nucleotide diversity was not steady. It was similar in localities on the same side of the two biogeographic barriers but differed across the barriers. Therefore, the pattern of nucleotide diversity variation along longitude had a stairstep pattern (Fig. 4).

We explored the direction and levels of gene flow (estimated as M = migration rate/mutation rate = m/μ,) in a greater detail using the coalescent approach implemented in Migrate (Beerli and Felsenstein 2001). In both Palearctic and Beringia gene flow was detected only between neighboring populations (Fig. 1). The direction of the gene flow was from south-east to north-west in Palearctic, and from south-west to north-east in Beringia. Both of these directions are consistent with the current migration routes and are likely representative of the routes of postglacial expansion. Gene flow across the Lena-Kolyma barrier was detected in south-west to north-east direction from south Siberia to Chukotka. The level of this gene flow, however, was 2–4 orders of magnitude lower than gene flow between localities within Palearctic and Beringia.

Discussion

Phylogeny of Arctic warbler haplotypes

Our ML analysis identified geographically concordant structuring of Arctic warbler ND2 haplotypes. First, it revealed two divergent (3.8% ML-corrected divergence) clades strongly supported by bootstrap (100%). One of these clades corresponded to birds sampled on Sakhalin Island and Kamchatka Peninsula. No haplotypes from this clade were found in neighboring regions to the west, north, or east of Sakhalin or Kamchatka (South Siberia, Magadan, Chukotka, Alaska) indicating a lack of matrilineal gene exchange between these regions. MK test indicated that selection was not responsible for the differences between the two clades. Therefore, our data may indicate a long-term isolation and independent evolutionary history of the Pacific clade that would justify upgrading it to a specific status (Pacific warbler *Ph. xanthodryas*). Chukotka should be excluded from the range of this taxon. Evolutionary independence of these clades, however, should be confirmed by independent nuclear loci data.

Second, our ML analysis revealed a recently derived clade composed of Beringian (Magadan, Chukotka, Alaska) haplotypes. This Beringian clade was embedded within Palearctic haplotypes and was supported by 63% bootstrap value. This clade had a star phylogeny (Rogers and

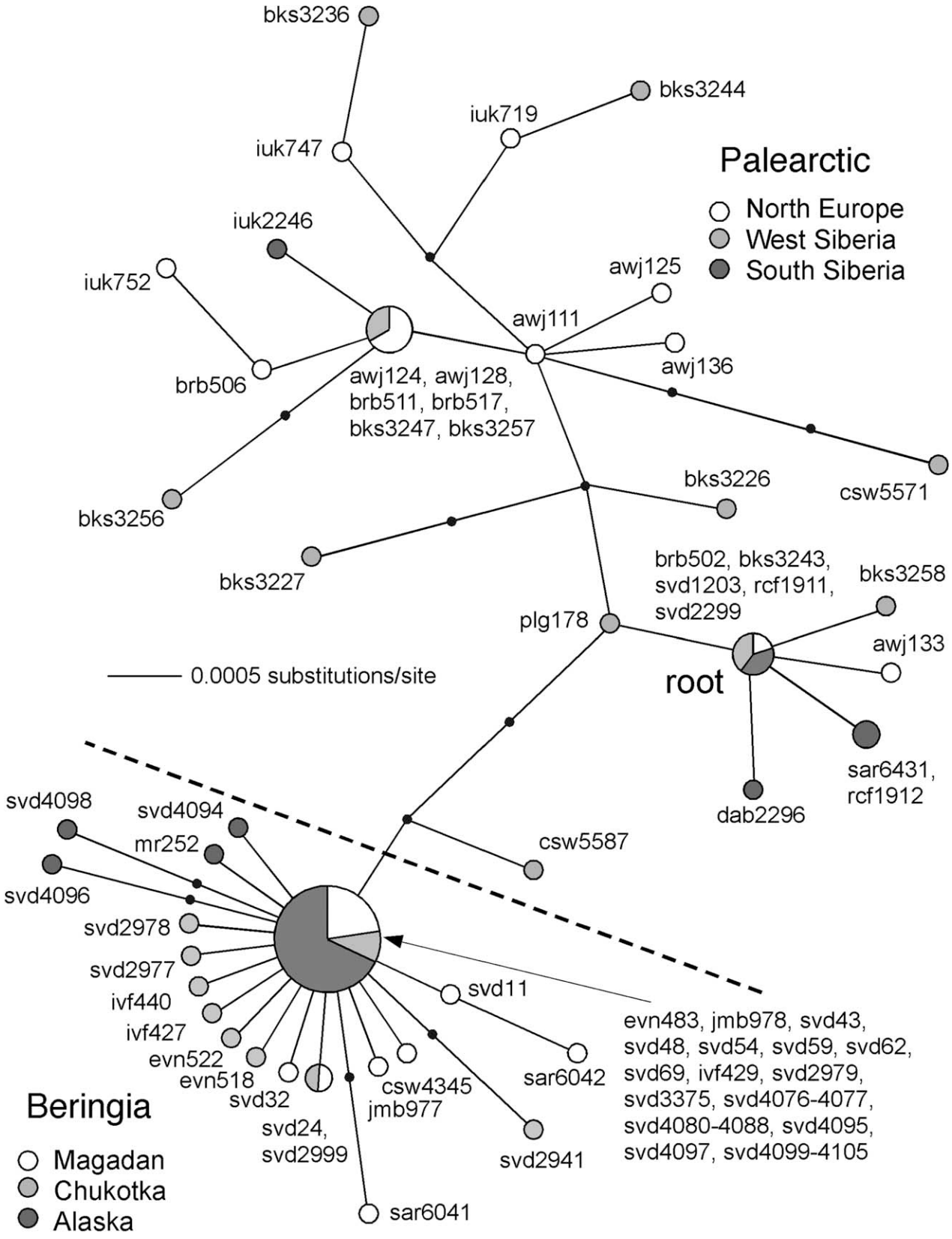

Figure 3. Unrooted ML tree of continental Palearctic and Alaskan haplotypes. Circle sizes represent frequency of haplotypes. Small black circles indicate unsampled haplotypes.

Table 1. Pairwise F_{st} (above diagonal) and their P-values (below diagonal), and Tajima's D and their P-values (diagonal).

Locality	North Europe	West Siberia	South Siberia	Magadan	Chukotka	Alaska
North Europe	−0.83 P = 0.23	0.03	0.32	0.70	0.66	0.78
West Siberia	0.17	−1.19 P = 0.13	0.10	0.54	0.51	0.65
South Siberia	0.00	0.08	−1.01 P = 0.19	0.70	0.66	0.82
Magadan	0.00	0.00	0.00	−1.88 P = 0.02	0.00	0.03
Chukotka	0.00	0.00	0.00	0.49	−1.96 P = 0.01	0.04
Alaska	0.00	0.00	0.00	0.02	0.01	−2.09 P = 0.01

Harpending 1992, Avise 2000) indicating a recent population expansion. The ancestral Beringian haplotype was the most common (31 of 50 individuals or 62%) and found in all three Beringian localities. It was at the center of the clade surrounded by low-frequency haplotypes that differ by 1–2 mutational steps from the ancestral haplotype.

In contrast to the Beringian clade, Palearctic (North Europe, West and South Siberia) haplotypes formed a complex phylogeny with many haplotypes of low frequency (1–6 individuals). The ancestral Palearctic haplotype was found in all three localities, however it had a low frequency (5 of 32 individuals or 15.6%). The complex tree of Palearctic haplotypes indicates a large, stable population inhabiting the area for a much longer time (Rogers and Harpending 1992; Avise 2000).

Population structure and history

Despite the differences in assumptions and algorithms, results of all our analyses had a great deal of agreement. They all identified a stepping-stone mode of gene flow within Palearctic and Beringia, presence of IBD, and an inhibiting effect of the Lena-Kolyma barrier on gene flow between Palearctic and Beringian localities. These results are consistent with the hypothesis of initial colonization of Beringia from Palearctic followed by period with restricted gene flow and, perhaps, existence of two separate refugia during the LGM. One of these refugia supplied colonists for formerly glaciated areas in Palearctic and the other initially for west and later east Beringia. This hypothesis is similar to that proposed for eastern yellow wagtail (Badyaev et al. 1998). The main difference between population histories of eastern yellow wagtail and Arctic warbler is the absence of IBD and founder effect in the Beringian population of the former (Pavlova et al. 2003). Our analyses point to another difference between eastern yellow wagtail and Arctic warbler. In the wagtail, nucleotide diversity is high and similar on both sides of the Bering Strait suggesting that during the LGM this species may have bred across the entire Beringia or unrestricted gene flow equalized the nucleotide diversity in western and eastern Beringia. Arctic warbler appears to have bred only in western Beringia during the LGM and crossed the Bering Strait more recently, when warming climate allowed arboreal vegetation to expand to the north and east in Chukotka. Perhaps, the limited dispersal ability of Arctic warbler, as indicated by strong IBD effects, resulted in a two-fold difference between nucleotide diversity in eastern and western Beringia pointing to a strong founder effect.

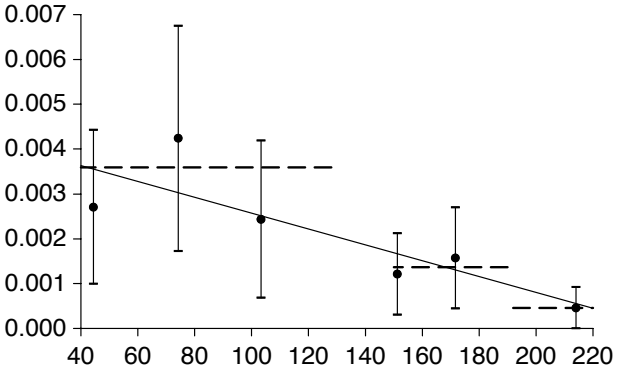

Figure 4. Plot of nucleotide diversity (π_n) versus longitude (degrees from the Greenwich meridian); $Y = 0.004–0.00001764X$ ($F_{(1,4)} = 10.25$, $R^2 = 0.72$; $P = 0.033$). Vertical bars represent one standard deviation above and below the estimated value of π_n. Horizontal dashed lines represent mean values of π_n and longitudinal extent of the Palearctic (left), western Beringian (middle), and Alaskan (right) parts of the sampled breeding range of Arctic warbler.

The only disagreement between our coalescent and F_{st}-based analyses was related to the assessment of gene flow across the Bering Strait. The F_{st}-value was small (0.036) but significant in the comparison of Chukotka and Alaska, but it was not significant in the comparison of Magadan and Chukotka. These results argue for presence of gene flow within western Beringia but absence of gene flow across the Bering Strait. In contrast, Migrate indicated the presence of gene flow across the Bering Strait and, in fact, its level is very similar to the level of gene flow from Magadan to Chukotka (Fig. 1). In our view, assessing gene flow between populations with substantially different levels of genetic variation using F_{st}, may produce misleading significant results. According to Slatkin (1991) $\Phi_{st} = \frac{f_0 - f_1}{1 - f_1} = \frac{\bar{t}_1 - \bar{t}_0}{\bar{t}_1}$; where f is the inbreeding coefficient, $\bar{t}$ is the mean coalescent time, 0 identifies values for two genes randomly drawn from the same population, and 1 identifies values for two genes randomly drawn from different populations. The coalescence time $\bar{t}_0$ is proportional to genetic variability of populations. When F_{st} is calculated for two closely related

populations with significantly different levels of genetic variability (source population and a recently established recipient population), this difference in genetic variability will inflate F_{st} value. The reason is that $\bar{t}_1$ will be similar to $\bar{t}_0$ of the source population ($\bar{t}_{0S}$) because most of the variation comes from within the source population. The value of $\bar{t}_0$ will be significantly smaller than $\bar{t}_{0S}$ as it will be similar to the average of $\bar{t}_{0S}$ and $\bar{t}_0$ of the recipient population ($\bar{t}_{0R}$): $\bar{t}_0 = \frac{t_{0S} + t_{0R}}{2}$ if the sampling is not significantly biased towards recipient population. In light of these considerations, we believe that the coalescent methods provide a more reasonable assessment of gene flow across the Bering Strait.

Migrate indicates that unlike the Lena-Kolyma barrier at the western border of Beringia, the Bering Strait does not appear to pose a significant geographic barrier for Arctic warbler beyond IBD found across the species' range. Despite the lack of IBD and apparently greater dispersal ability of eastern yellow wagtail, the response of both species to geographic barriers is very similar. Both species appear to be affected by the Lena-Kolyma barrier but not by the Bering Strait. Interestingly, neither species crossed the Mackenzie barrier at the eastern edge of Beringia. This suggests that Beringia may currently represent a single biogeographic unit. Historically, however, once western Beringia was colonized by Arctic warblers, they had to wait for arboreal vegetation to expand towards the Bering Strait before they could cross it. This delay could be responsible for the two-stage stepping-stone pattern of Beringia colonization we found in Arctic warbler. An interesting aspect of this study is that contrary to traditional wisdom, the last interglacial period, not the existence of the Beringian land bridge during the LGM, appears to facilitate crossing of the Bering Strait by Arctic warbler. Comparative phylogeographic studies of additional avian species with Holarctic and asymmetric intercontinental ranges and independent molecular markers are needed to test the viability and generality of this hypothesis.

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Appendix 1. Voucher specimen IDs, institutions that provided tissue samples, and collection dates and geographic coordinates.

Field ID	Collection and ID	Date	Lat., °N	Long., °E
Phylloscopus borealis				
AWJ 111	BMUM	15-Jun-02	65.72	44.37
AWJ 124	BMUM	19-Jun-02	65.72	44.37
AWJ 125	BMUM	19-Jun-02	65.72	44.37
AWJ 128	BMUM	20-Jun-02	65.72	44.37
AWJ 133	BMUM	22-Jun-02	65.72	44.37
AWJ 136	BMUM	25-Jun-02	65.72	44.37
BRB 502	BMUM	16-Jun-02	65.72	44.37
BRB 506	BMUM	17-Jun-02	65.72	44.37
BRB 511	BMUM	18-Jun-02	65.72	44.37
BRB 517	BMUM	20-Jun-02	65.72	44.37
IUK 719	BMUM	12-Jun-02	65.72	44.37
IUK 747	BMUM	14-Jun-02	65.72	44.37
IUK 752	BMUM	15-Jun-02	65.72	44.37
PLG 178	UWBM59484	28-Jun-97	66.77	66.52
BKS 3226	UWBM56531	13-Jun-96	63.48	74.87
BKS 3227	UWBM56532	13-Jun-96	63.48	74.87
BKS 3236	UWBM56539	15-Jun-96	63.48	74.87
BKS 3243	UWBM56545	16-Jun-96	63.48	74.87
BKS 3244	UWBM56546	16-Jun-96	63.48	74.87
BKS 3247	UWBM56549	17-Jun-96	63.48	74.87
BKS 3256	UWBM56556	17-Jun-96	63.48	74.87
BKS 3257	UWBM56557	17-Jun-96	63.48	74.87
BKS 3258	UWBM56558	18-Jun-96	63.48	74.87
CSW 5571	UWBM56717	10-Jun-96	63.48	74.87
CSW 5587	UWBM56733	11-Jun-96	63.48	74.87
SVD 1203	UWBM56969	20-Jun-96	63.48	74.87
RCF 1911	UWBM66539	29-Jun-00	50.77	91.52
RCF 1912	UWBM66540	29-Jun-00	50.77	91.52
SVD 2299	UWBM66701	29-Jun-00	50.77	91.52
DAB 2296	UWBM58016	11-Jun-97	48.12	100.37
IUK 2246	UAAAC	05-Jun-05	49.64	110.17
SAR 6431	UWBM47209	24-Jun-93	50.77	134.75
RYA 1385	UWBM	19-Jul-03	49.10	142.82
RYA 1349	UWBM	17-Jul-03	49.27	143.20
RYA 0966	UWBM	15-Jun-03	46.48	143.33
SVD 43	UWBM52560	26-Aug-92	60.08	150.78
SVD 48	UWBM52564	28-Aug-92	60.08	150.78
SVD 54	UWBM52570	29-Aug-92	60.08	150.78
SVD 59	UWBM52575	30-Aug-92	60.08	150.78
SVD 62	UWBM52578	30-Aug-92	60.08	150.78
SVD 69	UWBM52585	30-Aug-92	60.08	150.78
SVD 11	UWBM52527	15-Aug-92	59.73	150.87
SVD 24	UWBM52541	17-Aug-92	59.73	150.87
SVD 30	UWBM52547	17-Aug-92	59.73	150.87
CSW 4345	UWBM43818	26-Jun-92	59.13	151.92
JMB 977	UWBM44390	26-Jun-92	59.13	151.92
JMB 978	UWBM44391	26-Jun-92	59.13	151.92
SAR 6041	UWBM44417	26-Jun-92	59.13	151.92
SAR 6042	UWBM44418	26-Jun-92	59.13	151.92
CSW 4583	UWBM44024	21-Jul-92	53.08	157.77
SAR 6192	UWBM44555	21-Jul-92	53.08	157.77
SAR 6193	UWBM44556	21-Jul-92	53.08	157.77
EVN 518	BMUM	03-Jul-03	64.69	170.42
EVN 522	BMUM	04-Jul-03	64.69	170.42
IVF 427	BMUM	04-Jul-03	64.69	170.42
IVF 429	BMUM	05-Jul-03	64.69	170.42
IVF 440	BMUM	07-Jul-03	64.69	170.42
SVD 2977	BMUM	30-Jun-03	64.69	170.42

Appendix 1 (*Continued*)

Field ID	Collection and ID	Date	Lat., °N	Long., °E
SVD 2978	BMUM	30-Jun-03	64.69	170.42
SVD 2979	BMUM	30-Jun-03	64.69	170.42
SVD 2999	BMUM	02-Jul-03	64.69	170.42
EVN 483	BMUM	22-Jun-03	64.58	177.25
SVD 2941	BMUM	23-Jun-03	64.58	177.25
MR 252	UAAAC	13-Jun-07	63.08	214.40
SVD 3375	UAAAC	31-Jul-04	63.08	214.33
SVD 4076	UAAAC	15-Jun-07	63.05	213.19
SVD 4077	UAAAC	14-Jun-07	63.05	213.19
SVD 4080	UAAAC	14-Jun-07	63.07	213.83
SVD 4081	UAAAC	14-Jun-07	63.07	213.82
SVD 4082	UAAAC	14-Jun-07	63.07	213.85
SVD 4083	UAAAC	14-Jun-07	63.08	213.87
SVD 4084	UAAAC	14-Jun-07	63.08	213.88
SVD 4085	UAAAC	14-Jun-07	63.07	213.89
SVD 4086	UAAAC	14-Jun-07	63.08	213.88
SVD 4087	UAAAC	14-Jun-07	63.07	213.67
SVD 4088	UAAAC	14-Jun-07	63.08	213.89
SVD 4094	UAAAC	14-Jun-07	63.07	213.89
SVD 4095	UAAAC	14-Jun-07	63.05	214.05
SVD 4096	UAAAC	13-Jun-07	63.08	214.33
SVD 4097	UAAAC	14-Jun-07	63.05	214.17
SVD 4098	UAAAC	13-Jun-07	63.06	214.45
SVD 4099	UAAAC	13-Jun-07	63.05	214.47
SVD 4100	UAAAC	13-Jun-07	63.06	214.44
SVD 4101	UAAAC	13-Jun-07	63.09	214.38
SVD 4102	UAAAC	13-Jun-07	63.06	214.34
SVD 4103	UAAAC	14-Jun-07	63.05	214.18
SVD 4104	UAAAC	13-Jun-07	63.10	214.38
SVD 4105	UAAAC	14-Jun-07	63.04	214.13
Ph. borealoides				
RYA 1253	UWBM 82935	7-Jul-03	46.37	141.87
Ph.collybita				
IUK 768	BMUM	16-Jun-02	65.72	44.37
SVD 2824	BMUM	20-Jun-02	65.72	44.37
Ph.trochiloides				
RCF 2464	UWBM 78381	25-Jun-04	62.17	108.33
DOT 063	UWBM 78250	25-Jun-04	62.17	108.33
Ph.trochilus				
AF407050	NCBI			
CSW 5588	UWBM 56734	11-Jun-96	63.48	74.87

BMUM – University of Minnesota Bell Museum of Natural History, NCBI-National Center for Biotechnology Information; UAAAC – University of Alaska Anchorage Avian Collection, UWBM – University of Washington Burke Museum.