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Diversification at high latitudes: speciation of buntings in the genus *Plectrophenax* inferred from mitochondrial and nuclear markers

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Abstract

High-latitude diversification is a process characterized by speciation and extinction due to climatically driven vicariance and dispersal events. McKay's buntings (*Plectrophenax hyperboreus*) are high-latitude island endemic songbirds, and their global range is restricted to Beringia. Snow buntings (*P. nivalis*), their closest relatives, are distributed throughout the Holarctic, breeding in available habitat surrounding the island range of McKay's buntings. We sequenced 1123 base pairs of mitochondrial DNA for 40 individuals of each species and analysed a total of 913 AFLPs for 57 individuals. Both marker types suggested weak but significant genetic differentiation. Analysis of sequence data indicated divergence occurring when the current breeding range of McKay's buntings was a hill on the Beringian steppe (~18 400 to ~73 700 years before present), suggesting that snow buntings were restricted to lower latitudes by ice sheets. Ancestral effective population size estimates indicate a founder event in McKay's buntings followed by an expansion and then a reduction in effective size. Rising sea levels and asymmetric hybridization from McKay's buntings into the postglacially-colonizing population of snow buntings could account for this reduction. Reproductive isolation is likely maintained through differential arrival dates on breeding grounds and the high breeding density of McKay's buntings. This recent, high-latitude divergence best fits a model of founder event speciation driven by vicariance and oscillations in habitat due to climate change.

Keywords: AFLPs, climate change, coalescent analysis, cytochrome *b*, *Plectrophenax*, speciation, vicariance

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Introduction

Differentiation in allopatry has long been considered the dominant process of speciation (Jordan 1905; Mayr 1942, 1963; Coyne & Orr 2004; Phillimore *et al.* 2008; Price 2008). This hypothesis is especially relevant to diversification at high latitudes during the Quaternary through vicariant events caused by repeated glacial cycles, creating the mechanism for population isolation and divergence in glacial refugia (Hewitt 1996, 2000; Stewart & Lister 2001; Taberlet & Cheddadi 2002). With recent evidence suggesting that speciation may occur more rapidly at higher latitudes (Weir & Schluter 2004, 2007), determining how young species are formed in regions where reticulation of isolated populations is more likely due to increased effects of climatic cycles is important for comparative evolutionary biology. The genetic effects of refugial isolation and population expansion and contraction have been explored in an array of taxa with a variety of molecular markers (Zink & Dittmann 1993; Cooper *et al.* 1995; Santucci *et al.* 1998; Runck & Cook 2005; Li *et al.* 2009). Our research goes further by utilizing an Isolation with Migration model to estimate a number of demographic parameters for a mitochondrial DNA (mtDNA) dataset, and in

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corroborating the signal of divergence with a large nuclear dataset. With the advent of fine-scale genetic markers, complex modelling systems, and detailed climate history, we can uncover the genetic signal of recent large-scale vicariance, postglacial population expansion, genetic differentiation in the absence of substantial morphological differences, and the population histories of affected biota, ultimately assembling these into a geoclimatic context (Hewitt 2000). We expect such signals to be stronger with increasing latitude because of the direct evidence of profound recent fluctuations in available habitat.

Beringia includes large areas of northeastern Russia, southwestern, central, and northern Alaska, extreme northwestern Canada, and the shallow continental shelf and islands of the Bering Sea. Sea level fluctuations caused tremendous changes in this region during the Wisconsin glaciation (10 000–117 000 ybp), and Beringia remained ice free while isolated from North America by extensive ice sheets (Hamilton *et al.* 1986; Williams *et al.* 1998). In Beringia, differing signals of population divergence, contraction, extinction, expansion, and origins of postglacial colonists have emerged among a broad spectrum of taxa hypothesized to have been isolated in the region (Tremblay & Schoen 1999; Flagstad & Røed 2003; Eddingsaas *et al.* 2004; Galbreath & Cook 2004; Shapiro *et al.* 2004; Alsos *et al.* 2005; Pruett & Winker 2005, 2008; Van Houdt *et al.* 2005; Loehr *et al.* 2006). Despite the variance observed in population histories, these studies share a common thread: organisms that lived in Beringia during the last glacial maximum and organisms that live there today were significantly impacted by major climatic changes affecting the region.

Buntings in the genus *Plectrophenax* and the longspurs (*Calcarius*) represent a clade of comparatively high-latitude origin in the family Emberizidae (Klicka *et al.* 2003). The sister species McKay's buntings (*P. hyperboreus*) and snow buntings (*P. nivalis*) are the least-differentiated members of the clade, both morphologically and genetically (Lyon & Montgomerie 1995; Klicka *et al.* 2003). They have been considered reliably diagnosable using only a single plumage character: the amount of black on the back of males in breeding plumage (Fig. 1; Lyon & Montgomerie 1995). However, both sexes of the two species differ in juvenal plumage as well (Maley & Winker 2007), suggesting that genetic differences may be more complex than a simple situation in which variation in a single gene might account for the most recognized adult plumage differences. The two species have allopatric breeding distributions with strongly contrasting range sizes; McKay's buntings are restricted to St. Matthew Island and its small satellite Hall Island in the central Bering Sea, whereas snow buntings breed not only in Beringia on every other major island and the Russian and Alaskan coasts but also broadly across the rest of the Holarctic (Fig. 2; Paynter & Storer 1970; Gibson & Kessel 1997; Winker *et al.* 2002). Both species are highly mobile and capable of moderate-distance overwater migratory flight. St. Matthew Island and Hall Island (hereafter referred to as St. Matthew) became isolated from the mainland of Beringia by rising sea levels between 9000 and 11 000 years ago (Guthrie 2004). McKay's buntings represent an island population in two senses: not only is their small population endemic to St. Matthew, but they are also a tiny island of morphologically distinct buntings breeding allopatrically entirely within the range of snow buntings (Fig. 2). The wintering range of McKay's buntings is largely restricted to the western coast of Alaska, whereas snow buntings typically migrate much farther south in both Asia and North America (Lyon & Montgomerie 1995).

Fig. 1 Dorsal view of adult male and female buntings in alternate plumage. The specimens are in order from left to right as follows: male McKay's bunting, male snow bunting, female McKay's bunting, female snow bunting. The primary difference in the plumage of the two species is the amount of black on the back, wings, and tail.

In assessing reproductive isolation between these two taxa, we find limited evidence for secondary contact. There are reports of male McKay's buntings occurring on islands peripheral to their breeding range and possibly hybridizing with snow buntings (Sealy 1967, 1969). Snow buntings can be common on St. Matthew prior to and early in the breeding season, with most individuals leaving before the beginning of fledging (Winker *et al.* 2002). Only one pair of snow buntings has ever been recorded on the island during fledging (Winker *et al.* 2002). Considering the much larger population of snow buntings, rampant hybridization on St. Matthew is unlikely, because if so the McKay's bunting population would quickly become swamped.

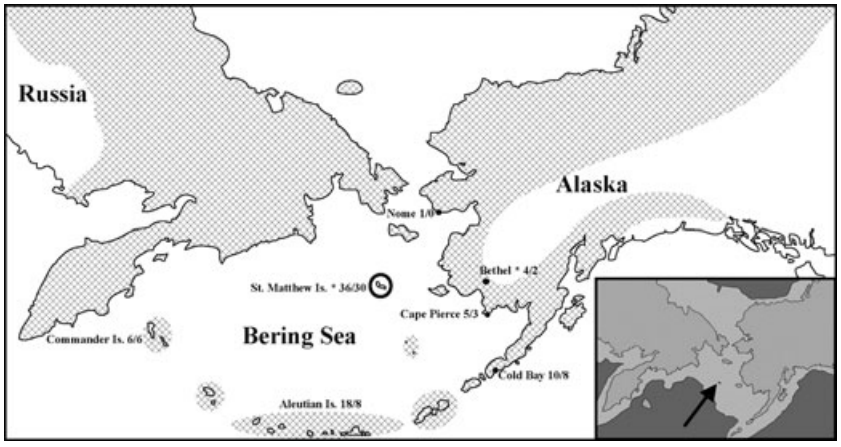

Fig. 2 Beringian range of *Plectrophenax* buntings, including sampling locations. Cross-hatching indicates the approximate breeding range of snow buntings; the circle in the centre indicates the breeding range of McKay's buntings. Sampling localities are labelled with the number of individuals used in cyt *b* analyses followed by a slash and the number of individuals included in AFLP analyses. An asterisk (*) indicates McKay's bunting sampling localities. Inset shows approximate extent of land exposed ~20 000 ybp (dark grey is ocean, light grey is presently submerged, intermediate grey is currently land); arrow is pointing to present day St. Matthew Island.

This closely related species pair represents an excellent system in which to examine the nature of high-latitude speciation in an area known for its dynamic vicariant history. We posed a series of questions to determine the history of speciation of McKay's buntings relative to snow buntings. First, they are morphologically distinct, but what is the level of genetic divergence? Second, does their divergence correspond with known climate history, such as the isolation of St. Matthew or the isolation of Beringia due to large ice sheets? Third, with dramatic regional sea level and land surface fluctuations in the relatively recent past, is there evidence of population expansion and contraction? Fourth, given potential hybridization (Sealy 1967, 1969), is there evidence of gene flow? Fifth, what is keeping McKay's buntings distinct from snow buntings? Finally, we synthesize the available evidence into hypotheses for how speciation occurred between these two lineages and what the current mechanisms might be that keep them separate.

Materials and methods

Mitochondrial sequence data and sampling

We sequenced 1123 bases of the mtDNA cytochrome *b* (cyt *b*) gene from 40 McKay's buntings and eight snow buntings. An additional 32 sequences of snow buntings were obtained from GenBank (Table 1; C. L. Pruett, unpublished data), selected from a variety of locations

Table 1 Species, locations, University of Alaska Museum (UAM) specimen voucher numbers, and GenBank accession numbers for cyt *b* data

Species	Location	Voucher numbers (UAM)	GenBank numbers
McKay's buntings	Bethel, AK	8473, 11864, 13166, 13167	DQ489335–489337, 489364
	St. Matthew Is, AK	7403–7407, 7524, 7746, 8198–8205, 8210, 8211, 8479, 8480, 8537	DQ489327–489334, 489338–489363
		8539, 10683, 17489, 17495–17499, 17502, 17547–17550, 17878, 17879	489365,489366
snow buntings	Attu Is, AK	7260, 7275, 7655, 8430, 9307	AY156428, 156430–156432, DQ489326
	Shemya Is, AK	9863, 9873, 9900	AY156433–156435
	Adak Is, AK	9319, 9320, 9864, 10038, 10039, 10046, 14610, 14675, 14676, 14712	AY156436–156445
	Cold Bay, AK	8474, 8476, 10043–10045, 11841–11843, 11855, 11856	AY156446–156455
	Cape Pierce, AK	7335, 7774, 7775, 7806, 14147	AY156461–156465
	Nome, AK	8621	AY156460
	Commander Is, Russia	17398, 17400, 17404, 17406, 17407, 17412	DQ489320–489325

surrounding the range of McKay's buntings, including Eurasia (Fig. 2), and including samples of both subspecies found in Alaska (*P. n. nivalis* $n = 16$; *P. n. townsendi* $n = 24$). The majority of McKay's buntings were collected from their breeding range; four were from phenotypically identified wintering individuals obtained near the Bering Sea coast (Bethel, Fig. 2). All sampled buntings are preserved as vouchered museum specimens at the University of Alaska Museum (UAM). Tissue extractions were done using a Qiagen DNeasy Tissue Kit following manufacturer's protocols. DNA amplifications followed standard PCR protocols using the primers L14851 (Kornegay *et al.* 1993) and H16064 (Harshman 1996). Cycle-sequencing reactions were run on an ABI 3100 genetic analyzer (ABI, Inc.). Sequences were aligned and trimmed using *Plectrophenax* cyt *b* sequences from GenBank. All variable sites were checked by eye using Sequencher v 3.1 (Gene Codes), and are deposited in GenBank (Table 1).

Amplified fragment length polymorphisms

We followed a modified protocol of Vos *et al.* (1995) to generate AFLP data. We primarily used reagents, protocols, and thermal cycle programs from the AFLP plant mapping kit for large genomes (ABI). The same 80 birds analysed using cyt *b* were initially chosen for AFLP analysis. DNA concentration was quantified on a spectrophotometer, and extractions were diluted to obtain 0.05 μg DNA per 6 μL. Samples were restricted and ligated in the same step. Preselective PCRs were conducted for each sample, followed by selective reactions using primers extended by two additional bases. For the selective reactions, primers were chosen at random without prior screening from the ABI AFLP kit (Table 2). The selective reactions were run using the selective step-down thermal cycle in the ABI protocol. We multiplexed all reactions by combining selective reactions that used 5-FAM and NED dye. We ran 2.0 μL of each sample with 0.5 μL ABI ROX 500 size standard and 7.5 μL of deionized formamide on an ABI 3100.

Scoring of amplified fragment length polymorphisms

All samples were binned using ABI GeneMapper®, and all peaks were scored by eye. Bins were created at a threshold of 10 fluorescent units so that all noise could be directly examined and to reduce the arbitrary nature of scoring based on peak height. We scored only unambiguously detectable fragments between the size of 75 and 400 bp. Overall quality was assessed on a relative scale incorporating height of peaks and missing sections of peaks of certain size (number of consecutively missing peaks that were monomorphic in all other samples). If the quality was determined to be low, then samples were either discarded entirely or rerun. Several samples were discarded that never amplified properly, apparently due to tissue degradation that is likely related to buffer-based preservation in remote field conditions. Twenty-three samples were discarded; resulting in 57 individuals included in the final analysis, 32 McKay's buntings and 25 snow buntings representing both subspecies found in Alaska (*P. n. nivalis* $n = 11$; *P. n. townsendi* $n = 14$).

Genetic differentiation and population structure

Before pooling all snow buntings into a single population for analyses, we first determined whether there was significant mtDNA genetic structure between the two subspecies. We examined potential differences between the subspecies by calculating pairwise Φ_{ST} and permuted the genotypes between populations 1000× to test for significance as implemented in Arlequin 3.0.1 (Excoffier *et al.* 1992). Because there was no significant structure

Table 2 AFLP amplification and scoring results for each primer pair and total

Primer pair extensions and dye			Both buntings			Within McKay's bunting			Within snow bunting		
*Eco*RI	*Mse*I	Dye	*T*	*P*	%*P*	*T*	*P*	%*P*	*T*	*P*	%*P*
–ACT	–CAA	FAM	157	99	63.1	146	85	58.2	148	84	56.8
–ACT	–CAT	FAM	146	98	67.1	131	80	61.1	136	80	58.8
–ACA	–CAG	FAM	120	79	65.8	114	67	58.8	119	71	59.7
–ACA	–CTT	FAM	118	79	66.9	102	61	59.8	109	62	56.9
–AAC	–CAC	NED	73	38	52.1	66	28	42.4	66	28	42.4
–ACC	–CTC	NED	99	80	80.8	87	63	72.4	95	73	76.8
–AGC	–CTA	NED	73	38	52.1	69	31	44.9	71	32	45.1
–AGC	–CTG	NED	127	69	54.3	119	53	44.5	125	64	51.2
Total			913	580	63.5	834	468	56.1	869	494	56.8

Total peaks (*T*), the number of polymorphic peaks (*P*), and the percentage of peaks that were polymorphic (%*P*).

(Φ_{ST} = 0.029, P = 0.16), we pooled the snow buntings, conducted the same test between species, and ran all further analyses assuming only two populations. A median joining network illustrating haplotype frequencies was generated using NETWORK 4.1.1.2 (Bandelt *et al.* 1999); mutational steps among haplotypes were added using Photoshop 10.0.1 (Adobe Systems, Inc.).

We analysed each fragment peak as a dominant marker locus with two states, presence or absence. We analysed 913 AFLP loci and found 580 (63.5%) were polymorphic when considering the total dataset, and similar levels of polymorphism, depending on primer pair, were observed when comparing calculations within species (Table 2). A binary matrix of polymorphic loci is available at http://hdl.handle.net/10255/dryad.1142. When analysing the AFLP data, we determined whether pooling the two snow bunting subspecies was appropriate using Arlequin 3.0.1 (Excoffier *et al.* 1992) to test for significant genetic differentiation. We calculated F_{ST} and permuted the haplotypes between populations 1000× to determine significance. The results (F_{ST} = 0.00, P = 0.99) allowed us to pool the data for all subsequent analyses, and we ran the same test between species.

We analysed the AFLP data under a Bayesian framework using MCMC simulations to determine the most likely number of populations involved and to assign individuals to populations using STRUCTURE 2.1 (Pritchard *et al.* 2000). Because AFLP data incorporate a large number of gene histories, the model must be independent of the mutational history of the loci used (Wang *et al.* 2003). This program uses a model-based clustering method to assign the individuals to a population and determine whether the genotype of each individual is admixed (Pritchard *et al.* 2000). Because of the forced dominance assumption inherent in AFLP data, each locus must be treated as a haploid allele. This treatment is considered valid under the no-admixture model (Pritchard *et al.* 2000). Preliminary runs indicated an appropriate burnin of 30 000 iterations. We then ran four independent simulations for 100 000 iterations using a no-admixture model with the number of populations (K) set from one to six, then calculated the likelihood of K given the data as $P(K|X)$. To assess the ability of the data to infer population structure, we did not use prior population origin information in the model, despite being able to use both phenotypic and geographic information to identify the individuals used in the study. We ran four replicates under the same conditions described above after determining the most likely number of populations. We used the program Distruct (Rosenberg *et al.* 2002) to transform and apply information from one STRUCTURE run to convert it into a figure.

Divergence time, effective population sizes, and gene flow

We ran a coalescent-based analysis of the mtDNA sequence data with a Markov Chain Monte Carlo (MCMC) approach under an Isolation with Migration (IM) model of divergence to estimate a variety of parameters (Nielsen & Wakeley 2001; Hey & Nielsen 2004; Hey 2005; Won & Hey 2005). θ is used to estimate effective population size, m is used to estimate gene flow from one population into another, t is used to estimate the time since divergence, and s is used to estimate the proportion of the ancestral population that split into each divergent population. Preliminary runs using very large priors were conducted to determine prior maxima on model parameter estimates. The priors were reduced in subsequent runs to determine the optimal priors to capture the distribution of the estimates without a long distributional tail. For our final analyses, we found that implementing Metropolis Coupling was not necessary and used a single chain; we used a burnin of 10^6 steps. We conducted four independent runs using different random number seeds with the following maximum priors: θ_1 = 1000, θ_2 = 350, $\theta_{ancestral}$ = 20, m_1 = 25, m_2 = 13, and t = 2.5. We also included the population splitting parameter, s, and used the full allowable range from 0 to 1 in preliminary runs. The parameter consistently converged close to 1, but to obtain a more robust overall fit of the model we constrained it to include only the range from 0.5 to 1. For each run, we used the HKY model of molecular evolution and an inheritance scalar of 0.25. After ensuring low autocorrelations following the burnin, we let each run proceed for more than 10^9 steps to achieve a minimum effective sample size of several thousand for any given parameter estimate. Because the results of the four runs were very similar, we only report the parameters estimated from a single run.

The model parameters estimated by IM are scaled to a neutral mutation rate; therefore to calculate demographic estimates we first calculated mutation rate estimates (Hey & Nielsen 2004). Although our best estimate of the substitution rate of cyt *b* in emberizids is 2.24% sequence divergence per million years (Myr; Weir & Schluter 2008), we used four different rates to incorporate uncertainty in this estimate (Lovette 2004; Ho *et al.* 2005; Weir & Schluter 2008) and to focus on the patterns in the parameter estimates, not the values themselves. We used the following rates for cyt *b*: 1, 1.6, 2.24, and 4% sequence divergence per Myr (Fleischer *et al.* 1998; Ho *et al.* 2005; Weir & Schluter 2008). An example of calculating the substitution rate per year for cyt *b* using 1% sequence divergence per Myr is 1123 bp × 0.005 substitutions/species/10^6 years = 5.62 ×

10^{-6} substitutions per species per year. These substitution rates were thus calculated as $\mu_{1\%} = 5.62 \times 10^{-6}$, $\mu_{1.6\%} = 8.98 \times 10^{-6}$, $\mu_{2.24\%} = 1.26 \times 10^{-5}$, and $\mu_{4\%} = 2.25 \times 10^{-5}$. Due to a lack of demographic data for these species, we assumed a generation time of one year in our calculations of effective population size (N_e). Because we only used nonrecombining, maternally inherited mtDNA for our model estimates, all N_e estimates are for females only. Following Hey (2005), using the estimates of θ we calculated N_e of snow buntings, McKay's buntings, and the ancestral population (N_1, N_2, and N_a, respectively), and using the estimates of m we calculated the effective number of migrants coming into a population from the other population per generation ($2N_1m_1$ and $2N_2m_2$). Using the estimate of t we calculated the time since divergence (t), and using the estimate of s multiplied by N_a we calculated the number of individuals from the ancestral population that founded each diverged population (sN_a and $[s\text{-}1]N_a$). To determine whether there was a genetic signal of expansion, we used Arlequin to calculate a pairwise mismatch distribution under a sudden expansion model for McKay's buntings to test for goodness of fit using 10 000 parametric bootstrap replicates (Schneider & Excoffier 1999).

Genetic diversity and selection

With the mtDNA data we used DnaSP 4.0 (Rozas *et al.* 2003) to calculate haplotype diversity (H), nucleotide diversity (π), and to conduct a χ^2 test of genetic differentiation based on haplotype frequencies following Nei & Chesser (1983). To test for a significant difference in nucleotide diversity between the two species, we used a simple χ^2 test with Yates' continuity correction as implemented in PopTools 2.6.9 (Hood 2005), an add-in for Microsoft Excel®. Using the AFLP data, we counted the number of peaks that were fixed in one species but polymorphic in the other and the number of peaks found in one species but not detected in the other. We conducted Pearson's χ^2 tests using PopTools 2.6.9 (Hood 2005) to test for significant differences in the number of fixed peaks in one species versus polymorphic in the other and the total number of peaks found in one species but absent in the other. We used TFPGA 1.3 (Miller 1998) to test for significant differences in overall peak frequencies between the two species using an exact test for population genetic differentiation. This program uses an MCMC simulation to provide an approximation of the exact probability of the differences observed in peak frequencies (Raymond & Rousset 1995). We ran 20 batches, 2000 permutations per batch, and 1000 dememorization steps to estimate the P-value (Miller 1998). We also searched for and estimated the number of outlier AFLP loci considered directly under or linked to loci that are under divergent selection by simulating two populations diverging by drift and mutation alone to determine neutral expectations using the program dfdist (Beaumont & Balding 2004).

Results

Genetic differentiation and population structure

There were no fixed base pair differences in cyt b sequence between snow and McKay's buntings. The haplotype network showed a common haplotype shared between species and several rare haplotypes unique to each species (Fig. 3). There were 13 different haplotypes found among snow buntings and seven found in McKay's buntings. Despite the high degree of haplotype sharing, snow and McKay's buntings showed significant mtDNA genetic differentiation (cyt b, $\Phi_{ST} = 0.078$, $P < 0.01$). The two species also showed significant genetic differentiation at the genome scale (AFLPs, $F_{ST} = 0.045$, $P < 0.01$). The exact test for AFLP peak frequency differences between the two species was also significant ($\chi^2 = 1484$, df = 1164, $P < 0.01$).

STRUCTURE analysis estimated that the most likely number of bunting populations involved in our samples was two ($\ln \Pr(X|K) = -12003.4$; $P(K|X) = \sim 1$). Two individuals were misassigned, one from each species. Populations were not substantially admixed, but there was more admixture from McKay's into snow buntings than from snow into McKay's buntings (Fig. 4). The snow bunting that was misassigned (UAM 7774) was estimated to have 92.9% of its genome originating from the McKay's bunting population. It was collected during the breeding season from Cape Peirce, the closest sampling locality for snow buntings to St. Matthew in our study (Fig. 2). The McKay's bunting that was misassigned (UAM 8199) was estimated to have 72.6% of its genome originating from the snow bunting population. It was collected during the middle of the breeding season from St. Matthew. We examined both specimen vouchers, but because they were both adult females in breeding plumage, definitive phenotypic identification was difficult. However, in examining them in a series they both fit phenotypically with their putative population of origin, rather than with their apparent genotypic population of origin.

Divergence time, effective population sizes, and gene flow

IM was able to estimate the high point and 90% highest and lowest posterior density (in parentheses) of the probability distribution for the following parameters: $\theta_1 = 540.5$ (162.5–999.5), $\theta_2 = 15.225$ (0.875–240.625),

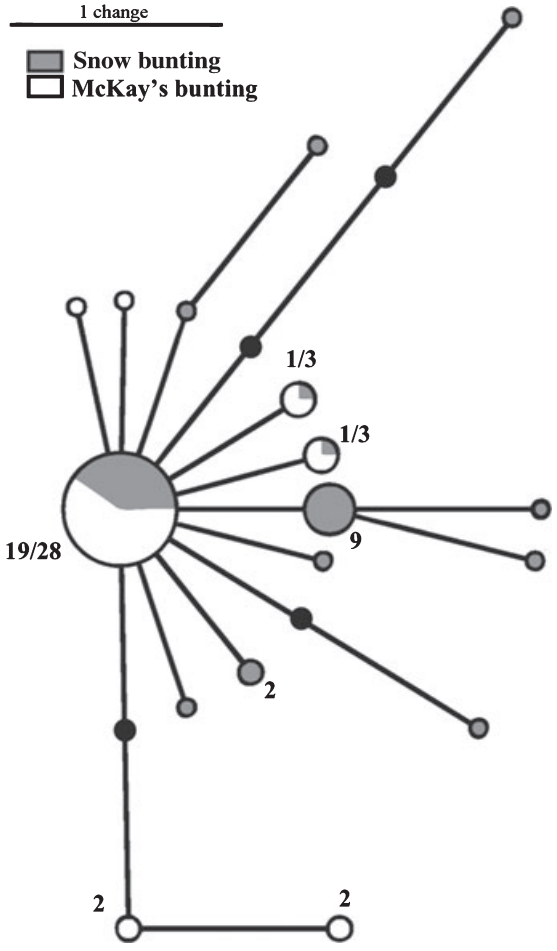

Fig. 3 Haplotype network depicting the number and relation of cyt *b* haplotypes of both species. Missing haplotypes are represented by black circles. Circles are scaled in size to the number of each haplotype sampled. For shared haplotypes, the first is number of snow buntings, followed by the number of McKay's buntings sharing that haplotype; those that are not numbered indicate a haplotype found once.

$\theta_{ancestral}$ = 0.13 (0.01–9.31), m_1 = 2.7875 (0.2375–11.7875), m_2 = 0.0065 (0.0065–4.765), s = 0.9925 (0.5645–0.9995), and t = 0.4138 (0.1462–1.0488). We then calculated the demographic parameters for the high points of each distribution (Table 3). The distribution of the posterior probability of t did not include zero (Fig. 5). Estimates of divergence time using four different substitution rates all suggested divergence prior to St. Matthew becoming isolated from mainland Beringia, and these estimates ranged from ~18 500 to ~73 700 ybp (Table 3). Only with the high rate of 4% sequence divergence per Myr did a substantial proportion of the probability distribution fall within or after the isolation of St. Matthew (Fig. 5).

Coalescent-based simulations were also used to estimate N_e of each species and the ancestral population

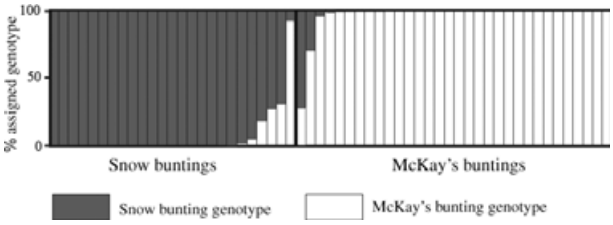

Fig. 4 The genotypic makeup of 25 snow buntings and 32 McKay's buntings inferred by STRUCTURE. A single bar represents each phenotypically or geographically identified individual. Two misassigned individuals are the closest to the boundary between the species, represented by a thick vertical black line.

(prior probability distributions not shown). N_e estimates of snow buntings were very high, ranging from ~6×10^6 to ~2.4×10^7 (Table 3). The estimates of N_e of McKay's buntings were smaller, ranging from ~1.7×10^5 to ~6.8×10^5 (Table 3). N_e estimates of the ancestral population ranged from ~1400 to ~5800 (Table 3). Estimates of the proportion of the ancestral population that founded each population suggested that very few individuals founded McKay's buntings (~11–43 females), whereas the majority of the ancestral population apparently established snow buntings (~1436–5745 females; Table 3). Nonsignificant results for the sum of squared deviation (SSD) of the test of goodness of fit for the mismatch distribution did not allow us to reject the sudden expansion model: P(Sim. SSD ≥ Obs. SSD) = 0.623 (Schneider & Excoffier 1999).

Coalescent-based analyses of cyt *b* showed highly asymmetric rates of introgression since divergence. The effective number of migrants per generation of McKay's buntings moving into the snow bunting population was estimated to be 753/year. The estimated effective number of migrants per generation of snow buntings into the McKay's bunting population was essentially zero according to these analyses using mtDNA alone (Table 3).

Genetic diversity and selection

McKay's buntings had lower cyt *b* haplotype diversity (H = 0.51) than snow buntings (H = 0.73; χ^2 = 28.73, df = 16, P = 0.026). Nucleotide diversity was also lower in McKay's (π = 0.00076) than in snow buntings (π = 0.001; χ^2 = 546, df = 1, P < 0.01). There were 33 AFLP loci fixed in McKay's buntings but polymorphic in snow buntings, and snow buntings had 42 fixed loci that were polymorphic in McKay's buntings; this level of polymorphism versus fixation did not differ significantly between the two species (χ^2 = 1.97, df = 2, P = 0.37). There were 80 AFLP loci present in snow buntings that were absent from McKay's buntings, and

Table 3 Demographic parameter estimates calculated from model parameters estimated using IM (Hey 2005)

Parameter	Substitution rate (μ)			
	$\mu_{1\%}$	$\mu_{1.6\%}$	$\mu_{2.24\%}$	$\mu_{4\%}$
Snow bunting effective population size	2.4×10^7	1.5×10^7	1.1×10^7	6.0×10^6
McKay's bunting effective population size	6.8×10^5	4.2×10^5	3.0×10^5	1.7×10^5
Ancestral effective population size	5788	3618	2583	1447
Migrants from McKay's into snow buntings*	753	—	—	—
Migrants from snow into McKay's buntings*	0.05	—	—	—
Time since divergence	73 695	46 060	32 899	18 424
Number of founders of snow buntings	5745	3590	2565	1436
Number of founders of McKay's buntings	43	27	19	11

Effective population size estimates of snow buntings, McKay's buntings, and the ancestral population, the number of migrants from McKay's into snow buntings per generation and from snow into McKay's buntings, and the likely number of ancestors that founded snow buntings and McKay's buntings are in units of individuals. The estimates of time since divergence are in years.
*These parameters are estimated independently of substitution rate.

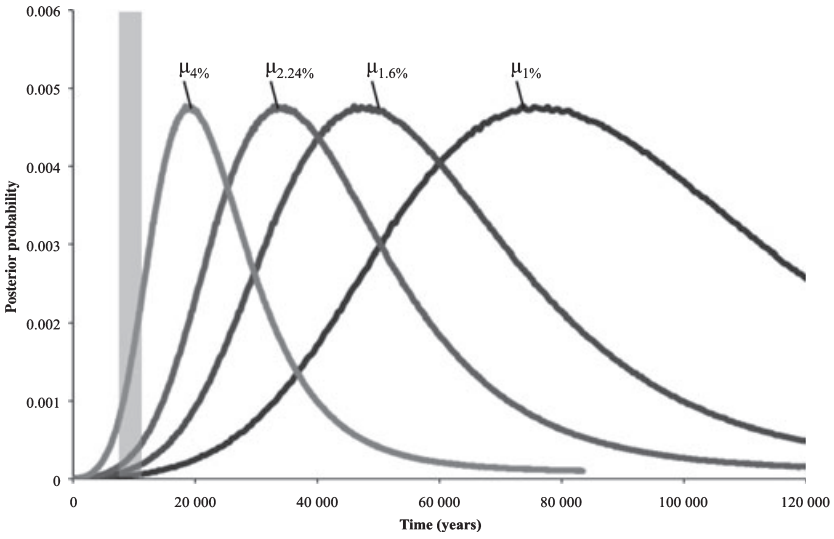

Fig. 5 Demographic parameter estimate prior probability distributions of time since divergence calculated using IM coalescent analysis. All curves were calculated from the same distribution of the model parameter estimate of *t* using four different substitution rates as labelled. The grey bar represents the approximate time that St. Matthew Island became isolated from the mainland (Guthrie 2004).

45 loci were present in McKay's buntings but missing in snow buntings; this difference (representing alleles newly arisen, recently lost, or unseen due to sampling) was significant between the two species ($\chi^2 = 11.8$, df = 2, $P = 0.003$). Three of 580 AFLP loci (0.5%) were detected as outliers when examined using dfdist (Beaumont & Balding 2004). The three loci had an unusually high F_{ST} and fell outside of the 99% confidence intervals. This result includes fewer loci than expected by chance (expected $N = 5.8$; 1%).

Discussion

The McKay's bunting/snow bunting species pair has many striking aspects, but three stand out in particular: these two species are very similar morphologically and genetically (Figs 1 and 3–4), the small island breeding population of McKay's buntings is completely surrounded by their highly mobile sister species (Fig. 2), and they are syntopic on St. Matthew during the breeding season but snow buntings apparently do not breed there (Winker *et al.* 2002). This high-latitude species pair diverged during the most recent major climatic oscillation, making it one of the youngest currently recognized species pairs in North America (Johnson & Cicero 2004). Despite the close genetic relationship, these two taxa are more distinct at both the mtDNA and nuclear genomic levels than we would expect by chance. The sharing of a common mtDNA haplotype with multiple haplotypes unique to each species is indicative of the earliest stage of divergence, neotypy (Omland *et al.* 2006).

Their divergence does correspond with recent climate history, but it appears to predate the formation of St. Matthew as an island (Fig. 5). Although this suggests that a population of ancestral buntings became isolated in Beringia due to ice sheets and not the formation of the

island specifically, we cannot rule out that the rough substitution rate estimates and high variance associated with estimating divergence times using only a single mtDNA marker in the face of gene flow could be misleading (Ballard & Whitlock 2005; Ho *et al.* 2005; Price 2008). Using mtDNA alone to estimate population parameters is not ideal, but finding sequence polymorphism in nuclear markers between recently diverged species is time-consuming, expensive, and may provide very little information (Zink & Barrowclough 2008). Additionally, levels of genetic differentiation vary considerably in the genome (Nosil *et al.* 2009), therefore we chose AFLPs to corroborate the pattern observed in the mtDNA data. The strongest evidence against the divergence occurring simply due to the isolation of St. Matthew from the mainland is that snow buntings nest on every other island in the Bering Sea and islands in the Arctic Ocean, with no apparent genetic or morphological differentiation (Fig. 2; Lyon & Montgomerie 1995). This is significant because these islands include some that were once part of the Bering Land Bridge and that are close to the mainland (e.g. Pribilof Islands) as well as some that have been isolated for much longer and are very far from any continent (e.g. western Aleutian Islands, Fig. 2). It is unlikely that a species with such high dispersal and colonization propensity would have been incapable of reaching St. Matthew long enough for reproductive isolation to evolve, unless their passage to the region was blocked, for example by ice sheets. We do not suggest that McKay's buntings diverged specifically in the area of St. Matthew, but rather that they were isolated in Beringia. McKay's buntings currently reside within Beringia year-round, whereas the majority of the snow bunting population migrates far south of Beringia. Additionally, while these buntings are highly mobile, and capable of covering moderate overwater migration distances, there is a substantial physiological difference between migrating several hundred miles over water and migrating several thousand miles over ice sheets.

In addition, our results suggest that McKay's buntings were founded by few individuals and subsequently expanded to a much larger population size. This large expansion would not have been possible on St. Matthew alone due to the small amount of available habitat from the time of island formation until the present. Evidence of reduced genetic diversity in both the mtDNA and nuclear genomes is consistent with a founder event, and evidence from both IM and the mismatch distribution support population expansion. This fits with a scenario of a small population becoming isolated on the mainland north of the ice sheets and subsequently filling the vast expanse of Beringia during the LGM. The IM estimate of N_e for McKay's buntings ($\sim 1.7 \times 10^5$ to $\sim 6.8 \times 10^5$, Table 3) greatly exceeds the census size of the modern population ($\sim$2800, Lyon & Montgomerie 1995; $\sim$31 200, Matsuoka & Johnson 2008), suggesting a recent population reduction (Alter *et al.* 2007) concordant with habitat reduction in Beringia due to rising sea levels (Fig. 2). Variance in this estimate using a single mtDNA marker may account for this disparity (Ballard & Whitlock 2005), but the observed difference of 1 to 2 orders of magnitude is striking.

Consistent with population reduction is the evidence of gene flow, which is asymmetric from McKay's buntings into snow buntings. While we cannot rule out retained ancestral polymorphism to explain this pattern, the model of Isolation with Migration is designed to distinguish between the effects of isolation and migration on gene frequencies in two recently diverged populations (Nielsen & Wakeley 2001), and was initially designed for a single, nonrecombining locus. There are several assumptions of the model outlined in the manual distributed with the software package and papers describing the model (Nielsen & Wakeley 2001; Hey & Nielsen 2004). The other major assumptions include a lack of recombination within loci, selective neutrality of the markers, and a lack of within-population substructure (Nielsen & Wakeley 2001), which these data also fit. To avoid the assumption of constant population size in the original model, we included the population splitting parameter, *s*, which allows each population to grow or shrink after divergence (Hey 2005). Typically, the estimate of ancestral effective population size is much larger than the estimate for the two descendant populations due to violations of the assumptions (Becquet & Przeworski 2009); our data do not show this pattern. To further explore the effects of potential ancestral polymorphism accounting for the asymmetric rate of migration of McKay's buntings into snow buntings, we ran an additional coalescent simulation using identical priors but removing the two snow buntings that share rare haplotypes with McKay's buntings (1/3 haplotypes in Fig. 3). We found similar estimates for all parameters, and migration rates were again asymmetric, with 426 McKay's buntings moving into snow buntings per generation and effectively zero migrants in the other direction. We would expect the signal of asymmetric gene flow if postglacially colonizing snow buntings, with a considerably larger population, successfully hybridized with McKay's buntings inhabiting Beringia everywhere except St. Matthew. Concordant with this scenario, evidence suggests that McKay's buntings have been reticulating into the snow bunting population, leaving a ghost genetic signal of a larger and more widespread population of McKay's buntings in Beringia. This signal of past hybridization and dominance of one species over another is not as strong as that seen in some other taxa (Rohwer *et al.* 2001), because these two

species were not isolated long enough for changes to accumulate and sort into reciprocally monophyletic lineages (Omland *et al.* 2006).

Considering their close relationship and evidence of gene flow, it is unlikely that effective postzygotic reproductive isolating mechanisms have evolved, and fertile hybrids would be expected (Grant & Grant 1997). One potential explanation for why McKay's buntings still exist despite evidence of historic mainland swamping by snow buntings is their shorter migration distance and corresponding heterochrony in arrival to St. Matthew during the spring. Male snow buntings fly to the breeding grounds earlier than females to establish territories (Lyon & Montgomerie 1995). McKay's buntings spend the winter relatively close to St. Matthew along the west coast of Alaska, and they have been observed flying to the island as early as 20 March (Irving *et al.* 1970). This is approximately the same time or just after snow buntings arrive in Interior Alaska from the south and overwintering birds begin moving in south-central and western Alaska (D. D. Gibson, personal communication). St. Matthew is a small island with a limited number of available territories, and McKay's buntings occur in very high densities on the island during the breeding season (KW, Pers. obs.; Matsuoka & Johnson 2008). The density of breeding McKay's buntings recently calculated by Matsuoka & Johnson (2008) is an order of magnitude higher than any recorded for snow buntings (Lyon & Montgomerie 1995). It is possible that McKay's buntings, arriving on St. Matthew earlier than snow buntings, occupy all available territories and effectively exclude snow buntings from breeding there. Hybrids may also face reduced fitness if they arrive on St. Matthew later than the majority of the McKay's bunting population. It should be noted that St. Matthew is among the most remote locations in the Bering Sea/Aleutian Archipelago axis and was also one of the first modern day Beringian islands to become isolated during the most recent warming period (Guthrie 2004). The Aleutian Islands are also remote, but they consist of a chain of islands with breeding snow buntings, and some birds remain on the Aleutians throughout the winter. Heterochrony in arrival to breeding areas has been implicated several times as a mechanism shaping patterns of hybridization and reproductive isolation in closely related species or populations of birds. Differential arrival time was cited as the mechanism driving reproductive isolation in populations of band-rumped storm-petrels (Friesen *et al.* 2007) and European blackcaps (Bearhop *et al.* 2005). This mechanism was also implicated as shaping mtDNA introgression patterns in hermit and Townsend's warblers (Rohwer *et al.* 2001), and in maintaining species boundaries between two closely related species of sapsuckers (Johnson & Johnson 1985).

The underlying causes for the evolution of reproductive isolation between these two species are difficult to uncover. Founder events have not been considered particularly contentious in species formation, but the genetic consequences of a founder event have been debated (Coyne & Orr 2004). Our evidence from mtDNA and AFLP data shows that a loss of genetic variation occurred and has persisted in McKay's buntings, concordant with Mayr's (1954) and Carson's (1971) predictions of the genetic consequences of a founder event. Strong genetic drift due to the loss of genetic variation is expected following a founder event (Carson & Templeton 1984). Under a model of genetic drift alone, we would expect reproductive isolation to take a long time to occur (Coyne & Orr 2004; Price 2008). The expected mode of speciation under a general model of peripatry involves either divergent selection or genetic drift coupled with a cessation of gene flow (Haldane 1930; Mayr 1954). We found that only 0.5% of AFLP loci might be under selection or linked to loci under selection, a value lower than expected by chance (1% at $\alpha = 0.01$). This proportion of loci is considerably lower than those found in other studies (Wilding *et al.* 2001; Campbell & Bernatchez 2004; Bonin *et al.* 2006; Nosil *et al.* 2009). Despite a lack of evidence for strong selection, we cannot rule it out as the mechanism responsible for the differences between these two species. Natural selection is certainly plausible given that organisms inhabiting the Arctic tend to have reduced pigment consistent with selective pressures for crypsis. However, snow buntings inhabit and breed on isolated Arctic islands at much higher latitudes than St. Matthew without a reduction of pigment. But considering that male McKay's buntings have substantially reduced pigment whereas females in alternate plumage have remained largely indistinguishable, the differences could be the result of sexual selection driving male McKay's buntings to lose pigment. Mutations at one or a few loci could reduce pigment production (Mundy *et al.* 2004; Steiner *et al.* 2007), and if females favor the resulting phenotypes then the mutations would rapidly spread through the population (Nadeau *et al.* 2007; Price 2008) without necessarily leaving a signature of strong selection. In the absence of field research into arrival times, female mate preferences, and heterospecific interactions on St. Matthew, the underlying mechanisms of reproductive isolation between these two species remain unknown.

Bunting speciation

Dramatic climate changes during the late Pleistocene had a major impact on many organisms (Hewitt 2000), and it is perhaps not surprising that these changes

caused the formation of new species of birds, particularly at high latitudes (Johnson & Cicero 2004; Weir & Schluter 2004, 2007). However, the mechanisms of putatively rapid, high-latitude speciation have not been thoroughly investigated. Our study, too, suggests that comparatively rapid speciation can occur at high latitudes due to recent climatic oscillations, and it advances our understanding of these events in part by providing a relatively narrow range of dates over which one of these splits has occurred. A small number of McKay's buntings became isolated within Beringia from ancestral snow buntings by massive ice sheets. During this process, morphological differentiation presumably became fixed in McKay's buntings, either through drift or selection. Following a presumed postglacial colonization of Beringia by snow buntings, the two taxa have been exchanging genes, but asymmetrically. In the face of asymmetric gene flow, the remaining population of McKay's buntings has retained its morphological and genetic integrity, likely through a process of heterochrony and high breeding density on St. Matthew. These processes likely resulted in the rapid evolution of extrinsic prezygotic reproductive isolating mechanisms. This divergence appears to be an example of recent, rapid speciation of a potentially small founding population isolated at high latitudes as a result of climate change.

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