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Northwestern song sparrow populations show genetic effects of sequential colonization

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Abstract

Two genetic consequences are often considered evidence of a founder effect: substantial loss in genetic diversity and rapid divergence between source and founder populations. Single-step founder events have been studied for these effects, but with mixed results, causing continued controversy over the role of founder events in divergence. Experiments of serial bottlenecks have shown losses of diversity, increased divergence, and rapid behavioural changes possibly leading to reproductive isolation between source and final populations. The few studies conducted on natural, sequentially founded systems show some evidence of these effects. We examined a natural vertebrate system of sequential colonization among northwestern song sparrows (*Melospiza melodia*). This system has an effectively linear distribution, it was probably colonized within the last 10 000 years, there are morphological and behavioural differences among populations, and the westernmost populations occur in atypical habitats for the species. Eight microsatellite loci from eight populations in Alaska and British Columbia (n = 205) showed stepwise loss of genetic diversity, genetic evidence for strong population bottlenecks, and increased population divergence. The endpoint population on Attu Island has extremely low diversity (H_E = 0.18). Our study shows that sequential bottlenecks or founder events can have powerful genetic effects in reducing diversity, possibly leading to rapid evolutionary divergence.

Keywords: Aleutian Islands, Aves, founder events, *Melospiza melodia*, population bottlenecks, song sparrow

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Introduction

The genetic consequences of single founder events or population bottlenecks have been examined through theoretical modelling (Nei *et al.* 1975; Chakraborty & Nei 1976; Barton & Charlesworth 1984), laboratory tests (Bryant & Meffert 1990; Meffert & Bryant 1991, 1992; Moya *et al.* 1995), and molecular genetic analyses of anthropogenically founded populations (Waldman *et al.* 1998; Seymour *et al.* 2001) and natural populations (Schwaegerle & Schaal 1979; Baker & Moeed 1987; Baker *et al.* 1990; Grant & Grant 1995; Tarr *et al.* 1998; Kuo & Janzen 2004; Pastor *et al.* 2004). Although models have predicted major reductions in genetic diversity and rapid differentiation between source and founder populations (Mayr 1954; Nei *et al.* 1975), laboratory (Moya *et al.* 1995) and natural systems (Clegg *et al.* 2002) often have not shown these effects.

The genetic consequences of cumulative founder events through sequential island colonization, or as steps in range expansions, have only recently been examined. Le Corre & Kremer (1998) used simulations to evaluate two models (island and one-dimensional stepping-stone models) of sequential founder events. They found rapid losses of genetic diversity, fixation of alleles, and increases in F_{ST} between neighbouring populations. This effect was most pronounced in the one-dimensional stepping-stone model in which individuals could only move between near populations. In the island model in which genes could be exchanged between any populations regardless of spatial orientation, gene flow eventually homogenized populations.

Very few natural systems have been examined to determine the effects of sequential founder events. However, several studies have implicated this process as leading to losses in diversity (Merilä *et al.* 1997; Moum & Árnason

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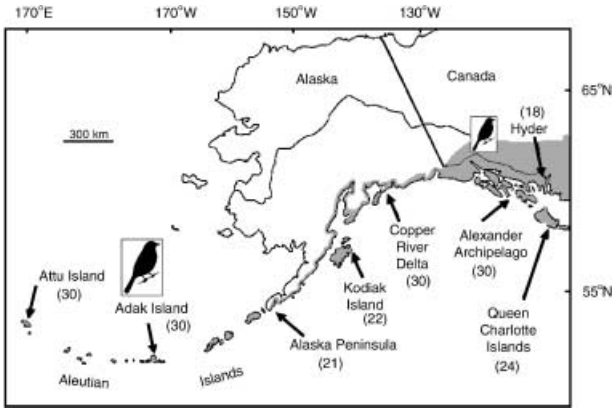

Fig. 1 Range of the song sparrow (shaded in grey) across the terminus of its distribution in northwestern North America. Song sparrow silhouettes represent relative differences in body size across Alaska. Sample sizes from each location included in parentheses (see Appendix I for museum voucher numbers).

2001; Onyabe & Conn 2001; Gautschi *et al.* 2002). In one case, Clegg *et al.* (2002) found that single founder events had little impact on genetic diversity or differentiation in the recent island colonization of silvereyes (*Zosterops lateralis*). Yet they also found that in sequential island colonizations via a stepping-stone process, there were significant losses of allelic diversity and increases in population divergences between the first island colonized and the fourth or fifth islands. In laboratory experiments, serially bottlenecked lines of houseflies (Meffert & Bryant 1991, 1992) showed marked differences in traits that might lead to premating isolation, including differentiation in courtship repertoire. Thus, sequential or serial reductions in population size could play a substantial role in the genetic and behavioural divergence of populations.

We sought to further examine the effects of cumulative founder events in the natural, sequentially colonized system of song sparrow (*Melospiza melodia*) populations that occurs in northwestern North America. Several factors make these populations an ideal system for examining the genetic consequences of sequential founding events including (i) an effectively linear geographical distribution; (ii) the likelihood that these populations were recently established through range expansion; (iii) morphological and behavioural differences among populations; and (iv) habitat differences among populations. Song sparrows are endemic to North America with the most northern and western populations distributed in a narrow strip along the Pacific coast and islands of Alaska (Fig. 1). The linear distribution of the Alaska populations extends for many thousands of kilometres, and they can be thought of as beads on a string that are increasingly distant from the species' continental distribution (Fig. 1).

Although the colonization history of these northwestern populations is unknown, past studies (with only limited sampling in Alaska) found that genetic variability was not partitioned geographically among North American song sparrows (Hare & Shields 1992; Zink & Dittmann 1993; Fry & Zink 1998). These studies suggested recent postglacial (< 10 000–12 000 BP) population expansions into previously glaciated areas throughout much of this region. Thus, it is likely that these populations would still show the effects of founder events because not enough time has ostensibly passed for new mutations to obliterate this record.

Song sparrows also exhibit remarkable geographical variation in morphology with seven (Gibson & Kessel 1997) to eight (Paynter 1970) phenotypically based subspecies described from Alaska — *Melospiza melodia maxima* from the western Aleutian Islands, *Melospiza melodia sanaka* from the eastern Aleutian Islands and Alaska Peninsula, *Melospiza melodia insignis* from the Kodiak Archipelago, *Melospiza melodia amaka* from Amak Island (but see Gibson & Kessel 1997; Pruett *et al.* 2004), *Melospiza melodia kenaiensis* from the Kenai Peninsula and Prince William Sound, *Melospiza melodia caurina* from the northern Gulf of Alaska, *Melospiza melodia rufina* from the Alexander Archipelago and Queen Charlotte Islands, and *Melospiza melodia inexspectata* from the southeast Alaska mainland (Gibson & Kessel 1997; Arcese *et al.* 2002). These morphological characters include variation in plumage colouration, bill length and width, and overall body size (Arcese *et al.* 2002). In the westernmost populations, body sizes are very large, showing masses of 40–55 g; individuals from other populations weigh, on average, half that amount (Rising 1996; Arcese *et al.* 2002; University of Alaska Museum, unpublished). The degree of morphological difference across the Alaska distribution is much greater than that found across the remaining continental range of this species (Gabrielson & Lincoln 1951). Also, populations west of central Alaska are not seasonally migratory, whereas most other song sparrow populations in Alaska and elsewhere are at least partially migratory (AOU 1957; Paynter 1970; Gibson & Kessel 1997). Thus, these western populations appear to exhibit traits that could be associated with the rapid differentiation of founded populations (Mayr 1954).

The westernmost song sparrow populations are found on the treeless Aleutian Islands and the Alaska Peninsula (Fig. 1), and they occur in a habitat different than that of other song sparrow populations in Alaska, which tend to be found in brushy and forested areas (Murie 1959; Aldrich 1984; personal observations). These treeless western regions might be thought of as marginal for this species (Murie 1959), and dramatic changes in morphology and a nonmigratory life history strategy suggest that these populations are under different selection pressures.

We used nuclear DNA microsatellite loci to evaluate colonization, population distinctiveness, genetic diversity, and gene flow among northwestern song sparrow populations. We asked several questions about cumulative

founder events and the genetic effects of this process in a natural vertebrate system: (i) Did song sparrows colonize the northwestern portion of their range via sequential (stepping-stone model) or a one-step (island model) event? (ii) What are the historic and current levels of gene flow among these populations? (iii) Are there sequential losses in genetic diversity and increases in genetic differentiation among populations? (iv) Is there evidence for population bottlenecks?

Materials and methods

Sampling and microsatellite data acquisition

Whole genomic DNA from the tissues of 205 song sparrows collected from eight western breeding populations (Fig. 1, Appendix I) was extracted following the procedure described in Glenn (1997). Sparrows were collected between April and November over several years. This sampling scheme, from the Queen Charlotte Islands to Attu Island, is equivalent to the distance between the US states of Florida and California (approximately 4000 km), which encompasses the breadth of the continental portion of this species' breeding range.

Eight microsatellite loci were amplified for all individuals using fluorescent dye-labelled primers developed for song sparrows (Jeffery *et al.* 2001) and for two other bird species (*Escu1*, Hanotte *et al.* 1994; *GF5*, Petren 1998) and were then genotyped using an ABI 373 A or 3100 automated sequencer. Because two of the loci are sex linked (*Mme3* and *Mme7*), we treated the females as having missing data for these two loci in analyses — females comprised approximately 30% of individuals for each population. Unequal sex ratios are caused by collection bias towards males. One locus (*Mme8*) had odd-sized alleles (some were only 1 bp different), so this locus was excluded from analyses that used a stepwise-mutation model (MIGRATE, GENECLASS, and M ratio). To ensure that this locus was correctly scored, we ran allele ladders made up of known size fragments on all gels.

Data analyses

Tests for Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium were performed using GDA (Lewis & Zaykin 2001). Two measures of genetic diversity were examined for each population, average expected heterozygosity and allelic richness, using BIOSYS-1 and FSTAT (Swofford & Selander 1981; Goudet 2002). Expected heterozygosities (H_E) were examined because this value combines information from allele frequencies and numbers of alleles. Expected heterozygosities are also commonly used to examine genetic variation, and the behaviour of this statistic following a bottleneck is well understood (Nei *et al.* 1975; Keller 2001). One-tailed *t*-tests were used to determine whether there were significant differences between neighbouring populations (those populations geographically nearest) for both measures. A bootstrapped (100 replicates), neighbour-joining (NJ) tree was developed using the programs SEQBOOT, GENDIST, NEIGHBOUR, and CONSENSE in the software package PHYLIP (Felsenstein 1993). Genetic distances (Nei 1972) were determined using GENDIST. Genetic differences between populations (F_{ST}) and whether these values differed significantly ($P < 0.05$) from zero were determined using the program ARLEQUIN version 2.000 (Schneider *et al.* 2000).

A Bayesian-clustering approach (STRUCTURE version 2, Pritchard *et al.* 2000; Falush *et al.* 2003) was implemented to examine how well the predefined populations corresponded to genetic groups (K). In this analysis, individual genotypes are assigned to clusters such that HWE and linkage equilibrium are achieved within each cluster. A Markov chain Monte Carlo (MCMC) approach is used to determine the number of clusters (K) that are most likely given the observed genotypes. We ran STRUCTURE two times for each user-defined $K(1 - 12)$ with an initial burn in of 10^5 and then 10^6 further iterations on the total data set. No prior information was used on the population of origin of each individual. We used the admixture model in which individuals may have mixed ancestry and the correlations model which takes into account that closely related populations might have correlated allele frequencies. When the K with the highest likelihood value was found, the proportion of membership of each predefined population (e.g. Attu Island, Adak Island) within each genetic cluster was determined.

We examined the relationship between genetic and geographical distances using a Mantel test. Rousset's (1997) genetic distance $[F_{ST}/(1 - F_{ST})]$ was used and geographical distances between collection locations were measured along the coast of Alaska in kilometres. Ten thousand random permutations of the geographical data matrix were employed. The program IBD (Isolation by Distance; Bohonak 2002) was used for all analyses.

Maximum-likelihood estimates of long-term gene flow (N_em) and θ ($4N_e\mu$) were determined using the program MIGRATE (version 1.6.9; Beerli & Felsenstein 1999), where N_e is the effective population size, m is the constant migration rate between population pairs, and μ is the rate of substitution per generation at the genetic locus considered. This program uses an expansion of the coalescent theory (Kingman 1982) to examine possible migration events and genealogies. Likelihood surfaces for each parameter were estimated by simulating gene genealogies based on the observed genotypes and estimates of θ and $4N_em$. Simulations were performed using an MCMC sampling approach. A Brownian motion approximation of the stepwise-mutation model was used to separately analyse each nuclear-encoded

locus. Ten short chains (10 000 gene trees sampled) and two long chains (100 000 gene trees sampled) were employed in analyses, with the first 10 000 trees ignored in each run to ensure parameter stability. The resulting integrated estimates of θ were used to initialize a second analysis. Likelihood surfaces were integrated across loci following parallel analysis of each locus on an SGI Origin 3800 computer (Silicon Graphics Inc.). We recognize that most natural systems violate an assumption of this program (constant population size over time; Beerli & Felsenstein 1999), thus we also used assignment tests as another indirect estimate of gene flow.

Assignment tests were used to determine whether sampled individuals were genetically unlikely to be from their population of origin (Cornuet *et al.* 1999). Sample sizes and the number of loci used in this study were similar to values that were shown in simulations to have a high likelihood of correctly assigning individuals to their population of origin (Cornuet *et al.* 1999). This method is an indirect means of examining recent instances of gene flow and is robust to violations of HWE. These analyses used likelihood-based Bayesian methods with 10 000 simulated individuals per population with the probability of exclusion threshold set to $P < 0.01$ (GENECLASS, Cornuet *et al.* 1999).

The program M was used to test for population bottlenecks (Garza & Williamson 2001). This test calculates the ratio (M) of the total number of alleles to the overall range in allele size within a population. When a population has experienced a reduction in size this ratio is expected to be smaller than in populations that are in mutation–drift equilibrium (Garza & Williamson 2001). Simulations were used to estimate the probability of the observed M ratio using Garza & Williamson's (2001) suggested values for proportion of one-step mutations (90%) and average size of non-one-step mutations ($\Delta_g = 3.5$). Various levels of θ ($4N_e\mu$) were used in simulations in which θ was set to 10, 25, and 40; these values correspond to equilibrium effective population sizes (N_e) before a bottleneck of 5000, 12 500, and 20 000, respectively, given a mutation rate of 5×10^{-4} (Goldstein & Schlötterer 1999). Current population sizes of song sparrows in Alaska are unknown but are likely to be smaller than 10 000 (personal observations). Thus, these values of θ are very conservative estimates. This technique should be able to detect past reductions in population size if they have occurred within the last 125–500 generations (Garza & Williamson 2001) and thus is preferable (in this system) to tests that can only identify very recent population reductions (Cornuet & Luikart 1996; Piry *et al.* 1999).

Results

Tests for HWE showed that two loci (*Mme1* from Attu Island and *Mme2* from Kodiak Island) were deficient in heterozygotes after adjustments for multiple comparisons. All loci were in linkage equilibrium.

The most likely number of genetic clusters (K) identified by the STRUCTURE analysis was nine [$\ln(X \mid K) = -4642$]. However, Pritchard *et al.* (2000) urged care in the estimation of K, especially when small differences in likelihood values are found. The likelihood of K being one to five clusters is small, but six to 12 clusters have similar likelihoods (Fig. 2). These values bracket the actual number of locations ($n = 8$) where song sparrows were collected for this study. When we examined the proportion of membership from each predefined population with $K = 9$, we found that greater than 50% of individuals from most of the populations assigned to one genetic cluster each (Table 1).

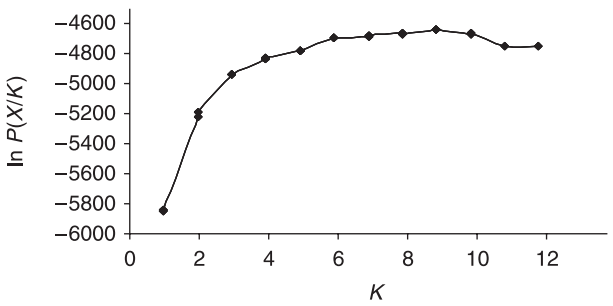

Fig. 2 Likelihood [$-\ln(X \mid K)$] of genetic population clusters (K) based on the STRUCTURE analysis of song sparrows from Alaska.

Table 1 Proportion of membership of individuals from each predefined population in each genetic cluster from STRUCTURE version 2

	Cluster								
Location	1	2	3	4	5	6	7	8	9
Attu	0.945	0.022	0.006	0.004	0.005	0.004	0.005	0.005	0.004
Adak	0.145	0.714	0.057	0.025	0.015	0.011	0.010	0.011	0.011
AK Pen	0.086	0.182	0.635	0.038	0.015	0.015	0.010	0.011	0.008
Kodiak	0.022	0.031	0.245	0.548	0.027	0.024	0.036	0.038	0.030
Copper R	0.019	0.024	0.049	0.098	0.549	0.056	0.087	0.085	0.033
Alex.	0.009	0.009	0.014	0.015	0.04	0.321	0.174	0.203	0.216
Hyder	0.019	0.009	0.014	0.017	0.05	0.102	0.397	0.286	0.106
QCI	0.011	0.011	0.010	0.015	0.011	0.117	0.145	0.189	0.492

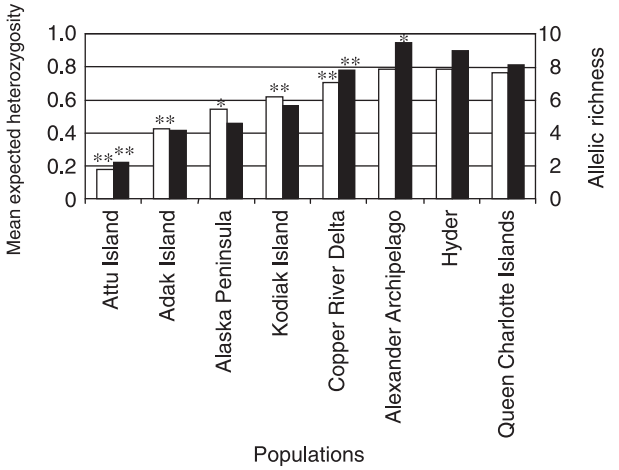

Fig. 3 Histogram of the mean expected heterozygosity (white bars) and allelic richness (black bars) for each song sparrow population. Significant *P* values for paired *t*-tests are indicated by either one ($P < 0.05$) or two ($P < 0.005$) asterisks appearing above the bar that is different from the population to the right.

Table 2 *T*-statistics (*T*), *P* values (*P*), and degrees of freedom (d.f.) for paired *t*-test comparisons of heterozygosity and allelic richness between neighbouring song sparrow populations (see Fig. 3)

	H_E			Allelic richness		
Locations	*T*	*P*	d.f.	*T*	*P*	d.f.
Attu vs. Adak	10.4	< 0.005	7	4.29	< 0.005	7
Adak vs. Alaska Pen.	4.87	< 0.005	7	1.40	> 0.100	7
Alaska Pen. vs. Kodiak	2.90	< 0.05	7	1.20	> 0.100	7
Kodiak vs. CRD	3.63	< 0.005	7	3.03	< 0.050	7
CRD vs. Alexander A.	5.67	< 0.005	7	2.50	< 0.050	7
Alexander A. vs. Hyder	0.12	> 0.400	7	0.28	> 0.400	7
Hyder vs. QCI	1.15	> 0.050	7	1.26	> 0.200	7

CRD, Copper River Delta, QCI, Queen Charlotte Islands.

Alexander Archipelago and Hyder populations showed evidence of admixture. Based on these outcomes, we feel that our predefined populations correspond fairly well to the groups identified using the STRUCTURE analysis. Thus, these groups were used in all other analyses.

Populations at the western periphery of the species' distribution in Alaska had significantly lower mean expected heterozygosities than neighbouring populations (Fig. 3, Table 2). Attu Island had both the lowest heterozygosity and allelic richness values, whereas populations in southeast Alaska and northern British Columbia had similarly high expected heterozygosities and allelic diversities. The Alexander Archipelago population had the highest allelic richness values, perhaps because this population sample comprised a grouping of several islands within the archipelago (Fig. 1, Appendix I). However, Attu Island and Alaska

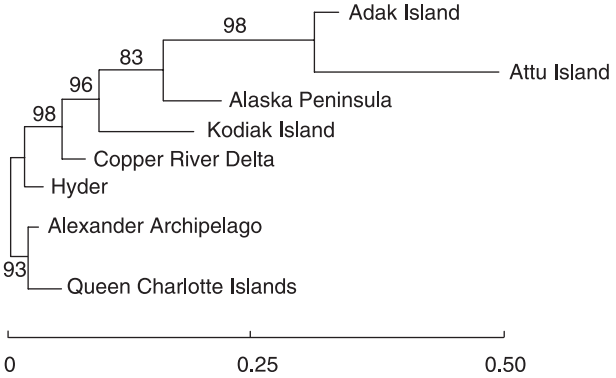

Fig. 4 Distance tree of song sparrow populations based on Nei's (1972) genetic distance using eight microsatellite loci. Genetic distance values are shown below the tree and bootstrap support for each branch are listed below that branch.

Peninsula samples comprised 2–3 locations (Appendix I) and did not exhibit inflated values. To determine whether a sampling bias was driving these values, we plotted the number of individuals sampled from a population against the average number of alleles from that population and found no correlation ($r^2 = 0.0046$, $P = 0.87$).

The NJ tree, based on Nei's (1972) genetic distance (Fig. 4), showed a sequential increase in genetic distance from east to west with the most distant population from a southeastern Alaska root being Attu Island (Fig. 4). Identical topologies were found when Cavalli-Sforza chord-distance (Cavalli-Sforza & Edwards 1967) and Reynolds distance (Reynolds *et al.* 1983) were used to build trees (POPDIST 1.1.1; Guldbrandtsen *et al.* 2002). All pairwise F_{ST} estimates were significantly different from zero ($P < 0.001$). The majority of pairwise F_{ST} and genetic distance estimates between populations showed a gradual increase in F_{ST} with each step eastward from the most western populations (Aleutian Islands; Table 3). An exception to this pattern was the higher F_{ST} and genetic distance estimates between Kodiak Island and the Aleutian Island locations than between these locations and the Copper River Delta. This suggests that Kodiak Island was not a source of colonists for some populations farther west. A pattern of isolation by distance was found among song sparrows for nontransformed distances ($Z = 33\,529.67$, $P = 0.001$).

Maximum-likelihood estimates of long-term gene flow (N_em) were all less than 10 (Table 4; –ln likelihood = 20 750). The highest pairwise gene flow estimates are consistently those between Copper River Delta and other sampled locations (Table 4). Directionality of gene flow was also examined by comparing east-to-west and west-to-east estimates based on MIGRATE results (Table 4). On average, gene flow has been higher from east to west (2.09) than west to east (1.76); however, a paired *t*-test showed no significant difference between these means ($P = 0.23$). Assignment tests

Table 3 Pairwise estimates of genetic differentiation (F_{ST}) and genetic distance (Nei 1972) between song sparrow populations above and below diagonal, respectively. All F_{ST} estimates are significantly different from zero ($P < 0.001$).

	Attu Is.	Adak Is.	Alaska Pen.	Kodiak Is.	CRD	Hyder	Alexander A.	QCI
Attu Is.	—	0.143	0.283	0.410	0.346	0.405	0.354	0.388
Adak Is.	0.064	—	0.079	0.216	0.200	0.224	0.217	0.237
Alaska Pen.	0.282	0.179	—	0.121	0.158	0.178	0.174	0.203
Kodiak Is.	0.527	0.391	0.212	—	0.092	0.106	0.109	0.107
CRD	0.409	0.365	0.376	0.257	—	0.047	0.050	0.080
Hyder	0.720	0.631	0.546	0.426	0.242	—	0.025	0.036
Alexander A.	0.628	0.624	0.550	0.426	0.232	0.186	—	0.025
QCI	0.755	0.756	0.774	0.509	0.397	0.279	0.146	—

CRD, Copper River Delta; QCI, Queen Charlotte Islands.

Table 4 Pairwise estimates of directional gene flow (N_em) using seven song sparrow microsatellite loci. Populations listed horizontally are receiving migrants; populations providing migrants are listed vertically. For example, the Alexander Archipelago population is receiving 1.73 migrants per generation from the Queen Charlotte Islands, and the latter is receiving 3.03 migrants from the former. See Fig. 1 for population locations. Confidence intervals for all estimates are within ± 0.5.

	Attu Is.	Adak Is.	Alaska Pen.	Kodiak Is.	CRD	Hyder	Alexander A.	QCI
Attu Is.	—	1.04	0.64	0.22	3.61	0.42	0.93	0.36
Adak Is.	1.56	—	1.20	2.63	4.97	1.64	2.07	0.42
Alaska Pen.	0.79	2.90	—	2.37	3.31	1.54	1.80	1.21
Kodiak Is.	0.22	0.37	3.96	—	5.07	2.47	1.26	0.27
CRD	3.34	3.92	6.97	1.37	—	1.75	3.96	1.31
Hyder	0.34	1.31	3.51	1.30	2.19	—	1.63	3.10
Alexander A.	0.37	1.89	2.50	0.38	3.92	2.66	—	3.03
QCI	0.38	0.29	2.97	0.53	1.07	2.24	1.73	—

CRD, Copper River Delta; QCI, Queen Charlotte Islands.

Population	N	Mean Bayesian probability	Genotypes not from population	Genotypes not from population	Most likely any population
Attu Island	30	0.58 ± 0.32	0	0	—
Adak Island	30	0.50 ± 0.34	1	0	Alex
Alaska Peninsula	21	0.43 ± 0.34	2	1	CRD
Kodiak Island	22	0.39 ± 0.33	1	1	—
Copper River Delta	30	0.27 ± 0.31	1	0	Hyder
Alexander Archipelago	30	0.22 ± 0.30	3	2	Hyder
Hyder	18	0.16 ± 0.26	2	1	CRD
Queen Charlotte Islands	24	0.23 ± 0.24	4	3	Alex

Table 5 GENECLASS assignment test results comparing individual microsatellite genotypes with their population of origin for eight song sparrow populations showing mean Bayesian probability of membership in that population, number of genotypes statistically not from that population, the number of individuals not assigned to any of the sampled populations, and population that the unassigned individuals are most likely from (Alex, Alexander Archipelago; QCI, Queen Charlotte Islands; and CRD, Copper River Delta).

corresponded with gene flow estimates (Table 5). Estimates of the numbers of individuals unlikely to have come from the sampled location were similar to gene flow estimates (within an order of magnitude) acquired using a coalescent approach. However, other nearby, nonsampled populations might be exchanging genes with these populations. This idea is supported by the genotypes that were not assignable to any sampled population (Table 5). None of the genotypes from Attu Island were excluded from that population, which indicates very low or nonexistent contemporary gene flow between this location and any other song sparrow population (Table 5). Other populations showed values suggesting higher levels of contemporary gene flow (N_em of 2–4 individuals per sampled population; Table 5). Individuals that did not assign to their population of origin ($P < 0.01$) but that had a high likelihood of assigning to another sampled location suggested current gene flow from eastern to western populations. The only exception

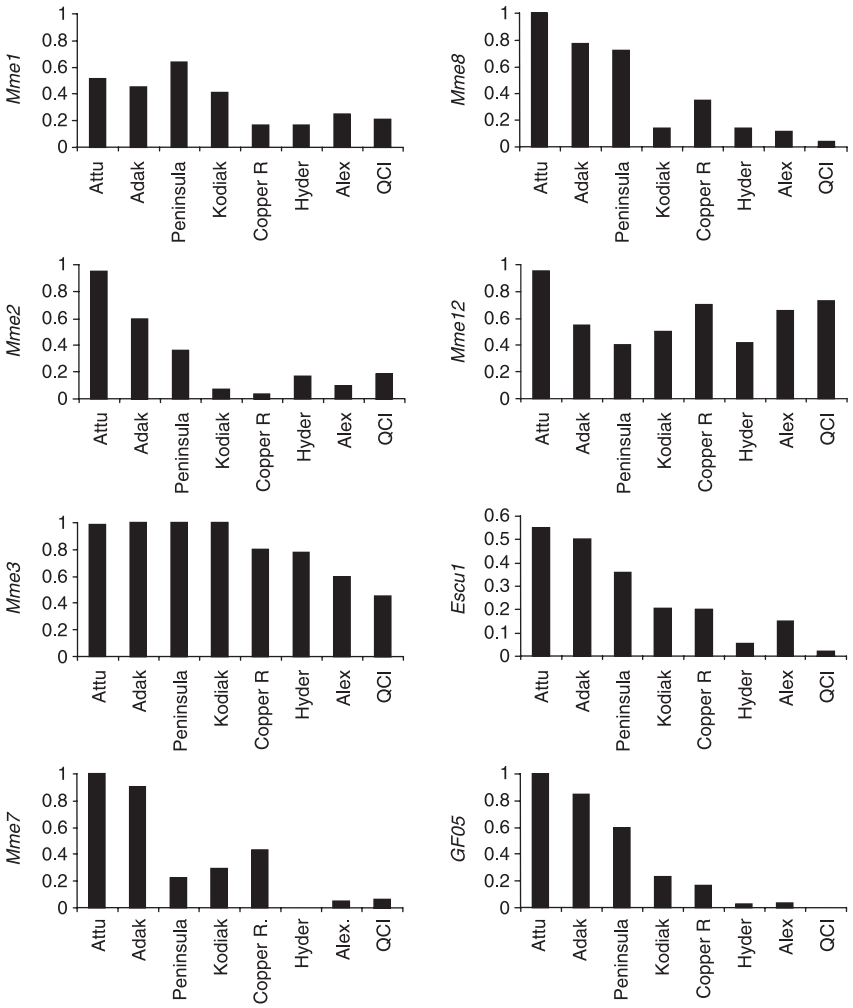

Fig. 5 Histograms of highest frequency allele for each of eight microsatellite loci in the Attu Island population and the frequencies of that allele in other sampled populations.

Table 6 Results of *M*-test for population bottlenecks in northwestern song sparrow populations. Significant bottleneck effects are indicated with asterisks ($P < 0.05$).

		P values given μ values		
Population	*M*	10	25	40
Attu Island	0.585	0.004*	0.021*	0.026*
Adak Island	0.624	0.023*	0.070	0.144
Alaska Peninsula	0.545	0.002*	0.010*	0.034*
Kodiak Island	0.588	0.014*	0.075	0.184
Copper River Delta	0.569	0.002*	0.010*	0.023*
Hyder	0.627	0.075	0.295	0.548
Alexander Archipelago	0.599	0.008*	0.028*	0.052
Queen Charlotte Islands	0.610	0.006*	0.094	0.208

was an individual from Hyder that was assigned with highest likelihood to the Copper River Delta population (Fig. 1, Table 5).

M-tests showed bottlenecks for most of the populations examined under various possible θ values (Table 6). These results indicated a reduction in population size for Attu Island, Alaska Peninsula, and Copper River Delta even if prebottleneck equilibrium population sizes were large. However, the Hyder population did not show a significant reduction in population size no matter how small the estimated θ was set for these tests (Table 6).

Comparisons of the frequency of the most common allele for each locus in the Attu Island population with the frequencies of this allele in all other song sparrow populations showed a general increase in allele frequency through each colonization step from southeastern populations to Aleutian Island populations (Fig. 5). Six of these eight alleles were fixed or had greater than 90% frequencies in the Attu Island population (Fig. 5).

Discussion

Genetic diversity

Two genetic consequences of founder events should be a substantial decrease in genetic diversity and an attendant

increase in genetic divergence (F_{ST}) from the most ancient to the most recent populations (Le Corre & Kremer 1998). Alaska song sparrows show both of these effects. There is a progressive loss of genetic diversity in both heterozygosity and numbers of alleles westward from the Alexander Archipelago (Fig. 3). This step-down process culminates with very low genetic diversity in the most western population (Attu Island) and with microsatellite heterozygosity values that are less than or similar to several endangered populations of vertebrates (Eldridge *et al.* 1999; Pope *et al.* 2000; Kretzmann *et al.* 2001; Whitehouse & Harley 2001). These levels are substantially lower than those found in other song sparrow populations by studies using these same microsatellite loci (Keller *et al.* 2001; Chan & Arcese 2002).

Colonization scenarios

The population genetics of northwestern song sparrows appear to fit a linear stepping-stone colonization model (Le Corre & Kremer 1998) from southeast to northwest. This is indicated by the genetic distance tree (Fig. 4) in that populations are genetically closest to their nearest geographical neighbours. In addition, historic gene flow based on coalescent estimates suggests that in many cases neighbours have had higher gene exchange (Table 4) than more distant populations. The Copper River Delta population consistently had the highest gene flow levels with song sparrow populations found east and west of its central location (Fig. 1). The Copper River Delta was probably a key component in the colonization of more western populations (Kodiak Island, Alaska Peninsula, and the Aleutian Islands). However, gene flow estimates are relatively low among all locations both currently (Table 5) and historically (Table 4), suggesting restricted gene flow over long timescales that has been insufficient to counteract genetic divergence.

These genetic relationships are also consistent with a one-step colonization of the entire range with subsequent divergence through isolation by distance. A comparison of geographical and genetic distances using a Mantel test supported an isolation-by-distance model, and a single colonization with subsequent population bottlenecks within each population would likely show a similar pattern. However, we do not believe that the preponderance of evidence supports such a scenario. In such a case, a random loss of diversity would be likely, especially given that many sampled populations are found on islands and thus have limited habitat availability and are more susceptible to catastrophic events. Thus, Adak and Kodiak islands, for example, would be just as likely to have extremely low diversity as the Attu Island sample. Instead, there is an apparently nonrandom decline in genetic diversity, wherein populations that are progressively farther away from the continental distribution of the species have progressively lower genetic diversity (Fig. 3). It seems most likely that a sequential series of colonizations would produce the pattern found.

In addition, the cline-like distribution of common Attu alleles across the study's geographical scope (Fig. 5) does not support a single colonization event. With a one-step event, all alleles in the founded populations would have an equal probability of being fixed because of genetic drift. However, alleles in our samples are not distributed stochastically. The alleles most common on Attu Island tend to be found at slightly lower frequencies on Adak Island and still lower frequencies on the Alaska Peninsula; this pattern tends to persist among populations farther away from Attu (Fig. 5). Also, many of these alleles are infrequent or absent in populations that are geographically distant from Attu Island (*Mme 2*, *3*, *7*, *8*, *Escu1*, and *GF05*; Fig. 5). It is possible that selection could be acting on portions of the genome close to these loci and that this could explain the cline-like pattern of allele frequencies. However, six of the eight loci examined would have to be under similar selection pressures and this seems unlikely given the neutral nature of most microsatellite variation (Goldstein & Schlötterer 1999).

Using the southeastern populations as the first step in the colonization of Alaska, pairwise F_{ST} estimates show an increase in genetic divergence (Table 3). A notable exception to this pattern is Kodiak Island. This population is more differentiated from Aleutian populations than these populations are from Copper River Delta. However, Kodiak Island is geographically closer to Adak and Attu islands than these locations are to the Copper River Delta (Fig. 1). This suggests colonization via the mainland for the Aleutian Island populations, with Kodiak Island undergoing its own colonization and differentiation slightly out of step with the most simplistic stepping-stone model of sequential colonization and differentiation. However, the distance tree suggests otherwise (Fig. 4). A possible explanation for this subtle apparent inconsistency is that the Alaska Peninsula population was colonized from two sources. Historic gene flow estimates (Table 4) suggest that the Alaska Peninsula received immigrants from Kodiak Island (*c.* 4/generation) and the Copper River Delta (~7/generation). Another possible explanation is that recent gene flow between the Alaska Peninsula and Copper River Delta (more than that occurring between Kodiak Island and Alaska Peninsula) has obscured the initial colonization event. Although this one step in the sequential process is a little unclear, the data suggest that most song sparrow populations in Alaska were colonized through a sequential stepping-stone process.

Fry & Zink (1998) suggested that song sparrows colonized their current distribution in North America from two or three glacial refugia: the east coast of North America, the

Queen Charlotte Islands, and southern California. In addition, there is genetic evidence for one or two Beringian refugia (possibly in the Aleutian Islands) for other bird species (Holder *et al.* 1999; Congdon *et al.* 2000; Pruett & Winker 2005). Our data seem to fit best a hypothesized sequential colonization of Alaska from the Queen Charlotte Islands refugium; we found no genetic evidence for colonization from a Beringian refugium for song sparrows. We would expect higher levels of diversity in refugial populations than in colonized populations (Hewitt 1996), as was found for the Queen Charlotte Island sample. We found the lowest genetic diversity in the Aleutian Island populations (possible location for the Beringian refugium; Pruett & Winker 2005). Unfortunately, we lack the ability to date these colonization events and our sampling scheme does not enable us to comment on possible colonization from refugia farther east or south.

Long-term drift vs. founder events

The study of Clegg *et al.* (2002) was useful for evaluating founder events because the colonization dates were known. They were thus able to differentiate between the effects of founding and longer-term genetic drift in very small populations because these populations were colonized recently (within the last 200 years). Although we do not have estimates of colonization dates for Alaska song sparrows, several factors suggest that the current population genetic structure is at least partially caused by founder events. Western populations have very low heterozygosities and allelic diversities; if they had existed for long periods, new mutations would likely have replenished some of this diversity.

Repetitive bottlenecks (Table 6) and losses in diversity (Fig. 3) also suggest that the actual number and genetic diversity of founders had a strong impact on this system of sequential foundings. The strongest effect occurs in the last step to the population on Attu Island (Fig. 3). This is probably caused by the limited diversity caused by each founding event in which fewer and fewer alleles were available for each colonization step.

Although the size of bottlenecked populations is difficult to determine, very few colonizing individuals probably founded these populations. Yet presently, all of these populations at a regional scale are at sizes of at least several thousand individuals each (personal observations), and thus it is unlikely that they are currently undergoing strong drift. With these relatively large current population sizes and the probable recent colonization, gene flow would be a more likely source of genetic replenishment. However, in this system gene flow appears to be too low to counteract the effects of the initial founder events and the unknown influences of longer-term drift occurring in possibly small historic populations.

Implications for sequentially founded populations

Overall, our findings support a stepping-stone founder model (Le Corre & Kremer 1998) for the song sparrow colonization of western Alaska. With each founding step, an increasing proportion of the genetic diversity available has been lost. In addition, genetic divergence increased with each step. These findings, coupled with the apparently limited gene flow and divergent morphology and behaviour found in populations in the Aleutian Islands, support many of the key expectations of founding events. These include a loss of genetic diversity, an increase in genetic divergence, and a rapid change in behaviour or morphology that might lead to reproductive isolation (Mayr 1954, 1982).

Our results show that in linear range expansions founder events can be amplified, and that the power of such amplifications can drastically alter the genetic structure of natural populations. Single-step founder events might not lead to 'genetic revolutions' in most populations (Barton & Charlesworth 1984; Moya *et al.* 1995; Clegg *et al.* 2002). But a system of sequential founding events might enable a rapid shift to new ecological optima in the genetically depauperate populations found at the end of linear distributions. As Mayr (1954; p. 175) stated, '… only a few [founded populations] will play a role in long-term evolution, perhaps one in fifty. The odds are very much against a successful passing through a bottleneck of reduced variability as well as the reaching of a new level of high variability and of an unoccupied ecological niche.' The genetic precursors to this continued process are present in western song sparrows through sequential founding events. In addition, they have successfully colonized an unoccupied niche, and thus far they have eluded extinction. If gene flow continues to be limited among these populations, only time will tell whether they persist, regain genetic variability and continue to diverge.

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This study is a portion of Christin Pruett's PhD research on the population genetics of Beringian landbirds. She is currently a Postdoc at the University of Alaska Fairbanks studying the genetics of birds as vectors of infectious disease. Kevin Winker is Curator of Birds and Associate Professor at the University of Alaska Museum, the Department of Biology and Wildlife, and the Institute of Arctic Biology. His research focuses on avian evolution and migration.

Appendix I

Location, subspecies, and voucher numbers for song sparrows used in this study

Locality	Subspecies	Catalogue numbers (all are University of Alaska Museum, UAM)
Alaska: Aleutian Islands, Attu Island*	*M. m. maxima*	8416–47, 9299–306, 9418, 9420, 9860, 11173–74, 11225–26, 11228, 11242, 11270–71, 11277, 11556, 11790, 1829, 12094, 12141, 13056, 13058, 13140
Alaska: Aleutian Islands, Adak Island	*M. m. maxima*	8460–61, 10040–42, 10167–68, 10170, 10172, 10179, 10188, 10942, 10946–47, 11048, 11175–78, 11267–69, 11501, 11511, 11827, 11850, 12143, 13057, 13059, 13161
Alaska: Alaska Peninsula†	*M. m. sanaka*	9321, 9328, 10091, 11230, 11362, 11365–66, 11381, 11389, 11823, 10090, 10171, 10187, 11276, 11379, 11390, 11585, 11713, 12142, 111238–239
Alaska: Kodiak Island	*M. m. insignis*	7522, 7661, 8776–78, 8807, 11871, 12139, 13899–900, 13914, 13939, 14001–10
Alaska: Copper River Delta	*M. m. caurina*	8922, 10652, 11101, 11103–04, 11141, 11180–82, 11210, 11223, 11231, 11233–34, 11245, 11247–48, 11272–73, 11329–32, 11361, 11367, 11382–84, 11388, 11583
Alaska: Alexander Archipelago‡	*M. m. rufina*	11360, 11516–17, 11712, 11824, 13287–88, 13438, 13463, 13542–43, 13886–87, 13911–13, 13915–16, 13936–38, 13941–47, 13952–53
Alaska: Hyder	*M. m. inexspectata*	7341–46, 8115, 8379, 8447–49, 8606–08, 10159–60, 10179, 13921
British Columbia: Queen Charlotte Is.	*M. m. rufina*	11172, 11179, 11542–53, 13079–80, 13908–10, 13917–20, 13940

*Includes individuals from Attu Island (27) and Shemya Island (3); †includes individuals from King Cove (10), Shumagin Islands (9), and Unalaska Island (2); ‡includes individuals from Prince of Wales Island (17), Gravina Island (8), Revillagigedo Island (2), Heceta Island (2), and Warren Island (1).

Appendix II

Allele frequencies for eight microsatellite loci from eight northwestern song sparrow populations

Locus/ alleles	Attu Island	Adak Island	Alaska Peninsula	Kodiak Island	Copper River Delta	Alexander Archipelago	Hyder	Queen Charlotte
Mme1								
130	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000
132	0.000	0.150	0.000	0.023	0.050	0.000	0.000	0.104
134	0.133	0.000	0.000	0.000	0.000	0.000	0.000	0.000
136	0.217	0.000	0.000	0.000	0.000	0.000	0.000	0.021
138	0.100	0.000	0.024	0.023	0.017	0.000	0.000	0.021
144	0.000	0.250	0.095	0.295	0.167	0.050	0.167	0.271
146	0.517	0.450	0.643	0.409	0.167	0.250	0.167	0.208
148	0.033	0.000	0.000	0.000	0.033	0.100	0.167	0.021
150	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.042
152	0.000	0.050	0.119	0.045	0.100	0.383	0.306	0.229
154	0.000	0.000	0.000	0.068	0.000	0.000	0.000	0.042
156	0.000	0.017	0.000	0.068	0.000	0.000	0.000	0.021
158	0.000	0.067	0.119	0.068	0.417	0.200	0.194	0.021
160	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000
164	0.000	0.000	0.000	0.000	0.050	0.000	0.000	0.000
Mme2								
120	0.000	0.000	0.024	0.000	0.000	0.017	0.000	0.000
124	0.000	0.000	0.000	0.000	0.000	0.100	0.000	0.000
126	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000
128	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000
136	0.000	0.000	0.000	0.023	0.000	0.000	0.000	0.104
138	0.000	0.000	0.024	0.091	0.317	0.167	0.444	0.271
140	0.000	0.000	0.000	0.000	0.000	0.133	0.028	0.000
142	0.950	0.600	0.357	0.068	0.033	0.100	0.167	0.188
144	0.017	0.000	0.000	0.000	0.033	0.000	0.028	0.000
146	0.000	0.133	0.000	0.045	0.017	0.050	0.000	0.063
148	0.000	0.183	0.381	0.091	0.033	0.050	0.000	0.000

Appendix II *Continued*

Locus/ alleles	Attu Island	Adak Island	Alaska Peninsula	Kodiak Island	Copper River Delta	Alexander Archipelago	Hyder	Queen Charlotte
150	0.033	0.033	0.048	0.091	0.117	0.100	0.083	0.104
152	0.000	0.017	0.000	0.318	0.133	0.017	0.056	0.021
154	0.000	0.033	0.143	0.114	0.05	0.050	0.000	0.021
156	0.000	0.000	0.000	0.159	0.017	0.033	0.028	0.021
158	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000
160	0.000	0.000	0.000	0.000	0.000	0.017	0.028	0.000
162	0.000	0.000	0.000	0.000	0.017	0.067	0.056	0.208
164	0.000	0.000	0.000	0.000	0.150	0.050	0.056	0.000
166	0.000	0.000	0.000	0.000	0.000	0.000	0.028	0.000
170	0.000	0.000	0.024	0.000	0.000	0.000	0.000	0.000
172	0.000	0.000	0.000	0.000	0.050	0.033	0.000	0.000
Mme3								
160	0.017	0.000	0.000	0.000	0.000	0.000	0.000	0.000
162	0.000	0.000	0.000	0.000	0.000	0.083	0.000	0.333
164	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000
170	0.000	0.000	0.000	0.000	0.083	0.083	0.056	0.063
172	0.983	1.000	1.000	1.000	0.800	0.600	0.778	0.458
174	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000
176	0.000	0.000	0.000	0.000	0.000	0.000	0.111	0.000
178	0.000	0.000	0.000	0.000	0.000	0.167	0.000	0.083
180	0.000	0.000	0.000	0.000	0.083	0.033	0.028	0.063
182	0.000	0.000	0.000	0.000	0.017	0.000	0.028	0.000
188	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000
Mme7								
100	0.000	0.000	0.000	0.000	0.000	0.000	0.139	0.000
110	0.000	0.000	0.024	0.000	0.000	0.000	0.000	0.000
112	0.000	0.000	0.024	0.000	0.000	0.000	0.000	0.000
114	0.000	0.000	0.119	0.091	0.100	0.067	0.111	0.000
116	0.000	0.000	0.000	0.000	0.000	0.000	0.028	0.000
118	0.000	0.017	0.024	0.000	0.017	0.017	0.056	0.000
120	0.000	0.000	0.119	0.045	0.15	0.033	0.056	0.021
122	1.000	0.900	0.167	0.295	0.433	0.05	0.000	0.063
124	0.000	0.033	0.524	0.523	0.100	0.033	0.000	0.000
126	0.000	0.017	0.000	0.000	0.000	0.200	0.167	0.375
128	0.000	0.033	0.000	0.000	0.067	0.083	0.139	0.083
130	0.000	0.000	0.000	0.045	0.083	0.267	0.083	0.292
132	0.000	0.000	0.000	0.000	0.000	0.150	0.000	0.104
134	0.000	0.000	0.000	0.000	0.033	0.050	0.028	0.063
136	0.000	0.000	0.000	0.000	0.000	0.033	0.167	0.000
138	0.000	0.000	0.000	0.000	0.017	0.000	0.028	0.000
140	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000
Mme8								
201	0.000	0.117	0.000	0.000	0.000	0.000	0.056	0.000
205	0.000	0.000	0.000	0.000	0.033	0.000	0.000	0.000
207	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000
208	0.000	0.000	0.000	0.000	0.000	0.033	0.028	0.021
210	0.000	0.000	0.000	0.045	0.000	0.150	0.056	0.271
211	0.000	0.000	0.000	0.000	0.000	0.000	0.028	0.000
213	1.000	0.767	0.714	0.136	0.350	0.117	0.139	0.042
215	0.000	0.067	0.024	0.000	0.200	0.133	0.056	0.021
217	0.000	0.017	0.143	0.250	0.200	0.283	0.250	0.292
218	0.000	0.000	0.000	0.023	0.000	0.067	0.000	0.042
219	0.000	0.000	0.000	0.023	0.067	0.067	0.167	0.125
220	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000
221	0.000	0.000	0.000	0.045	0.017	0.017	0.000	0.000

Appendix II *Continued*

Locus/ alleles	Attu Island	Adak Island	Alaska Peninsula	Kodiak Island	Copper River Delta	Alexander Archipelago	Hyder	Queen Charlotte
223	0.000	0.000	0.000	0.000	0.000	0.017	0.083	0.021
224	0.000	0.017	0.000	0.000	0.000	0.000	0.000	0.000
225	0.000	0.017	0.024	0.000	0.000	0.000	0.000	0.083
227	0.000	0.000	0.095	0.432	0.067	0.017	0.000	0.021
228	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000
229	0.000	0.000	0.000	0.045	0.033	0.067	0.111	0.063
234	0.000	0.000	0.000	0.000	0.017	0.000	0.028	0.000
Mme12								
182	0.000	0.000	0.095	0.000	0.000	0.000	0.000	0.021
188	0.950	0.550	0.357	0.500	0.700	0.650	0.417	0.750
200	0.000	0.000	0.000	0.000	0.000	0.067	0.000	0.063
206	0.000	0.000	0.000	0.000	0.017	0.067	0.028	0.000
212	0.033	0.050	0.000	0.023	0.017	0.033	0.195	0.000
218	0.000	0.000	0.262	0.250	0.183	0.083	0.111	0.000
224	0.017	0.333	0.095	0.091	0.033	0.033	0.167	0.063
230	0.000	0.067	0.190	0.136	0.033	0.033	0.056	0.021
236	0.000	0.000	0.000	0.000	0.017	0.033	0.028	0.083
Escu1								
128	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000
132	0.000	0.000	0.000	0.023	0.000	0.033	0.000	0.063
134	0.000	0.000	0.000	0.091	0.433	0.167	0.222	0.104
138	0.550	0.500	0.286	0.205	0.200	0.150	0.056	0.021
140	0.000	0.000	0.024	0.000	0.033	0.000	0.000	0.000
142	0.000	0.000	0.024	0.000	0.000	0.000	0.000	0.000
144	0.000	0.000	0.000	0.000	0.017	0.000	0.222	0.146
146	0.000	0.000	0.000	0.159	0.083	0.200	0.139	0.104
148	0.433	0.200	0.119	0.205	0.117	0.200	0.194	0.188
150	0.017	0.300	0.548	0.318	0.050	0.000	0.056	0.021
152	0.000	0.000	0.000	0.000	0.050	0.217	0.083	0.208
154	0.000	0.000	0.000	0.000	0.000	0.017	0.028	0.125
156	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.021
Gf05								
184	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000
186	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.083
192	0.000	0.000	0.024	0.000	0.000	0.017	0.000	0.000
194	0.000	0.000	0.000	0.000	0.05	0.067	0.111	0.000
196	0.000	0.000	0.000	0.000	0.000	0.000	0.111	0.021
198	0.000	0.050	0.333	0.682	0.267	0.117	0.139	0.229
200	0.000	0.017	0.000	0.000	0.000	0.050	0.000	0.042
202	0.000	0.000	0.000	0.000	0.000	0.283	0.139	0.208
206	0.000	0.000	0.000	0.000	0.000	0.017	0.056	0.021
208	0.000	0.000	0.000	0.023	0.000	0.000	0.083	0.000
210	0.000	0.033	0.000	0.045	0.067	0.050	0.056	0.000
212	0.983	0.850	0.571	0.227	0.167	0.033	0.028	0.000
214	0.000	0.033	0.000	0.000	0.000	0.017	0.000	0.083
216	0.017	0.017	0.048	0.000	0.000	0.083	0.028	0.083
218	0.000	0.000	0.000	0.023	0.150	0.117	0.028	0.000
220	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.104
222	0.000	0.000	0.000	0.000	0.017	0.000	0.167	0.000
224	0.000	0.000	0.000	0.000	0.033	0.033	0.000	0.125
226	0.000	0.000	0.000	0.000	0.133	0.017	0.028	0.000
228	0.000	0.000	0.000	0.000	0.017	0.033	0.028	0.000
232	0.000	0.000	0.000	0.000	0.083	0.000	0.000	0.000
234	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000
238	0.000	0.000	0.000	0.000	0.000	0.017	0.000	0.000