THE POPULATION AND EVOLUTIONARY GENETICS OF THE ANCIENT MURRELET (*SYNTHLIBORAMPHUS ANTIQUUS)*: A SUMMARY

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ABSTRACT

Genetic data can help answer questions about the basis of the contemporary population structure and evolutionary history of a species. Ancient Murrelets (*Synthliboramphus antiquus*) are north-Pacific seabirds that breed in a 9,000 km subarctic range from China to British Columbia. In this study, DNA sequence variation data from the cytochrome *b* gene and the mitochondrial control region are used to both estimate the extent of genetic differentiation, or structure, among current populations of the Ancient Murrelet, and to infer the evolutionary events responsible for this structure. Neither genotype frequencies nor sequence variation revealed any large-scale population structuring, suggesting that contemporary Ancient Murrelets form a single, panmictic population. Estimated rates of gene flow between colonies and regions are high enough to counter genetic drift. Genetic distances between haplotypes are small. Mismatch distribution and phylogenetic analysis are not consistent with an expanding population. Thus the null hypothesis that Ancient Murrelets are in genetic equilibrium with respect to mutation, migration and genetic drift cannot be rejected.

INTRODUCTION

ANCIENT Murrelets (*Synthliboramphus antiquus*) are small, migratory seabirds that breed in a 9,000 km arc around the northern rim of the Pacific Ocean (Fig. 1), becoming increasingly abundant from China to British Columbia (Gaston 1992). British Columbia supports about half of the world's breeding population of Ancient Murrelets, while the majority of the remaining 50% nest in Alaska. Over the past several decades, dramatic population declines have been reported worldwide for Ancient Murrelets, and the species has been designated as "Vulnerable" by the Committee on the Status of Endangered Wildlife in Canada (Gaston 1994). Much of this population decline has been attributed to the introduction of mammalian predators, including rats (*Rattus rattus* and *R. norvegicus*) and raccoons (*Procyon lotor*).

Little is known about the movements of Ancient Murrelets among different colonies, and there is no information about their global population genetic structure. Aspects of this population structure include gene flow (the loss or gain of genetic information from a population due to the immigration or emigration of fertile individuals) and effective population sizes (the number of breeding individuals in a particular area). Analysis of genetic population structure can also indicate whether populations from different geographic areas are genetically different from each other, and if so, how different. Banding studies have suggested that either Ancient Murrelets do not exhibit strong natal philopatry (birds do not necessarily return to the geographic location where they were hatched), or that they suffer high mortality (Gaston 1992).

Asian Ancient Murrelets remain close to Asia, which suggests that while gene flow may be high within Asia and North America, significant gene flow may not occur between North American and Asian Ancient Murrelets (Piatt and Gould 1994). Also, few Ancient Murrelets nest in the Aleutians, so Asian and North American populations are essentially geographically disjunct, or separate.

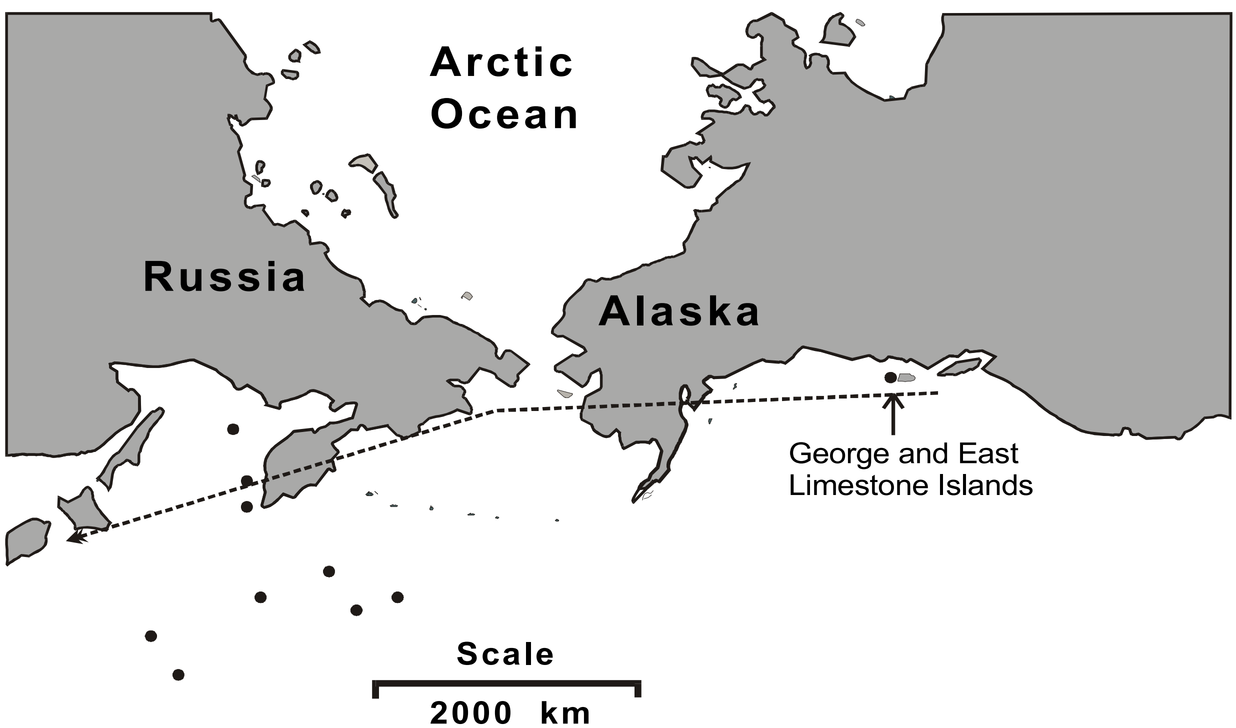

Figure 1. Ancient Murrelet breeding range (dotted line) and sampling sites (dots).

The goal of this study was to apply various statistical methods, including coalescent theory, to sequence variation data from the mitochondrial control region and cytochrome *b* gene in order to estimate gene flow between North American colonies of

Ancient Murrelets on George Island and East Limestone Island, and between North American and Asian populations. Mitochondrial DNA (mtDNA), found in mitochondria (a cellular organelle), is useful in populations genetics studies because it is maternally inherited, haploid (single-copy), and has a higher mutation rate than most nuclear DNA.

The coalescent theory is a novel populations genetics model that relates evolutionary processes to DNA sequence variation represented as a genealogy, or tree (Harding 1996). It can be used to infer population genetics quantities, such as gene flow and effective populations size (e.g. Slatkin and Maddison 1989, Beerli 1998, Beerli and Felsenstein 1999).). Estimators of migration rates based on the coalescent are potentially more accurate than traditional estimators, as they accommodate possible asymmetries in migration rates (for example, if two populations are considered, more individuals could be migrating from population A to population B than from population B to population A), and differences in population sizes (Beerli 1998, Beerli and Felsenstein 1999). From the large distance between North American and Asian breeding sites and the likelihood that these populations have separate wintering grounds, we predicted that little gene flow would occur between North America and Asian Ancient Murrelets. However, given evidence from banding encounters and the proximity of the two islands, we expected to find significant gene flow between the two North American colonies, in the Queen Charlotte Islands.

METHODS

Forty-five blood and tissue samples were taken from adult Ancient Murrelets caught in drift nets off the coast of the Kamchatka Peninsula during the spring of 1995 and 1996 (Fig.1). Birds were in breeding condition, so were probably near their breeding sites, however they could not be associated with any particular colony. Fourteen tissue samples were taken from adult Ancient Murrelets predated by rats and raccoons on East Limestone Island during the spring of 1996 (Fig.1), and eleven tissue samples were taken from eggs and predated Adult Ancient Murrelets on George Island during the spring of 1997 (Fig.1). These islands are located 70 km apart within the Queen Charlotte Islands. East Limestone Island is 48 ha and supports about 1,150 pairs of Ancient Murrelets, while George Island has an Ancient Murrelet population of approximately 11,600 pairs (Gaston 1992).

Laboratory methods

DNA was extracted from the samples. Parts of the mitochondrial DNA were amplified, including a portion of the cytochrome *b* gene, which codes for a protein involved in the electron transport chain, and the mitochondrial control region, which is a non-coding region of the mitochondrial DNA. The DNA of individuals with variation in these regions was sequenced.

Data analysis
Genetic differentiation among populations was indexed using gamma (γ), the mutational divergence, or difference, between populations. Gene flow and effective population size (N_e) were calculated using the program MIGRATE (Beerli and Felsenstein 1998), which uses the coalescent theory to estimate gene flow and population size.

The possibility that Ancient Murrelets underwent a recent population expansion, estimates of population sizes before and after the expansion, and time since population expansion were estimated using a mismatch distribution (Rogers and Harpending 1992, Rogers 1995). An expansion is when a population increases in size over a period of time. A maximum-likelihood tree was also compiled for mtDNA sequences with the program PHYLIP (Felsenstein 1995). For more specific materials and methods, please see Pearce et. al. (unpublished data).

RESULTS AND DISCUSSION

Structure and variability of the mtDNA sequence
Altogether, 1153 base pairs (bp) of the mitochondrial genome were analyzed. A total of 21 mtDNA haplotypes, or different sequences, defined by 21 variable sites and one 9 bp insertion, were found among 59 individuals. The structure and base composition of the Ancient Murrelet mtDNA sequenced in this study is similar to that found in other avian mtDNA genes (Wenink et al. 1994, Baker and Marshall 1997). Similar control region composition has also been found in non-avian species, such as the Humpback Whale (Baker et al. 1993).

Genetic structure between colonies and regions
No significant difference from a random distribution of variation was found either between colonies or between regions (Table 1). This means that the populations interbreed sufficiently frequently that there are no significant genetic differences between them.

Results from Migrate suggest that, with the possible exception of gene flow from Asia to East Limestone Island, gene flow both between George Island and East Limestone Island and between Asia and North America is high (Table 2, Fig. 2). Gene flow out of East Limestone Island greatly exceeds gene flow into this colony (Fig. 2). The effective population size at East Limestone appears to be much lower than at George Island. Estimates of effective population sizes were 1500 individuals for George Island, 70 individuals for East Limestone Island, and 15 000 individuals for Asia (Table 2).

There is no strong evidence to support a recent population expansion. Neither mismatch distribution analysis nor the maximum-likelihood tree fit the patterns expected for expanding populations. Rather than expanding, Ancient Murrelet population are likely in genetic equilibrium.

Evolutionary inferences
No evidence was found to support population genetic structure in Ancient Murrelets. This is not surprising given the high migration rates found in this study, and in light of Wright's (1931) theory that one migrant per generation is sufficient to prevent genetic divergence of populations through genetic drift (chance changes in the gene pool of populations). Gene flow both within British Columbia and Asia appears to be sufficient to counteract drift, and suggests

that East Limestone Island is acting as a net source of recruits for both George Island and Asia.

Table 1. Results of analysis of genotype frequencies and analysis of molecular variance for mtDNA haplotypes.

	Haplotypes	
	North American Colonies	Regions
G_{st}	0.032	0.009
γ	0.320	0.231
$N_em\,(G_{st})$	15	55
φ_{st}	0	-
φ_{sc}	0	-
φ_{ct}	-	0.021*
$N_em\ (\varphi_{sc})$	NA/EI	-
$N_em\,(\varphi_{ct})$	-	23

*P<0.01
NA/EI = not available but effectively infinite.
N_em given in number of females per generation
G_{st}–among-population genotype variation
γ–mutational divergence between populations
$N_em\,(G_{st})$–estimation of gene flow using G_{st} in the equation $N_em=(1/G_{st}-1)1/2$ (Crow and Aoki 1982)
φ_{st}–sequence variation among populations relative to variation from the whole species
φ_{sc}–sequence variation among populations within a region
φ_{ct}–sequence variation among groupings of populations relative to the entire species
$N_em\ (\varphi_{sc})$–estimation of gene flow using φ_{sc}
$N_em\,(\varphi_{ct})$–estimation of gene flow using φ_{ct}

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Table 2. Estimates of N_e (effective population size) derived from θ values from Migrate for three different values of μ (N_e=θ/4μ).

	George Island	East Limestone Island	Asia
θ	0.0078 (0.0027-0.042)	0.00016 (0.00011-0.00024)	0.039 (0.019-0.1)
N_e (μ=0.10/My)	19,500 (6800-105,000)	400 (275-600)	98,000 (48,000-250,000)
N_e (μ=0.05/My)	9,800 (3400-53,000)	200 (140-300)	49,000 (24,000-130,000)
N_e (μ=0.02/My)	3,900 (1400-21,000)	80 (55-120)	20,000 (9,500-50,000)

Values of μ (mutation rate) given in bases per million years

Ancient Murrelets probably underwent a population expansion in the past, possibly from a glacial refugium which could have arisen during the late Pleistocene, approximately 10,000 years ago. Studying a region of DNA with a slower mutation rate than mtDNA (e.g. nuclear introns) could provide more information about this historical expansion, such as when it may have occurred. However, as the mismatch distribution and maximum-likelihood tree do not fit the model of an expanding population, the assumption that Ancient Murrelets are now in genetic equilibrium cannot be discounted. Genetic equilibrium occurs when the same allelic frequencies (the relative numbers of different forms of a gene) persist over a series of generations. Genetic equilibrium infers that populations are in balance with respect to genetic mutation, migration rates, and genetic drift. If the populations are in equilibrium, estimates of migration rates determined from MIGRATE can be taken as contemporary, rather than historical.

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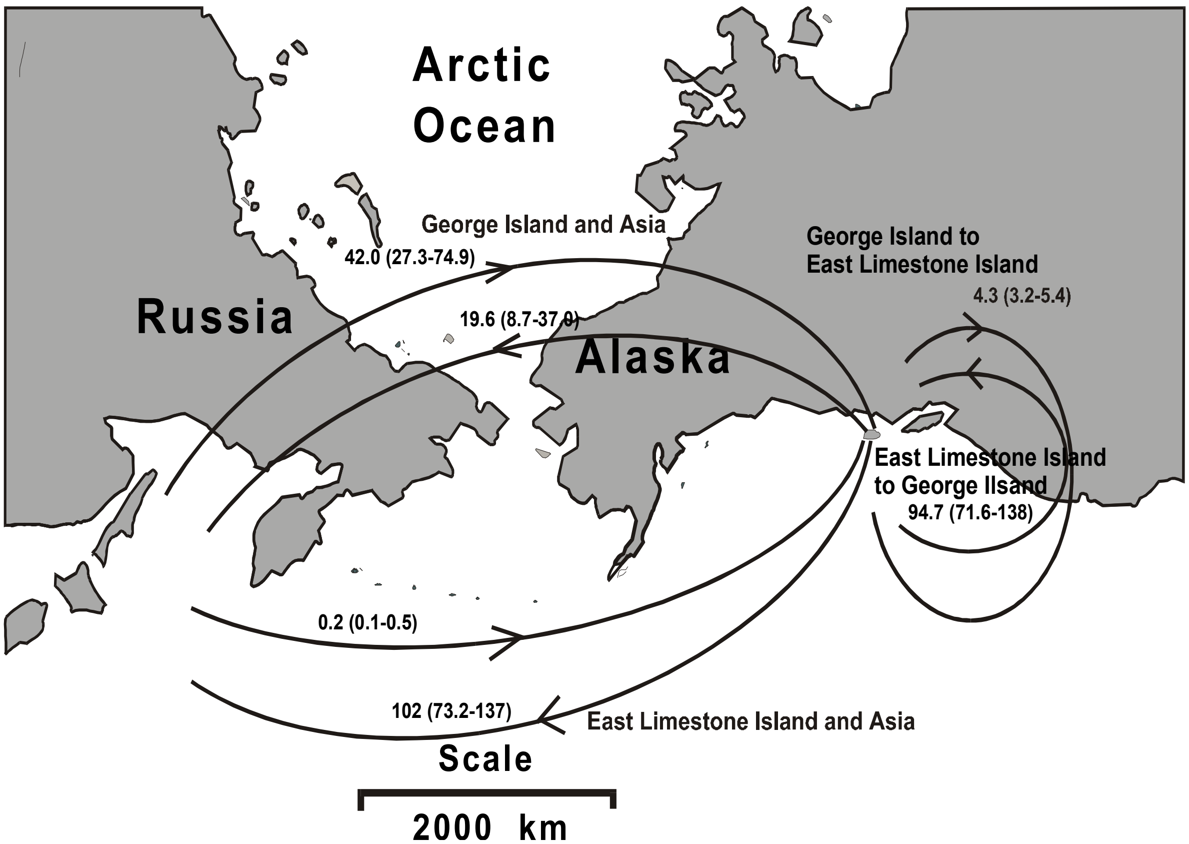
Figure 2. Migration rates among North American colonies and Asian populations of Ancient Murrelets, and estimates of effective population sizes.



Conservation Implications

When conservation biology is considered from a genetic perspective, two of its major goals are to preserve genetic diversity and evolutionary potential (Avise 1994). For this reason, studies of the population and evolutionary genetics of a particular species are useful in that they can provide information about the genetic diversity and evolutionary processes of the species (Avise 1994). By looking at the way genetic diversity is partitioned among populations of a threatened species, the genetic resources that conservation biology attempts to preserve can be assessed and characterized (Avise 1994). For example, if one population is significantly genetically different than three other populations which are not genetically different, it could be suggested that this population should have conservation priority over the other three populations. As there was no genetic structuring found in this study among Ancient Murrelet populations, from a genetic perspective it is likely that no real conservation priority can be assigned to any one Ancient Murrelet population.

Caveats

There are number of factors that may confound the results that were found in this study. In particular, the statistical

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methods used have several assumptions that may have been violated by the data used. However, violation of these assumptions does not undermine the general conclusions of this study. As several different types of analyses suggest high gene flow and no global population structuring among Ancient Murrelet populations, the inferences made in this paper are sound. Finally, in the future, data from more loci (DNA regions) could be used to obtain a better estimate of gene flow within the Queen Charlotte Islands.

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