Exxon Valdez Oil Spill
Restoration Project Annual Report

A Genetic Study to Aid in Restoration of Murres, Guillemots and Murrelets to the Gulf of Alaska

Restoration Project 97169
Annual Report

This annual report has been prepared for peer review as part of the *Exxon Valdez* Oil spill Trustee Council restoration program for the purpose of assessing project progress. Peer review comments have not been addressed in this annual report.

Vicki Friesen
John Piatt

Alaska Biological Sciences Center, USGS, 1011 E. Tudor Rd., Anchorage, AK 99503, USA

April 1998

A Genetic Study to Aid in Restoration of Murres, Guillemots and Murrelets to the Gulf of Alaska

Restoration Project 97169
Annual Report

Study History: Seabirds affected by the *Exxon Valdez* Oil Spill (EVOS) included common murres, pigeon guillemots, marbled murrelets and probably Kittlitz's murrelets. Although immediate impacts of the spill included mortality of large numbers of these birds, it is still not clear whether damages were restricted to local populations, or whether they were more widespread owing to flux of birds from colonies outside the spill zone at the time mortality occurred. Based on these concerns, the Pacific Seabird Group and the EVOS Trustee Council suggested that population genetics of seabirds within the spill area be examined to assess whether population structure could be resolved for any of these species in the spill zone. Restoration Project 97169 was initiated in FY97 to examine the genetic structure of murre, murrelet and guillemot populations in Alaska. This is the first annual report for the research initiated under Restoration Project 97169. Two manuscripts based on data collected under this project are in preparation for publication in scientific journals. The project is being continued under Restoration Project 98169, and a proposal is being submitted for a third year of research under Restoration Project 99169.

Abstract: An analysis of variation in mitochondrial DNA, microsatellite NDA and nuclear introns was initiated to aid in the restoration of common murres (*Uria aalge*), pigeon guillemots (*Cepphus columba*), marbled murrelets (*Brachyramphus marmoratus*) and Kittlitz's murrelets (*B. brevirostris*) to the Gulf of Alaska. In FY97, samples of tissue or blood were collected from all four species from several sites within the Spill area and adjacent sites. Laboratory protocols for the analysis of variation in the mitochondrial control region were refined for murres, and techniques for assaying variation in nuclear introns were perfected for murres and guillemots. Primers for amplification of microsatellites were developed for pigeon guillemots, and procedures for assaying variation in microsatellites in murres and guillemots using previously developed primers were refined. Variation in nine introns and three microsatellites was assayed in approximately 240 marbled murrelets, and variation in the mitochondrial control region and cytochrome *b* gene was assayed in approximately 100 common murres. Results for both species indicate that significant genetic differences exist among birds from some sites, and that some colonies may be genetically isolated. All results are preliminary, and no restoration recommendations can be made yet.

Key Words: common murre, cytochrome *b*, cryptic species, introns, gene flow, Kittlitz's murrelet, marbled murrelet, microsatellites, mitochondrial control region, pigeon guillemot, source and sink populations, SSCPs

Project Data: Data collected to date include frequencies and sequences of intron alleles for marbled murrelets, frequencies of microsatellite alleles for marbled murrelets, and frequencies and sequences of mitochondrial genotypes for common murres. Estimates of population genetic structure and gene flow, and phylogenetic hypotheses for mitochondrial genotypes have been derived. All data are preliminary, and should not be used or cited without prior permission of the authors.

Citation: Friesen, V.L. and J.F. Piatt. 1998. A genetic study to aid in restoration of murres, guillemots and murrelets to the Gulf of Alaska. *Exxon Valdez* Oil Spill Restoration Project Annual Report (Restoration Project 97169), Alaska Biological Sciences Center, U. S. Geological Survey, Anchorage, Alaska.

Table of Contents

Executive Summary

We are using state-of-the-art molecular methods to aid in the restoration of common murres, pigeon guillemots, and marbled and Kittlitz's murrelets to the Gulf of Alaska. For each species, we have three main objectives: (1) to determine the geographic extent of the populations affected by the Spill; (2) to identify source and sink colonies; and (3) to identify appropriate reference or 'control' sites for monitoring. To meet these objectives, we are comparing variation in two mitochondrial genes (the control region and cytochrome *b*), 6-8 microsatellite loci and 8-10 nuclear introns among approximately 30 birds from each of 12-15 colonies for each species. Results will be used to estimate the extent of genetic differentiation and gene flow among colonies, as well as genetic variability and inbreeding within colonies. DNA samples are being obtained primarily by J.F.P., and molecular analyses are being performed in V.L.F.'s laboratory at Queen's University.

We have just completed the first year of this project. Much of the year was spent refining laboratory protocols for each species; however, some preliminary screening was completed. Approximately 240 marbled murrelets were assayed for variation in nine introns and three microsatellite loci; results suggest that marbled murrelets from the western Aleutians are genetically different from those farther east, and that those from Belkofski Bay and Kachemak Bay may differ from those elsewhere. Approximately 100 common murres were screened for variation in the mitochondrial control region and cytochrome *b* gene; preliminary interpretations suggest that murres from Chisik Island and Kachemak Bay may be genetically differentiated from those elsewhere. Due to the preliminary nature of these data, interpretation of results in terms of the primary objectives of the project would be premature.

Introduction

Seabirds of the family Alcidae are highly vulnerable to marine oil pollution due both to the large amount of time they spend resting on the ocean surface, and to their dependence on marine fish and invertebrates for food. Many species of alcids suffered heavy mortality associated with the *Exxon Valdez* Spill; for example, the estimated mortality for common murres was in the hundreds of thousands. Although guillemots and murrelets were declining prior to the Spill, the accident probably increased their rate of decline. Common murres now appear to be recovering from the Spill, but pigeon guillemots and marbled murrelets apparently are not; the state of recovery of Kittlitz's murrelets is unknown. The reasons for the failure of these species to recuperate (as well as for the prespill declines) are unclear, but may be due to availability and quality of prey (currently being investigated through the APEX Predator Experiment and Nearshore Verterbate Predator Project), and/or genetic problems such as isolation of colonies or inbreeding. We propose to use state-of-the-art genetic techniques to aid in the restoration of these species.

Although the application of molecular methods to fisheries and wildlife management is common (e.g. Ryman and Utter 1987, Hansen and Loeschcke 1994, Allendorf and Waples 1996, Graves

1996), few if any studies have used genetic methods explicitly to aid in seabird conservation (Friesen 1997). Theoretically, measurement of genetic divergence and gene flow among populations of murres, murrelets and guillemots will aid restoration in the following three main ways:

Definition of the geographic limits of the affected populations.-Many seabirds killed by the *Exxon Valdez* Oil Spill were migrating: the 'affected' zone, or the populations that were affected by the Spill and require restoration effort, may be geographically different from the actual Spill zone. Genetic data should enable identification of breeding populations of birds killed by the Spill. Furthermore, genetic data should indicate if colonies are essentially panmictic and/or constitute metapopulations, in which case they should recover without assistance within a few generations. However, if colonies constitute numerous localized populations, they may not naturally recolonize sites affected by the Spill, and may require human assistance for recovery.

Identification of sources and sinks.-According to metapopulation theory, 'source' populations are populations that occur in optimal habitat and can act as exporters of recruits for populations elsewhere; 'sink' populations occur in suboptimal habitat and require immigration to maintain numbers (e.g. Pulliam 1996). Genetic data can provide measurements of rates of immigration into and emigration out of colonies, and thus enable identification of sources and sinks. For example, protein data suggest that rock shags (*Stictocarbo magellanicus*) on the Falkland Islands may have served as the main source of breeders for other colonies in southern South America (Siegel-Causey 1997).If colonies affected by the Spill represent sources, then their restoration will be critical. If a colony represents a sink, its restoration may be a waste of resources and may actually prevent recovery of the total population.

Environmental monitoring.-Demographic parameters may be very different for genetically divergent populations, even if they occur in ecologically similar or geographically proximate areas. For example, common murres breeding in Washington have different breeding chronologies from those at neighboring colonies in British Columbia, and may be genetically different (Warheit et al. unpubl. data). Genetic data may enable identification of appropriate reference or 'control' sites from which to obtain baseline data for monitoring, restoration and modeling, e.g. to determine if a seabird colony has recovered 'normal' functioning.

Three other types of information that are useful for conservation and restoration are produced incidentally by genetic studies:

Population uniqueness and cryptic species.-A colony's uniqueness (e.g. its endemicity or genetic distinctiveness) may be used to priorize restoration efforts. Most importantly, genetic data enable the identification of cryptic species - populations that are similar in appearance but that represent separate, non-interbreeding species (e.g. long-billed [*Brachyramphus perdix*] and marbled murrelets; Friesen et al. 1996a).

Small effective population size and inbreeding.-The 'effective size' of a population is the size of an idealized population that would have the same amount of genetic drift as the population being

considered; the effective size of a population may be one or two orders of magnitude lower than its census size due to such factors as unequal breeding success and population bottlenecks (Futuyma 1998). For example, the North Atlantic population of thick-billed murres (*Uria lomvia*) consists of approximately 2.5 million breeding pairs (Nettleship and Evans 1985), but appears to have a long-term effective size of only ~15,000 females (Friesen et al. 1996b). Theoretically, as a population's effective size decreases, individual fitness declines due to increased inbreeing (Allendorf and Leary 1986, Gilpen and Soulé 1986); several researchers have argued that if effective population size declines below a certain critical level, the population may enter an extinction vortex in which inbreeding, deleterious alleles and stochastic effects combine synergistically to accelerate extinction (Gilpin and Soulé 1986). Genetic information may be used to estimate effective population size (Nei and Li 1979), and thus to determine the extent to which small effective population sizes and inbreeding are preventing or slowing population recovery.

Translocations.-If breeding success within a colony is low due to inbreeding depression, or if recruitment is low, transplantation of small numbers of individuals from other sites may be desirable. Ideally, sources of animals for such introductions should be neighboring colonies within the same population or a closely related population. Genetic data are important for determining which colonies are genetically appropriate sources to prevent both inbreeding (Allendorf and Leary 1986) and outbreeding depression (Templeton 1986).

This project will require collection of blood, feather and/or tissue samples from birds breeding thoughout the Pacific basin, mostly in Alaska (Table 1). As much as possible, tissue will be obtained from museum specimens, and blood and blood feathers ('pin' or growing feathers) will be obtained from chicks or adults during banding. Birds being collected for ongoing diet studies in Alaska (J.F.P.) also will be used for tissue. In FY98 we hope to obtain samples from common murres from southeastern Alaska, Middleton Island, the eastern Aleutians and Japan, from marbled murrelets from Washington, Oregon and the central and eastern Aleutians, from Kittlitz's murrelets from the Bering Strait, and from guillemots from British Columbia and Kachemak Bay. Most samples will be obtained through contributions from researchers working at specific sites, but special collection trips will be made to British Columbia and Kachemak Bay (for guillemots), Middleton Island (for murres), and the Bering Strait (for Kittlitz's murrelets). Sampling efforts in 1999 will focus on remaining key sites (Table 1).

Objectives

The primary purpose of this project is to conduct a genetic analyses to aid in the restoration of common murres, pigeon guillemots, and marbled and Kittlitz's murrelets to areas affect by the *Exxon Valdez* Oil Spill. We have three main objectives for each species:

1) To determine the geographic extent of the populations affected by the Spill.

2) To identify source and sink colonies.

3) To identify appropriate reference or 'control' sites for monitoring.

As incidental results, we should also be able

4) To identify cryptic species or subspecies.

5) To measure coefficients of inbreeding and effective population sizes.

6) To identify appropriate source populations for translocations, if necessary.

Methods

We are comparing variation in two mitochondrial genes, 6-8 microsatellite loci and 8-10 nuclear introns among approximately 30 birds from each of 12-15 colonies for each species except Kittlitz's murrelets, for which samples are difficult to obtain (Table 1). For each species, we are testing the null hypothesis that colonies are panmictic (genetic structure is essentially absent) against the alternative hypothesis that significant genetic differences exist among birds from different colonies.

Sampling.-To obtain reliable estimates of genetic differentiation and gene flow within and between the Spill area and neighboring areas, as well as to define the geographic limits of the breeding populations, we are sampling 4-6 colonies of each species from the spill area, as well as 4-6 colonies each at increasing distances west and east of the Spill area. A minimum of 30 samples are required from each site for each species for reliable estimation of genetic variation within and between sites (Richardson et al. 1986, Weir 1996). Many of the necessary baseline samples were obtained opportunistically during previous projects through the assistance of other researchers.

Loci.-Much of southern Alaska was ice-covered during the Pleistocene glaciations, so most seabird colonies from the Spill area were probably only populated within the last ~10,000 years. Measurement of gene flow and genetic divergence among colonies of these birds therefore requires analysis of loci with high mutation rates. Mitochondrial DNA (mtDNA) has proven useful for studies of such populations since it has a relatively high mutation rate and is more sensitive to population bottlenecks and restricted gene flow than are nuclear loci (Wilson et al. 1985, Avise 1994, Avise and Hamrick 1996, Mindell 1997). The mitochondrial control region is especially useful for analysing recently isolated populations since it has a mutation rate 5-10x higher than the mean for mtDNA (Brown et al. 1986, Avise 1994, Avise and Hamrick 1996, Baker and Marshall 1997). The mitochondrial cytochrome *b* gene is also useful for estimating population genetic structure and effective population sizes in alcids since its mutation rate has been calibrated for this family (Friesen et al., submitted). However, mtDNA represents a single supergene whose pattern of inheritence is not typical of the rest of the genome (Wilson et al. 1985); results of analyses of mtDNA therefore need to be confirmed with analyses of nuclear loci. Microsatellite loci have mutation rates higher than those of mtDNA so are being used

increasingly for evolutionary studies (Avise 1994, Dowling et al. 1996,McDonald and Potts 1997). However, depending on the age of populations, microsatellite loci may contain high levels of homoplasies (back-, parallel and convergent mutations), which may result in inaccurate estimates of genetic differentiation and gene flow. Nuclear introns have mutation rates equivalent to those of mtDNA (Congdon et al. in prep. a), so are also useful for studying recent evolutionary events (Friesen et al. 1996; Congdon et al. in prep. b). Because microsatellites and introns are nuclear loci, they are less sensitive to population bottlenecks and restricted gene flow than are mitochondrial genes; Moore (1995) estimated that, due to the larger effective population size of nuclear genes, 8-16 nuclear loci are required to obtain information equivalent to that of one mitochondrial gene. Previous researchers (e.g. Richardson et al. 1986, Weir 1996) have also suggested that information from at least five to six nuclear loci are required to obtain reliable estimates (i.e. to derive robust error estimates) of genetic structure and gene flow. Thus, we are analyzing the mitochondrial control region and cytochrome *b* gene, as well as 8-16 nuclear loci, with the specific number of each class of marker depending on observed levels of variability.

Laboratory Assays.-Variation in number of repeating units in microsatellite loci are being assayed using standard protocols (Dowling et al. 1996). To reduce time and cost associated with assaying sequence variation in mitochondrial genes and introns, a two-step procedure is being used. Samples are first screened for mutations using analysis of single-stranded conformational polymorphisms (SSCPs; Friesen et al. 1996a, 1997). The exact nature of mutations is then determined by direct sequence analysis of at least one individual with each genotype detected from SSCPs. Previous experience indicates that this combination of techniques provides an efficient and sensitive method for comparing sequence variation variation among populations (Friesen et al. 1996a, 1997, Congdon et al. in prep. a). We estimate that a trained technician can process approximately 4500 sample-loci per year. Analysis of 20 loci (two mitochondrial genes, eight microsatellite loci and ten introns) for each of approximately 1200 samples (excluding 150 murrelets already analyzed by Congdon et al.) is expected to require approximately 5.5 person-years. Approximately 6700 sample-loci were analyzed in FY97, and 9000 sample-loci each will be analyzed in FY98 and FY99.

Statistical Analyses.-Data are being analyzed using standard methods developed for data from protein electrophoresis and sequencing (e.g. Swofford & Selander 1981; Swofford 1993), as well as using a few new techniques that capitalize on the power of combining genotypic and sequence data (e.g. Michalakis and Excoffier 1996):

1) To determine the geographic limits of populations affected by the Spill, the extent of genetic differentiation of colonies is being calculated using Wright's *F* statistics and its analogues (e.g. ϕ_{st}) and tested for significance using randomization procedures (e.g. Excoffier et al. 1992).

2) To identify source and sink colonies, the direction and magnitude of gene flow among colonies is being estimated using coalescence theory (Slatkin and Maddison 1989) and Hedrick's *U* statistic (Hedrick 1971, 1975).

3) Appropriate reference or 'control' sites for monitoring, as well as colony-specific markers for impact assessment, will be apparent from the results of objective (1).

4) Cryptic species are being inferred from (i) fixed allele differences, which indicate prolonged genetic isolation of populations, (ii) paraphyletic relationships among populations from different species, and/or (iii) high sequence divergences between the mitochondrial genomes of individuals from different populations.

5) Coefficients of inbreeding are being estimated from nuclear data using Wright's F statistics, and effective population sizes are being estimated from mitochondrial sequence data using the method of Nei and Li (1979).

6) Appropriate source populations for translocations will be apparent from the results of objective (1).

Results

We have just completed the first year of this project. Much of the year was spent refining laboratory protocols for each species; however, results of some preliminary assays are available and indicate that, despite the high dispersal potential of these birds, significant genetic differentiation exists within all species:

Marbled and Kittlitz's Murrelets.-In FY97 we refined protocols for assaying variation in nine introns and three microsatellite loci in murrelets, and screened samples collected previously from within the Spill area and neighboring sites. Results suggest that marbled murrelets from the western Aleutians are genetically different from those farther east, and that those from Belkofski Bay and Kachemak Bay may differ from those elsewhere (Appendix 1).

Common Murres.-In FY98, we refined protocols for analyses of mitochondrial control regions (mCR), microsatellites and introns in common murres, and screened samples from the Spill area and immediately adjacent sites for variation in the mCR and cytochrome *b*. Preliminary results indicate that murres from the western Aleutians, Chisik Island and Kachemak Bay may be genetically differentiated from those elsewhere (Appendix 2). Preliminary results of a concurrent study of variation in mCRs, microsatellites and introns in common murres from British Columbia south suggest that a murres from British Columbia differ from those from Oregon and Califronia (Warheit et al. unpubl. data).

Piegon Guillemots.-Previously, we surveyed variation in the mitochondrial control region among populations of guillemots from throughout the Northern Hemisphere and found colony-specific sequence differences (Kidd and Friesen 1998). In FY98 we refined protocols for analysis of variation in microsatellites and introns in guillemots. No screening was completed due to a turnover of personnel.

Discussion

Due to the preliminary nature of the present data, interpretation of results in terms of the primary objectives will be not addressed until the Final Report.

Acknowledgements

Many samples used in this study were obtained through the assistance of Vern Byrd and Dave Roseneau (Alaska Maritime National Wildlife Refuge), S. Kitaysky, Jay Pitocchelli, Tom van Pelt and Lindsey Hayes (Alaska Biological Sciences Center, USGS, Anchorage), Alex Pritchard (University of Alaska), Jan Hodder (Oregon Institute of Marine Biology) and Kathy Martin (Canadian Wildlife Service). Tim Birt, Brad Congdon, Christine Crossman, Shane Doran, Deb Harrison, Karen Holder, Gabriela Ibarguchi, Heather Jones, Kathy Kennedy, Monica Kidd, Vinay Lodha, Denise Michaud, Jeff Moy, Anoma Patirana, Lisa Veit and Jesse Wood provided technical help and valuable discussions. K. Warheit shared unpublished data.

Literature Cited

Allendorf, F.W. and R.F. Leary. 1986. Heterozygosity and fitness in natural populations of animals. Pp. 57-76 *in* M.R. Soulé (ed.), Conservation biology, the science of scarcity and diversity. Sinauer, Sunderland.

Allendorf, F.W. and R.S. Waples. 1996. Conservation and genetics of salmonid fishes. Pp. 238-280 *in* J.C. Avise and J.L. Hamrick (eds.), Conservation genetics. Chapman and Hall, New York.

Avise, J.C. 1994. Molecular markers, natural history and evolution. Chapman and Hall, New York.

Avise, J.C. and J.L. Hamrick (eds.). 1996. Conservation genetics. Chapman and Hall, New York.

Baker, A.J. and H.D. Marshall. 1997. Mitochondrial control region sequences as tools for understanding evolution. Pp. 50-82 *in* D.P. Mindell (ed.), Avian molecular evolution and systematics. Academic, London.

Brown, G.G., G. Gadeleta, G. Pepe, C. Saccone and E. Sbisá. 1986. Structural conservation and variation in the D-loop-containing region of vertebrate mitochondrial DNA. *J. Mol. Biol.* 192:503-511.

Dowling, T.E., C. Moritz, J.D. Palmer and L.H. Reiseberg. 1996. Nucleic acids III: analysis of fragments and restriction sites. Pp. 249-320 *in* D.M. Hillis, C. Moritz and B.K. Mable (eds.), Molecular systematics, 2nd ed. Sinauer, Sunderland.

Evans, P.G.H. 1987. Electrophoretic variability of gene products. Pp. 105-162 *in* F. Cooke and P.A. Buckley (eds.), Avian genetics. Academic, London.

Excoffier, L., P.E. Smouse and J.M. Quattro. 1992. Analysis of molecular variance inferred from metric distance among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 131:479-491.

Friesen, V.L. 1997. Population genetics and the spatial scale of conservation of colonial waterbirds. Colon. Waterbirds 20:353-368.

Friesen, V.L., A.J. Baker and J.F. Piatt. 1996a. Evidence from cytochrome *b* sequences and allozymes for a 'new' species of alcid: the long-billed murrelet (*Brachyramphus perdix*). *Condor* 98:681-690.

Friesen, V.L., B.C. Congdon, H.E. Walsh and T.P. Birt. 1997. Intron variation in marbled murrelets detected using analyses of single-stranded conformational polymorphisms. *Mol. Ecol.* 6:1047-1058.

Friesen,V.L., W. A. Montevecchi, A. J. Baker, R. T. Barrett and W. S. Davidson. 1996b. Population differentiation and evolution in the common guillemot *Uria aalge*. *Mol. Ecol.*5:793-805.

Futuyma, D. 1998. Evolutionary biology. Sinauer, Sunderland.

Gilpin, M.E. and M.E. Soulé. 1986. Minimum viable populations: processes of species extinction. Pp. 19-34 *in* M.R. Soulé (ed.), Conservation biology, the science of scarcity and diversity. Sinauer, Sunderland, MA.

Graves, J.E. 1996. Conservation genetics of fishes in the pelagic marine realm. Pp. 335-366 *in* J.C. Avise and J.L. Hamrick (eds.), Conservation genetics. Chapman and Hall, New York.

Hansen, M.M. and V. Loeschcke. 1994. Effects of releasing hatchery-reared brown trout to wild trout populations. Pp. 273-289 *in* V. Loeschcke, J. Tomiuk and S.K. Jain (eds.), Conservation genetics. Birkäuser Verlag, Basel.

Hedrick, P.W. 1971. A new approach to measuring genetic similarity. Evolution 25: 276-280.

Hedrick, P.W. 1975. Genetic similarity and distance: comments and comparisons. Evolution 29: 362-366.

Kidd, M.G. and V.L. Friesen. 1998. Analysis of mechanisms of microevolutionary change in *Cepphus* guillemots using patterns of control region variation. *Evolution,* in press.

McDonald, D.B. and W.K. Potts. 1997. DNA microsatellites as genetic markers at several scales. Pp. 29-49 *in* D.P. Mindell (ed.), Avian molecular evolution and systematics. Academic, London.

Michalakis, Y. and L. Excoffier. 1996. A generic estimation of population subdivision using distances between alleles with special reference to microsatellite loci. *Genetics* 112:629-647.

Mindell, D.P. (ed.). 1997. Avian molecular evolution and systematics. Academic, London.

Moore, W. S. 1995. Inferring phylogenies from mtDNA variation: mitochondrial -gene trees versus nuclear-gene trees. Evolution 49:718-726.

Nei. M., and W. H. Li. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Scienecs, USA 76:5269-5273.

Nettleship, D.N. and P.G.H. Evans. 1985. Distribution and status of the Atlantic Alcidae. Pp. 53-154 *in* D.N. Nettleship and T.R. Birkhead (eds.), The Atlantic Alcidae. Academic, New York, NY.

Patirana, A. 1998. A conservation genetic study of common murres (*Uria aalge*) in the *Exxon Valdez* spill area through comparison of mitochondrial control region and cytochrome *b* sequences. B.Sc. thesis, Queen's University, Kingston, Ontario.

Pulliam, H.R. 1994. Sources and sinks. *In* O.E. Rhodes, R.K. Chesser and M.H. Smith (eds.), Spatial and temporal aspects of population processes.

Richardson, B.J., P.R. Baverstock and M. Adams. 1986. Allozyme electrophoresis. Academic, London.

Rockwell, R.F. and G.F. Barrowclough. 1987. Gene flow and the genetic structure of populations Pp. 223-256 *in* F. Cooke and P.A. Buckley (eds.), Avian genetics. Academic, London.

Ryman, N. and F. Utter (eds.). 1987. Population genetics and fishery management. University of Washington Press, Seattle.

Siegel-Causey, D. 1997. Molecular variation and biogeography of Rock Shags. *Condor* 99:139-150.

Slatkin, M., and W. P. Maddison. 1989. A cladistic measure of gene flow inferred from the phylogenies of alleles. Genetics 123:603-613.

Swofford, D. L. 1993. PAUP: Phylogenetic analysis using parsimony. Version 3.1.1. Illinois Natural History Surveys, Champaign, IL.

Swofford, D. L., and R. B. Selander. 1981. BIOSYS-1: a Fortran program for the comprehensive analysis of electrophoretic data in population genetics and systematics. Journal of Heredity 72:281-283.

Templeton, A.R. 1986. Coadaptation and outbreeding depression. Pp. 105-116 *in* M. E. Soulé (ed.), Conservation biology. Sinauer, Sunderland.

Weir, B.S. 1996. Intraspecific differentiation. Pp. 385-405 *in* D.M. Hillis, C. Moritz and B.K. Mable (eds.), Molecular systematics, 2nd ed. Sinauer, Sunderland.

Wilson, A. C., R. L. Cann, S. M. Carr et al. (11 co-authors). 1985. Mitochondrial DNA and two perspectives on evolutionary genetics. Biol. J. Linn. Soc. 26:375-400.

Table 1. Sites, numbers of samples available, and numbers of samples needed for genetic analyses of murres, murrelets and guillemots.

Site	Available	Needed
COMMON MURRE		
California (Farallon Islands)	30	0
Washington (Clallam)	30	0
N. Vancouver Island	40	0
Southeastern Alaska	0	30
Prince William Sound (Cordova)	23	7
Middleton Island	30	0
Upper Cook Inlet (Kachemak Bay, Chisik I.)	48	0
Lower Cook Inlet (Barren Is.)	27	3
Alaska Peninsula (Semidi, Midun Is.)	18	12
Eastern Aleutians (Aiktak I.)	14	16
Western Aleutians (Attu, Agattu & Buldir Is.)	25	5
Bering Sea (Pribilof, St. Matthew, St. Lawrence Is.)	30	0
Chukchi Sea (Capes Lisburne & Thompson)	33	0
Sea of Okhotsk (Talan I., Magadanskaya)	30	0
Japan (Teuri I.)	0	30
MARBLED MURRELET		
California	40	0
Oregon	12	18
Washington	18	12
British Columbia (Queen Charlotte Is.)	30	0
Southeastern Alaska (Lemesurier I.)	20	10
Prince William Sound (Unakwik Fjord)	20	10
Cook Inlet (Kachemak Bay)	24	6
Kodiak Island	26	4
Mitrofania Bay	26	4
Shumagin Islands (Koniuji Is., Belkofski B., Yakutat P.)	22	8
Eastern Aleutians (Dutch Harbor)	12	18
Central Aleutians (Adak I.)	10	20
Western Aleutians (Attu I.)	18	12

Table 1, cont'd.

Site	Available	Needed
KITTLITZ'S MURRELET		
Prince William Sound	4	*
Kachemak Bay	18	*
Adak Island	6	*
Western Aleutians (Attu I.)	5	*
PIGEON GUILLEMOT		
California (Farallon Is.)	20	10
Oregon	25	5
British Columbia (Queen Charlotte Is.)	30	0
Southeast Alaska (Glacier Bay)	0	30
Prince William Sound (Jackpot & Naked Is.)	30	0
Cook Inlet (Kachemak Bay)	30	0
Kodiak Island	0	30
Alaska Peninsula (Semidi and Shumagin Is.)	7	23
Western Aleutians (Attu, Agattu Is.)	0	30
Kuril Is.	0	30
Bering Sea (Pribilof, St. Lawrence Is.)	0	30
Chukchi Sea (Capes Thompson and Lisburne)	0	30

*Samples will be obtained from Kittlitz's murrelets opportunistically.

NOTE: Every effort will be made to obtain samples non-destructively to minimize the need for collections, e.g. as feathers or blood samples collected during banding, or from museum specimens.

Products

The following papers relating to EVOS funded research on seabird population genetics have been prepared or published. Two of the most recent products (indicated by asterisks) resulting from work conducted entirely in FY97 are attached.

**Congdon, B.C., J.F. Piatt, K. Martin and V.L. Friesen. Rapid population expansion and peripheral isolation in marbled murrelets: contemporary vs historic processes. In prep. for *Evolution*.

Congdon, B.C. and V.L. Friesen. Population and evolutionary dynamics of introns. In prep. for *Genetics*.

Friesen, V.L., J.F. Piatt and A.J. Baker. 1996. Evidence from cytochrome *b* sequences and allozymes for a 'new' species of alcid, the long-billed murrelet (*Brachyramphus perdix*). *Condor 98:*681-690.

Kidd, M. G. and V. L. Friesen. 1998. Analysis of mechanisms of microevolutionary change in *Cepphus* guillemots using patterns of control region variation. Evolution, in press.

Kidd, M.G. and V.L. Friesen. 1998. Sequence variation in the guillemot (Alcidae: *Cepphus*) mitochondrial control region and its nuclear homolog. *Mol. Biol. Evol.*15:61-70.

Friesen, V.L. 1997. Population genetics and the spatial scale of conservation of colonial waterbirds. *Colon. Waterbirds* 20:353-368.

Friesen, V.L., B.C. Congdon, H.E. Walsh and T.P. Birt. 1997. Intron variation in marbled murrelets detected using analyses of single-stranded conformational polymorphisms. *Mol. Ecol* 6:1047-1058.

Friesen, V.L., J.F. Piatt and A.J. Baker. Evolution and speciation in the Alcidae (Aves: Charadriiformes). Submitted to *Auk,* Dec. 1997.

Friesen, V.L., B.C. Congdon, M.G. Kidd and T.P. Birt. General PCR primers for the amplification and sequencing of nuclear introns in vertebrates. In prep. for *Mol. Ecol.*

**Patirana, A. 1998. A conservation genetic study of common murres (*Uria aalge*) in the *Exxon Valdez* spill area through comparison of mitochondrial control region and cytochrome *b* sequences. B.Sc. dHonours dhesis, Queen's University.

RAPID POPULATION EXPANSION AND PERIPHERAL ISOLATION IN MARBLED MURRELETS: CONTEMPORARY VS HISTORIC EVOLUTIONARY PROCESSES

BRADLEY C. CONGDON[1,4], JOHN F. PIATT[2], KATHY MARTIN[3], VICKI L. FRIESEN[1]

[1]*Department of Biology, Queen's University, Kingston, Ontario, K7L 3N6, Canada.*

[2]*National Biological Service, Alaska Science Centre, Anchorage, Alaska 99503, USA.*

[3]*Pacific Wildlife Research Centre, Canadian Wildlife Service, Delta, British Columbia, Canada*

Abstract. - We examined the evolutionary processes responsible for intra-specific genetic divergence in marbled murrelets (*Brachyramphus marmoratus*, Charadriiformes: Alcidae) by comparing patterns of geographic variation at nuclear intron and microsatellite loci. We suggest that vicariance associated with Pleistocene glacial events is responsible for the origin but not the maintenance of population structuring in this species. Our results are consistent with the rapid expansion of murrelets from a single refugium during the late Pleistocene, the subsequent isolation and divergence of Aleutian Island populations during the final Pleistocene glacial advance, and secondary contact among island and mainland populations following the retreat of the Wisconsin ice sheets. Since the glacial retreat, limited introgression of mainland and island populations has occurred relative to dispersal along the North American coast. The intron and microsatellite marker systems provide alternative peripatric and parapatric explanations for this phenomenon. Patterns of intron variation suggest peripheral isolation due to habitat fragmentation is responsible. Microsatellite variation implicates disruptive selection associated with changes in nesting habitat. The contrast between these results highlights the need for comparative analyses among multipe loci that are evolving at different rates. Using only one marker system would have failed to identify one or other of these processes, and would not accurately reflect the evolutionary history of this species. Our results continue to emphasize vicariance due to glaciation as the dominant process promoting genetic divergence in Northern Hemisphere alcids. However, we have demonstrated that peripatric and/or parapatric modes of divergence have been influential in maintaining population structure over extended periods of time. Thus, while the specific

conditions under which non-allopatric processes promote further diversification may not be present in murrelets, we suggest that these processes may significantly influence divergence in others species where the requirements of non-allopatric models are satisfied.

Keywords. - *Brachyramphus marmoratus*, Marbled murrelet, peripheral isolation, habitat fragmentation, disruptive selection, phylogeography, introns, microsatellites, SSCP, AMOVA, mismatch distribution, population genetics.

Modes of divergence in broad-ranging highly mobile seabird species are problematic. Allopatric models of divergence require extrinsic barriers to dispersal usually associated with large-scale vicariant events (Mayr 1963). However, for the majority of seabirds vicariant events or extrinsic barriers to dispersal are either non-existent, or limited to a small number of geological events. Whether the level of philopatry in these species is sufficient to allow differentiation is generally unknown. However, a number highly philopatric species show little or no population genetic structuring (eg: Austin et al. 1994; Friesen et al. 1996a). Peripatric, parapatric and sympatric models of divergence are theoretical alternatives that do not emphasize prolonged geographic isolation (Maynard-Smith 1966; Mayr 1963; Bush 1975; Endler 1977; Barton and Charlesworth 1984; Carson and Templeton 1984). Peripatric divergence involves the fixation of new genetic variants in small peripheral populations. Effective population size and relative position are thought to be the primary factors promoting divergence. Alternative peripatric models may or may not invoke natural selection as a diversifying agent (Barton and Charlesworth 1984; Carson and Templeton 1984). In contrast, parapatric and sympatric models invoke disruptive selection as the primary mode of divergence. Parapatric or sympatric differentiation occurs in response to resource use without geographic isolation, if the intensity of selection is large relative to the level of inter-population gene flow (Endler 1977). Despite the theoretical plausibility of these alternatives, demonstrated examples of non-allopatric divergence are rare (Wood and Foote 1996). Controversy continues regarding the ability of non-allopatric processes to promote and maintain genetic divergence (Otte and Endler 1989; Duffy 1996; Wood and Foote

1996).

By examining whether alternative models of differentiation can explain current patterns of intra-specific variation, evaluation of their potential as modes of divergence is possible. Seabird species lacking extrinsic barriers to dispersal are particularly suited to this type of analysis. The spatial distribution of populations, and the resources used in specific locations, varies significantly throughout many seabird distributions. Such variation provides both the opportunity for diversification through multiple evolutionary processes, and allows divergence due to different processes to be partitioned and examined independently. We investigated the nature and stability of mechanisms promoting divergence in one such species, the marbled murrelet.

The marbled murrelet is a broad-ranging seabird distributed along the North American coast from central California to the outer Aleutian Islands (Piatt and Ford 1993; Ralf et al. 1995). Throughout this distribution murrelets nest predominantly in large trees in old-growth forest (Carter and Morrison 1992; Ralf et al. 1995). However, approximately 3% of birds nest on the ground in the Aleutian Islands and other non-forested areas of Alaska (Mendenhall 1992; Piatt and Ford 1993). On the basis of summer distributions, a shift from tree- to ground-nesting occurs at the end of the Alaskan Peninsula (Piatt and Ford 1993; Pitocchelli et al. 1995, Fig. 1). Murrelet nesting habitat becomes increasingly fragmented, and population sizes decline dramatically in the Aleutian Archipelago (Piatt and Ford 1993).

Preliminary evidence indicates a low but significant level of population structuring in marbled murrelets (Friesen et al. 1996b; Friesen et al. 1997). Based on variations in relative population size and the spatial distribution of nesting habitat, possible explanations for current genetic structuring in murrelets fall into four categories; (1) Secondary contact following historical vicariance, (2) peripatric isolation of small peripheral populations due to nesting habitat fragmentation, (3) parapatric divergence *in situ* due to disruptive selection associated with nesting habitat, or (4) isolation by distance associated with natal philopatry. These possibilities are not mutually exclusive. Our aim was to evaluate the applicability of these hypotheses to divergence in murrelets. This required separating the effects of habitat heterogeneity, isolation by distance, and relative population size and position, from divergence associated with historic isolation.

Separating historic from contemporary evolutionary processes requires a synthesis of phylogenetic and population genetic analyses across multiple loci that are evolving at different rates. Until recently the high resolution nuclear markers needed for this type of analysis have been unavailable. Combining the targeted amplification of nuclear introns (Palumbi and Baker 1994) with single stranded conformational polymorphism (SSCP) analysis (Lessa and Applebaum 1992) offers a potential solution to this problem. We have developed primers and protocols for rapid analysis of sequence variation in a range of nuclear introns (Friesen et al. 1997, Friesen et al. in prep, Congdon et al. in prep), and have established the relative rates of evolution in these markers (Congdon et al. in prep). We are currently developing microsatellite primers systems for comparative purposes. In this paper we compare the results obtained using these two marker systems. We aim to provide comparative data on the utility of these marker systems for use in evolutionary and conservation genetics studies, and at the same time obtain a detailed

temporal and spatial understanding of the evolutionary processes responsible for intra-specific divergence in marbled murrelets.

MATERIALS AND METHODS

DNA Samples

Tissue samples were obtained from 120 marbled murrelets from 10 locations throughout the breeding range (Fig. 1). Samples were collected at sea adjacent to breeding sites and usually comprised freshly frozen tissue taken from birds shot for dietary analysis. Because sampling for dietary analysis is kept to a minimum samples collected over eight years were combined in this study. Samples from Desolation Sound-British Columbia comprised whole blood stored in ethanol, collected from birds netted at sea. Samples sites included adjacent ground- and tree-nesting locations near the centre of the species range, peripheral ground-nesting locations in the Aleutian Islands, and multiple tree-nesting locations in mainland North America that could be compared at a number of different spatial scales. Tree-nesting sites in Alaska included the three principal breeding areas, the Kodiak Archipelago, Prince William Sound, and the Alexander Archipelago, plus smaller populations in Kachemak, and Mitrofania Bays.

Molecular techniques

Standard protease digestion and phenol/chloroform extraction techniques were used to obtain total cellular DNA from both tissue and blood samples (Friesen et al. 1997). Information on sequence variation at nine nuclear intron loci was obtained using a combination of targeted PCR amplification, single stranded conformational polymorphism (SSCP) analysis, and direct cycle sequencing of double stranded PCR products. Intron primer sequences and general protocols are given in Friesen et al. (in prep). Locus specific protocol variations and sequence details are given in Congdon et al. (in prep).

Allele frequencies at each sampling location were also obtained for three nuclear microsatellite loci. Microsatellite primer development was as per Otter et al. (in press). Two primer sets were designed and optimised using marbled murrelet DNA templates. A third primer pair designed for thick-billed murres (*Uria lomvia*) (Ibaguchi unpublished data) also produce usable markers in marbled murrelets. Murrelet primer sequences were: BMA 10-18F, 5'- GGT AGG AGC GGA GTA GGA GG -3', BMA 10-18R, 5'- GCA AAA TAA GGG TGA AGG CA -3'; BMA 9-28F, 5'- AGG TAG GAA GGA GGG AGG GT -3', BMA 9-28R, 5'- ACC CTG TTT GGT GAT TGG AG -3'.

PCR reactions were performed in 10 µl volumes which contained ≈ 50-100 ng of genomic DNA, 0.05 µmoles of forward primer end-labelled with γP^{33}- dATP, 0.05 µmoles of unlabelled forward primer, 0.1 µmoles of reverse primer, 0.2 mM dNTPs, 0.2 U Taq DNA Polymerase, 0.2 M Tris-Cl pH 8.3, 50 mM KCl, and 1.5 mM $MgCl_2$. Prior to thermal cycling samples were covered with a mineral oil overlay. PCR amplification was performed using a MJ Research PTC–100-60 thermal cycler with heated bonnet. The following thermal cycling conditions were used for the murrelet primer set BMA 10-18: 1 cycle- 94°C 2 m (denaturation); 35 cycles- 94°C 30 s (denaturation); 60°C 30 s (annealing), 72°C 40 s (extension) ; 1 cycle- 72°C 5 m (final extension). Thermal cycling conditions for the other murrelet primer set BMA 9-28 and the thick-billed murre primer set 14B-29 were identical, except that annealing

temperatures of 55°C and 58°C was used, respectively. Amplification products were resolved on 5% polyacrylamide denaturing gels containing 7.0 M urea. Gels were run at 40 W (20 cm) or 70 W (40 cm) dependent upon gel width. Dried gels were exposed to BIOMAX (Dupont) X-ray film overnight. At each locus, allele product sizes were determined for a set of reference individuals by comparing these individuals to a sequencing reaction of known template. Unknown alleles were sized by comparison to these reference individuals.

Data analysis

Introns.– Population structure in murrelets was examined using analysis of molecular variance (AMOVA) (Excoffier et al. 1992; Michalakis and Excoffier 1996). Initially, all populations were classified as a single group so that variation among locations could be established. Data on variable sites from all nine intron loci were combined into two pseudo-haplotypes for each individual. Alleles at each locus were randomized within individuals to avoid establishing pseudo-linkages across loci. Contiguous nucleotide insertions or deletions were treated as a single mutation event (Congdon et al. in prep). F_{ST} values between all site combinations were generated from pairwise sequence divergence and haplotype frequency differences. The significance of F_{ST} values was tested using Markov chain analysis as described by Raymond and Rousset (1995).

Isolation by distance was examined using a regression analysis of log M versus log geographic distance (Slatkin 1993), where M is an index of gene flow given by the equation $M = 0.25(1/F_{ST}-1)$. Linearized F_{ST} values (Slatkin 1995) were used in this analysis. Intersite geographic distances were calculated using Distance Finder (http://www.indo.com/distance/). All analyses were repeated for both shortest geographic distance, and shoreline distance between sites. F_{ST} values of 0 indicated panmixia between sampling locations ($M = \infty$) and were removed from these analyses. The significance of regression coefficients from each analyses was tested using non-parametric resampling techniques (Resampling Stats™) as per Congdon et al. (1997).

The components of genetic variation attributable to nesting habitat and colony position were determined by altering the *a priori* group definitions in further AMOVA (Stanley et al. 1996), and examining changes in the among-group variance components. To isolate nesting habitat and colony position effects the nine sampling locations were grouped in three different ways: tree- vs ground-nesting, mainland vs Aleutians, and mainland vs each Aleutian Island as a separate group. Levels of within- and among-group variation were also examined for other population groupings. Phylogeographic relationships among populations were examined using a UPGMA analysis of linearized F_{ST} values (Slatkin 1995). Support for UPGMA branches was determined by testing whether branch lengths (F_{ST} values) differed significantly from 0.

Evidence for potential bottlenecks and/or population expansions was obtained using a mismatch distribution of pairwise substitutional differences among pseudo-haplotypes (Slatkin and Hudson 1991, Rogers and Harpending 1992, Rogers et al. 1996). To undertake this analysis we assumed that recombination effects within each intron were negligible (Rogers et al. 1996). Tajima's *D* statistic was calculated to check for significant mutation rate heterogeneity among nucleotide sites (Aris-Brosou and Excoffier 1996).

Possible secondary contact between previously isolated populations was examined using Chakraborty's test of population amalgamation (Chakraborty 1990). Analyses were performed for each locus combining all populations, and populations within the most probable groupings generated by AMOVA. The Ewans-Watterson test of selective neutrality (Watterson 1978, Slatkin 1994, 1996) was used to determine whether observed sequence variation differed from neutral expectations. Neutrality tests were performed for each locus separately. Genotype frequencies at each locus were also tested for deviations from Hardy-Weinberg equilibrium. Hardy-Weinberg tests were carried out on genotype frequencies at each location, and for the most probable population groupings generated by AMOVA. All of the above analyses apart from the log-log regressions of isolation by distance were performed on Arlequin (Schneider et al. 1997). Hedricks -U was calculated using combined data from all nine loci (Siegel-Causey 1997). This statistic was used as an index of the direction of gene flow between major breeding areas .

Time since divergence between populations was estimated as $t = \delta\ 10^6 / r$ where $\delta = \pi_{xy} - 0.5 (\pi_x + \pi_y)$, π_{xy} is the mean pairwise nucleotide divergence between populations x and y, π_x and π_y are the mean pairwise divergences within populations x and y, and r is the nucleotide divergence rate for the gene being analyzed (Wilson et al. 1985). Mean pairwise divergences within and among populations were calculated using the pairwise distance matrices generated by AMOVA. Mutation rates at each locus were estimated as per Congdon et al. (in prep).

microsatellites. – AMOVA, pairwise F_{ST}, isolation by distance and Hedrick's-U analyses were repeated using allele frequency data from the three microsatellite loci. Hardy-Weinberg analyses were undertaken on each microsatellite locus at each location, and for the most probable population groupings obtained from AMOVA.

RESULTS

Introns. – Substitutional relationships among intron alleles, and allele frequencies at each locus for the nine murrelet populations are given in Tables 1 and 2a. AMOVA classifying all locations as one group indicated significant genetic structuring among murrelet populations (Table 3a). Significant pairwise F_{ST} values were observed for comparisons between Aleutian Island and mainland sites (Table 4a). Murrelets from Attu Island were genetically distinct from those at all mainland sites, whereas murrelets from Adak Island differed from murrelets at all sites east of Mitrofania Bay. The two Aleutian Island populations did not differ from each other. No significant F_{ST} values were observed among any two mainland populations.

Significant isolation by distance effects were observed from a log-log regression analysis using all sites ($F_{1,22} = 12.42$, $R^2 = 0.33$, $P < 0.001$, Fig. 2a). Shortest geographic distance explained 57% of the observed variation. This relationship was not significant when the two Aleutian Island populations were removed ($F_{1,7} = 0.13$, $R^2 = -0.12$, $P = 0.748$, Fig. 2b), but remained significant for a subsample of populations from Kachemak Bay to Attu Island ($F_{1,7} = 45.63$, $R^2 = 0.85$, $P = 0.02$, Fig 2c). Similar results were obtained for analyses of both shoreline and shortest geographic distance. Thus, distance effects were observed in the eastern half of the species' distribution, and were associated with ground-nesting vs tree-nesting comparisons. Distance effects among

ground-nesting populations only could not be tested. Distance did not correlate with divergence among tree-nesting populations. Importantly, Aleutian Island to mainland comparisons were significant over distances that comparisons among mainland were not.

All three AMOVA in which *a priori* group definitions were varied showed significant among-group variation, and no variation attributable to among-population differences within groups (Table 3a). Combining mainland populations but separating Aleutian Island populations maximized the among-group variation, and was considered the most probable population structuring. Analysis of tree-nesting vs ground-nesting populations explained little more among-group variation than analyses grouping all populations together. This was because ground-nesting populations on the Alaskan Peninsula were most similar to adjacent tree-nesting populations. All AMOVA groupings that partitioned tree-nesting populations showed significant among-population differences within groups (data not shown). UPGMA analysis confirmed the principal groupings of mainland versus Aleutian Island sites (Fig. 3a)

Genotype frequencies differed from Hardy-Weinberg expectations at two loci, aldolase ($P < 0.0001$) and lamin ($P = 0.038$), for analyses combining mainland populations. This was due to heterozygote deficiencies in British Columbia for aldolase ($P < 0.0001$), and at Prince William Sound ($P = 0.045$) for lamin. Genotype frequencies did not differ from Hardy-Weinberg equilibrium at any of the nine loci for the Aleutian Island populations, but the power of these tests was low due to small sample sizes and large numbers of alleles. Thus, deviations from Hardy-Weinberg do not appear to be responsible for the patterns of population structuring observed in the AMOVA.

A mismatch distribution of pairwise sequence divergence combining all populations produced a distinctly unimodal distribution approximately Poisson in form (Fig. 4a). While this curve was significantly different from a Poisson distribution ($P < 0.001$), its shape contrasted markedly with distributions generated in stationary populations at mutation-drift equilibrium (Slatkin and Hudson 1991; Aris-Brosou and Excoffier 1996). A negative but non-significant Tajima's D ($D = -0.012$, $P > 0.1$), suggests the shape of this curve results from a combination of rapid population expansion flowing either a bottle-neck or period of constant population size, and some mutation rate heterogeneity (Slatkin and Hudson 1991; Aris-Brosou and Excoffier 1996). The average mutation rate across all intron loci was estimated at ~ 0.5% Ma^{-1} and ranged between 0.15-0.8% Ma^{-1} (Congdon et al. in prep). Mutation rates at 3 loci differed substantially from the mean rate ($> \pm$ 0.2% Ma^{-1}), two loci evolving more rapidly and one more slowly (Congdon et al. in prep). Results of the mismatch analysis did not differ when these three loci were removed (Fig. 4b). Tajima's D decreased but remained non-significant ($D = -1.019$, $P > 0.1$). A similar unimodal distribution could also result from a selective sweep having occurred simultaneously across all nine intron loci; however, we consider this possibility unlikely because the Ewans-Watterson tests of selective neutrality revealed no evidence of selection at individual loci ($P =$ 0.06 to 0.65). In contrast, a mismatch analysis using only the three most rapidly evolving loci (> 0.6% divergence Ma^{-1}) produced a distinctly bimodal distribution (Fig. 4c). This second distribution suggests our sample contains individuals originating from multiple refugia. Because of differences in the

average mutation rate of loci used in these two analyses (0.43% and 0.73% respectively), the results do not conflict if a single bottleneck and range expansion, as measured by the more slowly evolving loci, had been followed by a second contraction and expansion involving two refugia. Chakraborty's test of population amalgamation showed significantly higher than expected numbers of alleles in the Aleutian Islands for five of the nine intron loci (P = 0.0001 to 0.035). Significant results were not obtained from either the total population analyses, or from analyses combining mainland populations. This result further suggests that Aleutian Island populations may originate from multiple refugia.

Using a mutation rate across all loci of ~0.5% Ma^{-1}, time since divergence between Aleutian Island and British Columbian populations was estimated at ~0.042 Ma (δ =2.1 x 10^{-4}). This places the divergence of these populations in the Pleistocene prior to the final glacial episode at 0.015-0.025 Ma (Hamilton 1994). Divergence between the Alaskan Peninsula and British Columbian populations was estimated at ~0.017 Ma (δ = 8.6 x 10^{-5}), or post this final glacial stade. Estimates of the relative timing of these events did not change when divergence rates of up to 1.0% Ma^{-1} were used.

Hedrick's-U statistic indicated that net gene flow was from east to west into the Aleutian Archipelago (Fig. 5). As with the isolation by distance results, gene flow among mainland populations appeared to be greater than gene flow among mainland and island populations over equivalent distances.

Microsatellites. – Allele frequencies at each microsatellite locus for the nine murrelet populations are given in Table 2b. Microsatellite AMOVA combining all populations into a one group indicated significant genetic structuring among sampling locations (Table 3b). Comparison of pairwise F_{ST} values did not produce an obvious population structure, but suggested that the Kachemak and Belkofski Bay sites were outliers in a general pattern of high inter-population gene flow (Table 4b). UPGMA independently clustered mainland and Aleutian sites, but also indicated that Belkofski and Kachemak Bay were outliers to this pattern (Fig. 3b). Analysis of ground-nesting vs tree-nesting populations with the Kachemak and Belkofski Bay sites held as independent groups, maximised the variation explained by among-group differences, and was considered the most probable population structure (Table 3b). A comparison of ground-nesting vs tree-nesting populations was also significant, but attributed less of the total variation to among-group differences. AMOVA comparing mainland to Aleutian Island groups produced marginally significant among-population differences within groups. This was due to allele frequency differences between ground-nesting populations on the Alaskan Peninsula and other mainland populations. This result directly conflicts with that obtained from the intron AMOVA. The preferential association of ground nesting sites on the peninsula with the Aleutian Islands, and the genetic discontinuity observed among mainland ground and tree-nesting locations over a relatively short distance, is consistent with restricted gene flow due to habitat differences. Elsewhere, the microsatellite AMOVA indicate relatively high levels of gene flow among mainland tree-nesting populations, with some divergence of Kachemak Bay.

No isolation by distance effects were observed from analyses of microsatellite allele frequencies ($F_{1,20}$ = 1.68, R^2 = 0.03, P = 0.946; Fig. 2d). No deviations from Hardy-Weinberg equilibrium were observed at any locus

in the Aleutian Island, Belkofski, or Kachemak Bay populations. Locus 114B-2929 was not in Hardy-Weinberg equilibrium in an analysis combining other tree-nesting populations. Deviations from equilibrium at this locus were due to heterozygote deficiencies at Mitrofania Bay and in the Alexander Archipelago. The reason for these deficiencies is unknown, but sampling error was considered the most likely explanation. Although AMOVA results did not appear to reflect deviations from Hardy-Weinberg at locus 14B-29, the micosatellite analyses were repeated using only data from the other two loci. Results of these analyses did not differ significantly from those previously given (data not shown). Hedrick's-U could only be calculated for microsatellite locus BMA 10-18 because genotypes at all other loci were shared across sites. Hedrick's-U indicated a net gene flow from east to west into the Aleutians. As with previous analyses, the level of gene flow between the mainland and Aleutian Island populations was lower than that observed among mainland populations over similar geographic distances (Fig. 5). Hedricks-U for microsatellites also indicated a relatively greater level of gene flow between peninsula and island populations than the U-value obtained from intron frequencies. This is consistent with the increase in gene flow between mainland ground-nesting sites and Aleutian populations observed in the microsatellite AMOVA.

DISCUSSION

Allopatric divergence

Overall, our results are consistent with rapid expansion of murrelets from a single refugium during an interglacial period ~0.05 Ma, the subsequent isolation and divergence of island populations in an Aleutian refugium during the final Pleistocene glacial episode, and secondary contact among island and mainland populations during a second range expansion following the retreat of the Wisconsin ice sheets ~0.017-0.011 Ma. This interpretation is consistent with a population bottleneck previously hypothesized from reduced levels of mtDNA variation (Friesen et al. 1996b), and with the timing of known late Pleistocene glacial events (Thorson and Hamilton 1986; Hamilton 1994; Hughes and Hughes 1994). Our scenario requires the presence of an unknown refuge in the outer Aleutians during the last Pleistocene glacial stade. A similar refugium is thought to have facilitated subspecific divergence in Pigeon Guillemots (*Ceppus columba*) 1.0-0.07 Ma (Kidd and Friesen 1998). An Aleutian refugium is not inconsistent with current geological data, but these data are scarce. The westward limit of the ice sheet that covered the Alaskan Peninsula and inner Aleutian Islands during the final Pleistocene glacial episode is unknown (Hamilton 1994). Therefore, the availability of nesting habitat is also unknown. Similarly, few data exist on the availability of food resources. Current information suggests that biological activity has been continuous in the south-west Bering sea for at least 50 ka (Morely and Robinson 1986). However, it is also thought that during the last Pleistocene glacial advance spring temperatures were cooler, the productive ice-free season was short, and perennial sea-ice was present around the south-east margins of the Bering basin (Sancetta and Robinson 1983).

Peripatric divergence

Despite the absence of barriers to dispersal since the glacial retreat, our

data suggest that introgression of mainland and Aleutian Island populations has been limited relative to dispersal along the North American coast. Given the relative size differences between mainland and island populations (Piatt and Ford 1993), and the period of time that has elapsed, it is unlikely that prior occupation of the Aleutians by a refugial population alone would have allowed this region to remain distinct. The slowly evolving intron loci provide no evidence that current population structure is maintained by disruptive selection associated with breeding habitat. Mainland tree-nesting birds appear to have effectively recolonized ground-nesting sites at the end of the peninsula. Populations on the peninsula are genetically and morphologically (Pitocchelli et al. 1995) indistinguishable from birds at other tree-nesting locations. Instead, the intron data suggest that distance effects associated with habitat fragmentation are responsible for restricting gene flow into the Aleutians. This implies that the small Aleutian Island populations are scattered over distances that are large relative to the average dispersal distances of murrelets. If so, most mainland emigrants would be unlikely to reach suitable breeding habitat. This process could significantly restrict gene flow into the islands, and would be intensified if Aleutian populations were at, or near, carrying capacity.

Parapatric divergence

In contrast, analysis of the more rapidly evolving microsatellite loci suggest disruptive selection associated with nesting habitat significantly restricts immigration into the Aleutians. The microsatellite AMOVA preferentially groups mainland ground-nesting populations with island populations, and shows a relatively abrupt genetic discontinuity between habitat types. A synthesis of both intron and microsatellite data suggests that historically birds nesting on the peninsula originate from mainland tree-nesting sources, but that ground-nesting sites in this region may act as sinks for the majority of these emigrants. Intense selection on mainland birds using these sites may act as a selective sieve, producing the allele frequency differences observed between this and other mainland sites. Only birds capable of passing through this sieve, or their offspring, may be able to successfully colonize further into the Aleutian system. Thus, colonization of the Aleutians by mainland immigration may only continue because of the ability of some tree-nesting birds to successfully use ground-nesting sites. The maintenance of this ability in the tree-nesting population may relate to ground-nesting being ancestral in *Brachyramphus* murrelets (Friesen et al. 1996b).

In summary, the maintenance of the Aleutians as a genetically distinct region may result from either habitat fragmentation and/or selection associated with nesting habitat. The contrast between results obtained using intron and microsatellite marker systems highlights the need for comparative analyses among multiply loci with different rates of evolution. Without both marker systems, one or other of these evolutionary processes would not have been observable. Even so, it is difficult to separate the relative contribution of each process using the current data set. At present, the microsatellite data must be interpreted cautiously due to the small number of loci involved in the analyses. Evaluation of potential selection effects will require fine scale ecological and genetic analysis of the transition zone on the Alaskan Peninsula. Similarly, a better understanding of the impact of fragmentation can be obtained by analysis of peripheral tree-nesting locations in Washington, Oregon and

California. Inclusion of these sites will allow fragmentation effects to be examined across populations where nesting habitat does not vary. This work is continuing.

If mutation rates in our analysis are incorrect, and recolonization of the outer Aleutians has occurred during the last 15 ka, habitat fragmentation and/or selection must also be responsible for the origin of current population structuring. We consider this possibility unlikely. Both Chakraborty's test of amalgamation, and the mismatch distribution for rapidly evolving loci, show evidence of admixture between previously isolated populations. A higher than expected number of alleles in the Aleutians, as indicated by Chakraborty's test, is not compatible with the divergence of small island populations by either intense drift or natural selection. Divergence by drift alone should reduce variation within islands, and increase variation among islands. This was not what we observed. Similarly, only diversifying selection acting directly on the intron loci used in this study, or linked loci, would produce higher than expected number of alleles. We found no evidence of selection at any intron locus. Also, diversifying selection would be expected to generate a 'star burst' phylogeny of alleles and unimodal mismatch distribution, not the bimodal distribution we obtained for island populations. Instead, our results suggest that mainland and island regions are slowly introgressing. Selection or habitat fragmentation may have slowed the rate of introgression, but at present these processes do not appear capable of establishing the original differences, or promoting further diversification.

Historic vs contemporary processes

Our results continue to emphasize vicariance due to glaciation as the dominant evolutionary process promoting genetic divergence in Northern Hemisphere alcids. Previous studies of alcids have implicated vicariance in speciation events (Friesen et al. 1996b), in the origin of inter-oceanic (Birt-Friesen 1992; Friesen et al. 1996a; Friesen et al. 1996b), and subspecific divergence (Kidd and Friesen 1997), and in the production of intra-specific population structure (Friesen et al. 1996a). Our results suggest that vicariance is also responsible for the origin of intra-specific structuring in marbled murrelets. Significantly, while the origin and magnitude of divergence is correlated with glacial vicariance, we suggest that the microgeographic spatial arrangement of populations and/or selection associated with nesting habitat, has been necessary for the maintenance of population structure over extended periods of time.

In summary, peripatric and parapatric modes of divergence have not produced intra-specifc structuring in marbled murrelets. However, we have demonstrated that one or both of these process has been influential in maintain divergence. Thus, while the specific conditions under which non-allopatric processes promote further divergence may not be present in murrelets, our results suggest that these processes may significantly influence divergence in others species where the requirements of non-allopatric models are satisfied.

Conservation / management implications

Marbled murrelets are in direct conflict with logging interests, suffer heavy mortality from oil pollution and gill netting (Sealy and Carter 1984; Carter and Kuletz 1995; Carter et al. 1995), and are officially listed as 'Threatened' or

Exxon Valdez Oil Spill
Restoration Project Annual Report

A Genetic Study to Aid in Restoration of Murres, Guillemots and Murrelets to the Gulf of Alaska

Restoration Project 97169
Annual Report

This annual report has been prepared for peer review as part of the *Exxon Valdez* Oil spill Trustee Council restoration program for the purpose of assessing project progress. Peer review comments have not been addressed in this annual report.

Vicki Friesen
John Piatt

Alaska Biological Sciences Center, USGS, 1011 E. Tudor Rd., Anchorage, AK 99503, USA

April 1998

'Endangered' throughout much of their distribution. Effective long-term management of this species requires detailed information on contemporary levels of gene flow. Unfortunately, the intron data provide little information on current levels of inter-population movement, particularly among tree-nesting populations. However, they do suggest that the microgeographic spatial arrangement of populations may significantly influence levels of genetic structuring, and provide a tentative scale at which this influence operates.

While, Aleutian populations are not evolving completely independently of mainland sites, they are genetically distinct, and may remain so well beyond the temporal scale of any management regime. For this reason they may warrant independent management status. The distribution of Alaskan murrelets attributable to Island and mainland refugia, correlates with the distributions of two pigeon guillemot subspecies (Storer 1952; Kidd and Friesen 1998). A third guillemot subspecies occurs south of our sampling locations (Storer 1952; Kidd and Friesen 1998). Divergence of this third subspecies is consistent with allopatric isolation in a southern refugium during the same time frame as our results (Kidd and Friesen 1998). Thus, murrelets populations south of our sampling locations may originate from a third refugium. This possibility requires further investigation.

The microsatellite data give more information on contemporary gene flow amongst mainland populations, although conclusions from these analyses must be tentative. Microsatellite results suggest that the four major tree-nesting regions examined in this study; the Kodiak Island Archipelago, Prince William Sound, the Alexander Archipelago, and Desolation Sound-British Columbia exchange large numbers of individuals and cannot be distinguish from a single interbreeding population. They also suggest that gene flow into Kachemak Bay as well as into ground-nesting sites on the Alaskan Peninsula may be significantly reduced. Reasons for the isolation of Kachemak Bay are unknown, but this result is consistent with a scarcity of murrelets in Cook Inlet relative to the available nesting habitat (Piatt and Ford 1993). Piatt and Ford (1993) suggest that this scarcity may be associated with suboptimal nesting sites, or poor foraging habitat in this region. More accurate estimation of current levels of gene flow between tree-nesting populations requires the use of additional microsatellite loci.

We conclude that historically marbled murrelets along the Canadian and Alaskan coastlines have not been distributed as small isolated breeding populations. However, extensive human fragmentation of nesting habitat in this region has the potential to mimic natural fragmentation of murrelet habitat in the Aleutians, and so isolate threatened populations at the southern end of the distribution.

ACKNOWLEDGMENTS

We especially thank D. Harrison for invaluable help in the laboratory. Thanks also to M. Kidd, J. Moy and H. Walsh for assistance with intron primer development; C. Crossman, D. Michaud and H. Jones for assistance with microsatellite primer development, G. Ibaguchi for allowing us to use unpublished primers, and S. Doran for laboratory analysis. We thank T. Van Pelt, V. Lodha, S. Kitaysky, J. Pitoccelli and the crew of the USFW research vessel 'Tiglax' for assistance with field collections. T. Birt, and K. Warheit provided helpful discussions. Financial support was provided by the

Environmental Innovations Program of Government Works and Public Services Canada grants to V. L. Friesen and T.P. Birt, the British Columbia Department of Forestry, and the Advisory Research Council of Queen's University. The research in this paper was also supported by , the *Exxon Valdez* Oil Spill Trustee Council. However the findings and conclusions presented by the authors are their own and do not necessarily reflect the views or position of the Trustee Council.

LITERATURE CITED

Aris-Brosou, S., and L. Excoffier. 1996. The impact of population expansion and mutation rate heterogeneity on DNA sequence polymorphism. Molecular Biology and Evolution. 13:494-504.

Austin, J. J., R. G. W. White, and J. R. Ovenden. 1994. Population genetic structure of a philopatric, colonial nesting seabird, the Short-tailed Shearwater (*Puffinus tenuirostris*). Auk 111:70-79.

Birt-Friesen, V. L., W. A. Montevecchi, A. J. Gaston, and W. S. Davidson. 1992. Genetic structure of thick-billed murres (*Uria lomvia*) populations examined using direct sequence analysis of amplified DNA. Evolution 46:267-272.

Barton, N. H., and B. Charlesworth. 1984. Genetic revolutions, founder effects, and speciation. Annual Review of Ecology and Systematics 15:133-164.

Bush, G.L. 1975. Modes of animal speciation. Annual Review of Ecology and Systematics 7:311-345.

Carson, H.L., and A. R. Templeton. 1984. Genetic revolutions in relation to speciation phenomena: The founding of new populations. Annual Review of Ecology and Systematics. 125:97-131.

Carter, H.R., and M. L. Morrison. 1992. Status and conservation of the marbled murrelet in North America. Proceedings of the Western Foundation for Vertebrate Zoology. Vol. 5.

Carter, H. R., and K. J. Kuletz. 1995. Mortality of marbled murrelets due to oil pollution in North America. Pp. 261-270 *in* C. J. Ralf, G. Hunt, M. Raphael and J. F. Piatt eds. Ecology and conservation of the Marbled Murrelet. General Technical Report PSW-GTR-152. Albany, California.

Carter, H. R., M. L. C. McAllister and M. E. Isleib. 1995. Mortality of marbled murrelets in gill nets in North America. Pp. 271-284 *in* C. J. Ralf, G. Hunt, M. Raphael and J. F. Piatt eds. Ecology and Conservation of the Marbled Murrelet. General Technical Report PSW-GTR-152. Albany, California.

Chakraborty, R. 1990. Mitochondrial DNA polymorphism reveals hidden heterogeneity within some Asian populations. American Journal of Human Genetics. 47:87-94.

Congdon, B. C., C. L. Lange, and A. R. Clarke. 1997. Geographic variation and gene flow in the eucalyptus defoliating beetle *Chrysophtharta bimaculata* (Coleoptera: Chrysomelidae). Journal of Applied Ecology 34:1287-1292.

Duffy, J. E. 1996. Resource-associated subdivision in a symbiotic coral-reef shrimp. Evolution. 50:360-373.

Endler, J. A. 1977. Geographic variation, speciation and clines. Princeton Univ. Press, Princeton.

Excoffier, L., P. E. Smouse, and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distance among DNA haplotypes: Application to human mitochondrial DNA restriction data.Genetics 131:479-491.

Friesen, V. L., W.A. Montevecchi, A.J. Baker, R. T. Barrett, and W.S. Davidson.1996a. Population differentiation and evolution in the

Common guillemot *Uria aalge*. Molecular Ecology 5:793-805.

Friesen, V. L., J. F. Piatt, A. J. Baker. 1996b. Evidence from cytochrome *b* sequences and allozymes for a 'new' species of alcid: The long-billed murrelet (*Brachyramphus peridix*). Condor 98:681-690.

Friesen, V. L., B. C. Congdon, H. E. Walsh, and T. P. Birt. 1997. Intron variation in Marbled murrelets detected using analysisi of single-stranded conformational polymorphisms. Molecular Ecology 6:1047-1058.

Hamilton, T. D. 1994. Late Cenozoic glaciation of Alaska. Pp. 813-884 *in* G. Plafker and H. Berg eds. The Geology of Alaska. The geology of North America, G-1. Geological Society of America, Boulder, Colorado.

Hughes, B. A., and T. J. Hughes. 1994. Transgressions: Rethinking Beringian glaciation. Palaeogeography, Palaeoclimatology, Palaeoecology 110:275-294.

Kidd, M. G, and V. L. Friesen. 1998. Analysis of mechanisms of microevolutionary change in Cepphus guillemots using patterns of control region variation. Evolution (in press)

Lessa, E. P., and G. Applebaum. 1992. Screening techniques for detecting allelic variation in DNA sequences. Molecular Ecology 2:121-129.

Mayr, E. 1963. Animal species and evolution. Harvard University Press, Cambridge, MA.

Maynard-Smith, J. 1966. Sympatric speciation. American Naturalist 100:637-650.

Mendenhall, V. M. 1992. Distribution, breeding records, and conservation problems of the Marbled murrelet in Alaska. Pp. 5-16 *in* H. R. Carter and M. L. Morrison eds. Status and conservation of the Marbled murrelet in North America. Proceedings of the West. Foundation of Vertebrate Zoology Vol. 5.

Michalakis, Y., and L. Excoffier. 1996. A generic estimation of population subdivision using distances between alleles with special reference to microsatellite loci. Genetics 112:629-647.

Morel, J. J., and S. W. Robinson. 1986. Improved method for correlating late Pleistocene/Holocene records from the Bering Sea: Applicationof a biosiliceous/geochemical stratigraphy. Deep-Sea Research 33:1203-1211.

Otte, D., and J. A. Endler. 1989. Speciation and its consequences. Sinauer, Sunderland.

Otter, K., L. Ratcliffe, D. Michard, and P.T. Boag. 1998. Do female Black-capped chickadees prefer high ranking partners. Behavioural Ecology and Sociobiology in press.

Palumbi, S. R., and C.S. Baker. 1994. Contrasting population structure from nuclear intron sequences and mtDNA of humpback wales. Molecular Biology and Evolution 11: 426-435.

Piatt, J. F., and R. G. Ford. 1993. Distribution and abundance of Marbled Murrelets in Alaska. Condor 95:662-669.

Pitocchell, J., J. Piatt, and M. Cronin.1995. Morphological and genetic divergence among Alaskan populations of *Brachyramphus* murrelets. Wilson Bulletin 107:235-250.

Ralf, C. J., G. Hunt, M. Raphael, and J. F. Piatt eds. Ecology and

conservation of the Marbled Murrelet. General Technical Report PSW-GTR-152. Albany, California.

Raymond, M., and F. Rousset. 1995. An exact test for population differentiation. Evolution 49:1280-1283.Rogers, A.R., and H.

Harpending. 1992. Population growth makes waves in the distribution of pairwise genetic differences. Molecular Biology and Evolution 9:552-569.

Rogers, A. R., A. E. Fraley, M. J. Bamshad, W. S. Watkins, and L. B. Jorde. 1996. Mitochondrial mismatch analysis is insensitive to mutational process. Molecular Biology and Evolution 13:895-902.

Sancetta, C., and S. W. Robinson. 1983. Diatom evidence on Wisconsin and Holocene events in the Bering Sea. Quaternary Research 20:232-245.

Schneider, S., J. -M. Kueffer, D. Roessli, and L. Excoffier.1997. Arlequin: A software for population genetic data analysis Ver 1.1. Genetics and Biometry laboratory, Department of Anthropology, University of Geneva.

Sealy, S. G., and H. R. Carter. 1984. At-sea distribution and nesting habitat of Marbled Murrelets in British Columbia: Problems in the conservation of a solitary nesting seabird. Pp. 737-756 *in* J. P. Croxall, P. G. H. Evans, and R. W. Schreiber eds. Status and Conservation of the worlds seabirds. International Commission for Bird Protection Technical Publication No. 2.

Siegel-Causey, D. 1997. Molecular variation and biogeography of Rock Shags. Condor 99:139-150.

Slatkin, M. 1993. Isolation by distance in equilibrium and non-equilibrium populations. Evolution 47:264-279.

Slatkin, M. 1994. An exact test for neutrality based on Ewans sampling distribution. Genetic Research 64:71-74.

Slatkin, M. 1995. A measure of population subdivision based on microsatellite allele frequencies. Genetics 139: 457-462.

Slatkin, M. 1996. A correction to the exact test based on Ewans sampling distribution. Genetic Research 68:259-260.

Slatkin, M. and R. R. Hudson. 1991. Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations. Genetics 123:603-613.

Stanley, H. F., S. Casy, J. M. Carnahan, S. Goodman, J. Harwood, and R. K. Wayne. 1996. World wide patterns of mitochondrial DNA differentiation in the Harbour seal (*Phoca vitulina*). Molecular Biology and Evolution 13:368-382.

Storer, R. W. 1952. A comparison of variation, behaviour and evolution in the sea bird genera *Uria* and *Cepphus*. University of California Publications in Zoology 52:121-222

Thorson, R. M., and T. D. Hamilton. 1986. Glacial geology of the Aleutians (Based on the contributions of Robert F. Black). Pp.171-192 *in* T. D. Hamilton, K. M. Reed and R. M. Thorson eds. Glaciation in Alaska - The Geological Record. Alaska Geological Society, Anchorage, AK.

Watterson, G. 1978. The homozygosity test of neutrality. Genetics 88:405-417.

Wilson, A. C., R. L. Cann, S. M. Carr, M. George, U. B. Gyllensten, K. M. Helm-Bychowski, R. G. Higuchi, S. R. Palumbi, E. M. Prager, and M. Stoneking. 1985. Mitochondrial DNA and two perspectives on evolutionary genetics. Biological Journal of the Linnean Society. 26:375-400.

Wood, C. C., and C. J. Foote. 1996. Evidence for sympatric genetic divergence of Anadromous and nonanadromous morphs of sockeye salmon (*Oncorhynchus nerka*). Evolution 1265-1279.

TABLE 1. Sequence variation in nine nuclear introns for Marbled murrelets. Site designations are nucleotide positions relative to the 5' primer.

Ornithine Decarboxylase

allele/ / site	1	2	4	3	7	5	8	9	15
1	A	T	A	C	T	I	G	A	T
2	A	T	A	C	C	I	G	A	T
3	G	T	A	C	T	I	G	A	T
4	A	T	G	T	T	I	G	A	T
5	A	T	A	C	T	-	G	A	T
6	G	T	A	C	T	I	A	G	G
7	G	T	A	C	T	I	A	G	T
8	A	A	A	C	T	I	G	A	T

Ribosomal Protien 40

	1	2	3	4	5	6	7	8	9	10
1	C	T	G	-	C	G	G	C	C	T
2	T	C	G	I	C	A	G	C	-	C
3	C	C	A	I	C	G	G	T	-	C
4	C	C	G	I	T	G	G	T	-	T
5	T	C	G	I	C	G	G	C	-	C
6	C	C	G	-	T	G	G	T	C	T
7	C	T	G	-	C	G	A	C	C	T
8	C	C	G	I	C	G	G	T	-	C

Tropomyosin

	1	2	3	7	15	20	21
1	A	C	T	C	T	C	C
2	A	C	T	C	C	T	C
3	G	C	C	C	C	C	C
4	A	C	T	C	C	C	T
5	A	C	T	C	C	C	C
6	A	C	T	T	C	C	C
7	A	T	T	C	C	C	C

MPP

	1	2	5
3	C	G	G
4	C	A	G
5	T	G	G
6	C	G	A

Lamin

	131	134	119	183	301	85
2	C	A	C	A	C	T
3	C	A	C	G	C	T
4	T	A	C	G	C	T
5	C	A	T	G	C	C
6	C	G	C	G	G	T

Enolase

	126	170	242	12
1	A	G	T	C
2	A	A	T	C
3	G	G	C	T
5	G	G	C	T
6	G	G	C	T

Aldolase

	42	76	8	9
3	G	C	C	T
4	A	C	C	C
2	A	C	G	C
6	A	A	C	C

Lactate dehydrogenase

	1	3	4	8	6	7	12
1	A	G	A	C	G	G	A
2	C	A	G	C	G	G	A
3	C	G	A	C	G	G	A
4	C	G	A	T	G	G	A
5	C	G	A	C	A	A	A
7	C	G	A	T	G	G	T

Glyceraldehyde-3-phosphate dehydrogenase

	95	123	124	232	277	278	279	220	12	100	216	219	163	175-187	247-250
2	G	T	A	A	T	G	C	T	C	C	A	A	G	CCTGCGTGGTTCA	-
3	G	T	A	G	T	G	C	T	C	C	A	A	G	CCTGCGTGGTTCA	TACT
4	A	T	A	G	T	A	C	T	C	C	A	A	G	CCTGCGTGGTTCA	TACT
5	A	T	A	G	T	G	C	T	C	C	A	A	G	CCTGCGTGGTTCA	TACT
6	A	C	G	G	T	G	C	T	C	C	A	A	G	CCTGCGTGGTTCA	TACT
7	G	T	A	G	T	G	T	T	T	C	A	A	G	CCTGCGTGGTTCA	TACT
8	G	T	A	A	T	G	C	T	C	C	A	A	G	CCTGCGTGGTTCA	TACT
9	G	T	A	G	T	G	C	T	C	C	C	A	G	CCTGCGTGGTTCA	TACT
10	A	T	A	G	T	G	C	C	C	C	A	A	G	CCTGCGTGGTTCA	TACT
11	A	T	A	G	T	G	C	T	C	G	A	A	G	CCTGCGTGGTTCA	TACT
12	A	T	A	G	C	G	C	T	C	C	A	A	G	CCTGCGTGGTTCA	TACT
13	G	T	A	G	T	G	C	T	C	C	A	G	G	CCTGCGTGGTTCA	TACT
14	A	T	A	G	T	G	C	T	C	C	A	A	G	-	TACT
15	G	T	A	G	T	G	C	T	C	C	A	A	C	CCTGCGTGGTTCA	TACT

TABLE 2a. Intron allele frequencies in populations of Marble murrelets. Refer to Fig. 1 for site locations

SITE / LOCUS	*Glyceraldehyde-3-phosphate dehydrogenase (Gapdh, EC No. 1.2.1.12)*														
	2	3	4	5	6	8	9	7	10	11	12	13	14	15	N
Attu Island	0.00	0.44	0.00	0.17	0.22	0.11	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	18
Adak Island	0.00	0.00	0.00	0.50	0.20	0.10	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10
Belkofski Bay	0.05	0.36	0.09	0.36	0.00	0.05	0.00	0.05	0.05	0.00	0.00	0.00	0.00	0.00	22
Mitrofania Bay	0.00	0.45	0.00	0.35	0.05	0.05	0.00	0.05	0.05	0.00	0.00	0.00	0.00	0.00	20
Kodiak Archipelago	0.00	0.38	0.12	0.35	0.08	0.04	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	26
Kachemak Bay	0.04	0.33	0.04	0.33	0.04	0.04	0.04	0.04	0.04	0.04	0.00	0.00	0.00	0.00	24
Prince William Sound	0.00	0.40	0.20	0.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	20
Alexander Archipelago	0.05	0.34	0.13	0.39	0.05	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	38
British Columbia	0.08	0.50	0.02	0.22	0.05	0.05	0.00	0.02	0.00	0.00	0.02	0.02	0.02	0.02	60

	Lamin						*Lactate dehydrogenase (Ldh)*						
	2	3	4	5	6	N	1	2	3	4	5	7	To
Attu Island	0.00	0.63	0.00	0.00	0.38	16	0.06	0.00	0.06	0.89	0.00	0.00	18
Adak Island	0.00	0.40	0.00	0.00	0.60	10	0.00	0.00	0.13	0.88	0.00	0.00	8
Belkofski Bay	0.00	0.74	0.14	0.00	0.14	22	0.00	0.00	0.00	0.91	0.09	0.00	22
Mitrofania Bay	0.00	0.72	0.11	0.06	0.11	18	0.05	0.00	0.10	0.70	0.10	0.05	20
Kodiak Archipelago	0.00	1.00	0.00	0.00	0.00	24	0.00	0.04	0.15	0.81	0.00	0.00	26
Kachemak Bay	0.00	0.83	0.08	0.00	0.08	24	0.04	0.00	0.25	0.71	0.00	0.00	24
Prince William Sound	0.05	0.70	0.10	0.10	0.05	20	0.00	0.00	0.17	0.83	0.00	0.00	18
Alexander Archipelago	0.00	0.78	0.03	0.08	0.13	40	0.08	0.10	0.00	0.80	0.03	0.00	40
British Columbia	0.04	0.75	0.04	0.05	0.13	56	0.00	0.07	0.09	0.83	0.02	0.00	58

	Myelin Proteolipid Protein					*Ornithine Decarboxylase*								
	3	4	5	6	N	1	2	3	4	5	6	7	8	N
Attu Island	0.56	0.00	0.44	0.00	16	0.72	0.00	0.22	0.00	0.00	0.06	0.00	0.00	18
Adak Island	0.50	0.10	0.40	0.00	10	0.50	0.00	0.10	0.00	0.30	0.10	0.00	0.00	10
Belkofski Bay	0.69	0.04	0.27	0.00	20	0.48	0.19	0.17	0.04	0.05	0.04	0.04	0.00	22
Mitrofania Bay	0.78	0.06	0.11	0.06	18	0.45	0.10	0.15	0.10	0.05	0.00	0.00	0.10	20
Kodiak Archipelago	0.79	0.21	0.00	0.00	24	0.65	0.00	0.19	0.04	0.08	0.00	0.00	0.04	26
Kachemak Bay	0.92	0.00	0.08	0.00	24	0.67	0.04	0.21	0.04	0.04	0.00	0.00	0.00	24
Prince William Sound	0.70	0.15	0.15	0.00	20	0.40	0.15	0.15	0.20	0.00	0.10	0.00	0.00	20
Alexander Archipelago	0.75	0.10	0.10	0.05	40	0.58	0.03	0.25	0.05	0.03	0.05	0.00	0.03	40
British Columbia	0.78	0.13	0.07	0.02	54	0.52	0.07	0.22	0.04	0.09	0.00	0.06	0.00	54

	Ribosomal Protien 40									*Aldolase (EC No. 4.1.2.13)*				
	1	2	3	4	5	6	7	8	N	3	4	2	6	N
Attu Island	0.00	0.44	0.06	0.50	0.00	0.00	0.00	0.00	18	0.00	1.00	0.00	0.00	16
Adak Island	0.20	0.50	0.00	0.30	0.00	0.00	0.00	0.00	10	0.00	1.00	0.00	0.00	10
Belkofski Bay	0.35	0.45	0.05	0.00	0.05	0.05	0.05	0.10	20	0.04	0.91	0.05	0.00	22
Mitrofania Bay	0.25	0.45	0.30	0.00	0.00	0.00	0.00	0.00	20	0.05	0.90	0.05	0.00	20
Kodiak Archipelago	0.19	0.23	0.27	0.04	0.04	0.00	0.15	0.08	26	0.09	0.91	0.00	0.00	22
Kachemak Bay	0.33	0.17	0.17	0.08	0.08	0.00	0.04	0.13	24	0.00	1.00	0.00	0.00	24
Prince William Sound	0.15	0.35	0.30	0.00	0.05	0.00	0.05	0.10	20	0.10	0.90	0.00	0.00	20
Alexander Archipelago	0.35	0.25	0.23	0.05	0.00	0.00	0.05	0.08	40	0.03	0.86	0.00	0.11	36
British Columbia	0.25	0.32	0.20	0.07	0.02	0.00	0.04	0.11	56	0.14	0.82	0.00	0.04	56

	Enolase (EC No. 4.2.1.11)						*Tropomyosin*							
	1	2	3	5	6	N	1	2	3	4	5	6	7	N
Attu Island	0.13	0.63	0.25	0.00	0.00	16	0.00	0.06	0.28	0.17	0.11	0.39	0.00	18
Adak Island	0.50	0.50	0.00	0.00	0.00	8	0.00	0.00	0.50	0.10	0.30	0.10	0.00	10
Belkofski Bay	0.04	0.59	0.34	0.00	0.04	16	0.00	0.09	0.42	0.25	0.09	0.05	0.10	22
Mitrofania Bay	0.19	0.31	0.44	0.06	0.00	16	0.06	0.06	0.50	0.13	0.13	0.06	0.06	16
Kodiak Archipelago	0.31	0.31	0.38	0.00	0.00	26	0.00	0.17	0.33	0.13	0.25	0.08	0.04	24
Kachemak Bay	0.08	0.29	0.63	0.00	0.00	24	0.00	0.25	0.40	0.10	0.30	0.05	0.00	20
Prince William Sound	0.20	0.15	0.65	0.00	0.00	20	0.00	0.06	0.33	0.22	0.22	0.11	0.06	18
Alexander Archipelago	0.08	0.33	0.60	0.00	0.00	40	0.00	0.10	0.63	0.08	0.18	0.03	0.00	40
British Columbia	0.10	0.33	0.57	0.00	0.00	58	0.00	0.09	0.57	0.09	0.12	0.07	0.03	58

TABLE 2b. Microsatellite allele freqences in each population of Marbled murrelets. Refer Fig. 1 for site locations

SITE / LOCUS	ULO 14B-29		BMA 9-28		BMA 10-18																		
	1	2	1	2	1	4	5	6	7	8	9	10	11	12	13	15	16	17	18	19	20	23	N
Attu Island	0.19	0.81	0.25	0.75	0.00	0.00	0.17	0.06	0.00	0.22	0.06	0.11	0.00	0.17	0.00	0.17	0.00	0.06	0.00	0.00	0.00	0.00	18
Adak Island	0.40	0.60	0.30	0.70	0.10	0.00	0.10	0.10	0.20	0.00	0.10	0.20	0.00	0.10	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.00	10
Belkofski Bay	0.72	0.28	0.40	0.60	0.23	0.00	0.05	0.00	0.09	0.05	0.23	0.09	0.00	0.05	0.09	0.14	0.00	0.00	0.00	0.00	0.00	0.00	22
Mitrofania Bay	0.61	0.38	0.22	0.77	0.05	0.00	0.05	0.30	0.05	0.20	0.15	0.05	0.00	0.00	0.00	0.10	0.05	0.00	0.00	0.00	0.00	0.00	20
Kodiak Archipelago	0.50	0.50	0.15	0.85	0.12	0.00	0.08	0.19	0.15	0.15	0.04	0.04	0.08	0.00	0.04	0.00	0.00	0.00	0.08	0.04	0.00	0.00	26
Kachemak Bay	0.29	0.71	0.25	0.75	0.00	0.05	0.09	0.18	0.09	0.00	0.36	0.05	0.00	0.00	0.09	0.09	0.00	0.00	0.00	0.00	0.00	0.00	22
Prince William Sound	0.70	0.30	0.33	0.66	0.05	0.00	0.00	0.30	0.20	0.20	0.05	0.00	0.00	0.00	0.05	0.10	0.05	0.00	0.00	0.00	0.00	0.00	20
Alexander Archipelago	0.57	0.43	0.41	0.58	0.10	0.00	0.05	0.20	0.05	0.20	0.13	0.05	0.03	0.05	0.03	0.10	0.00	0.00	0.00	0.00	0.00	0.03	40
British Columbia	0.62	0.37	0.40	0.60	0.00	0.02	0.03	0.21	0.09	0.22	0.09	0.10	0.05	0.02	0.07	0.02	0.02	0.02	0.03	0.00	0.02	0.00	58

TABLE 3. AMOVA results for different subdivisions of marbled murrelet populations for both Intron and microsateilite analyses. P values are based on 4970 permutations. AG, AP/WG, and WP are the among-groups, among-populations/within-groups, and within-population components of variation, respectively.

(a) Intron AMOVA

Groupings	Divisions	Variance Component	% Total Variance	F - Statistics	P
Global	None	AP	2.10	FST= 0.021	0.003 +- 0.001
	All populations	WP	97.90		
2 Habitat divisions	Ground-nesting	AG	3.04	FCT = 0.030	0.024 +- 0.002
	Tree-nesting	AP/WG	0.89	FSC = 0.009	0.106 +- 0.004
		WP	96.07	FST = 0.039	0.003 +- 0.001
2 Geographic divisions	Aleutian Islands	AG	8.94	FCT = 0.089	0.027+- 0.002
	Mainland North America	AP/WG	-0.19	FSC = -0.002	0.580 +- 0.007
		WP	91.25	FST = 0.088	0.005 +- 0.001
3 Geographic divisions	Attu Island	AG	9.45	FCT = 0.094	<0.001 +- <0.001
	Adak Island	AP/WG	-0.39	FSC = -0.004	0.714 +- 0.006
	Mainland North America	WP	90.94	FST = 0.091	0.004 +- 0.001

(b) Microsatellite AMOVA

Groupings	Divisions	Variance Component	% Total Variance	F - Statistics	P
Global	None	AP	1.49	FST= 0.015	0.019 +- 0.002
	All populations	WP	98.51		
2 Habitat divisions	Ground-nesting	AG	2.45	FCT = 0.029	0.024 +- 0.002
	Tree-nesting	AP/WG	0.53	FSC = 0.005	0.212 +- 0.005
		WP	97.02	FST = 0.030	0.019 +- 0.002
2 Geographic divisions	Aleutian Islands	AG	1.25	FCT = 0.013	0.199+- 0.006
	Mainland North America	AP/WG	1.18	FSC = 0.012	0.049 +- 0.003
		WP	97.57	FST = 0.024	0.022 +- 0.003
4 Geographic divisions	Aleutian Isalnds	AG	4.03	FCT = 0.040	0.002 +- <0.001
	Belkofski Bay	AP/WG	-0.83	FSC = -0.009	0.511 +- 0.008
	Mainland North America	WP	96.80	FST = 0.032	0.022 +- 0.002
	Kachemak Bay				

TABLE 4. Pairwise FST (upper number), and *P*-values (central number) for intron loci below diagonal, and microsatellite loci above diagonal. Inter-site distances (lower number) for shortest (below diagonal) and shoreline (upper diagonal) distance.

	Population								
	Attu Island	Adak Island	Belkofski Bay	Mitrofania Bay	Kodiak Arch.	Kachemak Bay	Prince William S.	Alexander Arch.	British Columbia
Attu Island		0.0000 0.5433 722	0.0425 0.0537 1746	0.0206 0.1885 1960	0.0343 0.0575 2446	0.0853 0.0046* 2593	0.0522 0.0414* 2799	0.0038 0.3466 3534	0.0237 0.0826 4269
Adak Island	0.0315 0.1897 722		0.0000 0.0763 1024	0.0059 0.4234 1238	0.0000 0.6829 1724	0.0129 0.2872 1871	0.0097 0.2880 2077	0.0000 0.5405 2812	0.0111 0.2796 3547
Belkofski Bay	0.1237 0.0016* 1641	0.0520 0.0766 1024		0.0483 0.0400* 214	0.0364 0.0388* 700	0.0369 0.0668 847	0.078 0.0078* 1053	0.0212 0.1090 1788	0.0647 0.0014* 2523
Mitrofania Bay	0.0871 0.0112* 1837	0.0545 0.0798 1236	0.0000 0.6459 214		0.0000 0.5299 486	0.0087 0.2927 633	0.0000 0.8169 839	0.0000 0.9802 1574	0.0000 0.7046 2309
Kodiak Archipelago	0.1541 0.0002* 2230	0.1271 0.0009* 1689	0.0074 0.2776 693	0.0000 0.5502 486		0.0494 0.0211* 147	0.0000 0.7153 353	0.0000 0.6329 1088	0.0000 0.6521 1823
Kachemak Bay	0.1343 0.0004* 2315	0.1104 0.0138* 1801	0.0003 0.3955 824	0.0000 0.5723 623	0.0000 0.9927 147		0.0453 0.0587 206	0.0283 0.0684 941	0.0474 0.0122* 1676
Prince William Sound	0.1189 0.0028* 2486	0.0899 0.0221* 1995	0.0098 0.2673 1030	0.0000 0.8706 829	0.0000 0.5918 349	0.0029 0.3421 206		0.0000 0.5981 735	0.0000 0.4693 1470
Alexander Archipelago	0.1257 0.0008* 3185	0.0873 0.0147* 2635	0.0007 0.3760 1617	0.0026 0.3546 1402	0.0000 0.9302 956	0.0000 0.9706 874	0.0067 0.2848 735		0.0000 0.6111 735
British Columbia	0.0936 0.0004* 3974	0.0758 0.0127* 3334	0.0000 0.4632 2333	0.0000 0.8487 2137	0.0033 0.3138 1810	0.0000 0.5431 1781	0.0000 0.4228 1700	0.0019 0.3434 735	

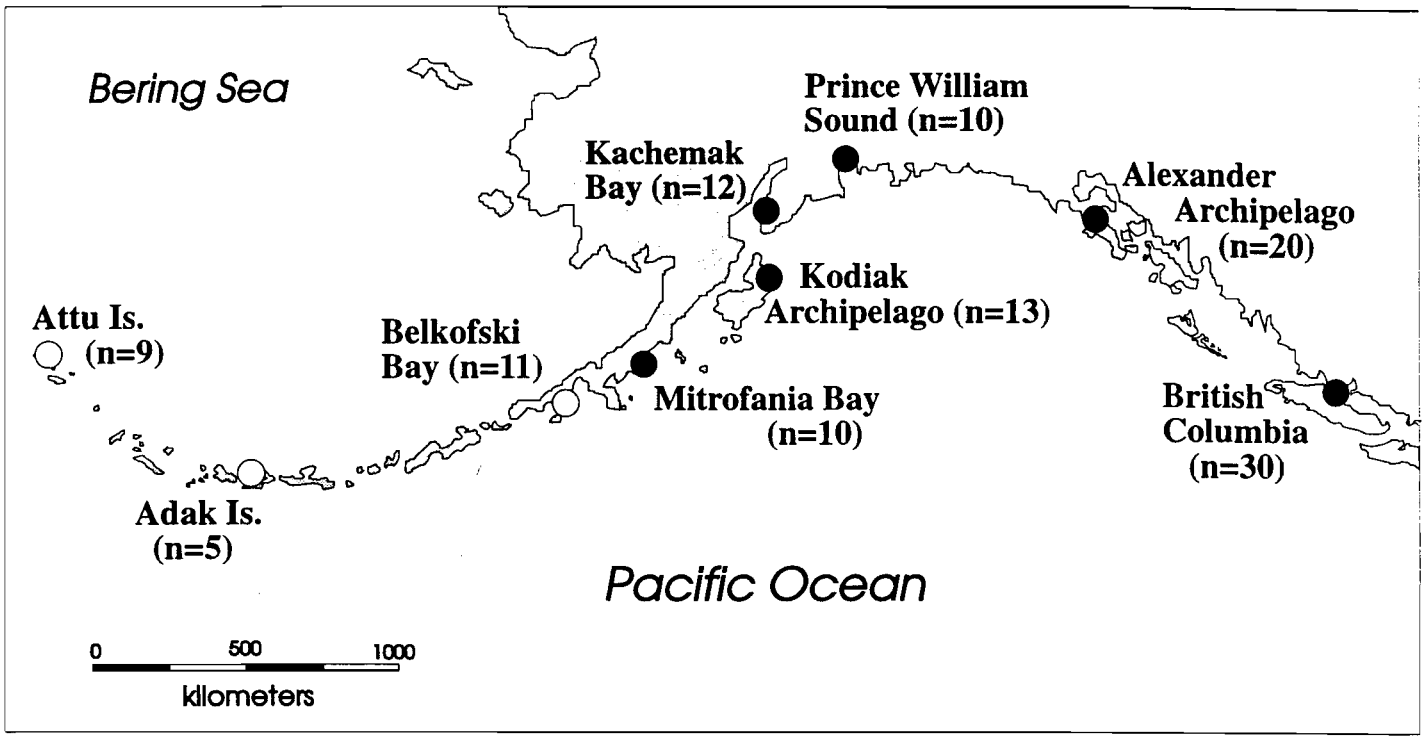
FIG. 1. Marbled murrelet sampling locations in Alaska and British Columbia. Sample sizes at each location are given in parenthesis. ● Tree-nesting locations, ❍ Ground-nesting locations.

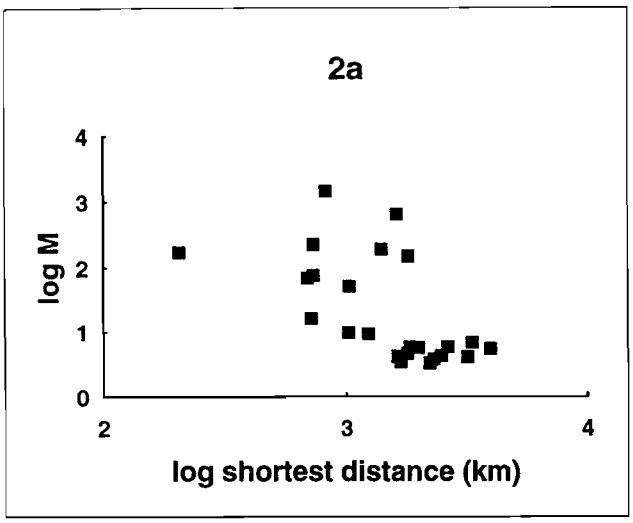
FIG. 2. Scatter plots of log (M) and geographic distance. a), b) and c) use M calculated from intron allele frequency differences among all sites, mainland sites, and sites from Kachemak Bay to Attu Island respectively. d) uses M calculated from microsatellite allele frequency differences among all sites.

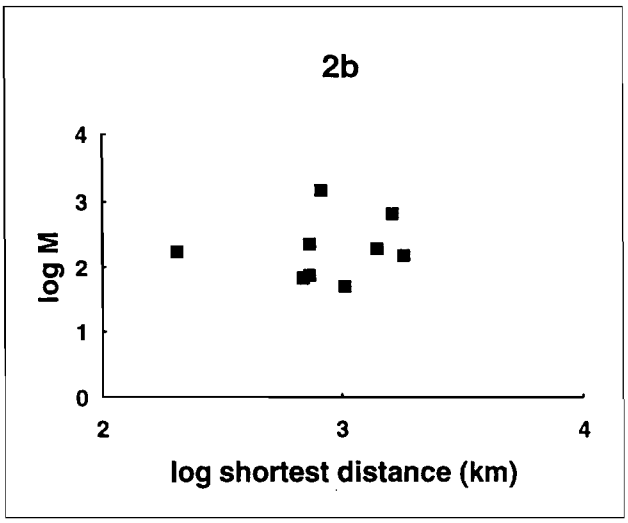
FIG. 3. UPGMA reconstruction of the relationships among populations based on Linearized F_{ST} values using a) intron allele frequencies and b) microsatellite allele frequencies. Branches in bold are significantly greater than 0 at α = 0.05.

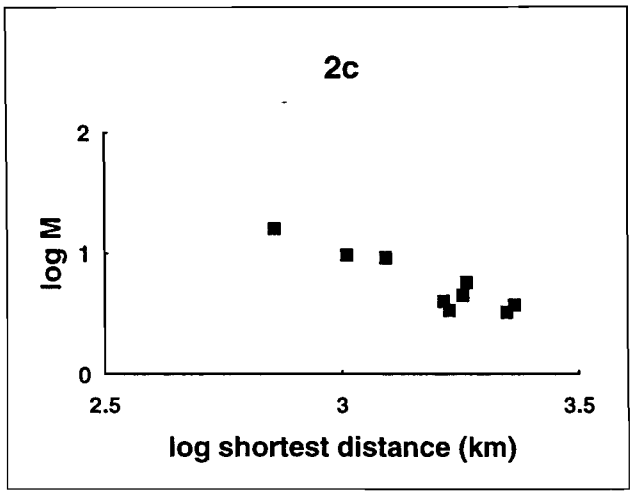
FIG. 4. Mismatch distributions of pairwise sequence differences among individuals for a) all populations and all intron loci combined, b) all population and loci with mutation rates from 0.35-0.65% Ma^{-1} c) for Aleutian Island populations and loci with mutation rates from >0.6% Ma^{-1}.

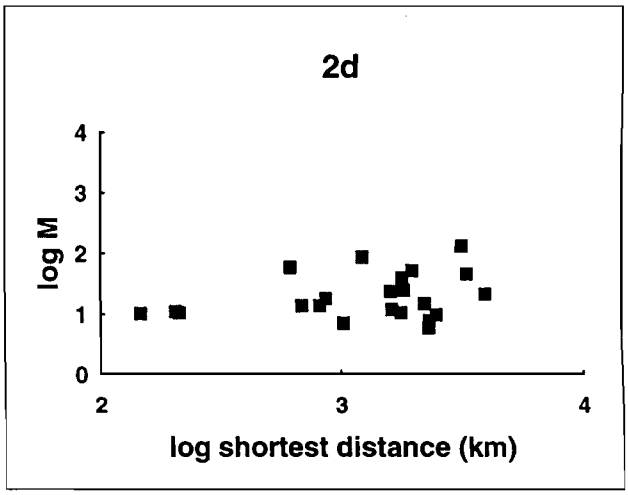
FIG. 5. Hedrick's-U statistic as an index of the relative direction and magnitude of gene flow among South-Eastern to Peninsula, and Peninsula to Aleutian Island populations. U-values were obtained using intron (Uint) and microsatellite genotypes (Umic) respectively.


Bering Sea
Prince William Sound (n=10)
Kachemak Bay (n=12)
Alexander Archipelago (n=20)
Kodiak Archipelago (n=13)
Attu Is. (n=9)
Belkofski Bay (n=11)
Mitrofania Bay (n=10)
British Columbia (n=30)
Adak Is. (n=5)
Pacific Ocean
0
500
1000
kilometers

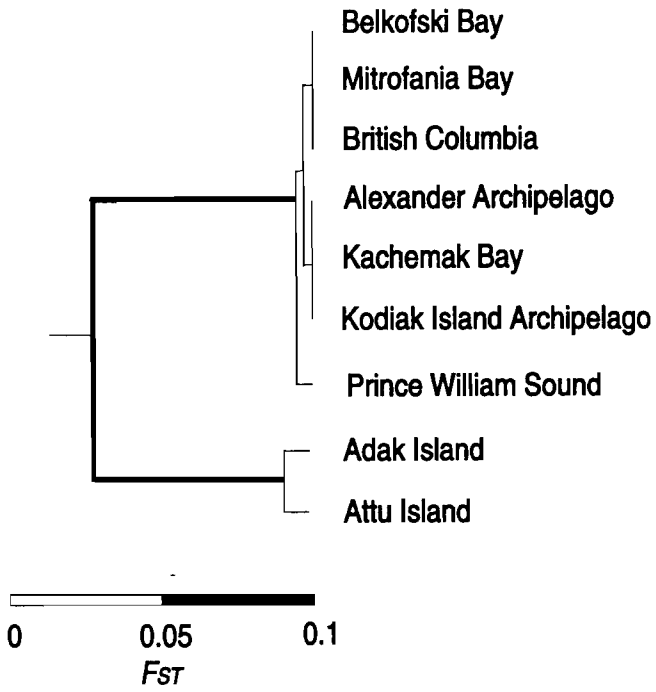

2a
log M
4
3
2
1
0
2
3
4
log shortest distance (km)


2b
log M
4
3
2
1
0
2
3
4
log shortest distance (km)

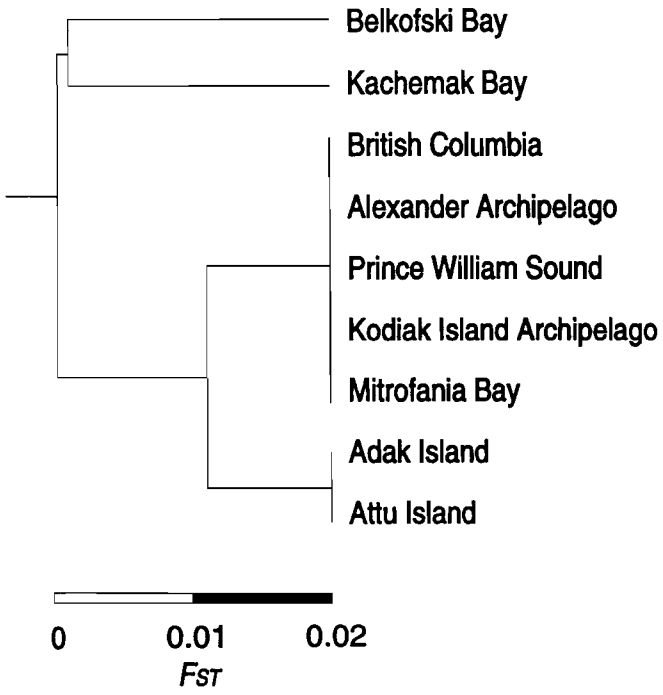

2c
log M
2
1
0
2.5
3
3.5
log shortest distance (km)

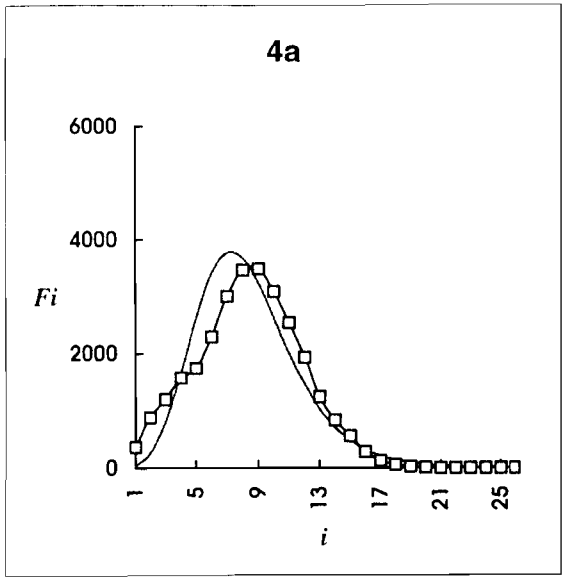

2d
log M
4
3
2
1
0
2
3
4
log shortest distance (km)

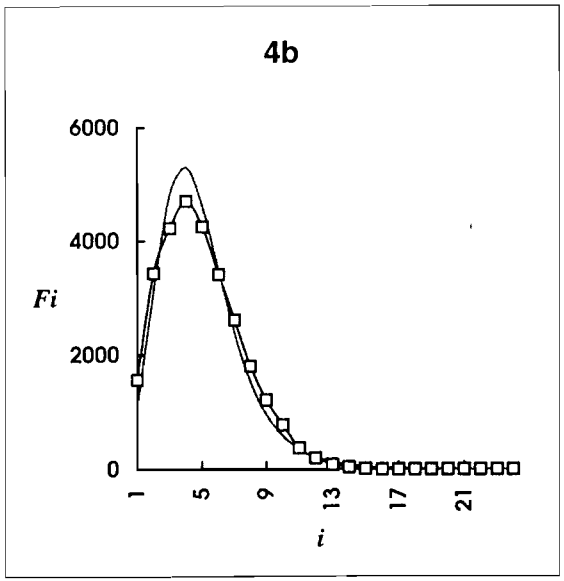
3a

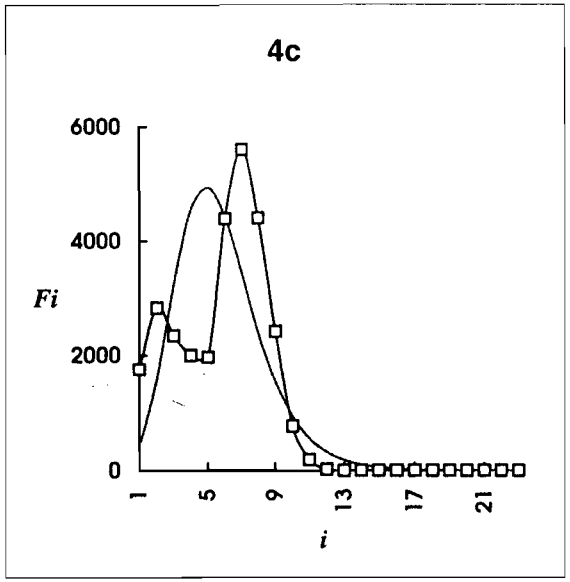

Belkofski Bay
Mitrofania Bay
British Columbia
Alexander Archipelago
Kachemak Bay
Kodiak Island Archipelago
Prince William Sound
Adak Island
Attu Island
0
0.05
0.1
FST

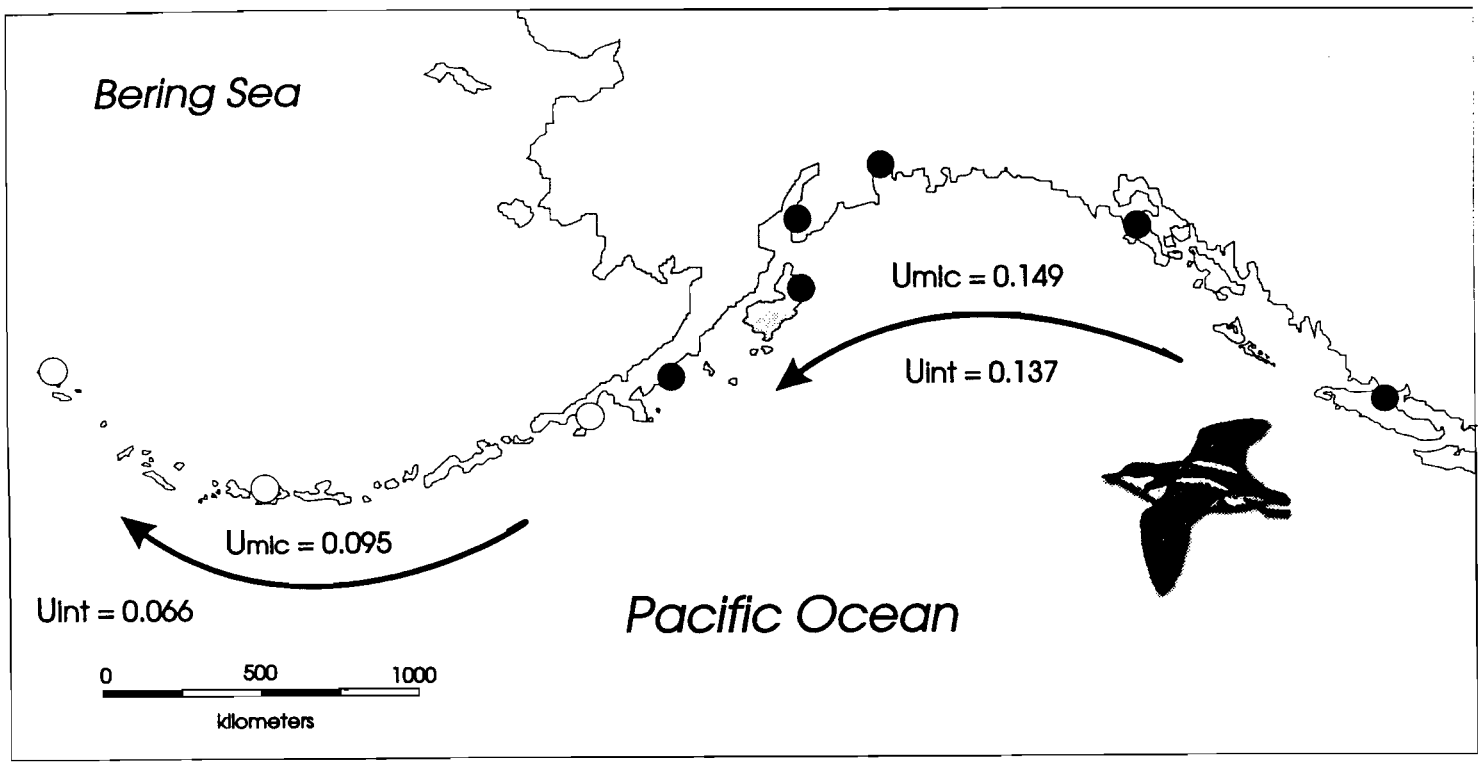
3b


Belkofski Bay
Kachemak Bay
British Columbia
Alexander Archipelago
Prince William Sound
Kodiak Island Archipelago
Mitrofania Bay
Adak Island
Attu Island
0
0.01
0.02
FST


4a
6000
4000
2000
0
Fi
1
5
9
13
17
21
25
i


4b
6000
4000
2000
0
Fi
1
5
9
13
17
21
i


4c
6000
4000
2000
0
Fi
1
5
9
13
17
21
i


Bering Sea
Umic = 0.149
Uint = 0.137
Umic = 0.095
Uint = 0.066
Pacific Ocean
0
500
1000
kilometers

A conservation genetic study of common murres (*Uria aalge*) in the *Exxon Valdez* spill area through comparison of mitochondrial control region and cytochrome *b* sequences

Anoma Patirana

A thesis submitted to the Department of Biology
in partial fulfillment of the requirements for
the degree of Bachelor of Science (Honours)

Department of Biology,
Queen's University, Kingston, Canada
March 23, 1998

ABSTRACT

Post spill monitoring of areas affected by the *Exxon Valdez* oil spill indicates that colonies of common murres (*Uria aalge*) from the Gulf of Alaska are failing to recover to pre-spill sizes. Design of an appropriate conservation strategy for the restoration of severely affected colonies requires information on the extent of genetic structuring of such populations. In this study, genetic differentiation and gene flow between affected populations in the Gulf of Alaska and surrounding areas were quantified using highly variable genetic markers: the mitochondrial control region, and cytochrome b gene. Genetic variation was assayed by SSCP analysis and direct sequencing of control region and cytochrome b haplotypes. Significant differences in 3' end of the control region and cytochrome b genotype frequencies among colonies, and non-random segregation of sequence variation for the 5' end of the control region provided evidence for genetic structuring among murre populations in the spill area and surrounding regions. Pair-wise analysis revealed significant genetic differentiation and isolation of colonies from East Amatuli Island from those in Chisik Island and Kachemack Bay, indicating low probability of natural re-colonization of these colonies. No significant genetic differentiation was found among colonies grouped into regions (Cook Inlet, the Semidi and Eastern and Western Aleutian Islands) although some evidence was found for possible isolation of murres from the Western Aleutians from those farther east . Phenetic analysis revealed no geographic sequestering of related haplotypes. However, a dichotomy of 5' end of the control region and cytochrome b haplotypes suggested that murres may have survived the last glaciations in two separate refugia. Estimates of N_em showed widespread gene flow among colonies and regions, indicating that failure of affected murre populations to recover from the *Exxon* spill may not be attributable to genetic isolation.

ACKNOWLEDGEMENTS

I would like to express my gratitude to my supervisor, Dr. Vicki Friesen for use of her laboratory facilities and more importantly, for providing me with the opportunity to conduct research at this level. Her patience and guidance in overseeing my work over the past few months is most sincerely appreciated. I would also like to express my gratitude to J. Piatt, J. Pitocchelli, T Van Pelt and G.V. Byrd for collecting tissue samples used in this study. I am also indebted to Tim Birt, Brad Congdon, Karen Holder, Hollie Walsh and Gabriela Ibarguchi for their invaluable advice and assistance. Technical assistance and moral support from the following fellow lab-mates are also acknowledged: Deb Harrison, Kathy Kennedy, Lisa Veit, Jeff Moy, Heather Jones, Vinay Lodha, Shane Doran and Jesse Wood. Funding for this project was supported by a grant from the *Exxon Valdez* Oil Spill (EVOS) Trustee Council awarded to Dr. Vicki Friesen, for which I am grateful.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION AND LITERATURE REVIEW

Background of problem

Oil contamination of the marine environment is a serious threat to many seabirds. Beginning early this century, oil pollution at sea from routine low level discharges from vessels and tanker accidents has resulted in the deaths of hundreds of thousands of seabirds (Butler, 1988). One incident that caused substantial seabird mortality was the *Exxon Valdez* oil spill in 1989, which occurred when the vessel ran aground on Bligh reef in Prince William Sound, Alaska, releasing 41 million litres of crude oil into the marine ecosystem (Piatt, 1990; Weins, 1996). More than 300,000 seabirds were killed by the spill and 30,000 oiled carcasses were retrieved in the initial months following the spill (Weins, 1996). Because the Gulf of Alaska, including Prince William Sound and Cook Inlet, hosts some of the world's largest populations of seabirds, approximately one million marine birds lived in the affected regions (Piatt et al., 1990). Common murres (*Uria aalge*) were among the most severely affected species and constituted 74% of the initial deaths (Parrish and Boersma,1995). Several years following this incident, post-spill monitoring indicates that murre populations in the affected areas are failing to recover to pre-spill levels. In addition, since many seabirds killed by the spill were migrating, the geographic range of populations affected by the spill is unknown. Information on the genetic structuring and population dynamics of murre colonies in the affected areas is

critical both for the restoration of severely affected colonies and for the successful management of the species as a whole.

Common murres in the Gulf of Alaska

Common murres (Charadariiformes: Alcidae) are colonial seabirds that breed along sub-arctic and temperate coastlines throughout the Northern Hemisphere. The breeding range of common murres extends from California through Alaska and the Aleutian Islands to Japan (Tuck, 1960). Murre colonies, which can range in size from several thousand to over one million breeding individuals, occur on cliffs and offshore islands, which provide safe breeding areas from mammalian predators (Nettleship and Birkhead, 1985). Murres tend to nest on open ledges on steep cliffs and stacks and lay their eggs on bare rock (Tuck, 1960). Despite the fact that they have low reproductive rates (having a clutch size of one), murres are the most abundant seabirds in the Northern Hemisphere (Tuck, 1960). However, in recent decades murre populations have become threatened due to human modification of their habitats by offshore oil drilling and commercial fisheries developments (Birkhead and Nettleship, 1980). Atlantic populations of murres have declined by more than 20-30 percent due to numerous factors, including mortality from oil contamination, drowning in gill nets, hunting and egging (Friesen, 1997). Due to their aquatic habitats and the large amount of time spent on the sea surface (at the air-sea-interface) murres are particularly vulnerable to contamination from oil spills. After contact with oil, birds

may die due to loss of thermoregulatory ability or toxicological effects following ingestion during preening (Fry and Lowenstine, 1985; Piatt et al., 1990).

Oil spills may affect seabird populations in several ways. For example, the loss of individual birds due to direct mortality or emigration from the spill area may alter either population size or structure, and may affect the reproductive performance of remaining birds (Weins, 1996). In the case of the *Exxon Valdez* spill, information about the origin of murres killed by the spill is largely lacking. Thus, despite the suspicion that particular colonies such as those on Barren Island at the mouth of Cook Inlet were severely affected, the geographic range of populations affected by the spill is unknown and may be much larger than speculated (Piatt, 1989). Since many seabirds killed by the Exxon spill may have been migrating, populations that require restoration may be geographically distant from the spill site. Genetic data may enable the identification of geographic limits of populations of common murres killed by the spill.

Importance of genetic information to conservation of murres

A fundamental goal of conservation biology is to preserve genetic diversity and to maintain the evolutionary process. Developing effective management plans for subpopulations of murres affected by the spill requires knowledge about specific genetic and demographic characteristics of such populations. In particular, information about the levels of genetic variability within each population, the extent of migration, and evolutionary relationships between populations is essential in determining the focus of conservation efforts. For example, genetic data can provide measurements of rates of immigration into

and emigration out of colonies, and thus enable the identification of 'source' and 'sink' populations (Friesen, 1997). A 'source' population is a colony of birds that has high productivity, thus serving as a net exporter of individuals (Friesen, 1997). A 'sink' population on the other hand, is one in which productivity is less than mortality. If colonies affected by the spill represent sources their restoration will be critical. Conversely, restoration of sink populations may be futile in the recovery of the species as a whole and may even accelerate extinction.

Genetic data also enable the identification of evolutionary significant units (ESU's) - sets of populations that have a distinct, long term evolutionary history that contribute substantially to the overall genetic diversity of a species (Mortiz, 1994). Identification of ESU's and genetically distinct colonies enables the prioritization of conservation efforts. In addition, genetic data can lead to the identification of cryptic species, which, although phenotypically similar, are genetically distinct. Identification of genetically isolated populations and/or cryptic species or subspecies would have important implications to a species' management. Specifically, such data enable the designation of management units (MU)- populations that have significant divergence of allele frequencies and that exchange so few migrants as to be considered genetically distinct (Moritz, 1994). Genetic studies of threatened species such as logger-head turtles *Caretta caretta* (Bowen et al., 1995), humpback whales *Megaptera novaengliae* (Palumbi and Baker, 1994), harbour seals *Phoca vitulina* (Stanley et al., 1996) and stellar sea lions *Eumetopias jubatus* (Bickham et al, 1996) all revealed strong population subdivisions requiring independent management. A similar finding of

genetic distinctiveness of murre colonies in the spill and surrounding areas would necessitate independent management of such populations for preservation of the species' diversity.

Gene flow (N_em) in individuals per generation, is the product of migration rate (m) and effective population size N_e (Hudson et al., 1992). Estimates of gene flow within and among murre populations in the spill and surrounding areas can offer an indication of the degree of genetic isolation of individual populations. For example, Friesen (1997) asserted that if gene flow among colonies of seabirds is restricted, then populations may essentially consist of individual colonies. However, if gene flow is more widespread, then populations can be considered to be panmictic (to include all members of a region or species). Either scenario could have important implications for the management of the species. For example, if populations of murres display widespread gene flow, re-colonization of affected colonies may occur within a few generations. Conversely, a finding of limited gene flow among populations may imply low probability of natural re-colonization of affected colonies.

From a theoretical perspective, low genetic variability in declining populations due to the loss of rare alleles and reduction in individual heterozygosity is harmful, because it decreases the ability of a population to cope with environmental perturbations (Lande, 1988). Thus, if individual murre colonies in the affected area are genetically differentiated, extirpation of a particular colony may result in a reduction of the overall genetic diversity of the species.

Genetic analyses of murre populations using genetic markers such as the mitochondrial control region and cytochrome *b* gene can be used to assess levels of gene flow among populations, genetic variability, and conservation value of specific colonies in terms of their genetic uniqueness. This information can be applied to restoration efforts by allowing the delineation of geographic limits of affected populations, identification of source and sink populations and identification of cryptic species and/or subspecies.

Nature of mitochondrial DNA

Due to a combination of intrinsic and technical features, mitochondrial DNA (mtDNA) has become a powerful tool in evolutionary and conservation biology studies (Wilson et al., 1985; Quinn, 1997). The utility of mtDNA in these areas of biology derives from its maternal mode of inheritance and haploidy, and smaller (¼) effective population size compared to nuclear genes (Wilson et al., 1985). Given these facts, mitochondrial DNA is more sensitive to effects of genetic drift, population bottlenecks, founder effects and restricted gene-flow (Brown et al., 1982; Wilson et al., 1985). MtDNA is also easily characterized due to its small genome size and lack of complicating features such as repetitive sequences or introns (Wilson et al., 1985). At the sequence level, mitochondrial DNA has been shown to evolve about 5-10 times faster than nuclear DNA, having an average sequence divergence rate of 2% per million years (Brown et al., 1979). Possible factors responsible for the higher mutation rate of mtDNA include an error-prone replication system, less efficient repair mechanisms and a

high turnover rate (Brown et al, 1982). A lack of recombination further enhances the attractiveness of this molecule both for studies of intraspecific sequence divergence, and for construction of gene trees within and among related species (Wilson et al., 1985).

Most importantly, different segments of the mitochondrial genome evolve at different rates, thus enabling the selection of an appropriate region for the resolution of phylogenetic relationships within or among taxa (Wenink et al., 1994). For example, slowly evolving cytochrome b sequences and ribosomal RNA genes have been valuable in reconstructing deep branches in the tree of life, whereas rapidly evolving mitochondrial DNA sequences such as the control region have revolutionized analyses of relationships among animals at the intraspecific level (Avise, 1994).

Mitochondrial control region and cytochrome b

The vertebrate mitochondrial genome is comprised of 13 protein - encoding genes, 22 tRNA genes, 2 rRNA genes and a control region and is inherited as a single genetic unit (Quinn, 1997). The advent of molecular tools such as the polymerase chain reaction (PCR) and direct sequencing have enabled researchers to determine nucleotide sequences of mitochondrial genes, thus allowing the detection and interpretation of intraspecific and interspecific genetic variation in natural populations (Avise, 1994). Since functional constraints vary over different segments of the mitochondrial genome, the rates of evolutionary change of mitochondrial genes vary as well. For example, the

cytochrome b gene, which is a protein of complex III of the mitochondrial oxidative phosphorylation system, evolves at a rate 10 times slower than the mitochondrial control region (Irwin et al., 1991). Because of its protein encoding nature, cytochrome b is relatively conserved and has been useful in providing phylogenetic resolution for both mammalian and avian taxa with divergence times of over one million years (Baker et al., 1994). In addition, the mitochondrially encoded cytochrome b gene has been well characterized in numerous avian taxa, and thus numerous universal primers are available for amplification of different segments (Moore and Defilippis, 1997).

Conversely, the mitochondrial control region is of particular use in tracing phylogeographic histories and population genetic structures of closely related lineages. The control region contains the heavy (H)-strand origin of replication as well as promoters for both heavy and light strand transcription (Hoelzel, 1993). It is regarded as the most rapidly diverging region of the mtDNA molecule (due to a lack of coding constraints), and evolves three to five times faster than any other mitochondrial gene (Quinn and Wilson, 1993). The avian control region is flanked by the genes for $tRNA^{phe}$ and $tRNA^{glu}$, and can be divided into three sub-regions: the hypervariable domains I and III (3' and 5' ends respectively) and a more conserved central domain II (Baker and Marshall, 1997). Analysis of mitochondrial control region variation has proved to be much more effective than allozymes and/or restriction fragment length polymorphisms (RFLP) in elucidating existing patterns of population level differentiation among a variety of vertebrate populations. For example, regional population structuring has been

demonstrated using hypervariable control region sequences in the dunlin *Calidris alpina* (Weinink et al., 1993), green-finch *Carduelis chloris* (Merila et al., 1997), sea-lion (Bickham et al., 1996), harbour seal (Stanley et al., 1996) and hump-back whale (Palumbi et al., 1994).

Study objectives

The purpose of this study was to investigate levels of genetic differentiation and rates of historical and contemporary gene flow (number of migrants) among common murre colonies in the *Exxon Valdez* spill area and neighbouring regions, using two hypervariable segments of the mitochondrial control region and part of the cytochrome b gene. A further goal was to assess patterns of differentiation and levels of gene flow among four geographically distant regions (Cook Inlet, the Semidi and Eastern and Western Aleutian Islands) for the purpose of examining levels of isolation and geographic structuring.

MATERIALS AND METHODS

Sampled populations

Tissue samples were obtained from 115 common murres between 1991 and 1996 from 13 populations ranging from the Western Aleutian Islands to the Gulf of Alaska (Figure 1). Samples from the 13 sites used in population analyses included four regions: Cook Inlet (spill area), the Semidi Islands, Eastern Aleutian Islands and Western Aleutian Islands (Table 1).

DNA extraction

DNA was extracted from tissue by protease digestion and phenol-chloroform extraction protocol (Friesen et al., 1997). Briefly, 0.5 g of ground tissue was incubated overnight at 65°C in 750 µl lysis buffer containing 100 mM Tris-Cl (pH 8.0), 10mM ethylenediaminetetra-acetic acid (EDTA; pH 8.0), 100 mM NaCl, 1% sodium dodecyl sulphate (SDS) and 1 µg proteinase K. Samples were mixed with 750 µl Tris saturated phenol (pH = 8.0) and centrifuged to separate phenol from template. The phenol extraction was repeated and the supernatant was mixed with 750 µl 24:1 (v/v) chloroform: isoamyl alcohol and centrifuged to remove phenol and chloroform residues. Prior to amplification, samples were diluted 1:10 with water.

Figure 1: **Map showing collection sites of common murre samples from the Gulf of Alaska and Aleutian Islands. The grouped localities from west to east are Western Aleutian Islands, Eastern Aleutians Islands, Semidi Islands and Cook Inlet (spill area).**

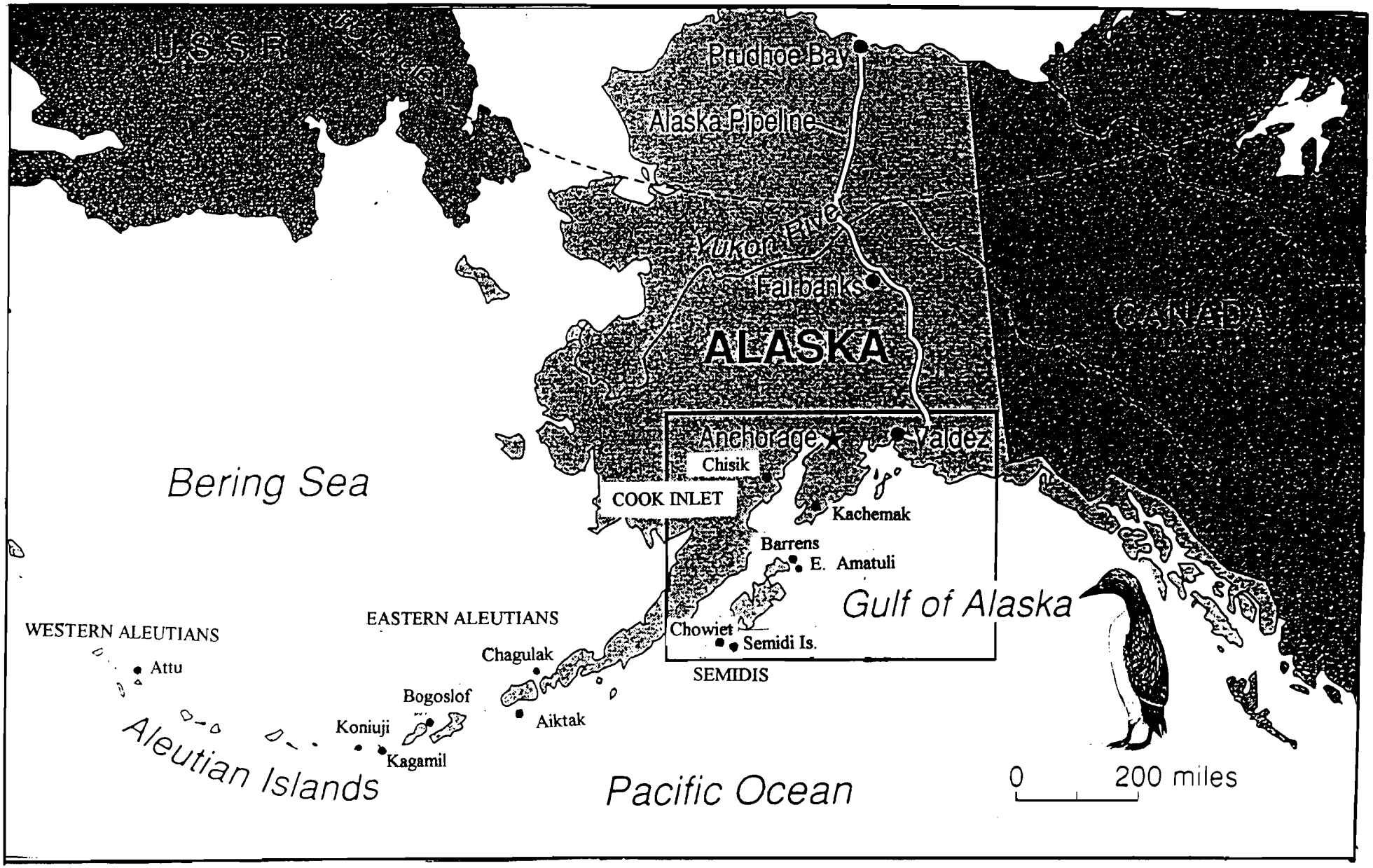

U.S.S.R.
Prudhoe Bay
Alaska Pipeline
Yukon River
Fairbanks
ALASKA
CANADA
Anchorage
Valdez
Chisik
COOK INLET
Kachemak
Barrens
E. Amatuli
Bering Sea
Gulf of Alaska
WESTERN ALEUTIANS
EASTERN ALEUTIANS
Attu
Chagulak
Bogoslof
Koniuji
Kagamil
Aiktak
Chowiet
Semidi Is.
SEMIDIS
Aleutian Islands
Pacific Ocean
0
200 miles

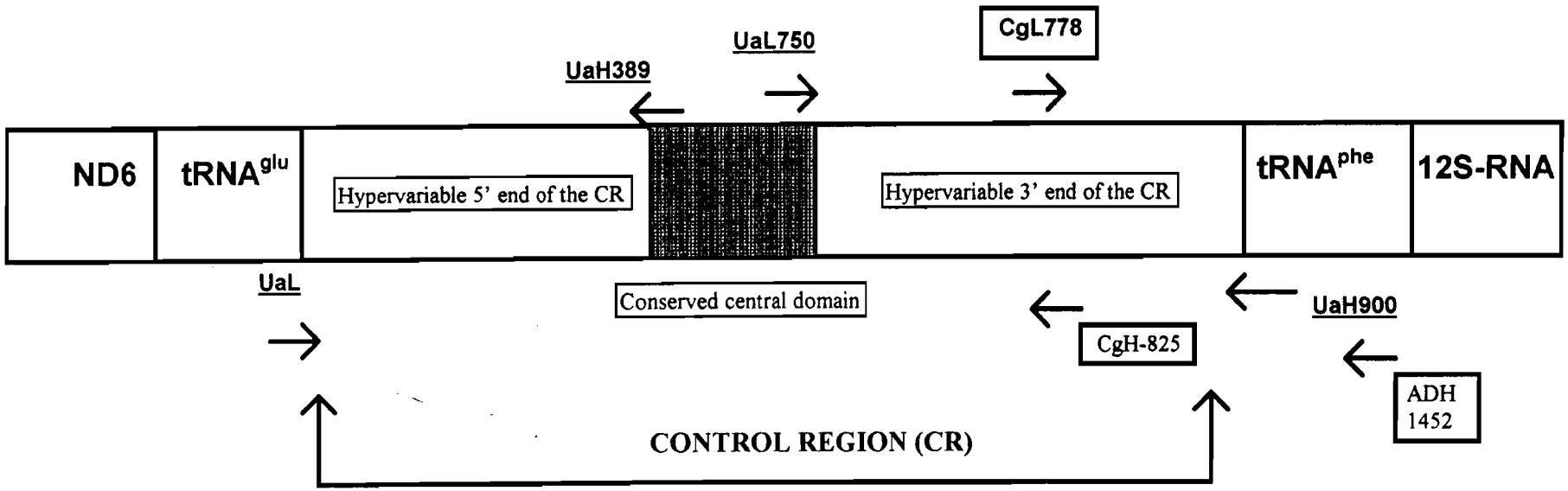
Table 1: Regional grouping of colonies used in statistical analyses and their sample sizes

REGION	*COLONY*	*SAMPLE SIZE*
COOK INLET	Barren Islands (n=8) Chisik Island (n=20) Kachemak bluff point (n=16) Homer Bluffs (n=11) E. Amatuli Island (n =10)	65
THE SEMIDIS	Chagulak Island (n =1) Semidi Island (n=1) Chowiet (n=5)	7
EASTERN ALEUTIANS	Koniuji (n=2) Kagamil Island (n=2) Bogoslof Island (n=4) Aiktak Island (n=13)	21
WESTERN ALEUTIANS	Attu Island (n=9)	9

Amplification of mtDNA

For both the control region and cytochrome b gene, 1 μl of diluted DNA was amplified in a 25 μl volume containing 10 mM Tris (pH 8.3 and pH 8.8), 50 mM KCl, 3.5 mM $MgCl_2$, 1.6 μM bovine serum albumin (BSA), 1% gelatin, 0.2 mM each of the four deoxynucleotide triphosphates (dNTP's), 0.4 μM each of two primers, and 0.5 units of *Thermus aquaticus* (Taq) DNA polymerase (Boehringer-Manneheim Canada; Laval, Quebec) in a PTC™ thermal cycler with a hot bonnet™. The two segments of the control region (I and III) and cytochrome b gene were amplified using the following temperature profile: denaturation at 94 ^{0} C for 90 seconds followed by 30 cycles of denaturation at 94 ^{0}C for 30 s, annealing at 55 ^{0} C for 30 s, extension at 72 ^{0} C for 45 s, and ending with an extension at 72^0 C for 3 minutes (Friesen et al., 1997). The control region was sequenced for six murres using the murre-specific forward primer UaL located near the 5'end of the control region and universal reverse primer ADH 1452 situated in the $tRNA^{phe}$ 3' to the control region (Figure 2). Double-stranded sequencing was conducted with Amplicycle ® cycle sequencing kits (Applied Biosystems) following the manufacturere's suggested protocol. Using these sequences, primers specific for common murres were designed to amplify the 5' and 3' hypervariable control region segments (Figure 2). Specifically, the primers used to amplify the 5' end of the control region were the forward primer UaL and the reverse internal primer UaH389. The 3' end of the control region was amplified using the internal forward primer UaL750 and reverse internal primer

Figure 2: **Diagram of the primers used to amplify the 5' and 3' ends of the mtDNA control region of common murres (underlined). Boxed arrows represent primers that were used to derive common murre specific primers for selective amplification of the hypervariable 3' and 5' ends of the control region. H and L refer to heavy and light DNA strands, respectively.**


UaH389
UaL750
CgL778
ND6
tRNA glu
Hypervariable 5' end of the CR
Hypervariable 3' end of the CR
tRNA phe
12S-RNA
UaL
Conserved central domain
UaH900
CgH-825
ADH 1452
CONTROL REGION (CR)

UaH900, located at the beginning of a microsatellite-type repeat at the 3' end of the control region (Figure 2). To amplify a 307 bp internal fragment of the cytochrome b gene, the primers L14841 and H15149 were used (Kocher et al., 1989). Amplified DNA segments were subjected to electrophoresis in 2% agarose gels with 1X TA buffer (0.04 M Tris-acetate, pH 7.6) and visualized under UV light in the presence of eithidium-bromide. All PCR products were run with negative controls to check for possible contamination of DNA. Bands in agarose gels were harvested and the DNA was extracted using the Gene Clean II kit (Bio 101 Inc.) according to the manufacturer's instructions.

SSCP analysis

Variation in the 5' and 3' ends of the control region and cytochrome b gene was assayed using single-stranded conformational polymorphisms, which provides a relatively easy method of mutational screening by comparing the secondary structures of single stranded DNA (Lessa and Applebaum; Friesen et al, 1997). For SSCP analysis, the 5' and 3' ends of the control and cytochrome b genes were amplified by PCR with direct incorporation of $\alpha^{33}P$, denatured by heating to 94 ^{0} C for 3 minutes, snap-cooled on ice and subjected to electrophoresis through non-denaturing polyacrylamide gels (MDE - J.T. Baker). The resulting products were visualized by autoradiography and the genotype for each individual was scored manually, based on the banding profile.

Sequence analysis of haplotypes

To determine the nature of variation in the 5' end of the control region segment and the cytochrome b segment, a representative of each haplotype was sequenced using standard protocols (Appendices I and II). In short, double stranded DNA was sequenced directly with the dideoxynucleotide chain-termination method (Sanger et al., 1977) using the Thermo Sequenase ™ radiolabelled terminator cycle sequencing kit (Amersham) according to the recommendations of the manufacturer. The 5' end of the control region and cytochrome b gene were sequenced with the forward primers UaL and L14841 respectively. The sequencing reaction products were electrophoretically separated in 5% polyacrylamide gels and visualized by autoradiaography. Nucleotide sequences for the 5' control region and cytochrome b were aligned manually using ESEE (Cabot and Beckenbach, 1989) and compared for base-pair differences using MEGA (Kumar et al., 1993).

Population genetic and phylogenetic analyses

Four different statistics were used to assess the degree of differentiation among populations and regions. Due to small sample sizes, five of the 13 populations (Chagulak, n=1; Koniuji, n=2; Kagamil n=2; Bogoslof, n=2; and Semidi Island, n=1) were excluded from these analyses. Genotype frequencies for cytochrome b, and the 5' and 3' ends of the control region were compared among colonies and regions using Chi-square (x^2) tests. Among-population and

among-regional genotypic variation was indexed using G_{st} using the program Rand-G_{st} (A. Lynch, unpublished program). Mutational divergence among genotypes was indexed using γ - the probability that any two randomly chosen individuals from different populations or regions will have different genotypes (Friesen et al., 1996a; A. Lynch, unpublished program). The program WINAMOVA (vers. 1.04; Excoffier 1992) was used to calculate ϕst - the extent to which sequence variation is partitioned among populations and regions. The significance of Gst, γ and ϕst was tested by randomization (1000 permutations). Gene flow (N_em, females per generation) was determined from G_{st} and ϕ_{st} values using the equation $N_em = \frac{1}{2}(1/G_{st} - 1)$ or $N_em = 1/2(1/\phi_{st} - 1)$, where N_e is the female effective population size and m is the female migration rate (Hudson et al., 1992).

Maximum parsimony analyses of sequences of the 5' end of the control region and cytochrome b were conducted on PAUP (version 3.1.1; Swofford, 1993) using the heuristic search algorithm with midpoint rooting and no specified outgroup. Neighbor-joining trees using Jukes-Cantor distances (Jukes and Cantor, 1969) were derived for sequences of the 5' end of the control region and cytochrome b using the program MEGA (Kimura et al., 1993).

RESULTS

Nucleotide sequences

Sequence analysis of a 365 bp segment of the 5' end of the control region of mt DNA revealed 32 haplotypes defined by 53 variable sites (of which 33 sites were phylogenetically informative) among 101 common murres sampled from Cook Inlet and the Semidi, Eastern Aleutian and Western Aleutian Islands (Tables 2 and 3). Of the 101 sampled common murres, 35 possessed genotype Ua24 and 30 had type Ua20; all other genotypes occurred in only one or two individuals each. Most genotypes differed by an average sequence divergence of 0.2%,with the exception of genotype Ua30, whose sequence diverged by approximately 8.7% (or 30 substitutions) from the two most common genotypes.

For a 307 bp cytochrome b fragment, four haplotypes defined by 21 variable sites (18 of these being phylogenetically informative) were identified (Tables 4 and 5). Seventy percent of the base substitutions were transitions (A→G; T→C) and the remaining 30% were transversions (ratio 2.2:1). Ninety-three of 102 individuals possessed haplotype BB, two held D and one each displayed haplotypes E and F. The sequences of haplotypes BB and F, and D and E diverged from each other by 0.9% and 0.6% respectively. However, the average sequence difference between the two groups of haplotypes (e.g BB/F and D/E) was 6%.

Table 2: **Frequency of genotypes for the 5' end of the control region for colonies and regions from which common murres were sampled.**

REGION	COLONY	GENOTYPE																																					SAMPLE SIZE
		Ua#....																																						
		1	2	3	4	5	6	7	8	9	11	12	13	14	15	16	17	18	19	20	21	22	24	25	26	27	29	30	31	32	34	35	36	37							
Cook Inlet	Barrens	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	3	0	0	0	0	0	0	0	1	0	1	0							8
	Chisik	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	7	0	1	9	0	0	0	1	0	0	0	0	0	0	1							20
	Bluff point	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	7	0	0	7	0	0	0	0	0	0	0	0	0	0	0							16
	Homer	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	3	0	0	2	0	0	1	0	0	0	1	0	0	0	0							10
	E. Amatuli	1	0	0	1	0	0	0	0	0	0	0	0	0	1	0	1	0	0	3	0	0	2	0	1	0	0	0	0	0	0	0	0	0							10
Semidis	Chagulak	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0							1
	Semidi Is.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0							1
	Chowiet	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0							5
Eastern Aleutians	Koniuji	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0							2
	Kagamil	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0							2
	Bogoslof	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0							4
	Aiktak	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	1	0	0	2	0	0	7	0	0	0	0	0	1	0	0	0	0	0							13
Western Aleutians	Attu Is.	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	4	0	0	3	0	0	0	0	0	0	0	0	0	0	0							9

Table 3: **Variable positions within a 365-bp segment of the 5' end of the control region among 101 common murres. Dots indicate identity with genotype Ua1.**

	NUCLEOTIDE POSITION					
	1111111	2222233333	8899999900	11 1111111111	1111111222	223
	1352456789	0234645689	8904568927	0137935867	3122302610	325
#ua1	CTGTCACACT	TCCCTGACTC	CCCTCCGTTT	CACACACTGG	ACCCTAAGGA	GCC
#ua2	........T.	....	....	...G...C..	..-.......	...
#ua3	.........-	.........-	....	.......C..	G.........	...
#ua5	....	....	....	......TC..	G.........	...
#ua6	....	....	....	...G...C..	....	...
#ua7	....	....	....	....	G.-.......	...
#ua8	....	....	....	T......C..	G.-.......	...
#ua9	....	....	....	....T..C..	..-.......	...
#ua11	....	....	....	.......C..	G.........	...
#ua12	....	....	....	.....G.C..	G.........	...
#ua13	....	........C.	.T........	........CA.	G.........	...
#ua14	....	....	....	.......C..	G.-.....C.	...
#ua15	..A-.....A	....	T....TA.AC	..T....A.T	..ATC.GA.G	...
#ua16	..ACACA..A	..T.......	.....TACAC	..T....A.T	..ATC.GA.G	...
#ua17	..A-.....A	.TT.......	T....TACAC	..T....A.T	..ATCG.A.G	...
#ua18	..A-.....A	..T.......	T....TACAC	..T....A.T	GA-TC..A.G	...
#ua19	....	.....AGACT	....	.G.....C..	....	...
#ua20	....	....	....	.......C..	....	...
#ua24	....	....	....	.......C..	G.-.......	...
#ua25	....	....	....	.......C..	G.........	.T.
#ua26	....	....	....	.......C..	G......A..	...
#ua27	....	.T........	....	.......C..	....	...
#ua28	....	....	....	T......A..	G..T......	...
#ua29	....	....	....	.......C..	G.........	.TA
#ua30	ACACACACAC	CT.TC.....	TT..TTACAC	.......A.T	G.ATC..A.C	C..
#ua31	....	....	..T.......	.......C..	....	...
#ua32	....	....	...C......	.......C..	G.........	...
#ua33	....	....	....	T......C..	....	...
#ua34	....	.........T	....	.......C..	....	...
#ua35	....	.........-	....	.......C..	....	...
#ua36	.CA......A	C.T.......	TT..TTACAC	.......A.T	G.ATC....G	...
#ua37	....	....	T.........	.......C..	G.........	...

Table 4: **Frequency of cytochrome b genotypes for colonies and regions from which common murres were sampled**

REGION	*COLONY*	*GENOTYPE*				*SAMPLE SIZE*
		BB	*D*	*E*	*F*	
Cook Inlet	Barrens	7	1	0	0	8
	Chisik	20	0	0	0	20
	Bluff point	16	0	0	0	16
	Homer	11	0	0	0	11
	E. Amatuli	8	1	0	1	10
Semidis	Chagulak	0	0	1	0	1
	Semidi Is.	1	0	0	0	1
	Chowiet	5	0	0	0	5
Eastern Aleutians	Koniuji	2	0	0	0	2
	Kagamil	2	0	0	0	2
	Bogoslof	4	0	0	0	4
	Aiktak	13	0	0	0	13
Western Aleutians	Attu Is.	9	0	0	0	9

Table 5: **Variable positions within a 307-bp segment of cytochrome *b* among 102 common murres. Dots indicate identity with genotype BB.**

NUCLEOTIDE POSITION

Genotype	*155 925*	*566 614*	*111 023 347*	*112 390 998*	*222 222 239*	*222 467 807*	*233 900 501*
BB	TAC	CTG	CAC	ACA	CAG	CCG	ATC
D	CCA	TCA	TCT	GTG	.GA	TTA	TAT
E	CCA	TCA	T.T	GTG	.G.	TTA	TAT
F	. . .	. . .	.C.	. AG	. . .	. . .	. . .

SSCP analysis revealed 20 haplotypes in an approximately 300 bp segment of the 3' end of the control region among 102 common murres, although these have yet to be sequenced to determine the nature of the variation (Table 6).

Gene flow and genetic differentiation of colonies:

The frequency distribution of genotypes differed significantly among colonies for cytochrome b and the 3' end of the control region but not for the 5' end of the control region, indicating genetic differentiation between populations (Table 7). G_{st} and γ values indicated no significant among site variation or mutational divergence of cytochrome b and 5' end of the control region genotypes between colonies. However, ϕ_{st} indicated non-random segregation of sequence variation for the 5' end of the control region among colonies. Differences among colonies accounted for only 8% of all variation, while variation within colonies accounted for 92%. Furthermore, pair-wise comparisons of populations (1000 permuations) using WINAMOVA revealed significant genetic between E. Amatuli Island and Chisik Island (P= 0.01), between E. Amatuli Island and Bluff Point (P= 0.007), and between E. Amatuli Island and Homer Bluffs (P = 0.026). N_em estimated from Φ_{st} indicated low gene flow between these colonies ($N_em \leq 2$).

Estimates of N_em from cytochrome b ϕ_{st} values indicated high levels of gene-flow among colonies while the more rapidly evolving 5' end of the control region yielded a lower estimate of exchange of migrants per generation (N_em=6).

Table 6: **Frequency of genotypes for the 3' end of the control region for colonies and regions from which common murres were sampled**

REGION	COLONY	GENOTYPE																				SAMPLE SIZE
		C	C1	C2	C3	B	B2	D	E	F	F2	G	H	I	J	J2	K	K2	L	M	N	
Cook Inlet	Barrens	4	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0	1	8
	Chisik	15	0	0	0	2	0	1	0	0	0	1	0	0	0	0	0	0	1	0	0	20
	Bluff point	11	1	1	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16
	Homer	8	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	1	0	0	0	11
	E. Amatuli	4	0	0	0	0	0	0	2	1	0	1	1	1	0	0	0	0	0	0	0	10
Semidis	Chagulak	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
	Semidi Is.	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
	Chowiet	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	5
Eastern Aleutians	Koniuji	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	2
	Kagamil	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	2
	Bogoslof	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
	Aiktak	11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	13
Western Aleutians	Attu Is.	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9

Table 7: Statistics indicating among population components of genotypic variation (X^2;G_{st}), genotypic divergence (γ), sequence variation (Φ_{st}) and gene flow (N_em, females per generation) for Alaskan colonies of common murres determined using cytochrome *b* and mitochondrial control region haplotypes

Sample Grouping	*Statistic*	*Cytochrome b*	*3' end of the control region*	*5' end of the control region*
Individual colonies	X^2	27.07*	135.58*	185.24
	G_{st}	0.096	0.043	0.01
	γ	0.00	0.00	0.095
	Φ_{st}	0.00	N/A	0.077**
	$N_m(G_{st})$	50	11	450
	$N_m(\Phi_{st})$	∞	N/A	6
Regions	X^2	17.2	30.7	123.9
	G_{st}	0.007	0.009	0.005
	γ	0	0	0.287**
	Φ_{st}	0	N/A	0
	$N_m(G_{st})$	71	55	100
	$N_m(\Phi_{st})$	∞	N/A	∞

* = P< 0.05; ** = P<0.01

Genetic differentiation among regions:

Genotype frequencies as indicated by X^2 and G_{st} values did not differ significantly for cytochrome b or the 5' and 3' ends of the control region among Cook inlet, the Semidi Islands, Eastern Aleutian Islands or Western Aleutian Islands (Table 7). However, γ values indicated non-random segregation of 5' end of the control region genotypes among the different regions. In addition, estimates of N_em from cytochrome b and 5' end of the control region G_{st} and Φ_{st} values indicated widespread gene-flow among the different regions.

Phylogenetic analysis

Phylogenetic reconstruction using maximum parsimony and neighbor-joining methods showed similar topologies for the 5' end control region haplotypes (figure 3, a & b). Both methods of reconstruction showed two major clades for both cytochrome b and 5' end control region genotypes (figure 3 a, b and c). This suggests that murres may have survived the last glaciations in two separate refugia. Levels of sequence differences within clades I and II for 5' end of the control region and cytochrome b genotypes were low, ranging from 0.6%-0.9% respectively, compared to that found between clades (3% and 6% respectively). A lack of representation of 5' end control region genotypes of clade I among individuals in the Western Aleutians (compare Table 2 and Figure 3 a & b) indicates possible isolation of murre populations in this region from ones farther east.

Figure 3 a: **Consensus of 981 shortest trees obtained by maximum parsimony analysis with mid-point rooting of haplotypes for the 5' end of the control region of common murres.**

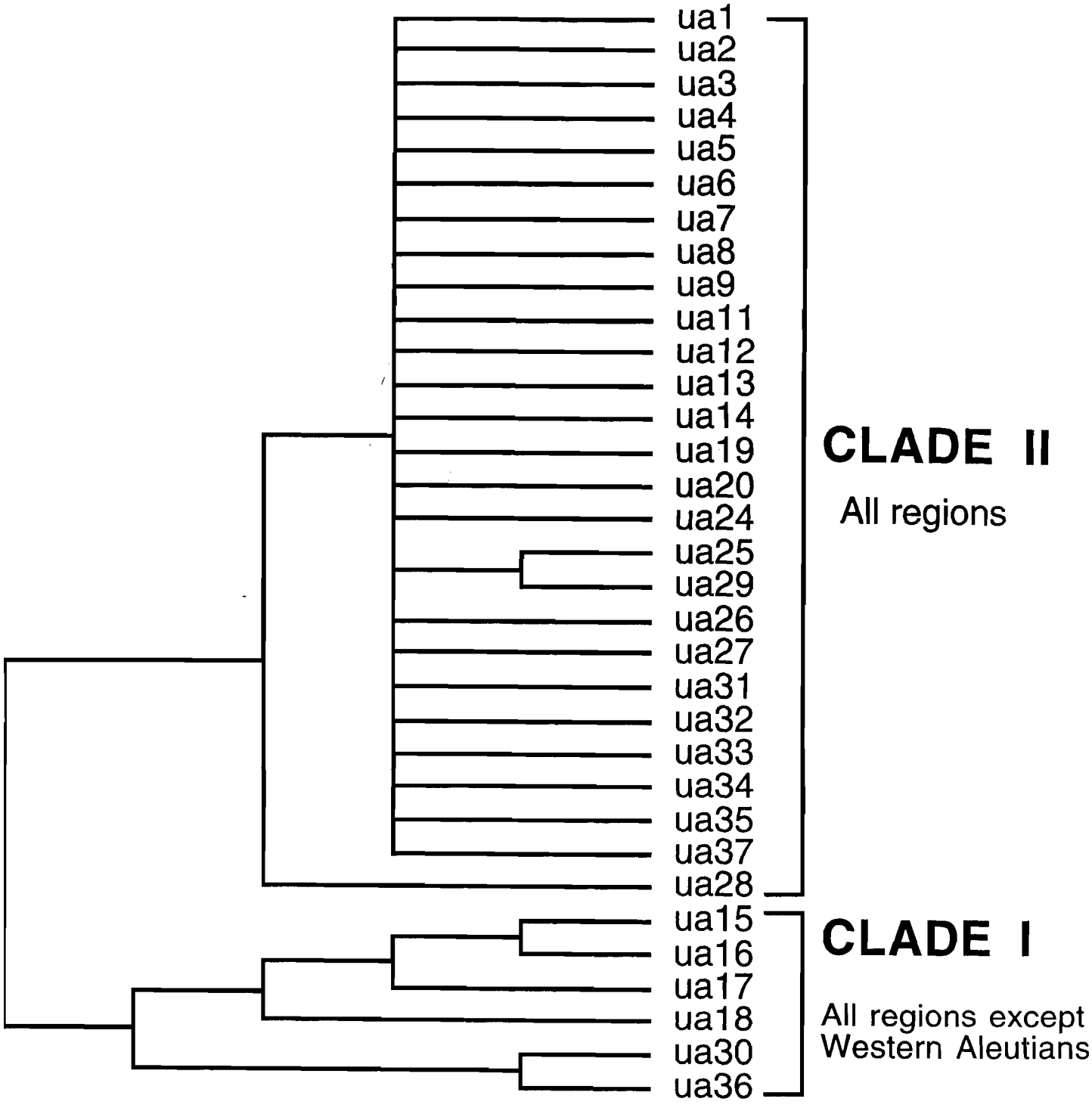

ua1
ua2
ua3
ua4
ua5
ua6
ua7
ua8
ua9
ua11
ua12
ua13
ua14
ua19
ua20
ua24
ua25
ua29
ua26
ua27
ua31
ua32
ua33
ua34
ua35
ua37
ua28
ua15
ua16
ua17
ua18
ua30
ua36
CLADE II
All regions
CLADE I
All regions except
Western Aleutians

Figure 3 b: **Neighbour joining tree based on Jukes-Cantor distances among genotypes for the 5' end of the control region of common murres. Numbers at nodes are indices of support from bootstrap analysis (100 replications). Branch lengths are proportional to numbers of substitutions.**

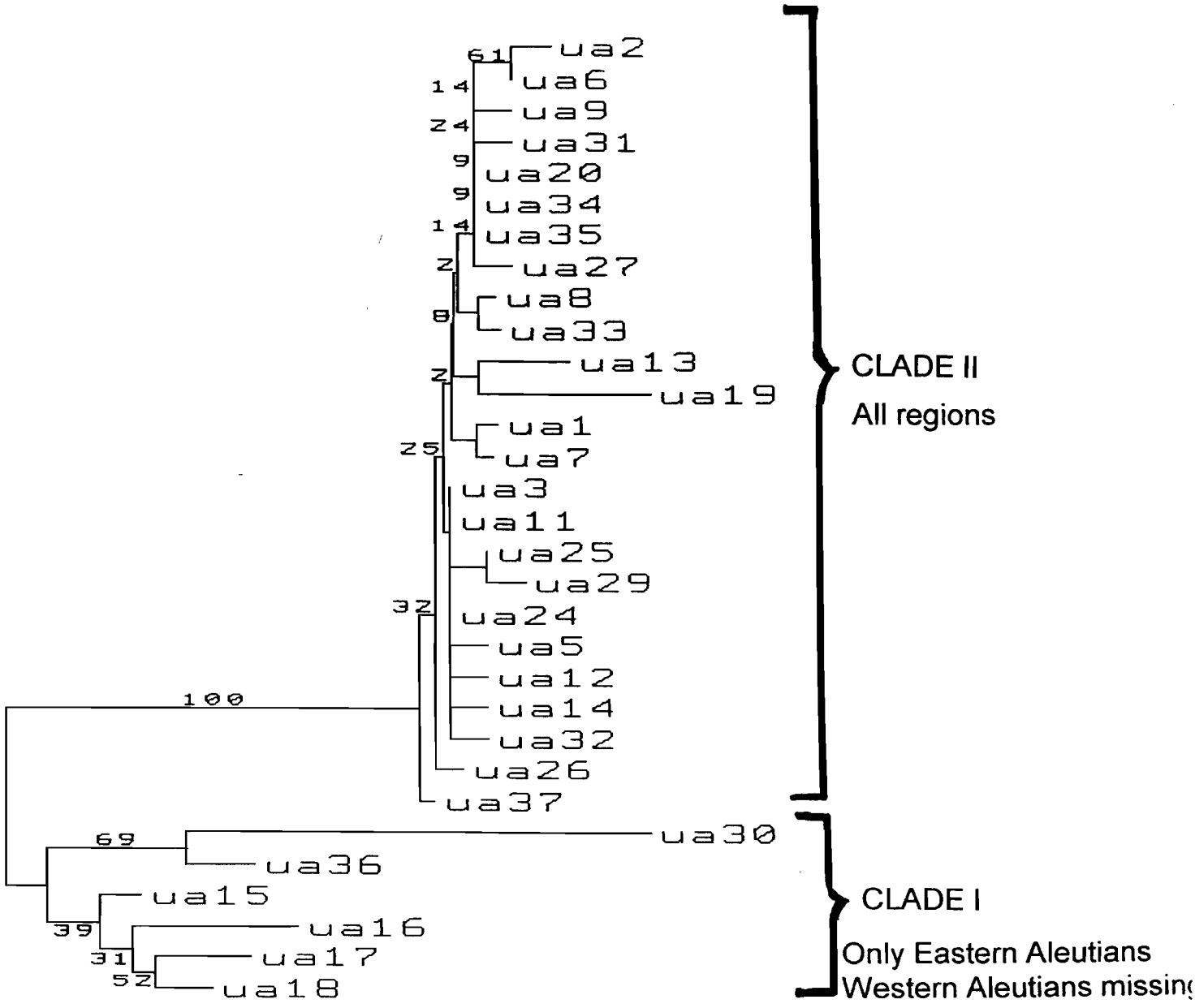
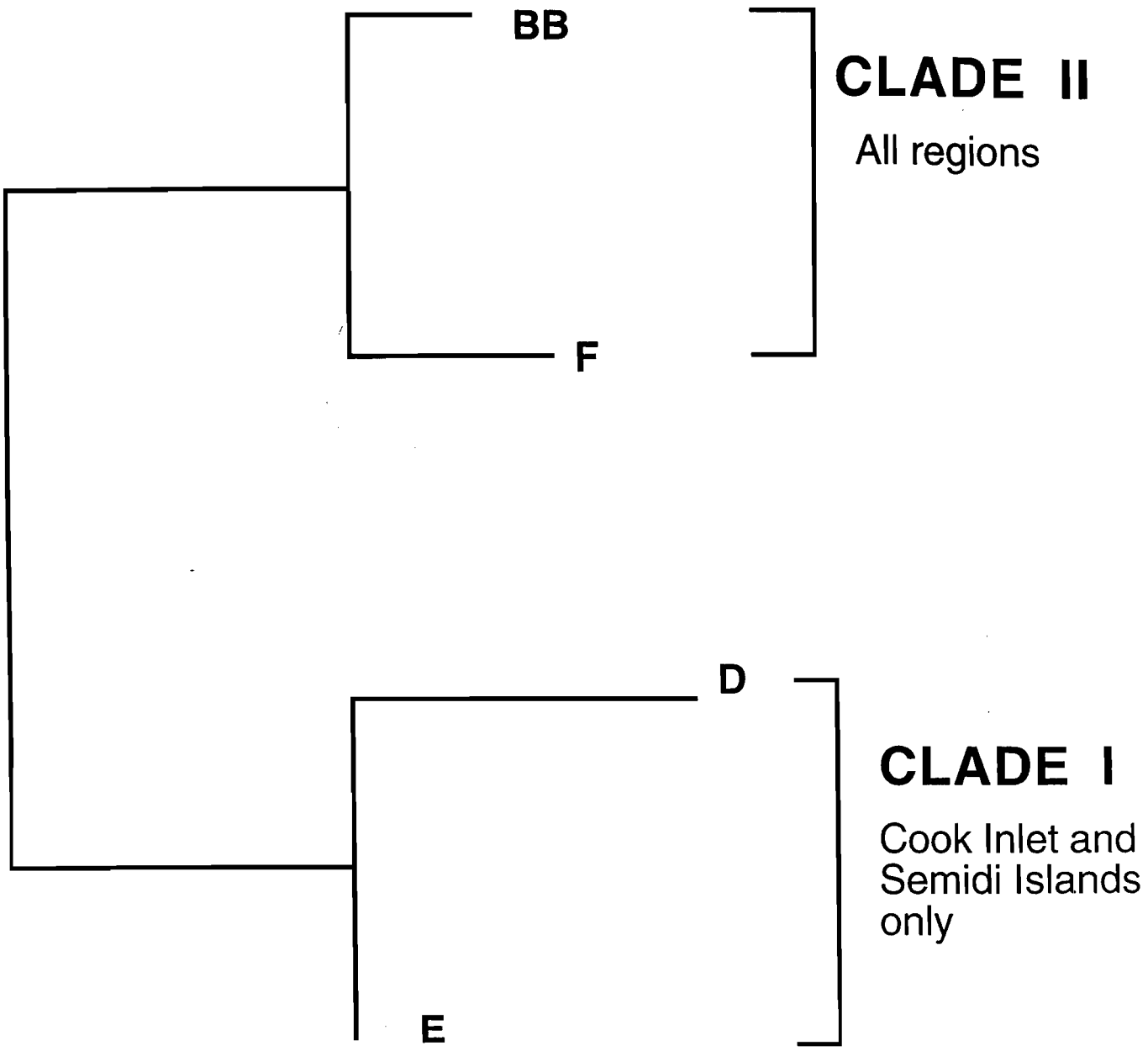

ua2
ua6
ua9
ua31
ua20
ua34
ua35
ua27
ua8
ua33
ua13
ua19
ua1
ua7
ua3
ua11
ua25
ua29
ua24
ua5
ua12
ua14
ua32
ua26
ua37
ua30
ua36
ua15
ua16
ua17
ua18
61
14
24
9
9
14
2
8
2
25
32
100
69
39
31
52
CLADE II
All regions
CLADE I
Only Eastern Aleutians
Western Aleutians missing

Figure 3c: **Maximum parsimony tree obtained by parsimony analysis with mid-point rooting of cytochrome b sequences of common murres. Branch lengths are proportional to numbers of substitutions.**


BB
F
CLADE II
All regions
D
E
CLADE I
Cook Inlet and
Semidi Islands
only

DISCUSSION

Genetic differentiation among murre colonies

Significant differences in frequencies of genotypes for the 3' end of the control region and non-random segregation of sequence variation for the 5' end of the control region provided some evidence for genetic differentiation among murre colonies in the spill area (in the Gulf of Alaska) and surrounding regions. This suggests that colonies do not constitute a single interbreeding population and that extirpation of a particular colony would result in a reduction in a portion of the species' genetic diversity. Colony specific differences reflect strong natal philopatry in common murres (Nettleship and Birkhead, 1985). Similar evidence of genetic structuring among Atlantic colonies of common murres also supports this possibility (Friesen et al., 1996b). Evidence for significant genetic differentiation and low gene flow (N_em =1) between murre colonies in E. Amatuli Island and Homer Bluffs and between Chisik Island and E.Amatuli Island were also found. This indicates that these colonies may be genetically isolated due to low dispersal and may possibly require independent management.

The apparent genetic structuring of colonies of common murres from the Gulf of Alaska indicated by control region analysis, contrasts with the genetic homogeneity found using the cytochrome b gene. This incongruity may be attributable to the highly conserved nature and thus much slower rate of evolution of cytochrome b, which results in lower levels of variability, making detection of existing sub-structuring among colonies more difficult. Rather, weak differentiation among cytochrome b haplotypes of murre colonies surveyed in this

study probably reflect historical association of populations. Specifically, the detection of two major cytochrome b clades through phylogenetic analysis indicates that murres may not trace their ancestry back to a single maternal ancestor. This suggests that murres may have survived the last glaciations in two separate refugia. Phylogenetic reconstruction also showed a similar dichotomy in haplotypes from the 5' end of the control region, providing further support for this hypothesis. Regional population subdivision by Pleistocene glaciers resulting in multiple glacial refugia has also contributed to population differentiation in a variety of marine species, including thick-billed murres *Uria lomvia* (Friesen et al., 1996b) *Cepphus* guillemots (Kidd and Friesen, in press), humpback whales (Baker et al., 1993), Stellar Sea lions (Bickham et al., 1996) and harbour seals (Stanley et al., 1996).

Genetic differentiation among regions and phylogeographic patterns

All but one index of genetic structure (γ -mutational divergence of 5' haplotypes) indicated no significant genetic structuring among common murre populations grouped into regions (Cook Inlet, Semidi, Eastern and Western Aleutian Islands). This finding implies that there is no appreciable geographic structure in genetic variation among murres when examined on a larger regional scale. No support was found for geographic sequestering of haplotypes, indicating non-significant differences in spatial frequencies of mtDNA clades (both cytochrome b and 5' haplotypes) among regions. Post-glacial dispersal resulting in the scattering of mitochondrial haplotypes across the surveyed

regions may explain the absence of geographic patterns in mtDNA. However, a lack of representation of 5' haplotypes of clade I (figure 3 a & b) in murres from the Western Aleutians (Table 2) suggests that they may be isolated from those farther east, indicating a possible restriction of gene flow. Separation of western populations from eastern ones by long distances, in combination with female-biased regional philopatry of murres, may result in such a restriction.

Gene flow among colonies and regions

Estimates of the number of female migrants per generation using the slowly evolving cytochrome b gene indicated high levels of gene flow among populations and regions. However during the last glaciations, from about 21,000 to 10,000 years ago, most of the Aleutian Island chain was covered by ice (Stanley et al., 1996). This suggests that murre colonies in the spill area (Gulf of Alaska) and Aleutian Islands were relatively recently founded by a large number of colonists. Thus, the presumed lack of genetic isolation among populations as indicated by high levels of inter-population migration probably reflect historical association of colonies as opposed to current circumstances. Conversely, estimates of number of female migrants per generation derived using the more rapidly evolving 5' end of the control region indicate a lower exchange of individuals (N_em =6) among populations, probably reflecting more contemporary conditions. Since common murres are considered to conform to a 2-dimensional stepping stone model of population structure (Friesen, 1997), whereby gene-flow is predominant between adjacent colonies (Rockwell and Barrowclough, 1987),

an exchange of 2-4 migrants per generation is considered to be sufficient to prevent differentiation through drift alone.

Results of this study indicate high rates of contemporary gene-flow, sufficient to prevent genetic differentiation between colonies. However, a finding of relatively high levels of contemporary gene-flow using control region sequences may not exclude the possibility of limited ongoing gene flow between colonies for several reasons. Firstly, as in the case of Atlantic populations of thick-billed murres, sampled Pacific populations of common murres may not have reached an equilibrium between migration, mutation and genetic drift (Friesen et al., 1996b). In other words, although sequence analysis of mitochondrial haplotypes suggests large amounts of genetic variation within colonies, either due to large founder populations or the sufficient elapse of time for evolutionary diversification of haplotypes, insufficient time may have passed since the populations achieved their present day distributions to allow gene flow to bring the distributions of all haplotypes to equilibrium. In addition, analysis of rapidly evolving control region sequences may not necessarily reflect present day gene-flow due to retention of a historical record of evolutionary events.

Finally, since banding data suggest low levels of gene-flow between Atlantic colonies of common murres (Friesen, 1997), a similar scenario may be plausible for Alaskan colonies of murres, owing to a high fidelity to their natal breeding sites. Therefore, murre colonies in sampled sites may be more isolated than is apparent through demographic parameters estimated from control region ariatin.

Population Genetic structure in common murres versus other avian species: a comparison

Due to their high dispersal potential, low levels of spatial-genetic differentiation are observed among most avian populations (Avise, 1994). However, many avian species have strong tendencies for nest-site-philopatry and exhibit gender biased dispersal, resulting in genetic differentiation among local demes (Birt-Friesen et al, 1992). Colony specific differences and significant genetic structuring detected among Pacific populations of common murres surveyed in this study may reflect the high propensity for female biased philopatry among members of this species. These findings are in concordance with genetic structuring found in several avian and mammalian species (using mtDNA) having similar life history patterns, such as the dunlin (Wenink et al., 1993), Canada goose *Branta canadensis* (Van Wagner and Baker, 1990), harbour seal (Stanley et al., 1996) and Stellar sea-lion (Bickham et al., 1996). Conversely, the extent of genetic structure detected in Pacific colonies of common murres sharply contrasts with the lack of genetic structure detected among Atlantic populations of the closely related thick billed-murre despite evidence of strong natal philopatry of this species (Friesen et al., 1996b). Similarly, Ball et al. (1988) attributed a lack of genetic differentiation among North American populations of red-winged blackbirds (*Agelaius phoeniceus*) to the highly vagile nature of these birds, resulting in homogenizing gene flow. An important consideration when attempting to decipher genetic structure of

populations is the nature of the genetic marker used to infer such information. Results of this study clearly support the notion that rapidly evolving DNA sequences such as the mitochondrial control region are capable of revealing patterns of subdivision among relatively recently evolved populations (Wenink et al., 1993). However, it is important to note that even with rapidly evolving sequences, thousands of generations may be required for isolated populations to diverge through the accumulation of mutations, and thus recent separation of populations may not be immediately apparent through analysis of such genetic markers (Mortiz, 1994).

Future research: nuclear loci

Despite the advantages of using mtDNA, are two problems. First, the mitochondrial genome is only a single locus. Due to a lack of recombination, the 37 different genes of the mitochondrial genome are linked as a single super-gene (Wilson et al., 1985). As pointed out by Palumbi et al. (1994), reliance on a single genetic locus diminishes the ability to detect spatial-temporal structure in populations since random changes in allele frequencies due to genetic drift will not affect two loci in the same manner. In addition, a phylogenetic tree derived using a single genetic locus may not accurately reflect genetic history of a population or species (Avise, 1994). Exclusive reliance on mtDNA data in reconstruction of phylogenetic patterns is also problematic because it only enables the reconstruction of maternal lineages (Wilson et al., 1985).

Concordance of phylogenetic patterns observed across several different independent loci is thus required to obtain a reliable perspective of genetic structure within local demes of common murres in the Gulf of Alaska and neighbouring regions. Further analysis of rapidly evolving nuclear genetic markers such as introns and/or microsatellites are needed to assess the extent of genetic structuring of common murres in the study areas. If evidence for genetic divergence of colonies in the spill area are further supported by nuclear loci, this may clearly indicate that extirpation of a colony may reduce the overall genetic diversity of the species.

Implications of findings to conservation of murres

Management units (MU) are defined as demographically independent breeding units, and are identified as populations having distinct allele frequencies (Mortiz, 1994). Results of this study can be used to define population units for conservation of murre colonies in the spill area in several ways. For example, the detection of widespread gene flow among colonies in the spill site and neighbouring regions would imply that most colonies of concern constitute a single population and thus form a single management unit. However, an important exception to this precept are colonies from Chisik, Kachemak and E.Amatuli Islands clustered within Cook Inlet (spill area), which appear to be extensively genetically differentiated and isolated from one other. Estimates of the number of female migrants per generation indicated reduced levels of gene flow between these sites, a finding that is rather unusual considering the close

geographic proximity of these islands. Thus, demographic parameters suggest that the probability of natural re-colonization between affected colonies in Chisik, Kachemak and E. Amatuli Islands is low. Due to these reasons, murre colonies from these sites may need to be managed as three demographically independent units. However, an overall high level of gene-flow between geographically disparate populations suggest that the failure of affected murre colonies to recover from the *Exxon* spill may not be a consequence of genetic isolation. Thus, the possible role of additional factors in impeding recovery, such as insufficient prey, lack of suitable habitats or reduced reproductive performance of birds, must be investigated.

REFERENCES

Avise, J.C. 1994. *Molecular Markers, Natural History and Evolution*. Chapman and Hall, New York.

Baker, A. J. and H. D. Marshall. 1997. Mitochondrial control region sequences as tools for understanding evolution Pages 51-79 in *Avian molecular evolution and systematics* (David P. Mindell, Ed.), Academic Press, London.

Baker, R. J., V. A. Taddel, J.L. Hudgeons and R.A. Van Den Bussche. 1994. Systematic relationships within *Chiroderma* (Chiroptera: Phyllostomidae) based on cytochrome b sequence variation. Journal of Mammology **75:**321-327.

Ball, R.M. Jr., F.C. James, S. Freeman, E. Bermingham and J.C. Avise. 1988. Phylogenographic population structure of Red-winged Blackbirds assessed by mitochondrial DNA. Proceedings of the National Academy of Sciences of the United States of America **85:**1558-1562

Bickham, J.W., J.C. Patton and T.R. Loughlin. 1996. High variability for control region sequences in a marine mammal: implications for conservation and biogeography of stellar sealions (*Eumetopias jubatus*). Journal of Mammology **77:**95-108

Birkhead, T.R and D.N. Nettleship. 1980. Census methods for murres, *Uria* Species: a unified approach. Canadian Wildlife Service Occasional paper number 43

Birt-Friesen, V.L. and W.A. Montevecchi, A.J Gaston, W.S. Davidson, 1992. Genetic structure of thick-billed murre (*Uria lomvia*) populations examined using direct sequence analysis of amplified DNA. Evolution **46:**267-272

Bowen, B. W., F.A. Abreu-Grobois, G.H. Balazs, N. Kamezaki, C.J. Limpus and R.J. Ferl. 1995. Trans-Pacific migrations of the loggerhead turtle (*Caretta caretta*) demonstrated with mitochondrial DNA markers. Proceedings of the National Academy of Sciences of the United States of America **92:**3731-3734

Brown, W.M., M. George Jr. And A.C. Wilson. 1979. Rapid evolution of animal mitochondrial DNA. Proceedings of the National Academy of Sciences of the United States of America **76:**1976-1971

Brown, Wesley M, E.M. Prager, A. Wang and A.C. Wilson. 1982. Mitochondrial DNA sequences of primates: Tempo and mode of evolution. Journal of Molecular Evolution **18:**225-239

Butler, R.G, A. Harfenist, F.A. Leighton and D.B. Peakall. 1988. Impact of sublethal oil and emulsion exposure on the reproductive success of Leach's Storm petrels: short and long-term effects. Journal of Applied Ecology. **25**:125-143

Cabot, E.L. and A.T. Beckenbach. 1989. Simultaneous editing of multiple nucleic acid and protein sequences with ESEE. Computing and Applied Biosciences **5:**233-234

Dunnet, G.M. 1987. Seabirds and North Sea oil. Philosophical Transactions of the Royal Society of London. **B 316:**513-524

Excoffier, L., P.E. Smouse, and J.M. Quattro. 1992. Analysis of molecular variance inferred from metric distance among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics **131:**479:491

Friesen, V.L. 1997. Population genetics and the spatial scale of conservation of colonial waterbirds. Colonial waterbirds **20:** 353-368

Friesen, V.L., B.C. Congdon., H.E. Walsh and T.P Birt. 1997. Intron variation in marbled murrelets detected using analyses of single-stranded conformational polymorphisms. Molecular Ecology **6:**1047-1058

Friesen, V.L., J.F. Piatt and A.J. Baker. 1996a. Evidence from cytochrome b sequences and allozymes for a 'new' species of alcid: The long-billed murrelet *(Brachyramphus perdix)*. The Condor **98:**681-690

Friesen, V.L., W.A. Montevecchi, A.J Baker, R.T Barretts and W.S. Davidson. 1996b. Population differentiation and evolution in the common guillemot *Uria aalge.* Molecular Ecology **5:**793-805

Fry, Michael D. and L. Lowenstine. 1985. Pathology of Common murres and Cassin's Auklets exposed to oil. Archives of Environmental Contamination Toxicology **14:** 725-737

Hoelzel, A.R. 1993. Evolution by DNA turnover in the control region of vertebrate mitochondrial DNA. Current Opinion in Genetics and Development **3:**891-895

Hudson, R. R., M. Slatkin and W. P. Maddison. 1992. Estimation of levels of gene flow from DNA sequence data. Genetics **132**: 583-589

Irwin, D.M., T.D. Kocher and A.C. Wilson. 1991. Evolution of cytochrome b gene of mammals. Journal of Molecular Evolution **32:**128-144

Jukes,T.H and C.R Cantor. 1969. Evolution of protein molecules. In H.N. Munro Ed., *Mammalian protein metabolism*, Pages 21-132, Academic Press, New York

Kocher, T.D., W.K. Thomas, A. Meyer, S.V. Edwards, S. Pääbo, F.X. Villablanca and A.C. Wilson. 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proceedings of the National Academy of Sciences of the United States of America **86**:6196-6200.

Kumar, S., K. Tamura and M. Nei. 1993. MEGA: Molecular Evolutionary genetics analysis, version 1.0, The Pennsylvania State University, University Park, PA

Lande, R. 1988. Genetics and demography in biological conservation. Science **241:**1455-1460

Lessa, E.P. and G. Applebaum. 1993. Screening techniques for detecting allelic variation in DNA sequences. Molecular Ecology **2:**119-129

Merila, J., M. Bjorklund and A.J. Baker. 1997. Historical demography and present day populations structure of the Greenfinch, *Carduelis chloris* - an analysis of mtDNA control-region sequences. Evolution **51:**946-956

Moore, W. S. and V.R. Defilippis. 1997. The window of taxonomic resolution based on mitochondrial cytochrome b. Pages 82-109 in *Avian molecular evolution and systematics* (David P. Mindell, Ed.), Academic Press, London.

Mortiz, C. 1994. Applications of mitochondrial DNA analysis in conservation: a critical review. Molecular Ecology. **3:**401-411

Nettleship, D.N. and T.R. Birkhead. 1985. *The Atlantic Alcidae: The evolution distribution and biology of auks inhabiting the Atlantic Ocean and adjacent water areas.* Academic Press, New York, New York.

Piatt, J. F. 1989. *Exxon Valdez* bird toll. Nature **342:**865-866

Piatt, J.F., C.J. Lensink, W. Butler, M. Kendziorek and D.R. Nysewander. 1990. Immediate impact of the 'Exxon Valdez' oil spill on marine birds. The Auk **107:**387-397.

Palumbi, S.R. and S. Baker. 1994. Contrasting population structure from nuclear intron sequences and mtDNA of Humpback Whales. Molecular Biology and Evolution **11:**426-435

Parrish, J. K. and P.D. Boersma.1995. Muddy Waters. American Scientist **83:**112-115

Rockwell, R.F. and G.F. Barrowclough. 1987. Gene flow and the genetic structure of populations. Pages 223-256 in *Avian genetics* (F. Cooke and P.A. Buckley, Eds.). Academic Press, London.

Sanger, F., S. Nicklen, and A.R. Coulson. 1977. DNA sequencing with chain terminating inhibitors. Proceedings of the National Academy of Sciences of the United States of America **74:**5463-5467

Stanley, H.F., S. Casey, J.M. Carnahan, S. Goodman, J. Harwood and R.K. Wayne. 1996. Worldwide patterns of mitochondrial DNA differentiation in the harbor seal (*Phoca vitulina*). Molecular Biology and Evolution **13:**368-382.

Tuck, L.M. 1960. *The murres: their distribution, populations and biology.* Canadian Wildlife Service Monograph No. 1, Ottawa, Ontario.

Van Wagner, C. E. and A.J. Baker. 1990. Association between Mitochondrial DNA and morphological evolution in Canada geese. Journal of Molecular Evolution. **31:**373-382

Weins., J.A. 1996. Oil, Seabirds and Science. Bioscience **46:**588-597

Wenink, P.W., A.J. Baker and M.G.J. Tilanus. 1993. Hypervariable control region sequences reveal global population structuring in a long distance migrant shorebird, the Dunlin (*Calidris alpina)*. Proceedings of the National Academy of Sciences of the United States of America **90:**94-98

Wenink, P.W., A.J. Baker and M.G. Tilanus. 1994. Mitochondrial control-region sequences in two shorebird species, the turnstone and the dunlin, and their utility in population genetic studies. Molecular Biology and Evolution **11:**22-31

Wilson, A.C., R.L. Cann., S.M. Carr., M. George., U.B. Gyllensten., K.M. Helm Bychowski., R.G. Higuchi., S.R. Palumbi., E.M. Prager., R.D. Sage and M. Stoneking. 1985. Mitochondrial DNA and perspectives on evolutionary genetics. Biological Journal of the Linnean Society **26:**375-400

Quinn, T.W. 1992. The genetic legacy of Mother Goose- phylogenographic patterns of lesser snow goose *Chen caerulescens caerulescens* maternal lineages. Molecular Ecology. **1:**105-117

Quinn, T.W. and A.C. Wilson. 1993. Sequence evolution in and around the mitochondrial control region in birds. Journal of Molecular Evolution **37**:417-425

Quinn, T.W. 1997. Molecular evolution of the mitochondrial genome. Pages 3-11 in *Avian molecular evolution and systematics* (David P. Mindell, Ed.), Academic Press, London.