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 99169
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.

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

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Study History: In the Final Report on project 96-038, the Pacific Seabird Group suggested that genetic variation within and among populations of common murres, pigeon guillemots, and marbled and Kittilitz's murrelets from the Gulf of Alaska and surrounding regions be examined both to assess the impact of the *Exxon Valdez* oil spill on these species and to aid in their restoration to the Gulf of Alaska. Restoration Project 97169 was initiated in FY97 to examine the genetic structure of populations of murres, guillemots and murrelets in the North Pacific. Annual Reports 97169 and 98169 documented results of sample collections and laboratory analyses for research conducted under Restoration Projects 97169 and 98169, respectively. The project was continued under Restoration Project 99169, and is being completed under Restoration Project 00169. One manuscript based on data collected under this project is in press in *Evolution,* and several others are in preparation. This is the third annual report for research initiated under Restoration Project 97169.

Abstract: Genetic data are needed 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. We are analyzing sequence variation in mitochondrial DNA, microsatellite DNA and nuclear introns in samples of each of these species from throughout the north Pacific. Preliminary analyses, including both traditional approaches and methods based on coalescent theory, indicate that (1) gene flow in guillemots is very restricted, guillemots from different regions are genetically distinct, and identification of the origin of guillemots killed by the Spill should be possible; (2) marbled murrelets from the Aleutian Islands are genetically different from those from 'mainland' sites, and gene flow between murrelets in the Aleutian Islands and those elsewhere is restricted, but tree- and ground-nesting murrelets are not genetically differentiated; (3) gene flow among common murres from throughout the North Pacific is high; (4) hybridization occurs between common and thick-billed murres (*U. lomvia*); and (5) little if any hybridization occurs between marbled and Kittlitz's murrelets. Restoration recommendations cannot be made yet, but will be addressed in the Final Report.

Key Words: coalescent theory, common murre, cryptic species, impact assessment, introns, gene flow, Kittlitz's murrelet, marbled murrelet, mitochondrial control region, pigeon guillemot, population genetics, source and sink populations

Project Data: Data collected include frequencies of intron and microsatellite alleles and mitochondrial haplotypes, and sequences of intron alleles and mitochondrial haplotypes for common murres, pigeon guillemots, marbled murrelets and Kittlitz's murrelets. Preliminary estimates of population genetic structure and gene flow, and phylogenetic hypotheses for

mitochondrial haplotypes have been derived. All results are preliminary, and should not be used or cited without prior written permission of the authors.

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Appendix 2. Pacheco, N. 2000. A molecular investigation of hybridization in Alaskan *Brachyramphus* murrelets. Unpubl. B.Sc. thesis, Queen's University, Kingston, Ontario.

Appendix 3. Poland, V. 2000. Genetic population differentiation in pigeon and spectacled guillemots (*Cepphus columba* and *C. carbo*): implications for recovery in the *Exxon Valdez* oil spill area. Unpubl. B.Sc. thesis, Queen's University, Kingston, Ontario.

Executive Summary

Common murres (*Uria aalge*), pigeon guillemots (*Cepphus columba*), and marbled (*Brachyramphus marmoratus*) and Kittlitz's murrelets (*B. brevirostris*) suffered heavy mortality associated with the *Exxon Valdez* oil spill, and have been slow to recover. Genetic data are needed to aid in their restoration to the Gulf of Alaska. We are using state-of-the-art molecular and analytical methods to compare variation in mitochondrial DNA, microsatellite loci and nuclear introns among approximately 30 birds from each of 12-15 colonies for each species (except for Kittlitz's murrelets, which are being sampled opportunistically). Results are being used to estimate the extent of genetic differentiation and gene flow among colonies, as well as genetic variability and inbreeding within colonies. 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.

In FY97-FY99, tissue and blood samples were collected from all four species from several sites within the spill area and adjacent sites. In FY97, protocols for screening variation in the mitochondrial control region were developed for murres, and techniques for assaying variation in nuclear introns and microsatellites were refined for murres. Variation in nine introns and three microsatellites was assayed in marbled murrelets, and variation in the mitochondrial control region and cytochrome *b* gene was assayed in common murres. In FY98, protocols for assaying variation in introns in guillemots were refined, and guillemot samples were screened for variation in five introns and one microsatellite locus. Common murres were screened for variation in the mitochondrial control region, five microsatellite loci and three introns, and murrelets were assayed for variation at one additional intron.

In FY99 we completed the third year of this project. Tissue samples were obtained from several new sites for most species. All available guillemot samples were screened for sequence variation in the mitochondrial control region, two new introns and three new microsatellite loci. New samples from common murres were screened for variation in the mitochondrial control region, five microsatellite loci and four introns. The mitochondrial control region of marbled murrelets was characterized, and PCR primers were designed for population-level screening. All available marbled murrelet samples were assayed for variation at two microsatellite loci, and new samples from marbled murrelets as well as all available samples from Kittlitz's murrelets were sampled for variation in cytochrome *b*, six introns and two microsatellites. Preliminary analyses suggest that gene flow among colonies of pigeon guillemots is very low, that gene flow between marbled murrelets from the Aleutians and 'mainland' sites is higher, and that gene flow both among marbled murrelets from 'mainland' sites as well as among colonies of common murres is high. Results also indicate that 5-10% of murres from the Gulf of Alaska are the descendants of hybridizations between common and thick-billed murres, but that the incidence of hybridization between marbled and Kittlitz's murrelets in this area is very low. The present approach should provide colony-specific markers (in the form of allele frequency differences at a number of loci) for identification of the sources of oiled birds, at least for guillemots. Restoration recommendations cannot be made at this time, but will be addressed in the Final Report.

Introduction

Seabirds of the family Alcidae are highly vulnerable to marine oil pollution due both to the large amount of time that 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* oil spill; for example, the estimated mortality for common murres was in the hundreds of thousands (Parrish and Boersma 1995). Although guillemots and murrelets were declining in the area prior to the spill, the accident probably increased their rate of decline. Common murres and marbled murrelets now appear to be recovering from the spill, but pigeon guillemots apparently are not; the state of recovery of Kittlitz's murrelets is unknown. The reasons for the difficulty of these species in recuperating (as well as for the prespill declines) are unclear, but may relate to availability and quality of prey (currently being investigated through the APEX Predator Experiment and Nearshore Vertebrate Predator Project), and/or genetic problems such as genetic isolation of colonies or inbreeding. We are using state-of-the-art molecular and analytical techniques to aid in the restoration of these species to the Gulf of Alaska.

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 can aid restoration in the following three main ways:

Definition of the geographic limits of the affected populations.-Many seabirds killed by the spill were migrating: the 'affected' zone, or the populations that were affected by the spill and require restoration effort, may be geographically distant 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 genetically 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 gene flow into and out of colonies, and thus aid in the 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 they represent sinks, their restoration may be a waste of resources and may actually prevent recovery of the species.

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.

Four 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 prioritize 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 long-term effective size of a population is the size of an idealized population that would have the same amount of genetic diversity as the population being considered; the long-term 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 historical 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 inbreeding (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). Application of a new body of theory known as coalescence theory to genetic information enables estimation of long-term effective population size (Beerli and Felsenstein 1999 and references therein). Thus the extent to which small effective population sizes and inbreeding are preventing or slowing population recovery may be inferred.

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

Hybridization and introgression.-Individuals from different species may interbreed, especially following habitat disturbance; if hybrids are viable and fertile, such hybridization events can result

in transfer of genetic material between species (genetic introgression, or interspecific gene flow). Hybridization can have either positive or negative effects: it can introduce new genetic variation into a species, thus increasing fitness and evolutionary potential; it can reduce the fitness of either or both species by disrupting adaptations; it can result in the genetic anihilation of one or both species; and it can interfere with legal protection of endangered species under the U.S. Endangered Species Act (e.g. Grant and Grant 1992, Avise 1994).

Objectives

The primary purpose of this project is to conduct genetic analyses to aid in the restoration of common murres, pigeon guillemots, and marbled and Kittlitz's murrelets to areas affect by the 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; and

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 long-term effective population sizes, and

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

A seventh objective, additional to those originally proposed, can also be addressed with the present data:

7) to measure the extent of hybridization and introgression between species.

Methods

We are comparing variation in mitochondrial DNA, microsatellite loci and 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 (i.e. that 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 the spill area and between the spill area and neighboring sites, 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 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 and 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 analyzing 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 also is useful for estimating population genetic structure and long-term effective population sizes in alcids since its mutation rate has been calibrated for this family (unpubl. data). However, mtDNA represents a single supergene whose pattern of inheritance is not typical of the rest of the genome (Wilson et al. 1985); results of analyses of mtDNA therefore need to be complemented 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 (unpubl. data), so are also useful for studying recent evolutionary events (Friesen et al. 1996; Congdon et al. 2000). 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 and gene flow.

Laboratory Assays.-Variation in the number of repeating units in microsatellite loci is 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 first are 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 among populations (Friesen et al. 1996a, 1997, Congdon et al. 2000).

Statistical Analyses.-Data are being analyzed using traditional methods developed for data from protein electrophoresis and sequencing (e.g. Swofford & Selander 1981; Swofford 1993), as well as using new techniques that capitalize on the power of combining genotypic and sequence data (e.g. Michalakis and Excoffier 1996, Beerli and Felsenstein 1999):

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 (including confidence limits) among colonies is being estimated using a maximum likelihood procedure based on coalescent theory (Beerli 1999, Beerli and Felsenstein 1999).

3) Appropriate reference or 'control' sites for monitoring, will be apparent from the results of objective (1); colony-specific markers (in the form of allele frequency differences at multiple loci) for impact assessment will be determined using SPAM (ADFG 1999) and Assign (M. Damus, unpubl. program).

4) Cryptic species will be 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 genes of individuals from different populations.

5) Coefficients of inbreeding will be estimated from nuclear data using Wright's F statistics and distance measures (e.g. d^2, Coulson et al. 1998), and long-term effective population sizes (including confidence limits) will be estimated from mitochondrial sequence data using the method of Beerli and Felsenstein (1999), which is based on coalescence theory.

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

7) Interspecific hybrids and their descendents can be identified by the presence within a species of nuclear or mitochondrial DNA sequences that either (1) otherwise occur only in another species, or (2) are more closely related to sequences in that occur in other species.

Results and Discussion

Blood, feather and/or tissue samples were collected from birds breeding throughout the Pacific basin, mostly in Alaska (Table 1). As much as possible, tissue was obtained from museum

specimens, and blood and blood feathers ('pin' or growing feathers) were obtained from chicks or adults during banding. Birds collected for ongoing dietary studies in Alaska (J.F.P.) also were used for tissue. In FY98 we obtained samples from common murres from the eastern Aleutians, from marbled murrelets from the central and eastern Aleutians, and from guillemots from British Columbia and Kachemak Bay. Most samples were obtained through contributions by researchers working at specific sites, but special collection trips were also made. Sampling efforts in 1999 and 2000 focused on remaining key sites (Table 1).

We have just completed the third year of laboratory work for this project. Special efforts were devoted to screening samples from guillemots and murrelets, and some preliminary analyses were conducted:

Common Murres.-Assays of sequence variation in the mitochondrial control region (treated as two fragments for screening), microsatellite loci and introns were begun in FY97 and were continued in FY98 and FY99, and presently are being completed (Birt and Warheit, unpubl. data). Data analyses are being conducted at two levels: (1) among samples from the spill area and neighboring sites; and (2) among regional samples from throughout the North Pacific. Preliminary results from all types of markers at both levels of analysis indicate that murres are genetically homogeneous (Table 2), and that gene flow is low but probably sufficient to counteract divergence of populations through genetic drift alone (Table 3). Significant asymmetries in gene flow may exist: for example, the colonies at Chowiet and Chisik Island export significantly more murres to the Barren Islands and Aiktak than they receive back, and the Bering Strait is a net importer of murres. Genetic diversity is high everywhere (Table 3), so the effects of inbreeding depression are probably negligible. Common murres from the Gulf of Alaska and surrounding sites can probably be treated as a single management unit, and populations should recover from the spill relatively quickly.

An unexpected finding was that six of 120 common murres sampled in the Gulf of Alaska carried mtDNA sequences of thick-billed murres and various combinations of nuclear alleles from common and thick-billed murres, suggesting that ~5% of common murres in this area are hybrids and back-crosses. (In a similar survey of thick-billed murres in the North Pacific, one was found to carry the mtDNA sequence of a common murre; M. Damus, unpubl. data.) Studies of the breeding biology and foraging ecology of murres in the Gulf of Alaska therefore should be interpreted in this light.

Marbled Murrelets.-Up to and including FY98, variation in nine nuclear introns and three microsatellites was assayed in 120 marbled murrelets sampled from British Columbia to Attu Island (Congdon et al. 2000). In FY98 and FY99, the mitochondrial control region of marbled murrelets was characterized, and PCR primers were designed for population screening (Gissing, unpubl. data). All species of charadriiforms that we have studied to date have nuclear copies of their mitochondrial control regions, and PCR primers have to be designed carefully and on a species-by-species basis to avoid amplifying the nuclear copy during analyses of the mitochondrial copy. Design of these primers has been particularly difficult for murrelets, but is now complete; population screening is now underway. In FY99, samples also were screened for variation at two

microsatellites. A paper based on variation in nine of the introns is now in press in *Evolution* (Appendix 1); results indicate that gene flow among marbled murrelets sampled from 'mainland' sites between British Columbia and the Alaskan Peninsula is relatively high, that gene flow occurs between tree- and ground-nesting murrelets, but that gene flow between murrelets from 'mainland' sites and the Aleutian Islands is restricted. Marbled murrelets probably represent at least two management units.

Kittlitz's Murrelets.-Sampling of Kittlitz's murrelets is insufficient to test for genetic differences among murrelets from different sites, although results from previous work suggest that Kittlitz's murrelets may represent two or more cryptic species (Friesen et al. 1996a). However, observations of plumage variation in marbled and Kittlitz's murrelets, as well as records of pairs consisting of one marbled murrelet and one Kittlitz's murrelet in Kachemak Bay (K. Kuletz, unpubl. data), suggest that marbled and Kittlitz's murrelets may hybridize. Variation in mitochondrial DNA and six nuclear introns in 17 Kittlitz's murrelets sampled from Kachemak Bay therefore was compared to variation in 130 marbled murrelets from throughout the North Pacific for evidence of hybridization and introgression (Pacheco 2000). No F_1, F_2, or backcross hybrids were found, suggesting that either hybridization between these species is rare, or hybrid offspring have low viability (Appendix 2).

Pigeon Guillemots.-In FY97 and 98, samples from pigeon guillemots were screened for variation at five introns and one microsatellite locus, but no variation was found (Moy, unpubl. data). In FY99, pigeon guillemots were screened for variation in the mitochondrial control region (Poland 2000), four microsatellites and two variable introns (Ibarguchi, unpubl. data). Preliminary analyses indicate that gene flow among local populations of guillemots is restricted, that local populations differ genetically (and independently of subspecies designations), and that guillemots probably include at least two management units (Appendix 3).

Conclusions

This project is proceeding according to the original schedule. The last of the data are currently being collected, and manuscripts are being prepared for submission to refereed journals over the next year. After all the data are gathered, the original objectives of the proposal will be addressed and management recommendations will be offered.

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Table 1. Numbers of DNA samples collected from common murres, pigeon guillemots, and marbled and Kittlitz's murrelets from various sites.

Site	Common Murres	Pigeon Guillemots	Marbled Murrelets	Kittlitz's Murrelets
California	40	37	25	-
Washington	30	29	8	-
Oregon	30	24	5	-
British Columbia	40	29	30	-
Southeastern Alaska	-	14	20	-
Prince William Sound	-	30	13	-
Middleton Island	30	-	-	-
Central Cook Inlet	48	31	26	19
Lower Cook Inlet	27	-	-	-
Central Alaska Peninsula	20	-	9	-
Western Alaska Peninsula	11	~5	12	-
Eastern Aleutians	27	~5	18	-
Central Aleutians	-	~5	15	-
Western Aleutians	13	-	8	3
Bering Sea	45	-	-	-
Chukchi Sea	34	-	-	-
Sea of Okhotsk	25	-	-	-

Table 2. Indices of population genetic structure among colonies of common murres from throughout the North Pacific.

Marker	F_{st}	γ
LDH Intron	0.021*	-
CRYS Intron	0.000	-
Control Region	0.000	0.00

*$P < 0.05$

F_{sr} = the proportion of variation due to differences among sites relative to the total sample (from Arlequin, Schneider et al. 1997).
γ = the probability that any two individuals, chosen at random from different populations, will have the same genotype (Lynch and Baker 1994).

Table 3. Estimates of migration (individuals per generation; derived from variation in five microsatellites using Migrate) among samples of common murres at two geographic scales.

a) Gulf of Alaska

	From				
To	Chisik	Kachemak	Barren Is.	Chowiet	Aiktak
Chisik	2.1 (1.7-2.4)	1.0 (0.8-1.2)	0.6 (0.4-0.8)	0.5 (0.4-0.6)	0.7 (0.5-0.8)
Kachemak	0.9 (0.7-1.1)	2.7 (2.3-3.1)	1.0 (0.8-1.2)	0.6 (0.7-0.8)	1.3 (1.3-1.6)
Barren	1.1 (0.8-1.4)	1.2 (0.9-1.5)	3.5 (2.9-4.2)	1.0 (0.7-1.2)	1.8 (1.5-2.1)
Chowiet	0.5 (0.4-0.7)	1.1 (0.9-1.3)	0.5 (0.3-0.6)	1.4 (1.1-1.7)	0.5 (0.4-0.6)
Aiktak	1.0 (0.8-1.2)	1.3 (1.1-1.5)	0.7 (0.6-0.9)	0.9 (0.7-1.1)	2.4 (2.0-2.8)

b) North Pacific

	From				
To	Kamchatka	Bering Strait	E. Aleutians	Gulf Alaska	California
Kamchatka	1.5 (1.3-1.7)	0.5 (0.4-0.6)	0.6 (0.5-0.7)	0.9 (0.8-1.1)	0.5 (0.4-0.6)
Bering Strait	1.6 (1.3-1.9)	3.8 (3.3-4.5)	1.1 (0.8-1.3)	3.3 (2.9-3.7)	0.8 (0.6-1.0)
E. Aleutians	0.7 (0.5-0.8)	0.7 (0.5-0.8)	2.0 (1.7-2.3)	1.6 (1.3-1.8)	0.5 (0.4-0.6)
Gulf Alaska	1.4 (1.2-11.6)	1.6 (1.4-1.8)	1.2 (1.1-1.5)	2.8 (2.5-3.0)	0.5 (0.4-0.6)
California	0.8 (0.6-0.9)	0.5 (0.4-0.7)	0.4 (0.3-0.6)	0.4 (0.3-0.6)	1.3 (1.1-1.6)

Products

The following papers, based on work funded entirely or in part by the EVOS Trustee Council, were published in FY9.

Friesen, V.L. and B.C. Congdon. 1999. Intron variation and population genetics of birds. Proceedings of the 22nd International Ornithological Congress.

Friesen, V.L., B.C. Congdon, M.G. Kidd and T.P. Birt. 1999. General PCR primers for the amplification and sequencing of five nuclear introns in vertebrates. *Molecular Ecology* 8:2147-2149.

The following papers, based on work funded entirely or in part by the EVOS Trustee Council, were accepted for publication in FY99.

Friesen, V.L. 2000. Introns. *In* A. J. Baker (ed.), Molecular Methods in Ecology, in press.

Congdon, B.C., J.F. Piatt, K. Martin and V.L. Friesen. 2000. Rapid population expansion and peripheral isolation in marbled murrelets: contemporary vs historic processes. *Evolution*, in press.

The following papers, based on work funded entirely or in part by the EVOS Trustee Council, were submitted for publication in FY98.

Friesen, V.L. Contributions of molecular genetics to the understanding of seabird ecology and evolution. *In* D.C. Duffy, Twenty-five years of seabird research.

Edwards, S.V., M.C. Silva, T. Burg, V.L. Friesen and K.I. Warheit. Molecular genetic markers in the analysis of seabird bycatch populations. *In* J. Parrish, Seabirds as Bycatch.

The following conference papers, based on work funded entirely or in part by the EVOS Trustee Council, were presented in 1999.

Friesen, V.L., A. Patirana, T.P. Birt, M. Damus and J.F. Piatt. 1999. Molecular evidence for hybridization between common and thick-billed murres in the Gulf of Alaska. *Ann. Meet. Pacific Seabird Gr.* Poster presentation.

Patirana, A., V.L. Friesen and J.F. Piatt. 1999. Conservation genetics of common murres in the *Exxon Valdez* spill area through comparison of mitochondrial DNA sequences. *Ann. Meet. Am. Ornithol. Union*. Poster presentation.

Patirana, A., J.F. Piatt and V.L. Friesen. 1999. Conservation genetics of common murres (*Uria aalge*) in the *Exxon Valdez* spill area through comparison of mitochondrial control region and cytochrome *b* sequences. *Soc. St. Evol. Ann. Meet.* Poster presentation.