Exxon Valdez Oil Spill
Restoration Project Final Report

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

EVOSTC Restoration Project 00169
Final Report

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October 2003

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Study History: In the Final Report on project 96038, the Pacific Seabird Group suggested that genetic variation within and among colonies 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 population genetic structure of common murres, pigeon guillemots and marbled 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 completed under Restoration Project 00169. One manuscript based on data collected under this project was published in *Evolution* (Congdon et al. 2000), and a second was published in *Conservation Genetics* (Pacheco and Friesen 2002). Several others are in preparation. This is the final report for research initiated under Restoration Project 97169.

Abstract: Genetic data are needed to aid in restoring several species of seabirds to the Gulf of Alaska. We analyzed sequence variation in mitochondrial DNA, microsatellite DNA and nuclear introns in samples of common murres (*Uria aalge*), pigeon guillemots (*Cepphus columba*) and marbled murrelets (*Brachyramphus marmoratus*) from throughout the North Pacific. Data were analyzed using traditional approaches, nested clade analyses and assignment tests. No cryptic species were found, and there was no strong evidence for inbreeding, low genetic variation, or source or sink regions in any of them. Pacific common murres constitute a single genetic management unit (MU), but hybridization occurs between common and thick-billed murres (*U. lomvia*). In contrast, gene flow in pigeon guillemots is very restricted and population genetic structure is very strong; guillemots from the spill area are part of a MU that extends from the Alaska Peninsula to somewhere between Prince William Sound and Vancouver Island. Marbled murrelets in the spill area are part of a MU that extends from the Alaska Peninsula to at least British Columbia; tree- and ground-nesting murrelets are not genetically differentiated. Little if any hybridization occurs between marbled and Kittlitz's murrelets.

Key Words: *Brachyramphus marmoratus*, *Brachyramphus brevirostris*, *Cepphus grylle*, common murre, gene flow, genetic variation, Gulf of Alaska, Kittlitz's murrelet, marbled murrelet, pigeon guillemot, population genetic structure, *Uria aalge*

Project Data: Data collected include frequencies of intron and microsatellite alleles and mitochondrial control region haplotypes, and sequences of intron alleles and mitochondrial haplotypes for common murres, pigeon guillemots, marbled murrelets and Kittlitz's murrelets. Data are kept in Excel spreadsheets and Asci files, archived at Queen's University. Data can be accessed by contacting Dr. Vicki Friesen (Department of Biology, Queen's University, Kingston,

Ontario K7L 3N6, Canada; phone 613-533-6156; fax 613-533-6617; email friesenv@biology.queensu.ca).

Citation:

Friesen, V.L. and J. F. Piatt. 2003. A genetic study to aid in restoration of murres, guillemots and murrelets to the Gulf of Alaska. *Exxon Valdez* Oil Spill Restoration Project Final Report (Restoration Project 00169), Alaska Biological Sciences Center, USGS, Anchorage, Alaska.

Table of Contents

Study History 4

Abstract 4

Key Words 4

Project Data 4

Citation 5

Table of Contents 6

List of Tables 8

List of Figures 9

Appendices 11

Executive Summary 13

Acknowledgements 14

Introduction 15

Objectives 17

Methods 18
- Sampling 18
- Mitochondrial Control Regions 18
- Introns 19
- Microsatellites 20
- Data Analyses 20

Common Murres 23
- Results 23
  - Mitochondrial Control Region 23
  - Nuclear Loci 24
- Discussion 26
  - Hybridization 26
  - Population Genetic Structure 26
  - Population History 26

Table of Contents, cont'd.

Pigeon Guillemots 27
Results 27
Mitochondrial Control Region 27
Nuclear Loci 28
Discussion 29
Population Genetic Structure 29
Population History and Gene Flow 30

Marbled Murrelets 32
Results 32
Mitochondrial Control Regions 32
Nuclear Loci 33
Discussion 35
Hybridization 35
Population Genetic Structure 35
Population History 36

Conclusions 37
Common Murres 37
Genetic Management Units 37
Source and Sink Regions 37
Cryptic Species 37
Genetic Variation and Inbreeding 37
Hybridization 38
Pigeon Guillemots 38
Genetic Management Units 38
Source and Sink Regions 38
Cryptic Species 38
Genetic Variation and Inbreeding 38
Marbled Murrelets 39
Genetic Management Units 39
Source and Sink Regions 39
Cryptic Species 39
Genetic Variation and Inbreeding 39
Hybridization 39
Kittlitz's Murrelets 39

Literature Cited 40

List of Tables

Table 1. Sampling regions, abbreviations, sites, and numbers (n) for common murres. 45

Table 2. Sampling regions, abbreviations, sites, and numbers (n) for pigeon guillemots. 46

Table 3. Sampling regions, abbreviations, sites, and numbers (n) for marbled murrelets. 47

Table 4. Sequences of PCR primers used to amplify the mitochondrial control region from murres, guillemots and murrelets. 48

Table 5. Introns surveyed for common murres (COMU), pigeon guillemots (PIGU), marbled murrelets (MAMU) and Kittlitz's murrelets (KIMU), and annealing temperatures for amplifications. 49

Table 6. Microsatellite primers and PCR annealing temperatures for common murres (COMU), pigeon guillemots (PIGU), and marbled murrelets (MAMU). 50

Table 7. Frequencies of control region haplotypes among common murres from 17 regions. . 51

Table 8. Variable sites within 761 bp of the mitochondrial control region of 328 common murres (not including haplotypes of putative hybrids). 55

Table 9. Estimates of nucleotide diversity (π) and Tajima's *D* for mtDNA, and average heterozygosity (H_E) for introns and microsatellites for common murres. 58

Table 10. Estimates of Slatkin's linearized F_{st} for pair-wise comparisons of regional samples of common murres, based on mitochondrial control region sequences (below diagonal), and mean F_{st} for nuclear loci (above diagonal). 59

Table 11. Conclusions from nested clade analysis of mitochondrial control region variation in common murres. 60

Table 12. Nuclear allele frequencies for common murres. 61

Table 13. Sequence variation among alleles for four nuclear introns in common murres. 67

Table 14. Numbers and origins of individual common murres with high probabilities of immigrant ancestry. 68

Table 15. Variable sites among 73 mitochondrial control region haplotypes of pigeon guillemots. 69

List of Tables, cont'd.

Table 16. Frequencies of control region haplotypes among within regional samples of pigeon guillemots. 72

Table 17. Estimates of nucleotide diversity (π) and Tajima's *D* for mtDNA, and average heterozygosity (H_E) for introns and microsatellites for pigeon guillemots. 74

Table 18. Estimates of Φ_{ST} (above diagonal) and corrected percent sequence divergence (δ; below diagonal) for mitochondrial control region sequences for pair-wise comparisons of regional samples of pigeon guillemot. 75

Table 19. Sequence variation among alleles for three nuclear introns for pigeon guillemots. . . 76

Table 20. Numbers of samples and regional allele frequencies for three introns and four microsatellites for pigeon guillemots. 77

Table 21. Estimates of F_{st} for pair-wise comparisons of guillemot regional samples based on introns (above diagonal, top number), all loci combined (above diagonal, lower number), microsatellites assuming an infinite alleles model of mutation (below diagonal, top number), and microsatellites assuming a step-wise mutation model (below diagonal, lower number). 79

Table 22. Variable sites among 51 control region haplotypes of 80 marbled murrelets. 80

Table 23. Frequencies of mitochondrial control region haplotypes among marbled murrelets from seven regions. Region abbreviations as in Table 3. 82

Table 24. Estimates of nucleotide diversity ($\pi \pm$sd) and Tajima's *D* for mitochondrial control regions, and average heterozygosity (H_E) for introns and microsatellites for marbled murrelets. 84

Table 25. Estimates of Φ_{st} (for mitochondrial control regions, above diagonal) and R_{st} (based on two microsatellite loci, below diagonal) for pair-wise comparisons of regional samples of marbled murrelets. 85

Table 26. Frequencies of alleles at five loci amplified with microsatellite primers within 11 regional samples of marbled murrelets. 86

Table 27. Estimates of F_{st} based on nine introns (above diagonal) and 14 nuclear loci (below diagonal) for pair-wise comparisons of regional samples of marbled murrelets. 88

Table 28. Indices of population genetic structure based on different types of loci in marbled murrelets. 89

List of Figures

Fig. 1. Approximate Pacific breeding distribution (heavy black lines; from Udvardy 1963), sampling regions (open dots) and subspecies ranges (dashed line) for common murres. 90

Fig. 2. Breeding distribution (heavy black lines; from Udvardy 1963), sampling sites (white dots) and subspecies ranges (dashed lines) for pigeon guillemots (from Udvardy 1963). . . . 91

Fig. 3. Breeding distribution (heavy black lines; from Udvardy 1963) and sampling sites (dots) for marbled murrelets. 92

Fig. 4. Map of the mitochondrial control region and neighboring genes for alcids, and approximate locations of PCR primers used in present study (Table 4). 93

Fig. 5 Alignment of the $tRNA^{glu}$ and $tRNA^{phe}$ genes and conserved sequence blocks within the mitochondrial control regions of the common murre (COMU, Moum and Johansen 1992 and present study), pigeon guillemot (PIGU, Kidd and Friesen 1998a), and marbled (MAMU) and Kittlitz's murrelets (KIMU, present study). 94

Fig. 6. Frequency of pair-wise sequence differences (mismatch distribution) for control region sequences of 328 common murres. 95

Fig. 7. Statistical parsimony tree showing the substitutional relationships among control region haplotypes of common murres (Table 8). 96

Fig. 8. Results of nested clade analysis of control region sequences of common murres, based on tree in Fig. 7. 99

Fig. 9. Sequences of the most common alleles for four nuclear introns for common (C1) and thick-billed murres (T1). 101

Fig. 10. Frequencies of pair-wise sequence differences (mismatch distribution) for control region sequences of pigeon guillemots. 102

Fig. 11. Statistical parsimony tree showing the substitutional relationships among control region haplotypes of pigeon guillemots (Table 15). 103

Fig. 12. Pair-wise estimates of (a) Φ_{st} (for control region sequences) and (b) F_{st} (for nuclear loci) versus geographic distance (km) between regional samples of pigeon guillemots. . . . 104

Fig. 13. Neighbor-joining trees based on Slatkin's linearized F_{st}s between regional samples of pigeon guillemots for (a) control region haplotypes, and (b) nuclear loci. 105

List of Figures, cont'd

Fig. 14. Results of nested clade analysis of control region sequences of pigeon guillemots. "No." indicates clade numbers. 109

Fig. 15. Summary of results of nested clade analysis for pigeon guillemots. 110

Fig. 16. Sequences of the most common alleles for three introns for pigeon guillemots. 111

Fig. 17. Frequency of pair-wise sequence differences (mismatch distribution) for control region sequences of 80 marbled murrelets. 112

Fig. 18. Statistical parsimony tree showing the substitutional relationships among control region haplotypes of marbled murrelets (Table 23). 113

Fig. 19. Results of the nested clade analysis for marbled murrelets. 114

Fig. 20. Slatkin's linearized F_{st} (for all nuclear loci) versus geographic distance (km) between regional samples of marbled murrelets. 115

Fig. 21. Neighbor-joining tree based on Slatkin's linearized F_{st} (for all 14 nuclear loci) between regional samples of marbled murrelets. 116

Appendices

Appendix I: Partial sequence of the mitochondrial control region of common murre haplotype 01. 117

Appendix II: Partial sequence of the mitochondrial control region of marbled murrelet haplotype 05. 118

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 as of 1996 were slow to recover. Genetic data were requested to aid in their restoration to the Gulf of Alaska. We used state-of-the-art molecular and analytical methods to compare variation in mitochondrial DNA, nuclear introns and microsatellite loci among birds from throughout the breeding ranges of each species (except for Kittlitz's murrelets, which were sampled opportunistically). Results were used to estimate the extent of genetic differentiation and gene flow among regions, as well as genetic variability and inbreeding within regions. We had three main objectives: (1) to determine the geographic extent of the populations affected by the spill; (2) to identify source and sink regions; and (3) to identify genetically appropriate reference or 'control' sites for monitoring. We also had four secondary objectives: (4) to identify cryptic species; (5) to measure coefficients of inbreeding and long-term effective population sizes; (6) to identify appropriate sources for translocations, if necessary; and (7) to measure the extent of hybridization and introgression between species.

Solid tissue and blood samples were collected from approximately 30 common murres, pigeon guillemots and marbled murrelets each from 8-17 regions, including several sites in and near the spill area. Samples also were obtained from 22 Kittlitz's murrelets. Protocols for screening genetic variation were developed for all four species. Common murres were screened for variation in the mitochondrial control region, for introns and five microsatellite loci; guillemots were screened for variation in the mitochondrial control region, three introns and four microsatellite loci; and marbled murrelets were assayed for variation at in the mitochondrial control region, nine introns and five microsatellite loci. All available samples from Kittlitz's murrelets were screened for variation in the mitochondrial cytochrome *b*, and five introns. Genotype frequencies and allele/haplotype sequences were used to derive estimates of genetic variability, population genetic structure, population history and gene flow.

Genetic variability was high in all molecular markers assayed in common murres. Weak isolation-by-distance appears to exist, but population genetic structure was otherwise essentially zero for all types of molecular markers. Assignment tests indicated that 4.6% or more of sampled birds are immigrants from other regions, and some may have dispersed a long distance between their natal and breeding regions. Pacific common murres apparently underwent a historical (probably post-Pleistocene) population expansion. Approximately 2.4% of murres appear to be descendants of recent hybridizations between common and thick-billed murres; hybrids were recovered from the Alaska Peninsula, and Chukchi and Bering seas.

Genetic variability in pigeon guillemots also is high. Population genetic structure is much higher than in common murres and higher than for most other species of birds, with global estimates of Wright's fixation index (F_{st}) ranging up to 0.34 for different molecular markers. Most regions that were sampled exhibited significant genetic differences from most other sites, with a strong isolation-by-distance effect. Assignment tests indicated that gene flow in pigeon guillemots is lower than in common murres, with 1.6% of sampled birds being migrants from other regions.

Nested clade analysis revealed a dynamic history, including historical (probably pre-Wisconsin) fragmentation, range expansion, long-range colonization, and isolation-by-distance.

Marbled murrelets also exhibited high genetic variability. Population genetic structure and gene flow were intermediate between murres and guillemots: global estimates of F_{st} between 0.02 and 0.09 for different types of markers, and assignment tests suggested that 2.5% of murrelets were immigrants. Murrelets appear to have undergone historical (post-Pleistocene) range expansions into the Aleutian islands and California; no evidence of historical fragmentation was found. The incidence of hybridization between marbled and Kittlitz's murrelets in Kachemak Bay is virtually zero.

No evidence for cryptic species was found in any species. Pacific common murres appear to constitute a single genetic management unit (MU), whereas pigeon guillemots in the spill area appear to be part of a MU that extends from Belkofski Bay (and possibly Adak) to Prince William Sound (and possibly southeastern Alaska); marbled murrelets in the spill area are part of a genetic MU that extends from the Alaska Peninsula to at least British Columbia. No strong evidence was found for either source or sink regions in any of these species; however, statistical power was often low, and the possibility that British Columbia is acting as a sink for marbled murrelets requires further investigation. Similarly, no consistent evidence was found for either inbreeding or low genetic diversity within any species in any region. Additional samples are needed for pigeon guillemots from the Aleutian islands and southeastern Alaska, marbled murrelets from Washington and Oregon, and Kittlitz's murrelets from throughout their range.

Acknowledgements

Samples used in this study were obtained through the assistance of and Yuri Artukhin, Tim Birt, Alan Burger, Vernon Byrd, Fred Cooke, Andy Derocher, George Divoky, Lindsey Hayes, Jan Hodder, Dennis Jelinski, Gary Kaiser, Sasha Kitaysky, Kathy Martin, Ken Morgan, Mike Kassera, Amy Marr, Jeff Moy, Anoma Patirana, Tom van Pelt, Jay Pitocchelli, Alex Pritchard, Dave Roseneau, Andrea Smith, Susan Speckman, Bill Sydeman, Hollie Walsh, Ken Warheit, Jeff Williams, and the Captain and Crew of the Tiglax. Help with data collected and interpretation was provided by Tim Birt, Peter Boag, Brad Congdon, Christine Crossman, Martin Damus, Shane Doran, Graeme Gissing, Deb Harrison, Karen Holder, Gabriela Ibarguchi, Heather Jones, Kathy Kennedy, Monica Kidd, Rhonda Kristensen, Vinay Lodha, Denise Michaud, Jeff Moy, Nicholas Pacheco, Veronica Poland, Anoma Patirana, Lisa Veit, and Hollie Walsh. K. Warheit shared unpublished data and analyses.

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 (*Uria aalge*) was in the hundreds of thousands (Parrish and Boersma 1995). Although pigeon guillemots (*Cepphus grylle*) and marbled murrelets (*Brachyramphus marmoratus*) were declining in the area prior to the spill, the accident probably increased their rate of decline. Common murres are now classified as 'recovered', pigeon guillemots as 'not recovering', and marbled murrelets as 'recovering'. The reasons for the slow recovery of these species (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. Our purpose was to apply state-of-the-art molecular and analytical techniques to aid in the restoration of common murres, pigeon guillemots and marbled murrelets 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 population genetic structure and gene flow in murres, murrelets and guillemots can aid restoration in the following three main ways:

Delineation of the geographic limits of the affected populations.-The geographic limits of populations[1] affected by the spill are important for recovery. If the geographic range of a population is small, gene flow is probably low, and the species may be slow to recover since there will be little immigration to supplement recruitment; furthermore, population decline associated with the spill may reduce the species' genetic diversity since a high proportion of the species' variation will be restricted to local populations. On the other hand, if the geographic range of a population is large, gene flow is probably high, and the species should recover more quickly; furthermore, population decline associated with the spill should have little affect on the genetic diversity of such species, since variation will be widely distributed. Molecular data enable delineation of the geographic extent of the populations that include the spill area.

Identification of sources and sinks.-According to metapopulation theory, 'source' populations are populations that occur in optimal habitat ('source habitat' or 'source regions') and can act as exporters of recruits for populations elsewhere; 'sink' populations occur in suboptimal habitat ('sink habitat' or 'sink regions') and require immigration to maintain numbers (e.g. Pulliam and Danielson 1991, 1988, Dias 1996). Molecular data can provide estimates of gene flow into and out of regions, and thus aid in the identification of sources and sinks. For example, protein data

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[1]Within this report, we use 'population' to refer to a group of individuals that interbreed and share a common gene pool, and are genetically differentiated from other such groups.

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 populations 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 different for genetically divergent populations, even if they occur in ecologically similar or geographically proximate areas. For example, K. Warheit (Washington State Fish and Wildlife Service) noted that common murres breeding in Washington (*U. a. californica*) have different breeding chronologies from those at neighboring colonies in British Columbia (*U. a. inornata*), and may be genetically different. Molecular data enable identification of genetically appropriate reference or 'control' sites from which to obtain baseline data for monitoring, restoration and modelling, e.g. to determine if a seabird colony has recovered 'normal' functioning.

Molecular data can also produce four other types of information that are useful for conservation and restoration:

Identification of cryptic species.-A population's uniqueness (e.g. its endemicity or genetic distinctiveness) may be used to prioritize restoration efforts. Most importantly, molecular data enable the identification of cryptic species: populations that are similar in appearance but that represent genetically distinct, non-interbreeding species (e.g. long-billed [*Brachyramphus perdix*] and marbled murrelets; Zink et al. 1995; Friesen et al. 1996a).

Estimation of 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). Molecular data may be used to infer the extent to which low effective population size and inbreeding are slowing population recovery.

Sources for translocations.-If breeding success within a colony is low due to inbreeding depression, or if recruitment is low, release of individuals from other sites may be desirable. Ideally, sources of animals for such introductions should be within the same genetic population or from a closely related population to prevent both inbreeding depression (Allendorf and Leary 1986) and outbreeding depression (Templeton 1986).

Identification of hybrid individuals.-Individuals from different species may interbreed, especially following habitat disturbance; if hybrids are viable and fertile, hybridization can result in transfer

of genetic material between species (genetic introgression, or interspecific gene flow). Hybridization can have both 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 parental species by disrupting adaptations; it can result in the genetic annihilation of one or both species; and it can complicate 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 was to conduct genetic analyses to aid in restoring common murres, pigeon guillemots, and marbled murrelets to areas affect by the spill. We had three main objectives for each species:

1) determine the geographic extent of the population affected by the spill;
2) identify source and sink regions; and
3) identify appropriate reference or 'control' sites for monitoring.

As secondary objectives, we also hoped to

4) identify cryptic species,
5) measure inbreeding and long-term effective population sizes, and
6) identify appropriate source populations for translocations, if necessary
7) measure the extent of hybridization and introgression between species.

Methods

Sampling

Tissue samples were obtained from 383 common murres, 204 pigeon guillemots and 184 marbled murrelets from throughout their breeding ranges (Tables 1-3, Figs. 1-3). Samples also were obtained from 19 Kittlitz's murrelets from Kachemak Bay and three from Attu Island. Most Alaskan samples consisted of solid tissue (heart, liver and/or striated muscle) from adults in breeding condition collected for dietary analyses in close proximity to colonies during the breeding season. Samples from Russia consisted of tissue from birds in breeding condition caught in gill nets during the breeding season. Samples from elsewhere generally consisted of blood samples from adults or chicks caught at nests. Samples are archived at Queen's University, the Royal Ontario Museum, the Burke Museum, the American Museum of Natural History and/or the University of Alaska Museum at Fairbanks. Samples were not available from birds killed by the spill. DNA was extracted using a standard protease-K phenol/chloroform technique (Friesen et al. 1997).

Mitochondrial Control Regions

The avian mitochondrial control region includes three sub-regions: the hypervariable Domains I and III (5' and 3' ends respectively) and a more conserved central Domain II (Baker and Marshall 1997; Fig. 4). The control regions of many species of charadriiform birds possess non-functional nuclear copies that often co-amplify with the target gene (e.g. Kidd and Friesen 1998a), and initial attempts to amplify the mitochondrial copy from each of the four species using generic primers (both in V.L.F.'s lab and elsewhere) failed to yield clean sequence. PCR primers specific to the mitochondrial copies of the control regions of each species therefore had to be designed:

Common murres.-A murre-specific forward primer (UaL50, situated near the 5' end of the control region; Table 4; Fig. 4) was designed from previously published mitochondrial DNA (mtDNA) sequences of murres (Moum and Johansen 1992), and a general reverse primer for birds (ADH1452, situated in the $tRNA^{phe}$ gene; Table 4; Fig. 4) was designed from previously published sequences of several other species of vertebrates. An ~1 kb fragment including most of the control region and the entire gene for $tRNA^{phe}$ then was amplified from one common murre and one thick-billed murre each from the Pacific using these primers under standard conditions (10 mM Tris pH 8.5, 50 mM KCl, 1.5 mM $MgCl_2$, 1.6 mM bovine serum albumin, 2% gelatin, 0.2 mM each of the four dNTP's, 0.4 mM each of the heavy and light strand primers, and 0.5 units of *Thermus aquaticus* [Taq] DNA polymerase [Boehringer-Manneheim]) in a PTC-100™ thermal cycler (MJ Research) with annealing at 55°C. Amplified DNA was subjected to electrophoresis through 2% agarose gels and purified using Gene Clean II™ kits (Bio 101 Inc.) according to the manufacturer's instructions. DNA was sequenced with Amplicycle™ cycle sequencing kits (Applied Biosystems) following the manufacturer's suggested protocol. DNA sequences were scored by hand and aligned using the alignment program, ESEE (Cabot & Beckenbach 1989). Based on these sequences, one new primer (UaH389; Table 4; Fig. 4) was designed to be used with UaL50 to amplify Domain I, and two additional primers (UaL750 and UaH900; Table 4; Fig. 4) were designed to amplify Domain III from murres. These primer pairs

were then used for population-level analyses (see below) under the above conditions with annealing at 54 and 58°C, respectively.

Pigeon guillemots.-PCR primers that preferentially amplify the mitochondrial copy of the mitochondrial control region were designed previously by Kidd and Friesen (1998a). Two overlapping fragments of the control region were amplified with primers CGL56 and CGH549 (Domain I and part of Domain II), and CGL486 and CGH1006 (Domain III and part of Domain II; Table 4; Fig. 4) following protocols detailed in Kidd and Friesen (1998b).

Murrelets.-Murrelet-specific PCR primers were developed by testing a suite of previously-designed PCR primers that anneal to conserved sequence blocks either within or flanking the mitochondrial control region of birds (V.L.F. unpubl. data). The 5' end of the control region was successfully amplified and sequenced from two marbled murrelets and one Kittlitz's murrelet using standard protocols (above) with primers ND6 (which anneals to the 3' end of the gene for ND6; Kidd and Friesen 1998a) and CgH825 (which anneals to Conserved Sequence Block 1 within Domain III of the control region, Table 4; Fig. 4). Sequence from these three samples was used to design two murrelet-specific primers (BmaH600 and BmaL650; Table 4; Fig. 4) that anneal to conserved regions within Domain II. BmaL650 was used in combination with a guillemot-specific primer, CgH1006 (Kidd and Friesen 1998a; Table 4; Fig. 4) to derive the remainder of the control region sequence for one murrelet. For population screening, BmaH600 was used in combination with ND6 to amplify a DNA fragment including the gene for $tRNA^{glu}$, all of Domain I and part of Domain II for 80 individuals.

For all species, population-level sequence variation was screened initially using single-stranded conformational polymorphisms (SSCPs; Hayashi 1991, Lessa and Applebaum 1993) with direct incorporation of α-^{33}P-dATP (detailed in Friesen et al. 1997). Individuals were assigned tentative haplotypes on the basis of banding profiles on autoradiograms. To determine the exact nature of variation, one or more representatives of each haplotype were then sequenced directly using either (1) the Thermosequenase radiolabelled Terminator™ cycle sequencing kit (Amersham) according to the manufacturer's recommendations, or (2) an ABI Prism™ 373 Automated Sequencing System (Mobix, McMaster University) with M13F-tailed primers. Analyses of base usage and substitution patterns were made using MEGA (version 1.0; Kumar et al. 1993).

Introns

Amplifications were attempted on four to six samples from each species with up to 30 pairs of PCR primers previously designed to amplify nuclear introns from vertebrates (Friesen et al. 1997, 1999, unpubl.); various annealing temperatures and concentrations of $MgCl_2$ and DMSO were tested to optimize amplifications. Loci for which clean amplification products could be derived consistently were then chosen for population screening (Table 5):

Common murres.-Amplification buffers for the introns for crystalline, ribosomal protein 40 and lactate dehydrogenase contained 2.5 mM $MgCl_2$, 10 mM Tris pH 8.5, and 50 mM KCl. The buffer for enolase also contained 5% DMSO.

Pigeon guillemots.-Amplification buffers for guillemots were similar to those for murres except that 62.5 µg/mL BSA and 0.01 mg/mL gelatin were included; 5% DMSO was included in amplifications for rhodopsin and cytochrome *c*.

Murrelets.-Protocols for marbled and Kittlitz's murrelets are detailed in Friesen et al. (1997, 1999) and Pacheco and Friesen (2002), respectively.

In all species, sequence variation was screened using a combination of SSCPs and direct sequencing, as described for mitochondrial control regions (above).

Microsatellites

Genomic libraries for common murres, pigeon guillemots and marbled murrelets were developed and screened for dinucleotide (CA) repeats following standard protocols (Ibarguchi et al. 2000). PCR primers were developed for two loci each for common murres, pigeon guillemots, and marbled murrelets (Table 6). DNA samples from each species were then tested for amplification using these primers as well as primers developed previously for thick-billed murres (Ibarguchi et al. 2000) and yellow warblers (*Dendroica petechia*; Dawson et al. 1997). $MgCl_2$ concentrations and annealing temperatures were optimized, and the presence of length variation was determined using standard protocols (Ibarguchi et al. 2000). All available samples from common murres, pigeon guillemots and marbled murrelets were screened for length variation in four to five loci (Table 6).

Data Analyses

Sample pooling.-The number of individuals sampled from several sites was too small for most types of data analysis (Table 1 to 3). We therefore tested for genetic differentiation within such areas using (1) Mantel's tests on percent sequence divergence between control region haplotypes of individuals versus geographic distance, and/or (2) analyses of molecular variance (AMOVA; detailed below). No evidence was found for differentiation within any of the regions defined in Tables 1 to 3; samples were pooled for further analyses. Genetic variability within regions was indexed using nucleotide diversity (percent sequence divergence among individuals, π, for mtDNA; Nei 1987) or expected heterozygosity (H_E, for nuclear loci), as calculated by ARLEQUIN (version 2.0, Schneider et al. 2000).

Tests of genetic assumptions.-Most methods for analyzing population genetic structure from molecular markers assume that populations are in Hardy-Weinberg equilibrium, that loci are in linkage equilibrium, and that variation is neutral to selection. These assumptions were tested using ARLEQUIN. Deviations from Hardy-Weinberg equilibrium were analyzed using an exact test based on contingency tables (Guo and Thomson 1992); linkage disequilibrium was analyzed using a likelihood ratio test (Slatkin and Excoffier 1996); and deviations from neutrality were analyzed using Ewens-Watterson's neutrality test (Ewens 1972, Watterson 1978) and Chakraborty's test of population amalgamation (Chakraborty 1990). Several analyses also assume that populations are in equilibrium between mutation and genetic drift (i.e., that populations are stable in size). This assumption was assessed in three ways: (1) by testing estimates of Tajima's *D* for significant deviations from zero (Tajima 1989); (2) by testing

mismatch distributions for significant deviations from distributions expected under a sudden population expansion (Rogers and Harpending 1992, Rogers 1995); and (3) by using nested clade analysis to test for historical range expansions (see below). Tajima's *D* and the mismatch distributions were tested both for regions and for the total sample for each species using ARLEQUIN.

Population genetic structure.-For each species and each type of molecular marker (mtDNA, introns, and microsatellites), AMOVA was used to calculate the proportion of genetic variation distributed among regions (F_{st}; Excoffier et al. 1992) using ARLEQUIN. F_{st} was first determined for the entire sample and for pair-wise comparisons of regions. Hierarchical F-statistics then were calculated to determine the distribution of variation within and among various potential groupings of sampling regions to find the grouping that maximized the proportion of variation due to differences among groups (F_{ct}) and minimized the proportion of variation due to differences among regions within groups (F_{sc}; Stanley et al. 1992). Statistical significance of F-statistics was tested by randomization using 10,000 permutations of the data with a rejection level (α) of 0.05. For control region sequences, Φ-statistics (derivatives of F-statistics that incorporate sequence differences between haplotypes; Excoffier et al. 1992) were used. For microsatellite loci, tests were run separately assuming either an infinite alleles model of mutation (F_{st}) or a step-wise mutation model (R_{st}; Slatkin 1995). For pair-wise comparisons of geographic regions, sequential Bonferroni corrections were applied (Rice 1989). Haplotype or genotype frequencies also were tested for deviations from a random distribution of using an exact test of population differentiation (Raymond and Rouset 1995) in ARLEQUIN.

To test for isolation by distance, shortest geographic distance between regions was calculated using the great circle distance calculator at http://www.wcrl.ars.usda.gov/cec/java/lat-long.htm. Slatkin's (1995) linearized estimates of Φ_{st} or F_{st} were then tested for correlation with geographic distance between regions using Mantel's tests (Smouse et al. 1986) in ARLEQUIN; significance was tested by randomization with 10,000 permutations of the data. When more than one sampling site was included in a region (Tables 1-3), the geographic midpoint of sampling sites was used.

If significant population genetic structure was uncovered using mtDNA, mean corrected percent sequence divergence among regions (δ; Wilson et al. 1985) was estimated using ARLEQUIN and was used to derive a population tree using the neighbor-joining method in MEGA. Divergence times (t, in generations) were estimated from δ using the equation $t = \delta / r$ (Wilson et al. 1985), where r is divergence rate.

Population history and gene flow.-Gene flow can be estimated indirectly using molecular markers from F_{st} and its derivatives (e.g. Slaktin 1987). However, most methods for estimating gene flow (including maximum likelihood methods based on coalescent theory) assume that populations are in equilibrium between mutation, migration and genetic drift (Beerli 1999). This assumption is difficult to test, since populations approach equilibrium asymptotically. Specifically, $t_{1/2} = \ln 2 / (2m + 1/N_e)$ (Birky et al. 1989), where $t_{1/2}$ is the time required for a population to go half way to equilibrium, m is the migration rate and N_e is the genetically

effective population size; however, N_e and m cannot be calculated without several, generally tenuous assumptions. Nested clade analysis (NCA) and assignment tests, which do not assume equilibrium, were therefore used to analyze general patterns of gene flow (Templeton 1998). For NCA, the method of statistical parsimony (Templeton et al. 1992) was used to derive haplotype trees for control region sequences using TCS (version 1.13; Clement et al. 2000). Ambiguous connections ("loops") were resolved using a hierarchy of decisions: (1) connections that increased the total number of transversions and indels were cut; (2) connections between rare haplotypes were cut so that these haplotypes became tips (Crandall and Templeton 1993); and (3) connections between geographically distant haplotypes were cut (since haplotypes are most likely to occur near their direct ancestor) (Damus and Friesen, in prep.). The tree was nested following the rules of Templeton et al. (1987), and clades with significantly small or significantly large geographic distributions were identified by analysis of variance using GEODIS (Posada et al. 2000) with 10,000 randomizations of the data. Instances of historical fragmentation, range expansion, long-distance dispersal and/or isolation by distance were identified using the inference key from Templeton (1998, updated at zoology.byu.edu/crandall_lab/geodis.htm).

For assignment tests, the probability that an individual originated from each of various populations is determined given its multilocus genotype and the genotype frequencies of the reference populations (e.g. Rannala and Mountain 1997; Palsbøll 1999; Wilson and Rannala 2003). For the present study, IMMANC (version 5.1; Rannala and Mountain 1997) was applied to the combined data for introns and microsatellites to identify individuals that probably did not originate in the population from which they were sampled; statistical significance was assessed using a Monte Carlo approach with 10,000 replications per test. To correct for the effect of repeated tests, a rejection level (α) of 0.001 was used (as recommended by B. Rannala, pers. comm.).

Common Murres

Results

Mitochondrial Control Region

Variability.-Sequence data were obtained for 902 bp of putative mtDNA from one common and one thick-billed murre each, including 831 bp of the control region and the complete gene for $tRNA^{phe}$ (Fig. 5, Appendix I). Sequence of 760 bp of putative mitochondrial control region, including 402 bp of the 5' end and 358 bp of the 3' end, was derived for 340 murres. Several lines of evidence indicate that these sequences represent the true mitochondrial gene rather than a nuclear copy (Kidd and Friesen 1998a): (1) Sequence for the $tRNA^{phe}$ formed the proper cloverleaf structure (Desjardins and Morals 1990) when analyzed with PCFOLD (Zuker 1989). (2) Control region sequences possessed a number of conserved motifs characteristic of other avian and mammalian taxa: in particular, sequences that shared 74-100% similarity with the F, D and C Boxes and Conserved Sequence Block-1 of the pigeon guillemot (Kidd and Friesen 1998a) and marbled murrelet (see below) were present (see also Baker and Marshall 1997) (Fig. 5). (3) As in other birds (including guillemots and murrelets), a poly-C repeat occurred at the start of the control region, a poly-T repeat occurred in Domain II, and a microsatellite-like motif ($[CAACAAA]_n$) occurred at the 3' end of Domain III (Quinn & Wilson 1993; Berg et al. 1995; Baker and Marshall 1997; Kidd and Friesen 1998a; not shown). (4) Base composition was biased towards Ts and against Gs (27% A, 26% C, 15% G, 32% T), as in control region sequences of other birds (e.g. Baker and Marshall 1997). And (5) sequences were highly variable, with the proportion of sites that were variable being highest in Domain I (39 [19%] of 210 sites) and lowest in Domain II (27 [6.3%] of 426 sites; Appendix I).

Eighty-five composite haplotypes, defined by 116 variable sites, were found among 340 common murres analyzed. Preliminary phylogenetic analyses revealed the presence of two highly divergent lineages (tree not shown); comparison of sequences with equivalent sequences from thick-billed murres (Patirana 1998; M. Damus, unpubl. data) indicated that one clade, including 12 or 3.5% of samples, grouped with thick-billed murres, and that some sequences were identical to those for thick-billed murres. Birds with these haplotypes (including four from the Barren islands, six from the Chukchi Sea, and two from the Alaska Peninsula, some of which also possessed unusual intron alleles; Table 7) probably represent hybrids or their offspring. The remaining 328 birds possessed 74 haplotypes. These haplotypes were defined by 76 variable sites, including 52 with a transition, 19 with a transversion, four with both a transition and a transversion, and one with both a transversion and an insertion/deletion (indel) (Table 8). Most (39) variable sites occurred within Domain I (Appendix I). Haplotypes differed from each other by one to 21 substitutions (Figs. 6, 7).

Chakraborty's test of population amalgamation indicated an excess of alleles within the sample from Cook Inlet ($P < 0.01$); otherwise, none of the tests for deviations from neutrality were statistically significant after Bonferroni corrections were applied (all $P > 0.10$). However, the mismatch distributions were distinctly wave-like and did not differ from the distributions expected under a sudden population expansion, either for the total sample (Fig. 6) or for individual regions (distributions not shown; all $P > 0.10$); furthermore, estimates of Tajima's D

were significantly different from zero both for the total sample (D = -2.62, $P < 0.001$) and for two regions (Table 9). Nucleotide diversity (π) was an order of magnitude higher within the sample from the Shumigan islands than for other regions, but otherwise was similar among regions (Table 9) and averaged 0.264% (se = 0.279%).

Population genetic structure.-Haplotype 01 occurred in 123 murres, and haplotype 02 occurred in 89 murres; both haplotypes were found within all regions, and other haplotypes were recovered in only one to four individuals each (Table 7). No evidence of population genetic structure was found. Global Φ_{st} was low (0.011) and not significantly different from zero (P = 0.078). None of the pair-wise estimates of Φ_{st} between regions was statistically significant (Table 10), and hierarchical AMOVAs with different groupings of regions did not result in significant values of either Φ_{ct} or Φ_{st} (all $P > 0.10$). Furthermore, haplotype frequencies did not differ significantly from a random distribution overall (exact P = 0.17), although haplotype frequencies differed significantly between some pairs of regions (Table 7). Log-linearized estimates of Φ_{st} tended to increase with distance between regions, but the correlation did not attain statistical significance (Mantel's test, r = 0.17, P = 0.08).

Population history and gene flow.-The statistical parsimony tree comprised two main "hubs" (haplotypes 01 and 02) from which most other haplotypes differed by only one or two substitutions (Fig. 7). The tree also contained a few longer branches with "missing" haplotypes (haplotypes that either are extinct or were not sampled). Haplotypes 01 and 02 had the highest root probabilities (0.08); the next highest probabilities were much lower (0.04; several haplotypes). No phylogeographic structure was evident. Nested clade analysis revealed one case of range expansion, and six cases of restricted gene flow with isolation by distance at different levels in the gene tree (Fig. 8; Table 11). Otherwise, the null hypothesis of no geographical association of haplotypes could not be rejected; given the comprehensive sampling, this result suggests that Pacific common murres are essentially panmictic (Templeton 1998).

Because the mismatch distributions and estimates of Tajima's D suggested that Pacific common murres have undergone a population expansion, neither the estimates of Φ_{st} nor coalescent theory were used to derive estimates of contemporary gene flow.

<u>*Nuclear Loci*</u>

Variability.-Sequences of the most common alleles for four introns for common murres differed from those of thick-billed murres by 0.95-2.30% (Fig. 9). All four introns were highly variable in common murres, with between 12 and 27 alleles (Table 12); after elimination of alleles found only in putative hybrid birds (see Results for Mitochondrial Control Regions, above), the number of alleles varied between eight and 21. Most differences between alleles involved transitions, but several involved insertions or deletions (up to 4 bp long; Table 13). For all four introns, one or two alleles were present at high frequency at most sites, and the remaining alleles occurred in only one or two individuals each (Table 12). An excess of homozygotes was found for the enolase intron among the samples from the Eastern Alaska Peninsula and Cook Inlet (both $P <$ 0.001); otherwise, no deviations from Hardy-Weinberg equilibrium were found. Genetic

diversity (heterozygosity) was high for all regions except Washington, where it was almost an order of magnitude lower than in other regions (Table 9).

All five putative microsatellite loci that were amplified from common murres exhibited the "stutter" typical of dinucleotide repeats on autoradiograms, had alleles that differed in size by increments of two (the size of the repeat units), and had levels of variability characteristic of microsatellite loci (between 8 and 25 alleles; Table 12). Locus Ulo12a12 exhibited significant excesses of homozygotes within five regions (all $P < 0.001$), suggesting the possible existence of a null (non-amplifying) allele; this locus was eliminated from further analyses. After exclusion of data from this locus and from putative hybrids, a homozygote excess was found in locus Ulo12a22 within the Californian samples, but no other deviations from Hardy-Weinberg expectations were found. As with the introns, one or two alleles were dominant throughout the species' range, and most other alleles occurred in only one or two individuals each (Table 12). Heterozygosity approached 100% in all regions (Table 9).

Linkage appeared to exist between alleles for the enolase, crystallin and P40 introns within the samples from Oregon, and between alleles for Ulo12a22 and Ulo14b29 within samples from the Sea of Okhotsk (all $P < 0.001$); otherwise, no deviations from linkage equilibrium were found.

Population genetic structure.-Little evidence of population genetic structure was found in either the introns or the microsatellites: global F_{st}s did not differ significantly from 0 ($F_{st} = -0.14$ for introns; $F_{st} = -0.02$ for microsatellites; $P > 0.50$); none of the exact tests of population differentiation were significant (all $P > 0.15$); and only four of 136 pair-wise estimates of F_{st} were significant after Bonferroni corrections were applied. Alternative groupings of regions did not result in significant estimates of F_{ct} in hierarchical AMOVAs for either type of locus. Mantel's test indicated a weak but statistically significant correlation between log-linearized estimates of F_{st} and linear distance between regions for microsatellites ($r = 0.20$, $P < 0.05$), but not for introns. Furthermore, no evidence of population genetic structure was found when data for introns and microsatellites were combined ($F_{st} = -0.19$, $P > 0.50$; exact $P = 1.00$), although exact tests for region pairs indicated significant differences in allele frequencies between samples from the Pribilof islands and most other regions (all $P < 0.001$; Table 10). No correlation between genetic and geographic distance between regions was detected using a Mantel's test ($r = -0.03$, $P = 0.59$).

Gene flow.-Of 366 individuals assayed for all eight nuclear loci, 17 (4.6%) had high probabilities (> 0.99) of being immigrants, although the origins of many of these birds could not be assigned with confidence (Table 14). Given that power for detecting migrants at $\alpha = 0.01$ was variable and often very low (range = 0.13 to 0.99), migration rates are probably higher than this estimate. No single region dominated as either a major source or a major recipient of migrants.

Discussion

Hybridization

Twelve (3.5%) of 340 common murres had mtDNA sequences of thick-billed murres; several of these individuals also had unusual intron and/or microsatellite alleles. All were found in areas of sympatry with thick-billed murres (the Chukchi Sea, Pribilof islands, Barren islands, and Eastern Alaska Peninsula), suggesting that the two species hybridize. Common murres with thick-billed murre mtDNA also have been found in the North Atlantic (Friesen et al. 1993; G. Ibarguchi, unpubl. data), and thick-billed murres with mtDNA sequences of common murres have been found in the North Pacific (M. Damus, unpubl. data).

Population Genetic Structure

Results of both the Mantel's test on microsatellites and the nested clade analysis suggest that some isolation-by-distance exists among Pacific common murres. Otherwise, results of the present analyses indicate that little or no population genetic structure exists among these birds: indices of global population genetic structure did not differ significantly from 0 for any locus; few of the pair-wise estimates of region differentiation were statistically significant (Table 10); nested clade analysis suggested that they are essentially panmictic; and assignment tests suggested that at least 4.6% of birds originated in regions other than where they were sampled. These results are similar to those for Atlantic common murres (Moum et al. 1991; Moum and Árnason 2001; M. Damus unpubl. data) and thick-billed murres in both the Atlantic and Pacific (Birt-Friesen et al. 1992, M. Damus unpubl. data), but contrast with results for black guillemots (*Cepphus grylle*, Kidd and Friesen 1998b), razorbills (*Alca torda*, Moum and Árnason 2001), marbled murrelets and pigeon guillemots (see below). The present results also question classification of Pacific common murres into two subspecies: *U. a. californica* (those breeding between California and Washington) and *U. a. inornata* (all others), suggesting that morphological differences between the subspecies either arose very recently, or do not have an underlying genetic basis. Indeed, the only real difference among these subspecies is a relatively slight difference in body size (Ainley et al. 2002), which could be explained as a simple north-south cline associated with differing environmental conditions.

Population History

Estimates of Tajima's *D*, the mismatch distribution (Fig. 6) and the nested clade analysis all suggest that Pacific common murres underwent an historical population expansion; similar results have been found in other species of seabirds, including Atlantic common and thick-billed murres (Birt-Friesen et al. 1992, Friesen et al. 1996b; Moum and Árnason 2001). Nested clade analysis revealed little evidence of historical restrictions in gene flow (e.g. multiple glacial refugia). This is similar to recent findings for Atlantic thick-billed murres (M. Damus, unpubl. data) and ancient murrelets (*Synthliboramphus antiquus*; Pearce et al. 2002), but differs from many other species of seabirds such as Atlantic common murres (Friesen et al. 1996b) and black guillemots (*Cepphus grylle*, Kidd and Friesen 1998b).

Pigeon Guillemots

Results

Mitochondrial Control Region

Variability.-Sequences obtained in the present study were similar to those published previously for guillemots (Kidd and Friesen 1998a), and contained the conserved sequence blocks typical of other species of birds (F, D, and C Boxes and CSB-1; Baker and Marshall 1997; Fig. 5). Base composition was biased against Gs and towards Ts (23.9% A, 26.1% C, 16.0% G, 34.1% T).

A total of 73 haplotypes, defined by 83 variable sites, was identified among 186 individuals (Table 15). Two variable sites involved insertions or deletions, 21 involved transversions, and 63 involved transitions. No variable sites were found in the conserved sequence blocks, 61 occurred in Domain I, 20 occurred in Domain II, and 2 occurred in Domain III. Haplotypes differed from each other by one to 41 differences (Figs. 10, 11).

Ewens-Watterson and Chakraborty tests did not reveal any deviations from expectations of selective neutrality for any region except for British Columbia, which had significantly more haplotypes than expected (16 *vs* 9.4; $P < 0.01$; all other $P > 0.10$). The mismatch distributions were slightly ragged but did not deviate from the distributions expected under a sudden population expansion, either for the total sample (Fig. 10) or for individual regions (not shown). Tajima's D was significantly less than zero for two regions, but otherwise did not differ from zero either for individual regions (Table 17) or for the entire sample ($D = -1.37$, $P = 0.08$). Nucleotide diversity (π) was similar among regions (Table 17) and averaged 1.08% for all samples combined (se = 0.06%).

Population genetic structure.-Population structure in guillemots was high compared to common murres. One of the 73 haplotypes was found in five of the seven regions; most other haplotypes occurred in only one or two regions, several occurred at high frequency, and with the exception of haplotype BH1 (which was shared between Prince William Sound and the Alaska Peninsula), shared haplotypes were always found in geographically adjacent regions (Table 16). Results from AMOVA indicated strong geographic structuring in sequence variation (global $\Phi_{st} = 0.34$, $P < 0.001$). Similarly, estimates of Φ_{st} were statistically significant for all pair-wise comparisons of regions except for Prince William Sound versus Kachemak Bay (Table 18). When regions were grouped by subspecies in a hierarchical AMOVA, the among-group component of variation was statistically significant ($\Phi_{ct} = 0.17$, $P < 0.05$) but a significant proportion of variation remained among regions within groups ($\Phi_{sc} = 0.31$, $P < 0.001$). The highest among-group component of variation was found when regions were classified into four groups: (1) Aleutian islands, (2) Alaska Peninsula, Kachemak Bay and Prince William Sound, (3) British Columbia, and (4) California and Oregon ($\Phi_{ct} = 0.37$, $P < 0.01$); however, a significant amount of variation still remained among regions within groups ($\Phi sc = 0.08$, $P < 0.001$), suggesting that variation is best explained when each region is treated separately (i.e. not grouped). Mantel's test indicated a significant correlation between genetic and geographic distance between regions ($r = 0.56$, $P = 0.004$; Fig. 12a). All pair-wise estimates of corrected percent sequence divergence (δ) were highly significant (Table 18). Neighbor-joining on Slatkin's linearized F_{st} indicated a major

genetic discontinuity between the Aleutian islands and other regions, with an isolation-by-distance pattern among the mainland samples (Fig. 13).

Population history and gene flow.-In the statistical parsimony tree, four haplotypes from the Aleutian islands (CD8, CE9, CN8 and CP9) formed a highly distinct monophyletic clade that exceeded the 95% connection limit (Fig. 11); maximum likelihood analysis using PHYLIP (version 3.4; Felsenstein 1989) suggested that this clade connects to the remaining haplotypes through haplotype CU15. Otherwise, the tree included several long branches, as well as numerous 'hubs' from which several haplotypes radiated. Some clades were geographically widespread, although other clades included haplotypes that were found either in a single region or in neighboring regions, suggesting weak phylogeographic structure. The haplotype with the highest outgroup weight in the Aleutian clade was CD8 (0.50); the haplotype with the highest outgroup weight in the rest of the tree was AE1 (0.09; found in California and Oregon). The haplotypes with the next highest weights were CP8 (0.25), and AN1 and AV1 (0.07), respectively.

The nesting design included five levels (Fig. 14). Clade 5-1 was restricted entirely to the Aleutian islands and Russia, whereas Clade 5-2 was restricted entirely to mainland North America (with one exception), suggesting historical fragmentation of guillemots into northwestern and eastern populations (Fig. 15). None of the tests within Clade 5-1 were significant. However, sample sizes were very small, and several clades nested within Clade 5-1 were restricted to individual islands or neighboring islands (not shown); thus, the possibility that significant phylogeographic structure exists within the Aleutian islands should be explored with larger sample sizes. Results of tests within Clade 5-2 reveal a dynamic demographic history, including isolation by distance (Clades 1-67, 3-13 and 5-1), range expansions (Clades 2-24, 3-11, 4-1 and 4-4), and long-distance dispersal events (Clades 3-12 and 4-1) (Figs. 14, 15). No indication of allopatric fragmentation within mainland North America was found.

<u>*Nuclear Loci*</u>

Variability.-Five to six alleles, defined by variation at four or five sites, were found within each of the three introns (Fig. 16; Table 19); alleles differed by one to three substitutions, most of which involved transitions. For all four putative microsatellite loci, alleles differed in size by increments of two, and autoradiograms exhibited the 'stutter' typical of microsatellites; numbers of alleles ranged from three to 11 (Table 20). For all seven nuclear loci, one or two alleles were present at high frequency at most sites, and the remaining alleles occurred in only one or two individuals each (Table 20). Significant deviations from linkage equilibrium were found among P40, cytochrome *c*, and rhodopsin within samples from British Columbia (all $P < 0.01$). However, no evidence was found for differentiation among sampling sites in either Prince William Sound or British Columbia, and no evidence was found for deviations from Hardy-Weinberg equilibrium for any locus after Bonferroni corrections were applied. Samples were therefore pooled into seven regions for further analyses (Table 2). Genetic diversity (heterozygosity) was high for all regions, and averaged 0.84 for introns, and 0.96 for microsatellites (Table 17).

Population genetic structure.-Estimates of global population genetic structure were weak but statistically significant both for introns (F_{st} = 0.03, $P < 0.001$) and for all loci combined (F_{st} = 0.067, $P < 0.001$); estimates for microsatellites were higher, and also significant (F_{st} = 0.11, $P < 0.001$; R_{st} = 0.09, $P < 0.001$). Pair-wise comparisons of regions both for microsatellites and for all loci combined indicated that guillemots from California, Oregon, and British Columbia are significantly different both from the Alaskan samples and possibly from each other (Table 21). In hierarchical AMOVAs based on all loci, variation was best explained when samples were placed in three groups: (1) Alaska; (2) British Columbia; and (3) Oregon and California (F_{ct} = 0.084, $P < 0.001$; F_{sc} = 0.005, ns). Grouping sampling sites by subspecies decreased the proportion of variation due to groups and significantly increased the proportion of variation among regions within groups (F_{ct} = 0.064, $P < 0.05$; F_{sc} = 0.035, $P < 0.001$). The neighbor-joining tree based on Slatkin's linearized F_{st} showed a similar structure, with a fundamental division between the Alaskan samples and those from farther south, and a deep split between samples from British Columbia vs. Oregon and Washington; guillemots from the Alaska Peninsula also were separated from samples from the rest of Alaska (Fig. 13b). Mantel's tests were highly significant for introns (r = 0.66, $P < 0.001$), microsatellites based on either the infinite alleles model (r = 0.75, $P < 0.01$) or the step-wise mutation model (r = 0.74, $P < 0.05$), and all loci combined (r = 0.82, $P < 0.01$; Fig. 12b).

Gene flow.-Of 187 individuals assayed for all seven nuclear loci, three (1.6%) had high probabilities (> 0.99) of being immigrants: one guillemot sampled in Kachemak Bay was probably from Prince William Sound, one sampled from Kachemak Bay was probably from California, and one sampled in Oregon was probably from the Aleutian islands. Given that power for detecting migrants at α = 0.01 was variable and often very low (range = 0.13 to 0.99), a higher proportion of birds may have been immigrants.

Discussion

Population Genetic Structure

Population structure in mtDNA in pigeon guillemots is strong relative to common murres (above), marbled murrelets (below), and most other bird species that have been studies (Friesen 1997): the estimate of global Φ_{st} for the mitochondrial control region was high (0.34) and statistically significant; almost all pair-wise estimates of Φ_{st} were significant (Table 18); pair-wise estimates of Φ_{st} were strongly correlated with geographic distance between regions (Fig. 12a); estimates of percent sequence divergence between regions were high and statistically significant (Table 18); and sampling sites tended to cluster on the gene tree (Table 16; Fig. 11). Marked population structuring was also indicated by nuclear DNA, although it was weaker than for mtDNA: estimates of global F_{st} were significant for all combinations of loci; most pair-wise comparisons of regions were significant (Table 21); and pair-wise estimates of F_{st} were strongly correlated with geographic distance between regions (Fig. 12b). Hierarchical AMOVAs both for the control region and for the nuclear loci suggested that genetic variation in pigeon guillemots is best explained when samples are placed in at least three groups: (1) Alaska, (2) British Columbia, and (3) California and Oregon. Control region sequences suggested that guillemots from the Aleutian islands also may be genetically distinct from those elsewhere, although this distinction was not apparent in any of the nuclear loci.

Current taxonomy appears to only partially reflect the distribution of neutral genetic variation in pigeon guillemots. Hierarchical AMOVAs indicate that guillemots in California and Oregon (*C. c. eureka*) are genetically different from those in British Columbia and Alaska (*C. c. adianata*), but those in British Columbia also appear to differ from those in Alaska (Prince William Sound and west), and possibly should be given separate subspecific status. The genetic distinctiveness of guillemots in the Aleutian islands (*C. c. kaiurka* and *C. c. adianata*), the Alaska Peninsula and southeastern Alaska (*C. c. adianata*) should be examined.

Population History and Gene Flow

Results of the nested clade analysis (Figs. 14, 15) suggest that guillemots from the Aleutian islands and Russia were historically isolated from guillemots elsewhere. Estimates of Φ_{st} and δ also were greatest between Aleutian Island and mainland samples (Table 18), and the Aleutian islands represented a basal split within the mtDNA population tree (Fig. 12a). Assuming a molecular clock and a divergence rate of ~11%/my for guillemot control regions (Vigilant et al. 1991), mean corrected sequence divergence between these two groups (1.22%) suggests a divergence date of 110,000 ya, i.e. prior to the Wisconsin glaciation. A divergence rate of 20%/my (Wenink *et al.* 1993) would date this divergence at ~60,000 ya; a rate of 2%/my (Shields and Wilson 1987) would place it at ~240,000 ya. Both estimates predate the last glacial maximum. Thus, differentiation of Aleutian islands versus mainland North American guillemots may be explained at least in part by historical fragmentation, probably by extensive Pleistocene ice fields that would have separated tracts of rocky coastline from each other. These findings agree with Udvardy's (1963) hypothesis that geographic variation in pigeon guillemots is due to isolation in multiple glacial refugia. However, guillemots from the Aleutian islands did not differ from those from the rest of Alaska in nuclear DNA, probably because of differences in mutation rates or effective population sizes between the two types of markers.

The nested clade analyses did not reveal any other instances of historical fragmentation, but suggested a complex and dynamic demographic history for guillemots within mainland North America, including clear cases of range expansion and long-distance dispersal (Figs. 14, 15):

Range expansion.-The range expansions indicated by the NCA are supported by indications of a population expansion by the mismatch distributions (Fig. 10), and several negative estimates of Tajima's *D*. The NCA suggested that the range expansion occurred from the south into Alaska, which is compatible with a northerly recession of glaciers in the northeastern Pacific during the Pleistocene.

Long-distance dispersal.-NCA revealed two cases of long-distance dispersal, including one from Kachemak Bay to the Alaska Peninsula/Aleutian islands (Clade 3-12; Figs. 14, 15). Long-distance dispersal also was indicated by the nuclear data: assignment tests suggested that two birds breeding in Kachemak Bay were immigrants (one from Prince William Sound and one from California). Banding data have also revealed a case of long-distance dispersal: one guillemot banded as a chick in California was found breeding in British Columbia (Ainley *et al.* 1990).

Despite historical range expansions and long-distance dispersal, contemporary gene flow is clearly restricted: population genetic structure is strong; NCA identified two cases of isolation by distance (Figs. 14, 15); Mantel's tests were significant for all molecular markers (e.g. Fig. 12); population trees suggested an isolation-by-distance pattern (Fig. 13); and assignment tests detected only three migrants out of 187 birds (although statistical power was low).

Marbled Murrelets

Results

Mitochondrial Control Regions

Variability.-Continuous sequence was derived for the $tRNA^{glu}$ for two marbled murrelets and one Kittlitz's murrelet, and for most of the mitochondrial control region for one marbled murrelet (Fig. 5, Appendix II). The $tRNA^{glu}$ sequences of the two species differed by only 1 bp (Fig. 5), and could be folded into a clover-leaf structure appropriate for a functional tRNA for glutamic acid (Desjardins and Morais 1990). Sequences of the F, D and C boxes and CSB-1 of the marbled murrelet differed little from those of the pigeon guillemot or common murre (Fig. 5). As in other birds (including guillemots and murres; Quinn & Wilson 1993; Baker and Marshall 1997; Kidd and Friesen 1998a), a poly-C repeat occurred at the start of the control region, a poly-T repeat occurred in Domain II (Appendix II), and a microsatellite-like motif ($[CAACAAA]_n$) occurred at the 3' end of Domain III (not shown). Base composition was biased against Gs (25% A, 29% C, 16% G. 29% T), as in other species of birds (Baker and Marshall 1997). Sequences were highly variable, with the proportion of sites that were variable being highest in Domain I (42 [12%] of 362 sites) and lowest in Domain II (9 [4.1%] of 218 sites; Appendix II).

Thirty-five haplotypes were identified within a 580 bp fragment of the 5' end of the control region among 80 marbled murrelets (Table 22). Haplotypes were defined by 51 variable sites, of which 38 involved transitions, 11 involved transversions, two included both a transition and a transversion, and none involved insertions or deletions (Appendix II). Most (42) variable sites occurred within Domain I (Appendix II). Haplotypes differed from each other by one to 17 substitutions (Figs. 17, 18).

Because sample sizes for the Aleutian islands and Alaska Peninsula were small, samples from these areas were pooled into two groups for further analyses: (1) Western and Central Aleutian islands, and (2) Eastern Aleutian and Shumigan islands and Mitrofania Bay. None of the Ewen-Watterson tests for selective neutrality or Chakraborty's tests for population amalgamation were significant, either for the total sample or for individual regions (all $P > 0.10$). However, the mismatch distributions were distinctly wave-like and did not differ from the distributions expected under a sudden population expansion, either for the total sample (Fig. 17) or for individual regions (distributions not shown; all $P > 0.10$). Tajima's D was significantly less than zero when all samples were pooled ($D = -1.72$, $P < 0.05$), but not for individual regions (Table 24). Genetic diversity (π) within Prince William Sound was 0 (only one haplotype was found); otherwise, variabilities were similar among sites (Table 24), and averaged 0.88 when all samples were pooled.

Population genetic structure.-Haplotype 05 was found in six of the nine regions; all other haplotypes occurred in only one or two regions, at frequencies between 6% and 100% (Table 23). Haplotype frequencies differed significantly from a random distribution both overall (exact $P < 0.001$) and between some pairs of regions (Table 25), and AMOVA indicated weak but significant population structure (global $\Phi_{st} = 0.090$, $P < 0.001$). However, none of the pair-wise

estimates of Φ_{st} were significant after Bonferroni corrections (Table 25). Hierarchical AMOVAs did not increase the proportion of variation that could be explained by differences among groups (all $\Phi_{ct} < 0.045$), and all groupings that were tested retained a significant proportion of variation among regions within groups (all $P < 0.05$), suggesting that variation is best described when regions are not grouped. Nesting habitat did not explain any of the variation ($F_{ct} = -0.009$, $P = 0.52$; $F_{sc} = 0.093$, $P < 0.001$). Estimates of Slatkin's linearized F_{st} were not correlated with geographic distance between regions (Mantel's test, $r = 0.05$, $P = 0.37$). Since none of the pair-wise estimates of Φ_{st} were significant (Table 25), a population tree was not generated.

Population history and gene flow.-The statistical parsimony tree included a number of hubs from which other haplotypes radiated, as well as several branches with missing haplotypes (Fig. 18). Haplotype 02 had the highest root probability (0.16), followed by haplotype 05 (0.15). Some tip clades had restricted ranges (Fig. 19), suggesting the existence of weak phylogeographic structure, but no major phylogeographic subdivisions were evident. Nested clade analysis did not provide any evidence of population fragmentation, but gave strong evidence for isolation-by-distance (clades 3-2, 3-4, 4-1 and 4-2) as well as range expansions into central California and the Aleutian islands (clades 2-4 and 2-10; Fig. 19).

Because the mismatch distributions, estimates of Tajima's *D* and nested clade analysis all suggested that marbled murrelets underwent an historical population expansion, neither the estimates of Φ_{st} nor coalescent theory could be used to derive estimates of contemporary gene flow.

<u>*Nuclear Loci*</u>

Variability.-Nine introns were screened in 120 murrelets; all nine introns were variable, with the number of alleles per locus ranging from 4 to 14 (Fig. 2 in Congdon et al. 2000). Most alleles differed by only one or two mutations, and most mutations involved transitions (Fig. 2 in Congdon et al. 2000). Other than an excess of homozygotes for the aldolase intron within British Columbia, genotype frequencies did not deviate from the expectations of Hardy-Weinberg equilibrium (all $P > 0.05$). Heterozygosity was high in all regions, and averaged 1.00 (Table 24).

Two of the five loci amplified using primers designed for microsatellites for alcids (Bma9-28 and Ulo14b-29; Table 6) did not exhibit patterns of variation typical of microsatellites: each possessed only two or three alleles (Table 26), and the autoradiograms did not exhibit the "stutter" typical of dinucleotide repeats. A third locus (Bma10-18) had many alleles that differed in size by increments of a single nucleotide; direct sequence analysis revealed that it contained a complex repeat, and so probably did not follow a simple step-wise mutation model. These three loci were excluded from analyses of population genetic structure and gene flow that assumed a step-wise mutation model, but were retained for analyses that assumed an infinite alleles model of mutation. The remaining two loci (Cco5-21 and Uaa5-8) had levels of variability characteristic of microsatellite loci (ten and six alleles each; Table 26), exhibited the typical "stutter" on autoradiograms, and had alleles that differed in size by increments of two. A significant excess of homozygotes was found at both loci within the sample from Kodiak Island ($P < 0.05$), a slight but significant excess of homozygotes was found for Cco5-21 for the total

sample, and a slight but significant deficit of homozygotes was found for Uaa5-8 for the total sample; otherwise, no evidence was found for deviations from Hardy-Weinberg equilibrium. Heterozygosity was high in all regions, and averaged 0.98 (Table 24). No evidence was found for linkage among any loci within any region.

Between two and eight alleles were found within each of five intron loci and the mitochondrial cytochrome *b* gene in Kittlitz's murrelets (Table 2 in Pacheco et al. 2002). No alleles were shared between samples of the two species for any locus. Alleles from the two species differed by three to 20 mutations, including up to ten transversions and up to three indels, and formed separate clusters within the gene trees for all six loci (Figure 1 in Pacheco et al. 2002).

Population genetic structure.-Most intron alleles were found in all regions (Congdon et al. 2000). None of the exact tests of population differentiation were significant for comparisons based on introns; however weak but statistically significant population structure was found (global $\Phi_{st} = 0.021$, $P < 0.01$; Congdon et al. 2000), and estimates of F_{st} between pairs of regions involving the Western and Central Aleutians tended to be significant (Table 27). Variation was best explained when regions were placed in three groups: (1) Western Aleutian islands, (2) Central Aleutian islands, and (3) mainland North America ($\Phi_{ct} = 0.094$, $P < 0.001$; $\Phi_{sc} = -0.004$, $P = 0.71$; Table 1 in Congdon et al. 2000). However, placing regions in two groups (Aleutian islands versus mainland North America) also resulted in a high value for Φ_{ct} (0.089, $P < 0.05$; $\Phi_{sc} = -0.002$, $P = 0.58$; Table 1 in Congdon et al. 2000). Grouping regions by nesting habitat did not improve the explanation of the variation ($\Phi_{ct} = 0.017$, $P < 0.01$; $\Phi_{sc} = 0.045$, $P < 0.01$). Pair-wise estimates of Φ_{st} were significantly correlated with geographic distance between regions ($r = 0.58$, $P < 0.001$; Figure 6 in Congdon et al. 2000).

Several alleles occurred at most or all regions for each of the microsatellite loci; the rest occurred in only one or two regions each (Table 26). AMOVA based on the two microsatellite loci that seemed to follow a step-wise mutation model indicated weak but statistically significant population structure ($R_{st} = 0.031$, $P < 0.01$). Estimates of R_{st} were significant for three pair-wise comparisons of regions, all of which involved California (Table 25). Exact tests of population differentiation also were significant for several comparisons involving California (Table 25). Hierarchical AMOVAs indicated that variation was best explained when regions were placed in three groups: (1) Western Aleutian islands, (2) California, and (3) all others ($F_{ct} = 0.075$, $P < 0.05$; $F_{sc} = -0.002$, $P = 0.50$). Grouping samples by nesting habitat did not increase the proportion of variation due to group ($F_{ct} = 0.023$, $P < 0.05$; $F_{sc} = 0.021$, $P < 0.01$). Mantel's test indicated a significant correlation between Slatkin's linearized F_{st} and distance between regions ($r = 0.45$, $P < 0.05$; not shown).

None of the exact tests of population differentiation based on all 14 nuclear loci (excluding the Eastern Aleutian islands and California, for which data were only available for two loci) were significant. However, AMOVA indicated weak but statistically significant population structure (global $F_{st} = 0.02$, $P < 0.001$); in pair-wise comparisons of regions, the Western and Central Aleutian islands appeared to be different from most other regions (Table 27). Variation was best explained when regions were placed in three groups: (1) Western Aleutian islands, (2) Central

Aleutian islands, and (3) all other regions (F_{ct} = 0.062, $P < 0.05$; F_{sc} = 0.004, ns); however, F_{sc} was only slightly lower if the Western and Central Aleutian islands were grouped together (F_{ct} = 0.060, $P < 0.05$; F_{sc} = 0.004, ns). Grouping regions by nesting habitat did not provide a satisfactory explanation of the variation (F_{ct} = 0.004, ns). Mantel's test indicated a significant correlation between Slatkin's linearized F_{st} and geographic distance between regions (Fig. 20). Neighbor-joining based on Slatkin's linearized F_{st} placed the two locations from the Aleutian islands together, apart from the other regions (Fig. 21).

Gene flow.-Power on assignment tests based on all 14 nuclear loci ranged from 0.36 to 1.00, but was generally greater than 0.80. Assignment tests suggested that three (2.5%) of 121 murrelets did not originate in the regions from which they were sampled: one murrelet sampled in Southeastern Alaska appeared to originate in the Western Aleutian islands; one sampled in British Columbia appeared to have originated in the Central Aleutian islands, and one murrelet sampled in British Columbia appeared to have originated in either the Western Aleutian islands or Southeastern Alaska.

Discussion

Hybridization

Intron sequences suggest that none of the murrelets screened in the present study were F_1 hybrids between marbled and Kittlitz's murrelets, and probably none of 121 murrelets screened for five or more introns are either F_2 (P = 0.001) or back-cross hybrids (P = 0.031). Given that 118 of these murrelets are from areas of sympatry, hybridization between the two species must not be common. More samples, especially from Kittlitz's murrets and from murrelets with apparent hybrid morphologies, are needed to exclude the possibility of recent hybridization.

The fact that alleles and haplotypes from the two species are reciprocally monophyletic on the gene trees (Figure 1 from Pacheco et al. 2002) indicates that little or no hybridization has occurred between these species historically. Sequence divergence between the species suggests that their gene pools have been independent for 1.8-5.7 mya (Pacheco et al. 2002).

Population Genetic Structure

Several results suggest that weak but significant population genetic structure exists within marbled murrelets, and that gene flow is partially restricted:

Estimates of F_{st} and its analogs for most molecular markers indicated that 0.08-0.09% of genetic variation is distributed among regions (Table 28). Results for introns and microsatellites both suggest that murrelets from the Western Aleutian islands are distinct from those elsewhere (Tables 25, 27); variation in the introns suggests that murrelets from the Central Aleutian islands also may be distinct, and variation in microsatellites indicates that murrelets from California may differ from those elsewhere.

Correlations between pair-wise estimates of population differentiation and geographic distance were significant both for introns (Congdon et al. 2000) and for microsatellites (present results).

Nested clade analysis indicated significant isolation-by-distance effects (Fig. 19).

Assignment tests detected only three immigrants among 121 birds sampled, despite relative high statistical power. Furthermore, estimates of migration rates based on coalescent theory for

introns indicated the gene flow is restricted, especially into peripheral areas (Congdon et al. 2000).

Population History

Previous analyses of the mitochondrial cytochrome *b* gene among 34 marbled murrelets from the Western Aleutian islands to Oregon suggested that marbled murrelets underwent an historical population expansion (Friesen et al. 1996a). Results of the present study support this hypothesis: the mismatch distributions for the control region and several of the introns were wave-like (Fig. 17; Fig. 3 in Congdon et al. 2000), and did not differ from the distributions expected under a sudden population expansion; some estimates of Tajima's *D* were significantly less than zero; and the nested clade analysis revealed at least two range expansions (Fig. 19). The fact that the range expansions detected by the nested clade analysis were at low nesting levels in the gene tree (level 2; Fig. 19) suggests that the expansions were relatively recent in evolutionary time.

Congdon et al. (2000) suggested that genetic differences between marbled murrelets from the Aleutian islands versus mainland North America could best be explained by historical isolation of murrelets in two or more Pleistocene refugia. They further suggested that current differences are maintained through isolation-by-distance combined with small population sizes and fragmented habitat in the Aleutian islands. The present analyses, which include additional results from mtDNA and microsatellites, support the hypothesis of isolation by distance. However, nested clade analysis did not provide any evidence for historical fragmentation, and suggested instead that population differentiation has arisen recently. Furthermore, results from both the nested clade analysis (present results) and methods based on coalescent theory (Congdon et al. 2000) indicated that gene flow into peripheral areas is restricted. Thus, genetic divergence of murrelets in the Western Aleutian islands and California is probably a result of peripheral isolation, rather than historical fragmentation.

Congdon et al. (2000) also argued that population genetic structure in marbled murrelets is probably not a result of selection associated with nesting habit. Analyses of allozymes (Friesen et al. 1996a), mtDNA (Pitocchelli et al. 1995, Friesen et al. 1996a, present results), introns (Congdon et al. 2000) and microsatellites (present results) all failed to detect genetic differences between murrelets from tree- versus ground-nesting areas. Together, these results suggest that nesting habit is more likely to be an environmentally induced decision than an evolutionary adaptation in murrelets. Note however that population subdivisions in neutral molecular markers do not necessarily correspond in space with differences in selective regimes (Endler 1977); thus, the possibility that nesting habit has a genetic basis cannot be excluded with the present data.

Conclusions

Four of the original seven objectives for this project are best addressed by delimiting evolutionarily significant units (ESUs) and genetic management units (MUs). An ESU is defined "as a set of populations with a distinct, long-term evolutionary history" (Moritz 1994), and equates with 'species' by many definitions. MUs are defined as "populations that exchange so few migrants as to be genetically distinct", such populations logically being demographically independent (Moritz 1994). Moritz advocated that ESUs be recognized on the basis of phylogeographically distinct alleles at mutliple loci, wheres MUs be delineated by signficiant divergences in allele frequencies. MUs equate with populations as defined in the Introduction, and so Objective 1 [to determine the geographic extent of the populations affected by the spill] is met by delineating MUs. Since birds from sites within the same MU will be genetically similar, delineation of MUs also meets Objectives 3 [to identify appropriate reference or 'control' sites for monitoring] and 6 [to identify appropriate sources for translocations]). Given the many assumptions involved in estimating genetically effective population size, genetic variation is best assessed by comparing indices of genetic diversity directly between species. Thus, the original restoration objectives will be addressed for each species under five headings: Genetic Management Units (Objectives 1, 3, 6); Source and Sink Regions (Objective 2); Cryptic Species (Objective 4); Genetic Variation and Inbreeding (Objective 5); and Hybridization (Objective 7).

Common Murres

Genetic Management Units

Results of the present work indicate that Pacific common murres do not include any cryptic species or subspecies, and constitute a single ESU which includes a single genetic MU. Loss or reduction of a local population, such as by an oil spill, will not have a major impact on the species' genetic resources, and the species should repopulate the Gulf of Alaska naturally. Any ecologically equivalent location within the North Pacific should serve as a genetically suitable control site for monitoring, or source for relocations.

Source and Sink Regions

The present data provided no strong evidence that any region is serving as either a genetic source or a genetic sink, although statistical power for assignments was often low - additional loci should be analyzed to increase statistical power.

Cryptic Species

Given the very weak population genetic structure within Pacific common murres, no cryptic species or subspecies are indicated. Furthermore, the current subspecies distinctions are not supported by neutral molecular variation.

Genetic Variation and Inbreeding

An excess of homozygotes was found for the enolase intron within the Eastern Alaska Peninsula and Cook Inlet, and in microsatellite locus Ulo12a22 within the Californian samples; otherwise, there was no consistent evidence for inbreeding within any region. Genetic diversity in introns was relatively low in Washington; this may related to the fact that the number of murres in

Washington crashed during the early 1980s and has never recovered (Manuwal et al. 2001). Otherwise, genetic variability in all markers was comparable to pigeon guillemots and marbled murrelets (Tables 17 and 24, respectively).

Hybridization

Pacific common and thick-billed murres appear to hybridize, with 2.4% of phenotypically common murres carrying DNA from thick-billed murres; this percentage may be even higher in areas of sympatry. The implications of hybridization for murres are unclear, although previous studies suggest that hybrid murres may be larger than either parental species, and that back-crossing and genetic introgression may occur (Friesen et al. 1993). Comparative studies of the morphology, behavior, breeding biology, reproductive success, and ecology of hybrids should be undertaken. Furthermore, the existence of hybrids may distort population censuses of murres, and should be accounted for.

Pigeon Guillemots

Genetic Management Units

Results of the present study suggest that pigeon guillemots do not include any cryptic species, and constitute a single ESU. However, genetic structure in this species is strong, and the North Pacific population includes at least three genetic MUs: (1) Alaska, (2) British Columbia, and (3) Oregon and California. The genetic affinities of guillemots from Southeastern Alaska are unknown at present, and guillemots in the Aleutian islands and/or Alaska Peninsula may constitute additional MUs - additional samples from these areas should be analyzed. Guillemots in the spill area appear to be part of a large MU that extends from Belkofski Bay (and possibly Adak) to Prince William Sound (and possibly Southeastern Alaska). Weak population genetic structure exists within each of these MUs and gene flow is probably low; however, the species should repopulate the Gulf of Alaska slowly from neighboring areas. Any location within the Alaskan MU should serve as a suitable reference site for guillemots in the spill area and/or as a source for reintroductions.

Source and Sink Regions

The number of recent migrants that could be detected with confidence using either NCA or genetic assignments was too low to be able to identify genetic source or sink regions- additional loci should be analyzed to increase statistical power.

Cryptic Species

No cryptic species were evident within pigeon guillemots from the northeastern Pacific. Management units defined by the present data suggest that subspecies boundaries should be reassessed.

Genetic Variation and Inbreeding

No consistent evidence was found for either inbreeding or low genetic variation within any population of pigeon guillemots, and genetic variabilities were comparable both to common murres (Table 9) and to marbled murrelets (Table 24; Congdon et al. 2000) for all molecular markers.

Marbled Murrelets

Genetic Management Units

The present results indicate that murrelets from the Western Aleutian islands should be considered a MU separate from mainland Alaska and British Columbia. The possibility that murrelets from the Central Aleutian islands and California constitute additional genetic MUs requires further investigation. The genetic affinities of murrelets from Washington and Oregon are unknown at present. Marbled murrelets in the spill area appear to be part of a large MU that extends from the western end of the Alaska Peninsula to Vancouver Island (and possibly farther west and east). Although weak population genetic structure exists within this MU and gene flow is probably low, the species should repopulate the Gulf of Alaska naturally (though probably slowly). Any location within this MU should serve as a suitable reference site for monitoring murrelets in the spill area and/or as a source for reintroductions. Additional samples from the Aleutian islands, Washington, Oregon and central California should be analyzed

Source and Sink Regions

Results from assignment tests suggest that two of 30 marbled murrelets sampled from British Columbia were immigrants. Although both the sample size and statistical power were low, this result suggests that dispersal into British Columbia may be high. Given that the number of murrelets in this area is declining and that breeding success is relatively low (Burger 2002), the possibility that British Columbia is acting as a sink requires further investigation. No potential sources were indicated, possibly due to low statistical power - additional loci should be screened.

Cryptic Species

No cryptic species of marbled murrelets were evident from the present study. The existence of genetic differences between marbled murrelets from the Aleutian islands and mainland North America, and the possibility of differentiation of those in California, suggest that classification of marbled murrelets into two or three subspecies should be considered.

Genetic Variation and Inbreeding

Genetic variability appears to be high within all regions sampled (Table 24), and is comparable to other species of alcids (Tables 9, 17). Although slight homozygote excesses were found at some loci within British Columbia and Kodiak, there was no consistent evidence for inbreeding in any region.

Hybridization

Results of the present study suggest that little or no hybridization or introgression occurs between marbled and Kittlitz's murrelets.

Kittlitz's Murrelets

Previous preliminary analyses of Kittlitz's murrelets suggest that population genetic structure in this species may be strong, and that it may consist of a number of MUs, as well as two or more ESUs or cryptic species (Friesen et al. 1996a). A comprehensive analysis of population genetic structure and gene flow in this species is needed urgently, especially given its current rate of decline (van Vliet 1993, Robards et al. 2002).

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Table 1. Sampling regions, abbreviations, sites, and numbers (n) for common murres.

Region	Abbrev-iation	Site	n
Sea of Okhotsk	Okho	at sea	25
Chukchi Sea	Chuk	Cape Lisburne	17
		Cape Thompson	17
Bering Strait	Ber	Fairway Rock	6
Pribilof islands	Prib	St. George Island	12
		St. Paul Island	33
Western Aleutian islands	WAle	Agattu Island	2
		Attu Island	9
		Buldir Island	2
Eastern Aleutian islands	EAle	Bogoslof Island	6
		Kagamil Island	2
Krenitzen islands	Kren	Aiktak Island	28
Western Alaska Peninsula	WAP	Midun Island	7
Shumigan islands	Shum	Koniuji islands	13
		Poperechenoi Island	1
Eastern Alaska Peninsula	EAP	Chowiet Island	21
Barren islands	Barr	East Amatuli Island	27
Cook Inlet	Cook	Chisik & Duck islands	21
		Gull Island	27
Northern Gulf of Alaska	NGA	Middleton Island	30
British Columbia	BC	Triangle Island	24
Washington	Wash	Cape Flattery	11
Oregon	Oreg	Newport	21
California	Cal	Farallon islands	21
Total			383

Table 2. Sampling regions, abbreviations, sites, and numbers (n) for pigeon guillemots.

Region	Abbrev-iation	Site	n
Eastern Russia	Ru	Eastern Kamchatka Peninsula	2
Aleutian islands	Ale	Adak Island	9
		Anangula Island	1
		Unalaska Island	2
		Aiktak Island	5
Alaska Peninsula	AP	Belkofski Bay	7
		Semidi islands	10
		Flat Island	3
		Shuyak Island	2
Kachemak Bay	Kach	Kachemak Bay	32
Prince William Sound	PWS	Naked& Jackpot islands	30
British Columbia	BC	Cleland Island & Seabird Rocks	9
		Mandarte Island	30
Oregon	Oreg	Coos Bay	24
California	Cal	Point Reyes National Seashore	1
		Southeast Farallon Island	35
		Ano Nuevo Island	2
Total			204

Table 3. Sampling regions, abbreviations, sites, and numbers (n) for marbled murrelets.

Region	Abbrev-iation	Sampling Site	n
Western Aleutian islands	WAle	Attu Island	9
Central Aleutian islands	CAle	Adak Island	13
Eastern Aleutian islands	EAle	Dutch Harbor	15
Shumigan islands	Shum	Belkofski Bay	6
		Shumagin islands	4
		Koniuji Strait	2
Mitrofania Bay	Mitr	Mitrofania Bay	10
Kodiak Island	Kodi	Shuyak Island	14
Kachemak Bay	Kach	Kachemak Bay	16
Prince William Sound	PWS	Unakwik Fjord	10
Southeastern Alaska	SEAK	Lemesurier Island	20
British Columbia	BC	Desolation Sound	30
California	Cal	Santa Cruz	35
Total			184

Table 4. Sequences of PCR primers used to amplify the mitochondrial control region from murres, guillemots and murrelets. See Fig. 4 for approximate priming locations.

Name	Sequence
ADH1452	5'-TGGCTAAAGCAAGGCGTC-3'
BmaH600	5'-CAAAAGTGCCAAAAAGGTCGGATGTCG-3'
BmaL650	5'-GGCGTCTTCAATAAACCCTTCCAGTGC-3'
CgH549[1]	5'-GTATCGGTGAAGTACAAGTTGAGAGG-3'
CgH825	5'-TATGCCCAACAAGCATTCARTAAATA-3'
CgL56[1]	5'-GYTCAATAACCATTAATATCAAACAG-3'
CgL486[1]	5'-AGCCCAACTTGCTCTTTTGCAC-3'
CgH1006[1]	5'-TTAATGAAACTCACTGCCGTTTGTAG-3'
ND6[1]	5'-CCTAGAAAAAGCACAAAATAAGTCAT-3'
UaH389	5'-CGGGTGAGATGGTGATGTATAGCCG-3'
UaH900	5'-CGTTCGAGTATATGAACGTAGGTTG-3'
UaL50	5'-CCATTAATACACACACAGACATAACC-3'
UaL750	5'-CAATAAACCCTTCCAGTGCACCG-3'

[1]Kidd and Friesen 1998a
"Y" = degenerate for C and T; "R" = degenerate for A and G.

Table 5. Introns surveyed for common murres (COMU), pigeon guillemots (PIGU), marbled murrelets (MAMU) and Kittlitz's murrelets (KIMU), and annealing temperatures for amplifications.

Gene	Intron	Abbrevi-iation	Annealing Temperature (°C)			
			COMU	PIGU	MAMU	KIMU
aldolase[1]	III & IV	Ald	-	-	59	-
crystallin[2]	VII	Cry	65	-	-	-
cytochrome *c*[2]	I	Cyt	-	53	-	-
α-enolase[1]	VIII	Enol	60	-	60	60
glyceraldehyde-3-phosphate dehydrogenase[1]	XI	Gpd	-	-	65	65
lactate dehydrogenase[3]	III	Ldh	55	-	59	59
lamin[1]	III	Lam	-	-	57	-
myelin proteolipid protein[3]	IV	Mpp	-	-	60	60
ornithine decarboxylase[3]	VI & VII	Od	-	-	62	-
ribosomal protein 40[3]	V	P40	59	63	65	65
rhodopsin[2]	III	Rhod	-	58	-	-
tropomyosin[3]	V	Trop	-	-	65	-

[1]Friesen et al. 1997
[2]V.L.F. unpubl. data
[3]Friesen et al. 1999
- = not analyzed

Table 6. Microsatellite primers and PCR annealing temperatures for common murres (COMU), pigeon guillemots (PIGU), and marbled murrelets (MAMU).

Locus	Primer Sequence (forward/reverse)	Annealing Temperature (°C) COMU	PIGU	MAMU
Bma9-28[2]	5'-AGGTAGGAAGGAGGGAGGGT-3'	-	-	55
	5'-ACCCTGTTTGGTGATTGGAG-3'			
Bma10-18[2]	5'-GGTAGGAGCGGAGTAGGAGG-3'	-	-	60
	5'-GCAAAATAAGGGTGAAGGCA-3'			
Cco5-9[2]	5'-TTCCTACCAGTAAAAGAGAGGA-3'	-	55	-
	5'-GTACCCCTTTCCTAATTCAAG-3'			
Cco5-21[2]	5'-TCAAGATGATGAAGACCCTAAT-3'	-	55	52
	5'-AGAGTTGCACAGGTTAAATACC-3'			
Dpu16[3]	5'-ACAGCAAGGTCAGAATTAAA-3'	-	61	-
	5'-AACTGTTGTGTCTGAGCCT-3'			
Uaa1-23[1]	5'-CCTGTGTTGAAAATAGAACAGA-3'	58	-	-
	5'-TTTAGCTGGTGAAGTTAGTCAG-3'			
Uaa5-8[1]	5'-CAGTTTCTTTAAGTCGTGCCAG-3'	53	60	50
	5'-CACTTAGGTCCAAAACCTAACC-3'			
Ulo12a12[1]	5'-TCTACGATTCTATGATTCCACA-3'	58	-	-
	5'-GATCTCTACCACATTCTCCCTA-3'			
Ulo12a22[1]	5'-TGAATGCAGTGTCAGTCAAG-3'	54	-	-
	5'-TATAGGCTTATGCCAGAGAGAC-3'			
Ulo14b29[1]	5'-GTATTATGTTCCGGAAAACTGT-3'	58	-	58
	5'-TACCCCTATATACAAACCCAAG-3'			

[1]Ibarguchi et al. 2000
[2]V.L.F. unpubl. data
[3]Dawson et al. 1997
- = not analyzed

Table 7. Frequencies of control region haplotypes among common murres from 17 regions. Region abbreviations as in Table 1.

	Okho	Chuk	Ber	Prib	WAle	EAle	Kren	WAP	Shum	EAP	Barr	Cook	NGA	BC	Wash	Oreg	Cal
1	8	8	2	11	4	3	13	4	2	3	9	16	9	10	4	7	10
2	7	5	3	15	6	1	6	2	1	7	2	10	4	6	1	9	4
3							1										
4							1										
5							1										
6							1		1								
8							1							1			
9							1										
10							1										
11		2					1										
12*											1						
13*											1						
14											1						
15											1						
16											3						
17										1	1						
18*											1						
19											1						
20					1	1											
21					1												
22											1				1		
23											1						
24											1						
25*											1						
26											1						
27									1								
28*									1								
29						1											1

Table 7, cont'd.

	Okho	Chuk	Ber	Prib	WAle	EAle	Kren	WAP	Shum	EAP	Barr	Cook	NGA	BC	Wash	Oreg	Cal
30		1		1	1					1		1					
31		1															
32		1															
33	2	1															1
34		1															
35*		1		1													
36		1															
38		1															
39		1							1			1	1				
40*		1															
41*		2															
42*		1															
43		1															
44		1															
45		1															
46*		1															
47												1					
48												1					
49												1					
50												2	2				
51												1					
52												1					
53										1							
54									1	1							
55*										1							
57										1							
58										1							
59	2								1								
									52								

Table 7, cont'd.

	Okho	Chuk	Ber	Prib	WAl	EAle	Kren	WAP	Shum	EAP	Barr	Cook	NGA	BC	Wash	Oreg	Cal
60	3			1													
61	1																
62	1																
63												1					
64												1					
65												1					
66												2					
67				1				1				1					
68												1					
69												1					
70				1					1								
71													1				
72													1				
73													1				
74													1				
75									1							1	
76				1													
77				1													
78										1							
80																	1
81																	3
82														1			1
83														2	1	1	
84																1	
85																1	
86																1	
87														2			
88															2		

Table 7, cont'd.

	Okho	Chuk	Ber	Prib	WAl	EAle	Kren	WAP	Shum	EAP	Barr	Cook	NGA	BC	Wash	Oreg	Cal
89															2		
Total	24	32	5	33	13	6	27	7	11	18	26	43	20	22	11	21	21

*Asterisks represent haplotypes that group with those of thick-billed murres in gene trees.

Table 8. Variable sites within 761 bp of the mitochondrial control region of 328 common murres (not including haplotypes of putative hybrids). Numbers refer to sites in Appendix I. Dots indicate identity with haplotype 01. Dashes represent insertions/deletions (indels).

	11111111	1111111111	1111111222	2223333344	4445555555	5555556666	777777
1133333888	8900011111	2334445555	5566779002	5680225624	4680244555	5568883344	111222
5901245456	9056712359	4142340234	8901085384	7926279042	3926212012	4545797904	014123
01 CCGACTCCCC	CTCCACCACA	ACCTTATGGG	AGGAACAGGG	ACAACATTAT	AGTACATAGT	CTTTGGCAAC	ACAACA
02	....	....	..A.......	....	....	....	......
03	....	.T........	...-......	....	....	....	......
04	....	....	........C.	....	....	....	......
05 ....T	....	....	....	....	....	....	......
06	....	....	..A.......	C	.A.G......	....	......
08	....	....	....	....	....TG....	....	......
09	....	....	..A.......	....	......C..C	T.........	CTG...
10 ....T..	....G.....	....	..A.......	....	....	T.........	......
11 ....T.	....	....	....	....	....	....	......
14 T.........	....	....	..A.......	....	....	....	......
15	....	....	..A.......	C	....	....	...CAC
16	....	....	....	....	..A.......	....	......
17	....	....	.......A..	....	....	....	......
19	....	....	..A.......	....	..A.......	....	......
20	....	....	...C......	....	....	....	......
21	........T.	....	..A.......	....	....	....	......
22	....	..A.......	....	....	....	....	......
23	....	....	..A.......	....	....	.........T	CTG...
24 ......T...	....	....	..A.......	....	C	T.........	CTG...
26	....	..T.......	..A.......	....	....	....	......
27	........T.	...CCCCA.A	.A....C..A	......C...	....	....	......
29 ......A...	....	....	....	....	....	....	......
30	....	....	..A.......	....	....	T.........	......

Table 8, cont'd.

	11111111	1111111111	1111111222	2223333344	4445555555	5555556666	777777
1133333888	8900011111	2334445555	5566779002	5680225624	4680244555	5568883344	111222
5901245456	9056712359	4142340234	8901085384	7926279042	3926212012	4545797904	014123
31	.....	.....	.....	..A.....	.....C..	.A.....	.....	.....
32	.....	..T.....	.....	..A.....	.....	C	.....	.....
33	.....	.....	.....	.....	.....	C	...C.....	.....
34	.....	.....	.....	..A.....	C	.....	..C.....	.....
36	.....T...	.....T....	.....A.	..A.....	.....	.....	.....	.....
38	.....	.....	.....	.....	.....	.....	.C.....	.....
39	.....	.....	.....	..A.....	.....	C	.....	.....
43	.....	.....	.....	..A.....	C	C	.....	.....
44	.....	..T.....	.....	..A.....	.....	.....	.....	.....
45	.....	.....	.....	G.A.....	.....	.....	.....	.....
47	.....C..T.	.....	.....	.....	.....	.....	.....	.....
48	.....	.....	.....	.....	C	.....	.....	...CAC
49	.....T..	.....	.....	...CC.....	.....A	.....	.....ATGT	.....
50	.....	.....	.....	..A.....	.....	....TG.C..	.....	.....
51	.....	.....	.....	..A.....	.....T.	.....	.....	.....
52	.....	.....	.....	.....	.T..A.....	.....	.....	.....
53	.....	.....G	.....	.....	.....	.....	.....	.....
54	.....	.....	.....	.....	.T.....	.....	.....	.....
57	.....	.....	.....	..A.....	G.....	.....	.....	.....
58	.....T..	....G.....	.....	..A.....	.....	....TG....	.....	.....
59	.....	.....	.....	.....	.....	C	.....	.....
60	.....	.....T.	.....	.....	.....	.....	.....	.....
61	.....	.....	..A.....	.....	.....	C.....	T.....	.....
62	.....	.....	.....	.....	.....	...G.....C	.....	.....
63	.....	...T.....	.....	.....	.....	.....	.....	.....
64	.....	.....	..T.....	.....	.....	.....C.	.....	.....

Table 8, cont'd.

	11111111	1111111111	1111111222	2223333344	4445555555	5555556666	777777
1133333888	8900011111	2334445555	5566779002	5680225624	4680244555	5568883344	111222
5901245456	9056712359	4142340234	8901085384	7926279042	3926212012	4545797904	014123
65	.....	.....	.....	..A.....	.....	C.....	T.....	.....
66	.T.....	.....	.....	..A.....	.....	.....	.....	.....
67	.....	.....G..	.....	..A.....	.....	.....	.....	.....
68	..AGACT...	....G.....	.....	..A.....	..CC.C....	.....	.....	.....
69	.....	.C.....	.....	.....	.....	.....	.....	.....
70	.....T..	....G.....	.....	..A.....	.....	.....	.....	.....
71	.....	.....	G.....	.....	.....	.....	.....	.....
72	.....	.....	.....	.....	.....	.....	T.....	.....
73	.....	.....T.	.....	.....	G.....	.....G..	.....	.....
74	.....	.....	.....	.....	C	C	.....	.....
75	.....	...T.....	..A.....	.....T....	.....	.....	.....	.....
76	.....	.....	.....	.....	.....	.....	....AA....	.....
77	.....	.....G..	.....	.....	.....	.....	.....	.....
78	......A...	.....	.....	..A.....	.....	.....	.....	.....
80	.....	....G.....	.....	..A.....	.....	....TG....	.....	.....
81	.....	.....	.....	.....	.....	...G.....	.....	.....
82	.....	.....	.....	..A.....	.....	.....	...C.....	.....
83	.....	.....	.....	.....	.....	.....	T....A....	.....
84	.....	.....	.....	..A.....	.....	.....	T....A....	.....
85	.....	T.....	.....	.....	.....	.....	.....	.....
86	.....	......T...	.....	..A.....	.....	.....	.....	.....
87	.....	.....	.....	..A.....	C	.....	.....	.....
88	.....	.....G	.....	..A.....	.....	.....	.....	.....
89	.....	....G.....	.....	..A.....	.....	.....	.....	.....

Table 9. Estimates of nucleotide diversity (π) and Tajima's *D* for mtDNA, and average heterozygosity (H_E) for introns and microsatellites for common murres. Region abbreviations as in Table 1.

Region	π (%)	Tajima's *D*	H_E	
			introns	microsat-ellites
Okho	0.199	-0.97	0.97±0.01	1.00±0.01
Chuk	0.266	-1.71	0.95±0.01	1.00±0.00
Ber	0.080	1.22	0.92±0.06	1.00±0.03
Prib	0.133	-1.48	0.76±0.03	0.98±0.02
WAl	0.129	-0.48	0.94±0.03	0.99±0.01
EAle	0.133	-0.93	1.00±0.10	0.98±0.03
Kren	0.237	-2.17*	0.94±0.02	0.99±0.01
WAP	0.114	0.21	0.74±0.08	0.97±0.04
Shum	1.310	-1.72	0.87±0.05	0.99±0.01
EAP	0.221	-1.75	0.97±0.02	0.99±0.01
Barr	0.319	-1.77	0.93±0.02	0.99±0.01
Cook	0.335	-2.37*	0.95±0.01	0.98±0.01
NGA	0.242	-1.23	0.92±0.03	0.99±0.01
BC	0.173	-1.04	0.65±0.07	0.99±0.01
Wash	0.232	-0.59	0.18±0.11	0.99±0.02
Oreg	0.194	-1.29	0.93±0.02	0.98±0.02
Cal	0.178	-1.16	0.97±0.02	0.98±0.01
mean	0.264	-1.13	0.86	0.99

Table 10. Estimates of Slatkin's linearized F_{st} for pair-wise comparisons of regional samples of common murres, based on mitochondrial control region sequences (below diagonal), and mean F_{st} for nuclear loci (above diagonal). Numbers that are underscored are significant at $\alpha = 0.01$; numbers in bold indicate significant differences in haplotype/allele frequencies from exact tests. Region abbreviations as in Table 1.

	Okho	Chuk	Ber	Prib	WAl	EAle	Kren	WAP	Shum	EAP	Bar	Cook	NGA	BC	Wash	Oreg	Cal
Okho		0	0	**0**	0	0	0	0	0	0	0	0	0	0	0.000	0.000	0.000
Chuk	0		0.1	**0**	0	0	0	0.000	0	0	0	0.000	**0.000**	0.000	0.000	0	0.000
Ber	0	0		0	0	0	0	0.000	0.000	0	0	0	0.000	0.000	0.000	0.000	0
Prib	0.1	0	0		**0**	**0**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
WAl	0.1	0	0	0		0	0	0.000	0	0	0.000	0.000	0.000	0.000	0.000	0	0
EAle	0	0	0.11	0.12	0.13		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0	0.000	0.000
Kren	0	0	0	0	0	0		0.000	0	0.000	0	0	**0.000**	0.000	0.000	0	0
WAP	0	0	0	0	0	0	0		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Shum	0	0.1	0	0.11	0	0	0.1	0		0.000	0	0.000	0.000	0.000	0.000	0.000	0.000
EAP	0.1	0	0	0	0	0.1	0	0	0		0	0.000	0.000	0.000	0.000	0.000	0.000
Bar	0	0	0	**0**	0	0	0	0	0	0		0	0.000	0.000	0.000	0	0.000
Cook	0	0	0	0	0	0	0	0	0.1	0	0		0.000	0.000	0.000	0.000	0.000
NGA	0	0	0	0	0	0	0	0	0	0	0	0		0.000	0.000	0.000	0.000
BC	0	0	0	0	0	0	0	0	0.1	0	0	0	0		0.000	0.000	0.000
Wash	0	0	0	**0**	0	0	0	0	0	0	0	0	0	0		0.000	0
Oreg	0.1	0	0	0	0	0.1	0	0	0.1	0	0	0	0	0	0		0
Cal	0	0	0	0.1	0.1	0	0	0	0	0.1	0	0	0	0	0	0	

Table 11. Conclusions from nested clade analysis of mitochondrial control region variation in common murres.

Nesting Clade	Steps in Inference Key	Conclusion
1-1	1-2-3-4-No	Isolation by Distance
1-18	1-2-3-4-No	Isolation by Distance
2-1	1-2-3-4-No	Isolation by Distance
2-9	1-2-3-4-No	Isolation by Distance
2-13	1-2-11-12-No	Contiguous Range Expansion
3-1	1-2-3-4-No	Isolation by Distance
3-4	1-2-11-17-No	Inconclusive
3-5	1-2-3-4-No	Isolation by Distance
4-1	1-2-11-17-No	Inconclusive
4-2	1-2-11-17-4-No	Isolation by Distance

Table 12. Nuclear allele frequencies for common murres. Region abbreviations in Table 1. Locus abbreviations in Tables 5 and 6.

	Okho	Chuk	Ber	Prib	WAle	EAle	Kren	WAP	Shum	EAP	Barr	Cook	NGA	BC	Wash	Oreg	Cal
Cry																	
C1	0.52	0.54	0.33	0.56	0.62			0.58	0.58		0.63	0.59	0.82	0.61			0.68
C2	0.48	0.29	0.58	0.35	0.27		0.31		0.35	0.29		0.34	0.13	0.28		0.41	0.25
C3		0.07		0	0.04			0.33					0.02				
C4							0.02										
C5											0.02						
C6					0.04												
C7							0.02			0.02	0.06						
C8*							0.02										
C9*											0.02						
C10*											0.02						
C11		0.01		0.01	0.04											0.09	0.04
C12*											0.04						
C13*											0.02						
C14*										0.02							
C15		0.01	0.08	0.04			0.02					0.06	0.02	0.06			0.04
C16*		0.01															
C17*		0.03															
C18*									0.04								
C19*									0.04								
C20								0.08		0.02							
C21*										0.05							
C22*		0.01												0.06			
C23				0.02													
C24*				0.02													
C25															0.5		
C26													0.02				
Total	50	68	12	84	26	4	48	12	26	42	54	96	56	18	2	34	28

Table 12, cont'd.

	Okho	Chuk	Ber	Prib	WAle	EAle	Kren	WAP	Shum	EAP	Barr	Cook	NGA	BC	Wash	Oreg	Cal
Enol																	
C1	0.48	0.24	0.50	1.00	0.46	0.33	0.43		0.50	0.39	0.43	0.34	0.50	0.67		0.53	0.50
C2	0.41	0.51	0.50		0.42	0.33	0.48		0.15	0.39	0.28	0.45	0.46	0.17		0.44	0.23
C3	0.04	0.01			0.08		0.02		0.04	0.03	0.11	0.05		0.17			0.04
C4	0.02								0.04	0.03	0.02						
C5	0.02	0.01					0.02			0.03		0.01					
C6	0.02	0.01			0.04				0.12			0.04	0.04			0.03	0.12
C7												0.02					
C8		0.04				0.17	0.02			0.03	0.04						
C9*							0.02				0.02						
C10*											0.02						
C11*		0.03									0.02						
C12											0.02	0.01					
C13												0.01					
C14											0.02	0.01					0.08
C15		0.07				0.17					0.02						
C16												0.02					
C17									0.04		0.02						
C18*		0.03															
C19									0.04								
C20												0.01					
C21									0.04			0.01					
C22									0.04								
C23																	0.04
C24										0.03		0.01					
C25		0.01															
C26*		0.01															
C27*										0.05							

Table 12, cont'd.

	Okho	Chuk	Ber	Prib	WAle	EAle	Kren	WAP	Shum	EAP	Barr	Cook	NGA	BC	Wash	Oreg	Cal
Total LDH	46	68	12	2	24	6	46	0	26	38	54	96	24	6	0	34	26
C1	0.94	0.88	0.92	0.96	1.00	0.75	0.93	1.00	0.92	0.95	0.85	0.95	0.89	0.96	0.91	1.00	0.96
C2	0.04			0.03			0.02				0.02	0.03	0.05		0.05		
C3	0.02						0.05				0.06			0.02			0.04
C4*										0.02							
C5*										0.02							
C6		0.08				0.25			0.08		0.06						
C7*											0.02						
C8		0.03											0.04	0.02			
C9		0.02		0.01								0.01					
C10													0.02				
C11			0.08									0.01					
C25*															0.05		
Total P40	48	66	12	74	26	4	42	10	24	40	54	96	56	48	22	40	24
C1	0.38	0.46	0.33		0.83				0.33	0.22	0.54	0.52	0.50				0.36
C2	0.12	0.06	0.67	0.10			0.42		0.67	0.22	0.04	0.17	0.09				0.09
C3	0.19	0.23			0.17					0.09	0.14	0.3					
C4		0.04								0.09	0.04		0.06				0.05
C5		0.06								0.04							
C6	0.05	0.06										0.01					
C7*		0.02															
C8*		0.02															
C9*		0.02															
C10	0.10			0.10		0.50					0.14		0.12				
C11	0.02			0.40		0.50							0.03				
C12		0.02		0.20			0.58			0.22							

Table 12, cont'd.

	Okho	Chuk	Ber	Prib	WAle	EAle	Kren	WAP	Shum	EAP	Barr	Cook	NGA	BC	Wash	Oreg	Cal
C13	0.02			0.10													
C14	0.05									0.04							
C15	0.02												0.03				
C16	0.05										0.04		0.15				
C17				0.10													
C18													0.03				
C19*										0.04							
C20*										0.04							
C21*											0.04						
C22*											0.04						
Total	42	48	12	10	12	2	24	0	6	23	28	84	34	0	0	20	22
Uaal-23																	
151*		0.03															
155		0.02							0.04		0.02						
157		0.02										0.01					
159									0.04		0.04						
161	0.02	0.05										0.01					
163*											0.02						
165*		0.03					0.02										
167	0.04	0.02		0.07								0.03				0.02	0.02
169	0.30	0.23	0.50	0.36	0.42	0.90	0.46	0.50	0.42	0.30	0.39	0.44		0.33	0.35	0.40	0.31
171	0.04	0.02	0.08		0.08		0.02	0.08	0.04	0.02	0.06	0.03		0.08	0.20	0.05	0.10
173											0.02						
175	0.07	0.12	0.17	0.07	0.12		0.04	0.17	0.08	0.02	0.06	0.04		0.04	0.05	0.05	0.10
177	0.09	0.09	0.08				0.07	0.08		0.13	0.06	0.06		0.06	0.10	0.14	0.10
179	0.07	0.02			0.04	0.10	0.05		0.08	0.05	0.06	0.05		0.06		0.05	
181	0.07	0.08		0.14			0.04		0.08	0.25	0.04	0.03		0.17	0.15	0.12	0.21
183	0.11	0.06			0.12		0.09		0.08	0.05	0.04	0.08		0.08	0.05	0.05	0.05

Table 12, cont'd.

	Okho	Chuk	Ber	Prib	WAle	EAle	Kren	WAP	Shum	EAP	Barr	Cook	NGA	BC	Wash	Oreg	Cal
185	0.11	0.03	0.08	0.07	0.04		0.05	0.08	0.04	0.10	0.07	0.10		0.13	0.05	0.07	0.02
187	0.09	0.09		0.21	0.15		0.09	0.08	0.08	0.07	0.04	0.07		0.02		0.05	0.05
189		0.02	0.08				0.04				0.02	0.02					
191		0.02		0.07			0.02		0.04		0.06				0.05		0.02
193		0.05															
195					0.04						0.02	0.01		0.02			
197		0.03									0.02						0.02
201		0.02															
205							0.02										
Total	46	66	12	14	26	10	56	12	26	40	54	96	0	48	20	42	42
Uaa5-8																	
106	0.09	0.03		0.04		0.14		0.07		0.07	0.04	0.01		0.02			0.08
108	0.15	0.19	0.17	0.04	0.12	0.07	0.14	0.07	0.04	0.07	0.13	0.07		0.08	0.20	0.10	0.24
110	0.20	0.33	0.25	0.38	0.31	0.36	0.23	0.14	0.38	0.36	0.20	0.35		0.40	0.40	0.57	0.37
112	0.04	0.03	0.08	0.04		0.07	0.02		0.08	0.10	0.04	0.02		0.04	0.10	0.02	
114							0.02				0.02	0.01					
116	0.39	0.36	0.33	0.50	0.54	0.14	0.41	0.64	0.42	0.31	0.44	0.42		0.44	0.30	0.30	0.26
118	0.13	0.03	0.17		0.04	0.21	0.14	0.07	0.04	0.10	0.07	0.10					0.05
119														0.22			
120		0.03					0.02		0.04		0.04	0.01					
122*							0.02				0.02						
Total	46	64	12	24	26	14	56	14	26	42	54	96	0	48	20	40	38
Ulo12a22																	
135		0.02					0.02		0.04		0.02						
137	0.03	0.07	0.17			0.06			0.04	0.07	0.11	0.02		0.04	0.09	0.02	0.02
139	0.16	0.15	0.17		0.32	0.31	0.12		0.12	0.13	0.15	0.15		0.08		0.07	0.10
141	0.81	0.63	0.67	0.90	0.64	0.56	0.76		0.77	0.80	0.61	0.78		0.73	0.82	0.79	0.85
143		0.08		0.10	0.05	0.06	0.10		0.04		0.11	0.05		0.15	0.09	0.12	0.02

Table 12, cont'd.

	Okho	Chuk	Ber	Prib	WAle	EAle	Kren	WAP	Shum	EAP	Barr	Cook	NGA	BC	Wash	Oreg	Cal
147*		0.03															
151*		0.02															
Total	32	60	12	20	22	16	50	0	26	30	54	94	0	48	22	42	40
Ulo14b29																	
127		0.04															
129	0.50	0.42	0.58	0.55	0.50	0.71	0.48		0.38	0.43	0.56	0.46		0.52	0.45	0.4	0.5
131		0.02			0.04		0.02			0.07							
132*											0.02						
133*		0.02									0.06						
134										0.07	0.02	0.01		0.02			0.05
135		0.02					0.02				0.02						
137		0.02				0.07	0.04			0.04		0.01		0.02			0.02
139	0.11	0.12		0.05	0.13	0.07	0.12		0.17	0.07	0.09	0.14		0.21	0.32	0.24	0.12
141	0.07	0.10	0.25	0.10	0.13		0.10		0.25	0.07	0.04	0.12		0.04	0.05	0.10	0.10
143	0.04	0.10		0.15		0.07	0.06			0.07	0.04	0.03			0.09	0.10	0.07
145	0.04			0.10	0.08		0.06		0.04	0.11	0.07	0.06				0.02	0.02
146												0.01					
147	0.07	0.02			0.08				0.08			0.03		0.04		0.05	0.05
149	0.04	0.06	0.08	0.05	0.04	0.07	0.02			0.07	0.04	0.07		0.04		0.02	0.02
151	0.07	0.02					0.02				0.02	0.03		0.06	0.05		0.05
153	0.04	0.02					0.04				0.11				0.05		
155							0.02				0.11			0.02		0.07	
157							0.02		0.04			0.01		0.02			
159	0.04		0.08						0.04								
161												0.01					
Total	28	48	12	20	24	14	52	0	24	28	54	94	0	48	22	42	42

*Asterisks denote alleles found only in hybrids.

Table 13. Sequence variation among alleles for four nuclear introns in common murres. Dots indicate identity with the first sequence; dashes indicate indels; "?" indicates an unresolved base. Alleles from possible hybrid individuals have been excluded. Locus abbreviations as in Table 5.

(a) Crys

```
                111122223
            788235622580
Allele      667570968406

C1          CCGCGCACGCCT
C2          ...G.A.T.-AC
C3          ...G.......C
C4          ...G.A.T.-AC
C5          G.AGTA.T.-AC
C6          ...........C
C7          ...G.A......
C11         ...GTA.T.-AC
C15         ......G.....
C20         ...G........
C23         .A..........
C25         ........C...
C26         ..AG.AGT.-AC
```

(b) Enol

```
                11111122222223
            36600367900224770
Allele      92307457016054269

C1          GCTGCTTTGCCACGCGA
C2          .A...C....T..C..C
C3          .A........T..C..C
C4          AA...C....T..C..C
C5          .A...C....T..T..C
C6          .A...C...TT..C..C
C7          .A..T.....T..C..C
C8          ..........T.TC..C
C12         .AC..C....T..C..C
C13         .A...C..C.T..C..C
C14         ............C...
C15         .......C..T..C..C
C16         .....C....T..C..C
C17         .A....C...TGTC..C
C19         ..........T..C.AC
C20         .A..............
C21         .....C..........
C22         .A...C....T..CT.C
C23         ...C............
C24         ................
C25         CA...C....T..C..C
```

(c) P40

```
                   1111112
               13999990000372
Allele         408067890123545

C1             CGCTAACCAACGTGG
C2             ..T............
C3             .AT.G.........A
C4             ..............A
C5             ....----...?..A
C6             ....----...?...
C10            .......----..A.
C11            .AT.G.......A.A
C12            ..T.G.......A.A
C13            ..T.G......AA.A
C14            ..T...........A
C15            .AT.G......AA.A
C16            T.TCG.......A.A
C17            .AT............
C18            ..T....----...A
```

(d) LDH

```
                11112333
            500092126
Allele      302585499

C1          GTTATCCCC
C2          .C.......
C3          ...C.....
C6          .....TTT.
C8          ........T
C9          ....C....
C10         ..C......
C11         A........
```

Table 14. Numbers and origins of individual common murres with high probabilities of immigrant ancestry. "?" indicates uncertain origin. Region abbreviations as in Table 1.

Origin	Bird Sampled From																
	Okho	Chuk	Ber	Prib	WAle	EAle	Kren	WAP	Shum	EAP	Barr	Cook	NGA	BC	Wash	Or	Cal
Okho												1					
Chuk	1																
Ber							1										
Prib											1				1		
WAl												1					
EAle																	
Kren		1															
WAP																	1
Shum																	
EAP		1															
Barr																	
Cook																	
NGA																	
BC																	
Wash		1															
Oreg																	
Cal											1						
?		1					1			3	1						
Total	1	4					2			3	3	2			1		1

Table 15. Variable sites among 73 mitochondrial control region haplotypes of pigeon guillemots. Numbers refer to positions of sites relative to the 3' end of the light strand primers. The first 132 bp for the 3' end were not sequenced for most samples. Dashes indicate insertions or deletions. ? denotes ambiguous sequence.

	5' end								3' end		
					11	1111111111	1111112222	22	1111111111	1222233334	4
	1111111	2222222334	4444455556	6677789901	1112222346	6788890111		15	4444555555	5112612777	8
	4891246789	1235678181	2346703450	4635613502	5681249900	8457893234		57	4578013478	9340141091	8
AA1	TCTTCTAGCC	ATTCTTCCAC	CTTTCAAAGG	TATTGCCCAA	GCTCCGGGGG	GGGGCTATTT	AA	TCTCATAGCC	TCGTGTGTTT	T	
AB1	....	....C.....	T.........	....A.....	..C.......	....	..	....	....	.	
AC1	....	....C.....	T.........	....A.....	..C......A	....	..	....	....	.	
AD1	....	....C.....	....	........G.	....	....	..	....	....	.	
AD6	....	....C.....	....	........G.	....	....	..	....	C	.	
AE1	....	....C.....	....	....	....	....	..	....	....	.	
AF1	.A..?...?.	....C.....	....	........G.	....	....	..	....	....	.	
AG1	....	....C.....	....	....	.........A	....	..	....	....	.	
AH1	....	....	....	.G........	....	....	..	....	....	.	
AI1	..?.T...T?	..C.C.....	T.........	....A.....	..C.......	....	..	....	....	.	
AJ1	CAA.T..?T.	??C.C..A..	T.........	....A.....	..C.......	....	..	....	....	.	
AK1	CAA.T..?T.	.C?.C..A..	T.........	....A.....	..C......A	A.........	..	....	....	.	
AL1	....	..C.C.....	TC........	....A.....	..C......A	....	..	....	....	.	
AL12	....	..C.C.....	TC........	....A.....	..C......A	....	..	....	......A...	.	
AM1	....	..C.C...G.	....	....A.....	..CT.A...A	....	..	....	....	.	
AN1	....	..C.C.....	TC........	....A.....	..C.......	....	..	....	....	.	
AO1	....	..C.......	T..CT.....	....A.....	.........AA	....	..	....	....	.	
AO5	....	..C.......	T..CT.....	....A.....	.........AA	....	..	....	...C......	.	
AP1	....T.....	....C.....	T.........	....A.....	..C......A	....	..	....	....	.	
AQ1	..?.T..T..	..?.C.....	....	........G.	....	....	..	....	....	.	
AR1	........?.	..C?.?....	T.........	....A.....	..C....A.A	....	..	....	....	.	
AS2	....	..C.C.....	T.........	....A.....	..C.......	....	..	....	.T........	.	
AT1	....	..C.C.....	....	....A.....	..CT.....A	....	..	....	....	.	

Table 15, cont'd.

	5' end								3' end		
					11	1111111111	1111112222	22	1111111111	1222233334	4
	1111111	2222222334	4444455556	6677789901	1112222346		6788890111	15	4444555555	5112612777	8
	4891246789	1235678181	2346703450	4635613502	5681249900		8457893234	57	4578013478	9340141091	8
AU1	........?.	..C?C?.A..	T.........	....A.....	..C.?.....		.....	..	.....	.....	.
AV1	.....	..C.C.....	T.........	....A.....	..C.....		.....	..	.....	.....	.
AW1	.....	..C.C.....	T.........	....A.....	..C.....		....T.....	..	.....	.....	.
AX3	....T.....	....C.....	.....	...C.....	.....		A.....	..	.....	..A.....	C
AY1	......GA..	..C.C.....	TC.....	....A.....	..C.....A		.....	..	.....	.....	.
AZ2	.....	..CTC.....	T.........	....A.....	..CT.A...A		.....	..	.....	.T.....	.
BA1	.....	..C.C.....	T.C.....	....A.....	..C.....		.....	..	.....	.....	.
BB1	.....	...TC.....	TA....G...	....A.....	..CT.A...A		A.....	..	.....	.....	.
BC1	.....	..C.C....T	T.........	....A.....	..C.....A		.....	..	.....	.....	.
BD1	....?...?.	..C.C....T	T.........	....A.....	..C.....A		A.....	.G	.....	.....	.
BE1	....?.....	..C.C.....	T.........	....A.....	..C.....A		....T.....	..	.....	.....	.
BF1	....T.....	...TC.....	TA....G...	....A.....	..CT.A...A		.....	..	.....	.....	.
BG1	....?.....	..CTC.....	T.....G...	....A.....	..CT.A...A		.....	..	.....	.....	.
BH1	.....	...TC.....	TA....G...	....A.....	..CT.A...A		.....	..	.....	.....	.
BI1	....?.....	..CTC.....	T....GG...	....A.....	..CT.A...A		.....	.G	.....	.....	.
BJ1	....?.....	...TC.....	TAC...G...	....A.....	..CT.A...A		....T.....	..	.....	.....	.
BK1	....T.....	..C.C....T	T.........	....A.....	..C.....A		.....	.G	.....	.....	.
BL1	.....	..C.C.....	T.........	....A.....	..C....A.A		.....	..	.....	.....	.
BM4	.AA.T..?T.	.C?TC..A..	T.....G...	....A.....	..CT.A...A		.....	..	.....	.....C.	.
BN1	.....	..C.C.....	.....	....A.....	..C.....A		.....?....	..	.....	.....	.
BO2	.....	..CTC..?..	T.........	....A.....	...T.A...A		.....	..	.....	.T.....	.
BP1	.....	..CTC.....	T.....G...	....A....G	..CT.A....		.....	..	.....	.....	.
BQ1	.?A.T..TTT	?CCTCA.A.T	T.........	....A.....	..C.....A		A.....	.G	.....	.....	.
BR1	.....	..C.C.....	TC.....	.....	.....A		.....	..	.....	.....	.
BS1	....T..T..	.CC?C.....	T.........	....A.....	..C.....A		.....	..	.....	.....	.
BT1	....T..?..	..CTC.....	TA....G...	....A.....	..CT.A...A		.....C....	..	.....	.....	.

Table 15, cont'd.

	5' end							3' end		
					11	1111111111	1111112222	22	1111111111	1222233334	4
	1111111	2222222334	4444455556	6677789901	1112222346	6788890111	15	4444555555	5112612777	8
	4891246789	1235678181	2346703450	4635613502	5681249900	8457893234	57	4578013478	9340141091	8
BU1	.T.....A..	..C.....	T.....	....A.....	..C.....A	.....	..	.....	.....	.
BV1	.....	..C.....	T..CT.....	....A.....	..C.....A	.....	..	.....	.....	.
BX1	.....	...TC.....	TA....G...	....A.....	..CT.A...A	.....C....	..	.....	.....	.
BZ2	....T...?.	..CTC.....	T.....	....A.....	..CT.A...A	.....	..	.....	.T.....	.
CB1	....T..?..	.CCTC..A.T	TA....G...	....A.....	..CT.A...A	.....	..	.....	.....	.
CC1	.....	..C.C.....	T.....	.....	.....A	A.....G...	..	.....	.....	.
CD8	.....	.....	T...T.G.AA	C...A.....	..CT.....A	.....	..	CTCATAGCTT	-.A.....	?
CD11	.....	.....	T...T.G.AA	C...A.....	..CT.....A	.-.....	..	.....	..A.A..C..	.
CE9	.....	.....	T...T.G.A.	C...A.....	..CT.....A	.....	..	?.CATAGCTT	-.A.AC....	?
CH1	.....	..C.C.....	T.....	....A.....	.....A	.....G...	..	.....	.....	.
CJ1	.....C....	..C.C.....	T.....	....A.....	..C.....A	.....	..	.....	.....	.
CK1	.....	?.C.C.....	T.....	.....	.....A	.....G...	..	.....	.....	.
CL1	.....	..C.C.....	T.....G..	.....G...	.....T..A	.....G...	..	.....	.....	.
CN8	.....	.....	T...T.G.AA	C...A..T..	..CTT.T..A	A.CC..GACC	T.	CTCATAGCTT	-.A.....	?
CP08	.....	.....	T..CT.G.AA	C...A.....	..CTT....A	A.....	..	CTCATAGCTT	-.A.....	?
CR1	.....	..C.C.....	T..CT.....	....A.....	..C.....	.....	..	.....	.....	.
CS01	.....	?.CTC....?	.....	....A.....	..CT.A...A	A.....	.G	.....	.....	.
CT01	.....	..C.C.....	T.....	..C.A..T..	..C.....A	.....	..	.....	.....	.
CU15	.....	.....	T...T.G.AA	C...A.....	..CTT....A	A.....	..	.....	..A.....	.
CV01	.....	..C.C.....	T..CT.....	.....	CG....T...	.....	..	.....	.....	.
CW01	.....?....	C.CAC?GA..	T.....	....A.....	..C...T..A	.....	..	.....	.....	.
CY1	??????????	???????...	TC.....	....AG....	..C.....A	.....	..	.....	.....	.
CZ16	...C.....	.....	T...T.G.AA	C...A.T...	..CTT....A	.....	..	.....T	..A.....	.
DA16	.....	.....	T...T.G.AA	C...A.T...	..CTT.....	.....	..	.....T	..A.....	.

Table 16. Frequencies of control region haplotypes among within regional samples of pigeon guillemots. Region abbreviations as in Table 2.

Haplo-type	Region						
	Ale	AP	Kach	PWS	BC	Oreg	Cal
AA1						6	3
AB1					5	2	3
AC1			1	1	1	1	1
AD1						3	8
AD6							1
AE1						2	2
AF1							7
AG1							7
AH1							2
AI1							1
AJ1							1
AK1							1
AL1					15	2	
AL12					1		
AM1					2	2	
AN1						4	
AO1					1		
AO5					1		
AP1						1	
AQ1						1	
AR1					1		
AS2				4			
AT1					1		
AU1					2		
AV1				1	2		
AW1					1		
AX3					1		
AY1					1		
AZ2				1			
BA1				2			
BB1				1			
BC1		2	1	3			
BD1		1	3	1			
BE1			2	2			
BF11			4	2			
BG1				3			

Table 16, cont'd.

Haplo-type	Region						
	Ale	AP	Kach	PWS	BC	Oreg	Cal
BH1	1	1		3			
BI1				1			
BJ1				1			
BK1				2			
BL1				2			
BM4			1				
BN1			1				
BO2			1				
BP1			1				
BQ1			4				
BR1			3				
BS1			1				
BT1			2				
BU1			2				
BX1			1				
BZ2			1				
CB1			1				
CC1		1					
CD8	1	1					
CD11	3						
CE9	1						
CH1		1					
CJ1		3					
CK1		1					
CL1		1					
CN8	1						
CP8	1						
CR1			1				
CS10		1					
CT10		1					
CU15	1						
CV1		1					
CW1		1					
CY1							
CZ16	1						
DA16	1						
Total	11	15	32	28	36	24	37

Table 17. Estimates of nucleotide diversity (π) and Tajima's D for mtDNA, and average heterozygosity (H_E) for introns and microsatellites for pigeon guillemots. Region abbreviations as in Table 2.

Region	$\pi \pm$ sd (%)	Tajima's D	H_E	
			introns	microsat-ellites
Ale	1.70±0.94	-0.56	0.91±0.03	0.98±0.01
AP	1.15±0.64	0.69	0.87±0.03	0.99±0.01
Kach	1.07±0.57	-1.58*	0.86±0.03	0.98±0.01
PWS	0.67±0.37	0.67	0.85±0.03	0.99±0.01
BC	0.47±0.27	0.13	0.86±0.02	0.93±0.01
Oreg	0.58±0.33	-1.56*	0.81±0.03	0.97±0.01
Cal	0.47±0.27	-0.48	0.73±0.05	0.91±0.02
mean	0.87	-0.38	0.84	0.96

* $P < 0.05$

Table 18. Estimates of Φ_{ST} (above diagonal) and corrected percent sequence divergence (δ; below diagonal) for mitochondrial control region sequences for pair-wise comparisons of regional samples of pigeon guillemot. Region abbreviations as in Table 2.

	Ale	AP	Kach	PWS	BC	Oreg	Cal
Ale		0.43**	0.50**	0.55**	0.68**	0.65**	0.72**
AP	1.02**		0.09*	0.09*	0.12**	0.21**	0.40**
Kach	1.20**	0.12**		0.04	0.18**	0.28**	0.42**
PWS	1.11**	0.07**	0.04**		0.16**	0.29**	0.48**
BC	1.21**	0.07**	0.17**	0.10**		0.22**	0.46**
Oreg	1.29**	0.18**	0.34**	0.26**	0.14**		0.11*
Cal	1.52**	0.37**	0.55**	0.51**	0.40**	0.06**	

*$P < 0.05$ after Bonferroni corrections; ** $P < 0.001$ after Bonferroni corrections.

Table 19. Sequence variation among alleles for three nuclear introns for pigeon guillemots. Dots indicate identity with the first sequence; dashes indicate indels. Locus abbreviations as in Table 5.

(a) Cytochrome *c*

Allele	11
	6915
	1329
1	GCGC
2	.T..
3	...A
4	.TA.
5	.T.T
6	AT..

(b) P40

Allele	1123
	5983
	4456
1	GTCC
2	A...
3	...T
4	..T.
5	.C..

(c) Rhod

Allele	112
	17799
	13707
1	TCGGC
2	.T...
3	..A.T
4	...A.
5	.T.A.
6	C....

Table 20. Numbers of samples and regional allele frequencies for three introns and four microsatellites for pigeon guillemots. Region abbreviations as in Table 2. Locus abbreviations as in Tables 5 and 6.

	Ale	AP	Kach	PWS	BC	Oreg	Cal
CytC							
1	0.28	0.56	0.55	0.55	0.55	0.65	0.85
2	0.53	0.38	0.29	0.34	0.30	0.30	0.15
3	0.03	0.08	0.11	0.00	0.15	0.00	0.00
4	0.06	0.03	0.03	0.00	0.00	0.00	0.00
5	0.11	0.03	0.02	0.10	0.00	0.04	0.00
6	0.00	0.00	0.02	0.00	0.00	0.00	0.00
N	14	20	66	58	74	46	48
P40							
1	0.69	0.85	0.73	0.81	0.76	0.74	0.87
2	0.25	0.12	0.27	0.19	0.22	0.26	0.13
3	0.03	0.03	0.00	0.00	0.01	0.00	0.00
4	0.03	0.00	0.00	0.00	0.00	0.00	0.00
5	0.00	0.00	0.00	0.00	0.01	0.00	0.00
N	14	20	66	58	74	46	46
Rhod							
1	0.33	0.47	0.45	0.50	0.47	0.35	0.23
2	0.53	0.47	0.55	0.50	0.51	0.65	0.77
3	0.08	0.03	0.00	0.00	0.00	0.00	0.00
4	0.00	0.03	0.00	0.00	0.00	0.00	0.00
5	0.03	0.00	0.00	0.00	0.00	0.00	0.00
6	0.03	0.00	0.00	0.00	0.01	0.00	0.00
N	14	20	66	58	74	46	44
Uaa5-8							
110	0.56	0.38	0.59	0.59	0.46	0.45	0.36
112	0.28	0.50	0.18	0.26	0.47	0.41	0.45
114	0.03	0.00	0.00	0.00	0.00	0.00	0.00
116	0.03	0.00	0.00	0.00	0.00	0.00	0.00
118	0.08	0.06	0.18	0.10	0.04	0.11	0.17
120	0.03	0.06	0.05	0.05	0.03	0.02	0.02
N	14	20	66	58	76	44	64

Table 20, cont'd.

	Ale	AP	Kach	PWS	BC	Oreg	Cal
Dpu16							
143	0.00	0.06	0.02	0.00	0.01	0.13	0.03
147	1.00	0.94	0.98	1.00	0.99	0.87	0.95
149	0.00	0.00	0.00	0.00	0.00	0.00	0.02
N	14	20	66	58	74	46	60
Cco5-9							
112	0.14	0.21	0.11	0.18	0.41	0.07	0.00
120	0.03	0.00	0.02	0.02	0.00	0.00	0.00
122	0.25	0.26	0.18	0.25	0.36	0.70	1.00
124	0.06	0.12	0.17	0.16	0.01	0.02	0.00
126	0.39	0.21	0.23	0.23	0.07	0.11	0.00
128	0.00	0.00	0.02	0.04	0.01	0.00	0.00
129	0.06	0.00	0.03	0.00	0.00	0.00	0.00
130	0.03	0.18	0.17	0.14	0.00	0.00	0.00
134	0.00	0.00	0.02	0.00	0.01	0.00	0.00
136	0.06	0.03	0.06	0.00	0.12	0.02	0.00
138	0.00	0.00	0.02	0.00	0.00	0.09	0.00
N	14	20	66	57	75	46	56
Cco5-21							
126	0.06	0.00	0.03	0.03	0.01	0.07	0.02
128	0.00	0.00	0.00	0.03	0.00	0.00	0.00
130	0.00	0.00	0.00	0.00	0.00	0.02	0.00
132	0.14	0.29	0.24	0.17	0.71	0.48	0.43
136	0.03	0.09	0.05	0.03	0.04	0.22	0.44
138	0.22	0.03	0.06	0.05	0.03	0.15	0.00
140	0.17	0.15	0.27	0.16	0.13	0.04	0.11
142	0.31	0.35	0.30	0.38	0.04	0.00	0.00
144	0.03	0.03	0.00	0.03	0.03	0.02	0.00
146	0.00	0.06	0.05	0.10	0.01	0.00	0.00
148	0.06	0.00	0.00	0.00	0.00	0.00	0.00
N	14	20	66	58	76	46	63

Table 21. Estimates of F_{st} for pair-wise comparisons of guillemot regional samples based on introns (above diagonal, top number), all loci combined (above diagonal, lower number), microsatellites assuming an infinite alleles model of mutation (below diagonal, top number), and microsatellites assuming a step-wise mutation model (below diagonal, lower number). Region abbreviations as in Table 2.

	Ale	AP	Kach	PWS	BC	Oreg	Cal
Ale	-	0.03	0.03	0.03	0.04	0.05*	0.18*
		0.03	0.02	0.02	0.10*	0.09*	0.17*
AP	0.02	-	0.00	-0.02	-0.01	0.01	0.09*
	-0.03		0.01	0.00	0.04*	0.06*	0.11*
Kach	0.01	0.02	-	0.00	-0.01	0.00	008*
	0.00	0.01		0.00	0.07*	0.08*	0.13*
PWS	0.00	0.01	0.00	-	0.00	0.01	0.09*
	-0.02	-0.02	0.02		0.08*	0.08*	0.14*
BC	0.14*	0.08*	0.13*	0.13*	-	0.01	0.08*
	0.14*	0.12*	0.19*	0.15*		0.05*	0.10*
Oreg	0.11*	0.08*	0.12*	0.12*	0.08*	-	0.03
	0.14*	0.12*	0.12*	0.15*	0.06		0.00
Cal	0.23*	0.17*	0.22*	0.21*	0.18*	0.02*	
	0.17*	0.09*	0.14*	0.14*	-0.06	-0.06	

*Significantly greater than zero after Bonferroni corrections at $\alpha = 0.05$.

Table 22. Variable sites among 51 control region haplotypes of 80 marbled murrelets. Numbers refer to position relative to the beginning of the control region. Dots indicate identity with haplotype 05 (the most common haplotype; Appendix II). Question marks indicate uncertain bases.

	1 1111111111	1111222222	2222222223	3344555555	5
	3445588880	1223445557	7899024456	6666777890	0506012566	7
	9494934582	8795670795	8925773553	4589245251	5696926628	0
05	GACAACGAGT	GCTCTGCTCC	TACCGTTATA	ACACATAAAC	ACGCTTTTAT	A
01	........A.	...TC...T.	....	....	.......???	?
03	....	....	C.........	....	...A......	.
04	.....T....	...T......	....	.......G..	....	.
06	........A.	...TCA..T.	........C.	....	....	.
07	....	.T.TC...T.	....	....	....	.
08	....	....C...T.	....	....	....	.
09	....GT.G..	....	......CG..	.....C....	....	.
10	....	...TC.T.T.	....	....	....	.
11	....	....	....	.........T	C.........	G
14	....	.T.TC...T.	....	....	....	.
15	....	...TC...T.	....	........G.	....	.
16	....	...T...CT.	C.........	....	....GCG???	?
21	......AG..	....	?.........	....	.......G..	.
24	........A.	....	......C...	....	....	.
25	...C......	...TC....T	....	....	....	.
26	....	...TC...T.	....	....	....	.
28	.G........	...T....T.	....	....	....	.
30	A........A	.T.T....T.	.G.T......	....	....	.
31	....	C.........	........C.	....	....	.
32	?.........	....	.........G	....	.........G	.
33	....	...T...CT.	....	....	....	.
34	....	....	....	....	..A.......	.
35	....	....	....	....	....	.
36	.....T.G..	....	....	CTGTGC....	....	.
37	....	...T....T.	....	....	....	.

Table 22, cont'd.

	1 1111111111 1111222222 2222222223 3344555555 5
	3445588880 1223445557 7899024456 6666777890 0506012566 7
	9494934582 8795670795 8925773553 4589245251 5696926628 0
38	....CA..T. ....
39	........A. ....G ....
40	.... .T..C...T. ....
41	........A. ...TC...T. ..T.....C. ....
42	........A. ...TC...T. ....C. ....
44	.... .TCT....T. ....G... .A........ .
46	..T....... ....T. ....T. .
47	.....G.... ...TC...T. ....G. .
48	.... .TCT....T. ....G... ....
50	....TC....T ....A..... ....
51	....TC...T. .....C.... ....??? ?

Table 23. Frequencies of mitochondrial control region haplotypes among marbled murrelets from seven regions. Region abbreviations as in Table 3.

	WAle/ CAle	EAle/ Shum/ Mitr	Kodi	Kach	PWS	SEAK	Cal
1		1					
3		1					
4		1					
5	2	1		1	5	3	2
6	1						
7	1					4	
8			2	3			
9				2			
10			1				
11							7
15						1	
16		1					
21						1	
24						1	
25						1	
26		1				1	
28						1	
30						2	
31						1	
32			2	1			
33			2				
34				2			
36			1				

Table 22, cont'd.

	WAle/ CAle	EAle/ Shum/ Mitr	Kodi	Kach	PWS	SEAK	Cal
37			1				
38			1				
39			1				
40	1						
41	1						
42	1						
44							3
46		2					4
47							1
48							1
50							5
51							1
Totals	7	8	11	9	5	16	24

Table 24. Estimates of nucleotide diversity ($\pi \pm sd$) and Tajima's *D* for mitochondrial control regions, and average heterozygosity (H_E) for introns and microsatellites for marbled murrelets. Region abbreviations as in Table 3. Locus abbreviations as in Tables 5 and 6.

Region	π (%)	Tajima's *D*	H_E	
			introns	microsat-ellites
WAle[1]	0.68±0.45	0.72	0.99	0.98
CAle	-	-	1.00	0.98
EAle[2]	0.86±0.53	-0.68	-	0.99
Shum	-	-	1.00	0.99
Mitr	-	-	1.00	1.00
Kodi	0.85±0.51	-0.92	0.99	0.98
Kach	0.76±0.47	0.14	1.00	0.97
PWS	0.00±0.00	0.00	1.00	1.00
SEAK	0.80±0.47	-0.96	1.00	0.99
BC	-	-	1.00	0.99
Cal	0.97±0.54	0.85	-	0.87[3]
mean	0.72	-	1.00	0.98

[1]WAle and CAle pooled for mitochondrial control regions.

[2]EAle, Shum and Mitr pooled for mitochondrial control regions.

[3]Two loci only.

Table 25. Estimates of Φ_{st} (for mitochondrial control regions, above diagonal) and R_{st} (based on two microsatellite loci, below diagonal) for pair-wise comparisons of regional samples of marbled murrelets. Estimates that are underscored are significant at $\alpha = 0.01$; numbers in bold indicate significant differences in haplotype/allele frequencies between populations according to exact tests of population differentiation. Region abbreviations as in Table 3.

	WAle	CAle	EAle	Shum	Mitr	Kodi	Kach	PWS	SEAK	BC	Cal
WAle			-	-	-	-	-	-	-	-	-
CAle	0.00			0.09	-	0.06	0.20	0.39	0.02	-	**0.13**
EAle	0.06	0.00		-	-	-	-	-	-	-	-
Shum	0.08	0.00	0.00		-	0.00	0.10	0.17	0.05	-	0.04
Mitr	0.08	0.00	0.00	0.00		-	-	-	-	-	-
Kodi	0.04	0.00	0.00	0.00	0.00		0.03	**0.11**	**0.03**	-	**0.09**
Kach	0.10	0.02	0.03	0.00	0.00	0.02		0.05	**0.13**	-	**0.13**
PWS	0.11	0.05	0.04	0.00	0.00	0.01	0.00		0.17	-	**0.09**
SEAK	0.06	0.01	0.02	0.00	0.00	0.00	0.00	0.00		-	-
BC	0.11	0.04	0.05	0.00	0.00	0.03	0.00	0.00	0.00		**0.08**
Cal	**0.22**	0.15	**0.10**	**0.06**	0.01	**0.10**	**0.06**	0.03	**0.08**	0.03	

Table 26. Frequencies of alleles at five loci amplified with microsatellite primers within 11 regional samples of marbled murrelets. Region abbreviations as in Table 3. Locus abbreviations as in Table 6.

	WAle	CAle	EAle	Shum	Mitr	Kodi	Kach	PWS	SEAK	BC	Cal
Bma9-28											
01	0.00	0.00	-	0.00	0.00	0.00	0.00	0.00	0.00	0.02	-
03	0.25	0.30	-	0.4	0.22	0.15	0.21	0.33	0.43	0.32	-
04	0.75	0.70	-	0.6	0.78	0.85	0.79	0.67	0.57	0.66	-
Total	16	10	0	20	18	26	24	18	42	56	0
Bma10-18											
01	0.00	0.10	-	0.23	0.05	0.12	0.00	0.05	0.10	0.00	-
04	0.00	0.00	-	0.00	0.00	0.00	0.04	0.00	0.00	0.02	-
05	0.17	0.10	-	0.05	0.05	0.08	0.09	0.00	0.05	0.03	-
06	0.06	0.10	-	0.00	0.30	0.19	0.26	0.30	0.22	0.21	-
07	0.00	0.20	-	0.09	0.05	0.15	0.09	0.20	0.05	0.09	-
08	0.22	0.00	-	0.05	0.20	0.15	0.00	0.20	0.17	0.22	-
09	0.06	0.10	-	0.23	0.15	0.04	0.30	0.05	0.15	0.09	-
10	0.11	0.20	-	0.09	0.05	0.04	0.04	0.00	0.02	0.10	-
11	0.00	0.00	-	0.00	0.00	0.08	0.00	0.00	0.02	0.05	-
12	0.17	0.10	-	0.05	0.00	0.00	0.00	0.00	0.07	0.02	-
13	0.00	0.00	-	0.09	0.00	0.04	0.09	0.05	0.02	0.07	-
15	0.17	0.10	-	0.14	0.10	0.00	0.09	0.10	0.10	0.02	-
16	0.00	0.00	-	0.00	0.05	0.00	0.00	0.05	0.00	0.02	-
17	0.06	0.00	-	0.00	0.00	0.00	0.00	0.00	0.00	0.02	-
18	0.00	0.00	-	0.00	0.00	0.08	0.00	0.00	0.00	0.03	-
19	0.00	0.00	-	0.00	0.00	0.04	0.00	0.00	0.00	0.00	-
20	0.00	0.00	-	0.00	0.00	0.00	0.00	0.00	0.00	0.02	-
23	0.00	0.00	-	0.00	0.00	0.00	0.00	0.00	0.02	0.00	-
Total	18	10	0	22	20	26	23	20	41	58	0

Table 26, cont'd.

	WAle	CAle	EAle	Shum	Mitr	Kodi	Kach	PWS	SEAK	BC	Cal
Cco5-21											
101	0.00	0.00	0.00	0.07	0.06	0.00	0.03	0.00	0.03	0.02	0.00
105	0.36	0.67	0.46	0.29	0.22	0.46	0.16	0.2	0.22	0.14	0.17
107	0.00	0.00	0.04	0.00	0.06	0.00	0.06	0.00	0.03	0.02	0.00
109	0.14	0.17	0.33	0.50	0.50	0.42	0.63	0.65	0.56	0.61	0.69
111	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.02	0.00
113	0.07	0.00	0.13	0.04	0.06	0.04	0.03	0.05	0.08	0.05	0.04
117	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00
121	0.21	0.17	0.04	0.07	0.06	0.04	0.06	0.05	0.08	0.14	0.09
123	0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
125	0.14	0.00	0.00	0.00	0.06	0.00	0.03	0.05	0.00	0.00	0.01
Total	14	6	24	28	18	24	32	20	36	56	70
Uaa5-8											
75	0.00	0.00	0.05	0.08	0.11	0.13	0.00	0.06	0.03	0.02	0.06
77	0.00	0.00	0.09	0.08	0.22	0.08	0.09	0.11	0.08	0.17	0.41
79	0.08	0.25	0.27	0.17	0.22	0.13	0.25	0.17	0.15	0.13	0.13
81	0.83	0.75	0.41	0.46	0.33	0.50	0.50	0.50	0.54	0.50	0.29
83	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04	0.06
85	0.08	0.00	0.18	0.21	0.11	0.17	0.16	0.17	0.21	0.13	0.04
Total	12	8	22	24	18	24	32	18	39	52	68
Ulo14b29											
01	0.19	0.40	-	0.73	0.61	0.58	0.71	0.70	0.57	0.62	-
02	0.81	0.60	-	0.27	0.39	0.42	0.29	0.30	0.43	0.38	-
Total	16	10	0	22	18	24	24	20	42	58	0

Table 27. Estimates of F_{st} based on nine introns (above diagonal) and 14 nuclear loci (below diagonal) for pair-wise comparisons of regional samples of marbled murrelets. Estimates that are underscored are significant at $\alpha = 0.01$. Region abbreviations as in Table 3.

	WAle	CAle	Shum	Mitr	Kodi	Kach	PWS	SEAK	BC
WAle		0.05	0.04	0.06	0.08	0.10	0.09	0.08	0.07
CAle	0.02		0.06	0.08	0.11	0.12	0.10	0.10	0.12
Shum	0.06	0.04		-0.01	0.01	0.03	0.01	0.01	0.02
Mitr	0.06	0.05	0.00		0.00	0.01	-0.02	0.00	-0.01
Kodi	0.07	0.07	0.01	0.00		0.00	0.00	0.01	0.02
Kach	0.09	0.08	0.01	0.00	0.00		0.01	0.01	0.02
PWS	0.09	0.07	0.00	-0.02	0.00	0.00		0.00	0.01
SEAK	0.06	0.06	0.00	0.00	0.01	0.01	-0.01		0.00
BC	0.07	0.08	0.01	-0.01	0.01	0.02	0.00	0.00	

Table 28. Indices of population genetic structure based on different types of loci in marbled murrelets. Region abbreviations as in Table 3.

Marker Type	No. samples	Sampling Range	No. Loci or Haplotypes	No. Alleles	Index
Allozymes[1]	36	WAle - Oregon	18	1 - 4	$F_{st} = 0.09$[2]
Introns	121	WAle - BC	9	4 - 14	$F_{ct} = 0.094$[2]
Microsatellites	174	WAle - Cal	2	6-10	$R_{st} = 0.075$[2]
Cytochrome *b*[1]	43	WAle - Oregon	1 (1045 bp)	13	$\Phi_{st} = 0.02$
Mitochondrial control region	79	WAle - Cal	1 (546 bp)	37	$\Phi_{ct} = 0.090$[2]

[1]Friesen et al. 1996a

[2]$P < 0.001$.

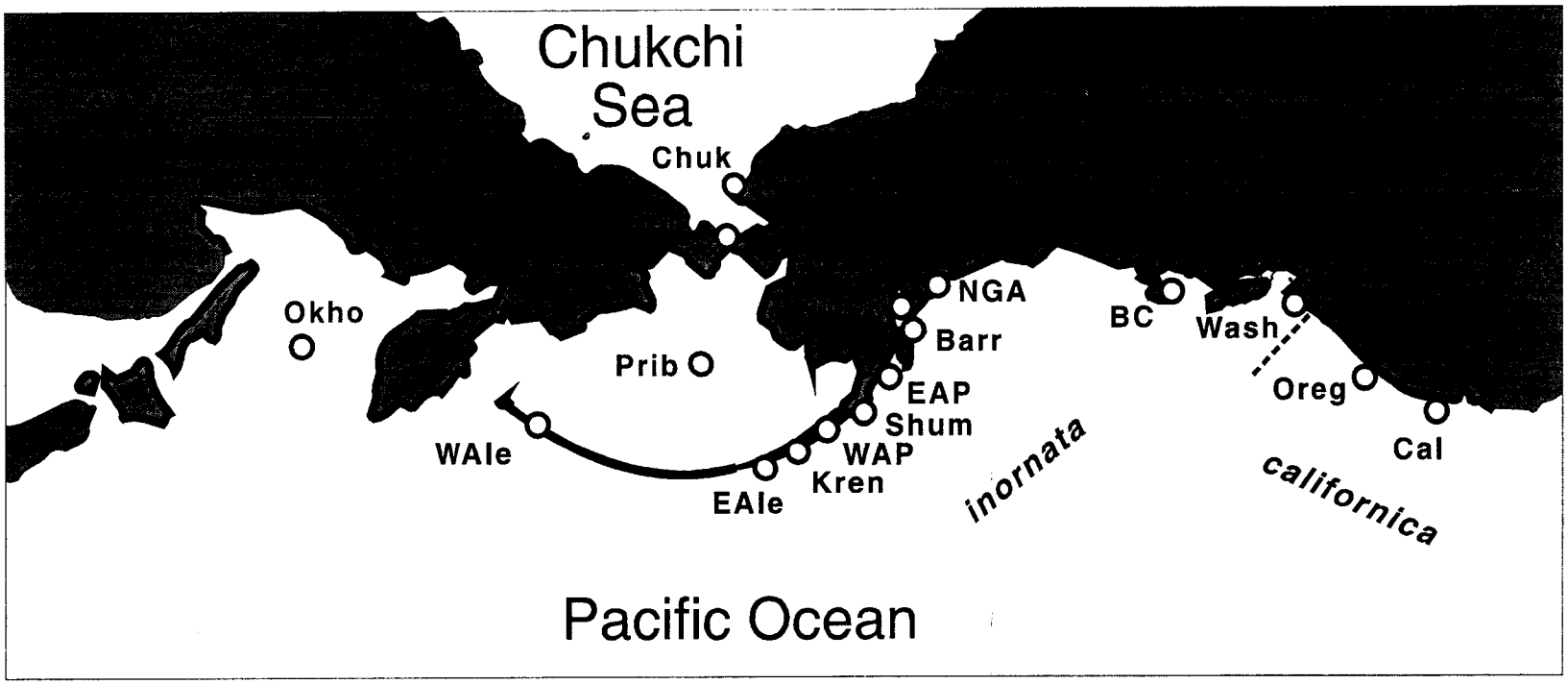


Fig. 1. Approximate Pacific breeding distribution (heavy black lines; from Udvardy 1963), sampling regions (open dots) and subspecies ranges (dashed line) for common murres. Region abbreviations as in Table 1.

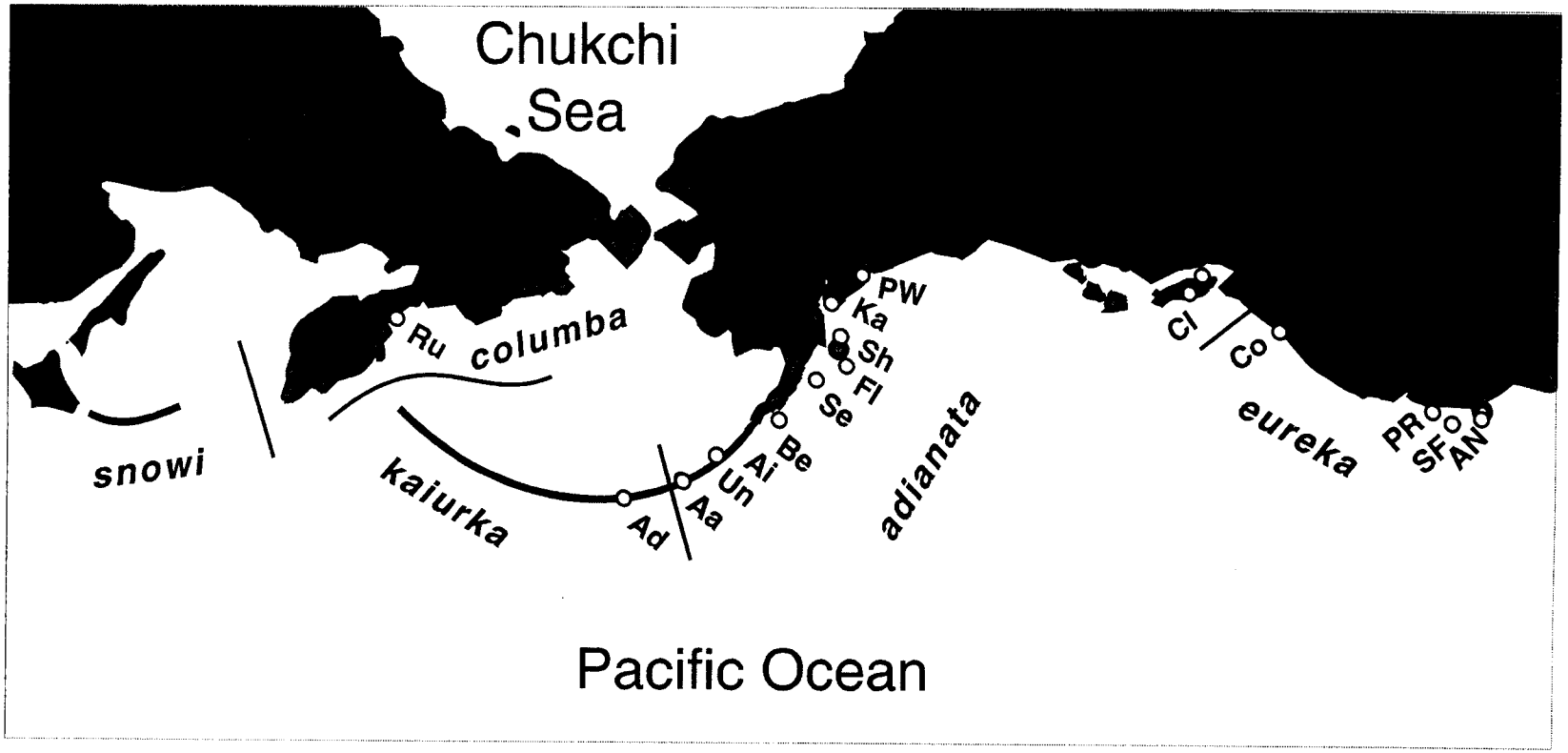


Fig. 2. Breeding distribution (heavy black lines; from Udvardy 1963), sampling sites (white dots) and subspecies ranges (dashed lines) for pigeon guillemots (from Udvardy 1963). Ru = Eastern Kamchatka Peninsula; Ad = Adak Island ; Aa = Anangula Island; Un = Unalaska Island; Ai = Aiktak Island; Be = Belkofski Bay; Se = Semidi & Suklik islands; Fl = Flat Island; Sh = Shuyak Island; Ka = Kachemak Bay; PW = Naked& Jackpot islands; Cl = Cleland Island & Seabird Rocks; Ma = Mandarte Island; Co = Coos Bay ; PR = Point Reyes National Seashore ; SF = Southeast Farallon Island; AN = Ano Nuevo Island.

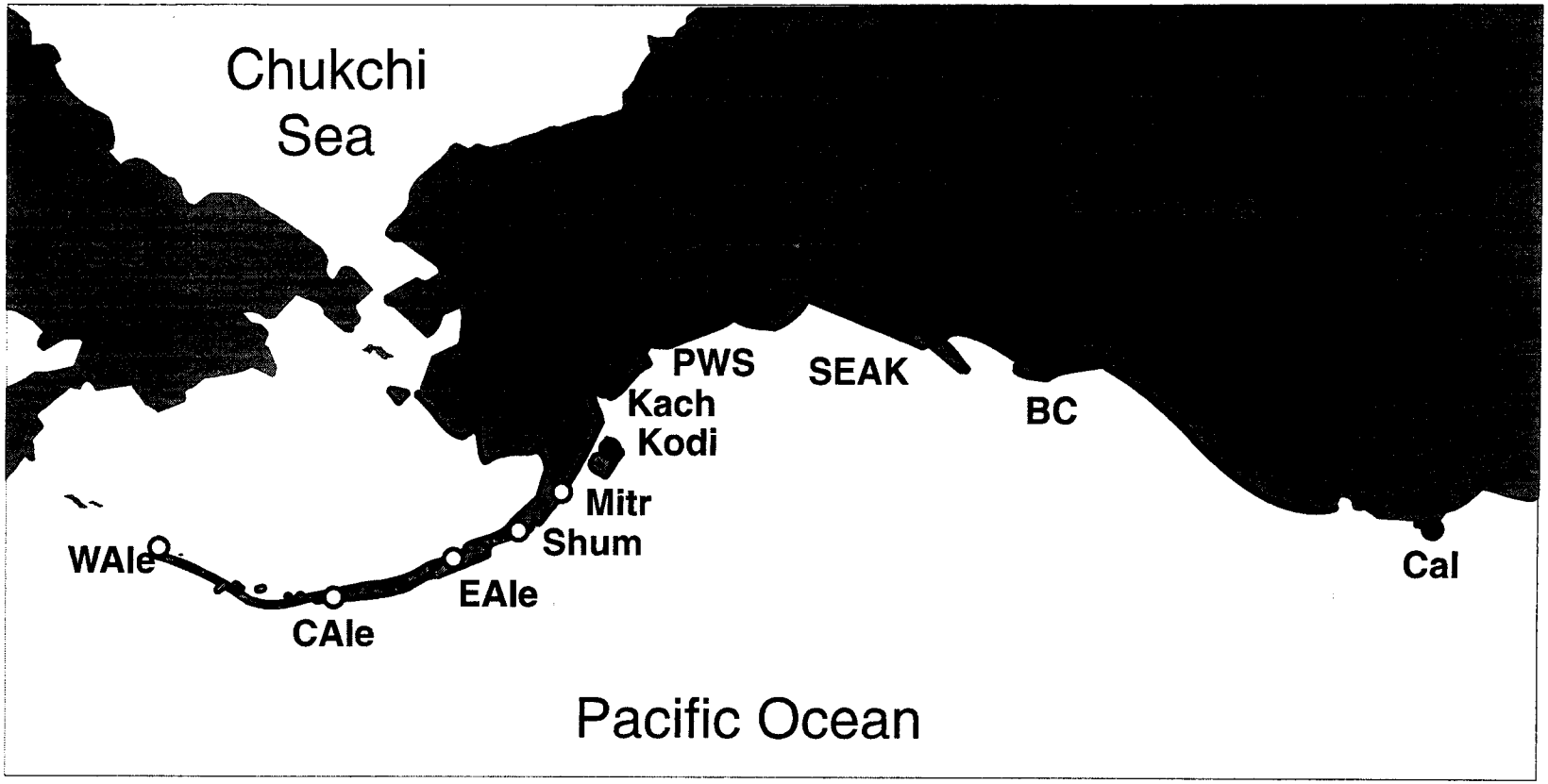


Fig. 3. Breeding distribution (heavy black lines; from Udvardy 1963) and sampling sites (dots) for marbled murrelets. Grey dots represent sites where murrelets breed in trees; white dots represent sites where murrelets nest on the groun. Abbreviations in Table 3.

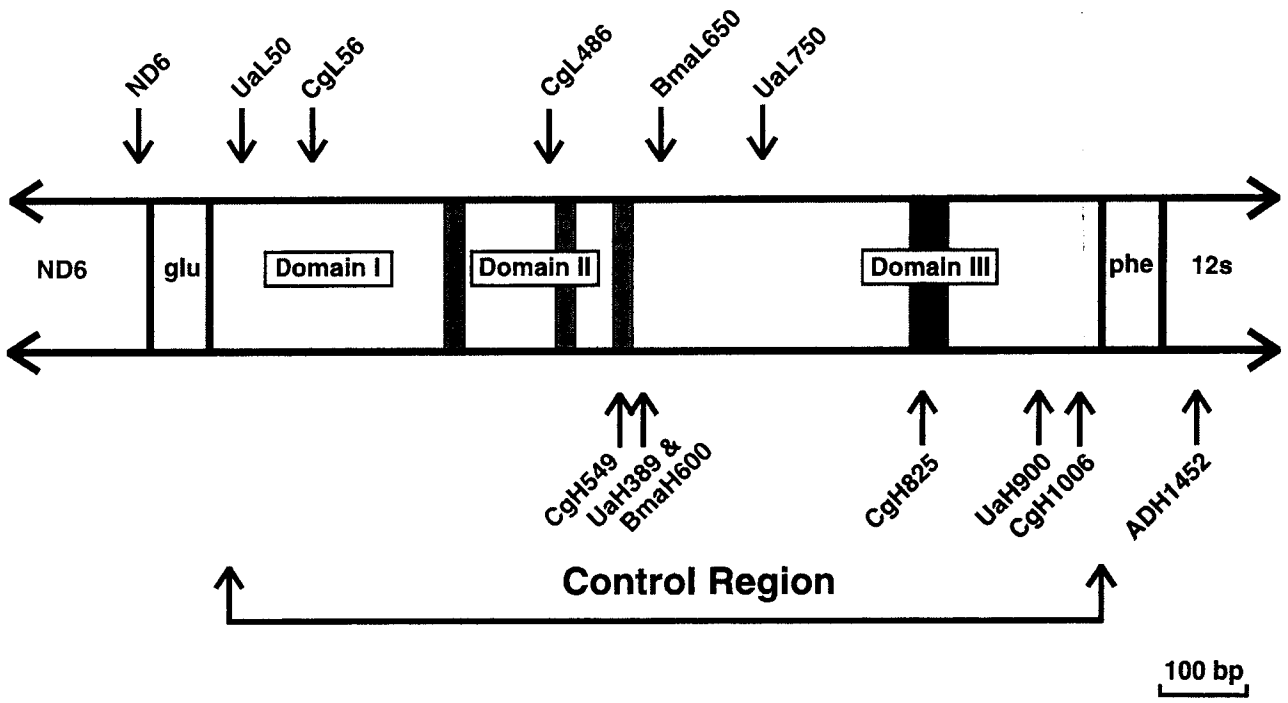


Fig. 4. Map of the mitochondrial control region and neighboring genes for alcids, and approximate locations of PCR primers used in present study (Table 4). "glu" = tRNA for glutamic acid; "phe" = tRNA for phenylalanine; "12s" = 12s rRNA; "F" = F Box; "D" = D Box; "C" = C Box, "1" = Conserved Sequence Block 1.

tRNA^{glu}

COMU gtttc**tgc**ttggcttttctccaagacccgcggcccgaaaagcc**act**gttgt**accac**

MAMU gtttc**ccg**ttggcttttctccaagacccgcggcccgaaaagcc**gtt**gttgt**caact**

KIMU gtttc**ccg**ttggcttttctccaagacccgcggcccgaaaagcc**gtc**gttgt**caact**

COMU **t**tcaactacagaaac

MAMU -tcaactacagaaac

KIMU -tcaactacagaaac

F Box

COMU g**a**gctcctcacgtgaaatcagcaacccg

PIGU g**t**gctcctcacgtgaaatcagcaacccg

MAMU g**a**gctcctcacgtgaaatcagcaacccg

D Box

COMU cctctggttcct**at**gtcagg**gcc**a**t**

PIGU cctctggttcct**cg**gtcagg**cat**a**a**

MAMU cctctggttcct**at**gtcagg**gcc**a**t**

C Box

COMU ttgtacttcaccgatacatctggtcggc

PIGU ttgtacttcaccgatacatctggtcggc

MAMU ttgtacttcaccgatacatctggtcggc

CSB-1

COMU tatttagtgaatgcttg**tt**ggg**g**cat**at**

PIGU tatttagtgaatgcttg**tt**ggg**g**cat**aa**

MAMU tatttagtgaatgcttg**ct**gg**a**cat**ga**

tRNA^{phe}

COMU gtctccgtagcttaacaatcaaagcatgcactgaagatgccaacatggccgccaca

COMU tgtacccgaagacaa

Fig. 5 Alignment of the tRNA^{glu} and tRNA^{phe} genes and conserved sequence blocks within the mitochondrial control regions of the common murre (COMU, Moum and Johansen 1992 and present study), pigeon guillemot (PIGU, Kidd and Friesen 1998a), and marbled (MAMU) and Kittlitz's murrelets (KIMU, present study). Dashes indicate insertions or deletions. Variable sites are shown in bold.

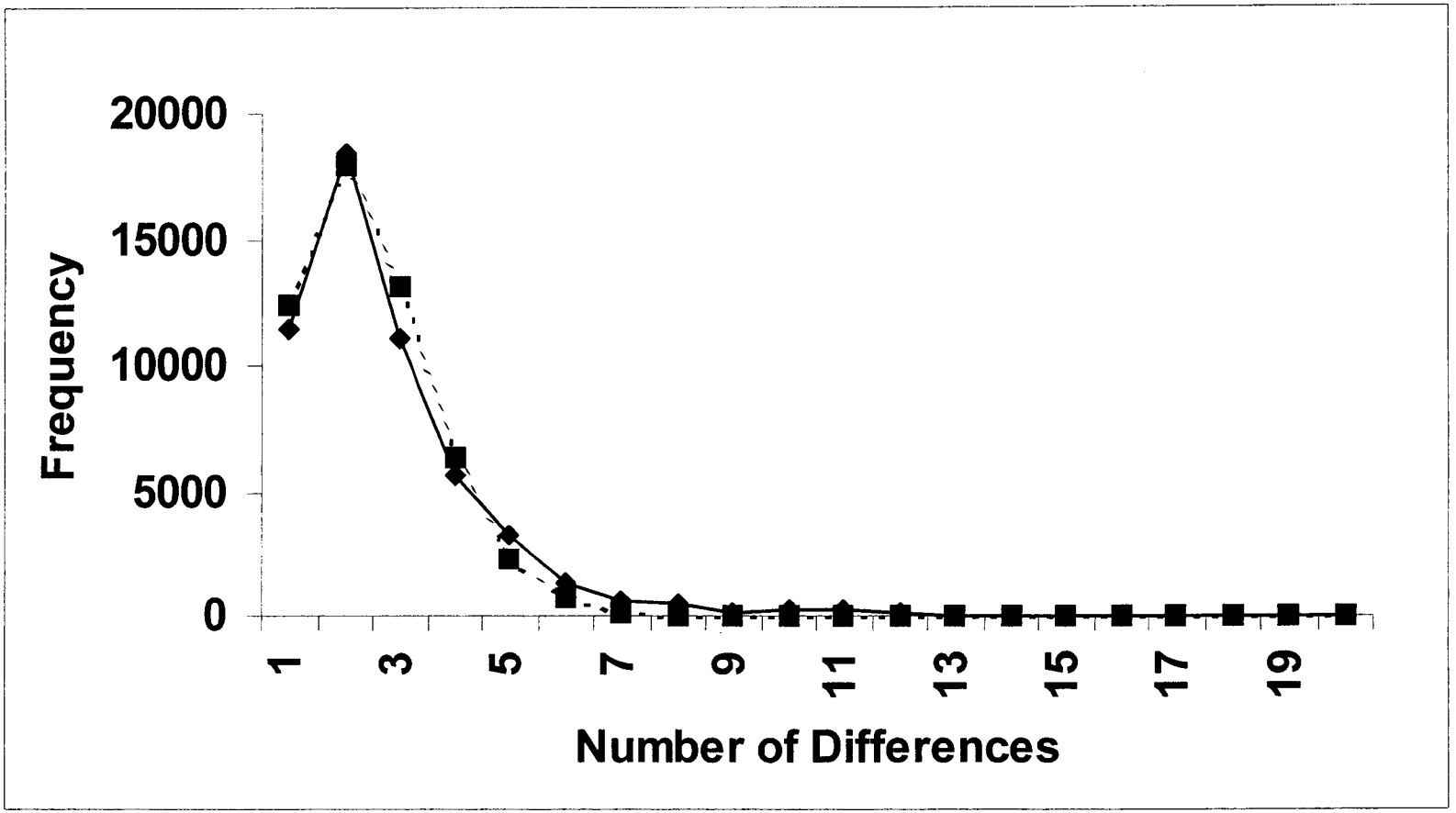


Fig. 6. Frequency of pair-wise sequence differences (mismatch distribution) for control region sequences of 328 common murres. Diamonds and solid line = observed distribution. Squares and dashed line = distribution expected under a sudden population expansion.

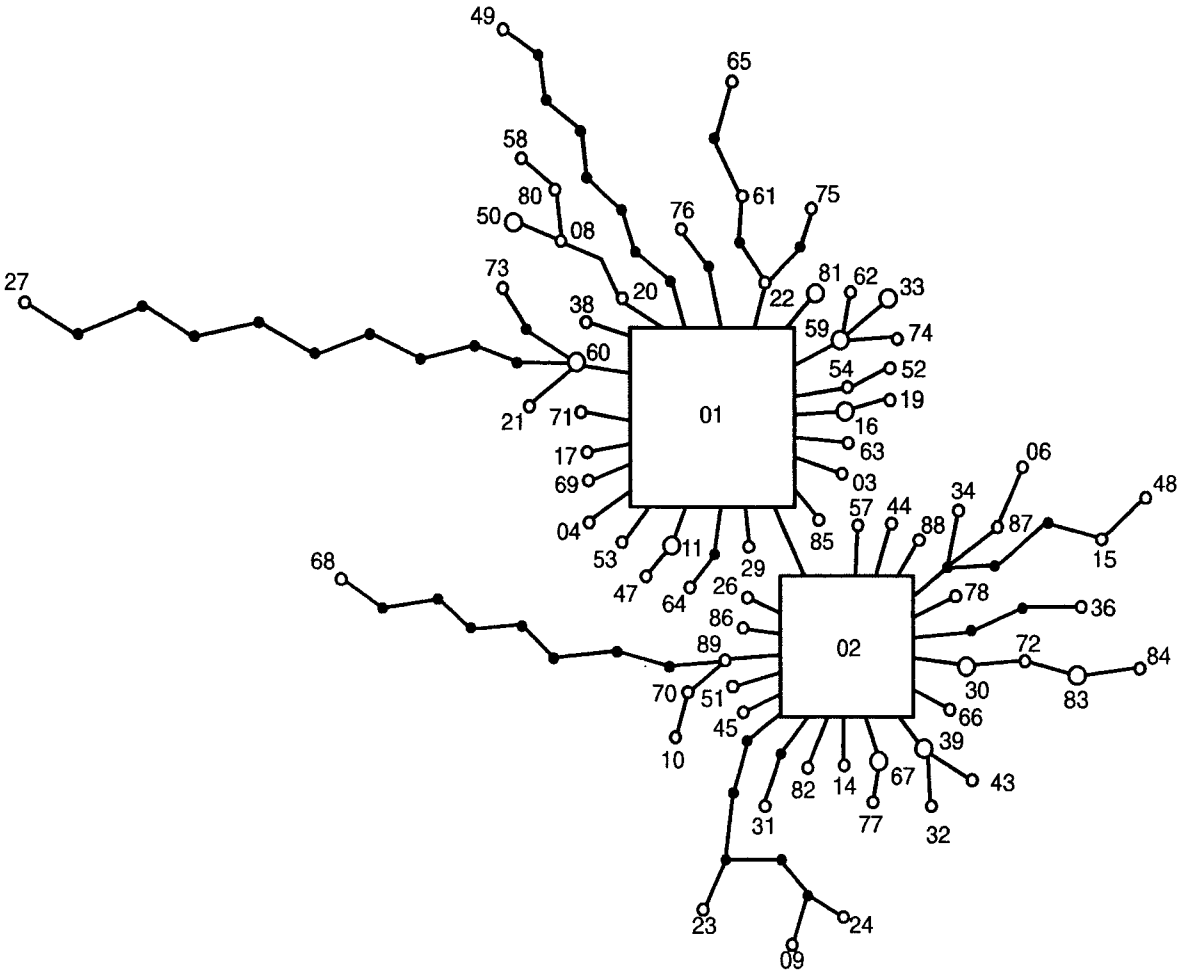
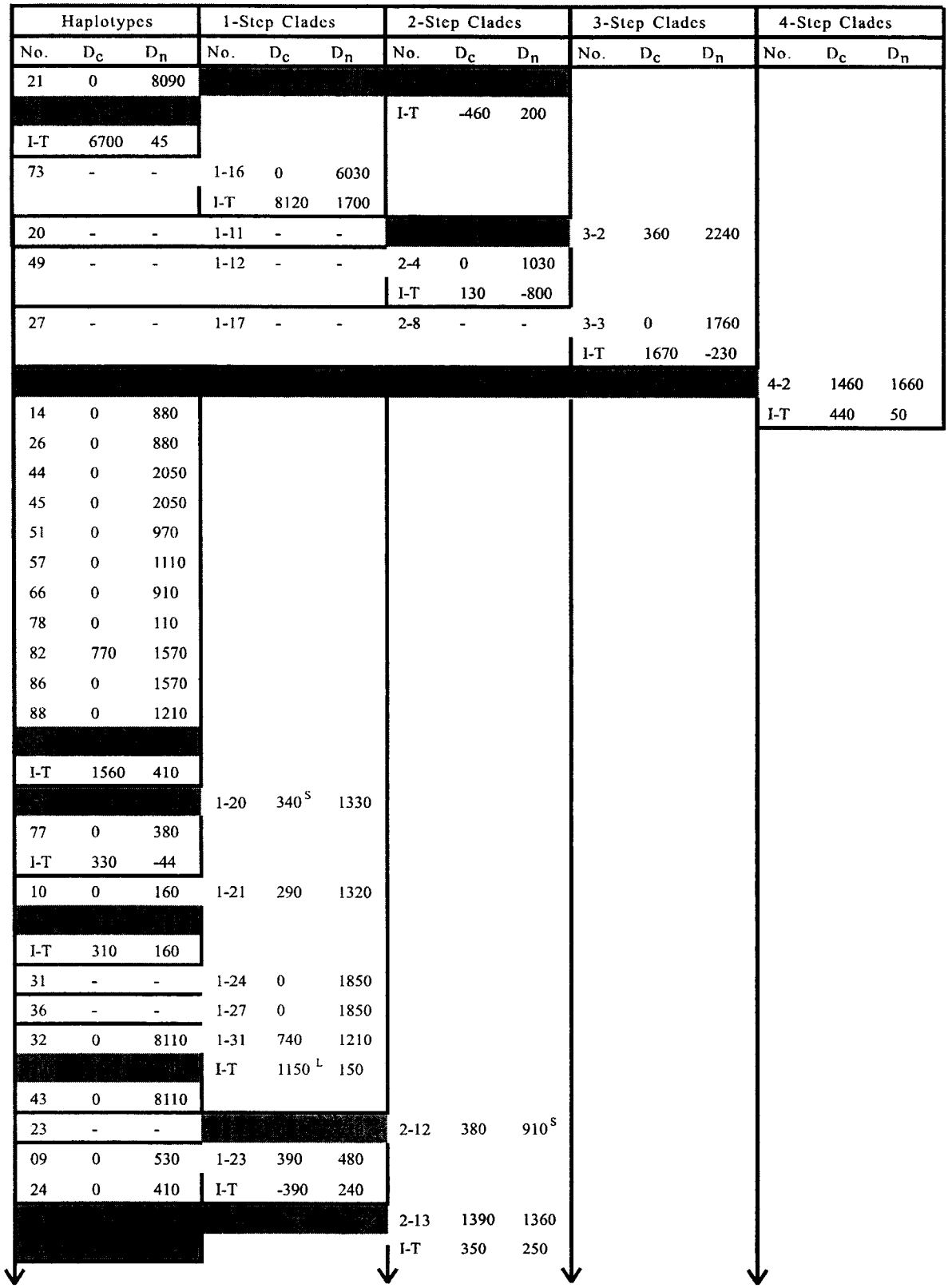


Fig. 7. Statistical parsimony tree showing the substitutional relationships among control region haplotypes of common murres (Table 8). Sizes of circles or squares are proportional to haplotype frequencies (Table 7). Squares indicate haplotypes with the highest root probabilities. Black dots indicate missing haplotypes.


Haplotypes
1-Step Clades
2-Step Clades
3-Step Clades
4-Step Clades
No. Dc Dn
21 0 8090
I-T 6700 45
73 - -
20 - -
49 - -
27 - -
14 0 880
26 0 880
44 0 2050
45 0 2050
51 0 970
57 0 1110
66 0 910
78 0 110
82 770 1570
86 0 1570
88 0 1210
I-T 1560 410
77 0 380
I-T 330 -44
10 0 160
I-T 310 160
31 - -
36 - -
32 0 8110
43 0 8110
23 - -
09 0 530
24 0 410
1-16 0 6030
I-T 8120 1700
1-11 - -
1-12 - -
1-17 - -
1-20 340S 1330
1-21 290 1320
1-24 0 1850
1-27 0 1850
1-31 740 1210
I-T 1150L 150
1-23 390 480
I-T -390 240
I-T -460 200
2-4 0 1030
I-T 130 -800
2-8 - -
2-12 380 910S
2-13 1390 1360
I-T 350 250
3-2 360 2240
3-3 0 1760
I-T 1670 -230
4-2 1460 1660
I-T 440 50

Haplotypes			1-Step Clades			2-Step Clades			3-Step Clades			4-Step Clades		
No.	D_c	D_n	No.	D_c	D_n	No.	D_c	D_n	No.	D_c	D_n	No.	D_c	D_n
03	0	1650												
04	0	1650												
05	0	1650												
17	210	1030												
29	1300	1940												
38	0	2110												
53	0	1110												
63	0	950												
69	0	950												
71	0	720												
81	0^S	2230												
85	0	1540												
I-T	1430	30												
64	-	-	1-2	0	110									
			1-3	720	1810									
47	0	580												
I-T	710	210												
16	-	-	1-4	0^S	1090									
19	-	-												
52	0	470	1-5	240	1390									
I-T	100	-310												
33	8110	7860	1-6	7570	2830									
62	0	8290												
74	0	7010												
I-T	2500	210												
75	-	-	1-7	1450	1720									
76	-	-	1-10	0	2130									
			I-T	-1450^S	-370^S									
						2-2	7450	2690						
65	-	-	1-9	0	6970									
			I-T	0	980									
50	-	-	1-13	170	520	2-5	270^S	1210						
58	0	1370												
			I-T	1430	1020									
I-T	0	510												

Haplotypes			1-Step Clades			2-Step Clades			3-Step Clades			4-Step Clades		
No.	D_C	D_n	No.	D_C	D_n	No.	D_C	D_n	No.	D_C	D_n	No.	D_C	D_n
			1-26	270 ^{S}	1560									
84	0	400	I-T	460	-380									
I-T	270	-120												
			1-28	-	-	2-14	70	330 ^{S}	3-5	890	1080			
48	0	70												
I-T	0	10												
34	0	1910												
						I-T	1030 ^{L}	760 ^{L}						
I-T	0	-970												
06	-	-	1-30	140	650									
			I-T	1090	930									
68	-	-	1-19	-	-	2-11	-	-	3-6	0	680			
									I-T	680	420 ^{L}			

Fig. 8. Results of nested clade analysis of control region sequences of common murres, based on tree in Fig. 7. "No." is clade number. "D_C" indicates clade distance. "D_N" indicates nested clade distance. Shading indicates interior clades. "I-T" indicates interior-tip distance. Supersript "S" indicates a distance that is significantly small; superscript "L" indicates a distance that is significantly large. Vertical lines enclose haplotypes that belong to the same clade (see Fig. 7). Hyphen indicates no result (no genetic variation and/or no geographc variation to test).

Crys

```
C1 TCAGAGCAAC AGCGTGGCTA GGAAAAAAAC ATCATGACAA ATGAAGCACC TGAATTCAGA  60
T1 ....C..... .......... .......... .......... .......... ..........  60

C1 AAACGTCACA AGGACCAAGA GGTAACGCTG GCCAAAGGAT GGAGTGTACA GGGAGCTGGC 120
T1 .......... .......... .......... .......... .......... .......... 120

C1 ATCACCTGCC CACTCAGTGC TTGAGTCCCC GGTTGGTCAG AGCCACACAG GAACATAGGG 180
T1 ....G..... .......... .........A A......... .......... .......... 180

C1 AGTTCCTCTT CCAGTTTGCA AGGTTAGCAC TGGTTTGTCA CCAACCTGAG GGGCCCCCTC 240
T1 ..G....... .......... .......... .......... .......... .......... 240

C1 AGAGGATGAT GCACTTCTAG GATCCAGCTG AGCAGGGCCC CAAGGGTGGA GGAGGTTTCC 300
T1 .......... .......... .......... .......... .......... .......... 300

C1 TCCAGTCAAG TGTGAACGGT GGTTACTGAG CAA                               333
T1 .......... .......... ......???? ???                               326
```

Enol

```
C1 AAAGGGTCTT CAATGGACAC CACTGTAGAG GGATACAAGG AGC-TTTAAG ACAACACCAC  60
T1 .......... .......... .......... .......... ...G...... ..........  60

C1 CCTGTGAAAG GTCTTGTTTG AAAGCAGTGG TACCAAAGTG CTGCAGCCCC AAAGAACTAC 120
T1 .......... .......... .......... ..T....... .......... .......... 120

C1 CCATCTCAAC ATTTTCTAAG ACCCCAAATA GGCTAGCCAT TTGTTCTGCT CTTGATTCCT 180
T1 .......... .......... .......... .......... .......... .......... 180

C1 CTTGTAAGAG AAGCTGCACT CTAACCCTTT CCTTCAGCAA TCACCCCTAT TTGCCACTAC 240
T1 .......... .......... .....T.... .......... .......... .......... 240

C1 TACGGCACTT TTTGGTTGAC AAGCAACTTA CCGGGGTAGC TCTTGACAAA GCCCTTGTAC 270
T1 ...CC..... .......... .......... .....T.... .A........ .......... 270

C1 AGGTCAGCAA GCTGATCAGG AGAAATGTAT CTGCTGGGAT CATCGGGGGA TTTGAAGTCC 320
T1 .....????? ?????????? ?????????? ?????????? ?????????? ?????????? 305
```

LDH

```
C1 CCCCTCTTGC TGACGAACTC CTGCAGTTAC TACCACAATC TTGGAGTTGG CCGTGACAGC  60
T1 .......... .......... .......... .......... .......... ..........  60

C1 ATAGTCTAAA ACACAGCATG GGGGA-GAGA TAGCAAACAT GTTTAATAGA CTTATCACTA 120
T1 .......... .......... .....C.... .......... .......... .......... 120

C1 ATTTCAATAG CATTTTGTTC ATAGAGGCAT TAT--CGGTA GCTTCTCCAC CATGCATACA 180
T1 .......... .......... .......... ...AT..... .......... .......... 180

C1 CTCAAACCCA AAAACCATGA GATATTAACC ATTAAAAGTA TTAACCCCTT GTTAGTGTAC 240
T1 .......... .......... .......... .......... .......... .......... 240

C1 TTCTGAAATC TCAGCCCAAT TCATACACTT TAACTTTTCA ATGCCAAGGC CTTTAGAGTT 300
T1 .......... .......... .......... .......... .......... .......... 300

C1 CTTTAAGATT ACACAACTAA CACTCCACCC TTAAGCAAAT TCACAGAATA CCAGTATTAA 360
T1 .......... .......... ........T. .......... .......... .......... 360

C1 ACAAAAGACG CTGAGGGTCA CCTTTGCCTG CCACAATCTT GTGAGTGTGA AGGACAGGCT 420
T1 .......... .......... .......... .......... .......... .......... 420

C1 GCCATGCTGT AGA                                                     433
T1 .......... ...                                                     433
```

P40

```
C1 CTCCTTTTCG ATCTGCAATG GAAGAGAGTA GCCTAAGTCT CCCACCAGAA ACACCTCACC  60
T1 .......... .......... .......... .......... .......... ..........  60

C1 ACCCTCACAC TATCACATAC CAGACAATAT GCACCGACCA AC-GACAAGA CTAATTAACC 120
T1 .......... .......... .......... .....A.... ..C....... ...C...... 120

C1 ACCACCCCTA ACTGTGAAGT GCAATCATTA TATTGGCAAA ACCTCAACAG CAGGCACACA 180
T1 .......... .......... .......... .......... ....?????? ?????????? 164

C1 GGACCATGGG GCAGGAGCAC AAGGAGGACC TCTCACAGAG CCACGCTTCC TTCCTGAGGT 240

C1 CTCCTACCTC CTCGGGATCC CTGTAGAAGT ACAAGTCAGG CATGACTTCC CATGGGTGCT 300

C1 CACGGGAGAT GGTGCCACGC ATGCGCAGGA CCTCCCGA                          338
```

Fig. 9. Sequences of the most common alleles for four nuclear introns for common (C1) and thick-billed murres (T1). Dots indicate identity with the sequence for the common murre. Dashes indicate insertions/deletions. Question marks indicate unknown bases. Variable sites for common murres are highlighted in bold.

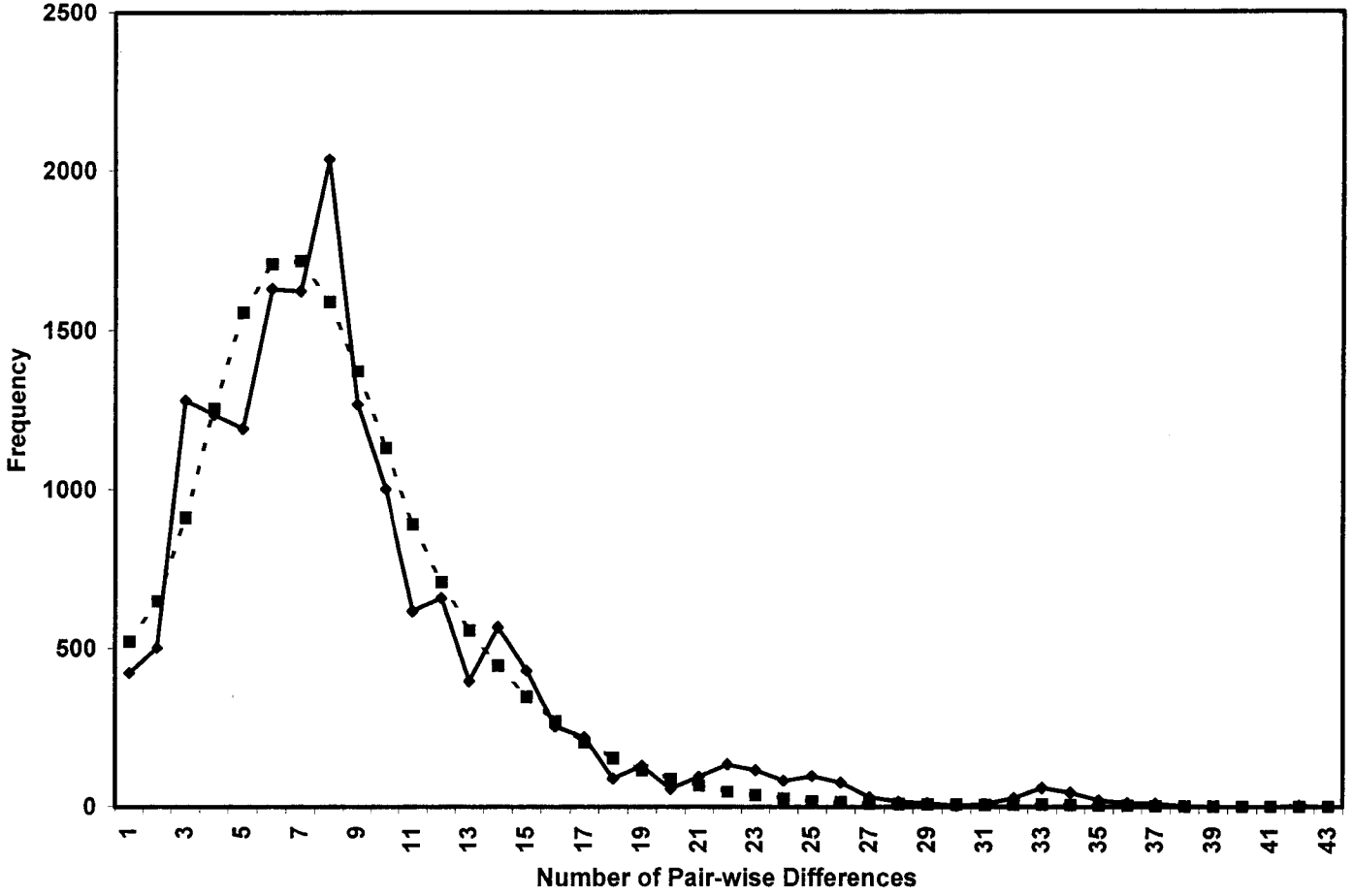


Fig. 10. Frequencies of pair-wise sequence differences (mismatch distribution) for control region sequences of pigeon guillemots. Solid line = observed distribution. Squares and dashed line = distribution expected under a sudden population expansion.

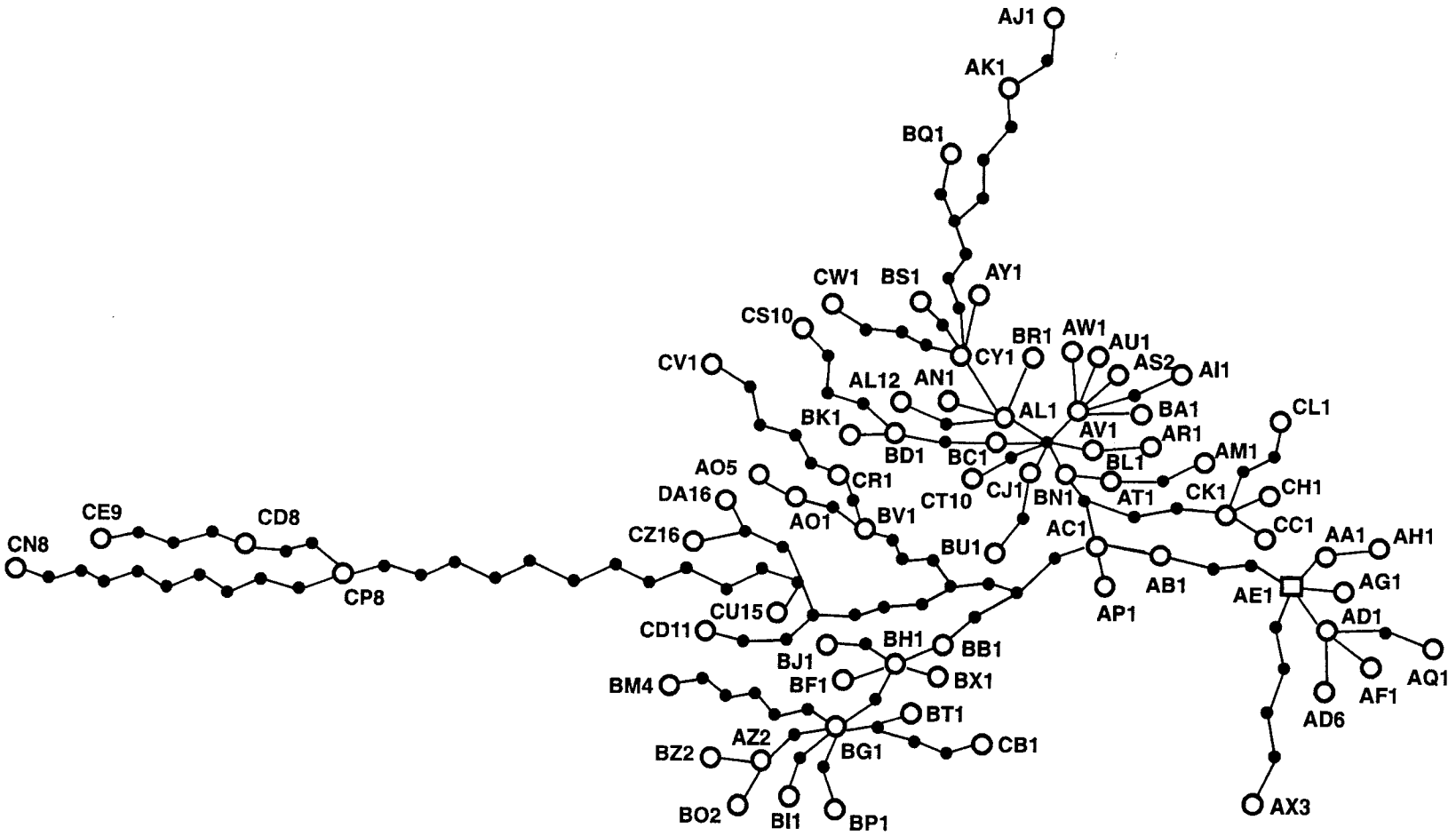


Fig. 11. Statistical parsimony tree showing the substitutional relationships among control region haplotypes of pigeon guillemots (Table 15). (Sizes of circles do not relate to haplotype frequencies.) Black dots indicate missing haplotypes. Square indicates the haplotype with the highest root probability.

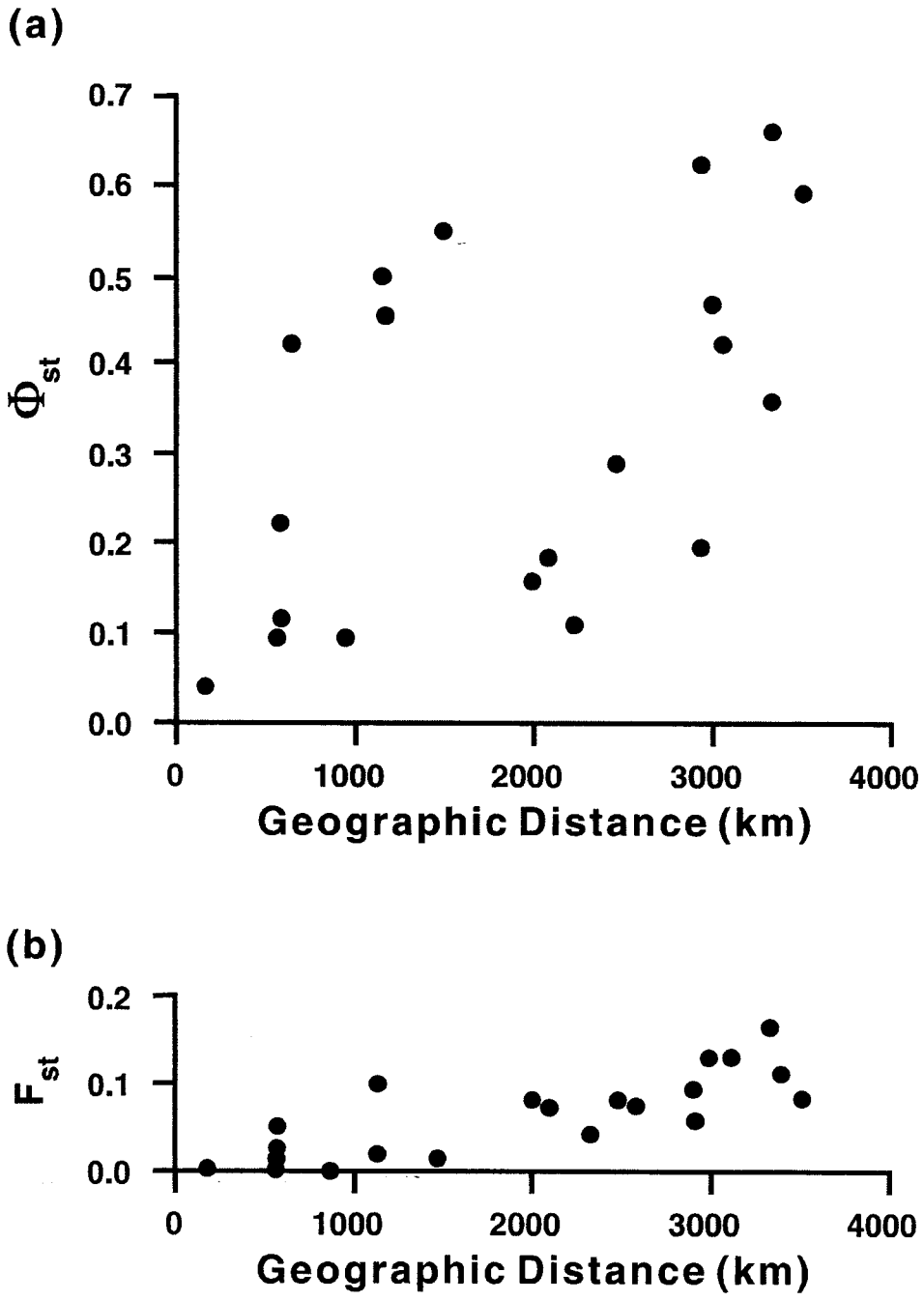


Fig. 12. Pair-wise estimates of (a) Φ_{st} (for control region sequences) and (b) F_{st} (for nuclear loci) versus geographic distance (km) between regional samples of pigeon guillemots.

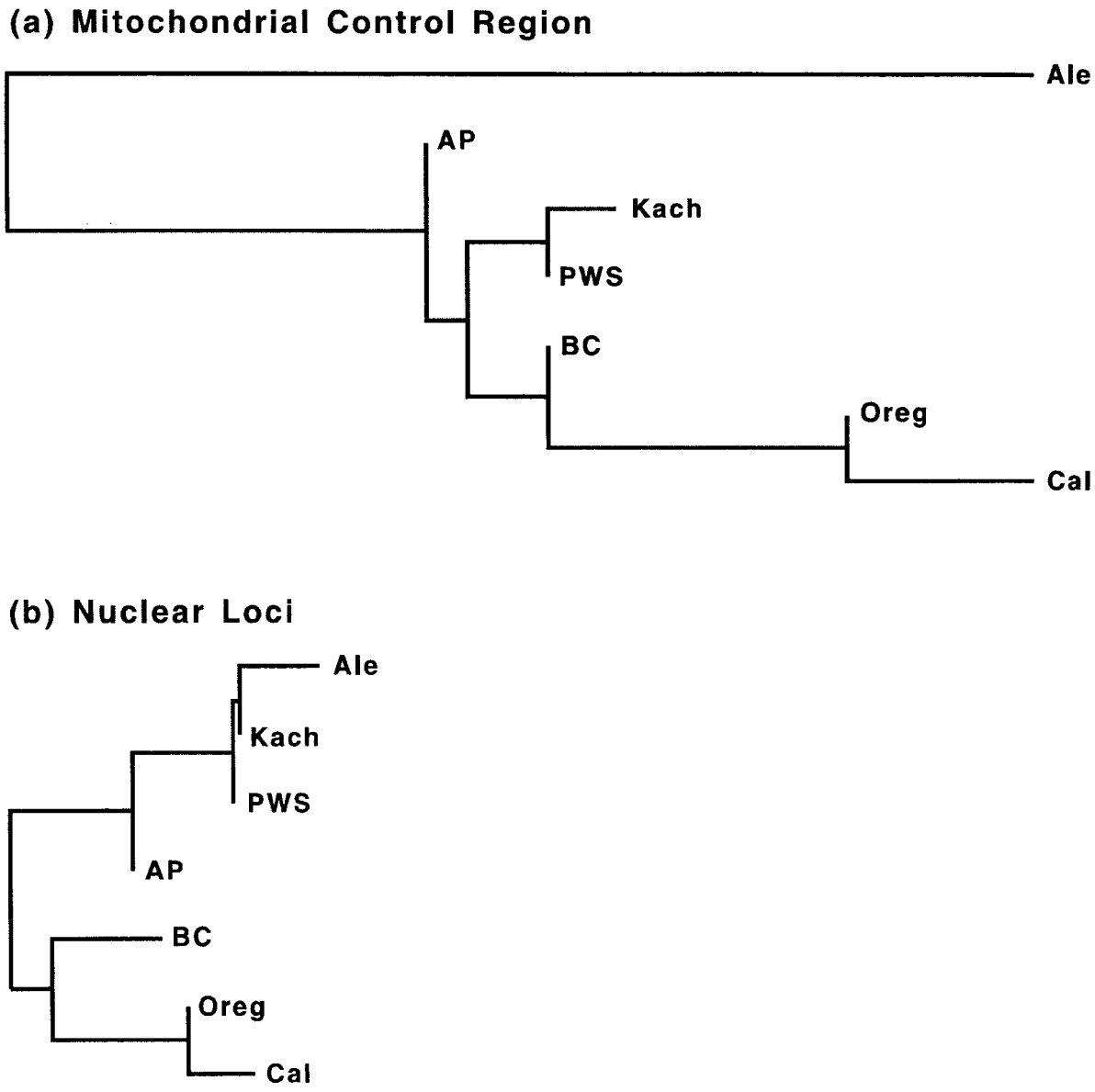
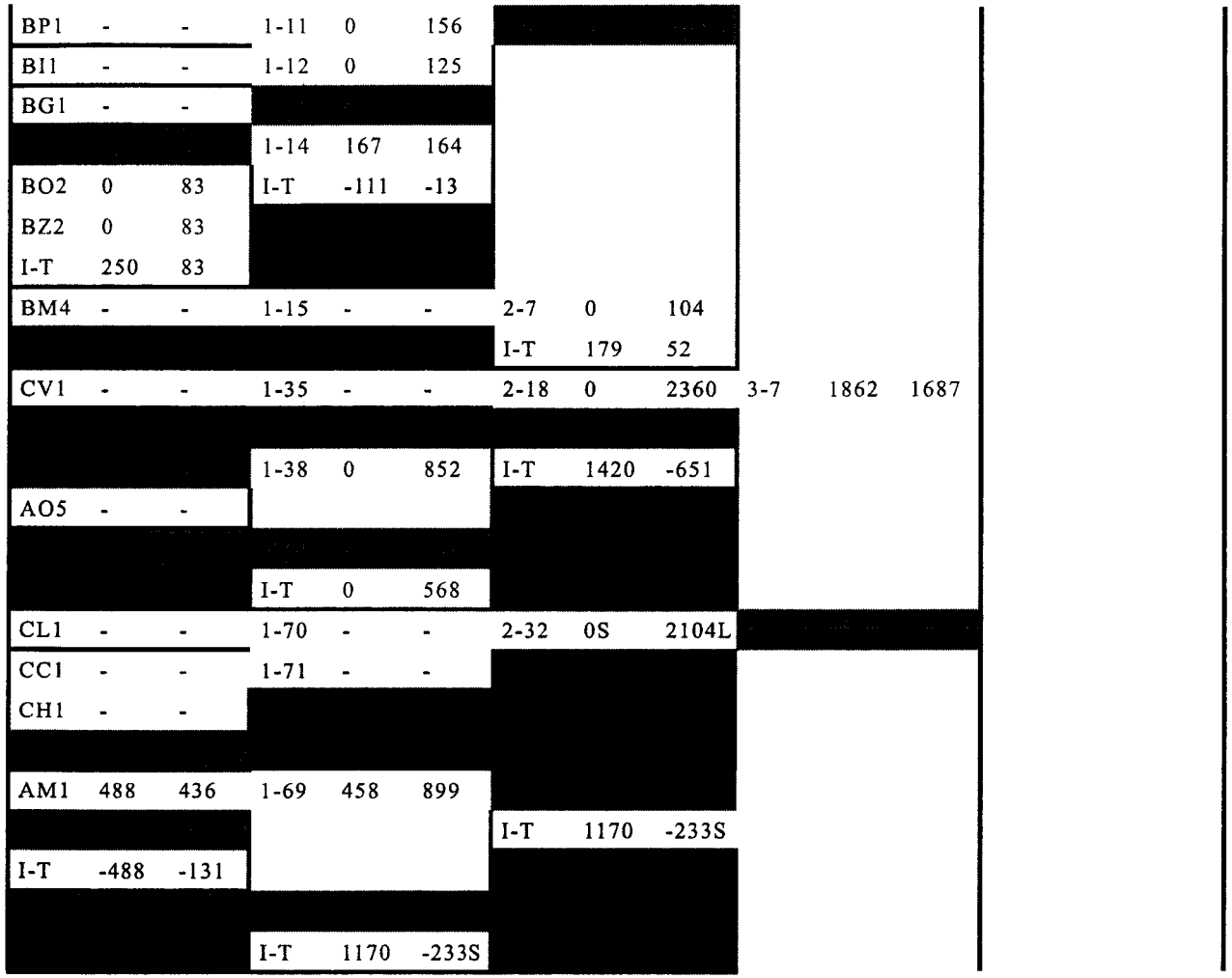
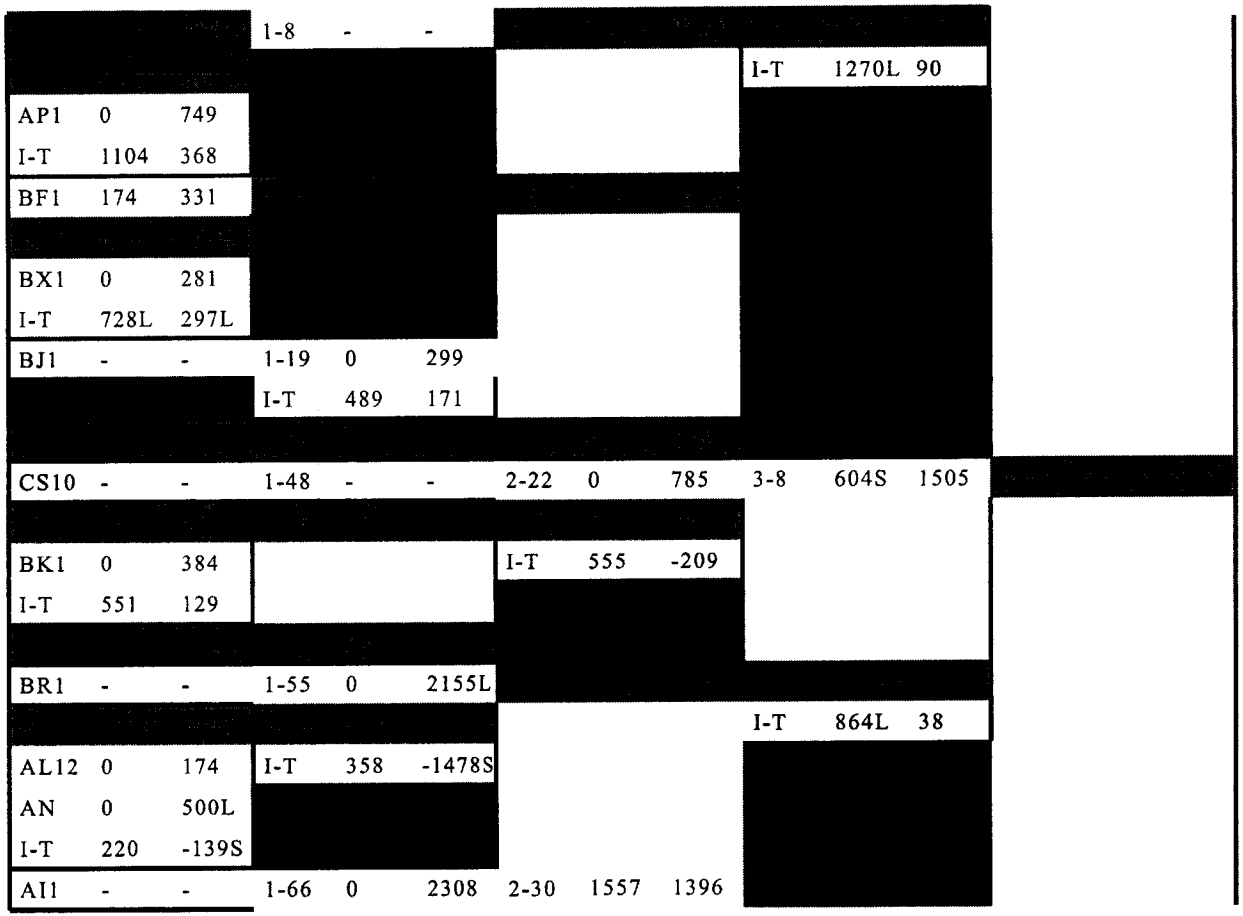


Fig. 13. Neighbor-joining trees based on Slatkin's linearized F_{st}s between regional samples of pigeon guillemots for (a) control region haplotypes, and (b) nuclear loci. See Table 2 for region abbreviations.

Haplotypes			1-Step Clades			2-Step Clades			3-Step Clades			4-Step Clades			5-Step Clades		
No.	D_c	D_n	No.	D_c	D_n	No.	D_c	D_n	No.	D_c	D_n	No.	D_c	D_n	No.	D_c	D_n
CN8	-	-	1-24	-	-	2-9	-	-	3-3	-	-	4-2	0	461	5-1	1765S	1804S
CE9	-	-	1-41	-	-	2-12	-	-									
CZ16	-	-	1-44	0	1000							I-T	1043	396			
DA16	-	-	I-T	0	500												
CD11	-	-	1-21	-	-												
			1-4	276	296	2-1	317	306	3-1	416S	1563S				5-2	900S	2507L
AD6	0	105															
AF1	0	126															
I-T	383	148															
AQ1	-	-	1-5	0	531												
AX3	-	-	1-1	-	-	2-2	0	1060L									
AG1	0	180				I-T	109	5									
I-T	504	365															
			1-6	445	379												
AH1	0	398	I-T	-134	-54												
I-T	420	22															
CB1	-	-	1-9	-	-	2-5	0	114	3-2	169S	1953L						
BT1	-	-	1-10	-	-												


BP1 - - 1-11 0 156
BI1 - - 1-12 0 125
BG1 - -
1-14 167 164
BO2 0 83 I-T -111 -13
BZ2 0 83
I-T 250 83
BM4 - - 1-15 - - 2-7 0 104
I-T 179 52
CV1 - - 1-35 - - 2-18 0 2360 3-7 1862 1687
1-38 0 852 I-T 1420 -651
AO5 - -
I-T 0 568
CL1 - - 1-70 - - 2-32 0S 2104L
CC1 - - 1-71 - -
CH1 - -
AM1 488 436 1-69 458 899
I-T 1170 -233S
I-T -488 -131
I-T 1170 -233S


1-8 - -
I-T 1270L 90
AP1 0 749
I-T 1104 368
BF1 174 331
BX1 0 281
I-T 728L 297L
BJ1 - - 1-19 0 299
I-T 489 171
CS10 - - 1-48 - - 2-22 0 785 3-8 604S 1505
BK1 0 384
I-T 551 129
I-T 555 -209
BR1 - - 1-55 0 2155L
I-T 864L 38
AL12 0 174
AN 0 500L
I-T 220 -139S
I-T 358 -1478S
AI1 - - 1-66 0 2308 2-30 1557 1396

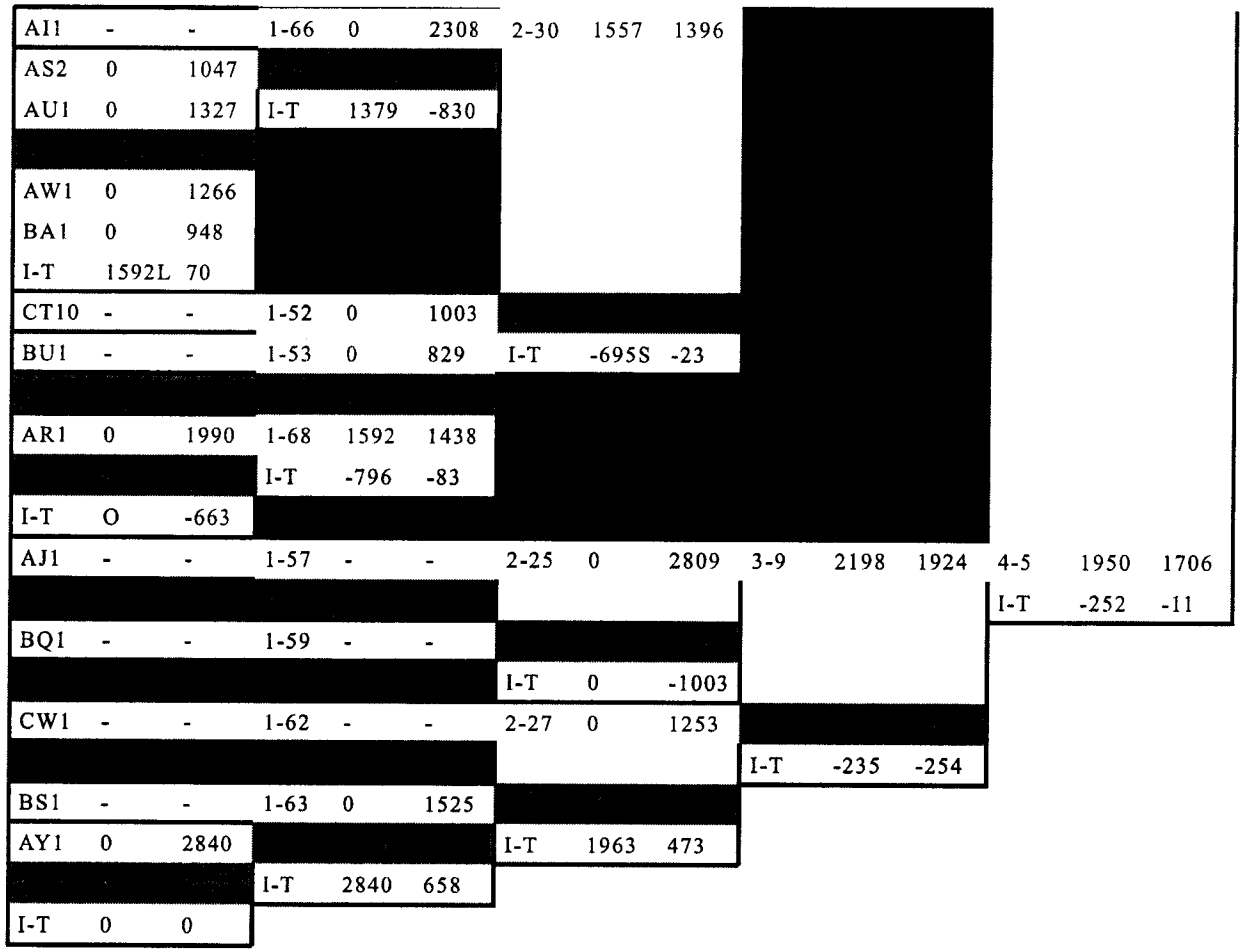


Fig. 14. Results of nested clade analysis of control region sequences of pigeon guillemots. "No." indicates clade numbers. "D_C" indicates clade distance. "D_N" indicates nested clade distance. Shading indicates interior clades. "I-T" indicates interior-tip distance. Supersript "S" indicates a distance that is significantly small; superscript "L" indicates a distance that is significantly large. Vertical lines enclose haplotypes that belong to the same clade (see Fig. 11).

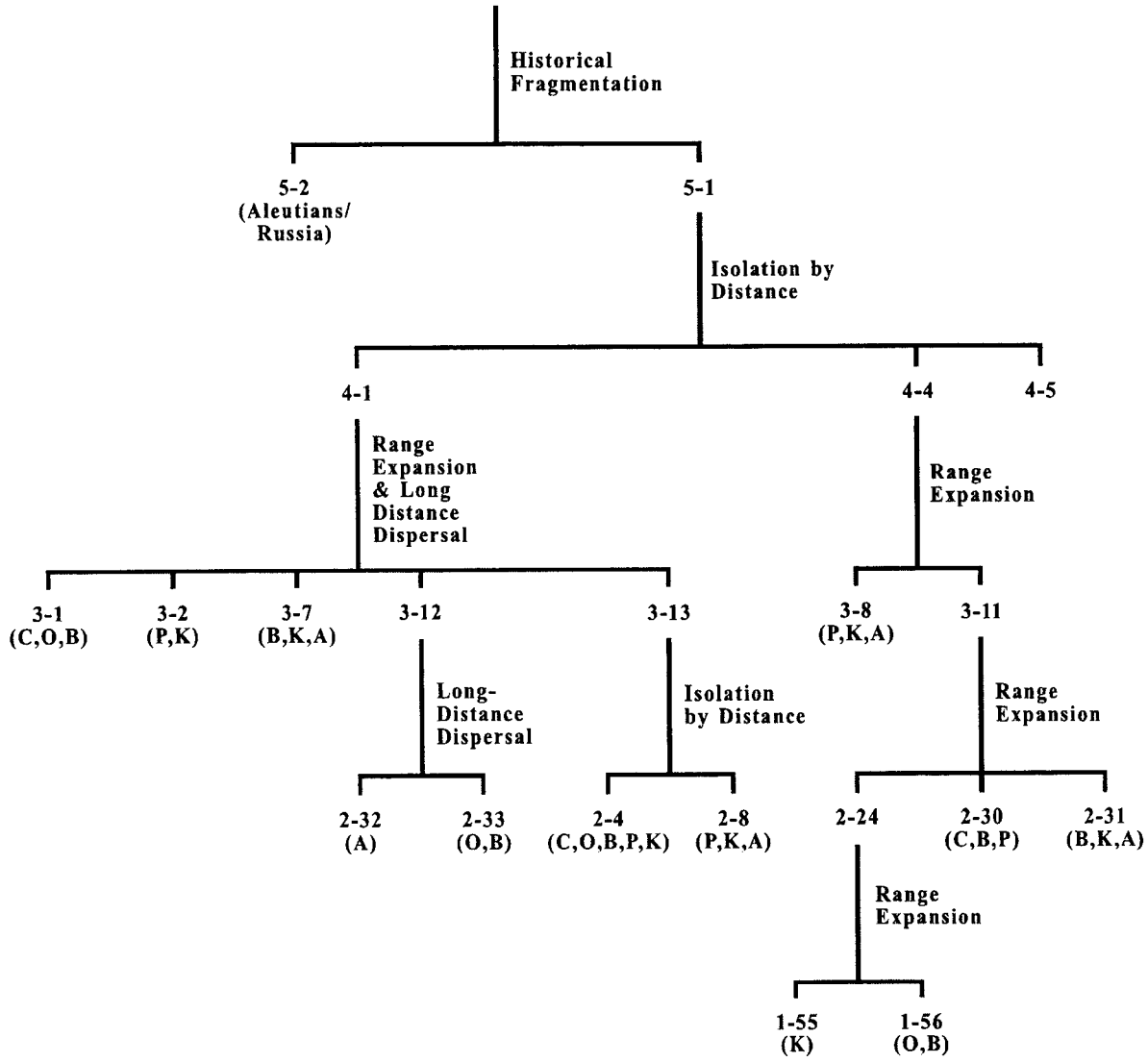


Fig. 15. Summary of results of nested clade analysis for pigeon guillemots. Numbers indicate clades from Fig. 14. Regions in which each clade was found are indicated: A = Aleutian islands; P = Alaska Peninsula; K = Kachemak Bay; P = Prince William Sound; B = British Columbia; O = Oregon; C = California.

Cytochrome c (1)

ACCCAACCTG AATGGCCTCT TTGGACGCAA GACTGGACAG GCTGAGGGCT TCTCTTATAC
GGATGCTAAT AAGAATAAAG GTAAACTTCA AG**C**TGTTCTT ATGAGTTACT A**G**GGACAAAA
*
ATACTCAGTT CTTTTCAGTA CTTTCCAGA- ACTACTGC**C**G TCTGCCCAAA AGTAAAATAA
TATGGAGGAA AAGAATATAA ATATGTCTGT TATTTTAA-A GTCACTGACC ATCTC--TTT
TCT-TCTTTC TAGGTATCAC CTGGGGTGAG GATACT
*

Ribosomal Protein 40 (1)

TCGGGAGGTC TTGCGCATGC GTGGCACCAT CTCCCGTGAG CACCCATGGG AAGTCATGCC
TGACTTGTAC TTCTACAGGG ATCCCGAGGA GGTAGGAGAC CTCAGGAAGG AAGCATGGCT
*
GTGTGAGATG TCCTCCTTGT GCTCCTGCCC CAT**G**GTCCTG CGTGCCTGCT GTTGAGGACA
GGTTTTGCCA ATA**T**AATGAT TACACTTCAC AGTTAGGGTG GTGGTTAATT AGTCTTGTC-
---GTTGGTT GGTGCGTGTT GTCTGGTATG TGATAGTGTG AGGG**C**GGTGA GGTGTTTCTG
GTGGGAGACT TAGGCTACTC TCTTCCGTTG CAGAT**C**GAAA AGGAG
*

Rhodopsin (2)

ATGATCCCGC **T**GATGGTCAT TTTCTTCTGC TACGGGAACC TGGTTTGCAC TGTCAAGGAG
GTGGGTACCT GC**T**AGTAGTG ATGGGCTG-G GG-----ACC ACCCCATG-C TGAGAAGGGT
*
CCCACACCAG GCTCCAGTCT GGTGACAGAA AGGGCCCTC- GGGGGCCCAG GCTGAC**G**CTC
CATAAAGGC**G** AATCAGCAAA TTCCAGATGT GCAGCTCAAC TGCCCCAATC CCTGACCCC-
TGTACCATGC CAGCACAGCC CTCCCCAGCT CCATTACGCC TCTGTTCCCT TCCACC**C**GCA
*

Fig. 16. Sequences of the most common alleles for three introns for pigeon guillemots. Variable sites are in bold. Asterisks are shown below the beginnings and ends of introns.

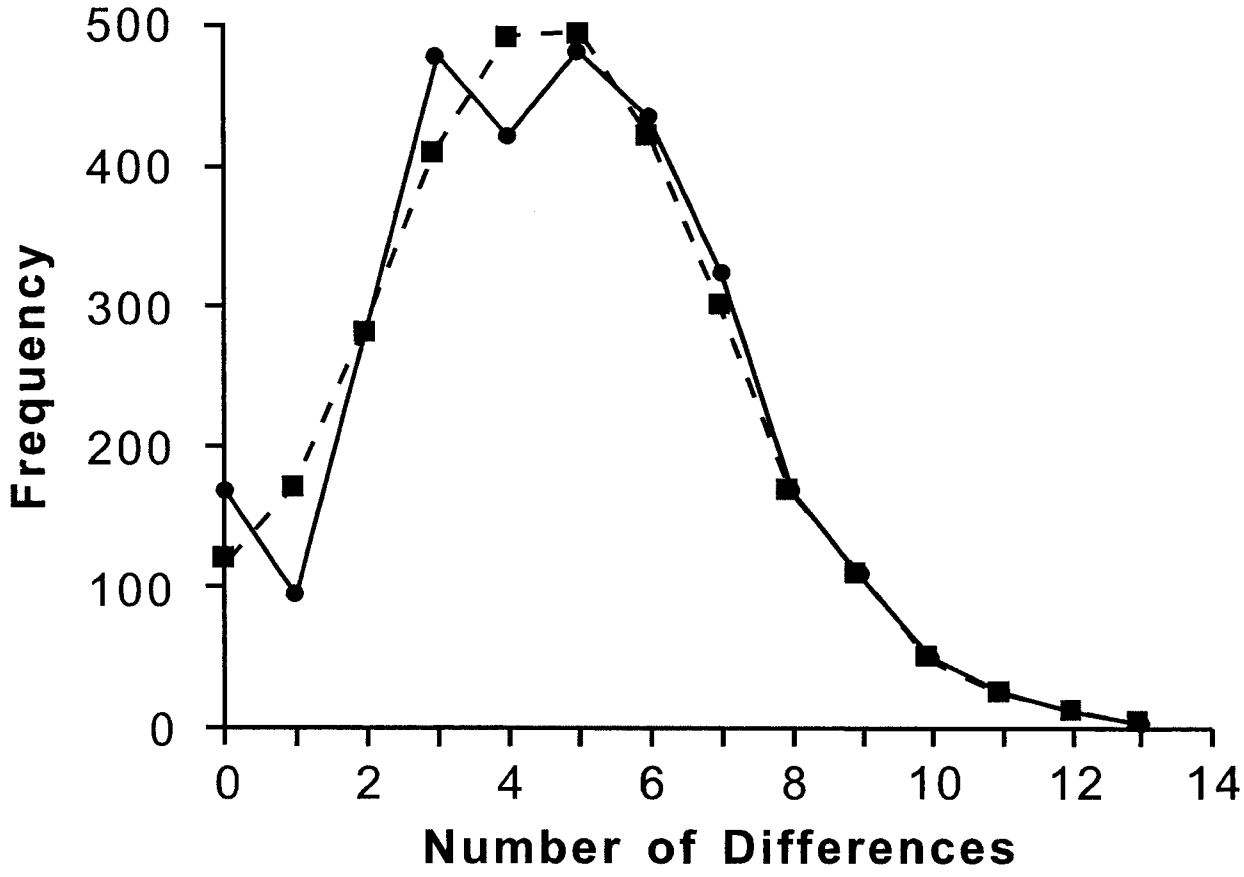


Fig. 17. Frequency of pair-wise sequence differences (mismatch distribution) for control region sequences of 80 marbled murrelets. Dots and solid line represent observed frequencies; squares and broken line represent frequencies expected under a sudden population expansion.

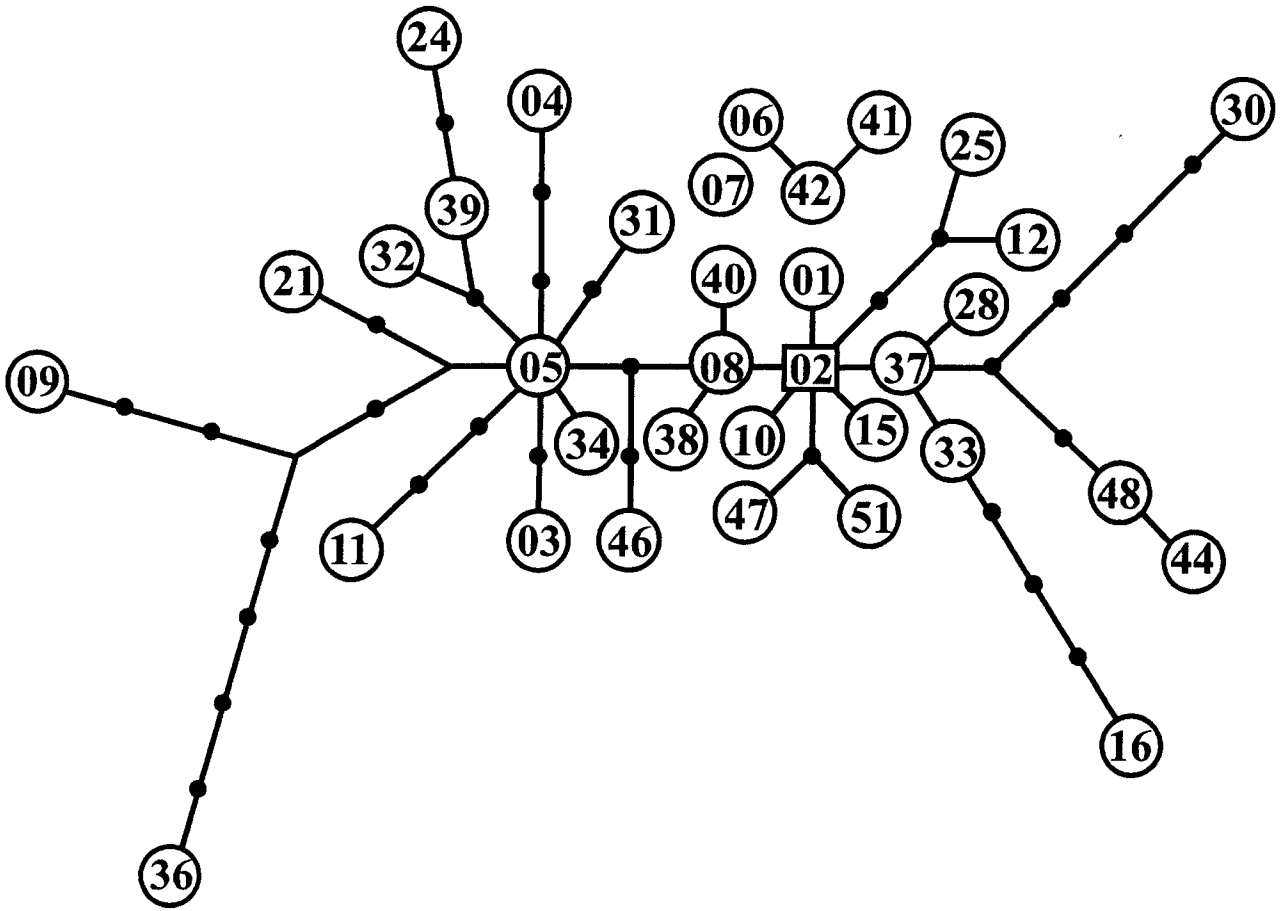


Fig. 18. Statistical parsimony tree showing the substitutional relationships among control region haplotypes of marbled murrelets (Table 23). Sizes of circles do not relate to haplotype frequencies. Black dots indicate missing haplotypes. The square indicates the haplotype with the highest root probability.

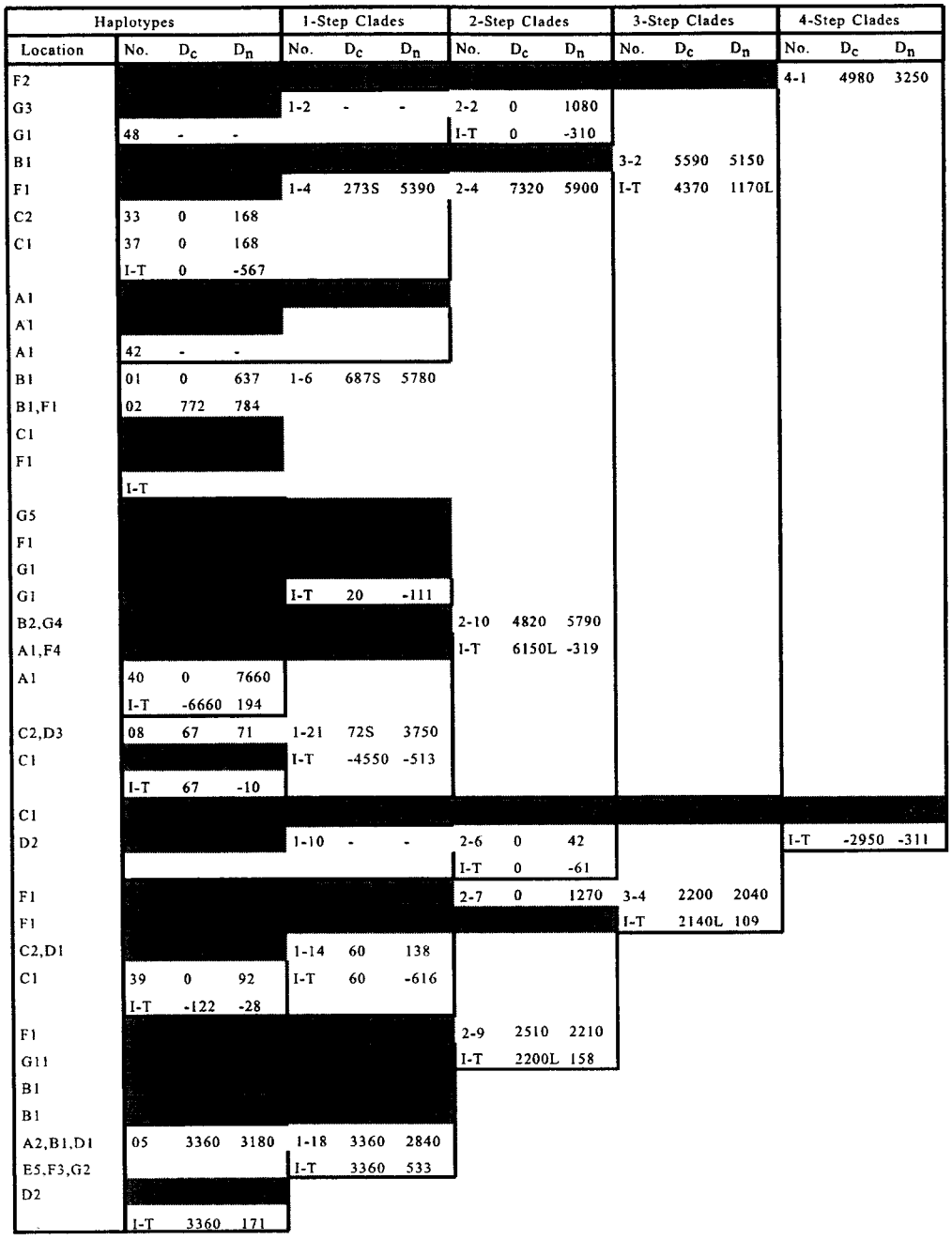


Fig. 19. Results of the nested clade analysis for marbled murrelets. "No." indicates clade numbers. "D_C" indicates clade distance. "D_N" indicates nested clade distance. Shading indicates interior clades. "I-T" indicates interior-tip distance. Supersript "S" indicates a distance that is significantly small; superscript "L" indicates a distance that is significantly large. Vertical lines enclose haplotypes that belong to the same clade (see Fig. 18). Locations: A = Western and Central Aleutian islands; B = Eastern Aleutian and Shumigan islands and Mitrofania Bay; C = Kodiak Island; D = Kachemak Bay; E = Prince William Sound; F = Southeastern Alaska; G = California; numbers = number of individuals in which a haplotype was found.

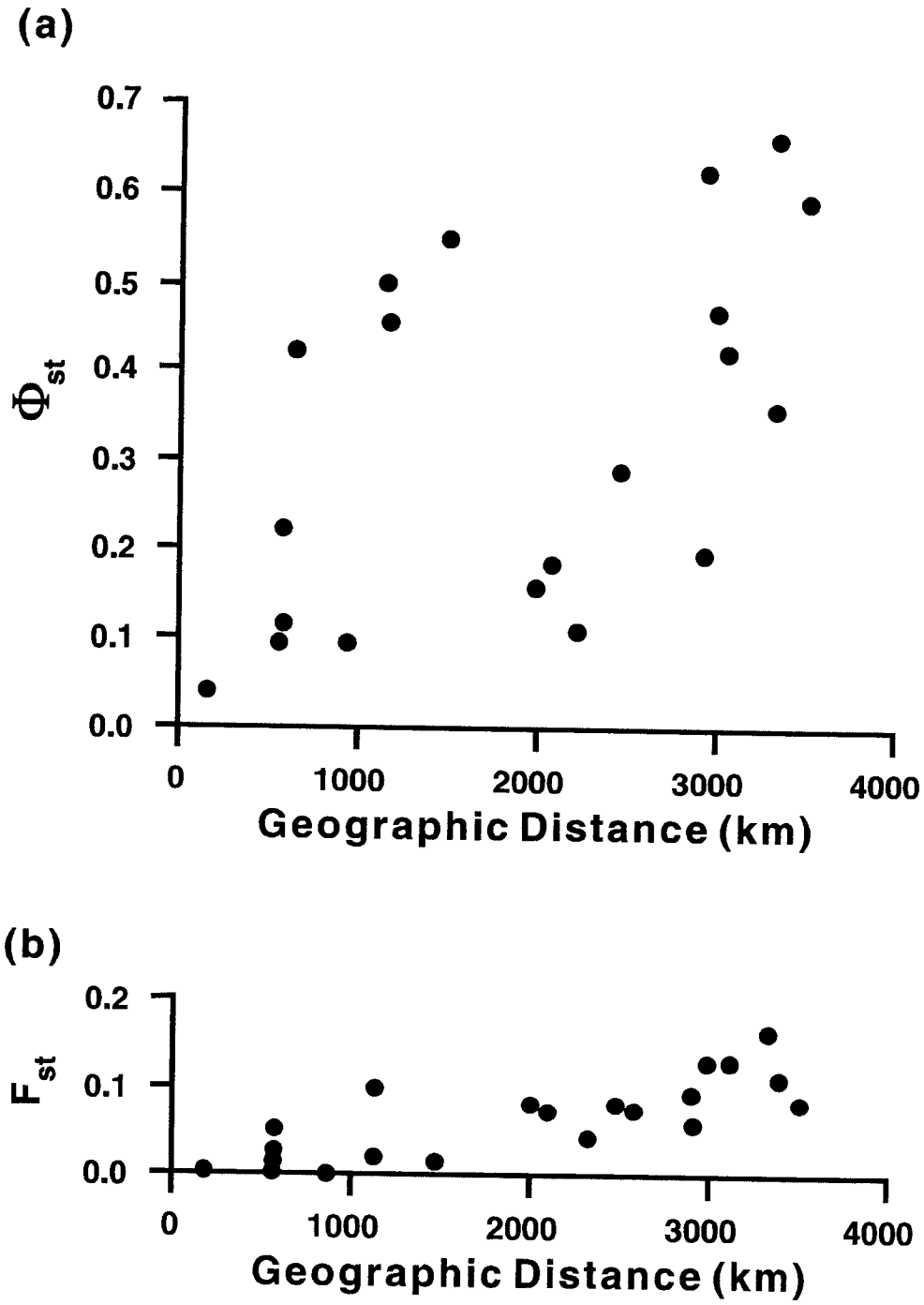


Fig. 20. Slatkin's linearized F_{st} (for all nuclear loci) versus geographic distance (km) between regional samples of marbled murrelets.

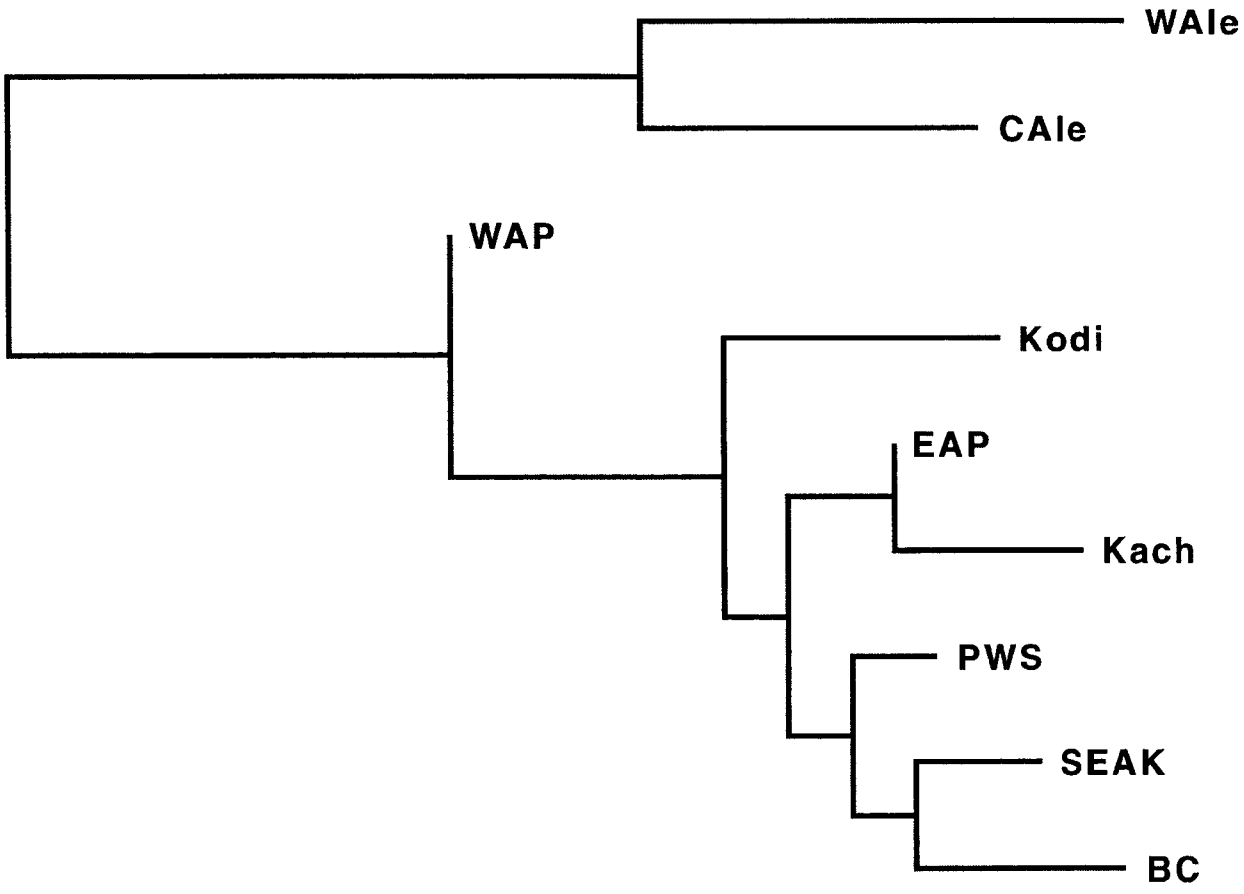


Fig. 21. Neighbor-joining tree based on Slatkin's linearized F_{st} (for all 14 nuclear loci) between regional samples of marbled murrelets. See Table 3 for region abbreviations.

Appendix I: Partial sequence of the mitochondrial control region of common murre haplotype 01. Variable sites are in bold. Conserved sequence blocks are in capitals. Sequence begins 141 bp from the beginning of the control region (Moum and Johansen 1992). Asterisks mark the beginnings and ends of the three Domains, and slashes mark the beginnings and ends of the two fragments that were amplified (sequence is not continuous between these fragments).

```
ctgaattttc cacacttccc cttcaagagg acctcccagc ccaatggatc cgaattccat  60
/

tacaatatcc gtactaatac catccctct ccagttttta cataccaact ccaacaagat 120
                      Domain I

acgacagtgc ctgcctacac cttatgtaat ggggtaaagg acatggccca tccaaaactt 180

ctcgaataca caaaagcttc gtgccaggtt atttattaat cGAGCTCCTC ACGTGAAATC 240
                               * *                    F BOX

AGCAACCcgg tgtttgaaag atcctacgct accagcttca gagaccatac tttcccccta 300

cacccactag cccatcttgc tcttttagcg CCTCTGGTTC CTATGTCAGG GCCATAActt 360
                                              D BOX

ggttagtcct ctcaacTTGT ACTTCACCGA TACATCTGGT aggtacacac aatctaagac 420
                      C BOX                / /

ctgagctttc cctggtattc gtacggattt tggccctcag gaatacctga atgtcaaggt 480
                      Domain II

ttaacgggtt gggggaatca tttttacact gatgcacttt gctttgcact tggttatgga 540

atctccgcaa gttcttattt atgttgtTAT TTAGTGAATG CTTGTTGGGC ATATtttatt 600
                                  CSB-1

atttttcatt tcctctaact ttttaaacaa cactagcaaa tttcattcaa aaacaaactg 660
                                       **

tgattttcat cacacatttt gtcatcgtca tcacacattt tatcatcaca cttatcatct 720
          Domain III

acaaacggca ctggaattcc attaaaaata aaggatattc a                    761
                                             /
```

Appendix II: Partial sequence of the mitochondrial control region of marbled murrelet haplotype 05. Variable sites are in bold. Conserved sequence blocks are in capitals. Sequence begins at the first base of the control region. Asterisks mark the beginnings and ends of the three Domains.

```
catgaaacta tatgtccccc cccctacccc cccgcacaga tatgtgcaca attacactac   60

attatcttcc cagctatgtg cgcgattgca ttcgattgtc ttccccataa atacatagag  120

tccatgcctc aatatcatta atatctgaac agacattacc ccgaatttcc acaaccccttc 180
                      Domain I

ttccagagaa cctccgcccc aatgggggcc gaaatccatt acaatatccg tactaatacc  240

atttactcgt taggttttac ataaccaact taatagatac gacagtgctt aggtacctcc  300

ctgcatgggg atgaagcatg accctccaaa ttttcctgag cgcataaagc tcgtaccagg  360

ttatttatta atcGAGCTCC TCACGTGAAA TCAGCAACCC GGtgtatgga agatcctacg  420
 **                        F Box

ttcccagctt caggaccatt ctttcccccc acacccctag cccatcttgc tcttttgcgC  480


CTCTGGTTCC TATGTCAGGG CCATaacttg gttagtcctc tcaacTTGTA CTTCACCGAT  540
   D BOX                                                 C BOX

ACATCTGGTC GGCtatatat caccatctca cccgtgatcg cgacatccga ccttttggca  600
                                              <---------- BmaH600---

cttttggttc ctttttttt tctggcgtct tcaataaacc cttccagtgc accgaggtaa  660
------                  ------- --BmaL650- --------->

atacaatcta tagacgtgga ccctccctgg tatccgtccg gttttggtcc tcaagaacgc  720
                      Domain II

cccggtgaga cggtttgcgg gttgggggaa tcatttttgc actgatgcac tttgttttac  780

atctggttat ggtctccccg caagctccta cttatgctgc TATTTAGTGA ATGCTTGCTG  840
                                                      CSB-1

GACATGAttt attacttttt atttcctcta gttttctaaa caacactagt aggtttcatt  900
                                                    **

tgaaaaatga accgtatttt ttcgtcaaaa caaatcgttt ttcatcacac attttatcat  960
                      Domain III

caccttcgtt at                                                      972
```