Exxon Valdez Oil Spill
Restoration Project Final Report

Differentiation and Interchange of Harlequin Duck Populations Within the North Pacific

Restoration Project 97161
Final Report

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Study History: Restoration Project 96161 was initiated in 1996 as the first year of a two- year study of harlequin duck demography in the *Exxon Valdez* oil spill affected area. Subsequently, additional information, made available through cooperators, provided contrasting views of other areas of the North Pacific. The project field work continued through 1997 under Restoration Project 97161. The 1998 and final project year, under Restoration Project 98161, culminated in draft publications titled Harlequin Duck Recovery From the *Exxon Valdez* Oil Spill: A Population Genetics Perspective and Limited Phylogeographic Structure in Discontinously Distributed Populations of Harlequin Ducks: A Species-wide Perspective With Conservation Implications.

Abstract: Concerns about constraints to harlequin duck (*Histrionicus histrionicus*) population recovery following the *Exxon Valdez* oil spill led biologists to ask whether birds in different molting and wintering areas belong to genetically distinct and, thus, demographically independent population segments. Because of the lack of direct observations of movements among marine areas, genetic markers, which differed in mode of inheritance (two sex-linked Z-specific microsatellite loci, four biparentally inherited microsatellite loci and maternally inherited mitochondrial deoxyribonucleic acid sequences), were used to evaluate the degree of genetic differentiation among wintering areas within Prince William Sound, Alaska Peninsula (Katmai National Park) and Kodiak Archipelago (Kodiak National Wildlife Refuge). We also used colored leg bands to detect population interchange within and among these regions. Our genetic results show that differences in genotype frequencies among wintering locations within Alaska were low and non significant for all three classes of markers. An analysis of genetic samples collected throughout the West Coast of North America revealed significant structuring at larger geographic scales. No interchange of banded birds was observed among regions and movements within regions were uncommon. Our failure to detect movements in banded birds may have been a function of the short duration of study (e.g., two years of banding with one year of recoveries and resightings), whereas our genetic analyses reflected demographic characteristics acquired over greater time scales. We conclude harlequin ducks are likely to recolonize or enhance populations in areas recovering from environmental damage via emigration of birds from unaffected areas. Our demographic results, and those from other studies, suggest that the levels of movements are low, and that population recovery by emigration may be a long-term process.

Key Words: Alaska, environmental impact, *Exxon Valdez*, harlequin duck, *Histrionicus*, microsatellites, mitochondrial DNA, oil pollution, oil spills, population genetics, sea ducks, waterfowl banding.

Project Data: Two data sets are available from this project: (1) sampling locations, morphological measures, and banding data are in digital (Microsoft Excel™) format and under the custodial responsibility of Dennis Zwiefelhofer, Kodiak National Wildlife Refuge, 1390 Buskin River Road, Kodiak, Alaska, 99615 (907/ 486-2600); and (2) genetics data are in digital

(Microsoft Excel™) format under the custody of Richard Lanctot, USGS-BRD Alaska Biological Science Center, 1011 East Tudor Road, Anchorage, Alaska 99503 (907/ 786-3609).

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Fig. 3. Neighbor-joining tree from gamma distances generated with Kimura's (1980) 2-parameter sequence evolution model showing phylogenetic relationships among mtDNA haplotypes in harlequin ducks sampled throughout Prince William Sound and the Alaska Peninsula/Kodiak Archipelago in 1996-1997. Numbers near tree branches represent the proportion of 500 simulations where two haplotypes (or groups of haplotypes) were clustered. Branch lengths represent genetic distances separating haplotypes. A transposed nuclear pseudogene sequence from a harlequin duck was used to root the tree.

Fig. 4. Neighbor-joining phenogram of genetic distances (Reynolds et al. 1983) for mtDNA describing relationships among eight populations of harlequin ducks distributed throughout Prince William Sound and the Alaska Peninsula/Kodiak Archipelago in 1996-1997. Birds sampled at Shemya, Aleutian Islands, Alaska, were used as an outgroup in this analysis.

EXECUTIVE SUMMARY

Harlequin ducks (*Histrionicus histrionicus*) are sea ducks inextricably tied to near shore marine habitats. Harlequin ducks are particularly vulnerable to oil effects due to their reliance on near shore habitats and associated invertebrate prey. They suffered direct oiling mortality during the initial stages of the *Exxon Valdez* oil spill. The spill affected area includes coastal areas of Prince William Sound, Kenai Peninsula, Kodiak Archipelago, and Alaska Peninsula. Direct effects of oil may continue to injure harlequin ducks, and the population (i.e., demographic) consequences of previous injury may continue to constrain recovery.

Population structure, movements, and relatedness among areas are poorly known for harlequin ducks. Understanding these basic aspects of harlequin duck life history is critical for interpreting ongoing studies, assessing recovery, and prescribing further restoration activities. We used genetic analyses and color-marking programs to assess the degree of population differentiation and movements among geographically separate groups of harlequin ducks within the spill affected area, as well as from other marine regions of the North Pacific for comparison.

No evidence of population interchange among sample sites was found from banding data. Some data depict small (3%) movements of ducks within, but not among, sampling areas. These data may not be at levels of significance useful to restoration planning, but they can have an effect on gene flow. Genetic analyses indicate a homogenous genetic character throughout the spill-affected region. These results may be a product of historical gene-flow and are not necessarily evidence of current emigration. We conclude that recolonization or enhancement of populations in spill injured areas will most likely occur naturally over a long-term period, but not within a sufficient timescale to serve as the primary restoration strategy.

INTRODUCTION

On 24 March 1989, the *T/V Exxon Valdez* ran aground on Bligh Reef in Prince William Sound, Alaska, spilling approximately 41 million liters of crude oil (Piatt and Lensink 1989, Piatt et al. 1990). Subsequent wind and ocean currents spread the oil southwest through Prince William Sound (PWS), and along the Kenai Peninsula, Alaska Peninsula and Kodiak Archipelago. Much of the oil was deposited in near shore intertidal and subtidal habitats (ADEC 1992, Morris and Loughlin 1994, Neff et al. 1995), which are of importance to a large number of vertebrates including molting and wintering waterfowl. Indeed, the near shore environment of PWS received about 40% of the oil spilled (Galt et al. 1991) with the remainder of oil being deposited in the Gulf of Alaska, including near shore zones of the Alaska Peninsula and Kodiak Archipelago (APKA). The impact of this spill to resident fish and wildlife has been dramatic (Piatt et al. 1990, ECI 1991) and the subject of extensive investigations for the past nine years.

Harlequin ducks (*Histrionicus histrionicus*) are year-round inhabitants of near shore environments within the spill zone (Isleib and Kessel 1973, Agler et al. 1994). Two-hundred and twelve

harlequin ducks were recovered from beaches after the spill (J. Piatt, pers. comm.). The actual number of harlequins that died of oiling is suspected to be much higher because the recovery of waterbirds was probably very low due to birds being scavenged, sinking or not being found along shorelines (Ford et al. 1987, ECI 1991). Adjusted estimates which correct for these recovery problems range from 1,298 to 2,650 harlequins killed from oiling (ECI 1991, Piatt and Ford 1996). Post spill studies suggest continuing constraints to recovery from the spill, based on differences in winter survival between oiled and unoiled areas (D. Esler, unpubl. data), declines in numbers of molting birds within the spill zone (D. Rosenberg, unpubl. data), and detectable levels of hydrocarbons in harlequin ducks and their prey from 1989-1993 (Patten 1995).

To understand the process of recovery of harlequin ducks from the spill, and to identify impediments to recovery, it is critical to determine whether aggregations of individuals within local marine areas are discrete and demographically independent (i.e., whether the population is structured within the spill region). For example, if birds located in oiled and unoiled areas belong to geographically different and genetically distinct population segments (i.e., management units; Moritz 1994), then certain segments of the population may have been (and continue to be) impacted disproportionately by oil. Alternatively, lack of evidence for spatial structure would imply that oil effects are distributed throughout a larger panmictic population. Information on the level at which populations of harlequin ducks are structured genetically would also aid in the interpretation of population demographic parameters which have been used as measures of recovery. For example, estimates of age and sex ratios are often used to infer productivity (Rosenberg et al. 1996). High juvenile to adult age ratios, for example, may represent high local recruitment.

Alternatively, if populations are not spatially structured, such ratios may simply indicate high immigration of juveniles from different segments of a larger, panmictic population. Population structure, in effect, determines the process and the rate by which recovery can occur. If harlequin ducks are structured as distinct population segments, then recovery of these segments can occur solely as a function of local recruitment. Conversely, if there is evidence of extensive movement and gene flow, then recovery can occur more rapidly as a function of both recruitment and immigration.

Several aspects of harlequin duck life history suggest that marine coastal areas utilized during molt and winter (e.g., areas within PWS) represent discrete, reproductively isolated populations. Demographic studies have shown that harlequin ducks spend most of their lives in the marine environment, leaving only as adults to breed in streams for a few months each summer (Bellrose 1980). Males and females are highly philopatric to breeding and wintering areas (Robertson 1997, D. Esler, unpubl. data) and pair formation occurs on the wintering grounds during early to midwinter (Robertson 1997). These factors suggest adults have a low probability of breeding with birds from other marine areas, and that genetically distinct groups could evolve within the marine molting/wintering sites.

Timing and location of pair bond formation can be of considerable importance for the evolution of spatial differentiation in gene frequencies (Ely and Scribner 1994), even for migratory species. Unfortunately, for harlequin ducks, very little is known about the level of movements (or gene flow) that occurs among areas of the marine environment, or the propensity of juvenile (hatching year) birds to return to the same marine region as their parents. Episodic pulses of dispersal or consistent low levels of gene flow could effectively homogenize gene frequencies, thereby resulting in a panmictic population.

In the absence of direct observational data, population discreteness can also be inferred using genetic markers (Slatkin 1985, 1995; Slatkin and Barton 1989). Use of different marker classes which differ in patterns of inheritance and rate of evolution can often yield valuable insights, particularly if the propensity for, or direction of, dispersal differs between males and females, as has been described for many waterfowl species (Anderson et al. 1992). In our study we used molecular genetic markers which are all evolving rapidly (mitochondrial (mt) deoxyribonucleic acid (DNA) control region and microsatellite nuclear DNA), but that differed in modes of inheritance (biparental, paternal and maternal) to estimate gene frequencies and levels of genetic variability among harlequin ducks wintering within PWS and APKA.

Differences in the mode of inheritance among the three markers we employed allowed us to determine the effect of gender-biased gene flow (as a function of sex-biased dispersal and natal philopatry) on genetic relationships among populations. For example, if females and not males are faithful to particular wintering locations, then analyses based on mtDNA will show strong matrilineal population structure (Avise 1994), whereas analyses based on paternal or biparental inherited markers will indicate no population structure. Similarly, if males and not females are faithful to particular wintering locations, then analyses based on paternally inherited markers will show strong paternal population structure, whereas analyses based on biparental or maternally inherited markers will indicate less population structure. In contrast, if both males and females move widely among winter areas, then analyses based on biparental, paternal and maternal inherited markers will show little or no population structure amongst wintering areas. Finally, if both males and females are faithful to particular wintering locations, then analyses based on biparental, paternal and maternal inherited markers will show strong population structure. Thus, analyses provided by all three markers may more accurately identify the level, direction and cause (i.e., male vs. female) of gene flow occurring among populations.

Genetic analyses assume that the degree of spatial variation in gene frequency for each marker is proportional to (1) the rate of inter-population gene flow, (2) the effective winter population size of each area, and (3) the amount of time since populations have been separated. We further assume those wintering aggregations are suitable for spatial genetic analyses because of the high degree of winter site fidelity and occurrence of pair-bond formation on wintering areas.

Along with genetic analyses, color-marking of birds provided additional information about movements. Leg bands persist over many years and may allow investigators to detect movements

in spill affected marine regions that are not detectable by genetic analysis (Slatkin 1985). Marked populations are also extremely valuable for assessing local movements and demographics.

The area of study encompasses the spill impacted areas of the northeast coast of the Alaska Peninsula along the Shelikof Straits (Katmai National Park), Prince William Sound and Kodiak Island Archipelago (Kodiak National Wildlife Refuge)(Figure 1). The banding portion of the study was restricted to these three primary study areas. Specimens for molecular genetics evaluation came from these three areas and were augmented by specimens from other areas in the North Pacific.

Our objective was to determine whether there was population structuring among nearby winter aggregations in coastal marine habitats of the PWS and APKA. Results could prove valuable for conservation purposes in other portions of the species' range where population numbers are extremely low and populations are threatened with extinction (i.e., populations in eastern Canada; Montevecchi et al. 1995).

OBJECTIVES

1. To assess spatial segregation and population differentiation of harlequin ducks from marine regions of the spill affected area and other North Pacific sites, using molecular genetic techniques.

2. To mark harlequin ducks with colored leg bands in three spill affected areas to provide opportunities for observations of direct evidence of movements and to test the feasibility of re-sighting colored leg bands.

METHODS

The collection of genetic specimens and the application of leg bands were integrated during harlequin duck drives into live-traps, during the molt in late August and early September of 1996 and 1997. We hypothesized that harlequin duck wintering aggregations represent distinct population units with little genetic exchange or movement of individuals. Our null hypothesis was that wintering aggregations are not distinct and harlequin ducks in the North Pacific represent a panmictic population.

Molecular Genetics

Sample collection - Blood sampling occurred during molting harlequin duck live-trap procedures. Blood was extracted from the jugular or tarsal vein and between 3-4 drops were placed in 1.5 ml tubes filled with a nonrefrigerated lysis buffer (100 mM Tris, pH 8.0, 100 mM EDTA, 0.5% SDS, 10 mM NaCl; Longmire et al. 1988). This buffer is amenable for storage in field conditions for extended periods. DNA was extracted from blood samples using Puregene DNA extraction kits

(Gentra Systems, Inc., Minneapolis, MN) and standard proteinase-K, phenol-chlorophorm methods (Sambrook et al. 1991).

Microsatellite analysis - Thirty-three microsatellite loci were examined for variation by screening two individuals from each of four populations on the west coast of North America. Of these, four biparentally inherited loci (Sfiμ4, Hhiμ2, Hhiμ5, and Bcaμ10) and two sex-linked loci (Sfiμ1, Bcaμ4) were variable. The sex-linked (Z-specific) loci essentially provide paternally inherited markers if sampling is conducting using females. Males have two gene copies (ZZ) for every single copy (ZW) in females. All six were cloned in our lab (Fields and Scribner 1997, Buchholtz et al. In press).

Microsatellite loci were assayed using polymerase chain reaction (PCR). One primer from each pair was end-labeled with gamma ^{32}P-ATP using T4 polynucleotide kinase according to manufacturer's specifications (Pharmacia, Piscataway, NJ). PCR reactions were carried out in a Stratogene (La Jolla, CA) thermalcycler (Robocycler) in 25-30 μl volumes. For five of the six primers (all but Sfiμ1), the reaction mix included 2.5 μl of 10x PCR buffer (500 mM KCl; 100 mM Tris-HCl, pH 8.5; 15 mM $MgCl_2$; 100 μg/ml nuclease-free BSA; 0.025% Tween-20), 2.5 μl of 2 mM dNTP mix, 10 μl of 10 μM unlabeled reverse primer, 0.9 μl of 10 μM unlabeled forward primer, 1.0 μl of 1.0 μM ^{32}P-labeled forward primer, 0.3 μl AmpliTaq polymerase (Perkin Elmer-Cetus, Foster City, CA), 3.0 μl (≈150 ng) template DNA and 13.8 μl ultrapure water. For the Sfiμ1 locus, we used 3.0 μl 10x PCR buffer, 0.2 μl of 2mM dNTP mix, 0.1 μl of 10 μM unlabeled reverse primer, 1.0 μl of 1.0 μM ^{32}P-labeled forward primer, 0.3 μl AmpliTaq polymerase, 4.0 μl template DNA and 21.4 μl ultrapure water. Thermal profiles consisted of initial denaturing for 2 min at 94°C; followed by 30 – 35 cycles of 1 min denature at 94°C, 2 min annealing at locus-specific temperature and 60 – 90 seconds extension at 72°C; followed by an additional extension for 5 min at 72°C. Optimal annealing temperatures for the Sfiμ4, Hhiμ2, Hhiμ5, Bcaμ10, Sfiμ1, and Bcaμ4 loci were 60, 55, 51, 55, 58 and 60°C, respectively. PCR products were mixed with 10 μl of a formamide loading mixture (95% formamide, 20 mM EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol), denatured for 5 min at 95°C, and then loaded and separated on a 6% denaturing polyacrylamide sequencing gel. A M13 sequencing reaction (Amersham Life Sciences Sequenase Kit, Arlington Heights, IL) and individual standards of known genotypes were run adjacent to the samples to provide an absolute-size marker for estimating the size of microsatellite alleles. Gels were dried and genotypes were resolved by autoradiography after exposure for 12-48 hrs using intensifying screens.

Amplification and sequencing of mitochondrial DNA - An ≅385 base-pair (bp) fragment of the 5' end of the hypervariable portion of the mitochondrial DNA control region was amplified using species- and mtDNA-specific primers designed in our laboratory for harlequin ducks. Mitochondrial DNA specific primers included HADUM1L: 5' TGC CCG AGA CCT ACG GCT C 3'; HADUM2L: 5' TCT AAA ATG ACT CAA CAG TGC C 3'; and HADUMITH: 5' TGA GTA ATG GTG TAG ATA TCG 3'. Nuclear specific primers included HADUN1L: 5' TAC CCG AGA CCT ACA GCT T 3' and HADUNUCH: 5' TGA GTT ATG GTG TAG ATA CTA 3'. MtDNA sequences were obtained by matching either the HADUM1L and HADUM2L

primers with the HADUMITH primer. Mitochondrial DNA specific primers were designed so that mtDNA sequences, and not nuclear DNA sequences originating from transposed mtDNA, would be amplified (Quinn 1992, Sorenson and Fleischer 1996).

Using the primers described above a 385 bp fragment of mtDNA was amplified in 50 µl reaction volumes using 1µl (50 ng) of non-purified double-stranded DNA, 5µl 10x PCR buffer (67 mM Tris-HCl, pH 8.0, 6.7 mM $MgCl_2$, 0.01% Tween-20), 5 µl of 10 mM dNTP mix, 5 µl each of 10 µM heavy and light strand primer, 0.2 µl of AmpliTaq DNA Polymerase (Perkin Elmer-Cetus, Foster City, CA) and 28.8 µl ultrapure water. Thermocycler parameters included a hot start of 80°C for 5 min before adding the AmpliTaq DNA Polymerase (Perkin Elmer, Foster City, CA), and running 40 cycles at 94°C for 1 min, 56°C for 1 min, and 72°C for 1 min. PCR products were visualized on a 1.5% agarose gel to select individuals suitable for sequencing. Amplified DNAs were purified using PEG precipitation to remove unincorporated nucleotides, primers, and salts. Specifically, ½ volume of precipitation buffer (30% PEG [MW 3350] and 1.5M NaCl) was added to the PCR reaction, precipitated for 10 min and centrifuged for 4 min at 14,000 rpm. The supernatant was removed and the pellet was washed in 75% ethanol, centrifuged for 4 min at 14,000 rpm, air dried and then resuspended in 15-30 µl of water.

Cycle-sequencing reactions were performed according to internal labeling (alpha ^{32}P-dATP) protocols using Sequitherm's EXCEL DNA Sequencing Kits (Epicentre Technologies, Madison, WI) and 1.5 mM of HADUMITH primer. Thermocycler parameters were set to 95°C for 5 min followed by 30 cycles of 95°C for 30 sec, 56°C for 30 sec, and 70°C for 1 min. Reactions were terminated by adding 3 µl of stop solution. Two to three µl of each sequencing reaction was electrophoresed on a 6% denaturing polyacrylamide gel at 70 watts. Gels were dried and sequences were visualized using autoradiography.

Statistical analysis - Genetic analyses were restricted to birds greater than 1-year of age. Data from males and females were used for the biparentally inherited loci, but were restricted to females for the maternally inherited mtDNA and sex-linked microsatellite loci.

For each population, all biparentally inherited loci were tested for linkage (all two-locus comparisons) and Hardy-Weinberg disequilibrium using the Fishers' Exact Test in the Genetics Data Analysis (GDA) program (Lewis and Zaykin 1998). We then estimated observed (H_o) and expected (H_e, under Hardy-Weinberg) heterozygosity and the mean number of alleles per locus (A) using the BIOSYS program (Swofford and Selander 1989). These estimates of H_o and H_e were used to generate inbreeding coefficients (F) for all loci using the formula $(1-[H_o/H_e])$ (Wright 1951). Observed and expected heterozygosity values were also calculated for each population-locus combination using GDA. Inbreeding coefficients were then generated and tested for significance as described in Li and Horivitz (1953).

Statistical analyses of spatial heterogeneity in gene frequency between regions and among populations within each region for each loci were assessed using hierarchical F-statistics (Weir 1996) in the GDA program. Allelic variance was partitioned among individuals within

populations, among populations within regions, and among regions. Values of F_{ST} range from 0 to 1 where a value of 1 for a specific locus implies that populations are 'fixed' for different alleles and a value of 0 implies that all populations share the same alleles in equal frequency. Significance of F_{ST} values was based on 95% confidence intervals determined by bootstrapping across loci. Confidence intervals that overlap zero were considered nonsignificant. A significant F_{ST} value implies that a significant portion of the total genetic variation at a specific locus is partitioned among populations. We also calculated genetic differences among populations in terms of a R_{ST} analogue, a measure which takes into account allele size differences within loci (Slatkin 1995), using the procedures described in Michalakis and Excoffier (1996) and the Analysis of Molecular Variance (AMOVA) program (Excoffier et al. 1992).

We then calculated Cavalli-Sforza and Edwards (1967) chord distances among the populations and constructed phylogenetic trees using the SEQBOOT, GENDIST, NEIGHBOR, and CONSENSE subroutines within the PHYLIP program (Version 3.572c, Felsenstein 1993). We present the neighbor-joining tree with consensus values illustrating the proportion of time two populations (or groups of populations) were clustered out of 500 replications. Moderate to low consensus values (e.g., below 50%) indicate unstable clusters. We used birds sampled at Shemya, on the outer Aleutian Islands of Alaska, as an outgroup in this analysis.

For the sex-linked microsatellite DNA (Sfiμ1 and Bcaμ4 loci), we calculated allele frequencies and a measure of genetic diversity (D), using the formula $1 - \sum x_i^2$ where x_i is the frequency of the i^{th} allele in each population (Nei 1987). Estimates were derived using the AMOVA program among individuals within populations (Φ_{SC}), among populations within regions (Φ_{CT}), and among region (Φ_{ST}) variability. These loci were, consequently, treated as the paternal equivalent of the maternally inherited mtDNA marker (see below).

Sequences for maternally inherited mitochondrial DNA in all individuals were aligned manually and haplotypes were assigned based on at least a single base pair substitution or insertion/deletion across the 163 bp segments scored. Genetic heterogeneity within samples was estimated using the haplotype ("h") and nucleotide diversity (π) indices for nonselfing populations (equations 8.1 and 10.4, respectively of Nei 1987), as calculated by the REAP program (McElroy et al. 1991) from pair-wise haplotype divergences calculated by the MEGA program (Kumar et al. 1993). The haplotype diversity index is approximately equivalent to the probability that two randomly chosen individuals will have different genotypes. The nucleotide diversity index (π) (Nei and Tajima 1981) was determined to measure the average pair-wise nucleotide difference between individuals within samples. The measurement π corrects h for the size of the nucleon examined (Nei 1987). Using the REAP program, Heterogeneity of genotype distribution among samples was tested with the Monte-Carlo chi-squared test of Roff and Bentzen (1989), which is suitable for genetic data matrices in which many or most elements are very small (<5) or zero. One-thousand resamplings of the data matrix were completed.

Estimates of regional, population and individual variance in haplotype frequency were assessed using the AMOVA program. F-statistics for mtDNA were estimated in two ways as discussed by

Excoffier et al. (1992). One approach considered haplotype frequencies weighted by the number of bp substitutions between the haploytpes. The other approach simply considered haplotype frequencies irrespective of evolutionary relationships.

Evolutionary relationships among haplotypes were inferred from mtDNA sequence data using gamma distances generated with Kimura's 2-parameter model of sequence evolution (Kimura 1980) with an alpha value of 0.5. All phylogenetic analyses were unweighted because transition substitutions accounted for all of the variable sites and the control region does not code for a specific gene product (Brown 1985). A distance tree was generated from the nucleotide sequence information using the neighbor-joining method and the computer program MEGA (Kumar et al. 1993). A sequence of a homologous region of a transposed nuclear pseudogene sequence from one harlequin duck was used to root the tree depicting the phylogenetic relationship among the haplotypes. Maximum parsimony analysis was not used to construct a tree since there were fewer parsimonious sites than haplotypes (Kumar et al. 1993). We next calculated Reynolds et al.'s (1983) coancestry coefficient distances among the populations using AMOVA and constructed a phylogeography tree using the neighbor-joining method within the PHYLIP program. Sequence data gathered from harlequin ducks sampled in Shemya, Alaska, were used as an outgroup for this tree.

Color Marking With Leg Bands

Capture - Molting harlequin ducks were captured at various locations within the three primary study sites (Figure 1) by driving flightless birds into a trap (Clarkson and Goudie 1994). Sea kayaks were used to slowly herd molting flocks toward a trap. The traps used in 1996 in all areas consisted of two 100' wings which led birds into a holding pen in shallow water. Wings were constructed of netting draped over aluminum poles. The holding pen was constructed of 1" Plastic pipe and netting. No top was required as the birds were flightless and a roof would have inhibited removal of birds from the trap. Decoys were used to decrease trap-avoidance responses. Sampling sites within PWS region included Bay of Isles, Green Island (Green, Little Green and Channel Islands), Foul Bay area (Foul Bay, Crafton Island and Main Bay) and Port Chalmers (Port Chalmers and Stockdale Harbor), whereas sampling sites within APKA region included Uganik (Terror Bay, Uganik Island, Uganik Passage), Uyak (Alf Island, Amook Bay, Browns Lagoon, Chief Cove), Afognak Island (Foul Bay, Bluefox Bay) and southern portions of the Alaska Peninsula (Amalik Islets, Takli Islands, Amalik Peninsula).

During 1997 field work, an experimental floating capture trap and wing setup was tested at Kodiak National Wildlife Refuge and Katmai National Park. The rectangular box trap was framed in PVC plastic pipe and wrapped with 1-inch stretched mesh fishing net. The exterior trap dimensions were approximately 2 m by 2 m by 3 m. The trap had a bottom but no top. Six closed-cell foam cylinders 15 cm diameter by 60 cm were attached to the sides of the trap to float it partially submerged to about 1-m, while allowing another meter of trap wall to extend above the water surface. A floating net (wing) was attached to the seaward side of the trap and set in an

acute angle relative to the shoreline to lead ducks into the trap. This wing net was about 20 m long and extended 10 m below the water surface. The wing net was fashioned from standard herring seine materials and designs, with the addition of a mesh fence attached to the cork line that extended above the waterline 60 cm. The trap was positioned at the waterline, in deep or shallow water. Additional net was used between the trap and the shore to prevent ducks from going overland to avoid the trap.

Marking - Harlequin ducks captured during molting drives were banded on the right leg with numbered metal bands obtained from the U. S. Fish and Wildlife Service, Office of Migratory Bird Management, Bird Banding Laboratory. Sex identification was based on plumage characteristics and age was determined by bursal probing. Adults (after third year, ATY) do not have a bursa; second year (SY) birds were distinguished from third year (TY) subadults by the depth of the bursa (SY bursa > 2 cm; TY bursa < 1 cm). Birds were released at the original point of capture.

Individually coded plastic tarsus bands were placed on the left leg of all captured harlequin ducks. The tarsus bands were oriented to be read from bottom to top as the bird is standing. Tarsus band color schemes were used to allow investigators to distinguish among the main study sites even without reading the unique code. Band background colors by area were orange and blue (PWS), white (Kodiak) and red (Katmai). The goal was to capture and band at least 200 birds in each area.

Band returns and recoveries - Band *returns* are recaptures at the original banding site. Band *recoveries* are captures of birds in other locations, reliable reports of color band sightings, hunter kills submitted for inspection or beached bird carcasses. The principle and most reliable instrument of band recovery were the drive-trapping band-returns of molting harlequin ducks in August-September of the second year of the study (1997).

From time of banding in 1996 through 1997, the feasibility of re-sighting the colored bands was tested during Trustee agency boat-based surveys or patrols and with the aid of other local-area cooperators. The feasibility of band color recoveries on free-flying and roosting harlequin ducks was tested to determine efficacy, and if the technique can be used to detect population interchange among the three primary areas.

RESULTS

Molecular Genetics

Biparentally inherited microsatellite DNA - Each population of harlequin ducks possessed between three and eight alleles at each biparentally inherited loci (Table 1). Measures of genetic variability across the four biparental microsatellite loci including mean number of alleles per locus across (3-4), and observed and expected heterozygosities (range: 0.427 and 0.494) were high and quite concordant across all populations (Table 2). In all instances, loci did not deviate

significantly from Hardy-Weinberg equilibrium (i.e., there was no heterozygote deficit) and no evidence of linkage was observed (i.e., when P-values were adjusted for the number of statistical tests).

The lack of a heterozygote deficit corresponds with the low alleles found within individual variations present for each loci and across all the loci (Table 1). Inbreeding coefficients (F) were low (range of population means across all loci: –0.056 to 0.080 (Table 2)). Inbreeding coefficients were more variable when analyzed for each population and locus combination (range: –0.266 to 0.262; mean = 0.008). These values were nonsignificant in all cases except for the APKA Katmai population for locus Sfiμ4 ($P < 0.05$).

Estimates of spatial variation in nuclear gene frequency for the four biparental microsatellite loci were very low (Table 1). No locus showed a significant difference in allele frequencies among populations or among regions (all $P>0.05$). When results were averaged across all loci, the overall probability that the allele frequencies differed among the populations within regions or among regions was also not significant. Estimates of Rst did not differ appreciably from Fst spatial variation values, and in no case was there any significant difference in allele frequencies among populations or among regions.

The lack of spatial variation among the populations was further evident based on low levels of bootstrap support for most nodes of the consensus neighbor-joining tree using the biparental loci (Figure 2). Only three nodes within the microsatellite tree received >50% bootstrap support. The fact that clusters were composed of populations from both regions, coupled with the lack of consistent clustering at most tree nodes, suggests weak population structuring due to either historic or ongoing gene flow. However, our ability to place accurate and precise bootstrap values on the various nodes of the tree may be compromised by the relatively small number of biparental loci used.

Sex-linked microsatellite DNA - Genetic diversity at both paternally inherited loci was high, especially for the Bcaμ4 locus where the number of alleles in any one population frequently exceeded ten (Table 2). The number of Bcaμ4 alleles (n=20) was seven times higher than the Sfiμ1 locus (n=3). This difference was due to the presence of rare Bcaμ4 alleles. Accordingly, the Sfiμ1 locus had lower genetic diversity scores than the Bcaμ4 locus.

Like the biparentally inherited loci, the paternally inherited markers suggest that male gene flow was and/or is currently high among populations and regions within the primary study area (Table 1). Indeed, F- statistic values were nonsignificant among populations within regions and between regions. The absence of population structure using the Bcaμ4 locus was potentially misleading, however, given the large number of rare alleles and the relatively low sample sizes for each population. Allele frequencies were generally very similar among populations for the Sfiμ1 locus.

Maternally inherited mitochondrial DNA - One-hundred twenty-seven harlequin ducks were sequenced from PWS (n = 63) and APKA (n = 64) regions (Table 1). Six of the 163 bp sites

were variable across all individuals; only two were parsimonious, and all occurred as transition substitutions (data not shown). When the nuclear DNA outgroup was included, 53 of the 163 bp sites were variable of which 16 were parsimonious. Forty-nine sites occurred as transition substitutions and the remainder were inversion/deletions (data not shown). This variation resulted in a total of seven unique haplotypes. Six of the seven haplotypes were found in both the PWS and APKA regions, and only one haplotype was detected in a single individual whereas the most common haplotype accounted for 52% of the samples.

Haplotype diversities ranged from 0.427 to 0.745 (Table 2), with the highest diversity located in the Port Chalmers and Afognak populations. Haplotype diversity was nearly significantly lower in PWS (0.702) than in the APKA (0.574) region (Monte Carlo simulation, $P = 0.08$). Haplotype diversity values were highly concordant across populations (Monte Carlo simulations indicated no significant differences across all eight populations or across four populations within each region; all $P > 0.05$). Nucleotide diversity corresponded closely to haplotype diversity. The relatively high and even number of samples sequenced from each population suggests we were unlikely to find additional novel haplotypes in any one population.

Phylogenetic analyses - The evolutionary relationship among haplotypes is presented in the form of a neighbor-joining consensus tree (Figure 3). Bootstrap support of the topology was weak; none of the haplotypes were clustered the majority of the time, and there was little evidence of phylogeographic concordance (i.e., particular haplotypes were not restricted to specific sampling sites). Branch lengths between haplotypes were also very short (maximum gamma distances were only 0.02 units).

Population genetic analysis - Hierarchical analyses conducted without information on haplotype evolutionary relationships (i.e., number of nucleotide substitutions) revealed no significant differences in mtDNA haplotype frequencies among individuals within populations, among populations within regions or among regions (Table 1). A similar analysis which used haplotype evolutionary relationships yielded similar results except a marginally significant difference in haplotype frequencies was found among regions ($P=0.0499$). The frequency of haplotypes "A" and "B" within each of the two regions differed, and haplotype G was only present in the APKA region. Haplotypes differed in sequence from each other by 1-2 bp substitutions.

The lack of structuring among the populations was also evident when we constructed a neighbor-joining tree using the maternally inherited mtDNA frequency information (Figure 4). Branch lengths were extremely short and it was usual for populations from the PWS and APKA to be clustered together.

A comparison of the population consensus tree generated from the four biparental microsatellite loci (Figure 2) and the branch length tree generated with mtDNA sequence information (Figure 4) revealed some similarities. First, the Bay of Isles and Katmai populations, and the Uyak and Uganik populations were grouped in both analyses. The Afognak population also appeared near the Uyak and Uganik populations in both trees, although this cluster differed in its relative

position within each of the trees. Overall, however, both figures provide little evidence for any significant phylogeographic relationship.

Color Marking With Leg Bands

Movements of harlequin ducks between regions were not detected during this study, and only minimal movements within regions were apparent. In the PWS region, site fidelity to molting and wintering areas was very high. Of 647 unique individuals marked during 1995 and 1996, 151 (23%) were recaptured in subsequent years. Of these, 145 (96%) were recaptured within 2 km of the original trap location. Of the 6 birds moving > 2 km between years, 5 were of the younger age classes (subadult and juvenile).

Rate of band returns at Kodiak for the Uganik and Afognak trapping locations combined was 9%, assuming all 138 harlequin ducks banded in 1996 (same area) were alive in 1997. Return rates of harlequin ducks in 1996 were similar for all 1997 Kodiak capture sites, with 11 of the 80 ducks (14%) captured from the Uganik site and one of 58 ducks (2%) from the Afognak sites. Based on the same assumptions, of Katmai's 38 ducks banded in 1996, 11% were returned with the 221 ducks trapped in 1997. In all areas we banded no less than 20 percent of the estimated population.

Nine hunter-kills of Kodiak banded harlequin ducks (males) were recovered during the period 1 September 1996 to 31 December 1997. Male mortality from sporthunting was 3 % (nine of 262). Six males banded in Uyak Bay were also harvested in Uyak Bay. Two were taken in outer Uyak Bay, approximately 26 km from the Uyak banding capture site on 23 November 1996 and 8 December 1997, respectively, while another was shot on 17 November 1997 at the original Uyak banding site. The fourth male's kill location was only identified as Uyak Bay. The remaining male kill locations were identified inexplicitly as Uyak Bay, and all occurred in December 1997. Hunters also collected one male bird each from the Afognak and Uganik banding sites. These birds were shot approximately 20 km and 3 km from the banding sites, respectively. The longest documented movement of a Kodiak banded harlequin duck was a male banded at Uyak, and recovered at Uganik Bay approximately 54 km (direct distance) from the capture site. The latter band return confirms movement of harlequin ducks between Uyak and Uganik Bays suggested by analysis of coastal harlequin survey data (Zwiefelhofer 1997a, 1997b).

During 4-5 June 1997, 13 male harlequin ducks were resighted and their individual color (white) band codes were read in the vicinity of both of the 1996 Uyak capture sites. Three additional colored (white) bands were seen (one female, two males) in the area but no codes could be read. At Kodiak a minimum of five banded (white/black codes) harlequin ducks were observed in Terror Bay on 9-10 April 1997. The banded harlequins (all adult males) were observed in flocks of 30-35 birds and were in the same location that the 1996 capture efforts had occurred. Additionally, four (e.g., a male and a female at Uyak and two males at Uganik) banded harlequin ducks were flushed during May 1997 coastal shoreline surveys, and three (one female at Uyak and

two males at Afognak) harlequins ducks with bands (white) were seen during the August surveys (Zwiefelhofer, 1997b). All the banded birds resighted at Kodiak were seen approximately 2-3 km from their 1996 capture locations.

Other efforts to observe bands and read the band codes during the period were not completely successful. Extreme wear of the outer portion of the Kodiak white-colored bands was evident after 1-year of service. This wear contributed to difficulties in obtaining individual band code observations and these worn white plastic bands were also hard to distinguish from the metal bands. At Katmai, the red-colored bands proved to be more durable and showed little wear a year later. No band returns or resightings were reported from the Katmai study area. Changes in National Park Service priorities and staff turn-over precluded dedicated or ancillary attempts at band resightings along the Katmai coast. Because of the remote nature of the Katmai coast, sport hunting, thus band recoveries, were not expected for harlequin ducks.

The experimental floating trap method greatly reduced the overall person hours expended to capture ducks and increased the sampling options over the study areas of the APKA. We could leave the vessel, set the trap, make the duck drive, catch the ducks, disassemble the trap, and transport the ducks back to the vessel for processing in slightly more than two hours. The conventional system often took at least 1-2 hours just for trap setup and needed, often rare, gradual beach slopes and bottom strata, and it requiring the timing of the capture operation with the tides. In the event of any mishaps, using this conventional method eliminated any chances to reset a trap until the next day.

The floating trap was more resilient to time frames (e.g., favorable tides were not needed) and it was more adaptable to a greater variety of shoreline types and slopes. These qualities are of significant value when working shores with high density populations of brown bear (*Ursus arctos*). At Katmai, where coastal brown bear populations are the highest on record (T. Smith, pers. comm.), interruptions, including the destruction of one trap, were common. The more mobile floating trap allowed us to avoid bear concentration areas and habituated individual bears.

We found one negative result from use of the floating trap. In areas subject to ocean swell action, the aerial movements of the trap apparatus appeared to repel a few ducks. However, the modified trap was clearly more efficient, cost effective and practical at sites where the conventional trap was unsuitable (e.g., deep water and steep shoreline gradients). This was the case at Katmai where the improved trap increased daily capture rates by 175% (8 to 22 ducks per day).

DISCUSSION

Our genetic analyses suggest those harlequin ducks wintering within PWS and APKA share population genetic characteristics consistent with those expected for organisms belonging to one panmictic population (i.e., there is little or no spatial population structuring). Indeed, all three classes of molecular genetic markers indicated there were no significant differences in allele and

haplotype frequencies among populations within regions, and only one of the three markers (mtDNA) indicated a marginally significant difference between PWS and APKA regions. Further, neighbor-joining consensus and branch length trees revealed few consistent population groupings, and similarities in nuclear and mitochondrial gene frequencies reflected the relatively small inter-population genetic distances and weakly resolved tree topologies. The mtDNA haplotype tree also failed to provide a strong phylogeographic signature whereby most closely related haplotypes are observed in only one region. These results suggest that male and female movement and gene flow occurs (or has historically occurred) at a sufficient level among populations and regions to homogenize allele and haplotype frequencies.

Our finding that harlequin ducks have little to no population structuring within PWS and APKA was surprising given that life history characteristics suggest the presence of discrete, reproductively isolated, populations (see introduction). This disparity may be explained in three ways. First, it is possible that reproductively isolating mechanisms have only recently become established within PWS and APKA and that insufficient time has elapsed for genetic differences to have evolved. This seems unlikely given the observational data on immature movement patterns recently collected (see below). Second, episodic dispersal may result as a consequence of birds responding to habitat alteration (e.g., cataclysmic events such as the 1912 Mt. Katmai volcanic eruption and the 1989 *Exxon Valdez* oil spill) resulting in periodic bouts of gene flow amongst populations. And third, adult or juvenile movement may still be occurring between populations and regions at a low level. This hypothesis is supported by recent band re-sighting data within APKA and PWS regions. Although such movements are relatively rare (e.g., the five birds in PWS represent only 3% of all resightings), they may, assuming these emigrants mate with local individuals, provide sufficient gene flow to homogenize gene frequencies. Indeed, gene flow via sub-adults and juveniles seems most likely given that they remain in the marine coastal environment for 2-3 years during maturation. This lack of natal philopatry may, in essence, be nullifying the effects of high adult fidelity to wintering sites. In all areas we banded no less than 20 percent of the estimated population. However, the general approach to banding studies requires a minimum banding of 300 birds in each age class with at least 5 years of banding (Brownie et al. 1985). Our results did not meet these criteria for optimum banding sample size.

If harlequins do not show genetic population structure within PWS and APKA, at what geographic scale is structure present? As part of a larger analyses involving the entire breeding range of harlequins, we compared gene frequencies of harlequin populations distributed throughout the West Coast and between the East and West coasts of North America. An analysis of these same markers across eight populations (Puget Sound in Washington, Straits of Georgia and Queen Charlotte Islands in British Columbia; PWS; APKA; and the Shemya, Pribolof and St. Lawrence Islands in Alaska) on the West Coast revealed significant differences in haplotype frequencies but not in allele frequencies. Only one of the four biparental microsatellite loci (Hhiμ5) had significant allele frequency differences among populations. When all biparental loci were combined, there was nearly a significant difference among population allele frequencies ($\underline{P}$ = 0.054). It is possible that the use of more biparental loci would reveal differences between

populations that we cannot detect now. In addition, there was no evidence of population structuring from the Sfiμ1 single sex-linked microsatellite marker.

The mitochondrial haplotype frequencies were, however, significantly different among populations. An examination of the neighbor-joining tree using the maternally inherited mtDNA sequence information (coancestry coefficient distances [Reynolds et al. 1983]) also indicates a geographic-based level of structuring. Two main groupings were present: a "Pacific Northwest" cluster represented by the Queen Charlotte Islands, Southern British Columbia and Washington populations, and a "Bering Sea" cluster represented by the Shemya, Pribolof and St. Lawrence populations. The APKA region was most closely related to the Bering Sea cluster, and the PWS region was distantly related to both clusters. These analyses suggest a moderate level of population structuring may be present in harlequin ducks along the West Coast of North America, and that this structuring is probably a function of limited female-biased dispersal (there was high population structuring in maternally inherited mtDNA but low in biparental and paternal inherited markers). The fact that adjacent populations share genotypes suggests that females may move between adjacent populations but that widely separated populations may be reproductively isolated (e.g., Bering Sea versus Pacific Northwest groupings).

Given the significant differences in gene frequencies among populations on the West Coast, it is not surprising that a similar comparison between East and West Coast harlequin ducks also revealed strong genetic differences. Indeed, allele frequencies for two of the four biparental loci (Hhiμ2, Sfiμ4) were significantly different and the mean F_{ST} across all loci was highly significant ($F_{ST} = 0.021$, $\underline{P} = 0.002$). Sample sizes were too small on the East Coast to compare sex-linked markers, but a comparison of the mtDNA haplotype frequencies between the two coasts was also highly significant ($Phi_{ST} = 0.102$, $\underline{P} < 0.002$). The large expanse of inappropriate breeding habitat in central North America (Bellrose 1980) appears to be effective at reducing or essentially eliminating contemporary movement of individuals.

Our inabilities to detect genetic population structure within and between PWS and the APKA regions does not mean, necessarily, that harlequin ducks belong to one large, demographically panmictic population. Indeed, our analyses were unable to differentiate between historic and contemporary gene flow. If contemporary demographic data is correct, then it is possible that distinct wintering groups exist and that these groups may have been disproportionately impacted by the spill. Thus, the lack of harlequin duck recovery in oiled areas could be due to low local recruitment and the lack of immigration into effected areas. On the other hand, if the absence of genetic structure is a reflection of high contemporary movements (probably by immature birds), then our results may undermine previous demographic studies that have used 'before-after' or 'treatment-control' comparisons to document harlequin duck recovery (Wiens 1995). Movements of harlequin ducks into and out of areas may lead to demographic changes between areas, either through time or between oiled and unoiled areas (see, e.g., Klosiewski and Laing 1994, Day et al. 1997, Wiens et al. 1996, Murphy et al. 1997). These demographic changes may, under this scenario, simply reflect changes in population constituents rather than major differences in local populations.

Our genetic analyses indicated that harlequin ducks sampled within PWS and the APKA share a recent common "ancestral" genetic background and that population segregation appears to have evolved only recently. These results indicate that oil-induced mortality of harlequin ducks from specific areas within PWS and APKA did not result in the loss of unique genetic variation. Indeed, inbreeding and outbreeding depression is unlikely to be a problem due to the loss of founders from a particular population or the influx of poorly adapted individuals into new habitats.

Finally, our study suggests those harlequin duck populations, at the local level, may have the capacity to recover from environmental perturbations. Movements within and between regions can result in recolonization or enhancement of damaged populations, although the time scale to full recovery as a result of these movements (especially if they are as rare as they appear to be) may be interminable. Of course, even with immigration, full recovery will not occur until residual, detrimental effects of the spill have disappeared.

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APPENDIX

TABLE 1. Harlequin duck (*Histrionicus histrionicus*) allele frequencies and F-statistics of four bi-parental, two sex-linked, and one maternally inherited marker from four populations each within two marine coastal wintering regions in south central Alaska.

										Variance Partitioning[a]			
		Prince William Sound				Alaska Peninsula and Kodiak Archipelago							
Inheritance/ Locus	Allele/ Haplotype	Bay of Isles	Foul Bay	Green Island	Montague Island	Katmai	Afognak	Uganik	Uyak	Alleles Within Individuals	Among Individuals Within Populations	Among Populations Within Regions	Among Regions
Bi-parental Microsatellite Loci													
Hhim2	94	0.278	0.241	0.294	0.358	0.297	0.294	0.306	0.361	-0.025	-0.028	-0.003	-0.001
	96	0.519	0.528	0.493	0.453	0.505	0.426	0.507	0.449				
	98	0.130	0.148	0.154	0.135	0.132	0.221	0.132	0.120				
	100	0.000	0.028	0.022	0.014	0.011	0.000	0.014	0.019				
	102	0.074	0.056	0.037	0.041	0.055	0.059	0.042	0.051				
	(N)	(27)	(54)	(68)	(74)	(91)	(34)	(72)	(79)				
Hhim5	138	0.000	0.000	0.000	0.000	0.000	0.000	0.007	0.000	-0.017	-0.011	0.006	0.000
	142	0.000	0.019	0.023	0.045	0.000	0.000	0.000	0.000				
	144	0.000	0.000	0.008	0.000	0.000	0.000	0.000	0.006				
	146	0.648	0.472	0.447	0.500	0.600	0.479	0.528	0.525				
	148	0.037	0.009	0.030	0.032	0.016	0.021	0.028	0.056				
	150	0.315	0.491	0.492	0.416	0.379	0.500	0.431	0.406				
	152	0.000	0.009	0.000	0.006	0.005	0.000	0.007	0.000				
	154	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006				
	(N)	(27)	(54)	(66)	(77)	(95)	(24)	(72)	(80)				
Bcam10	112	0.815	0.797	0.804	0.757	0.771	0.792	0.753	0.819	0.000	-0.005	-0.005	0.000
	114	0.148	0.144	0.145	0.158	0.182	0.139	0.185	0.138				
	116	0.037	0.059	0.051	0.086	0.047	0.069	0.062	0.044				
	(N)	(27)	(59)	(69)	(76)	(96)	(36)	(73)	(80)				
Sfim4	141	0.722	0.794	0.784	0.776	0.742	0.825	0.778	0.775	0.064	0.058	-0.006	-0.007
	143	0.278	0.206	0.216	0.224	0.258	0.175	0.222	0.218				
	145	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.007				
	(N)	(27)	(51)	(67)	(78)	(93)	(20)	(27)	(71)				
all bi-parental loci										-0.001 (NS)	-0.003 (NS)	-0.001 (NS)	0 (NS)

TABLE 1. Continued.

Inheritance/ Locus	Allele/ Haplotype	Prince William Sound: Bay of Isles	Prince William Sound: Foul Bay	Prince William Sound: Green Island	Prince William Sound: Montague Island	Alaska Peninsula and Kodiak Archipelago: Katmai	Alaska Peninsula and Kodiak Archipelago: Afognak	Alaska Peninsula and Kodiak Archipelago: Uganik	Alaska Peninsula and Kodiak Archipelago: Uyak	Variance Partitioning[a]: Alleles Within Individuals	Variance Partitioning[a]: Among Individuals Within Populations	Variance Partitioning[a]: Among Populations Within Regions	Variance Partitioning[a]: Among Regions
Sex-linked Microsatellite Loci[b]													
Sfim1	198	0.056	0.229	0.061	0.170	0.040	0.071	0.053	0.065	n/a	-0.004	0.021	0.017
females	200	0.222	0.200	0.184	0.226	0.160	0.143	0.211	0.226		(NS)	(NS)	(NS)
	202	0.722	0.571	0.755	0.604	0.800	0.786	0.737	0.710				
	(N)	(18)	(35)	(49)	(53)	(25)	(14)	(38)	(31)				
Bcam4	190/194	0.000	0.000	0.030	0.029	0.000	0.000	0.000	0.032	n/a	0.002	0.003	0.005
females[c]	196	0.059	0.031	0.061	0.235	0.222	0.143	0.056	0.097		(NS)	(NS)	(NS)
	198	0.059	0.094	0.030	0.059	0.000	0.000	0.028	0.032				
	200	0.000	0.031	0.030	0.000	0.000	0.000	0.028	0.000				
	202	0.176	0.094	0.061	0.029	0.074	0.143	0.083	0.194				
	204	0.118	0.094	0.182	0.147	0.037	0.143	0.167	0.065				
	206	0.118	0.219	0.091	0.059	0.259	0.286	0.250	0.161				
	208	0.235	0.094	0.242	0.118	0.111	0.143	0.139	0.129				
	210	0.118	0.219	0.091	0.059	0.148	0.143	0.111	0.129				
	212	0.118	0.094	0.091	0.059	0.000	0.000	0.083	0.000				
	214-238	0.000	0.031	0.091	0.205	0.148	0.000	0.056	0.162				
	(N)	(17)	(32)	(33)	(34)	(27)	(14)	(36)	(31)				
Maternal Mitochondrial DNA[d]													
	A	0.125	0.188	0.000	0.250	0.118	0.267	0.500	0.438	n/a	0.011	0.040	0.051
	B	0.063	0.188	0.200	0.188	0.118	0.200	0.063	0.063		(NS)	(NS)	(NS)
	C	0.000	0.000	0.067	0.063	0.059	0.067	0.000	0.063				
	D	0.063	0.000	0.000	0.063	0.059	0.000	0.063	0.000	n/a	0.02	0.035	0.055
	E	0.000	0.063	0.000	0.000	0.000	0.067	0.063	0.063		(NS)	(NS)	(P = 0.049)
	F	0.000	0.000	0.000	0.000	0.059	0.000	0.000	0.000				
	G	0.750	0.563	0.733	0.438	0.588	0.400	0.313	0.375				
	(N)	(16)	(16)	(15)	(16)	(17)	(15)	(16)	(16)				

TABLE 1. Continued.

[a] F-statistics for bi-parental loci, sex-linked (Z-specific) loci, and maternal mtDNA haplotypes. Nomenclature is as follows: Alleles within individuals represented by f, among individuals within populaitons represented by F and PhiSC values, among populations within regions represented by Theta-S and PhiCT values, and among region variability represented by FST and PhiST values. NS = non-significant (I.e., P>0.05). "n/a" indicates that no F-statistic was available for the sex-linked microsatellite loci and mitochondrial DNA data because they are haploid.

[b] Z-specific loci (Fields and Scribner 1997, Buchholz et al. 1998). Analyses restricted to females to provide a measure of male-mediated gene flow.

[c] Alleles present in low frequency were combined, including 190 and 194, and 214, 216, 218, 220, 222, 224, 226, 230 and 238.

[d] F-statistics are first presented using only haplotype frequencies and then with haplotype frequencies weighted by DNA sequence differences among haplotypes.

TABLE 2. Measures of genetic diversity estimated for harlequin ducks (*Histrionicus histrionicus*) from four populations each within two marine coastal wintering regions in south central Alaska.

Inheritance/ Variable	Prince William Sound				Alaska Peninsula and Kodiak Archipelago			
	Bay of Isles	Foul Bay	Green Island	Montague Island	Katmai	Afognak	Uganik	Uyak
Bi-parental Microsatellite Loci								
Observed Heterozygosity	0.491	0.479	0.483	0.494	0.463	0.427	0.462	0.482
Expected Heterozygosity[a]	0.465	0.465	0.471	0.492	0.474	0.464	0.481	0.470
Mean No. of alleles	3.00	3.75	3.75	3.75	3.50	3.00	3.75	4.00
Inbreeding Coefficient[b]	-0.056	-0.030	-0.025	-0.004	0.023	0.080	0.040	-0.026
Sex-linked Microsatellite Loci								
Genetic diversity[c] (no. alleles)								
Sfim1	0.426 (3)	0.582 (3)	0.392 (3)	0.555 (3)	0.333 (3)	0.357 (3)	0.410 (3)	0.441 (3)
Bcam4	0.851 (8)	0.857 (10)	0.865 (13)	0.852 (14)	0.821 (10)	0.816 (6)	0.856 (10)	0.861 (11)
Maternal Mitochondrial DNA								
Haplotype diversity (p) [d]	0.427	0.629	0.432	0.726	0.635	0.745	0.661	0.677
Nucleotide diversity (h) [d]	0.004	0.005	0.003	0.007	0.006	0.007	0.007	0.006
No. of haplotypes	4	4	3	5	6	5	5	5

TABLE 2. Continued.

[a] Assuming Hardy-Weinberg equilibrium.

[b] Wright (1951) inbreeding coefficient. Significance of F at a specific locus and population were tested as described by Li and Horvitz (1953).

[c] Genetic diversity Nei (1987). For the Bcam4 locus, alelles 190 and 194 were combined; and alleles 214, 216, 218, 220, 222, 224, 226, 230, and 238 were combined.

[d] Haplotype and nucleotide diversity indices for nonselfing populations (equations 8.4 and 10.4, respectively of Nei 1987), as calculated by the REAP program (McElroy et al. 1991) from pairwise haplotype divergences calculated by the MEGA program (Kumar et al. 1993).

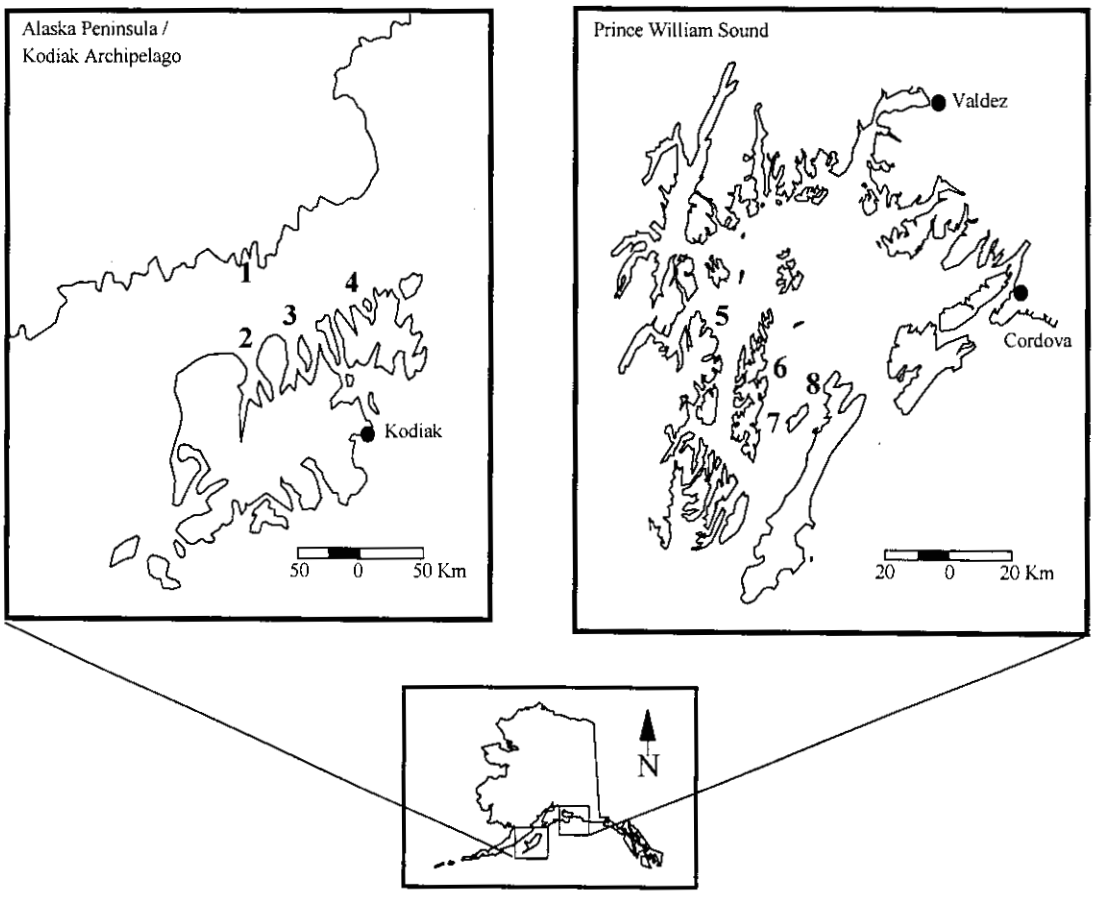

Fig. 1. Harlequin duck sampling locations in the Alaska Peninsula/Kodiak Archipelago (1 = Katmai, 2 = Uyak, 3 = Uganik, 4 = Afognak) and Prince William Sound (5 = Foul Bay, 6 = Bay of Isles, 7 = Green Island, 8 = Montague Island).

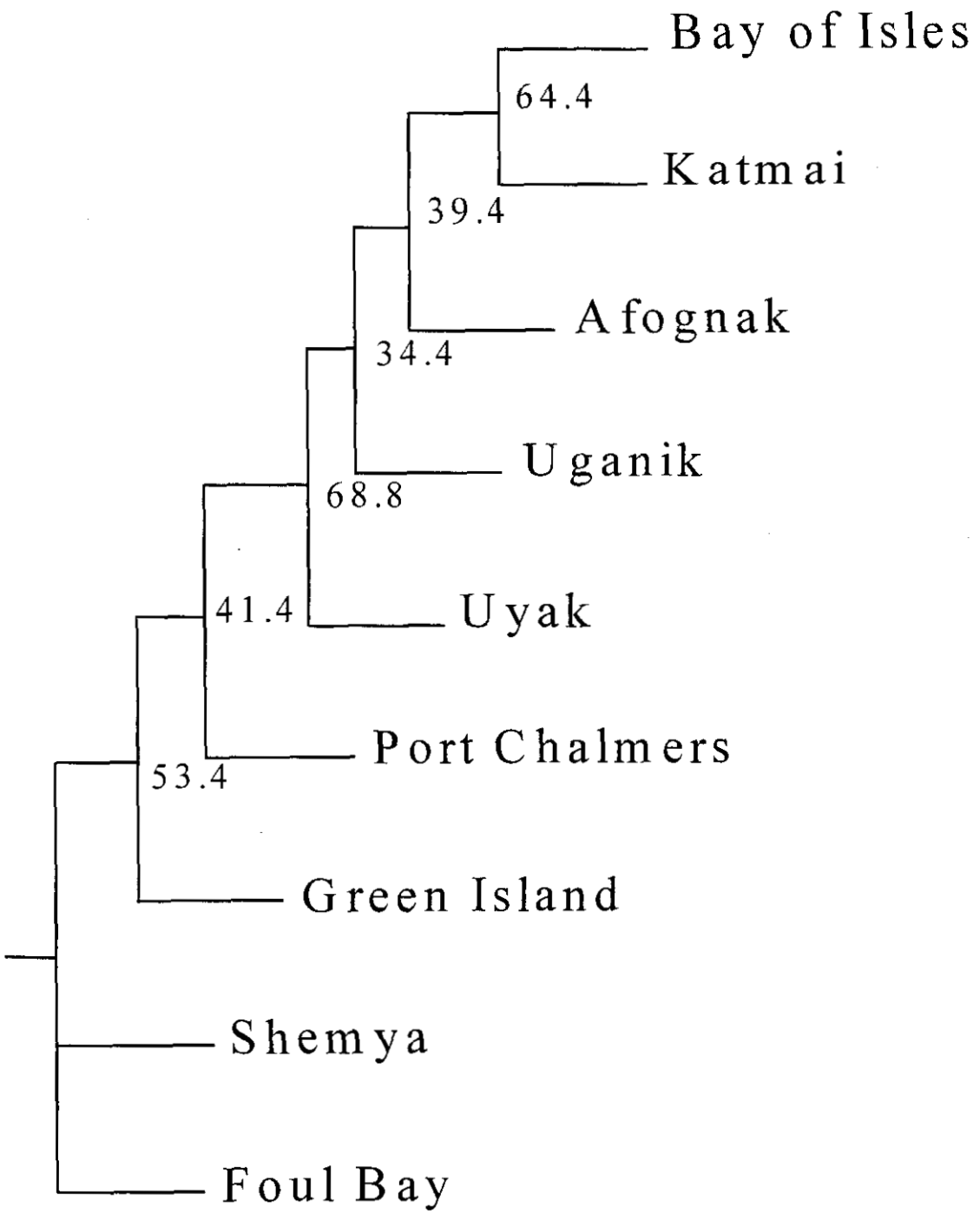

Fig. 2. Neighbor-joining phenogram of genetic chord distances (Cavalli-Sforza and Edwards 1967) for four bi-parentally inherited microsatellite loci describing relationships among eight populations of harlequin ducks distributed throughout Prince William Sound and the Alaska Peninsula/Kodiak Archipelago. Numbers near branch nodes represent the proportion of 500 bootstrap iterations where two populations (or groups of populations) were clustered. Birds sampled at Shemya, Aleutian Islands, Alaska, were used as an outgroup in this analysis. See text for details on methods.

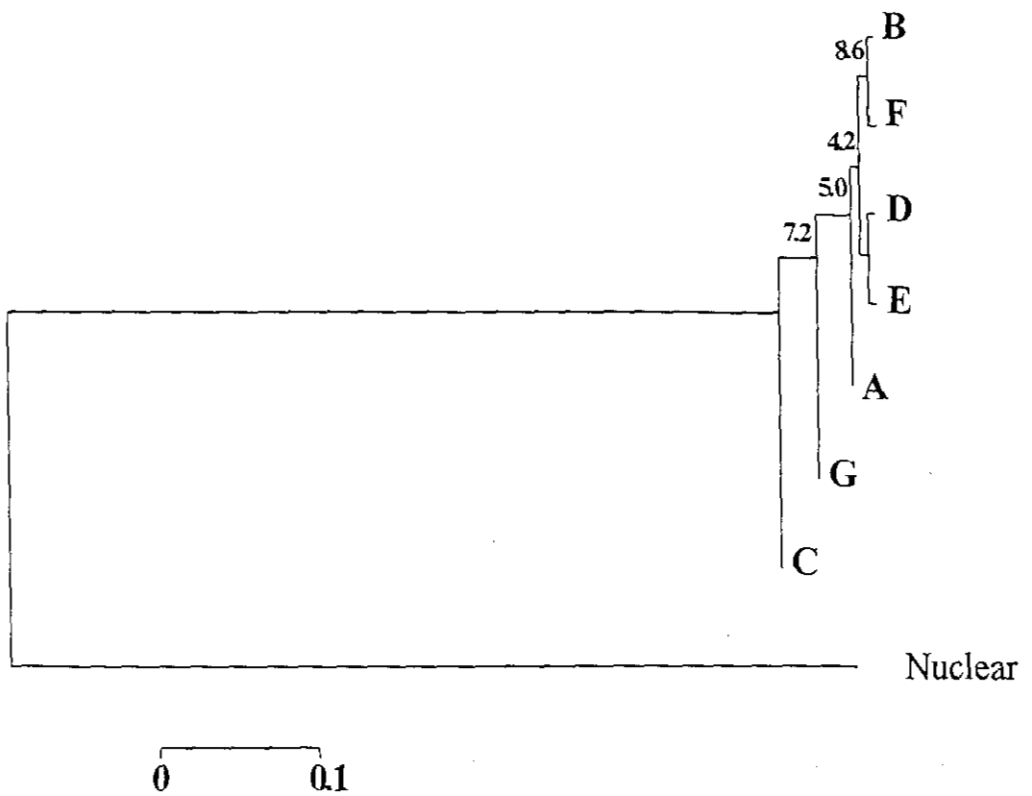

Fig. 3. Neighbor-joining tree from gamma distances generated with Kimura's (1980) 2-parameter sequence evolution model showing phylogenetic relationships among mtDNA haplotypes in harlequin ducks sampled throughout Prince William Sound and the Alaska Peninsula/Kodiak Archipelago. Numbers near tree branches represent the proportion of 500 bootstrap iterations where two haplotypes (or groups of haplotypes) were clustered. Branch lengths represent genetic distances separating haplotypes (see text). A transposed nuclear pseudogene sequence from a harlequin duck was used to root the tree.

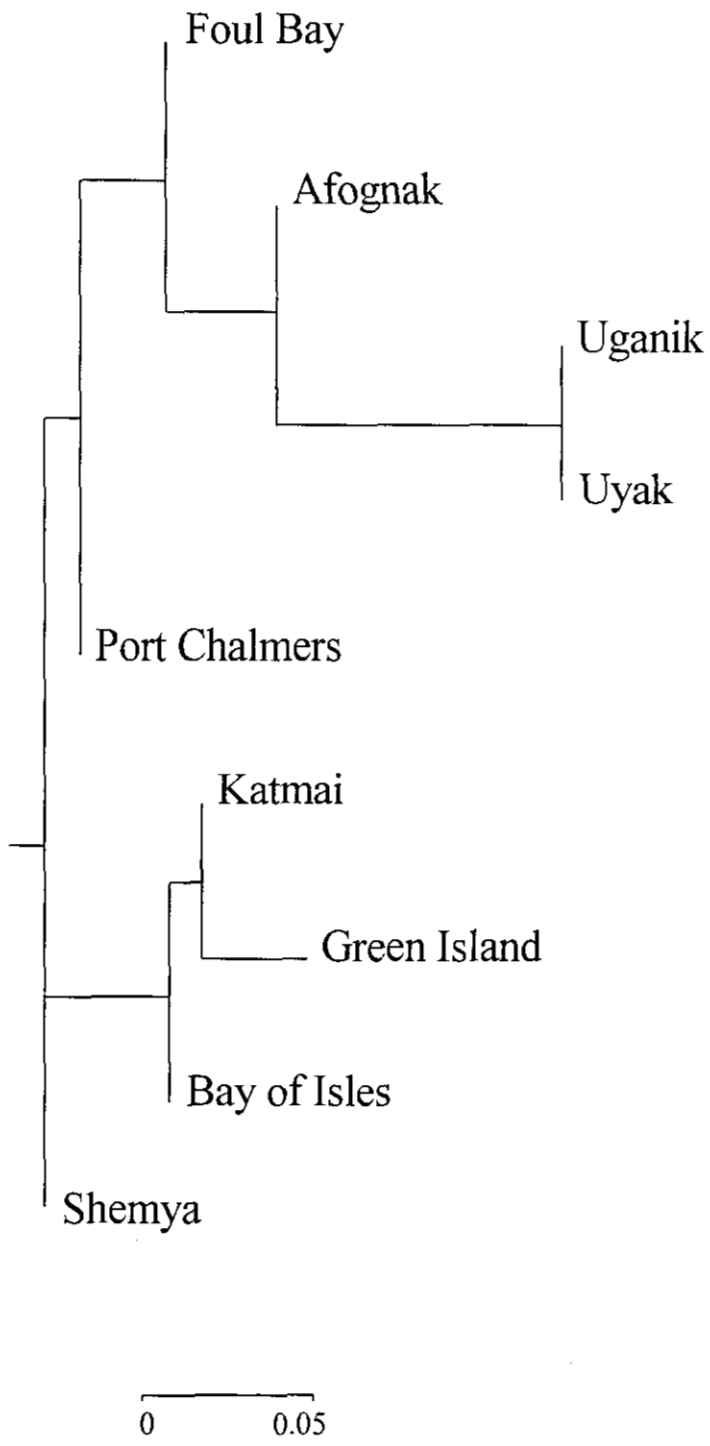

Fig. 4. Neighbor-joining phenogram of genetic distances (Reynolds et al. 1983) for mtDNA describing relationships among eight populations of harlequin ducks distributed throughout the Prince William Sound and the Alaska Peninsula/Kodiak Archipelago. Birds sampled at Shemya, Aleutian Islands, Alaska, were used as an outgroup in this analysis.