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HARLEQUIN DUCK RECOVERY FROM THE *EXXON VALDEZ* OIL SPILL: A POPULATION GENETICS PERSPECTIVE

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ABSTRACT.—Concerns about Harlequin Duck (*Histrionicus histrionicus*) population recovery following the *Exxon Valdez* oil spill led biologists to ask whether birds located in different molting and wintering areas belong to genetically distinct and, thus, demographically independent populations. Owing to the lack of direct observations of movements among marine areas, three classes of genetic markers that differed in mode of inheritance were used to evaluate the degree of genetic differentiation among wintering areas within Prince William Sound (PWS) and the Alaska Peninsula and Kodiak Archipelago (APKA). We could not reject the null hypothesis that the wintering aggregations within each region are composed of a single genetically panmictic population. Differences in genotype frequencies among wintering locations within PWS and APKA were low and nonsignificant for all three classes of markers. Furthermore, we saw no evidence for deviations in Hardy-Weinberg equilibrium or gametic disequilibrium between loci within a winter collection site as would be expected if these locales were composed of individuals from reproductively isolated (and genetically distinct) breeding locales. Finally, no evidence for significant structuring was noted between PWS and APKA. Lack of spatial genetic structuring could be due to the cumulative effects of low levels of gene flow over long time periods, low levels of gene flow by immature birds moving between marine habitats, or to episodic dispersal caused by habitat alteration (e.g. volcanic eruptions). Harlequin Ducks are likely to recolonize or enhance populations in areas recovering from environmental damage via emigration of birds from non-affected areas. Demographic studies suggest, however, that levels of movements are low, and that population recovery by emigration is a long-term process. *Received 11 May 1998, accepted 14 January 1999.*

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), along the Kenai and Alaska peninsulas, and along the Kodiak Archipelago. Much of the oil was deposited in nearshore intertidal and subtidal habitats (Neff et al. 1995), which are important to a large number of vertebrates, including molting and wintering waterfowl. Indeed, the nearshore environment of Prince William Sound received about 40% of the oil that was spilled (Galt et al. 1991). The effects of this oil spill on resident fish and wildlife have been dramatic (Piatt et al. 1990, ECI 1991) and the subject of extensive investigations.

Harlequin Ducks (*Histrionicus histrionicus*) are year-round inhabitants of nearshore environments within the oil spill zone (Isleib and Kessel 1973, Agler et al. 1994). Two hundred and twelve Harlequin Ducks were recovered from beaches after the oil spill (J. Piatt pers. comm.), but the actual number that died probably was much higher because the recovery rate of waterbirds was low due to birds being scavenged, sinking, or not being found along shorelines (Ford et al. 1987, ECI 1991). Adjusted estimates that correct for these recovery problems range from 1,298 to 2,650 Harlequin Ducks killed from oiling (ECI 1991, Piatt and Ford 1996, J. Piatt pers. comm.). Post-spill studies

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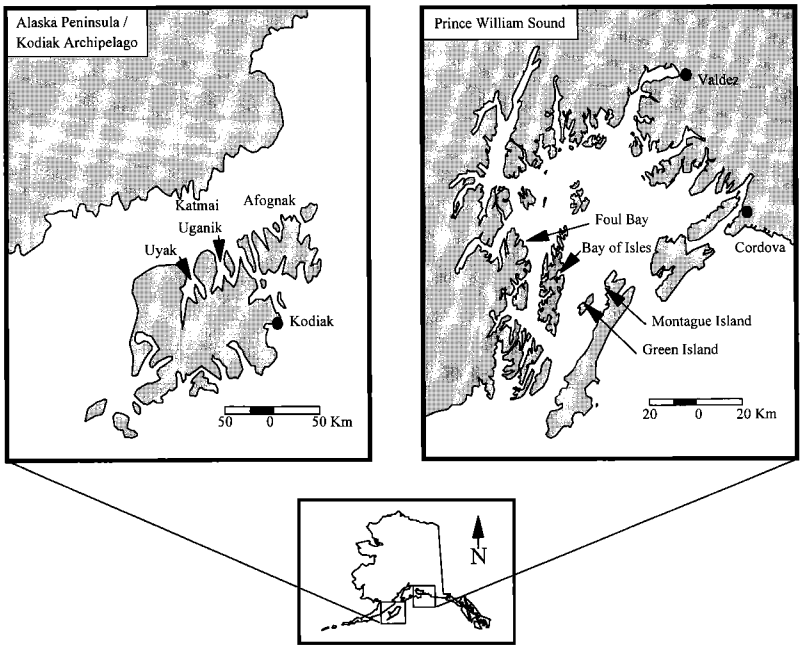

FIG. 1. Harlequin Duck sampling locations in the Alaska Peninsula/Kodiak Archipelago and Prince William Sound.

suggest that negative effects from the spill continue, 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 to 1993 (Patten 1995).

To understand the process of post-spill recovery of Harlequin Ducks, and to identify impediments to recovery, it is critical to determine whether aggregations of individuals within local areas of the marine environment are discrete and demographically independent, i.e. whether the population is structured within the oil spill region. For example, if birds located in oiled and unoiled areas belong to demographically distinct population segments (i.e. management units; Moritz 1994), then certain segments of the population may have been (and are continuing to be) influenced disproportionately by oil. Alternatively, a lack of spatial structure would imply that oil effects are distributed throughout a larger, panmictic population. Unfortunately, little is known about movements of Harlequin Ducks among areas of the marine environment. In the absence of direct observational data, population discreteness also can be inferred using genetic markers (Slatkin 1985, 1995; Slatkin and Barton 1989). In this study, we used three classes of molecular genetic markers that differ in their mode of inheritance (biparental, maternal, and sex-linked) to determine whether population structuring occurred among winter aggregations of Harlequin Ducks in coastal marine habitats of PWS and the Alaska Peninsula/Kodiak Archipelago (APKA). Information on population structure was then used to assess the potential constraints to recovery of Harlequin Ducks from the *Exxon Valdez* oil spill.

METHODS

We compared genetic characteristics of four aggregations of molting birds (referred to as populations) captured within each of two regions (PWS and APKA; Fig. 1). Because band resightings and radiotelemetry data indicate that Harlequin Ducks almost always winter at or near their molting sites (Robertson 1997, D. Esler unpubl. data), we considered these molting aggregations to represent discrete wintering populations. Sampling sites within the PWS region included Bay of Isles, Green Island, Foul Bay, and Montague Island, whereas sampling sites within the APKA region included Uganik and Uyak bays and Afognak Island of the Kodiak Archipelago, and Kat-

mai on the Alaska Peninsula. All of these sites were located inside the area affected by the oil spill.

Molting Harlequin Ducks were captured at each of the eight sampling sites during July to Sept of 1995, 1996, and 1997 by herding the flightless birds into pens (see Clarkson and Goudie 1994). The sex of each duck was determined by plumage characteristics and the age by bursal probing (Mather and Esler 1999). Blood was extracted from the jugular or tarsus veins and was preserved in lysis buffer (Longmire et al. 1988). DNA was extracted using Puregene DNA extraction kits and standard proteinase-K phenol-chloroform methods (Sambrook et al. 1991).

Thirty-three microsatellite loci (Fields and Scribner 1997, Buchholtz et al. 1998, Cathy et al. 1998) were examined for variation by screening two individuals each from 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 and Bcaμ4) were variable. The sex-linked (*Z*-specific) loci provide an estimate of male-mediated gene flow from the preceding generation if sampling is conducted using females. Microsatellite loci were assayed using the polymerase chain reaction and end-labeled (^{32}P-γATP) primers. Specific conditions for each locus are available from the senior author. Products were visualized on 6% denaturing polyacrylamide sequencing gel after autoradiography. An M13 sequencing reaction (Amersham Life Sciences Sequenase kit) and individual standards of known genotypes were run adjacent to samples to provide an absolute-size marker for determining the size of microsatellite alleles.

Primers specific for mitochondrial DNA (mtDNA) were designed so that mtDNA sequences, and not nuclear DNA sequences originating from transposed mtDNA (i.e. numts; Sorenson and Fleischer 1996), would be amplified. We verified this by sequencing DNA obtained from mitochondrial-rich heart tissue and mitochondrial-poor blood from the same individual as described in Sorenson and Quinn (1998). Using the mtDNA-specific primers, we amplified a fragment (ca. 385 base pairs) of the 5′ end of the control region that is comparable to the hypervariable region 1 of the mammalian mtDNA control region (Vigilant 1990, Wakely 1993). These primers included HADUM1L (L16744; Desjardins and Morais 1990): 5′ TGC CCG AGA CCT ACG GCT C 3′; HADUM2L (L12; Desjardins and Morais 1990): 5′ TCT AAA ATG ACT CAA CAG TGC C 3′; and HADUMITH (H737; Desjardins and Morais 1990): 5′ TGA GTA ATG GTG TAG ATA TCG 3′. Nuclear specific primers included HADUN1L (L16744; Desjardins and Morais 1990): 5′ TAC CCG AGA CCT ACA GCT T 3′ and HADUNUCH (H737; Desjardins and Morais 1990): 5′ TGA GTT ATG GTG TAG ATA CTA 3′. MtDNA sequences (GenBank accession numbers AF101372 to AF101381) were obtained by amplifying either the HADUM1L or HADUM2L primers with the HADUMITH primer. Mitochondrial DNA was PCR-amplified, purified, and sequenced using Sequitherm's EXCEL DNA sequencing kits (Epicentre Technologies) and 1.5 mM of HADUMITH primer. Sequences were visualized using autoradiography, manually scored, and aligned.

Genetic analyses were restricted to birds at least one year of age. Data from males and females were used for the biparentally inherited loci, and data 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 Fisher's exact test in the Genetics Data Analysis (GDA) program (Lewis and Zaykin 1998). *P*-values were adjusted for the number of statistical tests (Sokal and Rohlf 1995). Mean number of alleles per locus were calculated using BIOSYS (Swofford and Selander 1981). We estimated observed (H_o) and expected (H_e) heterozygosity under Hardy-Weinberg assumptions for each locus (BIOSYS) and for each population and locus (GDA). These estimates of H_o and H_e were used to generate inbreeding coefficients ($F = 1 - [H_o/H_e]$) combined across loci for each population (Wright 1951) and tested for significance as described in Li and Horivitz (1953). Statistical analyses of spatial heterogeneity in gene frequency between and within regions for each locus were assessed using hierarchical *F*-statistics (Weir 1996) in the GDA program at three levels: (1) among individuals within populations, (2) among populations within regions, and (3) between regions. We also calculated R_{st}, an analogue of F_{st} (Michalakis and Excoffier 1996), using the analysis of molecular variance (AMOVA) program (Excoffier et al. 1992). Significance of F_{st} values was based on 95% confidence intervals determined by bootstrapping across loci. Confidence intervals that included zero were considered nonsignificant. We used allele frequencies to calculate Cavalli-Sforza and Edwards (1967) chord distances among the populations and constructed bootstrapped population trees using subroutines within the PHYLIP program (version 3.572c; Felsenstein 1993). This distance metric has been described as producing robust tree topologies for groups separated evolutionarily over time periods comparable to the populations we studied (Takezaki and Nei 1996). We used birds sampled at Shemya, on the outer Aleutian Islands of Alaska, as an outgroup in this analysis. For the Sfiμ1 and Bcaμ4 sex-linked loci, we calculated allele frequencies and a measure of genetic diversity (*D*; Nei 1987: equation 8.3). Estimates of variance among individuals within populations (Φ_{sc}), among populations within regions (Φ_{ct}), and between regions (Φ_{st}) were derived using the AMOVA program.

Mitochondrial DNA sequence haplotypes were assigned based on at least a single base-pair substitution or insertion/deletion across the 163 base-pair

TABLE 1. Harlequin Duck allele frequencies and *F*-statistics of four biparental, 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				Alleles within individuals	Among individuals within populations	Among populations within regions	Among regions
Locus	Allele/ haplotype	Bay of Isles	Foul Bay	Green Island	Montague Island	Katmai	Afognak	Uganik	Uyak				
Biparental microsatellite loci													
Hhiμ2	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)				
Hhiμ5	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)				
Bcaμ10	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)				
Sfiμ4	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 biparental loci										−0.001 (NS)	−0.003 (NS)	−0.001 (NS)	0 (NS)

TABLE 1. Continued.

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]													
Sfiμl females	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
	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)				
Bcaμ4 females[c]	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
	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,e]													
	A	0.125	0.188	0.000	0.250	0.118	0.267	0.500	0.438	n/a	0.11	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)				

[a] *F*-statistics for biparental loci, sex-linked (Z-specific) loci, and maternal mtDNA haplotypes. Nomenclature is as follows: Alleles within individuals represented by *f*, among individuals within populations represented by *F* and *PhiSC* values, among populations within regions represented by *Theta-S* and *PhiCT* values, and among region variability represented by F_{ST} and Phi_{ST} values. NS = nonsignificant (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.

[e] GenBank accession numbers AF10372–374, 376, 378, 379, 381).

TABLE 2. Measures of genetic diversity estimated for Harlequin Ducks from four populations each within two marine coastal wintering regions in south-central Alaska.

Variable	Prince William Sound				Alaska Peninsula and Kodiak Archipelago			
	Bay of Isles	Foul Bay	Green Island	Montague Island	Katmai	Afognak	Uganik	Uyak
Biparental 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)								
Sfiμ1	0.426	0.582	0.392	0.555	0.333	0.357	0.410	0.441
	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
Bcaμ4	0.851	0.857	0.865	0.852	0.821	0.816	0.856	0.861
	(8)	(10)	(13)	(14)	(10)	(6)	(10)	(11)
Maternal mitochondrial DNA								
Haplotype diversity (π)[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

[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 Bcaμ4 locus, alleles 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).

segment. Pairwise haplotype distances were calculated by the MEGA program (Kumar et al. 1993) and used to estimate haplotype (h) and nucleotide diversity (π) indices for nonselfing populations (equations 8.1 and 10.4, respectively, of Nei 1987) using the REAP program (McElroy et al. 1991). We tested heterogeneity of genotype distributions among samples using Monte Carlo resampling and the chi-square test of Roff and Bentzen (1989). This approach is suitable for genetic-data matrices in which many or most elements are very small (<5) or zero. Estimates of regional, population, and individual variance (F-statistics) in haplotype frequency were assessed using the AMOVA program as discussed by Excoffier et al. (1992). We considered haplotype frequencies alone and by weighting the number of base-pair substitutions among haplotypes. Evolutionary relationships among haplotypes using the numt as an outgroup were estimated using distances generated under the Tamura-Nei model of sequence evolution (Tamura and Nei 1993). We generated an evolutionary tree using the neighbor-joining method of Saitou and Nei (1987). We next determined relationships among populations by constructing neighbor-joining phylogeographic trees using coancestry coefficient distances (Reynolds et al. 1983) generated from the AMOVA and PHYLIP programs. Harlequin Ducks (n = 16) sampled in Shemya were used as an outgroup for this tree.

RESULTS

Each population of Harlequin Ducks had three to eight alleles at each biparentally inherited locus (Table 1). The mean number of alleles per locus, and observed and expected heterozygosities, were moderately high and concordant across all populations (Table 2). The biparental loci did not deviate significantly from Hardy-Weinberg equilibrium (i.e. there was no heterozygote deficit), and no evidence of linkage was observed in any population. This lack of heterozygote deficiency corresponds with the low and nonsignificant values of f (estimate of the variation associated with how alleles are distributed among individuals) present for each locus and across all loci (Table 1). Inbreeding coefficients (F) were low when averaged across loci (Table 2) but were more variable when analyzed for each population and locus combination ($\bar{x}$ = 0.008, range −0.266 to 0.262; data not shown). These values were not significant in all cases except for the Katmai population for one locus (Sfiμ4; $P < 0.05$). Estimates of spatial variation for the four biparental microsatellite loci were low (Table 1). No locus

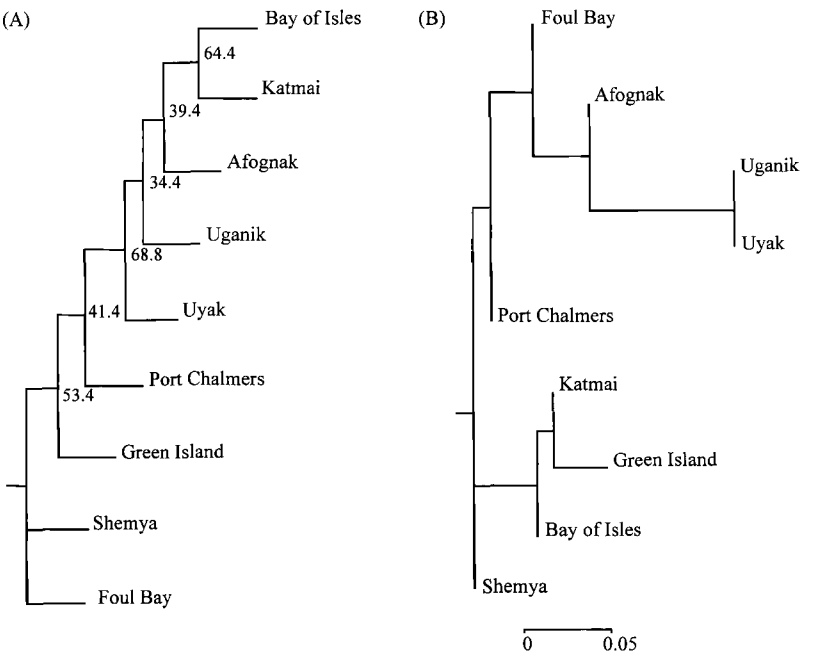

FIG. 2. Neighbor-joining phylogeographic trees describing the relationships among eight populations of Harlequin Ducks distributed throughout Prince William Sound and the Alaska Peninsula/Kodiak Archipelago for (A) four biparentally inherited microsatellite loci and (B) mitochondrial DNA. Branch distances are only meaningful in (B) where they represent Reynolds et al.'s (1983) coancestry coefficient distances. Numbers near branch nodes in (A) 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 both (A) and (B). See text for details on methods.

showed a significant difference in allele frequencies among populations within regions or between regions (all Ps > 0.05). When averaged across all loci, allele frequencies also did not differ significantly among populations within regions or between regions (Table 1). Estimates of R_{st} did not differ appreciably from F_{st} spatial-variation values and also showed no significant difference in allele frequencies among populations within regions or between regions (data not shown). The lack of spatial variation among populations was further evident based on the low levels of bootstrap support for most nodes of the consensus neighbor-joining tree using the biparental loci (Fig. 2A). 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, suggested weak population structuring. However, our ability to place accurate and precise bootstrap values on the various nodes of the tree may have been compromised by the relatively small number of biparental loci that we used (Takezaki and Nei 1996).

Genetic diversity was high for the Bcaμ4 sex-linked locus, where the number of alleles in any one population frequently exceeded 10 (Table 2). Genetic-diversity scores were much lower for the Sfiμ1 locus. Like the biparentally inherited loci, there were no significant differences in allele frequencies for sex-linked loci among populations within regions or between regions (Table 1). 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.

One hundred and twenty-seven Harlequin Ducks from the PWS (n = 63) and APKA (n = 64) regions (Table 1) were sequenced to test for variation in the mtDNA control region. Six of the 163 base-pair sites were variable across all

individuals; only two were present in more than one haplotype, and all occurred as transition substitutions. Haplotypes differed in sequence by one to two base-pair substitutions (data not shown). Fifty-three nucleotide-substitution differences occurred between the numt and the mtDNA haplotypes. Forty-nine sites occurred as transition substitutions and the remainder were insertions/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 ("F" in Table 1) was detected in a single individual, whereas the most common haplotype ("G" in Table 1) accounted for 52% of the samples.

Haplotype diversities ranged from 0.427 to 0.745 (Table 2), with the highest diversity located in the Montague Island and Afognak populations. Haplotype diversity was not significantly lower in PWS (0.574) than in the APKA (0.702) region (Monte Carlo simulation, $P = 0.08$). Haplotype diversities were highly concordant across populations and were not significantly different among populations or regions based on Monte Carlo simulations (all $P > 0.05$). Nucleotide diversity corresponded closely with haplotype diversity. Neighbor-joining phylogenetic trees based on distances between haplotypes failed to support phylogeographic relationships (i.e. particular haplotypes were not restricted to specific sampling sites). Indeed, phylogenetic relationships (tree not shown) determined by the neighbor-joining analysis were only weakly supported by the bootstrapping analyses. Hierarchical analyses conducted without information on haplotype evolutionary relationships (i.e. number of nucleotide substitutions) revealed no significant differences in mtDNA haplotype frequencies (Table 1). When haplotype evolutionary information was included, similar results were produced; however, a marginally significant difference in frequency of the two most common haplotypes ("A" and "G") was found among regions ($P = 0.049$; see Table 1). The lack of structuring among populations also was evident when we constructed a neighbor-joining tree using the mtDNA haplotype frequency information (Fig. 2B). Branch lengths were extremely short, and it was not unusual 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 and the branch length tree generated with mtDNA information (Fig. 2) revealed several similarities. First, the Bay of Isles and Katmai populations, and the Uyak and Uganik populations, clustered together in both trees. The Afognak population also clustered near the Uyak and Uganik populations in both trees, although this cluster differed in its relative position within each of the trees.

DISCUSSION

Our analyses show that Harlequin Ducks molting and wintering within PWS and APKA share population-genetic characteristics consistent with those expected for organisms belonging to one panmictic population (i.e. little or no spatial population structuring). Indeed, within regions, all three classes of genetic markers indicated no significant differences in allele and haplotype frequencies among populations. Further, the topologies of neighbor-joining consensus trees revealed a general lack of phylogeographic concordance (i.e. populations from different regions are more similar in allele and/or mtDNA haplotype frequency than they are to other locales within the same region). There was, however, a significant difference between PWS and APKA regions with the mtDNA marker (Table 1). Given the lack of information on evolutionary rates of microsatellite loci relative to the mtDNA, it is difficult to interpret these findings. Nevertheless, this regional difference was not found when only the frequencies of mtDNA haplotypes were compared, i.e. there was a notable paucity of private alleles within populations. Consequently, we interpret these results as a general lack of historic structuring within and across regions.

Our finding that Harlequin Ducks have little or no population structuring within PWS and APKA was surprising given that life-history characteristics suggest that discrete, reproductively isolated populations exist. Males and females are highly philopatric to molting and wintering areas (Robertson 1997, D. Esler unpubl. data), and pair formation occurs on the wintering grounds during early to mid-winter (Gowans et al. 1997, Robertson et al. 1998). Lack of population structuring may be explained in four ways. First, Harlequin Ducks in

PWS and APKA may represent a recent range expansion from a refugial population (Wenink et al. 1994). Second, barriers to gene flow may have become established only recently such that insufficient time has elapsed for genetic differences to have evolved. Third, habitat alterations (e.g. Katmai eruption [Rigg 1914], Great Alaskan earthquake of 1964 [Jacob 1986]) may result in episodic dispersal and possible gene flow among populations that are otherwise presently reproductively isolated. Finally, low levels of adult or juvenile movement may occur among populations and regions. Recent band-resighting data within the APKA and PWS regions indicate that individuals occasionally move between populations (although movements between regions have not been detected; D. Esler and D. Zwiefelhofer unpubl. data). If these emigrants mate with local individuals, they may provide sufficient gene flow to homogenize gene frequencies (Wright 1931). Indeed, subadults and juveniles are the most likely avenues for gene flow because they may remain in the marine coastal environment for several years before breeding (Cassirer et al. 1993), during which time they may stray from their parents' wintering sites. Lack of natal philopatry to molting and/or wintering sites may, in essence, nullify the effects of high adult fidelity to these same sites.

Although the specific reason for the absence of genetic structuring among these populations is not known, our results indicate that genetic variation was not lost as a result of the *Exxon Valdez* oil spill, and that the spill did not harm a genetically unique component of the species. This assumes, of course, that our genetic markers are representative of the overall genetic characteristics of the populations. Lack of recovery of Harlequin Ducks in oiled areas does not appear to be related to their population-genetic characteristics, but may instead be associated with unfavorable local environmental conditions. If movements of Harlequin Ducks within the area affected by the oil spill are as rare as the demographic data suggest, enhancement of populations via recolonization is likely to be a long process. Of course, even with immigration, full recovery will not occur until residual detrimental effects of the oil spill have disappeared. We cannot recommend the use of translocation to augment damaged populations at this time (even though genetic characteristics of the populations appear to be similar), given the paucity of information on the natural history of the species and the continuing negative environmental effects from the oil spill. Nevertheless, these genetic data do serve as an important baseline from which to assess the influence of future environmental perturbations on genetic characteristics.

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