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Spatial genetic structure and asymmetrical gene flow within the Pacific walrus

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Pacific walruses (*Odobenus rosmarus divergens*) occupying shelf waters of Pacific Arctic seas migrate during spring and summer from 3 breeding areas in the Bering Sea to form sexually segregated nonbreeding aggregations. We assessed genetic relationships among 2 putative breeding populations and 6 nonbreeding aggregations. Analyses of mitochondrial DNA (mtDNA) control region sequence data suggest that males are distinct among breeding populations ($\Phi_{ST} = 0.051$), and between the eastern Chukchi and other nonbreeding aggregations ($\Phi_{ST} = 0.336$–0.449). Nonbreeding female aggregations were genetically distinct across marker types (microsatellite $F_{ST} = 0.019$; mtDNA $\Phi_{ST} = 0.313$), as was eastern Chukchi and all other nonbreeding aggregations (microsatellite $F_{ST} = 0.019$–0.035; mtDNA $\Phi_{ST} = 0.386$–0.389). Gene flow estimates are asymmetrical from St. Lawrence Island into the southeastern Bering breeding population for both sexes. Partitioning of haplotype frequencies among breeding populations suggests that individuals exhibit some degree of philopatry, although weak. High levels of genetic differentiation among eastern Chukchi and all other nonbreeding aggregations, but considerably lower genetic differentiation between breeding populations, suggest that at least 1 genetically distinct breeding population remained unsampled. Limited genetic structure at microsatellite loci between assayed breeding areas can emerge from several processes, including male-mediated gene flow, or population admixture following a decrease in census size (i.e., due to commercial harvest during 1880–1950s) and subsequent recovery. Nevertheless, high levels of genetic diversity in the Pacific walrus, which withstood prolonged decreases in census numbers with little impact on neutral genetic diversity, may reflect resiliency in the face of past environmental challenges.

Key words: gene flow, genetic structure, *Odobenus rosmarus*, Pacific walrus

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Dispersal and philopatry can have profound effects on the spatial distribution of genetic variation (Avise 2000). Barriers to dispersal can produce genetic signatures of substructuring, even in species noted for their ability to move long distances (Swenson and Howard 2005; Weckworth et al. 2010). However, philopatry can produce a pattern of genetic structure even within species for which dispersal is not restricted by geographic barriers (Carreras et al. 2007; Sonsthagen et al. 2011). Dispersal propensity often differs between the sexes; in mammals, the classic pattern is female-biased philopatry and male-mediated dispersal (Greenwood 1980). Sex biases in dispersal can result in contrasting signatures of genetic structure between the genomes, because male dispersal tends to homogenize allelic frequencies in the nuclear genome and female philopatry promotes spatial variation in haplotypic frequencies in the mitochondrial DNA (mtDNA) genome (Avise 2000). Therefore, assaying molecular markers that differ in the mode of inheritance enables researchers to not only assess genetic structure within species but also gain valuable insight on philopatry and sex-biased dispersal (Avise 2000). Indeed, many studies have employed markers with different modes of inheritance and found genetic structure was weaker for nuclear loci than mitochondrial markers, consistent with a pattern of female philopatry and male-mediated dispersal (e.g., Andersen et al. 1998; Burg et al. 1999; Goldsworthy et al. 2000; Hoffman et al. 2006; Slade et al. 1998).

Marine mammals are often characterized as being highly vagile with distributions covering vast areas of ocean and limited barriers to dispersal. Many marine mammals in Arctic habitats are associated with sea ice at least during a portion of their annual cycle, including the narwhal (*Monodon monoceros*), beluga (*Delphinapterus leucas*), bowhead whale

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(*Balaena mysticetus*), ringed seal (*Pusa hispida*), bearded seal (*Erignathus barbatus*), walrus (*Odobenus rosmarus*), and polar bear (*Ursus maritimus*—Laidre et al. 2008). Current climate change predictions suggest that these species will likely encounter increased levels of anthropogenic and environmental stressors associated with the loss of Arctic sea ice (e.g., Amstrup et al. 2008; Jay et al. 2011). A greater understanding of historical structuring of diversity in ice-associated marine species is essential for predicting the future response and persistence of species to changing Arctic ecosystems.

Walruses are discontinuously distributed around the Arctic Basin. They typically occupy relatively shallow waters over continental shelves, where they feed on benthic invertebrates (Fay and Burns 1988; Gjertz et al. 2001). Two modern subspecies of walruses are generally recognized: the Atlantic walrus (*O. r. rosmarus*), which ranges from the central Canadian Arctic eastward to the Kara Sea (Reeves 1978); and the Pacific walrus (*O. r. divergens*), which ranges across the Bering and Chukchi seas (Fay 1982). Although geographically isolated and ecologically distinct, walruses from the Laptev Sea appear to be most closely related to Pacific walruses (Lindqvist et al. 2009). The Pacific walrus is the most abundant subspecies, comprising approximately 90% of walrus numbers worldwide (Fay 1985). Nearly the entire Pacific walrus subspecies occupies Arctic and sub-Arctic waters of the Bering and Chukchi seas and breeds in the Bering Sea. From the mid-19th to the mid-20th centuries, Pacific walrus numbers fluctuated wildly as a result of commercial harvest (Fay et al. 1989), reaching the lowest population size in the 1950s of between 50,000 and 100,000 individuals (Fay 1982; Fay et al. 1997). After that time, rigid restrictions placed on harvesting walruses in both Alaska and Russia have undoubtedly aided in their recovery (Fay 1982; Scribner et al. 1997). Pacific walruses now occupy a large proportion of their pre–19th century range (Scribner et al. 1997).

Annual seasonal migrations of up to 2,000 km demonstrate that Pacific walruses are capable of long-distance dispersal (Fay 1982; Jay and Hills 2005). During the January–March breeding season, walruses congregate in the Bering Sea pack ice in areas where open leads (fractures in the ice caused by wind drift or ocean currents), polynyas (enclosed areas of unfrozen water surrounded by ice), or thin ice allow access to water (Fay 1982; Fay et al. 1984). Although the specific location of winter breeding aggregations varies annually depending upon the distribution and extent of ice, breeding aggregations generally form southwest of St. Lawrence Island, south of Nunivak Island, and south of the Chukotka Peninsula in the Gulf of Anadyr (Fay 1982; Speckman et al. 2011; Fig. 1). Upon the retreat of seasonal sea ice, Pacific walruses migrate to sexually segregated summering (nonbreeding) aggregations in the Bering and Chukchi seas. Females and young exhibit a strong tendency to remain with the sea ice and migrate to the northeastern and western Chukchi Sea to haul out on ice floes. Most adult males remain in the Bering Sea or southwestern Chukchi Sea, where they forage from shore in the absence of sea ice, although some migrate with females to the northern and eastern Chukchi Sea. The level of fidelity to breeding and nonbreeding areas is unknown, although resightings of marked males at summer coastal haul-out areas suggest some site fidelity to nonbreeding areas (Jay and Hills 2005; Taggart 1987).

Studies investigating spatial structure of Pacific walruses based on genetic assays (Cronin et al. 1994; Scribner et al. 1997) and whole-tooth elemental assays (Jay et al. 2008) have produced contradictory signals; genetic studies failed to detect substructuring, whereas tooth elemental analyses identified at least 2 stocks. Cronin et al. (1994) compared mtDNA restriction fragment length polymorphism data between Atlantic walruses sampled from 3 sites around Greenland, and Pacific walruses sampled from 2 areas in the Chukchi Sea and found that each subspecies was reciprocally monophyletic. Furthermore, variations in haplotype frequencies among the sampling locations of Atlantic walruses were suggestive of genetic subdivisions; this was corroborated by more-targeted genetic studies of Atlantic walrus (Andersen and Born 2000; Andersen et al. 1998; Born et al. 2001). No evidence of genetic subdivision was observed between the 2 groups of Pacific walruses. Scribner et al. (1997) compared restriction fragment length polymorphism haplotypic frequencies among nonbreeding Pacific walruses harvested from 4 areas in the Bering Sea and found no significant differences among areas. However, frequency differences, although not significant, were observed between haplotypes uncovered in their study and those generated by Cronin et al. (1994), suggesting the possibility of differences between the nonbreeding (Chukchi Sea) and breeding (Bering Sea) groups. Examination of nuclear minisatellite data, however, failed to uncover evidence of geographic structuring within Pacific walruses (Scribner et al. 1997). Jay et al. (2008) compared elemental tooth concentrations among 2 breeding populations and 7 nonbreeding aggregations of Pacific walrus, which uncovered differing elemental tooth concentrations between breeding areas, providing evidence of separate stocks.

These contrasting signatures of spatial structure within the Pacific walrus warrant further investigation. Here, we employ fragment analyses of nuclear microsatellite loci, and nucleotide sequence data from the mtDNA control region, to examine spatial genetic structure of 2 breeding populations and 6 nonbreeding aggregates of Pacific walruses and assess levels and polarity of gene flow among breeding populations.

Materials and Methods

Sample collection.—Skin and muscle biopsies were collected from live or dead adult walruses between 1991 and 2009, or were obtained from the University of Alaska Museum (Appendix I). Sample collection followed guidelines of the American Society of Mammalogists (Sikes et al. 2011). Adverse weather, light, and ice conditions during the Pacific walrus breeding period (January–February) make it difficult to obtain samples from walruses during breeding. We obtained samples from presumably 2 separate breeding populations by collecting samples from individuals that were within, or very

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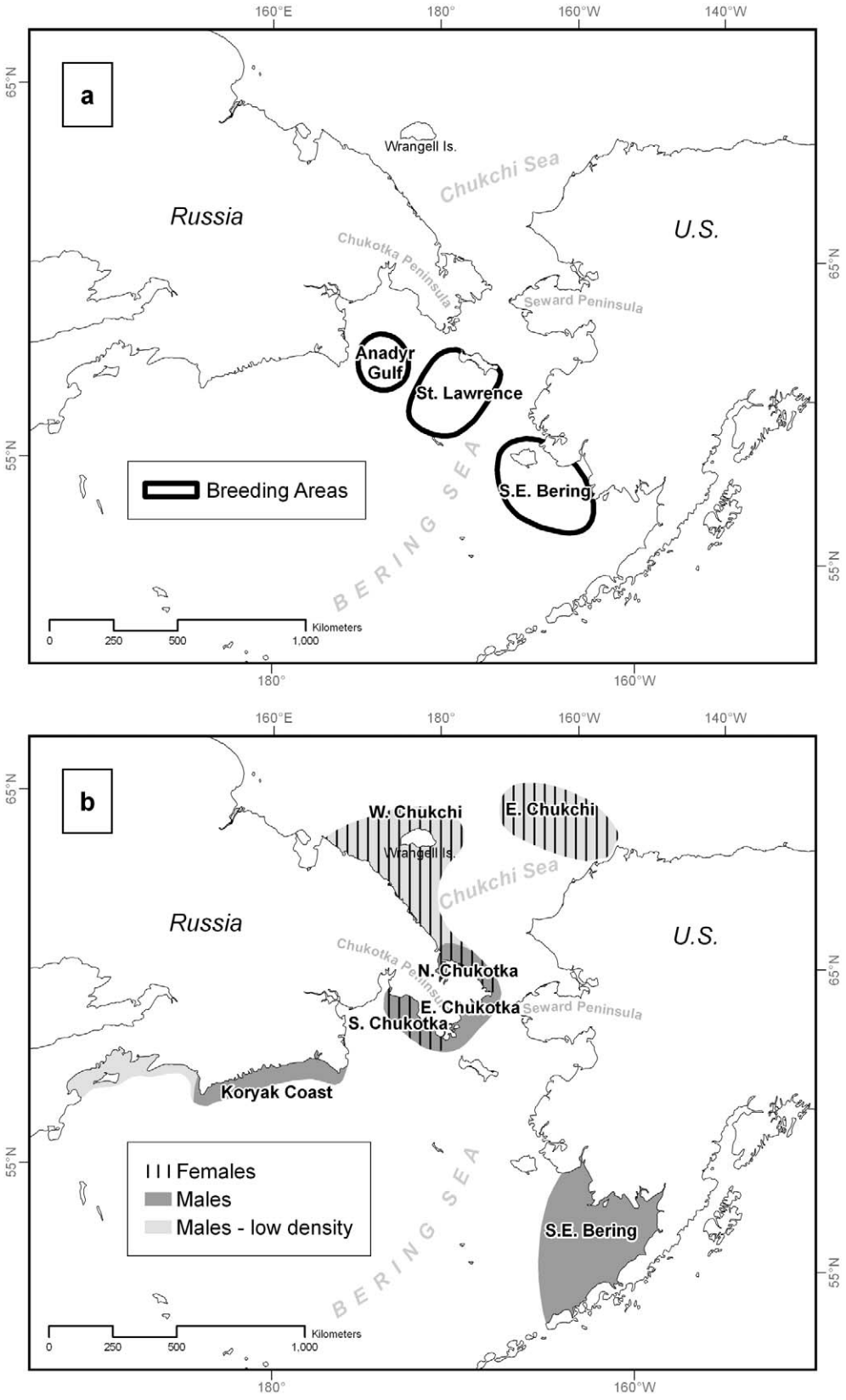


Fig. 1.—Areas of Pacific walrus occupation a) during breeding (January–February) and b) nonbreeding (August–October) periods (from Jay et al. 2008).

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Table 1.—Source of genetic samples assayed from breeding populations and nonbreeding aggregations of Pacific walruses in the Bering and Chukchi seas. E = eastern; N = northern; S = southern; SE = southeastern.

Group	Months	Source and year[a]	Female	Male	Total
Breeding					
SE Bering	March, April	ZS 1991, ST 2004, ST 2005	43	38	81
St. Lawrence	March	MA 2006, HL 2008, HL 2009	22	12	34
Nonbreeding					
E Chukchi	June, July	BA 2007	10	3	13
Bristol Bay	August, October	CP 1995, CP 1996, CP 1999, CP 2000	0	40	40
Koryak Coast	April	ZS 1991	0	36	36
E Chukotka	August, September, October	LO 2001, NC 2001, YA 2001	0	27	27
N Chukotka	August, September, October	IN 2001, EO 2001	6	20	26
S Chukotka	August, September	EN 2001, ME 2001	13	38	51
Total			94	214	308

[a] BA = Barrow—offshore; CP = Cape Pierce coastal haul-out mortalities; EN = Enmelen subsistence hunt; EO = Enurmino subsistence hunt; HL = USCG *Healy* cruise; IN = Inchoun subsistence hunt; LO = Lorino subsistence hunt; MA = R/V *Magadan* cruise; ME = Meechkyn Spit subsistence hunt; NC = Novo Chaplino subsistence hunt; ST = R/V *Stimson* cruise; YA = Yanrakynot subsistence hunt; ZS = *Zaslonovo* Russia–United States joint pinniped research cruise (Hills et al. 1991).

near, the southeastern (SE) Bering and St. Lawrence breeding populations immediately after the breeding period (Table 1; Fig. 1a). Samples were collected from nonbreeding individuals in areas of the eastern Chukchi Sea, Bristol Bay, Koryak Coast, eastern Chukotka, northern Chukotka, and southern Chukotka (Fig. 1b). Hereafter, we refer to individuals sampled from each of the breeding areas as "populations" and nonbreeding areas as "aggregations." We were unable to obtain samples from the Anadyr Gulf breeding population and the western Chukchi Sea nonbreeding aggregation. DNA was extracted from muscle samples according to Medrano et al. (1990), as modified in Sonsthagen et al. (2004). Extracted DNA was quantified using fluorometry and diluted to 50-ng/μl working solutions.

Genotyping and sequencing.—We determined the sex of all sampled individuals genetically using methods outlined in Fischbach et al. (2008). Genotype data were collected for 11 microsatellite loci (Orr2, Orr3, Orr7, Orr8, Orr11, Orr16, Orr21, Orr24 [Buchanan et al. 1998]; Hg6.1 [Allen et al. 1995]; 1GF-1 [Kirkpatrick 1992]; and SgPv9 [Goodman 1997]) from 308 walruses. Polymerase chain reaction amplifications were carried out in 5 multiplex reactions using procedures similar to those described in Cronin et al. (2009). Electrophoresis of polymerase chain reaction products, gel standardization, and sizing of microsatellite alleles follow Sonsthagen et al. (2004). Ten percent of the samples were genotyped in duplicate for the 11 microsatellite loci for quality control.

We amplified a 589- to 592-base pair (bp) segment of mtDNA comprising 106 bp of the cytochrome-*b* gene, 70 bp of the tRNA-thr, 65 bp of the tRNA-pro, and 348–351 bp of the hypervariable portion of the control region, with primer pairs L15774b (5′-GAATTGGAGGACAACCAGT-3′) and H00019 (Talbot and Shields 1996). Polymerase chain reaction amplifications, cycle-sequencing protocols, and postsequencing processing followed Sonsthagen et al. (2004). For quality control purposes, we extracted, amplified, and sequenced 10% of the samples in duplicate. No inconsistencies in nucleotide base calling were observed between replicates. Sequences are accessioned in GenBank (JQ964427–JQ964632).

Estimation of genetic diversity.—Given differences in the nonbreeding ecology of male and female walruses, we partitioned populations by sex for all analyses. Allelic richness, inbreeding coefficient (F_{IS}), observed and expected heterozygosities, Hardy–Weinberg equilibrium, and linkage disequilibrium were calculated for each microsatellite locus in GENEPOP 3.1 (Raymond and Rousset 1995) and FSTAT version 2.9.3 (Goudet 1995). Haplotype (h) and nucleotide (π) diversity for mtDNA data were estimated in ARLEQUIN 2.0 (Schneider et al. 2000). We performed tests of selective neutrality and historical fluctuations in population demography in ARLEQUIN using Fu's F_S (Fu 1997) and Tajima's D (Tajima 1989). Fu's F_S is sensitive to historical fluctuations in population size; therefore, critical significance values of 5% require a P-value below 0.02 for Fu's F_S (Fu 1997). An unrooted phylogenetic tree (1,000 bootstrap replicates) based on mtDNA control region was constructed in MEGA 4.0 (Tamura et al. 2007) using the minimum evolution optimality criterion to illustrate the relationship among Pacific walrus haplotypes. An unrooted phylogenetic network for mtDNA control region was constructed from haplotypes represented by breeding individuals in NETWORK 4.610 (Fluxus Technology, Clare, United Kingdom) using the reduced median method (Bandelt et al. 1995), to illustrate possible reticulations in the gene tree because of homoplasy.

Estimation of spatial genetic structure.—Spatial variance in allelic and haplotypic frequencies was calculated (F_{ST} and Φ_{ST}, respectively) in ARLEQUIN and population differentiation based on chi-square distributions of alleles and haplotypes in GENEPOP, adjusting for multiple comparisons using Bonferroni correction ($\alpha = 0.05$). Pairwise Φ_{ST} was calculated using a Hasegawa, Kishino, and Yano nucleotide substitution model (HKY—Hasegawa et al. 1985) as determined using Modeltest (Posada and Crandall 1998), and Akaike's information criterion (Akaike 1974). Hierarchical analyses of molecular variance were conducted in ARLEQUIN to examine whether genetic structure occurs between sexes or at the population level irrespective of sex.

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We further examined substructuring of walrus populations and aggregations using a Bayesian clustering approach (STRUCTURE 2.1—Pritchard et al. 2000) to infer the occurrence of population structure without a priori knowledge of putative populations and probabilistically assigning individuals to putative populations based on microsatellite allelic frequencies. Data were analyzed using an admixture model assuming correlated frequencies with 10,000 burn-in period, 500,000 Markov chain Monte Carlo iterations, and number of possible populations (K) ranging from 1 to 5; the analysis was repeated 5 times to ensure consistency across runs. The K that maximized the likelihood given the data was used to determine the most likely number of clusters (Pritchard et al. 2000).

Estimations of population demography.—Evidence for fluctuations in historical population demography was evaluated for the 11 microsatellite loci using BOTTLENECK 1.2.02 (Cornuet and Luikart 1996) and for the mtDNA sequence data using FLUCTUATE (Kuhner et al. 1995). BOTTLENECK compares the number of alleles and gene diversity at polymorphic loci under the infinite allele model (Maruyama and Fuerst 1985), stepwise mutation model (Ohta and Kimura 1973), and 2-phase model of mutation (Di Rienzo et al. 1994). Parameters for the 2-phase model of mutation were set at 79% stepwise mutation model with a variance of 9% (Garza and Williamson 2001; Piry et al. 1999), with 1,000 simulations performed for each population. Significance was assessed using a Wilcoxon sign-rank test, which determines if the average of standardized differences between observed and expected heterozygosities is significantly different from zero (Cornuet and Luikart 1996). Significant heterozygote deficiency values relative to the number of alleles indicate recent population growth, whereas heterozygote excess relative to the number of alleles indicates a recent population bottleneck (Cornuet and Luikart 1996). It is important to note that BOTTLENECK compares heterozygote deficiency and excess relative to genetic diversity, not to Hardy–Weinberg equilibrium expectation (Cornuet and Luikart 1996).

FLUCTUATE estimates a population growth parameter, g, incorporating coalescence theory (parameters: 10 short chains with 200 of 4,000 sampled trees, and 3 long chains with 20,000 of 400,000 sampled trees). Data were analyzed 5 times and parameters converged across runs. Positive values of g indicate population growth over time and negative values indicate population decline. Because this method incorporates aspects of genealogy, it is sensitive to changes in demography and may have an upward bias (Kuhner et al. 1998). Therefore, we used a conservative estimate of significance based on 99.9% confidence intervals for g to test for significant differences from zero (Waltari and Cook 2005).

Estimation of gene flow.—Estimates of gene flow (N_em or N_fm for nuclear or mtDNA, respectively) between breeding areas were calculated in MIGRATE 3.0.3 (Beerli and Felsenstein 1999, 2001). MIGRATE analyses included a full migration model (θ, the effective population size parameter [$4N_e\mu$], and M, the rate of gene flow [m/μ] were estimated individually from the data) that was compared to a restricted model (θ was averaged and M was symmetrical between populations) to test for asymmetry in gene flow. MIGRATE was run using maximum-likelihood search parameters; 10 short chains (1,000 used trees of 20,000 sampled), 5 long chains (10,000 used trees of 200,000 sampled), and 5 adaptively heated chains (start temperatures: 1, 1.5, 3, 6, and 12; swapping interval = 1). Models were run 3 times and parameters converged. We evaluated the alternative model for goodness-of-fit given the data using a log-likelihood ratio test (Beerli and Felsenstein 2001).

Results

Genetic diversity.—Multilocus genotypes were collected for 11 microsatellite loci and each individual had a unique genotype. The number of alleles per microsatellite locus ranged from 4 to 18, with an average of 8.7 alleles per locus. The average number of alleles and allelic richness per population or aggregation ranged from 3.0 to 7.2 and 3.0 to 5.4, respectively (Table 2). Private alleles (alleles only observed in a single population) were observed within the SE Bering Sea population and Bristol Bay and Koryak aggregations. Observed heterozygosity ranged from 59.8% to 65.9% for each population or aggregation, with an overall mean observed heterozygosity of 63.1% (Table 2). All loci were in Hardy–Weinberg equilibrium and linkage equilibrium.

We identified 206 mtDNA haplotypes among the 235 Pacific walruses sequenced among 2 breeding populations and 6 nonbreeding aggregations (Fig. 2A). Haplotypes were characterized by 99 polymorphic sites, in addition to 10 insertions–deletions (indels) in 2 regions (Supplemental Appendix S1), and all but 3 substitutions were transitions (2 C $\leftrightarrow$ A, 1 T $\leftrightarrow$ A; transitions : transversions [Ti/Tv] = 48.1). The high variation observed is largely attributable to the thymine repeat number at the 5′ end of the control region (Supplemental Appendix S1), which likely occurs through DNA replication slippage in cells (Levinson and Gutman 1987) and is considered a mutational hotspot (Stoneking 2000). Haplotypes ODRO10, 31, and 42 were the most widely distributed (observed in 3 populations or aggregations), although few individuals shared haplotypes (Fig. 2B). BLAST analyses (Altschul et al. 1990) of sequences accessioned in GenBank showed that 3 haplotypes recovered in the Pacific walrus (ODRO 80, 119, and 232; observed in nonbreeding aggregations) were genetically similar to Atlantic walrus haplotypes (97% genetic similarity) and were placed in their own clade with high bootstrap support (99%; Fig. 2A). Haplotype (h) and nucleotide diversity (π) ranged from 0.987 to 1.000 and 0.0104 to 0.0166, respectively (Table 2). No significantly positive values of Tajima's D or Fu's F_S were observed, confirming neutrality of the sequence data. However, a significantly negative Tajima's D was observed in SE Bering males and significantly negative Fu's F_S were observed in most populations (Table 2), suggestive of population growth.

Spatial genetic structure.—Spatial genetic structure (F_{ST}, Φ_{ST}, and χ^2) was observed among Pacific walrus populations and aggregations across marker types (Table 3). Breeding populations were differentiated for males based on mtDNA;

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TABLE 2.—Estimates of genetic diversity of Pacific walrus breeding populations and nonbreeding aggregations partitioned by sex (F = female; M = male), including average number of alleles with the number of private alleles in parentheses, allelic richness, observed (H_O) and expected (H_E) heterozygosities calculated from 11 microsatellite loci, haplotype (h) and nucleotide (π) diversity, Tajima's D, and Fu's F_S estimated from domain I of the mitochondrial DNA control region. Significant values are denoted by boldface type. E = eastern; N = northern; S = southern; SE = southeastern.

	Microsatellites				mtDNA				
	No. alleles	Allelic richness	H_O/H_E	n	h	π	Tajima's D	Fu's F_S	n
Breeding (winter)									
SE Bering (F)	6.7 (2)	5.2	62.6/62.7	43	0.998	0.0159	−1.395	**−24.438**	34
SE Bering (M)	6.9 (3)	5.4	63.4/62.4	38	0.997	0.0141	**−1.537**	**−19.780**	28
St. Lawrence (F)	6.2 (—)	5.4	64.9/64.5	22	1.000	0.0120	−1.248	**−11.223**	17
St. Lawrence (M)	5.1 (—)	5.1	65.9/65.0	12	1.000	0.0155	−0.539	−3.501	10
Nonbreeding (summer)									
E Chukchi (F)	5.4 (—)	3.2	64.6/64.0	10	1.000	0.0166	−0.735	−3.316	10
E Chukchi (M)	3.0 (—)	3.0	60.6/61.2	3	1.000	0.0104	0.000	0.703	3
Bristol Bay (M)	7.2 (4)	3.2	59.8/65.2	40	1.000	0.0159	−1.235	**−11.209**	19
Koryak (M)	6.5 (1)	3.0	63.9/62.2	36	0.996	0.0114	−0.988	**−14.531**	22
E Chukotka (M)	6.0 (—)	3.1	60.5/62.1	27	0.996	0.0153	−1.078	**−13.895**	24
N Chukotka (F)	4.2 (—)	3.0	62.1/59.6	6	1.000	0.0136	−0.760	−1.181	6
N Chukotka (M)	6.0 (—)	3.2	64.6/64.9	20	1.000	0.0128	−0.797	**−14.219**	20
S Chukotka (F)	5.0 (—)	3.0	60.1/63.2	13	0.987	0.0156	−0.878	−3.541	13
S Chukotka (M)	6.2 (1)	3.1	65.1/62.9	38	0.993	0.0138	−1.438	**−18.328**	29

however, significant comparisons were not observed between female breeding populations at either microsatellite or mtDNA loci (Table 3). Overall tests of spatial genetic structure indicated differentiation among non-breeding female aggregations based on microsatellite and mtDNA loci, and differentiation among nonbreeding male aggregations based on mtDNA (Table 3). Specifically, the eastern (E) Chukchi nonbreeding female aggregation was significantly differentiated from all other nonbreeding female aggregations at both marker types, and the E Chukchi nonbreeding male aggregation was differentiated from all other nonbreeding male aggregations, except the Bristol Bay aggregation, based on mtDNA (Table 3). Moreover, the SE Bering breeding female population was differentiated from the E Chukchi nonbreeding female aggregation based on microsatellite data (pairwise $F_{ST} = 0.019$, $P < 0.001$, data not shown); both breeding female populations also were differentiated from the E Chukchi nonbreeding female aggregation based on mtDNA (pairwise $\Phi_{ST} = 0.382$–0.444, $P < 0.001$, data not shown). Comparisons among E Chukchi and other populations should be interpreted with caution because the sample size is low ($n \leq 10$), albeit type I error is not sample-size dependent (Chittenden 2002). All comparisons of the distribution of haplotypes based on the chi-square statistic were significant; however, only the overall estimate for the female nonbreeding aggregation was significant (Table 3). Overall and interpopulation comparisons for the remaining Pacific walrus winter populations and summer aggregations were not significant (Table 3). STRUCTURE did not uncover genetic partitioning within Pacific walruses; the likelihood was maximized when $K = 1$ (Supplemental Fig. S1).

Hierarchical analyses of variance revealed genetic partitioning between female and male nonbreeding aggregates ($F_{ST} = 0.234$, $P < 0.001$) and among aggregates irrespective of sex ($F_{SC} = 0.010$, $F_{ST} = 0.114$), based on mtDNA when a nucleotide substitution model was applied to the data. The among-group variance (F_{CT}) did not significantly differ for any of the groupings indicating that sex or populations or aggregations are not significant partitions in haplotypic frequencies for Pacific walruses. No significant variance measures (F_{CT}, F_{ST}, or F_{SC}) were observed based on the microsatellite data (data not shown).

Fluctuations in population demography.—Evidence for contemporary and historical fluctuations in population demography was detected for breeding populations based on mtDNA. Breeding populations showed evidence of recent population stability under the 3 mutation models for microsatellite data. All populations exhibited a historical signal of population growth based on mtDNA sequence data (data not shown).

Gene flow.—Gene flow, as estimated by microsatellite data, was moderate and did not show strong biases in the directionality of gene flow between population pairs (Table 4). The number of migrants per generation (N_em) ranged from 2.3 to 7.0 and θ ranged from 0.955 to 1.264 (Table 4). Gene flow was greater into SE Bering from St. Lawrence than vice versa for the female, male, and pooled population estimates, although the signal for the females was weak (Table 4). Stronger asymmetry in gene flow estimates were observed based on mtDNA sequence data, with the number of female migrants per generation (N_fm) ranging from 0.0 to 72.0 with θ from 0.011 to 0.095 (Table 4). As with the microsatellite analyses, walruses exhibited evolutionary dispersal from St. Lawrence into SE Bering across mtDNA analyses (Table 4). Across all population groupings and marker types, full models (M and θ allowed to vary independently) had significantly higher ln-likelihoods than the restricted model (M and θ symmetrical), indicating asymmetric dispersal among populations (Table 4).

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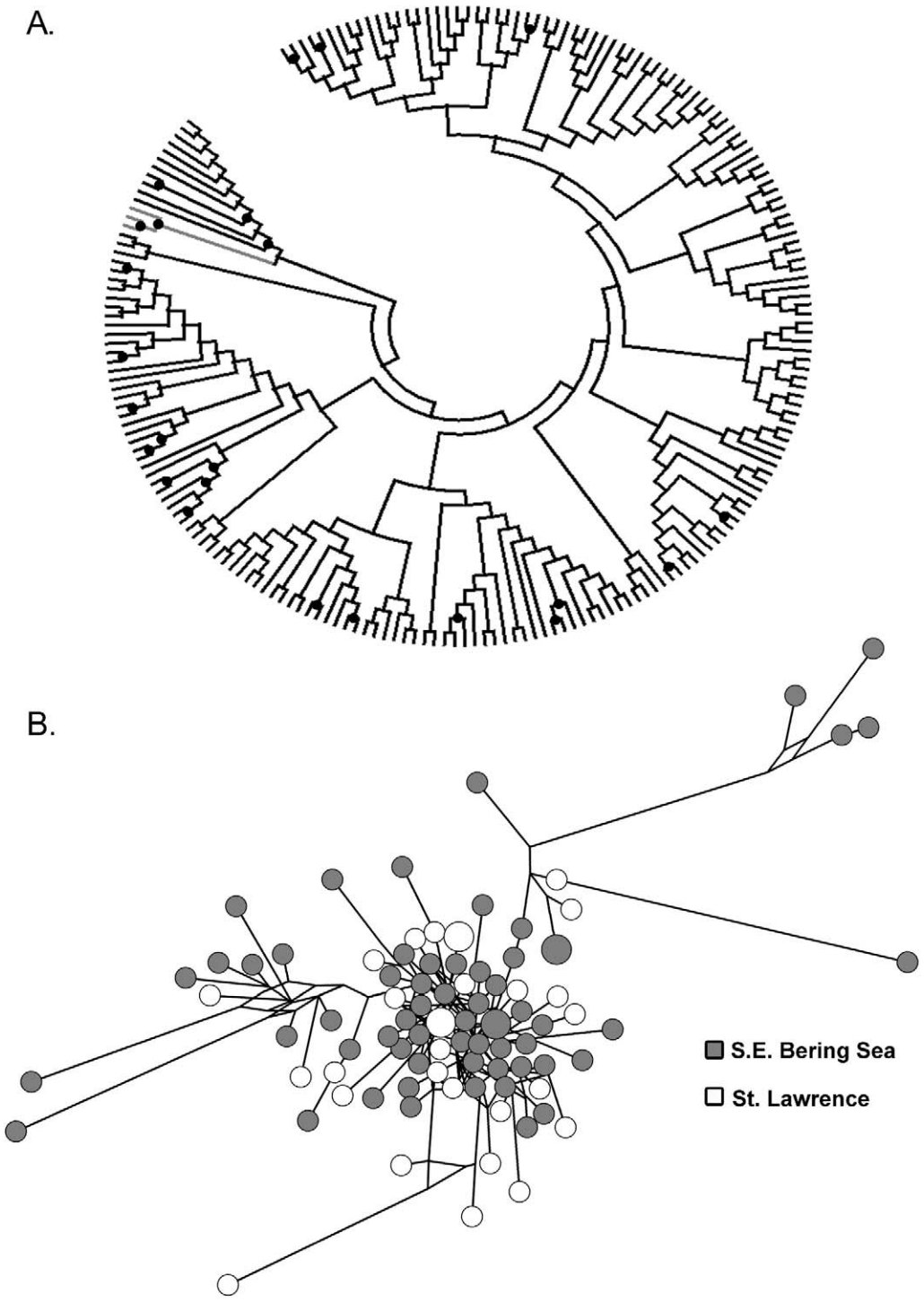


Fig. 2.—A) Minimum evolution phylogenetic tree of mitochondrial DNA control region haplotypes ($n = 206$) assayed from Pacific walruses. Pacific walrus haplotypes that are genetically similar to those of the Atlantic walrus are shown in gray. Nodes with $\geq$50% bootstrap support are denoted with black circles. B) A parsimony network of mitochondrial DNA control region haplotypes ($n = 87$) assayed from breeding populations only, with the size of the node corresponding to the frequency of each haplotype.

Discussion

Genetic diversity.—We found very high levels of mtDNA haplotype diversity within Pacific walruses (206 haplotypes among 235 individuals). Previous studies of Pacific walruses observed 6 and 9 unique haplotypes, but used conservative markers and methods for estimating mtDNA diversity (mtDNA restriction fragment length polymorphism—Cronin et al. 1994; Scribner et al. 1997), respectively. In addition, haplotype diversity reported by Cronin et al. (1994) indicated that Pacific walruses are predominately represented by a single haplotype,

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Table 3.—Pairwise and overall values of F_{ST} calculated from 11 microsatellite loci, and Φ_{ST} calculated from domain I of the control region of the mitochondrial DNA (mtDNA), for breeding populations and summer aggregations of Pacific walruses partitioned by sex (F = female; M = male). Results of chi-square analysis of the distribution of alleles and haplotypes also are given. Significant values are in boldface type. E = eastern; N = northern; S = southern; SE = southeastern.

	Microsatellite		mtDNA	
	F_{ST}	χ^2	Φ_{ST}	χ^2
Breeding (winter)				
SE Bering (F)				
–St. Lawrence (F)	−0.001	28.148	0.022	∞
Overall (F)	−0.001	28.493	0.022	∞
SE Bering (M)				
–St. Lawrence (M)	−0.015	18.527	**0.051**	∞
Overall (M)	−0.010	13.945	**0.051**	∞
Nonbreeding (summer)				
E Chukchi (F)				
–N Chukotka (F)	**0.035**	28.876	**0.389**	∞
–S Chukotka (F)	**0.019**	34.412	**0.386**	∞
N Chukotka (F)				
–S Chukotka (F)	0.007	26.427	−0.049	∞
Overall (F)	**0.019**	**34.863**	**0.313**	∞
E Chukchi (M)				
–Bristol Bay (M)	0.015	15.617	0.288	**14.2**
–Koryak (M)	0.003	22.200	**0.449**	**16.5**
–E Chukotka (M)	0.002	19.907	**0.336**	**17.6**
–N Chukotka (M)	0.010	20.314	**0.439**	**20.3**
–S Chukotka (M)	0.002	23.900	**0.336**	**20.3**
Bristol Bay (M)				
–Koryak (M)	0.003	29.208	0.001	∞
–E Chukotka (M)	−0.001	21.312	−0.020	∞
–N Chukotka (M)	−0.009	9.885	0.013	∞
–S Chukotka (M)	−0.004	21.400	0.000	∞
Koryak (M)				
–E Chukotka (M)	0.002	23.060	−0.001	∞
–N Chukotka (M)	−0.005	12.375	−0.011	∞
–S Chukotka (M)	0.002	25.736	0.004	∞
E Chukotka (M)				
–N Chukotka (M)	−0.003	20.875	0.006	∞
–S Chukotka (M)	−0.003	22.164	0.013	∞
N Chukotka (M)				
–S Chukotka (M)	−0.003	21.563	0.000	∞
Overall (M)	−0.001	22.653	**0.034**	∞

P1 ($n = 51/57$), with the remaining haplotypes only observed once or twice. In contrast, we observed few haplotypes shared among individuals. This difference may be largely attributable to marker selection and method of assessing haplotype variation. Cronin et al. (1994) assayed a more conserved portion of the mtDNA genome (reduced nicotinamide adenine dinucleotide phosphate) using restriction fragment length polymorphism techniques, whereas we assayed the mtDNA hypervariable region using direct sequencing. High variability also was reported by Lindqvist et al. (2009); 13 unique haplotypes were identified among 16 individuals by direct sequencing of the mtDNA control region. The high number of haplotypes observed for Pacific walruses, we believe, is noteworthy. Atlantic walruses were assayed for the same portion of mtDNA; 17 unique haplotypes were reported among 27 individuals (63%—Lindqvist et al. 2009). Levels of haplotype diversity observed here (87%) are higher than observed in the Atlantic population. This finding is consistent with the larger census population size, and presumably larger effective population size, of Pacific walrus relative to Atlantic walruses.

High levels of genetic diversity observed here are especially noteworthy, because Pacific walruses experienced what was considered a prolonged population bottleneck; nevertheless their genetic diversity does not appear to have been noticeably impacted. Historical estimates of the census population size are not available but researchers speculate that the Pacific walrus had to number at least 200,000 individuals to have withstood the high levels of harvest observed (Fay 1982). Despite a reduction in the census number of individuals, between 50% and 75% of the historical population, and the duration of exploitation (1880–1950s), we did not detect a genetic signature of a population decline in either the microsatellite allelic or mtDNA haplotypic diversity. Luikart (1997) suggests the detection of a bottleneck using the methods employed herein requires that the effective population size would have to drop to fewer than 25 individuals (for a severe bottleneck), that the bottleneck last longer than 2 generations, and that the bottleneck occur at least 2 generations past. The prolonged population bottleneck likely did last longer than 2 generations, but was apparently not severe enough to reduce the effective population size to below 25 individuals.

Similar to Pacific walruses, several other marine mammal populations have experienced large population declines that were not accompanied by detectable declines in levels of neutral genetic diversity (see O'Corry-Crowe 2008: tables 1 and 2). The ability of many marine mammals to withstand dramatic reductions in census population size, while maintaining neutral genetic variation, may be partly due to their relatively large historic population sizes, and consequently large effective population sizes. Large effective population sizes, coupled with high vagility, have likely provided many marine mammals with a buffer to adverse genetic effects (i.e., loss of genetic diversity) associated with prolonged population bottlenecks. In instances where populations became isolated, relatively lower levels of neutral genetic diversity were observed (e.g., St. Lawrence beluga whales—O'Corry-Crowe 2008). Therefore, connectivity among populations is likely an important component for the maintenance of genetic diversity and the ability of marine mammals to withstand periods of reduced census population size.

Population genetic structure.—Males were genetically differentiated between the breeding populations only at mtDNA, and at a low level. However, females were not differentiated among breeding populations, and given that mtDNA is maternally inherited, this suggests that Pacific walrus females are not philopatric. Based on the genetic signature from males, however, we can infer female gene flow from the previous generation, which suggests that at least some females were philopatric (although this is likely not an

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TABLE 4.—Full-model gene-flow matrix (allowing all parameters to vary independently) calculated from 11 microsatellite loci and mitochondrial DNA (mtDNA) control region for Pacific walrus breeding populations along with an evaluation of alternative models of gene flow using a log-likelihood (LnL) ratio test. Parameter estimates for number of migrants per generation (nuclear DNA N_em; mtDNA N_fm) and θ (nuclear DNA $N_e\mu$; mtDNA $N_f\mu$) are listed for each population pair. The 95% confidence intervals are given in parentheses. SE = southeastern.

	SE Bering into St. Lawrence Island		St. Lawrence Island into SE Bering				
Marker	θ	N_em or N_fm	θ	N_em or N_fm	Full (LnL)	Restricted (LnL)	*P*
Microsatellites							
Breeding females	0.961 (0.890–1.048)	5.1 (4.2–6.3)	1.037 (0.955–1.130)	6.5 (5.4–7.8)	−149.8	−155.5	0.022
Breeding males	0.955 (0.878–1.038)	2.3 (1.7–3.2)	1.143 (1.018–1.305)	5.6 (4.5–6.8)	−21.8	−61.5	<0.001
Pooled	1.012 (0.943–1.077)	4.9 (4.0–6.0)	1.264 (1.162–1.369)	7.0 (5.9–8.2)	−71.5	−94.6	<0.001
mtDNA							
Breeding females	0.022 (0.015–0.033)	0.0 (0.0–0.6)	0.016 (0.012–0.022)	13.9 (6.2–31.6)	8.6	−9.6	<0.001
Breeding males	0.023 (0.015–0.036)	0.0 (0.0–1.6)	0.011 (0.008–0.016)	10.4 (4.3–25.2)	25.6	16.2	<0.001
Pooled	0.095 (0.065–0.144)	1.3 (0.4–3.0)	0.034 (0.028–0.041)	72.0 (38.7–138.1)	38.2	−17.9	<0.001

evolutionarily long-term strategy for Pacific walruses). The lack of differentiation among females is consistent with other genetic studies of Pacific walruses (Cronin et al. 1994; Scribner et al. 1997); however, it differs from tooth elemental analyses. Three groups were identified, 2 of which corresponded to the 2 sampled populations of Pacific walruses and were characterized with a high degree of separation (classification error of 0.07 for both groups) based on the accumulation of elements in whole teeth (Jay et al. 2008). Elemental assays were conducted on adult individuals (average age 20 years, range 6–36 years) and premolars are replaced at age 1 year in walruses (Fay 1982). Therefore, elemental signatures reflect an individual's breeding and summering environment throughout its life, and do not include its natal site nor does it carry a signature from previous generations. A genetic signature of structure at mtDNA is only expected if a female is philopatric and would not be observed if females exhibit natal dispersal between breeding populations but still exhibit breeding site fidelity (Avise 2000). Although the elemental signatures in teeth are suggestive of some adult female breeding and summer site fidelity, a sufficient number of young female walruses appear to disperse among breeding populations to result in a genetic signature of panmixia at mtDNA over time.

Genetic structure observed among the nonbreeding E Chukchi aggregation and all sampled breeding populations and nonbreeding aggregations (except Bristol Bay), although with low sample size, is supportive of inferences that Pacific walruses are substructured (Cronin et al. 1994; Scribner et al. 1997). Previous genetic assessments observed haplotypic frequency differences, albeit not significant, between summer (western and eastern Chukchi Sea) and winter (Bering Sea) groups (Scribner et al. 1997). Interestingly, among the 3 groups identified by elemental signatures was an "other" group that may represent an unsampled population (Jay et al. 2008). Although breeding populations located in the SE Bering and St. Lawrence areas are not genetically differentiated from each other, congruence in genetic and elemental tooth signatures of substructure suggest that at least 1 genetically unique breeding population may exist. Samples from the only other known breeding population, the Gulf of Anadyr, are needed to test this hypothesis.

Variances in allelic and haplotypic frequencies between breeding populations of Pacific walruses are 10-fold lower than estimates among Atlantic walrus populations (pairwise mtDNA restriction fragment length polymorphism $\Phi_{ST} = 0.000–0.904$; pairwise microsatellite $F_{ST} = 0.024–0.058$ [Born et al. 2001]). Differences in the degree of genetic substructure within Pacific and Atlantic walruses suggest that they may differ in aspects of their breeding biology. Specifically, male and female Atlantic walruses may exhibit higher levels of philopatry than Pacific walruses. However, lower levels of genetic differentiation may be due to differences in the effective population size of Atlantic and Pacific walruses. Pacific walruses comprise approximately 90% of the world's population of walruses with approximately 9% being represented by Atlantic walruses (Fay 1985). Assuming similar life-history characteristics and breeding biology between the 2 subspecies, it would take approximately 10 times longer for genetic signatures of structure to be observed in Pacific walruses than in Atlantic walruses, following genetic admixture. Therefore, there may have been insufficient passage of time for the accumulation of genetic differences between Pacific walrus breeding populations, given recent perturbations in their population size and longevity of individuals.

Alternatively, differences in the degree of genetic structure between Pacific walrus populations may be attributable to the geographic proximity of breeding areas. SE Bering Sea and St. Lawrence Island sites are approximately 500 km apart. In years of minimal sea ice, the range of the 2 breeding areas is nearly continuous (see Fay et al. 1984:figure 18), likely facilitating dispersal between populations, and this is consistent with observed gene flow estimates between these 2 breeding populations. In contrast, geographic distances among Atlantic walrus populations sampled range from 900 to 2,800 km (Born et al. 2001). Among Atlantic walrus populations within relatively closer geographic proximity (eastern Greenland, Svalbard, and Franz Josef Island, <1,000 km apart), levels of genetic differentiation were comparable to those observed between Pacific walruses studied here (microsatellite $F_{ST} =$

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0.024–0.040 [Born et al. 2001]). Therefore, differences in the degree of substructuring within Pacific and Atlantic walruses may be more attributable to the proximity of breeding areas than differences in breeding biology between the 2 subspecies. Data from more geographically close populations of Atlantic walruses are needed to gain further insight into factors (i.e., differences in breeding biology, effective population size, or proximity of breeding populations) influencing the spatial distribution of allelic and haplotypic variation.

Implications for climate change.—When environmental conditions remain within species-specific physiological tolerances, the species' response to a changing Arctic climate will depend in part on various aspects of its biology, such as level of fidelity to breeding and summering areas, as well as plasticity in habitat and foraging requirements. Within historical times, Pacific walruses have apparently withstood a prolonged decrease in population numbers, with little impact on their neutral genetic diversity. Some researchers hypothesize that a reduction in sea ice may facilitate gene flow between Pacific and Atlantic walruses through the Canadian High Arctic (O'Corry-Crowe 2008). Increased gene flow among the walrus populations may reduce the genetic distinctiveness of Pacific and Atlantic walruses and potentially disrupt coadapted gene complexes; however, increased gene flow would likely increase genetic diversity across the species' broader distribution. This may further enable the species to withstand reductions to its census population size. However, it is also possible that secondary contact between previously isolated populations may facilitate the transmission of pathogens and parasites to naïve populations. Of particular interest is the potential for associated pathogens to shift their distribution or host affiliation, or increase in virulence as a consequence of climate change, or both (Hoberg and Brooks 2008; Jenkins et al. 2006; Kutz et al. 2005). Examination of genes associated with immune response may help elucidate whether there has been differential evolutionarily relevant and adaptive processes operating within and between the 2 walrus subspecies.

As for other high-latitude taxa (Hope et al. 2011), understanding the responses of walruses to climatic cycles of the Pleistocene may provide insight to how this species will respond to ongoing changes in Arctic climate. Community structure in Arctic ecosystems is temporally dynamic, but an initial understanding of which taxa have been historically associated with certain habitats may be uncovered by comparing historical demographies among species. Wide ranging organisms such as *O. rosmarus* highlight the inherent heterogeneity within Holarctic species, and may help to illustrate that changes in the climate can produce distinctive regional lineages and help predict regional response. Researchers have speculated that species able to shift ranges in response to rapidly changing climate conditions may have an advantage over persistent habitat specialists, and this process can be better understood by examining geographic regions where these 2 types come into secondary contact (Hope et al. 2011). In the case of walruses, then, at least some attention should be given to the High Arctic Canadian waters during the upcoming decades.

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Supporting Information

Appendix S1.—List of mitochondrial DNA control region haplotypes. Found at DOI:http://dx.doi.org/10.1644/11-MAMM-A-344.1.S1

Appendix Fig. S1. Found at DOI:http://dx.doi.org/10.1644/11-MAMM-A-344.1.S2

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Appendix I

List of University of Alaska Museum catalogue numbers (University of Alaska Museum Mammals) along with collection locality and date for Pacific walrus (*Odobenus rosmarus*) voucher specimens assayed for this study.

Catalogue no.	Collection locality	Collection date	Tissue type
53958	North Pacific Ocean, Bering Sea, southeast of Nunivak Island	30 March 1991	Muscle
53963	North Pacific Ocean, Bering Sea, southeast of Nunivak Island	30 March 1991	Muscle
53986	North Pacific Ocean, Bering Sea, southeast of Nunivak Island	30 March 1991	Muscle
53995	North Pacific Ocean, Bering Sea, southeast of Nunivak Island	31 March 1991	Muscle
54008	North Pacific Ocean, Bering Sea, southeast of Nunivak Island	31 March 1991	Muscle
54032	North Pacific Ocean, Bering Sea, south of Cape Navarin to Cape Olyutorsky	15 April 1991	Muscle
54050	North Pacific Ocean, Bering Sea, south of Cape Navarin to Cape Olyutorsky	21 April 1991	Muscle
54052	North Pacific Ocean, Bering Sea, south of Cape Navarin to Cape Olyutorsky	22 April 1991	Muscle
54075	North Pacific Ocean, Bering Sea, south of Cape Navarin to Cape Olyutorsky	15 April 1991	Muscle
54106	North Pacific Ocean, Bering Sea, south of Cape Navarin to Cape Olyutorsky	12 April 1991	Muscle
54108	North Pacific Ocean, Bering Sea, south of Cape Navarin to Cape Olyutorsky	12 April 1991	Muscle
54112	North Pacific Ocean, Bering Sea, south of Cape Navarin to Cape Olyutorsky	12 April 1991	Muscle
54119	North Pacific Ocean, Bering Sea, south of Cape Navarin to Cape Olyutorsky	25 April 1991	Muscle
54121	North Pacific Ocean, Bering Sea, south of Cape Navarin to Cape Olyutorsky	25 April 1991	Muscle
54126	North Pacific Ocean, Bering Sea, south of Cape Navarin to Cape Olyutorsky	25 April 1991	Muscle
54135	North Pacific Ocean, Bering Sea, south of Cape Navarin to Cape Olyutorsky	12 April 1991	Muscle
54155	North Pacific Ocean, Bering Sea, south of Cape Navarin to Cape Olyutorsky	14 April 1991	Muscle
54160	North Pacific Ocean, Bering Sea, south of Cape Navarin to Cape Olyutorsky	14 April 1991	Muscle
54163	North Pacific Ocean, Bering Sea, south of Cape Navarin to Cape Olyutorsky	12 April 1991	Muscle
54164	North Pacific Ocean, Bering Sea, south of Cape Navarin to Cape Olyutorsky	14 April 1991	Muscle
54180	North Pacific Ocean, Bering Sea, south of Cape Navarin to Cape Olyutorsky	14 April 1991	Muscle
54187	North Pacific Ocean, Bering Sea, south of Cape Navarin to Cape Olyutorsky	12 April 1991	Muscle
54208	North Pacific Ocean, Bering Sea, south of Cape Navarin to Cape Olyutorsky	25 April 1991	Muscle
54221	North Pacific Ocean, Bering Sea, south of Cape Navarin to Cape Olyutorsky	25 April 1991	Muscle
54243	North Pacific Ocean, Bering Sea, south of Cape Navarin to Cape Olyutorsky	15 April 1991	Muscle
54246	North Pacific Ocean, Bering Sea, southeast of Nunivak Island	5 April 1991	Muscle
54247	North Pacific Ocean, Bering Sea, southeast of Nunivak Island	5 April 1991	Muscle
54254	North Pacific Ocean, Bering Sea, south of Cape Navarin to Cape Olyutorsky	12 April 1991	Muscle
54304	North Pacific Ocean, Bering Sea, south of Cape Navarin to Cape Olyutorsky	14 April 1991	Muscle
54305	North Pacific Ocean, Bering Sea, south of Cape Navarin to Cape Olyutorsky	12 April 1991	Muscle
54336	North Pacific Ocean, Bering Sea, south of Cape Navarin to Cape Olyutorsky	12 April 1991	Muscle
54337	North Pacific Ocean, Bering Sea, south of Cape Navarin to Cape Olyutorsky	14 April 1991	Muscle
54365	North Pacific Ocean, Bering Sea, south of Cape Navarin to Cape Olyutorsky	25 April 1991	Muscle
54380	North Pacific Ocean, Bering Sea, south of Cape Navarin to Cape Olyutorsky	22 April 1991	Muscle
54383	North Pacific Ocean, Bering Sea, south of Cape Navarin to Cape Olyutorsky	20 April 1991	Muscle
54385	North Pacific Ocean, Bering Sea, south of Cape Navarin to Cape Olyutorsky	20 April 1991	Muscle
54396	North Pacific Ocean, Bering Sea, south of Cape Navarin to Cape Olyutorsky	14 April 1991	Muscle
54398	North Pacific Ocean, Bering Sea, south of Cape Navarin to Cape Olyutorsky	14 April 1991	Muscle
54399	North Pacific Ocean, Bering Sea, south of Cape Navarin to Cape Olyutorsky	14 April 1991	Muscle
54401	North Pacific Ocean, Bering Sea, south of Cape Navarin to Cape Olyutorsky	14 April 1991	Muscle
54404	North Pacific Ocean, Bering Sea, south of Cape Navarin to Cape Olyutorsky	14 April 1991	Muscle
54405	North Pacific Ocean, Bering Sea, south of Cape Navarin to Cape Olyutorsky	14 April 1991	Muscle
54408	North Pacific Ocean, Bering Sea, south of Cape Navarin to Cape Olyutorsky	25 April 1991	Muscle

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