POPULATION GENETIC STRUCTURE AND PHYLOGEOGRAPHY OF

COMMON EIDERS (*SOMATERIA MOLLISSIMA)*

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ABSTRACT

Population genetic structure of Common Eiders (*Somateria mollissima*) was assessed at increasing spatial scales (microgeographic [<1 km] to throughout their circumpolar distribution), using molecular markers with varying modes of inheritance and rates of evolution. Population genetic subdivision was observed at all spatial scales; however, the degree of structure differed among marker types. Relatively lower levels of spatial genetic structuring were observed at bi-parentally inherited markers, and high levels of structuring were observed at a maternally inherited locus. Differences in the degree of subdivision between marker types may be attributable to the breeding biology of eiders. Pair formation occurs on the wintering grounds; where several populations of eiders interact. Female eiders exhibit high natal and breeding philopatry; whereas, males accompany females back to breeding sites and may disperse long distances between breeding seasons. Significant structuring observed at microgeographic scales indicates that eiders may nest in kin groups. Though the underlying mechanism enabling female eiders to discriminate kin is unknown, waterfowl may achieve kin recognition indirectly through association during brood rearing. Genetic signatures of philopatry among Common Eider populations do vary among Alaskan populations. No genetic structuring at mitochondrial DNA (mtDNA) was observed among islands in the Beaufort Sea in close geographic proximity (1–49 km apart). However, high structuring was observed among island groups, suggesting females are philopatric to island groups rather than individual islands. In contrast, moderate levels of genetic partitioning for mtDNA were observed among Yukon-Kuskokwim Delta (YKD) colonies (9–63 km apart); therefore,

female eiders may be philopatric to individual colonies. MtDNA haplotypes representing Aleutian and YKD populations are more genetically similar to Canadian and Scandinavian populations than northern Alaska populations, indicating that southern Alaskan populations were colonized from central Canadian refugia. Data indicate that the North Slope may have been a refugium for eiders but contributed little to the post-glacial colonization of North America and Scandinavia. Finally, philopatry and winter site fidelity observed in waterfowl have predictable effects on population genetic structure. Researchers characterizing populations using molecular techniques could under- or over-estimate the degree of population genetic differentiation if estimates are based on a single marker type.

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INTRODUCTION

Microgeographic population structure is greatly influenced by natal and breeding dispersal. Here we define natal dispersal as the distance between an individual's natal site and the site of its first breeding attempt, and breeding dispersal as the distance an individual travels between each subsequent breeding attempt (Greenwood 1980). For many species, assessing natal and breeding dispersal is difficult, especially for mobile organisms that may travel long distances prior to first and between subsequent breeding attempts. Advances in molecular techniques have made it possible to assess genetic structure of natural populations and evaluate the roles of contemporary and past dispersal events among areas (Newton 2003). Birds are of particular interest because most species that breed in arctic or temperate regions migrate to other areas during the nonbreeding season, and thus may show less geographic structure than other vertebrate groups (Avise 1996). Lack of population structure has been attributed to environmental variability of arctic and temperate regions, which increases dispersal and migratory behavior in birds, and can homogenize genetic diversity (Winker et al. 2000). Conversely, many birds exhibit high natal and breeding site fidelity (e.g., natal and breeding philopatry), which is expected to restrict gene flow among neighboring populations (Avise 1996), leading to population subdivision.

Differences in the degree of philopatry also may exist between males and females. The most common pattern in birds is for females to disperse farther between natal and breeding sites than males (Greenwood 1980). However, female waterfowl typically show greater natal and breeding philopatry than males (Rohwer and Anderson 1988). Males

and females typically pair on the wintering grounds, and the male accompanies the female back to her natal area. Because ducks from different breeding areas frequently share a common wintering ground, males may disperse over long distances. As a result, pairbonds do not remain stable across breeding seasons, individual males breed in widely disparate locations from year to year (Anderson et al. 1992). This behavior is expected to cause genetic mixing among individuals from multiple breeding areas, which may explain why many species of ducks are morphologically monotypic across the Palearctic (Newton 2003).

Genetic consequences of philopatric behavior have been demonstrated in several species (e.g., Tiedemann et al. 1999, Scribner et al. 2001, Avise 2004, Pearce et al. 2005). Dispersal homogenizes allelic frequencies, whereas natal and breeding philopatry can lead to patterns of spatial genetic subdivision among populations. Unfortunately, dispersal is difficult to study as it often requires long-term demographic studies (Koenig et al. 1996), and, in species that are highly mobile, estimates may be more difficult to obtain as individuals may disperse out of the study site. Genetic studies often have been implemented to assess inter-generational dispersal (gene flow) among populations. However, most studies that have characterized population genetic structure have focused on assessing allelic frequency differences within and among populations, and often are not designed to detect local groupings of genetically related individuals within populations, which would be expected in species that exhibit restricted dispersal particularly in species that demonstrate high levels of natal and breeding philopatry (Double et al. 2005).

Researchers have postulated mechanisms promoting philopatric behavior within species, including: (1) selective advantages of increased assistance from relatives during the breeding season (Lessells et al. 1994); (2) decreased competition and aggression between related or familiar neighbors (Greenwood et al. 1979, Waldman 1988, Eason and Hannon 1994); and (3) site familiarity (Anderson et al. 1992). Kin association and philopatry may have different effects on spatial genetic structure at the inter-individual scale. Individuals preferentially breeding near more genetically-related individuals might create clusters of non-random genetic associations among individuals at fine-spatial scales (Fowler et al. 2004, Double et al. 2005). Conversely, if individuals are philopatric to an area alone, fine-scale spatial associations may not be observed.

Pleistocene glacial cycles also have influenced levels of genetic diversity and distribution of species breeding in northern latitudes (Hewitt 2004). Throughout the Arctic, colder climates and ice sheets displaced species to lower latitudes and ice-free high latitude areas during the last glacial maximum (Hewitt 2004). Fossil and molecular data, however, suggest that some areas of the Arctic, notably Beringia, were unglaciated. During glacial maxima, species' ranges contracted into refugia, and during inter-glacial periods expanded and colonized ice-free areas (Hewitt 2004a). Population expansion from glacial refugia has left predictable genetic patterns in recently colonized regions. Molecular data coupled with coalescent theory have enabled researchers to investigate historical species distribution and demography and identify areas that exhibit a signature of rapid population expansion (Lessa et al. 2004). Conversely, populations that do not

exhibit genetic signatures of expansion have aided in the identification and location of glacial refugia.

Despite the importance of glacial refugia in species persistence during glacial maxima and as sources of colonizers of the Arctic, the number, locations, and significance of refugia remain largely unknown (Byun et al. 1997, Demboski et al. 1999). Ploeger (1968) provided a comprehensive review of proposed ice-free areas during the last Pleistocene glacial period and hypothesized the relative importance of ice-free areas as potential refugia for arctic Anatidae based on current species distributions. High arctic ice-free areas proposed by Ploeger (1968) included Beringia, Canadian Arctic Archipelago, northern Greenland, Spitsbergen Bank near Svalbard, and northwest Norway. Proposed temperate ice-free areas included Newfoundland, western Greenland, Iceland, and western Europe. Without fossil evidence; however, it is difficult to determine whether ice-free areas were inhabited by arctic species and contributed to species persistence. More recently, molecular data coupled with coalescent theory have substantiated Beringia, Canadian Arctic Archipelago, and western Greenland as ice-free refugia for arctic vertebrates (Holder et al. 1999; 2000, Fedorov and Stenseth 2002, Fedorov et al. 2003, Flagstad and Røed 2003, Scribner et al. 2003, Waltari and Cook 2005). Convergence in genetic signatures of population expansion across arctic species could provide insights into the locations of proposed refugia and their relative importance as historical reservoirs of species genetic diversity.

Here we investigate the population genetic structure at microgeographic spatial scales, postglacial colonization of North America and Scandinavia by Common Eiders

(*Somateria mollissima*) using microsatellite genotypes, nucleotide sequences from the mitochondrial DNA (mtDNA) control region, and two introns, and the effect of analyzing a sex-linked microsatellite locus as autosomal. Common Eiders are an arctic-nesting seaduck, composed of 6–7 morphologically distinct subspecies that together have a circumpolar distribution (Goudie et al. 2000). As observed in other waterfowl, female Common Eiders are highly philopatric to natal and breeding sites, whereas males disperse among populations that share common wintering grounds. Both sexes, however, display winter site fidelity (Spurr and Milne 1976). Common Eiders are unusual among waterfowl, as they exhibit fine-scale spatial genetic structuring for both mtDNA and nuclear markers (Tiedemann et al. 1999). High levels of natal, breeding, and winter site philopatry, coupled with microgeographic genetic partitioning observed for Common Eiders, enabled us to investigate patterns of population subdivision and gain insight into the locations of potential Pleistocene refugia for Common Eiders and the contribution of refugia to the postglacial colonization of North America and Scandinavia. We evaluated localities that have been proposed as ice-free areas or glacial refugia for other arctic vertebrates and Common Eider, including: southern edge of the Bering Land Bridge, northern Beringia, High Arctic Canadian Archipelago, Newfoundland, Spitsbergen Bank, and northwest Norway.

In this study, we present the first analysis, using microsatellite, nuclear intron, and mtDNA loci, of genetic relationships among North American and Scandinavian Common Eiders. The primary goals of this study were to: (1) use multivariate, multilocus autocorrelation analyses and highly variable markers to investigate local genetic

associations among female Common Eiders nesting on two island groups in the Beaufort Sea; (2) examine the spatial population structure of Pacific Common Eiders breeding on 12 barrier islands in the Beaufort Sea using molecular techniques, coupled with banding and genetic recapture data; (3) genetically characterize Common Eiders breeding on the Yukon-Kuskokwim Delta; (4) examine the population genetic structure of Common Eiders breeding throughout North America and Scandinavia, using a multilocus approach to evaluate subspecies classifications, evaluate genetic diversity within populations to test for refugial populations and directions of post-glacial colonization; and (5) assess the effect of sex-linkage on an estimator of population differentiation.

CHAPTER 1

DO WATERFOWL NEST IN KIN GROUPS? EVIDENCE FROM THE COMMON EIDER (*SOMATERIA MOLLISSIMA*) BREEDING IN THE BEAUFORT SEA, ALASKA[1]

Abstract — We investigated local genetic associations among female Common Eiders nesting on two island groups in the Beaufort Sea, Alaska, in 2000–2003 using multivariate autocorrelation analyses and highly variable microsatellite markers. Global analyses revealed strong correlations between genetic and geographic distances among years and island groups (Pearson's $r = 0.534 - 0.813$, $P < 0.001$), and between genetic relatedness (r_{xy}) and geographic distance (Pearson's $r = -0.012$ to -0.181, $P < 0.001$), indicating that females are nesting in closer proximity to more genetically related individuals. Nonrandom genetic associations also were observed using a global spatial autocorrelation analyses for distance classes up to 1000 m in Simpson Lagoon but not Mikkelsen Bay. Nearest-neighbor analyses identified clusters of genetically related females in both Simpson Lagoon and Mikkelsen Bay. Differences in the degree of genetic structuring between island groups may be attributable to the availability or

[1]Sonsthagen, S.A., S.L. Talbot, R.B. Lanctot, and K.G. McCracken. Do Waterfowl Nest in Kin Groups? Evidence from the Common Eider (*Somateria mollissima*) Using Molecular Techniques. Prepared for submission to *Animal Behaviour*.

distribution of nesting habitat, as Simpson Lagoon has three islands with colonies, whereas Mikkelsen Bay has only one. Significant structuring observed at microgeographic scales indicates eiders may nest in kin groups. Though the underlying mechanism enabling female eiders discriminate kin is unknown, waterfowl may achieve kin recognition indirectly through association during brood rearing. Finally, clusters of positive genetic autocorrelation observed among nesting females in Simpson Lagoon and Mikkelsen Bay could make these eiders more susceptible to disturbance, as localized disturbance could potentially affect a kin group rather than a cluster of individuals randomly associated to each other.

INTRODUCTION

Genetic consequences of philopatric behavior have been demonstrated in several taxa (e.g., Tiedemann et al. 1999, Scribner et al. 2001, Avise 2004). Dispersal homogenizes allelic frequencies, whereas natal and breeding philopatry can lead to patterns of spatial genetic subdivision among populations. Unfortunately, dispersal is difficult to study because it often requires long-term demographic studies (Koenig et al. 1996), and in species that are highly mobile, estimates may be more difficult to obtain since individuals may disperse out of the study site. Genetic studies frequently have been used to assess intergenerational dispersal (gene flow) among populations. However, most studies that have characterized population genetic structure have assessed allelic frequency differences within and among populations and are not designed to detect local groupings of genetically related individuals within populations, which would be expected in species that exhibit restricted dispersal (Double et al. 2005).

Researchers have hypothesized several mechanisms promoting philopatric behavior within species, including selective advantages of increased assistance from relatives during the breeding season (Lessells et al. 1994); decreased competition and aggression between related or familiar neighbors (Greenwood et al. 1979, Waldman 1988, Eason and Hannon 1994); or site familiarity (Anderson et al. 1992). Kin association and philopatry may have different effects on spatial genetic structure at the inter-individual scale. Individuals preferentially breeding near more genetically-related individuals might create clusters of non-random genetic associations among individuals at fine-spatial scales (Fowler et al. 2004, Double et al. 2005). Conversely, if individuals

are philopatric to an area alone, fine-scale spatial associations may not be observed depending upon the size of the study area and density of the population.

Here we investigate microgeographic genetic structuring in Common Eiders (*Somateria mollissima*). Pacific Common Eiders (*S. m. v-nigrum*) breeding on coastal barrier islands in the Beaufort Sea, Alaska, nest in association with driftwood. Female Common Eiders either nest in dense colonies or scattered locations on islands because of the availability of nesting habitat (Goudie et al. 2000). As observed for other waterfowl, female Common Eiders exhibit high natal and breeding philopatry (Goudie et al. 2000), which promotes high levels of genetic partitioning among populations at mitochondrial DNA (mtDNA; Tiedemann et al. 1999, Tiedemann et al. 2004, Sonsthagen et al. submitted a, b). Furthermore, researchers investigating the colonial nesting of eiders breeding in Hudson Bay (*S. m. sedentaria*) hypothesized that eiders breeding in groups were composed of extended family, as some groups of Common Eiders exhibited greater nesting synchrony than expected by chance (Schmutz et al. 1983). Variance in egg shape among females within these groups suggested genetic relatedness. In addition, Common Eiders (*S. m. borealis*) breeding on Southampton Island in Hudson Bay arrive to the colony, nest, and brood rear in female kin-based social groups, which were determined using molecular techniques (McKinnon 2005).

We used a multivariate autocorrelation analyses developed by Double et al. (2005) to investigate local genetic associations among female Common Eiders breeding on 12 islands in the Beaufort Sea. Given evidence from previous studies in other subspecies of Common Eiders and high natal and breeding philopatry observed for

female Common Eiders, we predicted that the Beaufort Sea eiders nest in close association with more genetically related individuals than expected by chance. We also hypothesized that due to differences in nesting habitat spatial genetic associations may not be as pronounced as those observed within Hudson Bay colonies. Seasonal arctic storms in the Beaufort Sea dramatically modify island topology, changing where nesting habitat is located annually (Noel et al. 2005). In contrast, Hudson Bay Common Eiders nest on coastal wetland tundra habitat (Goudie et al. 2000) that has remained relatively unchanged across consecutive breeding seasons.

METHODS

SAMPLE COLLECTION

Blood or feather samples were collected from breeding female Common Eiders opportunistically during mark-recapture and monitoring efforts on barrier islands in the Beaufort Sea, Alaska, from 2000–2003. Samples were collected from two island groups, consisting of 12 islands in total. The western group, hereafter called Simpson Lagoon, consists of five islands: Stump (70.419°N 148.601°W), Wannabe (70.437°N 148.725°W), Egg (70.440°N 148.739°W), Long (70.480°N 148.937°W), and Spy (70.564°N 149.895°W) islands (Fig. 2.1A). The eastern group, hereafter called Mikkelsen Bay; consists of seven islands: Camp (70.172°N 146.226°W), Point Thomson (70.186°N 146.325°W), Mary Saches (70.200°N 146.207°W), North Star (70.225°N 146.347°W), Duchess (70.233°N 146.405°W), Alaska (70.233°N 146.559°W), and Challenge (70.237°N 146.640°W) islands (Fig. 2.1B). Distances between islands within each of the

two island groups ranged from 1.2–49.2 km, and distances between islands located in Simpson and Mikkelsen Bay ranged from 78.1–143.1 km. Two islands, Camp and Wannabe, are not official names of islands on any recognized maps, but were given these names for the purpose of identifying areas in this study.

Females were captured on nests using a dip net during initial nest searching efforts, or with a bow net during late-incubation (Sayler 1962). Blood was collected from the tarsal, brachial, or jugular veins and placed in lysis buffer (Longmire et al. 1988). Feather samples were collected from nest bowls from unsampled females and stored in silica gel desiccant at room temperature. After returning from the field, samples were archived at –80°C at the U. S. Geological Survey Molecular Ecology Laboratory, Anchorage, Alaska. Genomic DNAs were extracted using either a "salting out" protocol described in Medrano et al. (1990) with modifications described in Sonsthagen et al. (2004), or a QIAGEN DNeasy Tissue Kit (QIAGEN, Valencia, CA). Concentrations of genomic DNA extracts were quantified using fluorometry and diluted to 50 ng/μL working solutions.

Microsatellite Genotyping

Primers used for microsatellite genotyping of Common Eiders (n = 317) were obtained via cross-species screening of microsatellite primers developed for other waterfowl. We screened 12 Common Eiders at 50 microsatellite loci reported to be variable for other waterfowl species and selected 14 microsatellite loci found to be polymorphic: *Aph*02, *Aph*08, *Aph*20, *Aph*23 (Maak et al. 2003); *Bca*μ1, *Bca*μ11, *Hhi*μ3 (Buchholz et al. 1998);

*Cm*09 (Maak et al. 2000); *Sfi*μ10 (Libants et al. unpubl. data); *Smo*4, *Smo*7, *Smo*08, *Smo*10, and *Smo*12 (Paulus and Tiedemann 2003). Microsatellites were amplified using the polymerase chain reaction (PCR), and products were electrophoresed following protocols described in Sonsthagen et al. (2004) for tailed primers (*Aph*02, *Aph*08, *Aph*20, *Aph*23, *Cm*09, *Smo*4, *Smo*7, *Smo*08, *Smo*10, and *Smo*12) and Pearce et al. (2005) for direct-labeled primers (*Bca*μ1, *Bca*μ11, *Hhi*μ3, and *Sfi*μ10). For quality control purposes, 10% of the samples were randomly selected, re-amplified, and genotyped in duplicate.

Data Analysis

Allelic frequencies, and expected and observed heterozygosities for each microsatellite locus were calculated in GENEPOP 3.1 (Raymond and Rousset 1995) and FSTAT 2.9.3 (Goudet 1995, 2001). Hardy Weinberg Equilibrium and linkage disequilibrium were tested in GENEPOP using the default parameters (Markov chain parameters: dememorization number 1000, number of batches 100, and number of iterations per batch 10,000), adjusting for multiple comparisons using Bonferroni corrections ($\alpha = 0.05$). To determine if we could accurately identify individuals, and therefore assess levels of relatedness among individuals, probabilities of identity for a randomly mating population (PID) and among siblings (PID_{sib}) were calculated in Gimlet 1.3.3 (Valière 2002) using genotypes from the 14 microsatellite loci.

Queller and Goodnight's (1989) index of relatedness (r_{xy}) was calculated overall and among pairs of individuals breeding on each island group within a given year using

IDENTIX 1.1 (Belkhir et al. 2002). Relatedness values range from –1 to 1, where r_{xy} equals 0.5 for full-sibling relationships, 0.25 for half-sibling relationships, and 0 for unrelated individuals. Genetic discordance among sampled areas may cause incorrect relatedness values, as values measure genetic differences in overall allelic frequency (Queller and Goodnight 1989). Therefore, spatial analyses of individuals were partitioned by island groups because Sonsthagen et al. (submitted a) observed significant genetic differentiation between Mikkelsen Bay and Simpson Lagoon. Squared genetic distance was calculated between pairs of individuals within each island group following the method of Smouse and Peakall (1999) in GenAlEx 6 (Peakall and Smouse 2006). Geographic distances among sampled nests were calculated using Universal Transverse Mercator (UTM) coordinates.

Overall correlation between genetic similarity and geographic distance at the population level was assessed using Mantel tests implemented in the software zt 1.0 (Bonnet and Van de Peer 2002). Significance of Pearson correlation coefficients were assessed using a randomization procedure, where the original value of the statistic was compared to the distribution of a random reallocation of the distance values in one of the matrices (randomization = 10,000).

Global spatial autocorrelation analyses were conducted in GenAlEx to further investigate spatial partitioning of individuals within an island group in a given year, as weak or scattered patterns may not be detected using a simple Mantel analysis. Genetic and geographic matrices calculated in GenAlEx were used to determine spatial autocorrelation of Common Eider nests with increasing distance classes (10, 25, 50, 100,

250, 500, and 1000 m). Distance classes were used to determine the spatial scale at which genetic structure was detected. Distance intervals larger than actual spatial genetic structure would lead to failure to detect structure, whereas distance classes smaller than actual genetic structure would result in increased inter-individual variance and decrease the probability of detecting structure. Distance classes selected were based on nearest-neighbor distances calculated in GenAlEx for a given year and island group and the nesting biology of Common Eiders breeding in the Beaufort Sea.

Common Eiders either nest in dense colonies or are largely scattered throughout the islands depending on the availability of nesting habitat. Mean nearest neighbor values ranged from 47.2–672.5 m across island groups and years, with a minimum observed distance of 0.0 m and a maximum distance of 6.4 km. Distance classes were chosen in attempt to account for different nesting strategies among eider females (i.e., nesting in dense colonies or scattered among islands). Genetic correlation (r) was estimated using two approaches: permutation and bootstrap (1000). Significance was assessed by 95% confidence intervals estimated by bootstrap.

A two-dimensional local spatial analysis was implemented in GenAlEx as described by Double et al. (2005) to assess fine-scale non-random patterns in genetic structure. Barriers to dispersal and social structure, such as female natal and breeding philopatry, can create non-random genetic patterns. If females preferentially nested closer to relatives, we would expect to observe a significant correlation at finer spatial scales. In contrast, if female natal and breeding philopatry are sufficient to explain overall trends, then more genetically-related females would not be nesting in close

association with each other. Local autocorrelation (*lr*) is estimated based on *n* pairwise comparisons for a focal individual and its *n* nearest neighbors using genetic and geographic distances calculated in GenAlEx. This analysis was repeated for all individuals in the data set. We calculated the two-dimensional local spatial analysis for four, six, eight, and ten nearest neighbors (10,000 permutations). Geographic distances calculated in GenAlEx, as described above, were used to determine the four, six, eight, and ten nearest neighbors. The output of the two-dimensional spatial analysis was converted to bubble plots across the landscape.

RESULTS

Multi-locus genotypes were obtained for 317 individuals. The number of alleles per locus for the 14 polymorphic microsatellite loci ranged from 3–44 (Table 1.1), with an average 11.3 alleles per locus. The average number of alleles per island group in a given year ranged from 6.21–8.79 (Table 1.2). The observed heterozygosity for each area in a given year ranged from 56.1–60.6% with an overall value of 57.7% (Table 1.2). All loci did not significantly deviate from Hardy-Weinberg equilibrium and were in linkage equilibrium ($P_{adj.} > 0.05$).

We calculated an overall PID of 3.2×10^{-12} for a population composed of randomly mating individuals and 5.3×10^{-5} for siblings using genotypes collected from 14 microsatellite loci (Table 1.1). These denominator values are much larger than the population breeding on the western Beaufort Sea (approximately 500 nests found on the islands each year; Johnson 2000), which gave us confidence in identifying individuals

correctly among years. Overall r_{xy} values from Mikkelsen Bay and Simpson Lagoon ranged from –0.037 to –0.008, and –0.063 to –0.014, respectively (Table 1.2). Mean r_{xy} values close to zero indicates that, on average, Mikkelsen Bay and Simpson Lagoon are composed of unrelated females. Variances were large (Table 1.2), indicating populations are comprised of some highly related individuals, and some individuals that are not closely related.

Significant correlations between genetic distance and r_{xy} values with geographic distance were observed among years and island groups (Table 1.3), indicating that more genetically related individuals are nesting geographically closer to each other than expected by chance. Fine-scale spatial structure was observed in Simpson Lagoon at the 0–50 m distance class in 2001; 0–100, 0–250, 0–500, and 0–1000 m distance classes in 2002; and 0–10 and 0–25 m distance classes in 2003 (Fig. 1.2A). Nesting female Common Eiders in Mikkelsen Bay did not depart from a nonrandom distribution of genotypes at any distance class (Fig. 1.2B).

For Common Eiders nesting in Simpson Lagoon, 0–29% of the *lr* values calculated for the four nearest neighbors were positive (one-tailed *P*-values = 0.001–0.046; Table 1.4). Positive values clustered around Stump, Long, and Egg Islands (Fig. 1.3). Within Mikkelsen Bay, 0–14% of the *lr* values were positive (one-tailed *P*-values = 0.004–0.046; Table 1.4) for the four nearest neighbors. Positive *lr* values clustered around Camp, Duchess, Alaska, and Challenge Islands (Fig. 1.3). A similar number and distribution of positive values were observed among years and island groups for estimates based on four, six, eight, and ten nearest neighbors (data not shown).

DISCUSSION

Global correlation analyses revealed fine-scale genetic structure among nesting females in the Beaufort Sea, indicating that genetically related individuals nested closer to each other than expected by chance. The pattern of spatial genetic structure revealed by global autocorrelation analyses using distance class sampling was not strong. Low *r* values were observed for Simpson Lagoon in 2002 and 2003, and females nesting in Mikkelsen Bay did not deviate from a random distribution. Differences in the degree of genetic structuring between Simpson Lagoon and Mikkelsen Bay may be attributable to the availability or distribution of nesting habitat. Three islands (Egg, Long, and Stump) contain Common Eider colonies within Simpson Lagoon; approximately 50, 35, and 155 nests were found in 2003 on each, respectively (J. Reed unpubl. data). In Mikkelsen Bay, only one island, Duchess, contains a colony, with approximately 90 nests found in 2003 (J. Reed unpubl. data). On islands without colonies, approximately 10–20 nests were found scattered across each. McKinnon (2005) found that females nesting in high densities had higher levels of relatedness among focal females and the two nearest females than those nesting in low-density areas. Females in high density nesting areas may prefer to nest in closer proximity to more genetically-related individuals because of reduced aggression among kin (Greenwood et al. 1979, Waldman 1988, Eason and Hannon 1994). In contrast, on low nesting density islands there may not be an advantage to nesting in close association with kin due to presumably fewer interactions among

neighbors. Alternatively, females in Simpson Lagoon may simply be able to nest in closer proximity to kin because of the availability of suitable habitat.

Microgeographic genetic structure was uncovered by the two-dimensional local spatial autocorrelation analysis, indicating that females are nesting in association with more genetically related females. Clusters of non-random genetic associations were observed in Simpson Lagoon and in Mikkelsen Bay. Double et al. (2005) hypothesized that clusters of local positive genetic autocorrelation observed may exist because of an individual being more successful reproductively. In highly philopatric species, progeny from successful lineages might cluster around natal sites. Female Common Eiders have been reported to be philopatric to natal sites (Swennen 1990), areas within colonies (Couch 1965), and to exhibit fidelity to specific nest bowls (Bustnes and Erikstad 1993). Therefore, clusters of related females may be due to extreme natal and breeding philopatry coupled with high reproductive output.

Kin recognition among female Common Eiders also may contribute to the local clusters of positive genetic autocorrelation observed. Kin-based clusters have been postulated to occur among nesting females on the Belcher Islands, Hudson Bay (Schmutz et al. 1983). Furthermore, female eiders breeding on Southampton Island, Hudson Bay, form kin-based social groups during colony arrival, nesting, and colony departure, which suggests some form of kin recognition (McKinnon 2005). A variety of mechanisms enabling individuals to discriminate kin have been identified (Komdeur and Hatchwell 1999) and could be achieved indirectly though association (Hatchwell et al. 2001, Komdeur et al. 2004). In the highly philopatric Barnacle Goose (*Branta leucopsis*),

females preferentially nested in kin groups that were based on kin recognition rather than extreme natal philopatry, as females that bred away from natal sites nested in close geographic proximity to sisters that they were familiar as brood mates (van der Jeugd et al. 2002). If recognition among female Common Eiders influences nest site selection, this may explain, in part, why only some females nest in kin groups. In Common Eiders, females may rear broods alone or randomly form brood amalgamations (i.e., not kin-based; Öst et al. 2005). Therefore, Common Eiders may nest in close proximity to brood mates, independent of their genetic relatedness, because of decreased competition and aggression between related or familiar neighbors (Greenwood et al. 1979, Waldman 1988, Eason and Hannon 1994).

Asymmetrical gene flow between islands groups may explain, in part, differences between island groups in the degree of genetic structuring. Gene flow estimates, calculated from mitochondrial DNA control region, 14 microsatellite loci, and two nuclear introns, indicate that more individuals are dispersing from Mikkelsen Bay to Simpson Lagoon (Sonsthagen et al. submitted a). Asymmetrical gene flow between island groups could generate a pattern of lower genetic structuring in the "source" population and clusters of more genetically related individuals in the "receiving" population coupled with unrelated individuals, as observed in our study. In the source population, females may be less able to nest in close proximity to kin because genetically-related individuals may have dispersed to the other island group. In the receiving population, females may nest in close proximity to kin, creating clusters of

positive genetic autocorrelations; however, fewer clusters of positive autocorrelation may be observed because of random associations created with source population females.

Differences in genetic structure observed for Mikkelsen Bay between global distance class sampling and local autocorrelation analyses may be attributable to the spatial scale at which analyses were conducted. We may have not selected distance classes at an interval to detect structure among females. Distance intervals larger than actual spatial genetic structure would lead to failure to detect structure, whereas distance classes smaller than actual genetic structure would result in increased inter-individual variance and decrease the probability of detecting structure. Local autocorrelation analyses, however, are conducted among a focal female and her four nearest neighbors, irrespective of distance, and therefore, may be more biologically significant as analyses reflect genetic associations among females that are interacting with each other during nesting.

CONCLUSIONS

Common Eiders nesting on the coastal barrier islands in the Beaufort Sea nested in closer proximity to more genetically related individuals, creating clusters of non-random associations among individuals. Although we were able to detect significant microgeographic genetic structuring among nesting Common Eiders, this study likely underestimates the degree of relatedness, as not all females nesting on the study islands were sampled. Therefore, a female's nearest-neighbors for this study may not be the nearest individuals that a female interacted with during nest site selection. Finally, we

cannot exclude the possibility that Common Eiders are nesting in close proximity to kin because of extreme natal philopatry rather than preferentially nesting close to kin. Long-term demographic data coupled with molecular techniques are needed to determine if the pattern of fine-scale genetic structuring observed in Beaufort Sea Common Eiders is because of extreme natal philopatry or female kin association.

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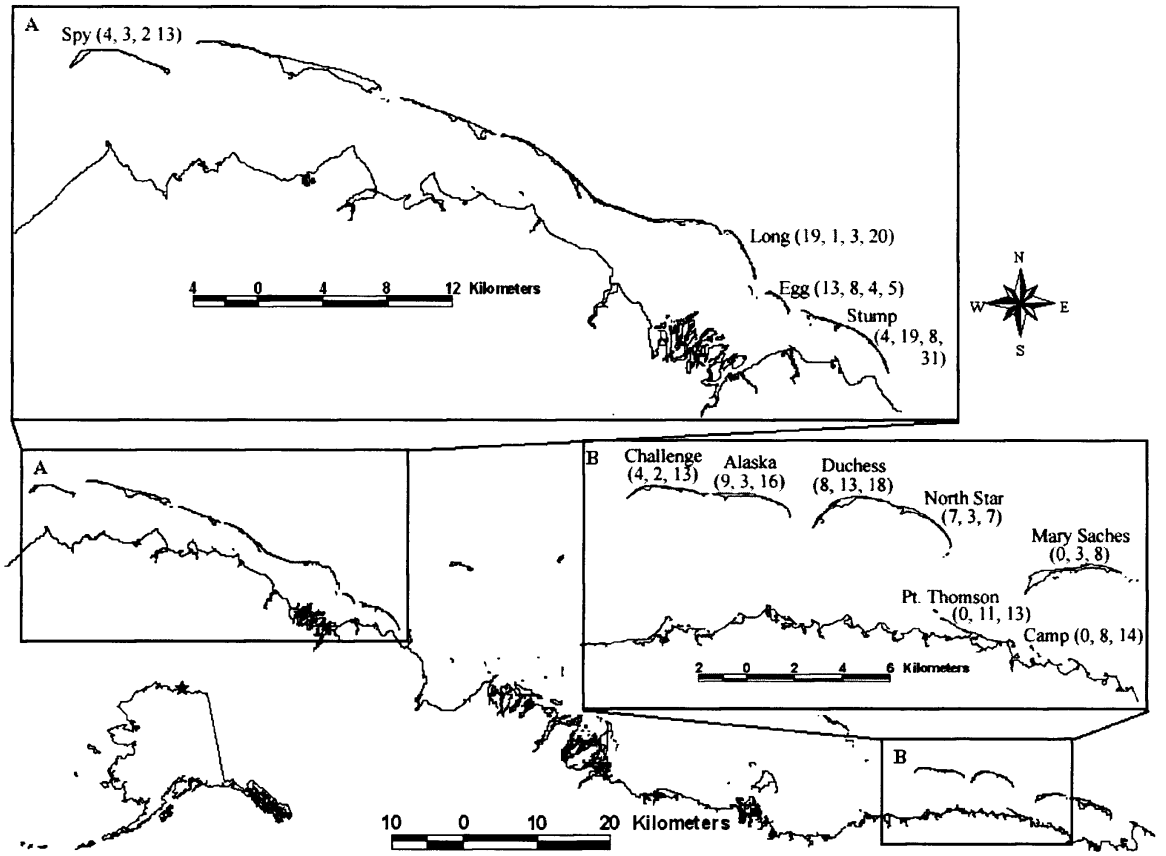

Figure 1.1. Beaufort Sea barrier islands located in (A) Simpson Lagoon (western group) and (B) Mikkelsen Bay (eastern group) with samples sizes for each island in a given year in parentheses; 2000–2003, respectively; no samples were collected in 2001 for Mikkelsen Bay. Camp designation is used by the authors and is not the official name of the island.

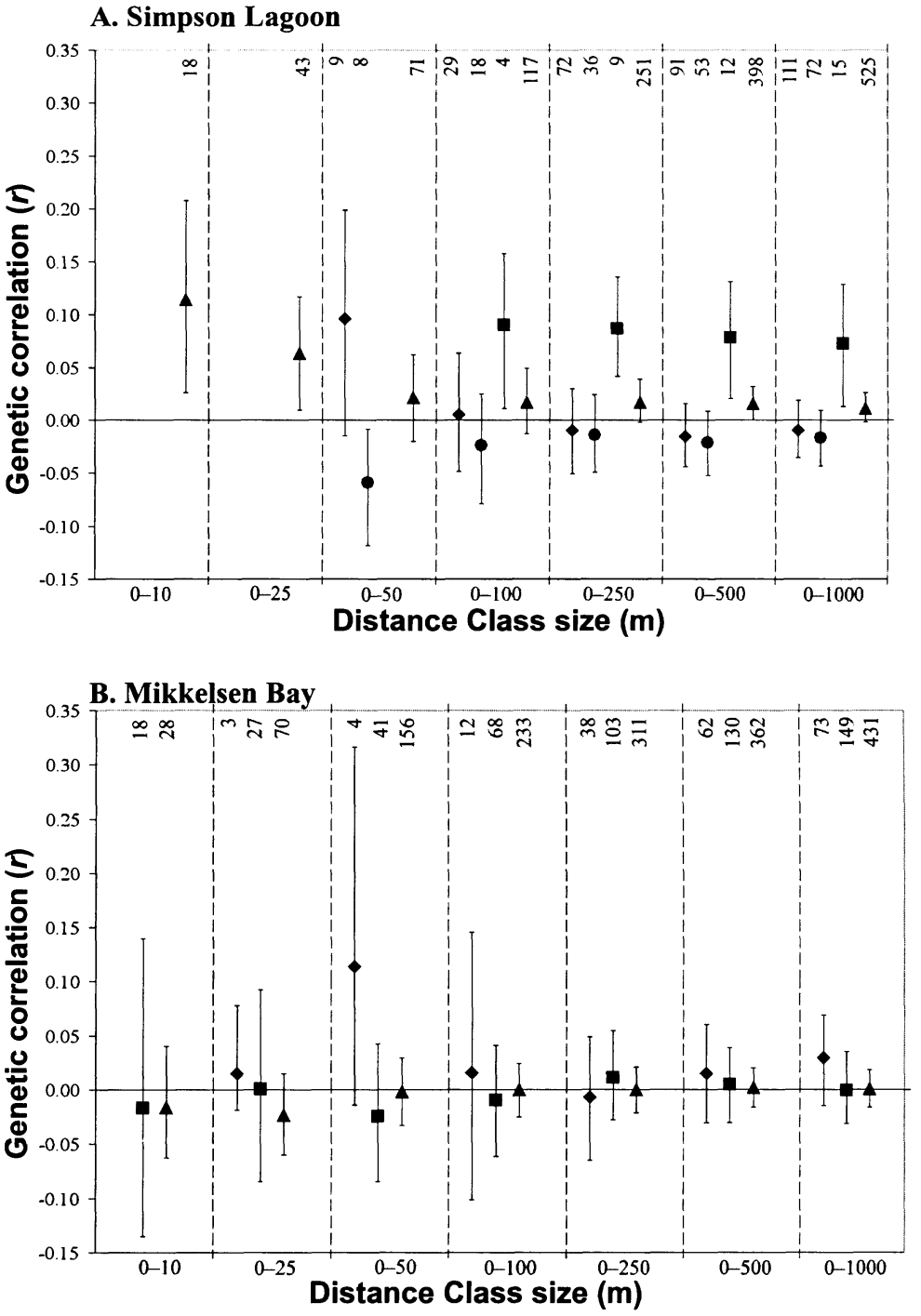

Figure 1.2. Genetic correlation (r) of females breeding in (A) Simpson Lagoon and (B) Mikkelsen Bay at increasing distance class size intervals. Symbols represent females breeding in 2000 (diamonds), 2001 (circles), 2002 (squares), and 2003 (triangles). Number of pairwise comparisons for each distance class is shown above the plotted values. The 95% confidence error bars about r were estimated by bootstrapping.

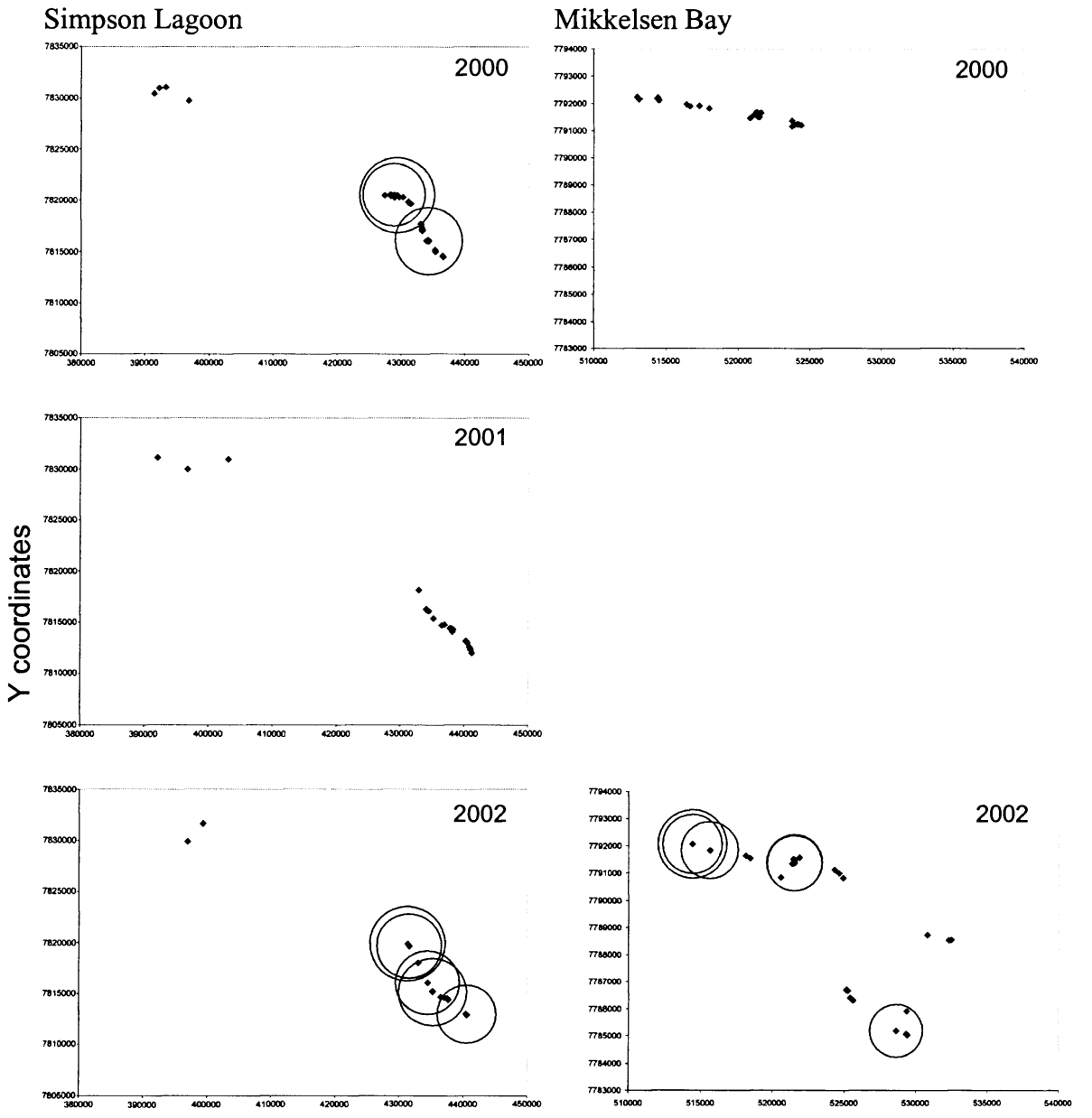

Figure 1.3. Bubble plots of two-dimensional local spatial autocorrelation analysis of Common Eider females nesting in Simpson Lagoon and Mikkelsen Bay in 2000–2003. Each plot shows the study area with squares indicating the nest location. Bubbles surround the nests with positive (solid lines) *lr* values, based on the four nearest neighbors, and within 5% tail of the permutated distribution. The size of the circle is proportional to the magnitude of *lr*.

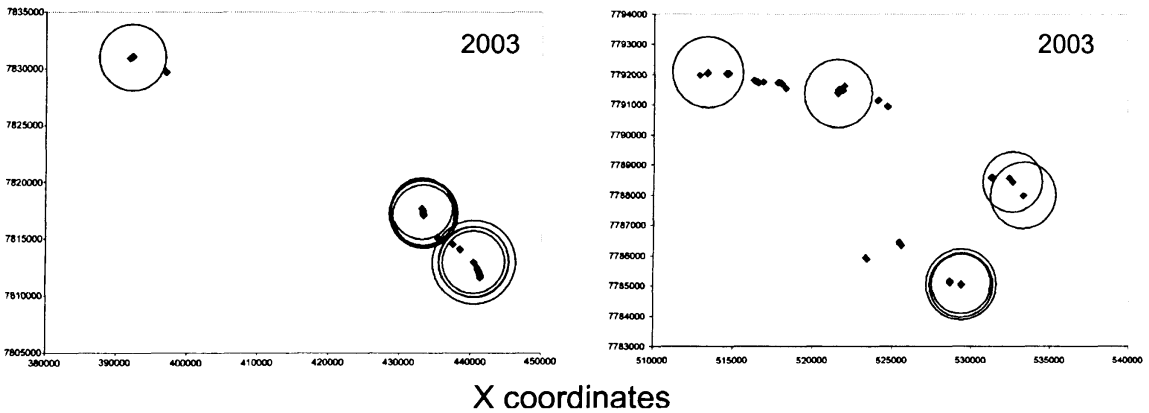

Figure 1.3 cont.

Table 1.1. Number of alleles, fragment length, observed heterozygosity (H_o), and probability of identity among randomly mating individuals (PID), and siblings (PID_{sib}) for 14 microsatellite loci.

Locus	Number of alleles	Fragment length	H_o	PID	PID_{sib}
*Aph*02	4	110–116	0.516	1/3.52	1/1.82
*Aph*08	3	138–142	0.459	1/2.53	1/1.61
*Aph*20	9	162–184	0.645	1/5.90	1/2.20
*Aph*23	7	206–218	0.599	1/5.10	1/2.60
*Cm*09	9	102–124	0.599	1/4.89	1/1.99
*Bcaμ*1	4	108–114	0.451	1/2.97	1/1.59
*Bcaμ*11	7	135–147	0.395	1/2.54	1/1.54
*Hhiμ*3	3	110–114	0.119	1/1.61	1/1.26
*Sfiμ*10	19	129–181	0.875	1/38.87	1/3.13
*Smo*4	44	155–257	0.918	1/251.38	1/3.63
*Smo*7	6	197–213	0.362	1/2.57	1/1.55
*Smo*8	7	115–127	0.625	1/4.90	1/2.00
*Smo*10	21	115–163	0.782	1/14.87	1/2.62
*Smo*12	15	100–117	0.729	1/11.84	1/2.50
Total loci	–	–	0.577	3.21×10^{-12}	5.34×10^{-5}

Table 1.2. Average number of alleles, observed and expected heterozygosities (H_o/H_e), overall relatedness values (r_{xy}; Queller and Goodnight 1989) with variances, and sample sizes (n) for Common Eiders breeding on two island groups (Simpson Lagoon and Mikkelsen Bay) in the Beaufort Sea, Alaska, in 2000–2003.

	No. alleles	H_o/H_e (%)	r_{xy}	variance	n
Simpson Lagoon					
2000	7.36	59.5/59.5	–0.026	0.033	40
2001	7.29	60.1/60.3	–0.033	0.029	31
2002	6.21	60.6/58.3	–0.063	0.039	17
2003	8.64	56.1/59.3	–0.014	0.037	69
Mikkelsen Bay					
2000	6.64	58.2/58.5	–0.037	0.036	28
2002	8.00	57.3/59.8	–0.021	0.042	43
2003	8.79	56.1/58.6	–0.008	0.037	89

Table 1.3. Pearson correlation values (r) for genetic distance (GD) and relatedness values (r_{xy}) and geographic distance for female Common Eiders nesting on two island groups (Simpson Lagoon and Mikkelsen Bay) in the Beaufort Sea, Alaska, in 2000–2003. Significant correlations ($P < 0.001$) are in bold text.

	2000	2001	2002	2003
Simpson Lagoon				
r–GD	**0.534**	**0.608**	**0.579**	**0.616**
r–r_{xy}	**–0.088**	**–0.047**	**–0.168**	**–0.033**
Mikkelsen Bay				
r–GD	**0.786**	–	**0.800**	**0.813**
r–r_{xy}	**–0.181**	–	**–0.032**	**–0.012**

Table 1.4. Local autocorrelation (*lr*) values and percent of nesting females from Simpson Lagoon and Mikkelsen Bay in 2000–2003 with positive genetic correlation among a focal individual and her four nearest neighbors.

	2000	2001	2002	2003
Simpson Lagoon				
Positive *lr*	0.141–0.176 8% (n = 3/40)	–	0.098–0.159 29% (n = 5/17)	0.137–0.250 13% (n = 9/69)
Mikkelsen Bay				
Positive *lr*	–	–	0.139–0.232 14% (n = 6/43)	0.124–0.180 8% (n = 7/89)

CHAPTER 2

POPULATION GENETIC STRUCTURE OF COMMON EIDERS (*Somateria mollissima*) BREEDING IN THE BEAUFORT SEA, ALASKA[1]

Abstract — We assessed the level of population subdivision within the Pacific Common Eider (*Somateria mollissima v-nigrum*) breeding on 12 barrier islands in the Beaufort Sea, Alaska, using molecular markers with differing modes of inheritance and rates of evolution, as well as recapture data. Common Eider populations exhibited fine-scale population structuring based on all marker types. Regional comparisons between two island groups, Mikkelsen Bay and Simpson Lagoon, revealed structuring at 14 microsatellite loci ($F_{ST} = 0.004$, $P = 0.016$), mitochondrial DNA (mtDNA) control region ($\Phi_{ST} = 0.082$, $P = 0.047$), and nuclear intron *lamin* A ($\Phi_{ST} = 0.022$, $P = 0.022$). Given the geographic proximity of island groups (approximately 90 km apart), these values are noteworthy. Recapture data revealed substructuring between island groups, as we did not detect any female dispersal between groups ($n = 34$). In addition, inter-population variation in allelic frequencies was observed within mtDNA ($\Phi_{ST} = 0.135$–0.271) and nuclear intron *lamin* A ($\Phi_{ST} = 0.089$–0.173). Gene flow estimates based on

[1]Sonsthagen, S.A., S.L. Talbot, R.B. Lanctot, K.T. Scribner, and K.G. McCracken. Population genetic structure of Common Eiders (*Somateria mollissima*) breeding in the Beaufort Sea, Alaska. Submitted to *Conservation Genetics*.

microsatellite, mtDNA, and nuclear intron loci indicate asymmetrical western dispersal has occurred between island groups. Asymmetrical western gene flow may be driven by females from Mikkelsen Bay stopping early on spring migration at Simpson Lagoon to breed. Alternatively, young females arriving later may be "forced" to nest in Simpson Lagoon due to distribution of available nest sites. These data suggest that areas of genetic discordance can exist over very small spatial scales relative to the species dispersal capabilities and known dispersal distances.

INTRODUCTION

Microgeographic population structure is greatly influenced by natal and breeding dispersal. Here we define natal dispersal as the distance between an individual's natal site and the site of its first breeding attempt, and breeding dispersal as the distance an individual travels between each subsequent breeding attempt (Greenwood 1980). For many species, detecting natal and breeding dispersal is difficult, especially for mobile organisms that may travel long distances prior to and between breeding attempts. Advances in molecular techniques have made it possible to assess genetic structure of natural populations and evaluate the roles of contemporary and past dispersal events among areas (Newton 2003). Birds are of particular interest because most species that breed in arctic or temperate regions migrate to other areas during the nonbreeding season, and thus, show less geographic structure than other vertebrate groups (Avise 1996). Lack of population structure has been attributed to environmental variability of arctic and temperate regions, which increases dispersal and migratory behavior in birds, and can homogenize genetic diversity (Winker et al. 2000). Conversely, many birds exhibit high natal and breeding site fidelity (e.g., natal and breeding philopatry respectively), which is expected to restrict gene flow among neighboring populations (Avise 1996), leading to population subdivision.

Differences in the degree of philopatry also may exist between males and females. The most common pattern in birds is for females to disperse farther between natal and breeding sites than males (Greenwood 1980). However, female waterfowl typically show greater natal and breeding philopatry than males (Rohwer and Anderson 1988). Males

and females typically pair on the wintering grounds, and the male accompanies the female back to her natal area. Because ducks from different breeding areas frequently share a common wintering ground, males may disperse over long distances. Additionally, males may not mate with the same female each year, resulting in individual males breeding in distant locations from year to year (Anderson et al. 1992). Male behavior, thus, is expected to cause genetic mixing among individuals from multiple breeding areas, which may explain why many species of ducks are morphologically monotypic across the Holarctic (Newton 2003).

Patterns of natal, breeding, and winter site fidelity may leave varying signatures in molecular markers. Accordingly, researchers using genetic tools to investigate levels of spatial structuring and gene flow should use markers that differ in their mode of inheritance. For example, if females exhibit high natal and breeding philopatry and males disperse over large distances; there should be genetic structuring at the maternally inherited marker and little or no structure at bi-parentally inherited nuclear markers. If data were collected from just one of these genomes, gene flow among populations might be grossly over- or under-estimated depending on which marker type was used (Avise 2004). However, combining markers with different modes of inheritance and rates of evolution, researchers may ask a wider range of questions involving species population genetic structure and behavior.

Common Eiders (*Somateria mollissima*) have a circumpolar distribution and inhabit coastal regions throughout the Holarctic. There are 6 or 7 recognized subspecies that have partially overlapping breeding ranges (Goudie et al. 2000). The Pacific

Common Eider (*S. m. v-nigrum*) breeds on the barrier islands of western Canada and Alaska (Johnson and Herter 1989, Johnson 2000), and has experienced a marked population decline (approximately 53%), since the mid-1970s (1996 population estimate 111,635 ± 42,440; Suydam et al. 2000). More recent surveys, however, indicate the population may have stabilized (R. Suydam, pers. comm.). Reasons for the decline are unknown. However, these birds are long lived with a low reproductive rate, which may be limiting population growth (Goudie et al. 2000). Long generation times could potentially increase extinction or extirpation risk (Marzluff and Dial 1991).

Rates of gene flow among breeding Pacific Common Eiders over scales relevant to conservation have not been measured. Satellite telemetry studies, however, indicate that adult female eiders that nest on islands in the Beaufort Sea may intermix with other eider populations during migration to wintering grounds in the Bering Sea south of the Chukotka Peninsula, Russia (Petersen and Flint 2002; L. Dickson, pers. comm.). Females that nest across large areas possibly pair with males from other breeding populations during winter, allowing gene flow through male-biased dispersal. Such gene flow would occur despite the observation that all transmittered females returned to their breeding areas in the western Beaufort Sea the following summer (Petersen and Flint 2002; L. Dickson, pers. comm.).

Data on degree of spatial population genetic structure are currently available only for the European Common Eider (*S. m. mollissima*) that breeds in the Baltic Sea. Tiedemann et al. (1999) found high levels of population structure among colonies in the Baltic Sea (133–1010 km) for maternally inherited mtDNA ($\Phi_{ST} = 0.262–0.343$, $P <$

0.001), and significant, but lower, levels for bi-parentally inherited microsatellite loci (F_{ST} = 0.009–0.029, $P < 0.05$). The authors attributed high levels of spatial structure assayed using mtDNA to high rates of female natal philopatry. Proportionally lower spatial variance in allelic frequency at microsatellite loci was attributed to non-random mating by males on the wintering grounds (i.e., males mate with females from the same locality more often than expected).

Information on degree of spatial population structure for Pacific Common Eiders in the western Beaufort Sea is of particular interest to management and industry agencies because of the close proximity of nesting areas to oil and gas development activities (Minerals Management Service 2003). The phylopatric nature of Common Eiders (Reed 1975, Swennen 1990) coupled with nesting proximity to major industry infrastructure (Flint et al. 2003) makes this population susceptible to human disturbance (i.e. aircraft flights, personnel, etc.). In addition, increases in numbers of avian and mammalian predators near oil development may adversely affect nest success and duckling survival (Johnson 2000). Effects of increased disturbances may be temporarily confounded as predators may avoid high use areas, potentially increasing nest success initially (Johnson 2000). Conversely, if increased disturbance causes females to flush from nests more readily, then nests would be more susceptible to predators. Subspecies risks can be exacerbated should genetically distinct populations occur in proximity to existing or proposed development. Thus, evaluation of population structure of Common Eiders in the western Beaufort Sea can provide means to assess potential risks of oil and gas exploration to the population or species.

We estimated levels of spatial population structure of Pacific Common Eiders breeding on the barrier islands in the western Beaufort Sea using microsatellite genotypes and sequence information from mtDNA control region and two nuclear introns, coupled with banding and genetic recapture data. Microsatellite and mtDNA loci have been used extensively to examine genetic discordance at fine spatial scales among waterfowl populations (e.g., Lanctot et al. 1999, Tiedemann et al. 1999, Scribner et al. 2001, Scribner et al. 2003, Pearce et al. 2004, Pearce et al. 2005). To our knowledge, however, this study is the first to use nuclear introns to assess levels of microgeographic population subdivision. Variation in nuclear introns, due to their higher effective population size relative to mtDNA and slower mutation rate, enables us to ask questions about historic processes influencing population subdivision (Hare 2001) occurring within the Beaufort Sea population. We hypothesized that the nuclear markers (microsatellites and intron sequences) would show little population genetic structure, because Common Eiders breeding on these islands share a common wintering ground with eiders from several other breeding areas. Over time, male dispersal among populations could homogenize allelic frequencies within the nuclear genome. However, we predicted that population structure would be observed at the maternally inherited mtDNA because of the high degree of female natal and breeding philopatry reported in other subspecies of Common Eider.

METHODS

SAMPLE COLLECTION

Blood or feather samples from breeding female eiders and egg samples from nests were collected during mark-recapture and monitoring efforts on the barrier islands in the Beaufort Sea, Alaska, between June and July of 2000–2003. Samples were collected from two island groups, consisting of 12 islands in total (Fig. 2.1). The western group, hereafter called Simpson Lagoon, consists of five islands: Stump (70.419°N 148.601°W), Wannabe (70.437°N 148.725°W), Egg (70.440°N 148.739°W), Long (70.480°N 148.937°W), and Spy (70.564°N 149.895°W) islands (Fig. 2.1A). The eastern group, hereafter called Mikkelsen Bay; consists of seven islands: Camp (70.172°N 146.226°W), Point Thomson (70.186°N 146.325°W), Mary Saches (70.200°N 146.207°W), North Star (70.225°N 146.347°W), Duchess (70.233°N 146.405°W), Alaska (70.233°N 146.559°W), and Challenge (70.237°N 146.640°W) islands (Fig. 2.1B). Distances between islands within each of the two groups ranged from 1.2–49.2 km, and distances between islands located in Simpson and Mikkelsen Bay ranged from 78.1–143.1 km. Two islands, Camp and Wannabe, are not official names of islands on any recognized maps, but were given these names for the purpose of identifying areas in this study.

Females (n = 198) were captured on nests using a dip net during initial nest searching efforts or with a bow net during late-incubation (Sayler 1962). Blood was collected from the tarsal, brachial, or jugular veins and placed in blood lysis buffer (Longmire et al. 1988). Feather samples (n = 114) were collected from nest bowls from unsampled females and stored in silica gel desiccant at room temperature. Egg samples

(n = 15 from 9 clutches) were collected opportunistically from abandoned or depredated nests or eggs that were cracked while trapping females. Egg membranes were placed in tissue preservation buffer (4.0 M Urea, 0.2 M NaCl, 10 mM EDTA, 0.5% N-Lauroyl-sarcosine, and 100 mM Tris-HCl [pH 8.0]; S. Talbot unpubl. data). Pectoral muscle and heart also were collected from eggs with developed embryos and stored in tissue preservation buffer.

After returning from the field, samples were stored at –80°C at the U. S. Geological Survey Molecular Ecology Laboratory. Genomic DNAs were extracted using either a "salting out" protocol described in Medrano et al. (1990) with modifications described in Sonsthagen et al. (2004), or a QIAGEN DNeasy Tissue Kit (QIAGEN, Valencia, CA). Genomic DNA extractions were quantified using fluorometry and diluted to 50 ng/μL working solutions.

MICROSATELLITE GENOTYPING

Primers used for microsatellite genotyping of Common Eiders (n = 327; Appendix 2.A) were obtained via cross-species screening of microsatellite primers developed for other waterfowl. We screened 12 Common Eiders at 50 microsatellite loci reported to be variable for other waterfowl species and selected 14 microsatellite loci found to be polymorphic: *Aph*02, *Aph*08, *Aph*20, *Aph*23 (Maak et al. 2003); *Bca*μ1, *Bca*μ11, *Hhi*μ3 (Buchholz et al. 1998); *Cm*09 (Maak et al. 2000); *Sfi*μ10 (Libants et al. unpubl. data); *Smo*4, *Smo*7, *Smo*08, *Smo*10, and *Smo*12 (Paulus and Tiedemann 2003). Microsatellites were amplified using the polymerase chain reaction (PCR), and products were

electrophoresed following protocols described in Sonsthagen et al. (2004) for tailed primers (*Aph*02, *Aph*08, *Aph*20, *Aph*23, *Cm*09, *Smo*4, *Smo*7, *Smo*08, *Smo*10, and *Smo*12) and Pearce et al. (2005) for direct-labeled primers (*Bcaμ*1, *Bcaμ*11, *Hhiμ*3, and *Sfiμ*10). For quality control purposes, 10% of the samples were randomly selected, re-amplified, and genotyped in duplicate.

MtDNA and Nuclear Intron Sequencing

We amplified a 545 bp portion of the control region domain I and II (Baker and Marshall 1996) using primer pairs L263 (5'–CCAAATYGCACRYCTGACAYTCCAAGC–3') and H848 (5'–GCCCCATTATRTAGGAGCTGCGG–3') approximately corresponding to positions 263 and 848 in the chicken mtDNA genome (Desjardins and Morais 1990). Only a subset of individuals, those for which we had blood samples, were sequenced (n = 98). PCR amplifications were carried out in a 50 μL volume reaction: 2–100 ng genomic DNA, 0.5 μM each primer, 1.0 μM dNTPs, 1X PCR buffer (Fisher Scientific, Pittsburgh, PA), 2.5 μM $MgCl_2$, and 0.2 units *Taq* Polymerase. PCR reactions began with 94°C for 7 minutes followed by 45 cycles each of 94°C for 20 s; 60°C for 20 s; 72°C for 1 min., concluded by a 7 min. extension at 72°C. PCR products were gel purified using a QIAGEN QIAquick Gel Extraction Kit and both strands were sequenced using Applied Biosystems BigDye v.3 Terminator Cycle Sequencing Kit diluted 4-fold on an ABI 3100 DNA sequencer (ABI: Applied Biosystems, Foster City, CA). Sequences from opposite strands were assembled using Sequencher 4.1.2 (Gene Codes Corporation, Ann Arbor, MI).

Due to the existence of nuclear pseudogenes in avian species (Sorenson and Fleischer 1996), we verified that the amplified sequences were mtDNA control region by comparing sequences from heart and blood samples from five putative mother and offspring groups. Since bird heart tissue is relatively rich in mtDNA and blood is relatively rich in nuclear DNA, any differences in sequences from mother/offspring groups are predicted to reflect the amplification of mtDNA and nuclear pseudogenes. Other studies also have compared heart, blood, and muscle to determine if primers are amplifying true mtDNA and not nuclear pseudogenes (Pearce et al. 2004). Common Eider sequences also were compared to those deposited in GenBank and individuals containing electropherogram double-peaks within mtDNA sequence data were re-sequenced. If co-amplified peaks were still detected at one of the 13 variable sites, presumably due to nuclear pseudogenes present in this species (Tiedemann and Kistowski 1998, S. Sonsthagen unpubl. data) or heteroplasmy, those individuals were removed (~10%). Sequences will be deposited in GenBank (http//:www.ncbi.nlm.nih.gov) upon publication of the results of this dissertation.

Six nuclear introns also were screened for polymorphism in Common Eiders: beta-fibrinogin (*bf*) intron 7 (BF7F2 5'-GTTAGCATTATGAACTGCAAGTAATTG-3'; BF7R2 5'-TTTCTTGAATCTGTAGTTAACCTGATG-3'; M. D. Sorenson unpubl. data), *lamin* A intron 3 (McCracken and Sorenson 2005), chromosome Z chromo-ATPase/helicase/DNA binding protein (*chd*1-W; Fridolfsson and Ellegren 1999), glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) intron 11 (McCracken and Sorenson 2005), and ornithine carboxylase (*od*) intron 7 (OD7F 5'-

TCGTTCAAGCCATTTCTGATGCC-3'; OD8R 5'-CCAGGRAAGCCACCAATRTC-3'; K. McCracken and M. Sorenson unpubl. data). Introns *bf*7, *od*7, and *chd*1-W showed very little variation within Common Eiders, with only 1–2 polymorphic sites in ten individuals. Two of the introns, *gapdh* (386–387 bp; McCracken and Sorenson 2005) and *lamin* A (280 bp; McCracken and Sorenson 2005) showed high levels of polymorphism (14 and 15 positions, respectively) and were sequenced using techniques described above with some modifications. PCR amplifications were carried out in a 50 μL volume; 2–100 ng genomic DNA, 0.5 μM each primer, and 25 μL AmpliTaq Gold PCR master mix (Applied Biosystems, Foster City, California). PCR reactions began with 94°C for 7 minutes followed by 45 cycles each of 94°C for 20 s; 64°C for 20 s; 72°C for 1 min., and ended with a 7 min. final extension at 72°C. Only sequences from the forward strand were collected on an ABI 3100 DNA sequencer because the PCR templates were short (280–387 bp) and sequences had a consistent electropherogram peak height throughout the length of the fragment. Sequences that contained double-peaks of approximately equal peak height, indicating the presence of two alleles, were coded with IUPAC degeneracy codes and treated as polymorphisms (Kulikova et al. 2004). Many sequences for *gapdh* contained a single recurring one base pair indel. To obtain data from the entire fragment for individuals that were heterozygous (~73%) for these alleles, we also sequenced the reverse strand. Sequences will be deposited in GenBank (http//:www.ncbi.nlm.nih.gov) upon publication of the results of this dissertation.

ESTIMATION OF GENETIC DIVERSITY

To determine if the same individual was sampled across multiple years (between feather and blood or feather and feather samples), probabilities of identity for a randomly mating population (PID) and among siblings (PID_{sib}) were calculated in Gimlet 1.3.3 (Valière 2002) using genotypes from the 14 microsatellite loci. Samples with identical genotypes ($n = 9$) across the 14 loci and mother/offspring groups ($n = 9$) were removed from the analyses. For females that switched breeding islands among years ($n = 13$; 38%), we designated the island where the first capture occurred as a female's breeding population to maintain independence among samples.

We sequenced a subset of individuals for mtDNA and the two introns. Islands with low sample sizes were pooled based on geographic proximity (not greater than 3 km) of nests to neighboring islands. Samples from Challenge Island were pooled with Alaska Island, samples from Mary Saches Island were pooled with North Star Island, and samples from Wannabe Island were pooled with Egg Island. Allelic phases for *lamin* A and *gapdh* introns were inferred from diploid sequence data using PHASE 2.0 (Stephens et al. 2001). This program uses a Bayesian approach to reconstruct haplotypes from population genotypic data, and allows for recombination and the decay of linkage disequilibrium (LD) with distance. The PHASE analysis (parameters: 1,000 iterations with a 1,000 burn-in period) was repeated three times to ensure consistency across runs, as suggested by Stephens et al. (2001).

Using only adult breeding females, we calculated allelic frequencies, inbreeding coefficient (F_{IS}), and expected and observed heterozygosities for each microsatellite

locus, mtDNA, and nuclear introns in GENEPOP 3.1 (Raymond and Rousset 1995) and FSTAT 2.9.3 (Goudet 1995, 2001). Hardy Weinberg Equilibrium (HWE) and linkage disequilibrium (LD) were tested in GENEPOP using the default parameters (Markov chain parameters: dememorization number 1,000, number of batches 100, and number of iterations per batch 10,000).

MtDNA control region and nuclear introns *lamin* A and *gapdh* sequences were tested for selective neutrality and historical fluctuations in population demography, using Fu's *Fs* (Fu 1997) and Tajima's *D* (Tajima 1989) in ARLEQUIN. Critical significance values of 5% required a *P*-value below 0.02 for Fu's *Fs* (Fu 1997). Unrooted phylogenetic trees for each gene were constructed in TCS 1.18 (Clement et al. 2000), which estimates genealogies using 95% statistical parsimony probabilities as defined by Templeton et al. (1992). *Lamin* A and *gapdh* intron sequences were also analyzed in NETWORK 4.1.0.8 (Fluxus Technology Ltd. 2004) using the Reduced Median network (Bandelt et al. 1995), to illustrate possible reticulations in the gene trees due to homoplasy or recombination.

Estimation of Population Subdivision

The degree of population subdivision among islands and between each island group were assessed by calculating global and pairwise F_{ST}, R_{ST}, and Φ_{ST} for microsatellite genotype and sequence data in FSTAT 2.9.3 and ARLEQUIN 2.0 (Schneider et al. 2000), adjusting for multiple comparisons using Bonferroni corrections ($\alpha = 0.05$) or permutations (3000) in FSTAT and ARLEQUIN, respectively. Fixation indices (F_{ST}, R_{ST}, and Φ_{ST}) mentioned

above differ in the underlying model used to calculate values; such that, F_{ST} uses the island model, R_{ST} uses the stepwise mutation model developed for microsatellites, and Φ_{ST} uses a nucleotide substitution model that best fits the sequence data. Inter-haplotypic and inter-allelic sequence divergences were used to calculate pairwise Φ_{ST} (Excoffier et al. 1992). MODELTEST 3.06 (Posada and Crandall 1998) was used to determine the minimum parameter nucleotide substitution model that best fit the mtDNA and intron sequence data under the Akaike Information Criterion (Akaike 1974). Pairwise genetic distances between unique haplotypes and alleles were calculated in PAUP* 4.0 (Swofford 1998) for mtDNA and ARLEQUIN for nuclear introns. *P*-values were corrected for multiple comparisons using protocols described in each program. Additionally, a hierarchical analysis of molecular variance (AMOVA) was performed using ARLEQUIN to determine the magnitude of spatial variance in haplotypic and allelic frequencies among populations within and among island groups. An isolation by distance analysis was performed in IBD (Bohonak 2002), with microsatellite data and nuclear intron data using genotypic data inferred from the PHASE analysis, to determine if more geographically distant population pairs are also more genetically differentiated. IBD tests the statistical significance of the relationship between genetic and geographic distance using a Mantel test and calculates slope and intercept from RMA regressions following Sokal and Rohlf (1981) with confidence limits.

Finally, microsatellite data were analyzed in STRUCTURE 2.1 (Pritchard et al. 2000) to detect the occurrence of population structure without *a priori* knowledge of putative populations. Data were analyzed using an admixture model assuming correlated

frequencies to probabilistically assign individuals to putative populations with 10,000 burnin period, 100,000 Markov chain Monte Carlo iterations, number of possible populations (K) ranging from 1–10; this analysis was repeated five times to ensure consistency across runs.

Estimation of Gene Flow Among Populations

We used MIGRATE 2.0.3 (Beerli 1998, 2002, Beerli and Felsenstein 1999) to calculate the number of migrants per generation (N_em) for microsatellite and nuclear intron data and number of female migrants per generation (N_fm) for mtDNA between the two island groups. Full models, θ ($4N_e\mu$, composite measure of effective population size and mutation rate), and all pairwise migration parameters were estimated individually from the data and compared to restricted island models for which θ and pairwise migration parameters are symmetrical among populations.

MIGRATE was run using maximum likelihood search parameters: ten short chains (1,000 used trees out of 20,000 sampled), five long chains (10,000 used trees out 200,000 sampled), and five adaptively heated chains (start temperatures: 1, 1.5, 3, 6, and 12; swapping interval = 1). Full models were run three times to ensure the convergence of parameter estimates. Restricted models were run once. Competing models were evaluated for the goodness of fit given the data using a log-likelihood ratio test. The resulting statistic from the log likelihood ratio test is equal to a χ^2 distribution with the degrees of freedom equal to the difference in the number of parameters estimated in the two models (Beerli and Felsenstein 2001).

RESULTS

GENETIC DIVERSITY

Bi-parentally inherited nuclear microsatellites

The number of alleles per locus at the 14 polymorphic microsatellite loci ranged from 3–44, with an average of 11.3 alleles per locus. The average number of alleles per population ranged from 4.93–8.21. The observed heterozygosity for each population ranged from 11.9–91.8% with an overall value of 57.7%. The inbreeding coefficient (F_{IS}) ranged from –0.071 to 0.060 across all islands with an overall value of 0.027. None of the inbreeding coefficients were significantly different from zero ($P_{adj.} > 0.05$).

Bi-parentally inherited nuclear introns

Twenty-five alleles for nuclear intron *lamin* A were reconstructed from 108 individuals in PHASE (Fig. 2.2B; Appendix 2.B). Sixty (56%) individuals were homozygous at all variable sites, and 22 (20%) individuals were heterozygous at one site. Using PHASE, probabilities of reconstructed haplotypes for individuals that were heterozygous for more than one site ranged from 0.82–0.99 ($n = 22$), except for two individuals with haplotype probabilities of 0.62, and 0.68. PHASE calculated the background recombination rate (ρ) as 0.50, with factors exceeding ρ ranging from 0.58–1.94 between 14 variable sites.

For nuclear intron *gapdh*, PHASE reconstructed 22 alleles from 88 individuals (Fig. 2.2B; Appendix 2.B). Six (7%) individuals were homozygous at all variable sites, and one (1%) individual was heterozygous at one site. Probabilities of all other reconstructed haplotypes ranged from 0.92–1.00 ($n = 57$) and 0.43–0.87 ($n = 24$), which

may be attributable to potentially high levels of recombination occurring within this marker (0.39–4.41 factors exceeding $\rho = 0.05$, between 15 variable sites). There were seven variable sites that exceeded ρ by one or more factors: 2.12 factors between sites 16 and 22, 1.42 factors between sites 22 and 26, 1.12 factors between sites 48 and 49, 1.02 factors between sites 136 and 145, 1.11 factors between sites 165 and 170, 1.36 factors between sites 186 and 192, and 4.41 factors between sites 232 and 252.

Haplotype (h) and nucleotide (π) diversity ranged from 0.600–0.915 and 0.005–0.009, respectively, for *lamin* A, and from 0.874–0.954 and 0.006–0.009, respectively, for *gapdh* (Table 2.1). Observed and expected heterozygosity for *lamin* A was 41.6% and 87.9%, respectively, which significantly deviated from HWE ($P = 0.004$). Observed and expected heterozygosity for *gapdh* was 92.4% and 89.9%, respectively, which also significantly deviated from HWE ($P < 0.001$). Observed and expected heterozygosity for *lamin* A and *gapdh* combined was 67.8% and 88.6%, respectively, which significantly deviated from HWE ($P = 0.004$). Significantly negative values for Fu's *Fs* ($P < 0.02$) were observed for North Star and Mary Saches (*lamin* A –2.690; Table 2.1), Duchess (*lamin* A –4.704; *gapdh* –3.602; Table 2.1), and Long (*lamin* A –4.943; Table 2.1) islands, suggestive of population expansion.

Maternally inherited mtDNA

Eleven unique mtDNA control region haplotypes were resolved from 83 individuals (Fig. 2.2C; Appendix 2.B) defined by 13 variable sites. Haplotype and nucleotide diversity was high for most populations with values for haplotype (h) and nucleotide (π) diversity

ranging from 0.000–0.891 and 0.000–0.009, respectively (Table 2.1). Spy Island was monotypic for mtDNA control region variation. Other islands were represented by 2–6 unique haplotypes, with Duchess Island having the highest number of unique haplotypes (Table 2.1). Neutrality tests found no evidence for selection (Fu's $Fs = 0.090–2.139$, $P > 0.02$; Tajima's $D = –1.295–0.591$, $P > 0.05$; Table 2.1).

POPULATION STRUCTURE

Bi-parentally inherited nuclear microsatellites

After removing mother/offspring groups and identical genotypes ($n = 18$) at 14 microsatellite loci, the overall F_{ST} (global 0.004, $P = 0.007$) was significant. However, we did not observe a significant level of differentiation using a R_{ST} based approach (global –0.004, $P > 0.05$). Our overall estimate of population subdivision was low, and was not detected using the Bayesian clustering method implemented by the program STRUCTURE. The most likely model generated from the microsatellite data was maximized when the total number of populations was one. In addition, we did not detect any significant pairwise F_{ST} and R_{ST} comparisons among islands. However, the comparison between Mikkelsen Bay and Simpson Lagoon was significant ($F_{ST} = 0.004$, $P = 0.016$; Table 2.2). Moreover, a hierarchical analysis of molecular variance uncovered low but significant variance within populations and populations within a group using the F_{ST} based approach (Table 2.2). Finally, we found no evidence of isolation by distance correlations between genetic and geographic distances ($r = 0.012$, $P = 0.46$).

Bi-parentally inherited nuclear introns

Levels of spatial genetic structure for nuclear intron sequences were calculated using a nucleotide substitution model. MODELTEST indicated that the nucleotide substitution model that best fit the intron sequence data was the Tamura-Nei (1993) model with an invariant site parameter for both *lamin* A and *gapdh*. We detected significant differences in the spatial distribution of allelic frequencies for *lamin* A (global $\Phi_{ST} = 0.023$, $P = 0.02$) among islands. AMOVA detected significant variance among populations within groups and within populations (Table 2.2). A pairwise comparison between Mikkelsen Bay and Simpson Lagoon island groups also was significant for *lamin* A ($\Phi_{ST} = 0.022$, $P = 0.02$; Table 2.2). Inter-island comparisons indicated large significant pairwise differences within *lamin* A ($\Phi_{ST} = 0.089$–0.173; Table 2.3), but not for *gapdh* (global $\Phi_{ST} = -0.071$, $P = 0.94$; Tables 2, 3). As with the microsatellite data, we detected no significant correlations between genetic and geographic distances for *lamin* A and *gapdh* combined ($r = 0.096$, $P = 0.28$) or analyzed separately (*lamin* A $r = -0.012$, $P = 0.050$; *gapdh* $r = 0.142$, $P = 0.20$).

Since we observed significant pairwise comparisons within *lamin* A, we also calculated F_{ST} values for each polymorphic site in FSTAT. Significant F_{ST} values occurred at one of the 14 polymorphic positions: site 116 ($F_{ST} = 0.153 \pm 0.084$). Significant pairwise comparisons among islands were calculated for position 116, with significant F_{ST} values ranging from 0.053–0.352 (Table 2.4). However, site 116 is monomorphic for all islands in Mikkelsen Bay, therefore, the combination of several sites

and the presence of rare alleles may be driving the observed population differentiation within Mikkelsen Bay.

Maternally inherited mtDNA

Population subdivision estimates also were calculated using a nucleotide substitution model. MODELTEST indicated that the nucleotide substitution model that best fit the data was the Tamura-Nei (1993) model with an invariant site parameter (substitute rate matrix: R[A–C] = 1.0000, R[A–G] = 34.6051, R[A–T] = 1.0000, R[C–G] = 1.0000, R[C–T] = 23.3368, R[G–T] = 1.0000, p–inv. = 0.8325, A = 0.2179, C = 0.3064, G = 0.1940, T = 0.2817). Mean inter-population variance in haplotypic frequency was low (global Φ_{ST} = 0.070, P = 0.05), along with a pairwise comparison between Mikkelsen Bay and Simpson Lagoon (Φ_{ST} = 0.082, P = 0.05; Table 2.2). Given the geographic proximity of these island groups, significant inter-population variances in haplotypic frequency (Φ_{ST}) of 0.05 are noteworthy (Wright 1951). In addition, we observed high levels of genetic discordance between Duchess (Mikkelsen Bay) and all four islands located in the Simpson Lagoon (Φ_{ST} = 0.135–0.271; Table 2.4). Finally, an AMOVA detected significant variance within populations and among populations within each group (Table 2.2), consistent with female philopatry over relatively short geographic distances (Scribner et al. 2001).

ESTIMATES OF GENE FLOW

Analyses using the software STRUCTURE detected no population subdivision among samples, however, we tested a two-population model in MIGRATE based on geographic proximity of islands. Individuals breeding on islands located in Mikkelsen Bay and Simpson Lagoon were treated as separate populations (Fig. 2.1, Table 2.5). There appears to be asymmetrical dispersal between island groups in the Beaufort Sea across all marker types, though some comparisons (mtDNA and nuclear introns) are not significant based on overlapping 95% confidence intervals. The biases in the variances and the means indicate that, on average over generations, gene flow is greater from Mikkelsen Bay to Simpson Lagoon than vice versa (Table 2.5). N_em and θ values calculated in MIGRATE from microsatellite genotypes, mtDNA, and nuclear intron sequence data ranged from 5.1–24.2 migrants per generation from Simpson Lagoon to Mikkelsen Bay with θ ranging from 0.001–0.683, and 24.4–34.2 migrants per generation from Mikkelsen Bay to Simpson Lagoon with θ ranging from 0.006–0.635 (Table 2.5).

The full model (all parameters allowed to vary independently) was found to have significantly higher likelihoods than the restricted island model (equal inter-population migration rate and equal θ across populations) for gene flow estimates based on microsatellite allele, mtDNA haplotype, and nuclear intron allele frequencies ($P < 0.001$; Table 2.5), indicating gene flow is asymmetric between Mikkelsen Bay and Simpson Lagoon.

FEMALE SITE FIDELITY

Analyses using the software Gimlet calculated an overall PID of 3.2×10^{-12} for a population composed of randomly mating individuals and 5.3×10^{-5} for siblings using genotypes collected from 14 microsatellite loci. These denominator values are much larger than the population breeding on islands in the western Beaufort Sea (approximately 500 nests found on the islands each year; Johnson 2000), which gave us confidence that identical genotypes for samples taken from different years were the same individual. Nine females were found to have identical genotypes; 56% had matching genotypes with feather samples taken on the same island in later years. Additionally, 25 females were captured in multiple years. The majority of these (n = 16/25; 64%) were recaptured on the same island. The remaining females switched breeding islands in subsequent years.

Throughout the course of the 4-year study, 34 females were detected breeding in two different years (based on observations of banded individuals and genetic techniques). Most (n = 21/34; 62%) nested on the same islands, whereas 13 (38%) females switched breeding islands. Inter-nest distances between breeding attempts ranged from 1.1–12.1 km using band recapture data (J. Reed unpubl. data), and 1.1–12.5 km using genetic recapture data. We found no evidence for female dispersal between Mikkelsen Bay and Simpson Lagoon. Females that did disperse to a different nest site between years generally moved to an adjacent island within the same island group to breed (9 of 13; 69%). However, three females breeding in Mikkelsen Bay moved from islands in the bay to islands closer to the coast (Alaska to Pt. Thomson Island, 12.1 and 12.5 km; Duchess

to Camp Island, 10.2 km). One female breeding in Simpson Lagoon dispersed three islands east of her original nest site (Long to Stump Island, 10.0 km).

DISCUSSION

Population Genetic Structure

Population subdivision was uncovered at all marker types. Comparability higher levels of structure were observed at maternally inherited mtDNA than bi-parentally inherited nuclear introns and microsatellite loci for inter-island comparisons, which is consistent with our prediction and known patterns of dispersal. The magnitude of differentiation decreased for mtDNA and nuclear intron *lamin* A when islands were combined into island groups, but it increased for microsatellites. Patterns of genetic structure were similar to those observed in Tiedemann et al. (1999); however, higher differentiation among colonies of similar geographic distance was observed. Tiedemann et al. (1999) proposed that the main mechanism promoting genetic subdivision among populations in the Baltic Sea was differences in migration phenology among geographic regions coupled with a selective advantage of early pair formation. However, differences in migratory phenology do not appear to occur among island groups in the Beaufort Sea, as satellite telemetry data indicate that there is no difference in the start of autumn migration among eiders breeding in the Beaufort Sea and Yukon-Kuskokwim Delta, Alaska (approximately 1250 km southwest of Beaufort Sea population; Petersen and Flint 2002). Lack of differences in migration phenology between island groups may explain, in part,

the lower levels of differentiation observed as island groups likely admix on the wintering grounds.

Low levels of population structure resolved based on microsatellite markers were expected, mainly due to aspects of Common Eider breeding and wintering biology. Although female Common Eiders are reported to be highly philopatric to natal and breeding sites (*S. m. dresseri*, Reed 1975; *S. m. mollisima*, Swennen 1990), male eiders have large natal and breeding dispersal distances (0–1270 km; Swennen 1990). Tiedemann et al. (1999) reported evidence of non-random mating within Common Eiders breeding in the Baltic Sea, based on significant F_{ST} values, such that males tended to pair with females from the same breeding area among populations. We did not observe significant inter-population comparisons; however, our overall F_{ST} was significant. Additionally, we did detect significant genetic discordance in allelic frequencies between Simpson Lagoon and Mikkelsen Bay. Significant structuring, albeit low, at this marker could be a result of high female philopatry to island groups (Reed 1975, Swennen 1990). However, random mating on the wintering ground should homogenize gene frequencies in the nuclear genome through male-mediated gene flow (Scribner et al. 2001, Pearce et al. 2004).

Based on the number of nests found each year, Simpson Lagoon appears to support a larger number of breeding birds than Mikkelsen Bay (Johnson 2000, S. Sonsthagen per. obs.). Scribner et al. (2001) indicated that unequal population sizes among studied sites could bias estimates of population subdivision. Individuals from populations that are larger would appear to mate assortatively, given the higher

probability of mating with an individual from the same colony. Assortative mating among sites would have an upward bias on estimators of subdivision. Therefore, there could be greater gene flow than F_{ST} reflects. Though significant, our estimate of population structure is low enough to allow for high levels of gene flow among sampled sites. Satellite telemetry data from breeding female eiders, likewise, show that eiders from the Beaufort Sea share wintering areas with eiders breeding on the Kent Peninsula, Canada, western Alaska, and eastern Russia (Petersen and Flint 2002; L. Dickson, pers. comm., M. Petersen pers. comm.). Because individuals winter in admixed groups, consisting of many breeding populations, there is a potential for pairing of females from the Beaufort Sea with males from other areas.

The high level of population structure we observed within nuclear intron *lamin* A is surprising because we observed low to no population structure in the microsatellite markers and nuclear intron *gapdh*. As mentioned previously, our estimates of population structuring could be biased due to the assumption of equal breeding population sizes among islands (Scribner et al. 2001). Alternatively, we may not have observed high levels of population subdivision in the microsatellite data due to fragment size homoplasy. However, for the mutation process, and therefore homoplasy, to have an effect on estimators of population subdivision, subpopulations need to have different ratios of coalescent times of genes long enough to have two or more mutational events to occur (Estoup et al. 2002). Since our estimate of subdivision for F_{ST} (assumes migration is driving subdivision) was greater than R_{ST} (assumes mutation is the driving subdivision; O'Reilly et al. 2004) and because of the relatively close geographic proximity of the

islands, mutation, and therefore homoplasy, is likely not playing a major role in differentiating populations of eiders breeding in the western Beaufort Sea. Moreover, PHASE estimated relatively low rates of recombination between variable sites within *lamin* A and higher recombination rates between variable sites within *gapdh*. The higher recombination rate observed within *gapdh* may have masked population structure among islands. *Lamin* A may be an "outlier" locus, as it may be in LD with a target of natural selection, which may have inflated Φ_{ST} (Storz et al. 2004). Charlesworth et al. (1997) stated that local adaptation tends to increase population differentiation at loci under selection, and very high F_{ST} values may be observed at closely linked neutral loci. *Lamin* A, thus, may be under balancing selection and coupled with genetic drift could create more alleles than what would be expected by chance. Low observed heterozygosity (H_o = 0.416, H_e = 0.879) and the larger number of alleles reconstructed by PHASE relative to *gapdh* (*lamin* A 70 alleles and *gapdh* 48 alleles; S. Sonsthagen unpubl. data) are consistent with this hypothesis. However, we did not find any evidence to indicate that *lamin* A is not selectively neutral (Table 2.1).

Comparatively higher levels of population subdivision assayed using mtDNA than nuclear DNA also could be attributed to lineage sorting. MtDNA has a lower effective population size relative to nuclear DNA. Therefore, when mutation rate and selection are held constant, genetic drift has a larger effect on mtDNA than nuclear DNA (Avise 2004), translating in higher estimates of population subdivision (F_{ST}). The effects of lineage sorting and sex-biased differences in philopatry on spatial genetic subdivision are not mutually exclusive and both may be playing a role in the degree of population

structure observed. However, microsatellite loci have a high rate of mutation relative to mtDNA control region (Avise 2004) resulting in new mutations arising more frequently within populations. By chance alone, one would expect new mutations to increase in frequency among isolated populations and dampen the effects of incomplete lineage sorting within microsatellite loci. Given differences in the degree of philopatry in Common Eiders between the sexes and congruence in results between microsatellite and nuclear intron loci, differences in estimates of population subdivision may be more attributable to male dispersal and high natal and breeding philopatry in females rather than incomplete lineage sorting for the Beaufort Sea population.

We observed high levels of population structure within the maternally inherited mtDNA control region. Significant population subdivision was observed between Duchess Island, located in Mikkelsen Bay, and all islands located in Simpson Lagoon. While we did not detect significant pairwise comparisons among all islands in Mikkelsen Bay and Simpson Lagoon, we believe that the significant structuring observed is noteworthy given that Duchess Island is the only island that contains a colony of breeding Common Eiders in Mikkelsen Bay. Nests on the remaining islands were scattered with relatively few nests per island. The presence of a colony on Duchess Island is likely driving the significant pairwise comparisons observed at this marker. Common Eiders are typically colonial nesters (Goudie et al. 2000) and the low-density nesters could be "overflow" from Duchess Island, though demographic data are needed to confirm this hypothesis. Although there are also colonies on three islands in Simpson Lagoon (Egg, Long, and Stump islands), these colonies occur on islands that are adjacent to each other

and thus unlikely to be genetically isolated as birds may disperse among islands. While we do not have natal dispersal data for this population, we do have breeding dispersal distances from recaptured individuals. Given that no breeding females dispersed between Mikkelsen Bay and Simpson Lagoon, we hypothesize that females breeding in the western Beaufort Sea are strongly philopatric to island groups rather than to a particular island. This differs from observations of *S. m. dresseri* breeding in Maine (Wakely and Mendall 1976). Distances among islands in Maine are similar to those observed in our study (1.7–24.3 km apart), however, 71% of females returned to their previous breeding island, and only 2% dispersed to neighboring islands (estimated 27% mortality rate; Wakely and Mendall 1976). Over many generations, females dispersing among neighboring islands would have a homogenizing effect within island groups while maintaining population subdivision between island groups.

Behavioral responses to a more stochastic arctic environment may play a role in the differences in the degree of breeding philopatry observed between Maine and Beaufort Sea eiders. Common Eider nests in the western Beaufort Sea are associated with driftwood (Goudie et al. 2000, Johnson 2000), and changes in driftwood locations will affect where eiders nest. Storms dramatically modify the shape and topography of these barrier islands, thus changing where available habitat is located annually (Noel et al. 2005, S. Sonsthagen pers. obs.). Finally, eiders breeding on the Beaufort Sea postpone nesting attempts until the island is surrounded by open water, reducing predation risk (Schamel 1977). Islands located in the same vicinity may not be surrounded by water at the same time (S. Sonsthagen pers. obs.). Therefore, in years

when ice break-up is late, eiders may initiate nesting on the first "suitable" island regardless of where they nested in previous years or hatched from because of the presumed selective advantage to nesting early (Milne 1974).

GENE FLOW

We do not completely understand the factors that influence the degree of migratory and homing behavior in eiders. Eiders appear to move the minimum distance to wintering areas (Petersen and Flint 2002), and the degree of movement is likely environmentally induced (Swennen 1990), which may explain, in part, the directionality of gene flow observed at microsatellite and mtDNA markers.

Microsatellite and nuclear intron loci indicate significant asymmetrical gene flow, such that, on average, more individuals were dispersing from Mikkelsen Bay to Simpson Lagoon (i.e., east to west) over evolutionary time. Since eiders breeding in the Beaufort Sea share a wintering area with eiders from other populations, there may be clinal variation of allele frequencies occurring across populations that share wintering areas. Clinal variation at nuclear-based characteristics that may be under selection (e.g., plumage) has been observed in waterfowl and other avian species (Cooke et al. 1988, Smallwood et al. 1999). However, we did not observe a significant correlation between genetic and geographic distances. Therefore, samples from a larger geographic area are needed to confirm this hypothesis.

Asymmetrical gene flow from east to west observed for mtDNA appears to be consistent with estimates based on nuclear loci. Young female birds or failed breeders

from the previous year from Mikkelsen Bay, returning from the wintering grounds to breed may stop earlier on their migration and attempt to breed at Simpson Lagoon. Islands become ice-free about two weeks earlier in Simpson Lagoon than in Mikkelsen Bay, likely due to the large volume of water flowing out of the Kuparuk River. This appears to expedite ice break-up on the nearby barrier islands (Schamel 1977, S. Sonsthagen pers. obs), enabling eiders to initiate nests and hatch broods sooner. Islands that first become free of ice produce the earliest broods in other populations (Ahlén and Andersson 1970). Should females from Mikkelsen Bay stop early on spring migration at Simpson Lagoon and successfully hatch young, they may be more likely to nest in Simpson Lagoon in succeeding years (Milne 1974). Thus, earlier nest initiation and previous nest success may be factors influencing females that hatched in Mikkelsen Bay to breed in Simpson Lagoon for the first time and then return there in successive years to breed. It is important to note that this pattern of westward dispersal would have to occur over many generations to be observed genetically. Thus, increasingly early ice break-up in Simpson Lagoon may have driven the westward bias in dispersal over evolutionary time. However, evidence from Common Eiders and other arctic nesting waterfowl suggests that young females initiate nesting later than older females (Johnson et al. 1992). Thus, open water may be present in both areas when young females are ready to nest, depending on the timing of ice break-up in a given year. Alternatively, Common Eiders may be dispersing west due to the distribution of available nest sites. Within the two study areas, Simpson Lagoon has more available nesting habitat relative to Mikkelsen Bay, based on the number of nests found in each island group in a given year (Johnson

2000, S. Sonsthagen pers. obs.). Female eiders hatched from Mikkelsen Bay arriving later to the breeding ground, such as first time breeders, may simply "choose" or be forced to nest in Simpson Lagoon due to unavailability of nest sites in Mikkelsen Bay. This may be particularly true for young female eiders that tend to arrive later from the winter grounds as females arriving earlier on the breeding grounds may have already secured many suitable nest sites. Over evolutionary time, the limited availability of nest sites could also be influencing the dispersal pattern observed. It is important to note that one bout of random dispersal per generation among individuals breeding in the western Beaufort Sea could homogenize gene frequencies among islands. Therefore, western biased dispersal must have occurred over many generations.

COMPARISON TO OTHER WATERFOWL

The fine-scaled spatial genetic structuring that we observed in Common Eiders breeding on island groups 90 km apart in the western Beaufort Sea is exceptional, especially when compared to other arctic nesting waterfowl. Pearce et al. (2004) examined levels of population subdivision within the Holarctic nesting King Eider (*S. spectabilis*) using mtDNA cytochrome *b* sequence data and genotypes from six nuclear microsatellite loci. Estimates of inter-population allelic and haplotypic frequencies were not significantly different indicating panmixia across sampled sites in Russia, Alaska, and Canada. Stable isotope data from King Eiders suggest high levels of dispersal among western and eastern arctic populations (Mehl et al. 2004), which the authors contended is likely homogenizing gene frequencies among sampled sites. Levels of population structure also were assessed

for Harlequin Ducks (*Histrionicus histrionicus*) breeding in Alaska (Lanctot et al. 1999). The authors did not detect any significant genetic discordance among sampled sites at four autosomal microsatellite loci, two *Z*-specific microsatellite loci, and mtDNA control region. Lack of structure was attributed to recent range expansion and thus insufficient time for genetic differences to evolve, stochastic events causing episodic dispersal, and low levels of dispersal among regions.

Among other waterfowl, population subdivision has been documented to varying degrees. Pearce et al. (2005) assessed population genetic structuring at seven microsatellite loci and cytochrome *b* mtDNA sequence among Steller's Eiders (*Polysticta stelleri*) breeding in Alaska and Russia. Low inter-population estimates of subdivision were observed at microsatellite loci (F_{ST} = 0.002–0.007). However, estimates based on mtDNA were not significant. In contrast, Scribner et al. (2001) documented high levels of differentiation in mtDNA among sampled sites in Spectacled Eiders (*S. fisheri*, Φ_{ST} = 0.242). However, the authors did not detect any differences in allelic frequencies within the nuclear genome at five autosomal microsatellite loci and one *Z*-linked microsatellite locus. Canada Geese (*Branta canadensis*) also exhibit high levels of genetic differentiation among sampled sites at five autosomal microsatellite loci (F_{ST} = 0.077), one *Z*-linked microsatellite locus (F_{ST} = 0.116), and mtDNA control region (Φ_{ST} = 0.177; Scribner et al. 2003). While these studies all documented significant differences in gene frequencies among sampled sites, studies were conducted at much larger spatial scales than our study.

Differences in the degree of population subdivision could be attributed to behavioral characteristics of individual species. Several aspects of the biology of many of these species are similar to that of Common Eiders, including: (1) exhibition of some degree of breeding site fidelity, (2) seasonal migratory behavior, (3) population admixture in large winter aggregations, (4) formation of pair-bonds in winter months with males following females back to winter sites, and (5) seasonal monogamy. In contrast to Common Eiders, many waterfowl species are monotypic across their range and show little to no population structuring (Newton 2003). Species that exhibit fine scale spatial structure, likely have high natal, breeding, and winter site philopatry, as has been indicated for Common Eiders (Goudie et al. 2000).

CONCLUSIONS

It appears that Common Eiders breeding in Simpson Lagoon are genetically differentiated relative to those breeding in Mikkelsen Bay, as we observed significant levels of population subdivision across all marker types. Therefore, Common Eiders breeding in each area may host populations that are demographically independent, though more demographic data are needed to confirm this hypothesis. Common Eiders appear to have high natal and breeding philopatry, as shown by the high inter-population variance estimates (Φ_{ST}) calculated for mtDNA and restricted female dispersal between island groups as shown by recapture data. In the event that a breeding area was extirpated it may be unlikely that the area would be easily re-colonized naturally by females hatched elsewhere, despite high levels of gene flow mediated by male dispersal (Wakely and

Mendall 1976, Avise 2004). Additionally, these data illustrate an important point. Genetic discordance can exist on very small spatial scales relative to the species dispersal capabilities and known male dispersal distance. High natal philopatry observed in waterfowl, as seen in Common Eiders, can have demonstrable effects on the degree of genetic partitioning among populations.

This study was the first, to our knowledge, to use nuclear introns in assessing inter-population variation in allelic frequencies at a microgeographic scale. Introns pose new challenges to phylogenetic and population genetic analysis, such as recombination impeding gene tree reconstruction (Hare 2001) and selective sweeps potentially confounding gene flow estimates (Storz et al. 2004). However, high levels of variation found in loci potentially under balancing selection can provide valuable insight on historic processes influencing population demography. Advancements in analytical tools have enabled researchers to address issues of recombination (e.g., PHASE) and selective sweeps (DetSel, Vitalis et al. 2003; Fu's *Fs*, Tajima's *D* in ARLEQUIN) and use these types of markers for population genetic analyses. Finally, these data provide further evidence for the need to use multiple marker types with varying modes of inheritance. If researchers were to restrict their investigation to either nuclear or mtDNA markers when genetically characterizing populations, studies could under or over estimate levels of population structure. As seen in Common Eiders, nuclear and mtDNA markers show varying levels of genetic spatial partitioning. Not utilizing molecular markers with different modes of inheritance and evolution could mislead researchers characterizing the genetic variation within this population.

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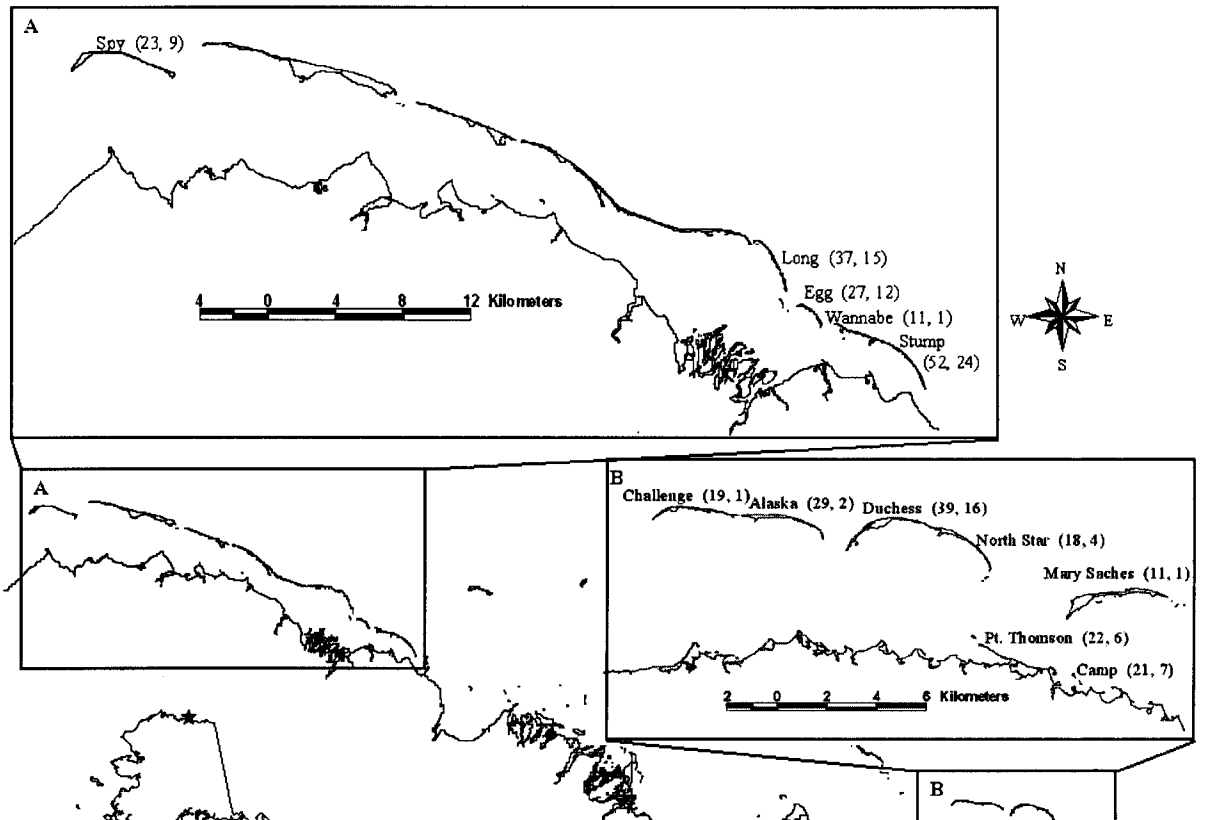

Figure 2.1: Beaufort Sea barrier islands located in (A) Simpson Lagoon (western group) and (B) Mikkelsen Bay (eastern group) with sample sizes for each island in parentheses, the first value is the number of samples (blood and feather) genotyped at 14 microsatellite loci and the second value is the number of samples (blood) sequenced for mtDNA and two nuclear introns. Wannabe and Camp islands are designations used by the authors and are not official names of islands.

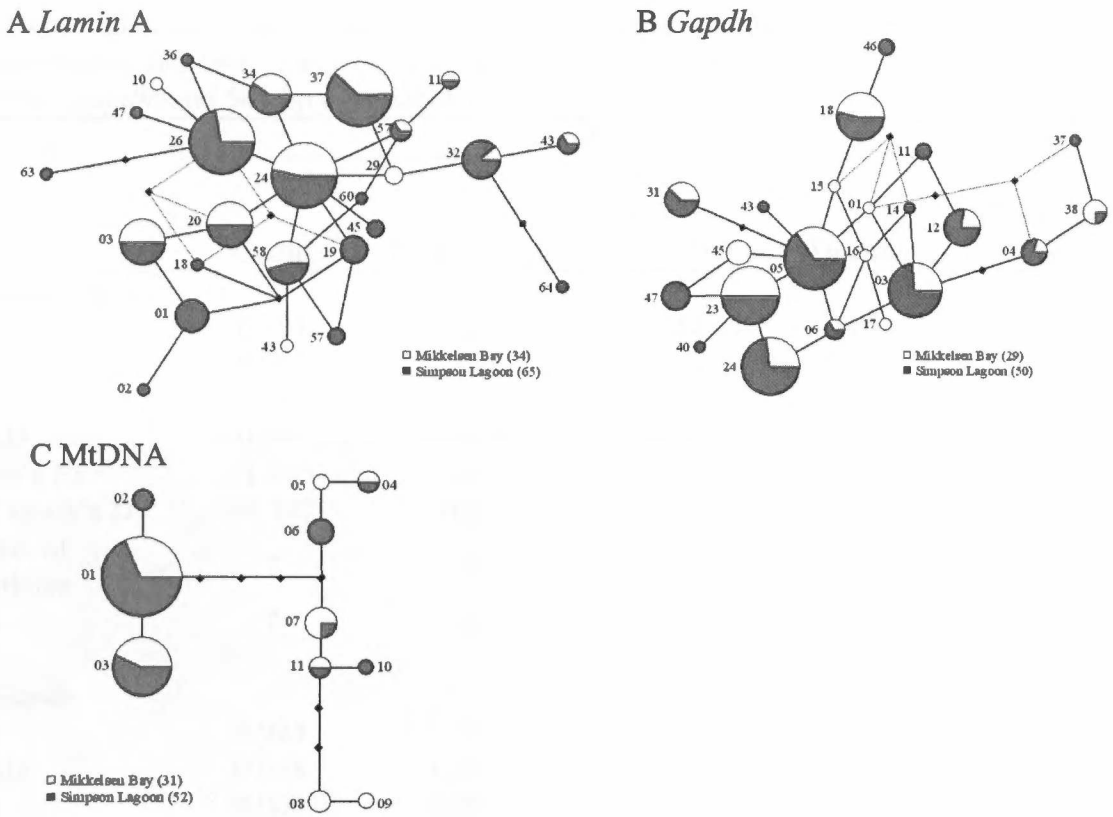

Figure 2.2: Unrooted parsimony tree illustrating relationships of (A) 25 *lamin* A alleles, (B) 22 *gapdh* alleles, and (C) 11 mtDNA control region haplotypes. The 95% probability set of parsimony trees are illustrated with bold branches, with the size of the circle node corresponding to the frequency of each allele. Gray lines indicate alternative branching patterns and possible reticulations. Small black squares indicate intermediate ancestral alleles that were not sampled. Mikkelsen Bay (eastern group) alleles are illustrated in white and Simpson Lagoon (western group) alleles are illustrated in gray. Sample sizes are shown in parentheses.

Table 2.1: Estimates of genetic diversity, including; nucleotide (π) and haplotype (h) diversity (including the standard deviation SD), number of unique haplotypes per population, and sample size (n), for 280 bp of nuclear intron *lamin* A, 387 bp of nuclear intron *gapdh*, and 545 bp of mtDNA control region.

	Mikkelsen Bay				
	Camp	Pt. Thomson	Mary Saches & North Star	Duchess	Challenge & Alaska
***Lamin* A**					
h	0.901	0.864	0.844	0.884	0.600
SD	0.047	0.072	0.103	0.034	0.215
π	0.009	0.006	0.005	0.007	0.005
SD	0.006	0.004	0.004	0.005	0.004
Fu's *Fs* *	–1.503	–2.004	**–2.690**	**–4.704**	0.381
Tajima's *D*	–0.147	–0.212	–0.682	0.226	0.338
No. of alleles	7	6	6	11	3
n	7	6	5	14	3
Gapdh					
h	0.924	0.911	0.927	0.909	0.933
SD	0.058	0.077	0.084	0.037	0.122
π	0.006	0.009	0.007	0.006	0.008
SD	0.004	0.006	0.005	0.004	0.006
Fu's *Fs* *	–1.584	–0.907	–0.930	**–3.602**	–1.466
Tajima's *D*	–0.323	–0.810	–0.856	–0.989	–0.631
No. of alleles	8	7	6	11	5
n	6	5	4	11	3
MtDNA					
h	0.333	0.800	0.400	0.891	0.667
SD	0.215	0.172	0.237	0.063	0.314
π	0.002	0.007	0.001	0.009	0.001
SD	0.002	0.004	0.001	0.005	0.001
Fu's *Fs* *	2.139	0.567	0.090	0.543	0.201
Tajima's *D*	–1.295	–0.516	–0.817	0.591	0.000
No. of haplotypes	2	3	2	6	2
n	6	6	5	11	3

* Significant *P*–values are in bold text; Fu's *Fs* ($P < 0.02$) and Tajima's *D* ($P < 0.05$).

Table 2.1 cont.

	Simpson Lagoon			
	Stump	Wannabe & Egg	Long	Spy
***Lamin* A**				
h	0.849	0.818	0.915	0.876
SD	0.036	0.052	0.027	0.045
π	0.008	0.007	0.009	0.008
SD	0.005	0.004	0.006	0.005
Fu's *Fs* *	–3.499	–3.033	**–4.943**	–1.896
Tajima's *D*	–0.169	–0.126	–0.119	0.070
No. of alleles	12	10	13	8
n	24	13	15	9
Gapdh				
h	0.900	0.874	0.886	0.954
SD	0.024	0.032	0.036	0.047
π	0.006	0.006	0.007	0.007
SD	0.004	0.004	0.004	0.005
Fu's *Fs* *	–2.850	–0.773	–0.996	–2.162
Tajima's *D*	–0.853	–0.836	–0.306	–0.964
No. of alleles	13	8	10	9
n	18	13	13	6
MtDNA				
h	0.579	0.526	0.654	0.000
SD	0.114	0.152	0.106	0.000
π	0.003	0.003	0.003	0.000
SD	0.002	0.002	0.002	0.000
Fu's *Fs* *	0.183	0.868	0.399	–
Tajima's *D*	–0.869	–0.167	–1.249	0.000
No. of haplotypes	5	4	4	1
n	19	13	13	7

* Significant *P*–values are in bold text; Fu's *Fs* ($P < 0.02$) and Tajima's *D* ($P < 0.05$).

Table 2.2: Hierarchical analysis of molecular variance (AMOVA) of allelic and haplotypic frequencies for islands within Mikkelsen Bay and Simpson Lagoon. Significant comparisons are in bold text.

Source of Variation	d.f.	Variance components	% of total variation	Φ	*P*–value
Microsatellite – F_{ST}					
Variance among group	1	0.003	0.08	0.001	0.247
Variance among pop. within group	10	0.011	0.34	**0.003**	**0.042**
Variance within populations	614	3.251	99.58	**0.004**	**0.016**
Total	625	3.264	–	–	–
Microsatellite – R_{ST}					
Variance among group	1	0.575	0.39	0.004	0.140
Variance among pop. within group	10	–0.106	–0.07	–0.001	0.652
Variance within populations	614	145.949	99.68	0.003	0.449
Total	625	146.418	–	–	–
***Lamin* A**					
Variance among group	1	–0.004	–0.34	–0.003	0.478
Variance among pop. within group	7	0.027	2.50	**0.025**	**0.012**
Variance within populations	193	1.066	97.84	**0.022**	**0.022**
Total	201	1.089	–	–	–
Gapdh					
Variance among group	1	–0.000	–1.00	–0.000	0.685
Variance among pop. within group	7	–0.020	–1.89	–0.019	0.933
Variance within populations	149	1.061	101.90	–0.019	0.929
Total	157	1.042	–	–	–
Mitochondrial DNA					
Variance among group	1	0.000	2.78	0.028	0.202
Variance among pop. within group	7	0.000	5.38	**0.055**	**0.030**
Variance within populations	74	0.002	91.84	**0.082**	**0.047**
Total	82	0.002	–	–	–

Table 2.3: Estimates of pairwise inter-population variance in allelic frequency (Φ_{ST}) values calculated for *lamin* A (above diagonal) and *gapdh* (below diagonal) for Common Eiders from each pair of nine islands breeding in the Beaufort Sea. Significant pairwise comparisons ($\alpha = 0.05$) are in bold text.

		Mikkelsen Bay				Simpson Lagoon			
Population [a]	Camp	Pt. Thomson	Mary Saches & North Star	Duchess	Alaska & Challenge	Stump	Wannabe & Egg	Long	Spy
Camp	–	–0.034	–0.007	0.001	0.088	0.074	0.027	0.026	0.052
Pt. Thomson	–0.027	–	0.034	0.009	**0.173**	0.037	0.059	0.024	**0.089**
Mary Saches & North Star	–0.025	–0.013	–	–0.029	0.119	–0.025	0.003	0.003	0.033
Duchess	0.002	0.026	–0.053	–	0.024	–0.007	–0.002	0.007	0.053
Alaska & Challenge	–0.110	–0.126	–0.056	–0.020	–	0.003	–0.042	0.024	**0.148**
Stump	–0.029	0.024	–0.031	0.023	–0.039	–	–0.012	0.022	**0.092**
Wannabe & Egg	–0.036	–0.032	–0.021	0.000	–0.071	0.002	–	0.025	**0.106**
Long	–0.037	–0.042	–0.040	–0.009	–0.082	0.003	–0.028	–	0.022
Spy	–0.060	–0.059	–0.035	0.002	–0.093	–0.023	–0.054	–0.049	–

[a] Islands are listed East to West.

Table 2.4: Pairwise Φ_{ST} values calculated for 545 bp of mtDNA control region (above diagonal) and *lamin* A site 116 (below diagonal) for Common Eiders breeding from each pair of nine islands breeding in the Beaufort Sea. Significant pairwise comparisons ($\alpha = 0.05$) are in bold text.

		Mikkelsen Bay				Simpson Lagoon			
Population [a]	Camp	Pt. Thomson	Mary Saches & North Star	Duchess	Alaska & Challenge	Stump	Wannabe & Egg	Long	Spy
Camp	–	–0.085	–0.018	0.097	–0.058	–0.083	–0.082	0.000	0.028
Pt. Thomson	–	–	0.032	–0.024	–0.174	–0.024	–0.082	0.026	0.127
Mary Saches & North Star	–	–	–	0.201	–0.299	–0.066	0.040	–0.095	0.073
Duchess	–	–	–	–	0.116	**0.183**	**0.135**	**0.230**	**0.271**
Alaska & Challenge	–	–	–	–	–	–0.141	–0.002	–0.199	0.300
Stump	–0.035	–0.042	–0.051	–0.012	–0.092	–	–0.010	–0.023	0.008
Wannabe & Egg	–0.032	–0.040	–0.050	–0.007	–0.091	–0.024	–	0.037	0.068
Long	0.121	0.102	0.078	**0.205**	–0.006	0.217	**0.169**	–	0.029
Spy	0.208	**0.182**	**0.151**	**0.330**	**0.053**	**0.352**	**0.284**	–0.086	–

[a] Islands are listed East to West.

Table 2.5: Comparison of alternative models of Common Eider gene flow between Mikkelsen Bay and Simpson Lagoon. Full model migration matrix (allowing all parameters to vary independently) and restricted model (symmetrical gene flow) migration rates calculated from 14 microsatellite loci, *lamin* A and *gapdh*, and mtDNA control region, were evaluated for significance using a log likelihood ratio test. Ninety-five percent confidence intervals are in parentheses.

				Simpson Lagoon to Mikkelsen Bay		Mikkelsen Bay to Simpson Lagoon	
Marker	Hypothesis	Ln(L)	*P*–value	$N_f m$ or $N_e m$	θ	$N_f m$ or $N_e m$	θ
Microsatellites	Full	–8782.1	<0.001	18.8	0.683	27.1	0.635
				(17.8–20.3)	(0.650–0.717)	(25.4–29.6)	(0.612–0.659)
	Restricted	–8888.0		78.3	2.247	78.3	2.247
Nuclear introns	Full	–401.8	<0.001	24.2	0.003	34.2	0.010
				(18.6–31.5)	(0.003–0.004)	(26.7–43.7)	(0.009–0.011)
	Restricted	–468.9		22.3	0.006	22.3	0.006
MtDNA	Full	1.9	<0.001	5.1	0.001	24.4	0.006
				(0.9–28.1)	(0.000–0.002)	(2.4–95.9)	(0.005–0.015)
	Restricted	–12.0		12.3	0.003	12.3	0.003

Appendix 2.A: Latitude and longitude of Common Eider (*Somateria mollissima*) samples** analyzed in this study.

USA: Alaska, North Slope, Bodfish, Spy Island 70.564°N, 149.895°W
NS27325, NS27441, NS27442, NS27443, NS76480, NS76481, NS76482, NS82136, NS82164, NS82232, NS82234, NS82235, SP001, SP002, SP003, SP017–1, SP035, SP085, SP087, SP088, SP089, SP092, SP093, SP144–2

USA: Alaska, North Slope, Bodfish, Long Island 70.480°N, 148.937°W
LO001, LO002, LO003, LO004, LO008, LO009, LO010, LO011, LO012, LO014, LO017, LO018, LO019, LO020, LO021, LO023, LO141, LO033, LO035, NS82101, NS82109, NS82117, NS82118, NS82119, NS82120, NS82121, NS82122, NS82123, NS82129, NS82130, NS82137, NS82138, NS82153, NS82160, NS82161, NS82162, NS82163

USA: Alaska, North Slope, Bodfish, Egg Island 70.440°N, 148.739°W
EG1, EG10–2, EG2, EG2–2, EG3, EG3–2, EG4, EG5, EG7, EG9, EG9–2, NS76478, NS82102, NS82104, NS82106, NS82107, NS82112, NS82127, NS82141, NS82146, NS82147, NS82151, NS82152, NS82156, NS82157, NS82158, NS82221

USA: Alaska, North Slope, Bodfish, Wannabe Island* 70.437°N, 148.725°W
NS27405, NS27406, NS76487, NS82150, NS82154, WA031, WA127, WA128, WA129, WA130, WA131

USA: Alaska, North Slope, Bodfish, Stump Island 70.419°N, 148.601°W
JAR144, JAR136, NS27321, NS27322, NS27323, NS27324, NS27351, NS27401, NS27402, NS27404, NS27407, NS76483, NS76485, NS76490, NS76491, NS76492, NS76493, NS76494, NS76495, NS76496, NS76497, NS76498, NS76499, NS76500, NS76551, NS76552, NS76553, NS76554, NS76555, NS76556, NS76557, NS76558, NS76559, NS76560, NS82133, NS82134, NS82135, NS82142, NS82143, NS82144, NS82145, NS82165, NS82166, NS82167, NS82168, NS82204, NS82205, NS82207, NS82209, NS82211, NS82224, NS82225, NS82237, ST024–2, ST024

USA: Alaska, North Slope, Flaxman, Challenge Island 70.237°N, 146.640°W
CH116, CH118, CH119, CH121, CH124, CH131, CH201, CH261, NS27280, NS27281, NS27282, NS27286, NS52252, NS52281, NS52282, NS52283, NS52284, NS52285, NS52286

USA: Alaska, North Slope, Flaxman, Alaska Island 70.233°N, 146.559°W
AK132, AK134, AK135, AK136, AK137, AK138, AK139, AK140, AK142, AK143, AK150, AK151, AK240, NS27252, NS27253, NS27260, NS27272, NS27273, NS27279, NS27291, NS27292, NS27293, NS272xx, NS52253, NS52287, NS52288, NS52289, NS76453, NS76471, NS76472

Appendix 2.A cont.

USA: Alaska, North Slope, Flaxman, Duchess Island 70.233°N 146.405°W
DU136, DU210, DU227, JAR136, JAR144, NS24351, NS27251, NS27256, NS27264, NS27274, NS27275, NS27276, NS27277, NS27284, NS27337, NS27338, NS27339, NS27340, NS27351, NS27354, NS27422, NS27423, NS27424, NS27425, NS52256, NS52257, NS52258, NS52259, NS52260, NS52261, NS52262, NS52263, NS52264, NS52265, NS52266, NS52267, NS52268, NS52269, NS52270, NS52271, NS52291

USA: Alaska, North Slope, Flaxman, North Star Island 70.225°N, 146.347°W
JAR204, NS100, NS202, NS203–1, NS204, NS218, NS219, NS220, NS222, NS223, NS27268, NS27269, NS27270, NS27271, NS27278, NS27304, NS27305, NS27336, NS27420, NS52272

USA: Alaska, North Slope, Flaxman, Point Thomson 70.186°N, 146.325°W
NS27341, NS27342, NS27343, NS27344, NS27345, NS27346, NS27417, NS52251, NS52273, NS52274, NS52275, PT102, PT103, PT105, PT109, PT110, PT111, PT114, PT222, PT223, PT225, PT226

USA: Alaska, North Slope, Flaxman, Camp Island* 70.172°N, 146.226°W
CA1–1, CA149, CA150, CA152, CA153, CA156, CA159, CA162, NSCAMP1–1, NS24348, NS27347, NS27348, NS27349, NS27350, NS27418, NS27419, NS52254, NS52255, NS52276, NS52277, NS52278, NS52279, NS52280

USA: Alaska, North Slope, Flaxman, Mary Saches Island 70.200°N, 146.207°W
MS224–2, MS226–2, MS227, MS230–5, MS231, MS233, MS235, MS262, MS303, NS27352, NS52290

* Camp Island and Wannabe Island are not official names of locations on any recognized maps, but were given these names for the purpose of identifying areas in this study.
**Samples are located in non–museum research collections.

Appendix 2.B: Number of haplotypes per sampled island for *lamin* A, *gapdh*, and mtDNA control region.

	Number of haplotypes per island								
	Camp	Pt. Thomson	Mary Saches & Northstar	Duchess	Alaska	Stump	Wannabe & Egg	Long	Spy
***Lamin* A**									
01	–	–	–	–	–	–	–	3	4
02	–	–	–	–	–	1	–	–	–
03	2	1	1	2	–	2	2	1	1
10	–	–	–	1	–	–	–	–	–
11	–	–	–	1	–	–	–	–	1
18	–	–	–	–	–	–	–	1	–
19	–	–	–	–	–	–	1	4	–
20	–	1	1	4	–	4	1	1	–
24	2	4	4	4	4	6	4	2	4
26	2	–	2	7	–	16	14	5	4
28	–	–	–	1	–	1	–	–	1
29	–	–	1	1	–	–	–	–	–
32	–	–	1	–	1	5	3	–	–
34	–	2	–	1	–	2	2	2	–
36	–	–	–	–	1	–	1	–	–
37	3	2	–	5	–	6	5	6	1
43	1	–	–	–	–	2	–	–	–
45	–	–	–	–	–	–	–	2	–
47	–	–	–	–	–	–	–	1	–
56	1	–	–	–	–	–	–	–	–
57	–	–	–	–	–	–	–	–	2
58	3	2	–	1	–	2	3	–	–
60	–	–	–	–	–	1	–	–	–
63	–	–	–	–	–	–	–	1	–
64	–	–	–	–	–	–	–	1	–
n	14	12	10	28	6	48	36	30	18
Gapdh									
01	1	–	–	–	–	–	–	–	–
03	2	–	1	1	1	6	5	2	1
04	1	–	–	–	–	–	–	3	1
05	1	3	–	5	1	6	6	6	1
06	–	–	–	1	–	1	–	0	1
11	–	–	–	–	–	1	–	1	–
12	–	1	–	1	–	2	2	1	2
14	–	–	–	–	–	1	–	–	–

Appendix 2.B cont.

	Number of haplotypes per island								
	Camp	Pt. Thomson	Mary Saches & Northstar	Duchess	Alaska	Stump	Wannabe & Egg	Long	Spy
15	–	–	–	1	–	–	–	–	–
16	1	–	–	–	–	–	–	–	–
17	–	–	–	1	–	–	–	–	–
18	1	1	1	3	1	2	3	2	1
23	3	1	2	4	2	2	5	1	2
24	2	1	1	2	–	6	2	6	2
31	–	1	1	1	–	–	2	2	1
37	–	–	–	–	–	–	1	–	–
38	–	2	–	–	1	1	–	–	–
40	–	–	–	–	–	1	–	–	–
43	–	–	–	–	–	1	–	–	–
45	–	–	2	2	–	–	–	–	–
46	–	–	–	–	–	–	–	2	–
47	–	–	–	–	–	6	–	–	–
n	12	10	8	22	6	36	26	26	12
MtDNA									
01	5	3	4	2	2	12	9	7	7
02	–	–	–	–	–	–	1	1	–
03	–	1	1	3	1	4	–	4	–
04	–	–		1	–	–	–	1	–
05	–	1	–	–	–	–	–	–	–
06	–	–	–	–	–	1	2	–	–
07	1	–	–	2	–	1	–	–	–
08	–	–	–	2	–	–	–	–	–
09	–	–	–	1	–	–	–	–	–
10	–	–	–	–	–	1	–	–	–
11	–	–	–	–	–	–	1	–	–
n	6	6	5	11	3	19	13	13	7

CHAPTER 3

GENETIC CHARACTERIZATION OF COMMON EIDERS (*Somateria mollissima*) BREEDING ON THE YUKON-KUSKOKWIM DELTA, ALASKA[1]

Abstract — We assessed population genetic subdivision among four colonies of Common Eiders (*Somateria mollissima v-nigrum*) breeding on the Yukon-Kuskokwim Delta (YKD), Alaska, using microsatellite genotypes and DNA sequences with differing modes of inheritance. Significant levels of genetic differentiation, albeit low, were observed between mainland populations and Kigigak Island for nuclear intron *lamin* A (Φ_{ST} = 0.026, P = 0.000) and mitochondrial DNA (mtDNA) control region (F_{ST} = 0.086, P = 0.000). Inter-colony variation in haplotypic frequencies also was observed at mtDNA (F_{ST} = 0.074–0.187, $P < 0.05$). Positive growth signatures assayed from microsatellite, nuclear introns, and mtDNA indicate recent colonization of the YKD, and may explain low levels of structuring observed. Gene flow estimates based on microsatellite, nuclear introns, and mtDNA, suggest asymmetrical gene flow between mainland populations and Kigigak Island, with more individuals on average dispersing from mainland populations to Kigigak Island than vice versa. Directionality of gene flow observed could be

[1]Sonsthagen, S.A., S.L. Talbot, and K.G. McCracken. Genetic characterization of Common Eiders (*Somateria mollissima*) breeding on the Yukon-Kuskokwim Delta, Alaska. Prepared for submission to *Condor*.

attributed to colonization of YKD from northern glacial refugia or YKD metapopulation dynamics.

INTRODUCTION

Natal, breeding, and winter site fidelity can leave varying signatures in molecular markers. For example, in a population where females exhibit high natal and breeding philopatry and males disperse among populations, spatial genetic structuring is expected at maternally inherited markers with little or no population subdivision at bi-parentally inherited markers. Studies that characterize populations using data from only one genome (nuclear or mtDNA) might grossly over- or under-estimate levels of spatial genetic structure among populations, depending on the marker type used (Avise 2004). Therefore, researchers examining the spatial genetic structure among populations should employ a suite of molecular markers that differ in their mode of inheritance in order to ask a wider range of questions involving species population genetic structure and behavior.

Pacific Common Eiders (*Somateria mollissima v-nigrum*) breed along coastal waters of the Beaufort, Bering, and Chukchi Seas (Goudie et al. 2000). Females within this species exhibit high levels of natal and breeding philopatry (Wakely and Mendall 1976, Swennen 1990, Bustnes and Erikstad 1993, Goudie et al. 2000), which may create genetically distinct breeding areas. Pair formation occurs on the wintering grounds (Spurr and Milne 1976), where several populations of Common Eiders likely intermix. Male Common Eiders accompany females back to breeding sites, and therefore, males may exhibit large natal and breeding dispersal distances (0–1270 km; Swennen 1990). Male-biased dispersal among breeding colonies likely homogenizes allelic frequencies of

genes within the nuclear genome (Scribner et al. 2001) among populations that share wintering grounds.

The Common Eider population breeding in the Yukon-Kuskokwim Delta (YKD), Alaska, is composed of partial migrants; some winter along the coast of the YKD, whereas others winter in the near shore waters of Bristol Bay, 325 km south of YKD (Petersen and Flint 2002). Numbers of Common Eiders breeding on the YKD have declined >90% in the past 40 years (Stehn et al. 1993). While the reason for the decline is unknown, other populations of birds wintering in the Bering Sea also have exhibited population declines; Common Eiders (Suydam et al. 2000), King Eiders (*S. spectabilis*; Dickson et al. 1997, Suydam et al. 2000), Spectacled Eiders (*S. fischeri*; Stehn et al. 1993, Ely et al. 1994), and Steller's Eiders (*Polysticta stelleri*; Kertell 1991). Satellite telemetry data indicate that birds breeding in YKD may be relatively isolated from other eiders breeding in Alaska. Only 6% (2 of 36) of females studied on the Beaufort Sea, Alaska, used the same wintering area as YKD eiders (Petersen and Flint 2002). Common Eiders breeding on the Aleutian Islands are believed to be residents (Goudie et al. 2000), and, therefore, likely do not intermix with individuals from the YKD. With the limited contact with other breeding Common Eider populations, the YKD population may be genetically distinct from other populations in Alaska. However, two females from the Beaufort Sea wintered in the same area as the YKD and potentially formed pair bonds with male YKD birds; male dispersal may provide an avenue for sex-biased gene flow between these regions.

Microgeographic population genetic structure has been observed in other populations of Common Eiders. Among European Common Eider (*S. m. mollissima*) colonies breeding in the Baltic Sea (133–1010 km apart), high levels of spatial population genetic structuring were observed for mitochondrial DNA (mtDNA Φ_{ST} = 0.262–0.343, $P < 0.001$), and significant, but lower levels for bi-parentally inherited microsatellite loci (F_{ST} = 0.009–0.029, $P < 0.05$; Tiedemann et al. 1999). Pacific Common Eiders breeding on 12 barrier islands (1–143 km apart) in the Beaufort Sea, Alaska, exhibited spatial structure at mtDNA and nuclear DNA (mtDNA Φ_{ST} = 0.135–0.271, $P < 0.05$; nuDNA Φ_{ST} = 0.089–0.173, $P < 0.05$; Sonsthagen et al. submitted). Population genetic structure assayed using maternally inherited mtDNA was attributed to the high levels of natal and breeding philopatry by female Common Eiders. Lower levels of population structure observed at bi-parentally inherited markers were attributed to non-random mating by males on the wintering grounds (i.e., males mate with females from the same locality more often than expected; Tiedemann et al. 1999).

We assessed population structure of Common Eider populations breeding in the YKD using three types of molecular markers with different modes of inheritance and rates of mutation: bi-parentally inherited microsatellite genotypes and sequence information from maternally inherited mtDNA control region and two bi-parentally inherited nuclear introns. Microsatellite and mtDNA loci have been used extensively to examine genetic discordance at fine spatial scales among waterfowl populations (e.g., Lanctot et al. 1999, Tiedemann et al. 1999, Scribner et al. 2001, Scribner et al. 2003, Pearce et al. 2004, Pearce et al. 2005, Sonsthagen et al. submitted). Nuclear introns are

useful because they enable researchers to ask questions about historic processes influencing population subdivision, because of their larger effective population size relative to mtDNA and slower mutation rate (Hare 2001). Analyzing markers with varying rates of mutation provide insight on population demography through evolutionary time, with microsatellite data reflecting more recent processes and mtDNA and nuclear intron data reflecting more historic processes. We hypothesized that the nuclear markers (microsatellites and intron sequences) would show little population genetic structure, as Common Eiders breeding on the YKD admix with other populations of Common Eiders on the wintering grounds. Male-mediated dispersal among YKD colonies could, over time, homogenize allelic frequencies within the nuclear genome. In contrast, we predicted that population subdivision would be observed at maternally inherited mtDNA among YKD colonies due to the philopatric nature of female Common Eiders and the fine-scale spatial genetic differentiation assayed for eiders breeding in the Beaufort Sea, Alaska.

METHODS

Sample Collection

Blood samples from adult breeding females (n = 125) were collected opportunistically through mark-recapture efforts on the YKD from 1997–2002 at four sites: Big Slough, Hock Slough, Kigigak Island, and Tutakoke (8.9–63.4 km apart; Fig. 3.1; Appendix 3.A). Samples were stored in lysis buffer (Longmire et al. 1988) and archived at –80°C at the Molecular Ecology Laboratory, U.S. Geological Survey, Anchorage, Alaska. Total

genomic DNA was extracted using the "salting out" procedure described in Medrano et al. (1990), with modifications described in Sonsthagen et al. (2004). Genomic DNA concentrations were quantified using fluorometry and diluted to 50 ng/μL working solutions.

MICROSATELLITE GENOTYPING

Twelve individuals initially were screened at 50 microsatellite loci known to be variable in other waterfowl species, and 14 polymorphic loci were selected for further analysis: *Aph*02, *Aph*08, *Aph*20, *Aph*23 (Maak et al. 2003); *Bca*1, *Bca*11, *Hhi*3 (Buchholz et al. 1998); *Cm*09 (Maak et al. 2000); *Sfi*μ10 (Libants et al. unpubl. data); *Smo*4, *Smo*7, *Smo*08, *Smo*10, and *Smo*12 (Paulus and Tiedemann 2003). Microsatellite loci were amplified using the polymerase chain reaction (PCR). Fluorescently-labeled PCR products were electrophoresed following protocols described in Sonsthagen et al. (2004, submitted) for tailed primers (*Aph*02, *Aph*08, *Aph*20, *Aph*23, *Cm*09, *Smo*4, *Smo*7, *Smo*08, *Smo*10, and *Smo*12) and Pearce et al. (2005) for direct-labeled primers (*Bca*1, *Bca*11, *Hhi*3, and *Sfi*μ10). Ten percent of the samples were re-amplified and genotyped for the 14 loci in duplicate for quality control.

MTDNA AND NUCLEAR INTRON SEQUENCING

We amplified 545 base pairs (bp) from the 5'-end of the mtDNA control region using primers developed by Sonsthagen et al. (submitted). PCR amplifications were carried out in a 50μL volume; 2–100ng genomic DNA, 0.5μM each primer, 1.0μM dNTPs, 1X PCR

buffer (Fisher Scientific, Pittsburgh, PA), 2.5μM $MgCl_2$, and 0.2 units Taq Polymerase (Fisher Scientific, Pittsburgh, PA). PCR reactions began with 94°C for 7 min. followed by 45 cycles of 94°C for 20 s, 60°C for 20 s, and 72°C for 1 min. with a 7 min. final extension at 72°C. PCR products were gel-purified using a QIAquick Gel Extraction Kit (QIAGEN, Valencia, CA), and both strands were sequenced using ABI's BigDye v.3 Terminator Cycle Sequencing Kit diluted 4-fold on an ABI 3100 DNA sequencer (Applied Biosystems, Foster City, CA). Sequences from opposite strands were reconciled using Sequencher 4.1.2 (Gene Codes Corporation, Ann Arbor, Michigan). Individuals containing double peaks within mtDNA sequence data were re-sequenced. If polymorphisms were still detected, these individuals were removed (~10%) due to the high number of nuclear pseudogenes present in this species (Tiedemann and Kistowski 1998, S. Sonsthagen unpubl. data) or heteroplasmy. Sequences will be deposited in GenBank (http//:www.ncbi.nlm.nih.gov) upon publication of the results of this dissertation.

Six nuclear introns were screened for variability in Common Eiders, and two polymorphic introns were selected for sequencing, *lamin* A and *gapdh* (280 bp and 386–387 bp, respectively; McCracken and Sorenson 2005). PCR amplifications of the introns were carried out in a 50 μL volume; 2–100ng genomic DNA, 0.5 μM each primer, and 25 μL AmpliTaq Gold PCR master mix (Applied Biosystems, Foster City, CA). PCR reactions began with 94°C for 7 min. followed by 45 cycles each of 94°C for 20 s; 64°C for 20 s; 72°C for 1 min. with a 7 min. final extension at 72°C. Only sequences from the forward strand were collected on an ABI 3100 DNA sequencer because the PCR

templates were short (280–387 bp) and sequences had a consistent peak height throughout the length of the fragment. Sequences that contained double-peaks of approximately equal peak height, indicating the presence of two alleles, were coded with IUPAC degeneracy codes and treated as polymorphisms (Kulikova et al. 2004). Several sequences for *gapdh* contained a single recurring one bp insert/deletion. To obtain data from the entire fragment for individuals that were heterozygous (~66%) for these alleles, we also sequenced the reverse strand. Sequences will be deposited in GenBank (http//:www.ncbi.nlm.nih.gov) upon publication of the results of this dissertation.

Statistical Analyses

Estimation of Genetic Diversity. Allelic phases of nuclear introns *lamin* A and *gapdh* were inferred from diploid sequence data using PHASE 2.0 (Stephens et al. 2001). PHASE uses a Bayesian approach to reconstruct haplotypes from population genotypic data and allows for recombination and the decay of linkage disequilibrium (LD) with distance. The PHASE analysis (1000 iterations with a 1000 burn-in period) was repeated three times to ensure consistency across runs, as suggested by Stephens et al. (2001).

We calculated allelic frequencies, inbreeding coefficient (F_{IS}), and expected and observed heterozygosities for each microsatellite locus, mtDNA, and the two nuclear introns in GENEPOP 3.1 (Raymond and Rousset 1995) and FSTAT 2.9.3 (Goudet 1995, 2001). Hardy Weinberg equilibrium (HWE) and LD were tested in GENEPOP (Markov chain parameters: dememorization number 1000; number of batches 100, and number of

iterations per batch 10 000), adjusting for multiple comparisons using Bonferroni corrections ($\alpha = 0.05$).

Estimation of Population Demography. Evidence for historical fluctuations in population demography was evaluated for 14 microsatellite loci using BOTTLENECK 1.2.02 (Cornuet and Luikart 1996) and for sequence data using FLUCTUATE 1.4 (Kuhner et al. 1995). BOTTLENECK compares the number of alleles and gene diversity at polymorphic loci under the infinite allele model (IAM; Maruyama and Fuerst 1985), stepwise mutation model (SMM; Ohta and Kimura 1973), and two-phased model of mutation (TPM; Di Rienzo et al. 1994). Parameters for the TPM were set at 79% SMM with a variance of 9% (Piry et al. 1999, Garza and Williamson 2001) with 1000 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 within the past several generations (Cornuet and Luikart 1996, Luikart 1997). It is important to note that heterozygote deficiency and excess calculated in BOTTLENECK differ from values calculated in other population genetic programs. As mentioned previously, BOTTLENECK compares heterozygote deficiency and excess relative to genetic diversity, not to Hardy-Weinberg equilibrium expectation (Cornuet and Luikart 1996). FLUCTUATE was run using maximum likelihood search parameters: ten short chains

(200 used trees out of 4000 sampled) and three long chains (20 000 used trees out 400 000 sampled). Data were analyzed three times to ensure convergence of parameters across runs. Sequence data were tested for selective neutrality and historical fluctuations in population demography, using Fu's *Fs* (Fu 1997) and Tajima's *D* (Tajima 1989) in ARLEQUIN. For critical significance, values of 5% required a *P*-value below 0.02 for Fu's *Fs* (Fu 1997). Unrooted phylogenetic trees for each gene were constructed in TCS 1.21 (Clement et al. 2000), which estimates genealogies using 95% statistical parsimony probabilities (Templeton et al. 1992). *Lamin* A and *gapdh* intron sequences also were analyzed in NETWORK 4.1.0.8 (Fluxus Technology Ltd. 2004) using the Median Joining network (Bandelt et al. 1999), to illustrate possible reticulations in the gene trees due to homoplasy or recombination.

Estimation of Population Subdivision. To assess levels of population subdivision among sampled sites, pairwise F_{ST}, R_{ST}, Φ_{ST}, global *F*-statistics, and *R*-statistics were calculated in FSTAT version 2.9.3 (Goudet 1995, 2001) or ARLEQUIN 2.0 (Schneider et al. 2000), adjusting for multiple comparisons using Bonferroni corrections ($\alpha = 0.05$) or permutations (3000) in FSTAT and ARLEQUIN, respectively. Inter-haplotypic and inter-allelic sequence divergences were used to calculate pairwise Φ_{ST} (Excoffier et al. 1992). MODELTEST 3.06 (Posada and Crandall 1998) was used to determine the minimum parameter nucleotide substitution model that best fit the mtDNA and intron sequence data under the Akaike Information Criterion (Akaike 1974). Pairwise genetic distances between unique haplotypes and alleles were calculated in PAUP* 4.0 (Swofford 1998) for mtDNA and ARLEQUIN for nuclear introns. Additionally, pairwise

comparisons were performed using ARLEQUIN to determine the magnitude of spatial variance in haplotypic and allelic frequencies between mainland and island populations. The mainland group is composed of Big Slough, Hock Slough, and Tutakoke, and the island group is composed of Kigigak Island. An isolation by distance analysis was performed in IBD (Bohonak 2002), with microsatellite data and nuclear intron data using genotypic data inferred from the PHASE analysis, to determine if more geographically distant population pairs are also more genetically differentiated. IBD tests the statistical significance of the relationship between genetic and geographic distance using a Mantel test and calculates slope and intercept from RMA regressions following Sokal and Rohlf (1981) with confidence limits.

Finally, microsatellite data were analyzed in STRUCTURE 2.1 (Pritchard et al. 2000) to detect the occurrence of population structure without *a priori* knowledge of putative populations. Data were analyzed using an admixture model assuming correlated frequencies to probabilistically assign individuals to putative populations with 10 000 burnin period, 100 000 Markov chain Monte Carlo iterations, number of possible populations (K) ranging from 1–10; this analysis was repeated five times to ensure consistency across runs.

Estimation of Gene Flow. Number of migrants per generation (N_em) for nuclear microsatellite and nuclear intron loci or number of female migrants per generation (N_fm) for mtDNA were calculated in MIGRATE v2.0.6 (Beerli 1998, 2002: Beerli and Felsenstein 1999) among sampled sites. Full models, θ ($4N_e\mu$ or $N_f\mu$, composite measure of effective population size and mutation rate) and all pairwise migration parameters

were estimated individually from the data and were compared to restricted island models for which θ and pairwise migration parameters are equal among populations.

MIGRATE was run using maximum likelihood search parameters; ten short chains (1000 used trees out of 20 000 sampled), five long chains (10 000 used trees out 200 000 sampled), and five adaptively heated chains (start temperatures: 1, 1.5, 3, 6, and 12; swapping interval = 1). Full models were run three times to ensure the convergence of parameter estimates. Restricted models were run once. The alternative model was evaluated for goodness-of-fit given the data using a log-likelihood ratio test. The resulting statistic from the log-likelihood ratio test is equivalent to a χ^2 distribution with the degrees of freedom equal to the difference in the number of parameters estimated in the two models (Beerli and Felsenstein 2001).

RESULTS

GENETIC DIVERSITY

Bi-parentally inherited nuclear microsatellites. Multilocus genotypes were collected for 125 individuals sampled in the YKD for 14 microsatellite loci. Most of the microsatellite loci (79%) appeared to conform to the SMM; three loci (*Smo*04, *Smo*07, and *Smo*12) had at least one allele that had a length change different from the repeat unit (1 bp difference). Number of alleles per locus ranged from 2–38, with an average 9.9 alleles per locus. The average number of alleles per population ranged from 5.6–7.6. The observed heterozygosity ranged from 17–96% for each population with an overall observed heterozygosity of 58%. The inbreeding coefficient (F_{IS}) ranged from –0.328 to 0.662

across sampled sites with an overall mean of 0.032. None of the inbreeding coefficients were significantly different from zero ($P_{adj} > 0.05$). Significant fluctuations in population demography were detected by BOTTLENECK (Table 3.1). Heterozygote deficiency was observed under the SMM in Kigigak, Hock Slough, and Tutakoke, and the global YKD estimate suggesting recent population growth in the past several generations (Table 3.1). However, Tutatoke and global YKD estimates also had significant heterozygote excess under IAM suggesting a recent population bottleneck (Table 3.1).

Bi-parentally inherited nuclear introns. PHASE reconstructed 31 unique alleles from 107 individuals for *lamin* A (Fig. 3.2A). Forty-seven (44%) individuals were homozygous at all variable sites, and 26 (24%) were polymorphic at one site. Probabilities of reconstructed haplotypes ranged from 0.82–1.00, except for five individuals that had probabilities ranging from 0.50–0.73. PHASE calculated the background recombination rate (ρ) as 0.50, with factors exceeding ρ ranging from 0.58–1.94 between 14 variable sites.

Twenty-one unique alleles for 85 individuals were reconstructed by PHASE for *gapdh* (Fig. 3.2B). Seven (8%) individuals were homozygous at all variable sites, and two (2%) individuals were polymorphic at only one site. Probabilities of reconstructed haplotypes ranged from 0.82–1.00, except for 14 individuals that had probabilities ranging from 0.50–0.76, which we attribute to potentially high levels of recombination occurring within this sequence (0.39–4.41 factors exceeding $\rho = 0.05$, between 15 variable sites). There were seven variable sites that exceeded ρ by one or more factors: 2.12 factors between sites 16 and 22, 1.42 factors between sites 22 and 26, 1.12 factors

between sites 48 and 49, 1.02 factors between sites 136 and 145, 1.11 factors between sites 165 and 170, 1.36 factors between sites 186 and 192, and 4.41 factors between sites 232 and 252.

Haplotype (h) and nucleotide (π) diversity ranged from 0.849–0.912 and 0.008–0.009, respectively, for *lamin* A, and from 0.875–0.923 and 0.006–0.008, respectively, for *gapdh* (Table 3.2). Observed and expected heterozygosity for *lamin* A was 60% and 90%, respectively, which deviated significantly from HWE ($P < 0.05$). Observed and expected heterozygosity for *gapdh* was 94% and 90%, respectively, which also deviated from HWE ($P < 0.05$). Significantly negative values for Fu's *Fs* were observed for Hock Slough and Tutakoke (Table 3.2). Significant population growth rates (g) were detected by FLUCTUATE in all populations for both *lamin* A and *gapdh* (Table 3.1).

Maternally inherited mtDNA. Nine unique haplotypes were identified from 75 individuals with 13 variable sites (Fig. 3.2C). Haplotype (h) and nucleotide (π) diversity was high for most populations with values ranging from 0.464–0.697 and 0.004–0.006, respectively (Table 3.2). The number of haplotypes per population ranged from 3–6. Neutrality tests found no evidence for selection (Table 3.2). We did not detect any significant fluctuations in population demography for each population analyzed separately; however, a significant population growth rate (g) was detected when all colonies were combined (Table 3.1).

POPULATION SUBDIVISION

Bi-parentally inherited nuclear microsatellites. We did not detect any significant variations in allelic frequencies for the 14 microsatellite loci (Table 3.3), suggesting panmixia among sampled localities. In addition, there were no significant pairwise population comparisons for F_{ST} or R_{ST} (Table 3.3). The Bayesian clustering method, implemented by the program STRUCTURE, indicated the most likely model generated from the microsatellite data was maximized when the total number of populations was one, which supports the interpretation of panmixia. In addition, the regional comparison between the mainland populations and Kigigak Island was not significant (Table 3.3). Finally, we found no evidence of isolation by distance correlation between genetic and geographic distances ($r = 0.33$, $P = 0.30$).

Bi-parentally inherited nuclear introns. The nucleotide substitution model that best fit the *lamin* A and *gapdh* intron sequence data was Tamura-Nei (1993) model with an invariant site (TrN+I) parameter. We did not detect any significant differences in the allelic distribution among sampled localities for *lamin* A or *gapdh* (Table 3.3). However, a regional comparison showed low levels of structuring between mainland populations and Kigigak Island for *lamin* A but not *gapdh* (Table 3.3). As observed with the microsatellite data, we did not detect any significant correlations among geographic and genetic distances for the nuclear introns when analyzed combined ($r = 0.58$, $P = 0.18$) or *gapdh* separately ($r = -0.44$, $P = 0.70$). However, when *lamin* A was analyzed separately, there was a significant correlation among the log Rousset's genetic distance ($\log[F_{ST}/(1-F_{ST})]$; Rousset 1997) and geographic distance ($r = 0.89$, $P = 0.04$; intercept =

–4.15 ± 1.01, slope = 0.04 ± 0.03, R^2 = 0.80). However, Rousset (1997) suggests not log-transforming either axis for a one-dimensional stepping stone model and only log transforming the geographic distance for a two-dimensional stepping stone model.

Maternally inherited mtDNA. The nucleotide substitution model that best fit the mtDNA data was Tamura-Nei (1993) model with an invariant site parameter (TrN+I; substitute rate matrix: R[A–C] = 1.00, R[A–G] = 20.22, R[A–T] = 1.00, R[C–G] = 1.00, R[C–T] = 7.25, R[G–T] = 1.00, p–inv. = 0.83, A = 0.22, C = 0.31, G = 0.20, T = 0.28). Mean inter-population variance in haplotypic frequency was significant (Table 3.3). In addition, there were significant inter-population comparisons between Hock Slough and all other populations, and Kigigak and Tutakoke (Table 3.3). However, when we applied the TrN+I model to the data set, inter-population comparisons were no longer significant (Table 3.3). A regional comparison between mainland populations and Kigigak Island uncovered moderate levels of genetic subdivision (Table 3.3). However, when we applied the TrN+I model to the data set, this regional comparison was no longer significant (Table 3.3).

GENE FLOW

Although, the Bayesian clustering program STRUCTURE did not uncover population subdivision among YKD colonies, we tested a two-population geographic model between mainland (Big Slough, Hock Slough, and Tutakoke) populations and Kigigak Island based on our inference of genetic partitioning at nuclear intron *lamin* A and mtDNA. N_em and θ values calculated in MIGRATE from microsatellite genotypes, mtDNA, and

nuclear intron sequence data ranged from 13.1–26.0 migrants per generation from mainland populations to Kigigak Island with θ ranging from 0.002–1.026, and 1.5–18.8 migrants per generation from Kigigak Island to mainland populations with θ ranging from 0.006–0.548 (Table 3.4). The full model (all parameters allowed to vary independently) had significantly higher likelihoods than the restricted island model (symmetric inter-population migration rates and thetas) across all marker types (Table 3.4) indicating asymmetric gene flow between mainland populations and Kigigak Island.

DISCUSSION

Population Structure and Fluctuations

Low levels and lack of spatial population genetic subdivision at bi-parentally inherited nuclear markers might be attributed to aspects of Common Eider breeding and wintering biology. Common Eiders breeding in the YKD winter in near shore waters of western Alaska within 400 km of nesting sites (Petersen and Flint 2002). In the winter aggregations, females may form pair bonds randomly (Spurr and Milne 1976) with males from different breeding colonies. Male dispersal among breeding areas, thus, is expected to homogenize genetic diversity in the nuclear genome (Scribner et al. 2001). However, we observed low, albeit significant, inter-population variance between mainland populations and Kigigak Island for nuclear intron *lamin* A. Significant population subdivision between the mainland and Kigigak Island may be the result of assortative mating occurring on the wintering grounds such that females preferentially form pair bonds with males from the same colony.

Non-random mating on the wintering grounds has been observed in Common Eiders (*S. m. mollissima*) breeding in the Baltic Sea (Tiedemann et al. 1999). Assortative mating on the wintering grounds could result from individuals from the same colony (1) arriving earlier on the wintering ground coupled with a selective advantage for early pair formation (Spurr and Milne 1976), or (2) wintering in different localities within the coastal waters of western Alaska and consequentially pairing with individuals from the same colony. Satellite telemetry data from individuals breeding in Big Slough indicate that individuals from this colony winter in different locations (Petersen and Flint 2002). Although one YKD female ($n = 39$) wintered in an area not used by other marked birds (Petersen and Flint 2002), wintering areas were likely an admixture of YKD colonies, creating the potential of females pairing with males from different colonies in the YKD. Winter site fidelity has been observed in Common Eiders (Spurr and Milne 1976), and polynya (openings in the ice) have been reported to occur regularly in coastal Alaskan waters (Petersen and Flint 2002). High winter site fidelity would have to occur over many generations to be detected genetically. Therefore, mainland colonies and Kigigak Island would have to winter in different areas over evolutionary time, as one bout of random mating per generation between mainland populations and Kigigak Island would contribute to homogenization of allelic frequencies among colonies. Finally, unequal population sizes among mainland populations and Kigigak Island might bias estimators of population subdivision (Scribner et al. 2001). Individuals from colonies with larger population sizes would appear to mate assortatively simply due to the higher probability

of mating with an individual from the same population, thus, leading to upward bias in estimates of F_{ST}.

Moderate levels of population structure were observed within the maternally inherited mtDNA control region. Genetic partitioning occurred at this locus among all sampled sites, except between Big Slough and Kigigak Island and Big Slough and Tutakoke, suggesting female natal and breeding philopatry over relatively short geographic distances. Banding data also indicate high breeding site fidelity, as only one breeding female has been observed to disperse between studied colonies (Tutakoke to Hock Slough; P. Flint pers. comm.). However, significant spatial genetic structuring was not evident when the nucleotide substitution model (TrN+I model) was applied to the data set, suggesting that colonies have not been subdivided long enough for mutation to be driving differentiation among populations (Scribner et al. 2001). Evidence for recent range expansion is supported by significant positive growth rates observed at nuclear markers. Although we did not observe significant positive population growth rates for mtDNA, when sequence data were combined across all sampled sites, YKD as a whole had a positive growth signature, suggesting a recent colonization (Waltari et al. 2004) of YKD by Common Eiders.

Low to high inter-population variances in haplotypic frequencies have been observed in another population of Pacific Common Eider. Common Eiders breeding in the Beaufort Sea exhibited population structuring within mtDNA (pairwise $\Phi_{ST} = 0.135$–0.271) among islands approximately 85–135 km apart (Sonsthagen et al. submitted). However, genetic discordance was not observed among islands within the same island

group indicating that Beaufort Sea females are philopatric to island groups rather than particular islands. The degree of philopatry differs among YKD females, as moderate levels of population subdivision were observed among colonies approximately 10–63 km apart. In contrast to the Beaufort Sea, females breeding in the YKD appear to be philopatric to individual colonies (e.g., Wakely and Mendall 1976, *S. m. dresseri*). Differences in the degree of philopatry among Beaufort Sea and YKD females could be attributed to a more stochastic arctic environment. Seasonal arctic storms in the Beaufort Sea alter where nesting habitat is available annually, potentially causing females to disperse among adjacent islands (Sonsthagen et al. submitted). Conversely, YKD Common Eiders nest in sedges on a coastal wetland tundra habitat (Kincheloe and Stehn 1991, Flint et al. 1998) that remains relatively unchanged across seasons (Stehn et al. 1993). Spatial stability in the availability of nesting habitat through evolutionary time would enable females to nest in the same area across years. Coupled with high natal philopatry, temporal and spatial stability of nesting habitat would create genetic discordance among adjacent colonies within mtDNA despite gene flow through male-mediated dispersal.

Inter-island comparisons in haplotypic frequencies (Φ_{ST}) among Beaufort Sea Common Eiders were an order of magnitude greater than inter-colony estimates observed in the YKD assayed from mtDNA (Sonsthagen et al. submitted). Larger estimates of population subdivision may be explained, in part, by the historic population demography of the northern Alaskan population. The Beaufort Sea population did not exhibit a genetic signature of population expansion, suggesting that northern Beringia was a glacial

refugium for Common Eiders during the Pleistocene (S. Sonsthagen unpublished data). Long-term persistence of Beaufort Sea Common Eiders in a Pleistocene refugium, coupled with female natal philopatry, would result in greater spatial population genetic structure assayed for mtDNA when compared to the potentially recently colonized YKD.

Comparatively higher levels of population subdivision assayed using mtDNA than nuclear DNA could also be attributed to lineage sorting. MtDNA has a lower effective population size relative to nuclear DNA. Therefore, when mutation rate and selection are held constant, genetic drift has a larger effect on mtDNA than nuclear DNA (Avise 2004), translating in higher estimates of population subdivision (F_{ST}). The effects of lineage sorting and sex-biased differences in philopatry on spatial genetic subdivision are not mutually exclusive and both may be playing a role in the degree of population structure observed. However, microsatellite loci have a high rate of mutation relative to mtDNA control region (Avise 2004) resulting in new mutations arising more frequently within populations. By chance alone, one would expect new mutations to increase in frequency among isolated populations and dampen the effects of incomplete lineage sorting within microsatellite loci. Given differences in the degree of philopatry in Common Eiders between the sexes and congruence in results between microsatellite and nuclear intron loci, differences in estimates of population subdivision may be more attributable to male dispersal and high natal and breeding philopatry in females rather than incomplete lineage sorting for the YKD population.

Population bottleneck signals observed with microsatellite loci under the IAM are consistent with demographic data indicating population decline (Stehn et al. 1993).

Differences in population fluctuations among models (IAM and SMM) may be because of underlying assumptions of mutation models, such that IAM does not allow for homoplasy (i.e. each mutation results in a new allele) and SMM allows for mutations to existing allelic states (homoplasy). When mutation rate is held constant, IAM will have more distinct allelic states and a higher expected heterozygosity under mutation drift equilibrium, and therefore may be better able to detect population declines. Simulation data indicate that IAM may better detect weak population bottlenecks than SMM (Cornuet and Luikart 1996), and empirical data suggest SMM may not be as able to detect recent population declines (hairy-nosed wombats; Cornuet and Luikart 1996). Therefore, differences in directionality of population fluctuations between mutation models may reflect differences in population size over evolutionary time, with IAM detecting the recent population decline observed by Stehn et al. (1993) and SMM detecting long-term population growth.

GENE FLOW

Common Eiders breeding in the YKD appear to be exhibiting asymmetrical gene flow between mainland colonies and Kigigak Island across all marker types, with more individuals dispersing from mainland populations to Kigigak Island. Asymmetrical gene flow observed at bi-parentally inherited nuclear markers could be attributed to clinal variation in allelic frequencies among populations that share common wintering areas, as Common Eiders breeding in the Beaufort Sea exhibited significant asymmetrical westerly gene flow at nuclear markers (Sonsthagen et al. submitted). Additionally, we observed

significant positive correlation between log Rousset's genetic distance and geographic distance in nuclear intron *lamin* A. However, it is important to note that Rousset (1997) did not recommend log-transforming either distances for a one-dimensional stepping stone model, and only log transforming the geographic distance for a two-dimensional stepping stone model. Therefore, over evolutionary time, individuals could be dispersing from northern portions of their range along the coast of Alaska.

Colonization of the YKD during the last glacial retreat also could explain, in part, the directionality of gene flow observed. Significant positive growth rates and changes in effective population size across all marker types indicate that the YKD was colonized recently. Ploeger (1968) hypothesized that during the last glacial period, Common Eider breeding distribution was restricted to the southern edge of the Bering Land Bridge, southwest of YKD. Assuming YKD was colonized by eiders residing in the southern edge of the Bering Land Bridge, we would expect to observe more individuals dispersing south to north. However, females breeding in the YKD are exhibiting asymmetrical gene flow from north to south. Therefore, we hypothesize that the YKD was likely colonized by Common Eiders from glacial refugia north of the current breeding site (S. Sonsthagen unpublished data). However, molecular data from a larger geographic scale are needed to confirm this hypothesis.

Unsampled populations may have effects on the estimation of population size (θ) and immigration rates (M; Beerli 2004). Simulation data indicate that estimates of θ and M show an upward bias with increasing immigration from unsampled populations and biases in the estimate of number of migrants per generation closely follow biases of θ

(Beerli 2004). Despite upward biases in estimators of gene flow in the absence of samples from larger, more influential, populations, directionality of gene flow does not appear to be affected in this study. Furthermore, in source-sink populations, which may describe the population dynamics observed on the YKD, θ is estimated accurately (Beerli 2004). Therefore, the directionality of gene flow observed between mainland and Kigigak Island is likely not influenced by unsampled mainland populations. However, the magnitude of gene flow may have an upward bias if unsampled populations on the YKD have a larger influence on the exchange of alleles among populations than sampled mainland populations.

CONCLUSIONS

Common Eiders breeding on the YKD are genetically differentiated, as we observed low levels of spatial genetic subdivision for nuclear intron *lamin* A and low to moderate levels for mtDNA control region. Male-biased dispersal among YKD colonies appears to be homogenizing allelic frequencies in the nuclear genome, as we did not detect population subdivision among sampled colonies for 14 microsatellite loci and nuclear intron *gapdh*. Moderate levels of population genetic structure observed for mtDNA are consistent with observations that female Common Eiders are exhibiting high levels of natal and breeding philopatry. Spatial genetic structuring, although low, observed for Common Eiders breeding on the YKD at such a microgeographic scale is noteworthy, especially when compared to other arctic breeding waterfowl. Studies of King Eiders (*S. spectabilis*; Pearce et al. 2004) and Harlequin Ducks (*Histrionicus histrionicus*; Lanctot

et al. 1999) did not detect significant levels of population genetic structure among sampled sites for microsatellite genotype or mtDNA sequence data. Steller's Eider (*Polysticta stelleri*) breeding in Alaska and Russia exhibited low levels of population differentiation among sites at microsatellite loci (Pearce et al. 2005). Higher levels of population genetic subdivision were observed among breeding populations of Spectacled Eiders (*S. fisheri*; Scribner et al. 2001) for mtDNA and Canada Geese (*Branta canadensis*; Scribner et al. 2003) for mtDNA and nuclear microsatellite loci. However, these studies were conducted at much larger spatial scales relative to our study.

Maximum-likelihood analyses based on the coalescent allow estimation of asymmetry in migration rates and subsequent identification of potential source and sink populations. However, source populations are more easily identified, since removal of individuals from sink populations (those with negative population growth) may be due to emigration, mortality, or both. These migration data are not sufficient to determine whether a population size is decreasing due to high mortality and would go extinct in the absence of immigration. Nevertheless, candidates for extinction can be identified as populations with small effective sizes and high immigration rates. Such populations can be targeted for additional studies on direct measures of population growth, based on mark-recapture techniques that verify results of the molecular analyses. In this study, Kigigak Island may be identified as a potential sink for most population pairwise comparisons for which exchange is estimated and has a lower effective population size relative to mainland populations based estimates calculated from microsatellite loci. The interpretation of Kigigak Island as a potential sink may be confounded since Kigigak

Island colony does not have lower levels of genetic variation when compared to mainland colonies and recent molecular population demography estimates and contemporary demographic data indicate recent population growth (P. Flint pers. comm.). However, given the geographic proximity of Kigigak Island to mainland populations, genetic diversity would not be reduced as short movements allow populations to retain genetic diversity during founding events (Hewitt 1996). In addition, stochastic events may influence populations with small effective numbers causing local extinction; however, source populations can recolonize the habitat (Hanski 1991, Hanski and Simberloff 1997). Positive population growth observed could be a result of a recent population reduction, or extinction, of Kigigak Island, followed by a recovery stage of the local metapopulation dynamics. It is difficult to determine if Kigigak Island is a population sink with analyses used in this study; therefore, long-term demographic data are needed to evaluate results of molecular analyses.

These data provide further evidence for the need to use multiple marker types with different modes of inheritance to assess levels of spatial genetic subdivision. As seen in Common Eiders, nuclear and mtDNA markers show varying levels of genetic partitioning among breeding sites. High levels of natal and breeding philopatry observed in waterfowl do have predictable effects on population genetic structure, and these results confirm that genetic discordance can occur on relatively small spatial scales relative to known dispersal distances and capabilities. Researchers characterizing populations using genetic techniques could under- or over-estimate the degree of population genetic differentiation if estimates are based on a single marker type. Therefore, not utilizing

molecular markers with varying modes of inheritance could mislead researchers characterizing genetic variation within populations.

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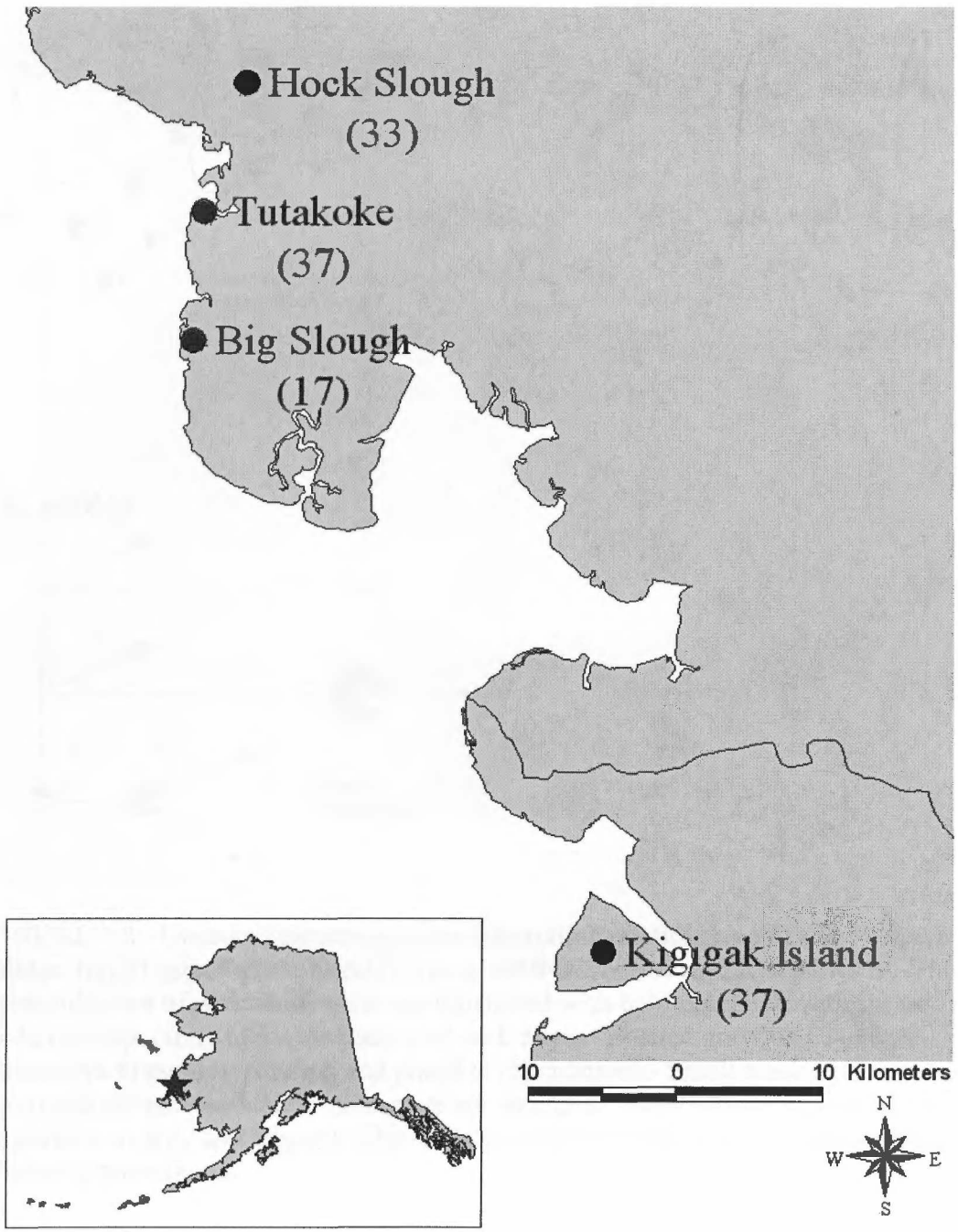

FIGURE 3.1: Locations of breeding Common Eiders in the Yukon-Kuskokwim Delta, Alaska, with sample sizes in parentheses.

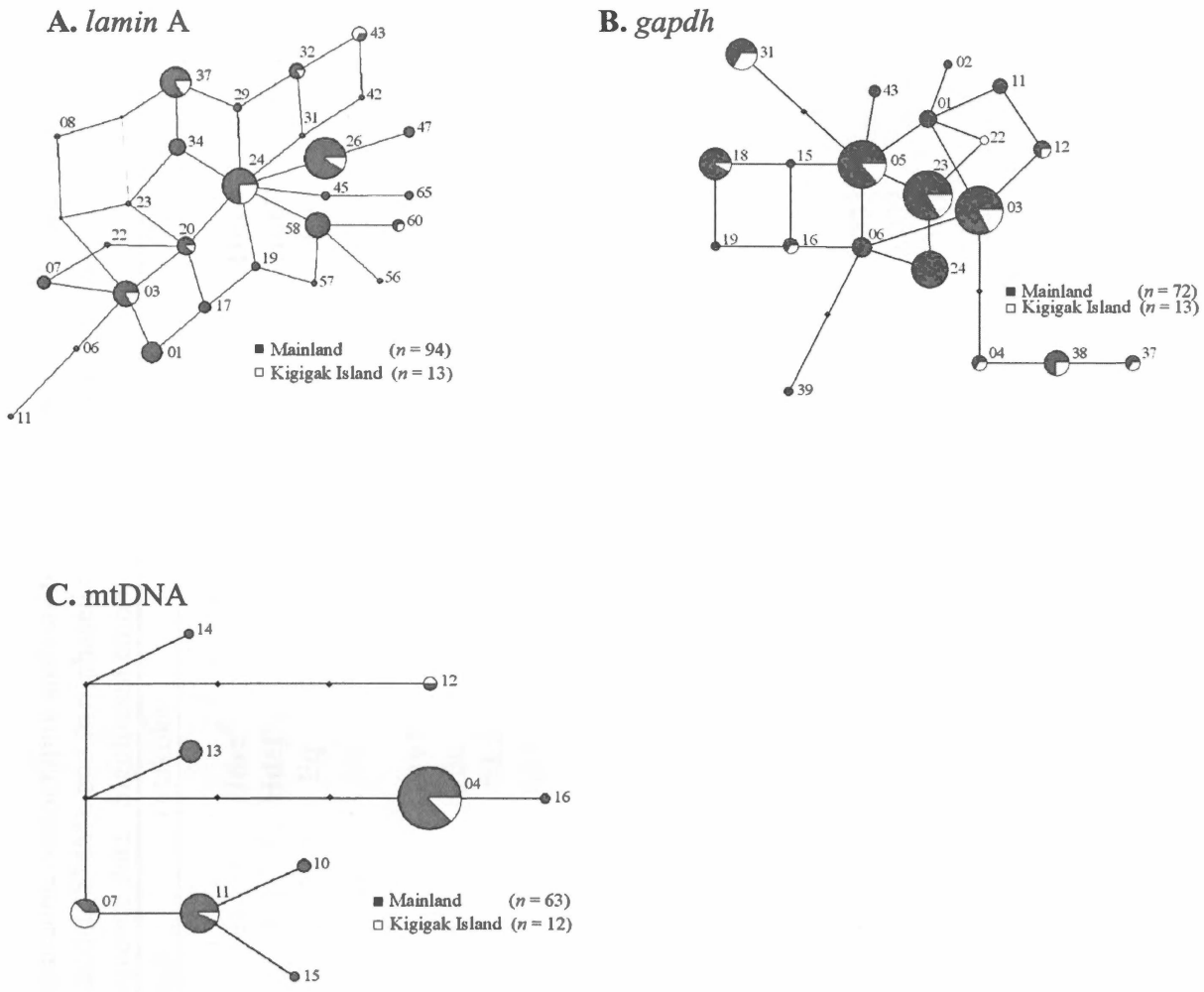

FIGURE 3.2: Unrooted parsimony trees illustrating relationships of (A) 31 *lamin* A alleles, (B) 21 *gapdh* alleles, and (C) nine mtDNA control region haplotypes. The 95% probability set of parsimony trees are illustrated with bold branches, with the size of the node corresponding to the frequency of each allele. Dashed gray lines indicate alternative branching patterns and possible reticulations. Small black squares indicate intermediate ancestral alleles that were not sampled. Mainland population alleles are illustrated in gray and Kigigak Island alleles are illustrated in white. Sample sizes are shown in parentheses.

TABLE 3.1: Results of historical fluctuations in population demography analysis for 14 microsatellite loci tested using the infinite allele model (IAM), stepwise mutation model (SMM), and two-phased model of mutation (TPM) and for nuclear introns, *lamin* A and *gapdh*, and mtDNA sequence data. Significant comparisons are in bold text.

	Big Slough	Hock Slough	Tutakoke	Kigigak	YKD
Microsatellite					
IAM	Eq	Eq	**Hexc**[a]	Eq	**Hexc**[a]
SMM	Eq	**Hdef**[a]	**Hdef**[a]	**Hdef**[a]	**Hdef**[a]
TPM	Eq	**Hdef**[a]	Eq	Eq	Eq
***Lamin* A**					
θ[c]	0.080	**0.167**	**0.193**	**0.016**[b]	**0.122**
SD	0.051	0.023	0.005	0.006	0.007
g[c]	**632.1**	**975.1**	**392.2**	**407.8**[b]	**550.8**
SD	142.3	55.8	82.8	136.9	61.9
Gapdh					
θ[c]	**0.028**	**0.015**[b]	**0.027**	**0.019**	**0.047**
SD	0.007	0.005	0.004	0.004	0.003
g[c]	**1126.9**	**385.5**[b]	**1066.5**	**322.2**[b]	**400.0**
SD	160.0	166.0	170.3	116.4	50.8
MtDNA					
θ[c]	**0.005**[b]	**0.005**	**0.004**	**0.005**[b]	**0.023**
SD	0.002	0.001	0.001	0.002	0.002
g[c]	104.5	-38.0	98.9	-18.2	**534.1**
SD	228.9	119.6	173.3	117.3	71.4

[a] Significant heterozygote deficiency (Hdef) indicates population growth, heterozygote excess (Hexc) indicates a population bottleneck, and non-significant values are assumed to be at equilibrium (Eq).

[b] Significant to $P < 0.05$, all others $P < 0.003$.

[c] Parameter estimates θ ($N_f\mu$ for mtDNA, $4N_e\mu$ for nuclear DNA) and exponential growth rate (g) plus the standard deviation (SD) for each population.

TABLE 3.2: Estimates of genetic diversity, including; nucleotide (π) and haplotype (h) diversity with standard deviation in parentheses, number of unique alleles and haplotypes per population, and sample size (n),for *lamin* A, *gapdh*, and mtDNA control region.

	Big Slough	Hock Slough	Tutakoke	Kigigak
***Lamin* A**				
h	0.912 (0.049)	0.910 (0.022)	0.895 (0.013)	0.849 (0.041)
π	0.009 (0.005)	0.008 (0.005)	0.009	0.008 (0.005)
Fu's *Fs*	–2.700	**–9.764**[a]	**–10.182**[a]	–1.187
Tajima's *D*	–0.196	–0.478	–0.423	–0.157
Unique alleles	8	23	22	8
n	7	33	37	13
Gapdh				
h	0.895 (0.033)	0.923 (0.018)	0.875 (0.017)	0.899 (0.030)
π	0.007 (0.004)	0.007 (0.004)	0.006 (0.003)	0.008 (0.005)
Fu's *Fs*	–1.079	–1.023	–3.785	–2.643
Tajima's *D*	–0.379	–0.321	–1.072	–0.183
Unique alleles	11	14	19	11
n	13	20	37	13
MtDNA				
h	0.464 (0.200)	0.626 (0.067)	0.638 (0.082)	0.697 (0.090)
π	0.004 (0.003)	0.006 (0.004)	0.005 (0.003)	0.005 (0.003)
Fu's *Fs*	1.612	3.220	0.757	1.953
Tajima's *D*	–0.561	0.324	1.062	–0.014
Unique haplotypes	3	5	6	4
n	8	19	36	12

[a] Significant *P*–values for Fu's *Fs* ($P < 0.02$) and Tajima's *D* ($P < 0.05$) are in bold text.

TABLE 3.3: Pairwise F_{ST} and R_{ST} calculated from 14 microsatellite loci and Φ_{ST} from mtDNA control region, *lamin* A, and *gapdh* sequence data for each population pair. Significant inter-population variances are in bold text.

Population pairs	F_{ST}	R_{ST}	*Lamin* A Φ_{ST}	*Gapdh* Φ_{ST}	mtDNA F_{ST}	mtDNA Φ_{ST}
Big Slough vs. Kigigak	0.009	–0.001	0.030	–0.021	0.076	0.083
Big Slough vs. Hock Slough	0.002	–0.019	0.014	–0.013	**0.187**	0.136
Big Slough vs. Tutakoke	0.004	–0.016	0.004	–0.004	–0.007	0.001
Kigigak vs. Hock Slough	–0.001	0.000	0.019	–0.011	**0.156**	–0.020
Kigagak vs. Tutakoke	0.002	–0.004	0.014	0.004	**0.080**	0.027
Hock Slough vs. Tutakoke	–0.003	–0.010	–0.004	–0.002	**0.074**	0.040
Mainland vs. Kigigak	0.002	0.003	**0.026**	0.006	**0.086**	0.008
Global Statistics	0.001	–0.009	0.003	0.005	**0.086**	0.034

TABLE 3.4: Comparison of alternative models of Common Eider gene flow between mainland populations and Kigigak Island. Full model migration matrix (allowing all parameters to vary independently) and restricted model (symmetrical gene flow) migration rates calculated from14 microsatellite loci, *lamin* A and *gapdh*, and mtDNA control region.

				Mainland to Kigigak Is.		Kigigak Is. to Mainland	
Marker	Hypothesis	Ln(L)[a]	*P*–value	N_fm or N_em[b]	θ[b]	N_fm or N_em[b]	θ[b]
Microsatellites	Full	–1454.4	<0.001	22.7	1.026	18.8	0.548
				(20.2–25.2)	(0.962–1.093)	(16.8–21.2)	(0.516–0.584)
	Restricted	–1606.0		17.4	0.787	17.4	0.787
Nuclear introns	Full	–141.7	<0.001	13.1	0.002	5.5	0.006
				(5.3–22.6)	(0.002–0.002)	(3.3–8.4)	(0.004–0.007)
	Restricted	–154.7		9.6	0.004	9.6	0.004
MtDNA	Full	15.5	<0.001	26.0	0.013	1.5	0.011
				(6.2–191.7)	(0.010–0.017)	(0.5–4.1)	(0.004–0.055)
	Restricted	–16.0		14.8	0.012	14.8	0.012

[a] Likelihood of the data under each hierarchical model is listed and was evaluated for significance using a log likelihood ratio test (Beerli and Felsenstein 2001).

[b] Parameter estimates for number of migrants per generation (N_em for nuclear DNA, N_fm for mtDNA) are listed for each population along with θ ($N_e\mu$ for nuclear DNA, $N_f\mu$ for mtDNA) for population migration rates and 95% confidence intervals are in parentheses.

Appendix 3.A: Localities of Common Eiders (*Somateria mollissima*) sampled** in this study.

USA: Alaska, Yukon-Kuskokwim Delta, Big Slough* 61.170°N, 165.590°W
BS76451, BS76452, BS76454, BS76455, BS76456, BS76457, BS76459, BS76460, BS76461, BS76462, BS76463, BS76464, BS76465, BS76466, BS76467, BS76469, BS76470

USA: Alaska, Yukon-Kuskokwim Delta, Tutakoke River 61.248°N, 165.617°W
YK54748, YK54774, YK76123, YK76151, YK76154, YK76155, YK76159, YK76162, YK76166, YK76192, YK76194, YK76195, YK76263, YK76264, YK76267, YK76268, YK76271, YK76275, YK76282, YK76316, YK76420, YK76421, YK76423, YK76424, YK76425, YK76432, YK76464, YK76665, YK76851, YK76854, YK76862, YK76863, YK76865, YK77451, YK77452, YK77551, YK77552, YK77553, YK77555, YK77557, YK77558, YK77560, YK77563, YK77565, YK77566, YK77568, YK77569, YK77570, YK77571, YK77572, YK77573, YK77575, YK77576, YK77578, YK77580, YK77581, YK77582, YK77584, YK77585, YK77656

USA: Alaska, Yukon-Kuskokwim Delta, Hock Slough* 61.067°N, 165.370°W
YK54775, YK57894, YK76112, YK76115, YK76117, YK76119, YK76120, YK76122, YK76124, YK76156, YK76157, YK76158, YK76161, YK76163, YK76164, YK76165, YK76167, YK76168, YK76169, YK76170, YK76171, YK76172, YK76173, YK76174, YK76177, YK76178, YK76179, YK76181, YK76182, YK76186, YK76187, YK76193, YK76672

USA: Alaska, Yukon-Kuskokwim Delta, Kigigak Island 60.846°N, 164.982°W
KIG1308, KIG1321, KIG1331, KIG1333, KIG1336, KIG1339, KIG1340, KIG1341, KIG1342, KIG1343, KIG1344, KIG1346, KIG1347, KIG2253, KIG2254, KIG2255, KIG2261, KIG2262, KIG2264, KIG2265, KIG2266, KIG2267, KIG2271, KIG2278, KIG2279, KIG2280, KIG2283, KIG2285, KIG2293, KIG2296, KIG2298, KIG2299, KIG6334, KIG6915, KIG6927, KIG6944, KIG6999

*Big Slough and Hock Slough are not official names of locations on any recognized maps, but were given these names for the purpose of identifying areas in this study.
**Samples are archived at the Molecular Ecology Laboratory, U.S. Geological Survey, Anchorage, Alaska.

CHAPTER 4

MULTILOCUS PHYLOGEOGRAPHY AND POPULATION STRUCTURE OF COMMON EIDERS BREEDING IN NORTH AMERICA AND SCANDINAVIA[1]

Abstract — We investigated the population genetic structure, subspecies classification, and postglacial colonization of Common Eiders (*Somateria mollissima*) breeding in North America and Scandinavia and evaluated localities of proposed glacial refugia using microsatellite genotypes, mtDNA control region, and intron sequences from two autosomal nuclear genes. Common Eiders exhibited high levels of structuring at all marker types. Variance in molecular data was better accounted for when populations were grouped by subspecies for nuclear markers, supporting subspecies classifications. Furthermore, populations grouped by subspecies for both principle components analysis and a Bayesian clustering program using microsatellite genotype data. In contrast to nuclear data, mitochondrial DNA (mtDNA) variance was better accounted for when populations were grouped based on geographic proximity indicating a stepwise post-glacial colonization of North America and Scandinavia. Historical population demographic data suggest that Common Eiders were restricted to four glacial refugia

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during the last glacial maxium; Belcher Islands, Newfoundland, Alaskan North Slope, and Svalbard. Newfoundland, North Slope, and Svalbard localities coincide with previously identified glacial refugia; Beringia (northern Alaskan shelf), Newfoundland Bank, and Spitsbergen Bank, respectively (Ploeger 1968). The Belcher Islands population may have retreated with the Laurentide ice sheet to its present day location. Southern refugia appear to have served as the main source populations for postglacial colonization of Canada, southern Alaska, and Scandinavia by Common Eiders. Beringia (North Slope) contributed little to colonizing deglaciated regions and remain genetically differentiated from southern Alaskan, Canadian, and Scandinavian populations.

INTRODUCTION

Pleistocene glacial cycles have influenced genetic diversity and distribution of species breeding in northern latitudes (Hewitt 2004a). Throughout the Arctic, colder climates and ice sheets displaced species to lower latitudes and high latitude ice-free areas during the last glacial maximum (Hewitt 2004a). Fossil and molecular data, however, suggest that some areas of the Arctic, notably Beringia, were unglaciated. Species' ranges contracted into refugia during glacial maxima, and during inter-glacial periods expanded and colonized ice-free areas (Hewitt 2004a). Population expansion from glacial refugia has left predictable genetic patterns in recently colonized regions. Molecular data coupled with coalescent theory have enabled researchers to investigate historical species distribution and demography and identify areas that exhibit a signature of rapid population expansion (Lessa et al. 2004). Conversely, populations that do not exhibit genetic signatures of expansion have aided in the identification and location of glacial refugia.

Despite the importance of glacial refugia in species persistence during glacial maxima and as sources of colonizers of the Arctic, number, locations, and significance of refugia remain largely unknown (Byun et al. 1997, Demboski et al. 1999). Ploeger (1968) provided a comprehensive review of proposed ice-free areas during the last Pleistocene glacial period and postulated the relative importance of ice-free areas as potential refugia for arctic Anatidae based on current species distributions. High arctic ice-free areas proposed by Ploeger (1968) included Beringia, Canadian Arctic Archipelago, northern Greenland, Spitsbergen Bank near Svalbard, and northwest

Norway. Proposed temperate ice-free areas included Newfoundland, western Greenland, Iceland, and western Europe. Without fossil evidence, however, it is difficult to determine whether ice-free areas were inhabited by arctic species and contributed to species persistence. More recently, molecular data coupled with coalescent theory has substantiated Beringia, Canadian Arctic Archipelago, and western Greenland as ice-free refugia for arctic vertebrates (Holder et al. 1999; 2000, Fedorov and Stenseth 2002, Fedorov et al. 2003, Flagstad and Røed 2003, Scribner et al. 2003, Waltari and Cook 2005). Convergence in genetic signatures of population expansion among arctic species could provide insights into the locations of proposed refugia and their relative importance as historical reservoirs of species genetic diversity.

In addition to climatic oscillations during the Pleistocene, patterns in the degree of natal, breeding, and winter philopatry also leave varying signatures in molecular markers (Avise 2004). Female natal and breeding philopatry can lead to high levels of spatial genetic subdivision at maternally inherited mitochondrial DNA (mtDNA). Conversely, males dispersing large distances may homogenize gene frequencies among populations at bi-parentally inherited markers present in the nuclear genome (Scribner et al. 2001). If data were collected from just one of these genomes, gene flow among populations might be grossly over or under-estimated depending on which marker type was used (Avise 2004). However, by combining markers with different modes of inheritance and rates of evolution, researchers may ask a wider range of questions involving species population genetic structure and behavior.

Here we investigate the postglacial colonization of North America and Scandinavia and population genetic structure of Common Eiders (*Somateria mollissima*) using microsatellite genotypes, mtDNA control region, and intron sequences from two autosomal nuclear genes. Common Eiders are an arctic-nesting seaduck, composed of 6–7 morphologically distinct subspecies that have a circumpolar distribution (Goudie et al. 2000). As observed in other waterfowl, female Common Eiders are highly philopatric to natal and breeding sites, whereas males disperse among populations that share common wintering grounds. Both sexes, however, display winter site fidelity (Spurr and Milne 1976). Common Eiders are unusual among seaducks, as they exhibit fine scale spatial genetic structuring for both mtDNA and nuclear markers (Tiedemann et al. 1999, Sonsthagen et al. submitted a). High levels of natal, breeding, and winter site philopatry coupled with microgeographic genetic partitioning observed for Common Eiders, enabled us to investigate patterns of population subdivision and gain insight into the locations of potential Pleistocene refugia for Common Eiders and the contribution of refugia to the postglacial colonization of North America and Scandinavia. We evaluated localities that have been proposed as ice-free areas or glacial refugia in other arctic vertebrates and Common Eider, including; the southern edge of the Bering Land Bridge, northern Beringia, High Arctic Canadian Archipelago, Newfoundland, Spitsbergen Bank, and northwest Norway.

We present the first analysis to assess genetic relationships among North American and Scandinavian eiders that uses microsatellite, nuclear intron, and mtDNA loci. The primary goals of this study were threefold. First, we aimed to use a multilocus

approach to evaluate subspecies classifications. Second, we evaluated genetic diversity within populations to test for refugial populations and directions of post-glacial colonization. Third, we estimated gene flow among populations within and between subspecies

METHODS

LABORATORY TECHNIQUES

We collected data from 12 microsatellite loci (*Aph*08, *Aph*20, *Aph*23; Maak et al. 2003; *Bcaμ*1, *Bcaμ*11, *Hhiμ*3; Buchholz et al. 1998; *Sfiμ*10; Libants et al. unpubl. data; *Smo*4, *Smo*7, *Smo*08, *Smo*10, and *Smo*12; Paulus and Tiedemann 2003), mtDNA control region (545–563 bp; Sonsthagen et al. submitted a), 280 base pairs of intron 3 of *lamin* A, and 386–387 base pairs of intron 11 of *gapdh* (McCracken and Sorenson 2005) from 716 Common Eiders sampled from five subspecies (Fig. 4.1, Appendix 4.A; Sonsthagen et al. submitted a, b): *S. m. v-nigrum* (Alaska and western Canada), *S. m. borealis* (northern Canada and Svalbard, Norway), *S. m. sedentaria* (southern Hudson Bay, Canada), *S. m. dresseri* (eastern Canada), and *S. m. mollissima* (Scandinavia).

Genomic DNA was isolated from blood, feather, or frozen tissues. Methods for DNA extraction, polymerase chain reaction (PCR) amplification, electrophoresis, and cycle sequencing are described in Sonsthagen et al. (submitted a). For quality control purposes, 10% of samples were randomly selected, re-amplified, and genotyped at the 12 microsatellite loci in duplicate. Three primer pairs were used for amplification and sequencing of the mtDNA control region: L263 and H848 (Sonsthagen et al. submitted

a), L263rev (5'–CCAAACTGCGCACCTGACATTCC–'3) and H848, and L319 (5'–TGAATGCTCTAAGAYCCAAACTGC–'3) and H848. MtDNA PCR products were sequenced in both directions and assembled using Sequencher 4.1.2 (Gene Codes Corporation, Ann Arbor, MI). Only sequences from the forward strand of the nuclear introns were collected because PCR templates were short (280–387 bp) and sequences had a consistent electropherogram peak high throughout the length of the fragment. Nuclear sequences that contained double-peaks of approximately equal height, indicating the presence of two alleles, were coded with IUPAC degeneracy codes and treated as polymorphisms. Individuals that were heterozygous (48%) for a single one base pair indel occurring in *gapdh* were also sequenced with the reverse strand to obtain data from the entire fragment. Sequences will be deposited in GenBank (http//:www.ncbi.nlm.nih.gov) upon publication of the results of this dissertation.

Statistical Analyses

Genetic diversity

Allelic phases for *lamin* A and *gapdh* introns were inferred from diploid sequence data using PHASE 2.0 (parameters: 1,000 burn-in period followed by 1,000 iterations; Stephens et al. 2001), which uses a Bayesian approach to reconstruct haplotypes from genotypic data and allows for recombination and the decay of linkage disequilibrium with distance. The PHASE analysis was repeated three times to ensure consistency across runs. Unrooted phylogenetic trees for each gene were constructed in TCS 1.18 (Clement et al. 2000), which estimates genealogies using 95% statistical parsimony probabilities as

defined by Templeton et al. (1992). *Lamin* A and *gapdh* sequences also were analyzed in NETWORK 4.1.0.8 (Fluxus Technology Ltd. 2004) using the Reduced Median network (Bandelt et al. 1995), to illustrate reticulations in the gene trees due to homoplasy or recombination.

Allelic frequencies, inbreeding coefficient (F_{IS}), and expected and observed heterozygosities for microsatellite, mtDNA, and nuclear intron loci were calculated in GENEPOP 3.1 (Raymond and Rousset 1995). Nucleotide and haplotype diversity for each population was estimated in ARLEQUIN 2.0 (Schneider et al. 2000). Tests of selective neutrality and historical fluctuations in population demography were performed in ARLEQUIN using Fu's *Fs* (Fu 1997) and Tajima's *D* (Tajima 1989). Critical significance values of 5% required a *P*-value below 0.02 for Fu's *Fs* (Fu 1997).

Population genetic structure

Estimates of inter-population variance in allelic and haplotypic frequencies (F_{ST}, R_{ST}, and Φ_{ST}) were calculated in ARLEQUIN and FSTAT 2.9.3 (Goudet 1995, 2001). Significance levels were adjusted based on 3,000 permutations or Bonferroni correction ($\alpha = 0.05$), respectively. We used MODELTEST 3.06 (Posada and Crandall 1998) and the Akaike Information Criterion (Akaike 1974) to determine the minimum parameter nucleotide substitution model that best fit the mtDNA and intron sequence data. Pairwise genetic distances between unique alleles and haplotypes were calculated in PAUP* (Swofford 1998) for mtDNA and ARLEQUIN for nuclear introns. Chi-square tests were conducted to determine if allele or haplotype groups were associated with a particular

locality or region. Hierarchical analyses of molecular variance (AMOVA) were conducted in ARLEQUIN to assess genetic diversity among and within populations grouped based on (1) subspecies classifications (groups: Aleutians, Bodfish, Flaxman, Kent Peninsula, YK Delta; Baffin, Belcher, Hudson Straits, Mansel, Southampton, Svalbard; New Brunswick, Nova Scotia; and Soderskar, Tromsø), and (2) geographic proximity (groups: Aleutians, Bodfish, Flaxman, YK Delta; Baffin, Hudson Straits, Kent Peninsula, Mansel, Southampton; Belcher, New Brunswick, Nova Scotia; and Soderskar, Svalbard, Tromsø) using the nucleotide substitution model that best fit the alleles and haplotypes. Principle components analysis (PCA) was performed on microsatellite genotype data to illustrate overall trends. In addition, Bayesian clustering method implemented by STRUCTURE 2.1 (Pritchard et al. 2000) was used to infer the occurrence of population structure without *a priori* knowledge of putative populations and probabilistically assign individuals to putative populations based on microsatellite allelic frequencies. Data were analyzed using an admixture model assuming correlated frequencies with 10,000 burnin period, 100,000 Markov chain Monte Carlo iterations, and number of possible populations (K) ranging from 1–13; the analysis was repeated three times to ensure consistency across runs. To determine if more geographically distant population pairs are also more genetically differentiated (isolation by distance), simple Mantel tests were performed in zt 1.0 (Bonnet and Van de Peer 2002). Significance of Pearson correlation coefficients (r) was assessed using a randomization procedure, in which the original value of the statistic was compared to the distribution of

a random reallocation of the distance values in one of the matrices (randomization = 10,000).

To assess the relative contributions of refugia for Common Eiders as possible source populations for sampled populations, we conducted hierarchical analyses of variance and grouped populations based on proximity to potential refugia. Given the high level of natal and breeding philopatry reported for female Common Eiders, AMOVAs were conducted on mtDNA haplotype data because gene flow among populations through male mediated dispersal may make it difficult to distinguish between contemporary and historical dispersal among populations. Population groups that maximized the variance among groups (Φ_{CT}) were predicted to indicate source populations for colonized areas.

Historical demography and gene flow

We assessed evidence for historical fluctuations in population demography of Common Eider populations to determine if populations were located in potential refugia. Population growth rates were estimated in BOTTLENECK 1.2.02 (Cornuet and Luikart 1996) for microsatellite loci and FLUCTUATE 1.4 (Kuhner et al. 1995) for sequence data. BOTTLENECK compares the number of alleles and gene diversity at polymorphic loci under the infinite allele model (IAM; Maruyama and Fuerst 1985), stepwise mutation model (SMM; Ohta and Kimura 1973), and two-phased model of mutation (TPM; Di Rienzo et al. 1994; parameters: 79% SMM, variance 9; Piry et al. 1999, Garza and Williamson 2001). One thousand simulations were 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 relative to the number of alleles indicates recent population growth, whereas heterozygote excess relative to the number of alleles indicates a recent population bottleneck (Cornuet and Luikart 1996, Luikart 1997). It is important to note that heterozygote deficiency and excess calculated in BOTTLENECK differs from values calculated in other population genetic programs. As mentioned previously, BOTTLENECK compares heterozygote deficiency and excess relative to allelic diversity, not to Hardy-Weinberg equilibrium expectation (Cornuet and Luikart 1996). FLUCTUATE estimates a population growth parameter, g, incorporating coalescence theory (parameters: ten short chains with 200 out of 4,000 sampled trees, and three long chains with 20,000 out of 400,000 sampled trees). Positive values of g indicate population growth over time and negative values indicate population decline. Data were run three times to ensure convergence of parameters across runs. Finally, mismatch distributions of mtDNA haplotype data were calculated in ARLEQUIN to gain further insight into historical population demography. Distributions multimodal in shape indicate a population that is at demographic equilibrium, whereas unimodal distributions suggest that a population has undergone a recent demographic expansion (Rogers and Harpending 1992).

We examined the influence of current and historical processes on population genetic structure by performing a nested clade analysis (NCA) of mtDNA sequence data

(Templeton et al. 1995, Templeton 1998). The haplotype network inferred by TCS was used to define nested series of clades according to Crandall and Templeton (1993). Clades were analyzed in GeoDis 2.0 (Posada et al. 2000), and demographic events were inferred based on an inference key (Templeton 1998, Posada and Templeton 2001).

To further assess gene flow among populations, number of migrants per generation (N_em) and number of female migrants per generation (N_fm) were calculated for nuclear microsatellite and intron loci and mtDNA, respectively, in MIGRATE v2.0.6 (Beerli 1998, 2002, Beerli and Felsenstein 1999) among sampled localities. Full models, θ ($4N_e\mu$ or $N_f\mu$) and all pairwise migration parameters were allowed to vary and estimated individually from the data, and were compared to restricted island models for which θ and pairwise migration parameters were equal among populations (symmetrical gene flow). MIGRATE was run using maximum likelihood search parameters; ten short chains (2000 out of 400,000 sampled trees), five long chains (10,000 out of 2,000,000 sampled trees), and five adaptively heated chains (start temperatures 1, 1.5, 3, 6, and 12; swapping interval = 1). Full models were run three times to ensure the convergence of parameter estimates. Restricted models were run once. Alternative models were evaluated for goodness of fit given the data using a log-likelihood ratio test. The resulting statistic from the log likelihood ratio test is equivalent to a χ^2 distribution with the degrees of freedom equal to the difference in the number of parameters estimated in the two models (Beerli and Felsenstein 2001).

RESULTS

GENETIC DIVERSITY

Bi-parentally inherited microsatellite loci

The number of alleles at the 12 polymorphic microsatellite loci ranged from 3–49, with an average of 13.8 alleles per locus (Appendix 4.B). The average number of alleles per population ranged from 2.7–9.9. Observed heterozygosity ranged from 44.5–57.7% for each population with an overall heterozygosity of 54.3% (Table 4.1). The inbreeding coefficient (F_{IS}) ranged from –0.005 to 0.445 among sampled sites with an overall value of 0.030. None of the inbreeding coefficients were significantly different from zero ($P_{adj.} > 0.05$).

Bi-parentally inherited nuclear introns

Seventy alleles were reconstructed for *lamin* A from 592 individuals in PHASE with 22 variable sites (Fig. 2A, Appendix 4.C). Two hundred seven (40%) individuals were homozygous, and 147 (25%) were heterozygous at one site. Probabilities of reconstructed haplotypes for 77% ($n = 184$) of individuals that were heterozygous for more than one site exceeded 0.85, and the probabilities for the remaining individuals ranged from 0.71–0.84 ($n = 30$, 13%), 0.50–0.68 ($n = 23$, 10%) and 0.34 ($n = 1$, 0.4%). The background recombination rate (ρ) was 0.50, with factors exceeding ρ ranging from 0.40–1.94 between 22 variable sites.

For nuclear intron *gapdh*, 48 alleles were reconstructed from 474 individuals with 22 variable sites (Fig. 2B, Appendix 4.D). Seventy-five (16%) individuals were

homozygous at all variable sites, and 48 (10%) were heterozygous at one site. Probabilities of 77% (n = 272) of reconstructed haplotypes that were heterozygous for more than one site exceeded 0.90, and the probabilities for remaining individuals ranged from 0.71–0.87 (n = 26, 7%) and 0.43–0.68 (n = 53, 15%), which we attribute to potentially high levels of recombination occurring within this locus (0.39–4.41 factors exceeding $\rho = 0.05$, between 22 variable sites). There were two variable sites that exceeded ρ by two or more factors: 2.12 factors between sites 16 and 22, and 4.41 factors between sites 232 and 252.

Haplotype (h) and nucleotide (π) diversity ranged from 0.733–0.901 and 0.005–0.009, respectively for *lamin* A, and 0.506–0.897 and 0.004–0.007, respectively for *gapdh* (Table 4.1). Observed heterozygosity ranged from 32.1–89.2% and 35.3–96.4% for *lamin* A and *gapdh*, respectively (Table 4.1). Significant Fu's Fs ($P < 0.02$) were observed for Aleutian Islands, YK Delta, Bodfish, Flaxman, Kent Peninsula, Belcher Islands, New Brunswick, Nova Scotia, Svalbard, Tromsø, and Soderskar (Table 4.1). We did not observe any significant Tajima's D values (Table 4.1).

Maternally inherited mtDNA

Sixty-four unique haplotypes were identified from 456 individuals with 36 variable sites; 78% of variable sites were located within the first 174 bp (e.g., domain I; Marshall and Baker 1997) of mtDNA control region, and 22% of variable sites were located within the remaining 239 bp (central domain and domain II; Fig. 4.2C, Appendix 4.E). Haplotype (h) and nucleotide (π) diversity ranged from 0.230–1.000 and 0.001–0.009, respectively

(Table 4.1). Significant Fu's *Fs* were observed for Baffin Island, Hudson Straits, and Soderskar (Table 4.1). Nova Scotia also had a significant Tajima's *D* (Table 4.1).

POPULATION GENETIC STRUCTURE

Bi-parentally inherited microsatellite loci

Overall estimates of population subdivision were significant ($F_{ST} = 0.060$, $P < 0.000$; $R_{ST} = 0.020$, $P = 0.010$). Significant estimates of inter-population variance in microsatellite allelic frequency primarily were observed among but not within subspecies, except among the Aleutian Islands and the other *S. m. v-nigrum* populations (Table 4.2). Estimates of F_{ST} generally were higher than R_{ST}, with values ranging from 0.021–0.166 and 0.021–0.203, respectively (Table 4.2). AMOVA revealed partitioning among groups, among populations, and within populations (Table 4.3). More variation was accounted for when populations were grouped by subspecies classification rather than by geographic proximity for both F_{ST} and R_{ST} (Table 4.3).

PCA grouped populations by subspecies classification into four clusters with Belcher Islands (*S. m. sedentaria*) grouping with *S. m. dresseri* (Fig. 4.3). The Bayesian clustering method implemented in STRUCTURE indicated that the likelihood generated for the microsatellite data was maximized when the total number of populations was four (data not shown). Results were similar to the PCA; however, *S. m. v-nigrum* populations were subdivided into two clusters. *S. m. mollissima* and *S. m. borealis* populations clustered together along with *S. m. dresseri* and *S. m. sedentaria* populations (Table 4.4).

Finally, there was a positive correlation between genetic (F_{ST}/[1-F_{ST}] and R_{ST}/[1-R_{ST}]) and geographic distances (F_{ST} r = 0.822, P = 0.001; R_{ST} r = 0.655, P = 0.001).

Bi-parentally inherited nuclear introns

The nucleotide substitution model that best fit *lamin* A and *gapdh* sequence data was the Tamura-Nei (1993) model with an invariant site parameter. Our overall estimate of spatial variance in allelic frequencies (Φ_{ST}) was significant for *lamin* A and *gapdh*, 0.072 and 0.075, respectively. Moreover, inter-population comparisons (Φ_{ST}) showed moderate levels of genetic differentiation with values ranging from 0.014–0.290 and 0.017–0.220 for *lamin* A and *gapdh*, respectively (Table 4.2). Inter-population comparisons calculated from *lamin* A sequence data were lower within subspecies, whereas most significant variances in *gapdh* allelic frequency occurred between *S. m. v-nigrum* and all other subspecies (Table 4.2). Alleles in each of the two-allele groups observed for *lamin* A and *gapdh* are not equally distributed among populations (Fig. 4.2; *lamin* A χ^2 = 86.9, d.f. = 13, $p < 0.001$; *gapdh* χ^2 = 159.6, d.f. = 13, $p < 0.001$). More *v-nigrum* individuals are present in one allele group with the remaining subspecies predominately in the other allele group.

We also calculated F_{ST} values for each of the 22 polymorphic single nucleotide polymorphisms (SNPs). Significant ($P < 0.05$) variance in *lamin* A allelic frequency occurred at five SNPs; 55 (F_{ST} = 0.049), 116 (F_{ST} = 0.040), 174 (F_{ST} = 0.124), 179 (F_{ST} = 0.152), and 195 (F_{ST} = 0.057). Significant overall allelic frequency variance was also observed at six SNPs for *gapdh*; 122 (F_{ST} = 0.131), 129 (F_{ST} = 0.050), 165 (F_{ST} = 0.151),

170 (F_{ST} = 0.058), 232 (F_{ST} = 0.122), and 258 (F_{ST} = 0.142). Within *lamin* A, it does not appear that a single nucleotide position is driving the significant pairwise comparisons observed, and no single SNP accounted for any of the variance observed among populations (data not shown). However, site 258 of *gapdh* appears to account for discordance among *S. m. v-nigrum* and the other subspecies as all populations had significant pairwise comparisons except New Brunswick and Mansel Island and accounted for 54.1% of the variance among populations (data not shown). *Gapdh* sites 122, 165, and 232 are likely driving the differentiation observed between New Brunswick and *S. m. v-nigrum* populations and accounted for 0.148, 0.064, and 0.082 of the variance among populations, respectively (data not shown). The remaining positions (129 and 170) did not account for any of the variance observed among populations.

AMOVA indicated that variance among groups was better accounted for by *lamin* A and *gapdh* when populations were grouped based on subspecies classifications (Table 4.3). There was also a significant positive correlation between genetic ($\Phi_{ST}/[1-\Phi_{ST}]$) and geographic distances assayed using nuclear intron sequence information (*lamin* A; r = 0.706, P = 0.001; *gapdh*; r = 0.791, P = 0.001).

Maternally inherited mtDNA

The nucleotide substitution model that best fit the mtDNA data was the Tamura-Nei (1993) model with an invariant site parameter (substitute rate matrix: R[A–C] = 1.0000, R[A–G] = 31.1491, R[A–T] = 1.0000, R[C–G] = 1.0000, R[C–T] = 32.3007, R[G–T] = 1.0000, p–inv. = 0.9187, A = 0.2248, C = 0.3065, G = 0.1907, T = 0.2780). Our overall

estimate of population subdivision was very high ($\Phi_{ST} = 0.497$, $P < 0.000$), and inter-population comparisons (Φ_{ST}) ranged from 0.051–0.927 (Table 4.2). Few significant comparisons were observed among the Hudson Bay eiders (Baffin Island, Hudson Straits, Southampton Island, Mansel Island, and Belcher Islands; Table 4.2). Populations that predominately were represented by haplotypes located at the tips of the haplotype network (Aleutian Islands, Bodfish, Flaxman, and Soderskar; Fig. 4.2c), exhibited very high levels of structuring among populations (Table 4.2). Haplotypes in each of the two-haplotype groups observed for mtDNA are not equally distributed among populations (mtDNA $\chi^2 = 263.6$, d.f. = 13, $P < 0.001$; Fig. 4.2). More Bodfish and Flaxman individuals are present in one haplotype group with the remaining populations predominately in the other haplotype group. There also was a significant positive correlation between genetic ($\Phi_{ST}/[1-\Phi_{ST}]$) and geographic distances for mtDNA haplotype ($r = 0.705$, $P = 0.001$).

In contrast to the nuclear loci, variance among groups in mtDNA haplotypic frequencies was better accounted for when populations were grouped based on geographic proximity rather than subspecies (Table 4.3). Among group variance (Φ_{CT}) was higher when North Slope (Bodfish and Flaxman) populations were grouped together exclusively than groups based on geographic proximity (Table 4.3, 5), indicating that the Aleutian Islands and YK Delta populations may be more genetically similar to Canadian populations. Moreover, more of the variation among groups was accounted for when North Slope populations were in one group and all remaining populations were in another group (Table 4.5). Among-group variance was also higher when Tromsø was grouped

with New Brunswick and Nova Scotia, indicating Tromsø may be genetically more similar to eastern Canadian than Scandinavian populations.

Historical Demography and Gene Flow

Population fluctuations

Evidence for significant fluctuations in historical population demography was detected based on genotypic data from 12 microsatellite loci. Under the IAM, YK Delta, Bodfish, Flaxman, Nova Scotia, and New Brunswick, showed excess heterozygosity suggestive of a population bottleneck (Table 4.6) and is consistent with band and resight data indicating population declines in Alaskan localities (Stehn et al. 1993, Suydam et al. 2000). Population growth, based on heterozygote deficiency, was observed for all populations except Mansel Island, Nova Scotia, and New Brunswick under the SMM (Table 4.6). Heterozygote deficiency was also observed under the TPM for three populations; Southampton Island, Tromsø, and Soderskar (Table 4.6).

Significant population growth based on nuclear intron sequences was detected using FLUCTUATE for most populations except; Mansel Island, Belcher Islands, and Soderskar with *lamin* A, and Baffin Island, Hudson Straits, Svalbard, Tromsø, and Soderskar with *gapdh* (Table 4.6). Theta ($4N_e\mu$) ranged from 0.009–0.138 for *lamin* A and 0.003–0.047 for *gapdh* (Table 4.6).

Populations showing positive growth rates using mtDNA were Aleutian Islands, YK Delta, Kent Peninsula, Baffin Island, Hudson Straits, Southampton Island, Mansel Island, Tromsø, and Soderskar (Table 4.6). Theta ($2N_f\mu$) ranged from 0.004–0.050.

Mismatch distributions did not reject the sudden expansion model based on sum of squared deviation statistic, with the exceptions of YK Delta, Kent Peninsula, and New Brunswick. Mismatch distributions did not reject the sudden expansion model based on Harpending's raggedness index for any population (Harpending 1994). Parameter estimates for time of expansion (τ) ranged from 0.497–7.877, with the smallest values observed for Aleutian Islands and Soderskar and larger estimates calculated for Kent Peninsula and the other Alaskan populations (Table 4.6).

Nested clade analysis

Thirteen clades had significant correlations among haplotypes and geography (Fig. 4.4), and NCA inferences for these clades are shown in Table 4.7. Continuous range expansion was supported by two clades; (1) Aleutian Islands/Kent Peninsula to Flaxman, and (2) Tromsø to Baffin Island and Nova Scotia. Three clades (I-4, I-12, II-5; Table 4.7) were indicative of past fragmentation and/or long distance colonization involving all analyzed regions. Restricted gene flow with isolation by distance was supported by four clades (I-10, I-15, II-1, II-2; Table 4.7) and with long distance colonization supported by three clades (I-21, II-3, II-4; Table 4.7) including all populations.

Dispersal

Gene flow among populations was estimated by grouping populations based on geographic proximity and subspecies designation, and among populations that exhibited a genetic signature of population stability, to examine historic and contemporary dispersal

among proposed refugial populations. Full models (all parameters allowed to vary independently) had significantly higher ln likelihoods than the restricted island model (symmetric gene flow) across all population groupings and all marker types, indicating asymmetric dispersal among analyzed populations (data not shown).

Gene flow estimates based on microsatellite loci are, in general, higher than estimates based on nuclear intron and mtDNA loci (Table 4.8). Among refugial populations, number of migrants per generation (N_em or N_fm) ranged from 4.53–15.22, 0.27–34.95, and 0.00–3.95 N_em for estimates based on microsatellites, introns, and mtDNA loci, respectively (Table 4.8). Asymmetrical gene flow, as indicated by non-overlapping 95% confidence intervals, was observed among all populations analyzed. Calculations of N_em from nuclear DNA indicated that, on average across generations, more individuals dispersed from Belcher Islands to New Brunswick, North Slope to New Brunswick, and Svalbard to Belcher Islands, New Brunswick, and North Slope (Table 4.8). Most gene flow estimates based on mtDNA control region were low and suggested symmetrical gene flow among populations, with several exceptions. More females dispersed from New Brunswick to North Slope and Svalbard (Table 4.8).

S. m. v-nigrum populations' gene flow estimates ranged from 10.79–25.50, 0.01–85.90, and 0.00–3.17 N_em for estimates based on microsatellites, introns, and mtDNA loci, respectively (Table 4.8). Asymmetrical gene flow was observed among all *S. m. v-nigrum* populations for nuclear loci, with more individuals dispersing from North Slope to Aleutian Islands and Kent Peninsula, and YK Delta to all populations. Directionality of dispersal differed between microsatellite and intron estimates for the Aleutian Islands

and Kent Peninsula. Microsatellite-based calculations indicated more individuals dispersed from Kent Peninsula to Aleutian Islands, whereas estimates based on introns suggested the reciprocal (Table 4.8). Gene flow estimates based on mtDNA control region were low and indicated symmetrical gene flow among populations, with two exceptions. More females dispersed from Aleutian Islands to Kent Peninsula, and North Slope to Kent Peninsula (Table 4.8).

Among Central Canadian and Svalbard populations, N_em ranged from 5.47–31.81, 0.00–55.88, and 0.00–41.37 for estimates based on microsatellites, introns, and mtDNA loci, respectively (Table 4.8). Asymmetrical gene flow was observed among all Central Canadian and Svalbard populations for nuclear loci, with more individuals dispersing from Belcher Islands to Baffin, Kent Peninsula to Baffin, Hudson Straits to Baffin, and Kent Peninsula, Southampton to Belcher Islands, and Svalbard to Baffin, Belcher Islands, Hudson Straits, and Kent Peninsula. Directionality of dispersal differed between microsatellite and intron estimates for Baffin, Hudson Straits, and Kent Peninsula, and Southampton. Microsatellite-based calculations indicated more individuals dispersed from Southampton to Baffin, Hudson Straits, and Kent Peninsula. Conversely, estimates based on introns indicated more individuals dispersed from Baffin, Hudson Straits, and Kent Peninsula to Southampton Island (Table 4.8). Gene flow estimates based on mtDNA control region indicated more females dispersed from Hudson Straits to Southampton, and Southampton to Belcher Islands; however populations have overlapping 95% confidence intervals (Table 4.8).

Gene flow estimates among southern Canadian populations ranged from 7.66–19.26, 3.27–21.35, and 0.21–8.93 N_em for estimates based on microsatellites, introns, and mtDNA loci, respectively (Table 4.8). Asymmetrical gene flow was observed between two populations, more individuals dispersed from Belcher Islands to New Brunswick based on nuclear introns (Table 4.8). Gene flow estimates based on mtDNA control region indicated asymmetrical gene flow among most populations. More females dispersed from New Brunswick to Belcher Islands and Nova Scotia (Table 4.8).

Among Scandinavian populations, N_em ranged from 9.20–24.08, 3.31–15.31, and 0.00–12.96 for estimates based on microsatellites, introns, and mtDNA loci, respectively (Table 4.8). N_em estimates among populations based on nuclear DNA indicated more individuals dispersed from Soderskar to Svalbard and Tromsø, and Svalbard to Tromsø (Table 4.8). Gene flow estimates based on mtDNA are congruent with nuclear DNA estimates, except N_fm estimates between Svalbard and Tromsø have overlapping 95% confidence intervals, but the variances suggest that more individuals are dispersing from Svalbard to Tromsø (Table 4.8).

DISCUSSION

Population Subdivision

Low to moderate levels of spatial genetic structuring observed for bi-parentally inherited nuclear markers were not surprising, due to aspects of Common Eider breeding and wintering biology. Pair formation occurs in coastal waters during non-breeding months, where admixture of several breeding populations of Common Eiders likely occurs. Male

eiders follow females back to breeding sites, and males have been reported to have high natal and breeding dispersal distances (Wakely and Mendall 1976, Swennen 1990). Male dispersal, therefore, is expected to homogenize allelic frequencies in the nuclear genome (Scribner et al. 2001). The overall lack of population subdivision observed within subspecies and more significant comparisons between subspecies assayed using microsatellite and nuclear intron loci, supports the hypothesis that male gene flow among populations homogenizes gene frequencies in the nuclear genome, as populations within the same subspecies share common wintering grounds (Ploeger 1968, Tiedemann and Noer 1998, Petersen and Flint 2002). Aleutian Islands were the only locality with significant inter-population comparisons among populations within the same subspecies. We attribute significant comparisons observed to *S. m. v-nigrum* populations wintering in disparate locations in the Bering Sea. Eiders breeding on the Aleutian Islands are believed to be residents, because eiders are observed year-round in near shore waters of the Aleutian chain (M. Petersen pers. comm.). In contrast, eiders from Bodfish, Flaxman, Kent Peninsula, and YK Delta are migratory to varying degrees and winter in the near shore waters of Chukotka Peninsula, Bristol Bay, and Yukon-Kuskokwim Delta (Petersen and Flint 2002, L. Dickson pers. comm.). Eiders from *S. m. v-nigrum* populations likely wintered in different locations over evolutionary time, allowing the accumulation of genetic differences among populations.

Genetic discordance observed among Common Eider populations appears to be driven more by migration rather than mutation, as our overall estimate of subdivision F_{ST} was higher than R_{ST} assayed from 12 microsatellite loci. Furthermore, inter-population

variances in allelic frequency were higher, along with more significant comparisons observed for F_{ST} based estimates. Populations could not have been subdivided long enough to accumulate two or more mutational events (Estoup et al. 2002). However, concordance between the rapidly evolving microsatellite loci and the more slowly evolving nuclear intron loci in the lack of population structure within subspecies, suggests that populations have been subdivided long enough for mutations to accumulate. Low levels of contemporary gene flow probably are occurring among populations, despite high winter site philopatry reported for waterfowl (Robertson and Cooke 1999). A *S. m. borealis* male was collected from Point Barrow, Alaska, during fall migration (07 August 1994, University Alaska Museum specimen UAM6631), which is part of the *S. m. v-nigrum* migratory route (Petersen and Flint 2002, L. Dickson pers. comm.). Occasional male dispersal among populations that do not normally share common wintering grounds may provide enough gene flow among wintering areas to limit the accumulation of genetic differences among populations resulting in dispersal playing a larger role in population differentiation rather than mutation.

Subspecies classifications for the five subspecies represented in this study are strongly supported by nuclear data. Few significant inter-population comparisons were observed within subspecies for both microsatellite and nuclear intron loci based on intra-subspecies comparisons, variance in allelic frequencies among groups, and PCA. Moreover, *S. m. v-nigrum* appears to be well supported by the Bayesian clustering method implemented in STRUCTURE, which assigned a majority of *S. m. v-nigrum* individuals to two of the four clusters almost exclusively. However, *S. m. borealis* and *S.*

m. mollissima individuals were grouped in one cluster and *S. m. dresseri* and *S. m. sedentaria* individuals were assigned to another cluster. Groupings could be a result of colonization of populations from the same glacial refugia or contemporary gene flow among populations. Common Eiders breeding in central and eastern Canada and Scandinavia may exhibit low levels of winter site fidelity. In areas where subspecies distributions overlap, individuals may winter in areas that are geographically closer and intermix with another subspecies, rather than migrating farther to winter with populations of the same subspecies. For example, *S. m. borealis* is reported to winter in the coastal waters of northern Norway and Labrador (Ploeger 1968), adjacent to *S. m. mollissima* and *S. m. dresseri* breeding sites. Because there is little overlap among *S. m. v-nigrum* distribution and other subspecies, the split between *S. m. v-nigrum* and the other subspecies would be expected. Therefore, individuals breeding in Canada and Scandinavia may be more likely to intermix with populations of different subspecies, resulting in lower inter-subspecies comparisons.

High spatial genetic structure assayed for mtDNA control region support banding data, which clearly indicate that female Common Eiders exhibit high natal and breeding philopatry (Goudie et al. 2000). In contrast to microgeographic population subdivision assayed between Alaskan North Slope populations (~90 km apart) and between *S. m. dresseri* populations (~200 km apart), few significant inter-population comparisons were observed between *S. m. borealis* and *S. m. sedentaria* populations. Female dispersal among Hudson Bay populations would be expected to homogenize mtDNA haplotype frequencies. Researchers have hypothesized that if suitable habitat is available, first-time

female breeders may nest near their wintering grounds rather than returning to natal sites (Tiedemann et al. 2004). Alternatively, *S. m. borealis* and *S. m. sedentaria* populations could have been recently colonized populations expanding from the same glacial refugium. Given significant positive growth rates observed at nuclear and mtDNA markers, except for Belcher Islands and Svalbard populations, Hudson Bay populations probably were colonized more recently and have not had sufficient time for genetic differences to evolve among populations.

Comparatively higher levels of population subdivision observed in mtDNA relative to nuclear DNA, alternatively could be attributed to lineage sorting. MtDNA has a lower effective population size relative to nuclear DNA; therefore, when mutation rate and selection are held constant, genetic drift has a larger effect on mtDNA than nuclear DNA (Avise 2004), translating to higher estimates of population subdivision (F_{ST}). The effects of lineage sorting and sex-biased differences in philopatry on spatial genetic subdivision; however, are not mutually exclusive and both factors may be playing a role in the degree of population structure observed. However, microsatellite loci have a high rate of mutation relative to mtDNA (Avise 2004); as a result, new mutations arising more frequently within populations. By chance alone, one would expect new mutations to increase in frequency among isolated populations and, over time, dampen the effects of lineage sorting within microsatellite loci. Given differences in the degree of philopatry in Common Eiders between the sexes and congruence in results between microsatellite and nuclear intron loci, we suggest that differences in estimates of population subdivision

probably are more attributable to male biased dispersal and high natal and breeding philopatry in females, rather than lineage sorting for sampled populations.

Differences in the degree of population subdivision observed between mtDNA and nuclear DNA also may be attributable to homoplasy. Not identifying unique alleles because fragments of the same length may have different sequence information, or have mutated back to the ancestral state are issues with fragment analyses (Estoup et al. 2002). Both types of homoplasy could pose problems when assessing population structure based on detecting allelic frequency differences among populations, where not identifying unique alleles among populations may lower population subdivision estimates (Estoup and Cornuet 1999). However, Rousset (1996) showed that there are no simple effects of homoplasy on population differentiation estimators (F_{ST} and R_{ST}) for loci evolving under the stepwise mutation model or an island model of migration. For the mutation process, and therefore homoplasy, to have an effect on estimators of population subdivision, subpopulations need to have different ratios of coalescent times of genes long enough to have two or more mutational events to occur (Estoup et al. 2002). Because our estimate of subdivision for F_{ST} was greater than R_{ST}, mutation, and therefore homoplasy, does not appear to be playing a major role in differentiating populations of Common Eiders.

Phylogeography and Postglacial Colonization

Concordance in allele and haplotype groups among nuclear microsatellite loci, introns, and mtDNA control region sequences suggest that Common Eiders were subdivided into populations occupying at least two long-term glacial refugia during the Pleistocene: *S. m.*

v-nigrum (exclusively North Slope populations for mtDNA) and the other four subspecies. The presence of a distinctive northern group suggests a historical split into an arctic refugium northwest of the continental ice sheets and subarctic refugia south of the ice sheets, a pattern identified in mammals by Nadler and Hoffmann (1977). The vicariant event that resulted in the divergence among North Slope populations and the other Alaskan, Canadian, and Scandinavian populations appears to have been maintained through evolutionary time, as few populations share similar haplotypes with the North Slope populations.

Estimates of genetic diversity appear incongruent with demographic analyses, as populations in formerly glaciated regions do not have significantly lower genetic diversity (based overlapping 95% CI) than proposed refugial populations. Populations residing in previously glaciated regions are expected to have lower haplotype diversity due to successive founder events (Hewitt 1996). However, admixture of mtDNA haplotypes from different Pleistocene refugia may have increased genetic diversity of Common Eiders in formerly glaciated areas (Fedorov et al. 1999). Regions where distinct haplotype groups co-occur, such as Kent Peninsula, support admixture of individuals from different arctic and subarctic refugia. Presence of a contact zone between Beringian and eastern Canadian haplotypes in Kent Peninsula provides further evidence for the MacKenzie River suture zone (Hewitt 2004b). MacKenzie River coincides with the eastern and western extent of the North American Cordilleran and Laurentide ice sheets and likely formed an eastern boundary for the Beringian refugium (Hewitt 2004b). Concordance for contact zones in other arctic vertebrates in the

MacKenzie River region indicates that this region was a strong geographic barrier limiting dispersal from Beringian populations (e.g. Collared lemming, *Dichrostonyx* ssp., Fedorov and Stenseth 2002; True lemming, *Lemmus* spp., Fedorov et al. 2003; Rock Ptarmingan, *Lagopus mutus*, Holder et al. 1999; 2000). Our data suggest that this region also may have contributed to divergence between eiders from the Alaskan North Slope and eiders inhabiting all other regions.

Historical population demographic data suggest that Common Eiders were restricted to four glacial refugia during the last glacial maxium; Belcher Islands, Newfoundland, North Slope, and Svalbard. Three regions exhibit a signal of a demographically stable population based on mtDNA estimates, North Slope, *S. m. dresseri*, and Svalbard populations, and coincide with previously identified glacial refugia; Beringia (northern Alaska shelf), Newfoundland Bank, and Spitsbergen Bank, respectively (Ploeger 1968). The proposed location of the Beringian refugium for Common Eiders; however, differs from Ploeger (1968), who hypothesized that Common Eiders where restricted to the southern edge of the Bering Land Bridge. Our data suggest that eiders in this region may have occupied an arctic refugium, north of the land bridge. Moreover, populations sampled from the Aleutian Islands and YK Delta have a genetic signature of relatively recent population expansion, and therefore, it is unlikely that these areas served as refugia for Common Eider populations. Belcher Islands did not exhibit a signal of population growth, despite Hudson Bay being glaciated during the Wisconsin glacial. Given the central position of haplotypes within the mtDNA network representing *S. m. sedentaria* individuals, it is likely that Belcher Island haplotypes are more historic

relative to the other sampled haplotypes (Alsos et al. 2005). *S. m. sedentaria* might have been restricted south of the Laurentide ice sheet, as proposed for other arctic vertebrates (Flagstad and Røed 2003, Scribner et al. 2003), and slowly colonized behind with the retreating ice sheet to its present day location. Shorter movements from a location south of the ice sheet to present day locations would allow populations to retain genetic diversity because effective population sizes would not be reduced (Hewitt 1996), especially if colonization occurred over a long period. Maintenance of genetic diversity while colonizing recently glaciated areas would, therefore, not be expected to produce a genetic signature of population expansion because this signature assumes low-diversity founder populations (Galbreath and Cook 2004).

Beringian populations (Bodfish and Flaxman) apparently contributed little to the postglacial colonization of North America, as few populations share North Slope haplotypes. Limited post-glacial colonization of unglaciated regions by Beringian populations has been observed in other arctic vertebrates (*Lemmus* ssp., Fedorov et al. 2003). Analyses suggest that Common Eiders dispersed west to Kent Peninsula and northern Hudson Bay, and some long distance dispersal to Scandinavian populations may have occurred. Contact zone between arctic and subarctic refugia in the vicinity of Kent Peninsula may explain why data for eiders in this region do not fit the sudden expansion model, as population subdivision violates the assumption of the sudden expansion model (Marjoram and Donnelley 1994). Surprisingly, few North Slope individuals appear to have dispersed to the Aleutian Island and YK Delta. Minimal dispersal from the Beringian refugium to southern Alaska could be attributed to the longer persistence of the

Laurentide ice sheet relative to the Cordilleran ice sheet (Westgate et al. 1987). The presence of the Cordilleran ice sheet may have inhibited colonization of southwest Alaska by Beringian Common Eiders due to the unavailability of habitat and is supported by the relatively late estimated time of population expansion (τ) for the Aleutian Islands.

The central position of southern refugia haplotypes (Belcher Islands and Newfoundland) indicate that populations expanding out of the southern refugia likely colonized formally glaciated areas of Canada, southern Alaska, and Scandinavia. Though the time of divergence (τ) calculated for New Brunswick is recent relative to other populations, mismatch distributions may not be a good estimator of population expansion, as New Brunswick did not fit the sudden expansion model. The Newfoundland populations, in particular, share haplotypes with most sampled sites suggesting this region was a main source for colonizers during glacial retreats through both range expansion and long distance colonization. Low genetic diversity estimates calculated for Nova Scotia may have been caused by a reduction in effective population size through founding events, as peripheral expanding populations are expected to have lower genetic diversity relative to central populations (Nei et al. 1975). Belcher Island haplotypes appear more restricted in their geographic range and this region may have been the main source of colonizers for Hudson Bay and southern Alaska. Slower colonization through short dispersal may have acted as a barrier to colonization (Runck and Cook 2005) of Hudson Bay and southern Alaska by other refugia. Belcher Island and Newfoundland haplotypes are shared with a majority of populations and are located

centrally in the mtDNA haplotype network; therefore, these regions were likely important refugia for Common Eider postglacial colonization.

The Spitsbergen Bank (Svalbard) refugium also appears to have played a role in the colonization of glaciated areas in Canada and Scandinavia. The peripheral location of Svalbard haplotypes in the mtDNA haplotype network suggests that this region was not a main source of colonizers for Canadian populations. Svalbard was likely the main colonizer of Soderskar because Soderskar shares few haplotypes with other regions. Shared haplotypes from Canadian and Scandinavian refugia suggest that Tromsø may be a contact zone for these regions. Evidence of ice-free areas in northern Norway during the last glacial is controversial (Ploeger 1968). However, eider relics dating to approximately 115,000 years ago have been identified from northern Norway (Lauritzen et al. 1996). If ice-free areas did occur, this may explain, in part, the distribution of haplotypes observed for Tromsø and the relatively moderate time of divergence estimate. Tromsø could have been colonized initially by the Newfoundland refugium and later came in contact with Svalbard colonizers through range expansion during glacial retreat. Northern Norway has been identified as a contact zone for other vertebrates (*Microtus agrestis*, Jaarola and Searle 2002; *M. oeconomus*, Brunhoff et al. 2003). In addition, Tiedemann et al. (2004) examined the post-glacial colonization of Europe by Common Eiders and hypothesized that Europe was colonized through range expansion by populations expanding from a single refugium via range expansion located in southern Norway. This scenario is consistent with our findings. Eiders could have colonized

southern Norway from the Newfoundland refugium, which was a main source population for the subsequent colonization of Europe during glacial retreat.

Conclusions

High levels of natal and breeding philopatry and winter site fidelity observed in waterfowl have predictable effects on population genetic structure, and researchers characterizing populations using genetic techniques could under- or over-estimate the degree of population genetic differentiation if estimates are based on a single marker type. As seen in Common Eiders, nuclear and mtDNA markers show varying levels of genetic partitioning among breeding sites. Therefore, not utilizing molecular markers with varying modes of heritance could mislead researchers characterizing genetic variation within populations.

Concordance of proposed glacial refugia utilized by Common Eiders with other arctic species indicates that arctic and subarctic refugia northwest and southeast of the ice-sheets, respectively, were important reservoirs of genetic diversity during the Pleistocene. Southern refugia appear to have served as the main source populations for postglacial colonization of Canada, southern Alaska, and Scandinavia as proposed for other vertebrates (Flagstad and Røed 2003, Scribner et al. 2003). Data suggest a stepwise postglacial colonization of North America and Scandinavia by Common Eiders with some bouts of long distance dispersal. Restricted gene flow expanding out from proposed refugia is supported by the increase in genetic differentiation with distance (Kimura and Weiss 1964). In contrast to Common Eiders restricted to southern refugia,

eiders residing in Beringia (and those on the North Slope of Alaska in particular) contributed little to colonizing deglaciated regions and remain genetically differentiated from Canadian and Scandinavian populations. Minimal colonization by the Beringian refugium is particularly evident in that geographically close populations (Aleutian Islands, Kent Peninsula, and YK Delta) share few haplotypes with North Slope Common Eiders and appear to be more genetically similar to central and eastern Canadian populations. Genetic discordance among populations residing in Beringia and other refugia has been maintained through evolutionary time despite contemporary gene flow among populations through male dispersal.

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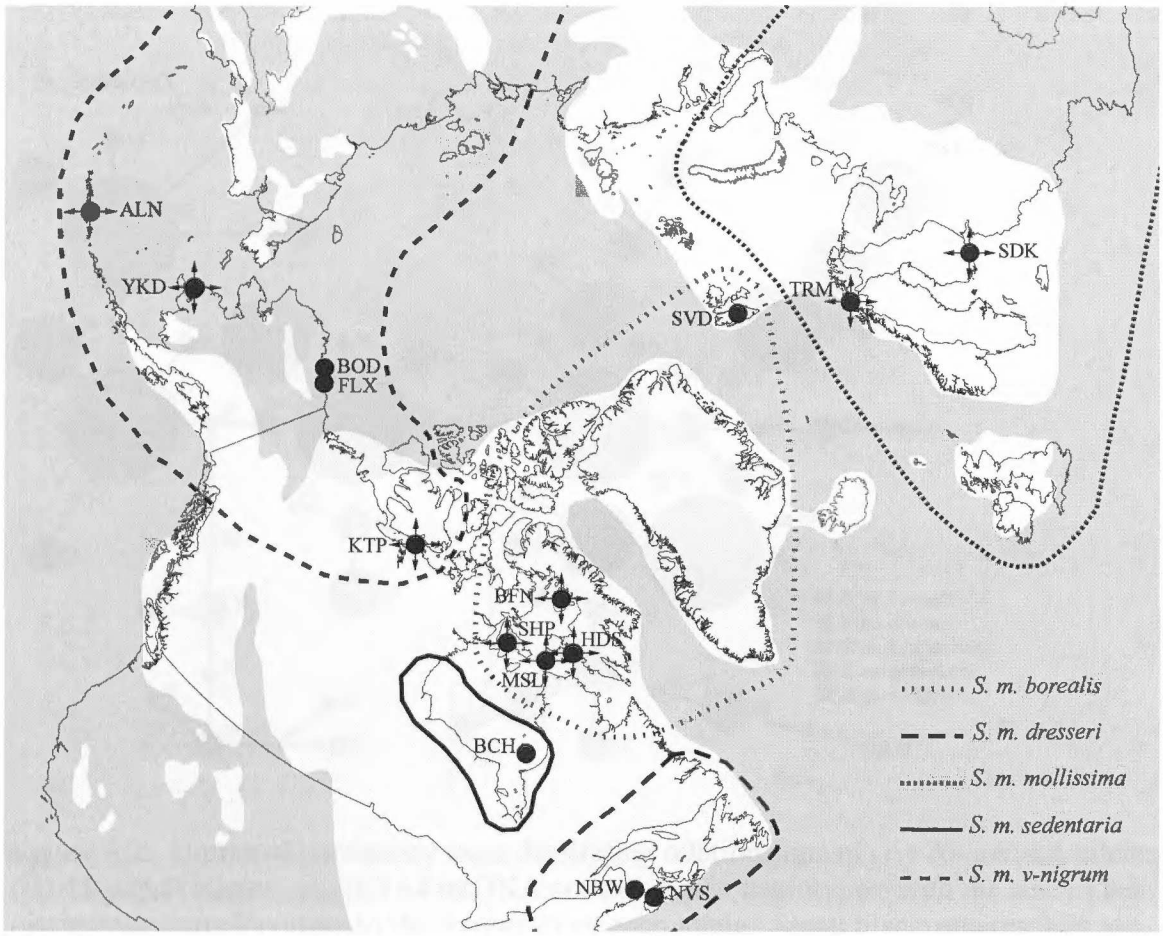


Figure 4.1: Subspecies distribution and localities of the 15 Common Eider populations sampled in this study: *S. m. borealis*; Baffin Island (BFN), Hudson Straits (HDS), Mansel Island (MSL), Southampton Island (SHP), and Svalbard (SVD), *S. m. dresseri*; New Brunswick (NBW), and Nova Scotia (NVS), *S. m. mollissima*; Soderskar (SDK), and Tromsø (TRM), *S. m. sedentaria*; Belcher Islands (BCH), and *S. m. v-nigrum*; Aleutian Islands (ALN), Bodfish (BOD), Flaxman (FLX), Kent Peninsula (KTP), and Yukon-Kuskokwim Delta (YKD). Arrows indicate populations with a positive growth signature. Extent of the most recent last glacial ice sheets are illustrated in white, and unglaciated regions are illustrated in gray (Hewitt 2004b).

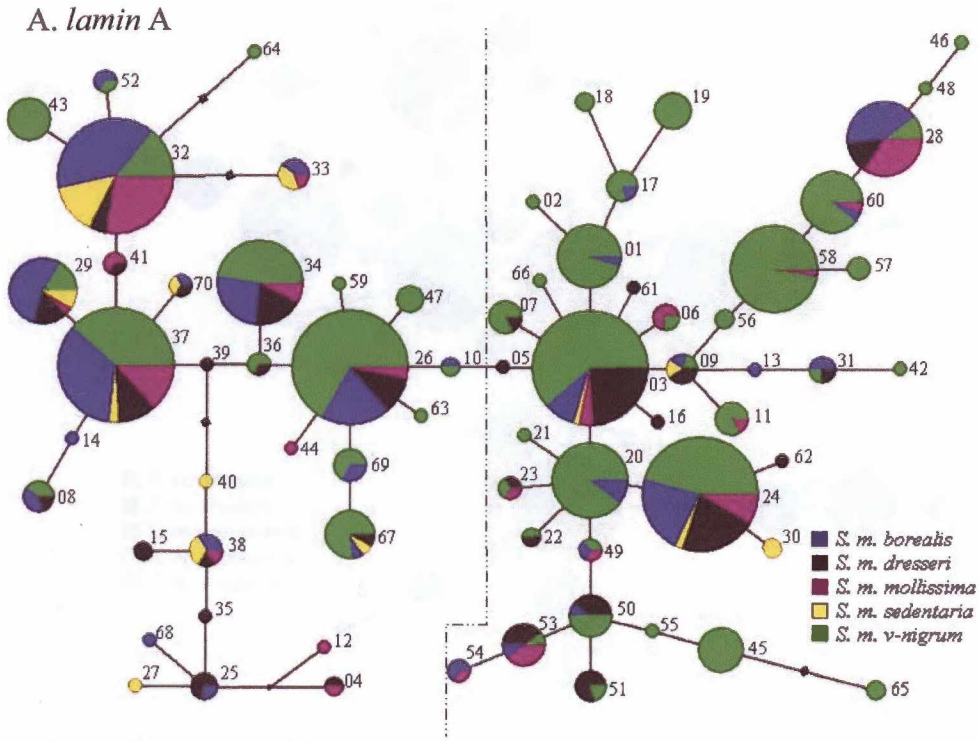
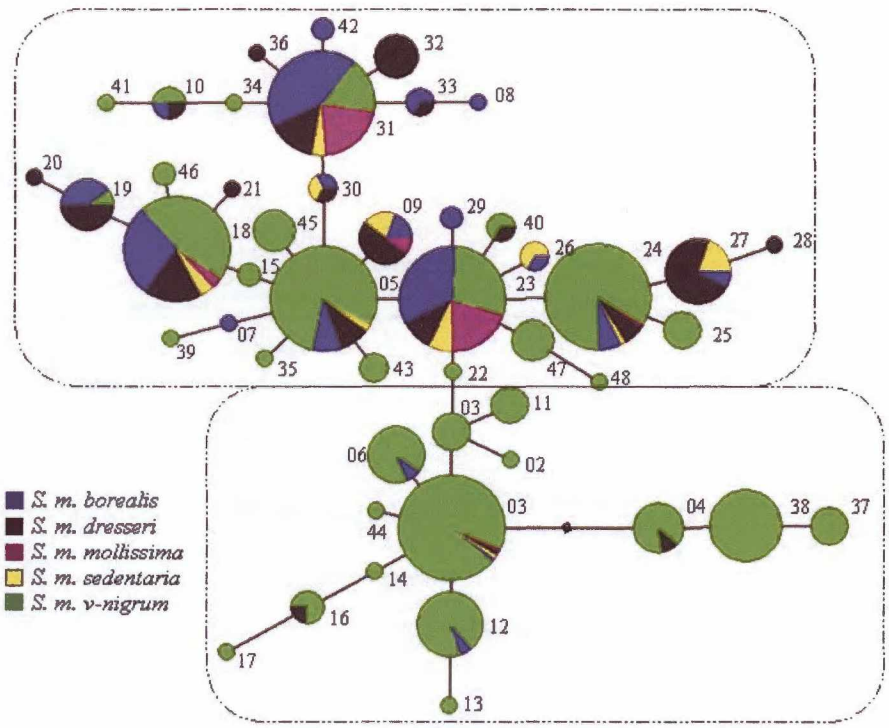


Figure 4.2: Unrooted parsimony trees illustrating relationships of (A) 70 *lamin* A alleles, (B) 48 *gapdh* alleles, and (C) 64 mtDNA control region haplotypes, with the size of the circle node corresponding to the frequency of each allele. Small black squares indicate intermediate ancestral alleles that were not sampled. Each sampled subspecies has a unique color.

B. *gapdh*



C. mtDNA control region

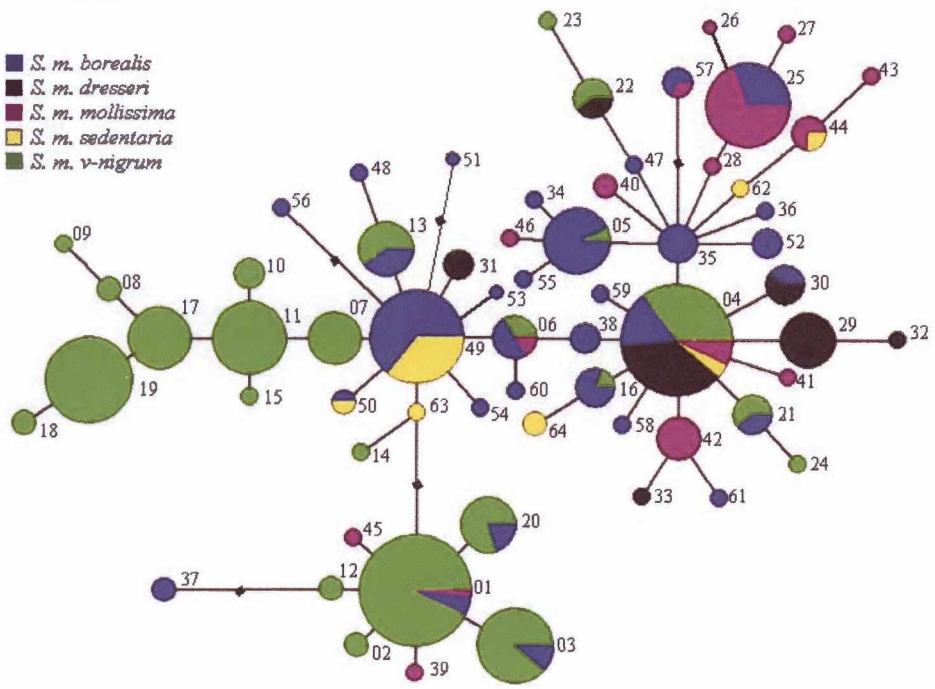


Figure 4.2 cont.

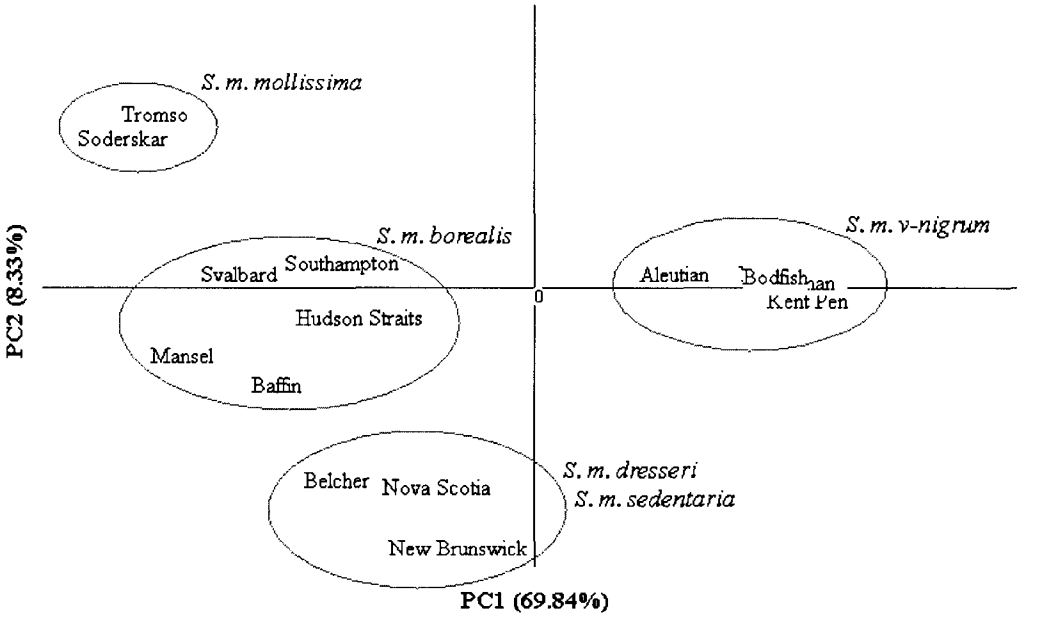


Figure 4.3: Canonical plot of the first two principal components illustrating the partitioning of overall F_{ST} variance among Common Eider populations. Ellipses illustrate the relative positions of populations from each of the five sampled subspecies.

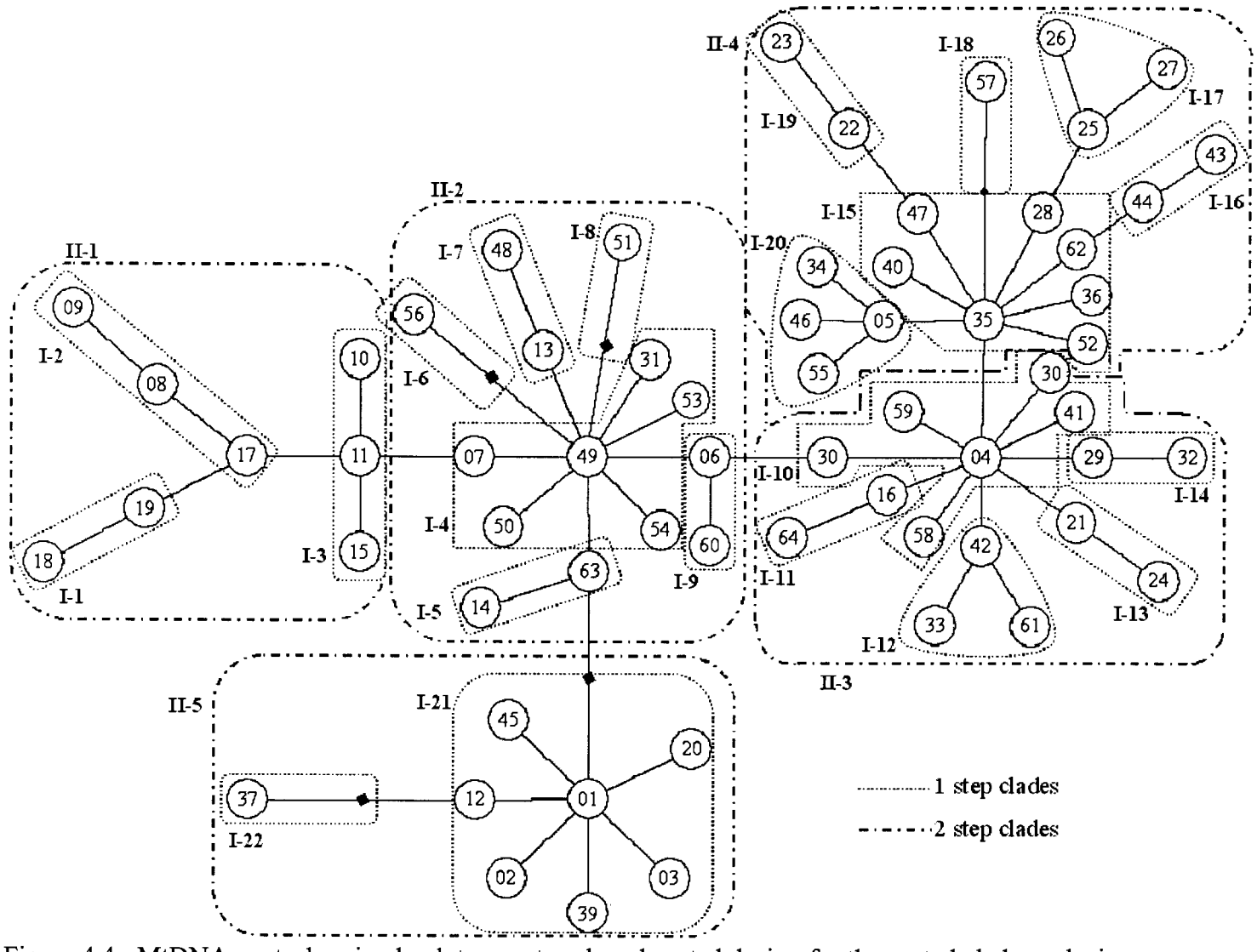


Figure 4.4: MtDNA control region haplotype network and nested design for the nested clade analysis.

Table 4.1: Observed (Ho) and expected heterozygosities (He), haplotype (*h*) and nucleotide (π) diversity, with standard deviation (SD), mean number of alleles or number of unique haplotypes per population, and sample size (*n*), for 12 microsatellite loci, *lamin* A, *gapdh*, and mtDNA control region.

	ALN	YKD	BOD	FLX	KTP	BFN	HDS	SHP
Msats								
Ho	0.516	0.577	0.578	0.563	0.549	0.478	0.548	0.535
He	0.537	0.595	0.602	0.587	0.602	0.487	0.540	0.537
Mean no. alleles	6.75	9.50	8.86	8.93	7.43	5.50	6.50	8.33
n	50	124	100	99	41	15	28	52
***Lamin* A**								
Ho	0.804	0.563	0.375	0.487	0.484	0.643	0.321	0.462
He	0.879	0.899	0.871	0.871	0.901	0.870	0.825	0.835
h	0.879	0.899	0.871	0.871	0.901	0.870	0.825	0.835
SD	0.019	0.010	0.019	0.019	0.019	0.039	0.023	0.020
π	0.007	0.008	0.008	0.007	0.009	0.007	0.007	0.007
SD	0.004	0.005	0.005	0.004	0.005	0.005	0.004	0.004
Fu's *Fs*	**–7.07***	**–18.03***	**–9.91***	**–6.16***	**–6.97***	–3.22	–0.26	–3.92
Tajima's *D*	–0.43	–0.29	–0.29	–0.26	0.37	0.01	1.32	0.60
No. Alleles	17	31	22	15	17	10	8	14
n	92	220	128	74	62	28	56	104
Gapdh								
Ho	0.918	0.918	0.920	0.931	0.964	0.857	0.900	0.778
He	0.821	0.889	0.897	0.897	0.840	0.773	0.669	0.781
h	0.821	0.889	0.897	0.897	0.840	0.773	0.669	0.781
SD	0.022	0.010	0.013	0.019	0.026	0.052	0.050	0.031
π	0.005	0.007	0.007	0.007	0.006	0.005	0.005	0.006
SD	0.003	0.004	0.004	0.004	0.004	0.003	0.003	0.003
Fu's *Fs*	**–5.76***	–2.55	–2.74	–2.29	–0.68	0.33	2.51	–0.68
Tajima's *D*	–0.94	–0.44	–0.48	–0.58	–0.52	1.60	1.37	0.88
No. Alleles	15	21	17	15	12	7	7	13
n	98	170	100	58	56	28	40	72

Table 4.1 cont.

	ALN	YKD	BOD	FLX	KTP	BFN	HDS	SHP
MtDNA								
h	0.638	0.667	0.510	0.701	0.862	0.923	0.933	0.881
SD	0.057	0.049	0.078	0.076	0.052	0.050	0.040	0.036
π	0.002	0.005	0.003	0.006	0.009	0.004	0.005	0.006
SD	0.001	0.003	0.002	0.003	0.005	0.003	0.003	0.003
Fu's *Fs*	–0.19	0.82	–1.68	0.14	–0.22	**–2.87***	**–4.97***	–4.91
Tajima's *D*	–0.30	0.17	–1.06	–0.26	0.89	–0.36	–0.64	–0.67
No. haplotypes	5	9	8	8	10	9	14	15
n	46	78	52	31	26	14	21	37

* Significant *P*–values for Fu's *Fs* ($P < 0.02$) and Tajima's *D* ($P < 0.05$)

Table 4.1 cont.

	MSL	SVD	BCH	NBW	NVS	TRM	SDK
Msats							
Ho	0.472	0.500	0.496	0.550	0.519	0.445	0.456
He	0.517	0.481	0.534	0.543	0.538	0.444	0.454
Mean no. alleles	2.67	6.92	6.50	6.50	7.33	5.83	5.75
n	3	37	22	40	40	38	27
***Lamin* A**							
Ho	0.333	0.892	0.400	0.821	0.692	0.730	0.846
He	0.733	0.866	0.782	0.903	0.853	0.861	0.856
h	0.733	0.866	0.782	0.903	0.853	0.861	0.856
SD	0.155	0.020	0.063	0.017	0.030	0.023	0.029
π	0.005	0.007	0.008	0.008	0.007	0.009	0.007
SD	0.004	0.005	0.005	0.005	0.005	0.005	0.005
Fu's *Fs*	0.46	**–8.89***	**–4.96***	**–11.35***	**–15.87***	**–6.16***	**–5.51***
Tajima's *D*	0.60	–0.24	–0.20	–0.14	–0.40	–0.05	–0.45
No. Alleles	3	18	13	21	23	17	14
n	6	74	40	78	78	74	52
Gapdh							
Ho	0.667	0.714	0.650	0.879	0.840	0.704	0.353
He	0.733	0.684	0.754	0.886	0.873	0.587	0.506
h	0.733	0.684	0.754	0.886	0.873	0.587	0.506
SD	0.155	0.056	0.065	0.016	0.028	0.048	0.069
π	0.006	0.005	0.005	0.007	0.006	0.005	0.004
SD	0.004	0.003	0.003	0.004	0.004	0.003	0.003
Fu's *Fs*	1.31	–0.48	–2.57	–1.41	–3.39	1.57	3.13
Tajima's *D*	0.37	–0.12	0.28	0.87	–0.40	0.71	0.72
No. Alleles	3	9	10	14	14	6	3
n	6	56	40	66	50	56	34

Table 4.1 cont.

	MSL	SVD	BCH	NBW	NVS	TRM	SDK
MtDNA							
h	1.000	0.900	0.726	0.714	0.230	0.877	0.380
SD	0.272	0.032	0.092	0.058	0.110	0.043	0.134
π	0.004	0.006	0.004	0.003	0.001	0.006	0.001
SD	0.003	0.003	0.003	0.002	0.001	0.003	0.001
Fu's *Fs*	–0.69	–2.54	–0.81	–0.22	–1.46	–3.76	**–2.07***
Tajima's *D*	0.00	–0.31	0.04	–0.89	**–2.09***	–0.38	–1.42
No. haplotypes	3	11	7	6	4	12	4
n	3	27	20	31	25	26	19

Table 4.2: Pairwise F_{ST}, R_{ST}, and Φ_{ST} values for 12 microsatellite loci, *lamin* A, *gapdh*, and mtDNA control region for 15 Common Eider populations. Significant pairwise comparisons ($\alpha = 0.05$) are in bold text, and populations representing the same subspecies are shaded in gray.

	Msat–F_{ST}	Msat–R_{ST}	*Lamin* A	*Gapdh*	MtDNA
Aleutians vs.					
—YK Delta	**0.021**	**0.018**	**0.027**	**0.019**	**0.580**
—Bodfish	**0.024**	0.019	**0.049**	0.012	**0.820**
—Flaxman	**0.025**	0.007	**0.028**	**0.017**	**0.677**
—Kent Pen.	**0.029**	0.021	**0.034**	**0.032**	**0.610**
—Baffin	**0.085**	0.017	**0.207**	**0.119**	**0.819**
—Hudson Straits	**0.067**	**0.050**	**0.165**	**0.199**	**0.749**
—Southampton	**0.069**	0.006	**0.158**	**0.128**	**0.718**
—Mansel Is.	**0.092**	**0.195**	**0.290**	0.097	**0.823**
—Svalbard	**0.088**	0.014	**0.108**	**0.095**	**0.758**
—Belcher	**0.088**	**0.085**	**0.285**	**0.087**	**0.797**
—New Brunswick	**0.062**	0.015	**0.054**	**0.154**	**0.848**
—Nova Scotia	**0.050**	0.022	**0.049**	**0.107**	**0.892**
—Tromsø	**0.122**	**0.050**	**0.127**	**0.138**	**0.760**
—Soderskar	**0.148**	**0.123**	**0.208**	**0.135**	**0.927**
YK Delta vs.					
—Bodfish	0.002	0.007	0.006	0.000	**0.579**
—Flaxman	0.001	–0.008	0.001	0.002	**0.424**
—Kent Pen.	0.003	–0.007	0.004	–0.005	**0.185**
—Baffin	**0.090**	**0.043**	**0.158**	**0.093**	**0.146**
—Hudson Straits	**0.068**	0.028	**0.095**	**0.170**	**0.142**
—Southampton	**0.072**	0.000	**0.095**	**0.110**	**0.121**
—Mansel Is.	0.096	0.046	**0.206**	0.066	**0.308**
—Svalbard	**0.098**	**0.034**	**0.081**	**0.103**	**0.204**
—Belcher	**0.083**	**0.046**	**0.224**	**0.105**	**0.173**
—New Brunswick	**0.061**	0.003	**0.015**	**0.129**	**0.246**
—Nova Scotia	**0.056**	**0.020**	**0.020**	**0.081**	**0.256**
—Tromsø	**0.132**	**0.086**	**0.088**	**0.139**	**0.155**
—Soderskar	**0.145**	**0.135**	**0.154**	**0.143**	**0.573**
Bodfish vs.					
—Flaxman	0.001	0.000	0.000	–0.005	**0.051**
—Kent Pen.	0.001	0.003	0.005	0.001	**0.300**
—Baffin	**0.091**	**0.032**	**0.141**	**0.085**	**0.714**
—Hudson Straits	**0.067**	0.009	**0.071**	**0.173**	**0.545**
—Southampton	**0.073**	0.006	**0.070**	**0.109**	**0.532**
—Mansel Is.	0.102	–0.055	**0.187**	0.058	**0.637**
—Svalbard	**0.100**	0.024	**0.071**	**0.092**	**0.623**
—Belcher	**0.084**	0.018	**0.209**	**0.090**	**0.626**
—New Brunswick	**0.060**	0.009	**0.014**	**0.122**	**0.771**

Table 4.2 cont.

	Msat–F_{ST}	Msat–R_{ST}	*Lamin* A	*Gapdh*	MtDNA
—Nova Scotia	**0.058**	0.017	**0.027**	**0.072**	**0.827**
—Tromsø	**0.134**	**0.045**	**0.079**	**0.134**	**0.654**
—Soderskar	**0.145**	**0.057**	**0.141**	**0.134**	**0.851**
Flaxman vs.					
—Kent Pen.	0.002	–0.008	–0.004	0.002	**0.155**
—Baffin	**0.102**	**0.021**	**0.170**	**0.055**	**0.514**
—Hudson Straits	**0.077**	0.001	**0.088**	**0.158**	**0.331**
—Southampton	**0.082**	–0.005	**0.082**	**0.086**	**0.361**
—Mansel Is.	0.110	–0.052	**0.245**	0.031	**0.304**
—Svalbard	**0.107**	0.013	**0.073**	**0.074**	**0.453**
—Belcher	**0.091**	0.010	**0.246**	**0.076**	**0.406**
—New Brunswick	**0.069**	–0.003	0.004	**0.094**	**0.633**
—Nova Scotia	**0.061**	0.004	0.012	**0.043**	**0.690**
—Tromsø	**0.146**	**0.040**	**0.086**	**0.123**	**0.486**
—Soderskar	**0.159**	**0.058**	**0.160**	**0.124**	**0.725**
Kent Pen. vs.					
—Baffin	**0.096**	**0.051**	**0.143**	**0.114**	**0.180**
—Hudson Straits	**0.076**	0.034	**0.070**	**0.220**	**0.071**
—Southampton	**0.082**	0.000	**0.068**	**0.139**	**0.091**
—Mansel Is.	**0.106**	0.034	**0.180**	0.109	0.086
—Svalbard	**0.111**	**0.040**	**0.059**	**0.134**	**0.153**
—Belcher	**0.081**	**0.047**	**0.210**	**0.137**	**0.150**
—New Brunswick	**0.073**	0.006	0.009	**0.141**	**0.330**
—Nova Scotia	**0.067**	**0.025**	0.016	**0.084**	**0.377**
—Tromsø	**0.157**	**0.095**	**0.062**	**0.186**	**0.185**
—Soderskar	**0.166**	**0.142**	**0.135**	**0.195**	**0.506**
Baffin Is. vs.					
—Hudson Straits	0.000	0.006	**0.038**	0.037	**0.079**
—Southampton	0.011	0.015	**0.051**	–0.012	0.036
—Mansel Is.	0.024	**0.148**	–0.035	–0.096	**0.410**
—Svalbard	0.017	–0.010	**0.053**	–0.004	0.019
—Belcher	0.014	**0.044**	–0.008	0.013	**0.131**
—New Brunswick	**0.041**	**0.026**	**0.124**	–0.014	**0.153**
—Nova Scotia	**0.037**	0.013	**0.127**	–0.012	**0.242**
—Tromsø	**0.061**	0.001	0.030	0.023	0.006
—Soderskar	0.061	**0.035**	0.029	0.030	**0.597**
Hudson Straits vs.					
—Southampton	0.001	0.010	–0.010	0.005	–0.013
—Mansel Is.	0.006	0.027	0.014	–0.076	0.122
—Svalbard	0.004	0.002	**0.034**	**0.041**	0.044
—Belcher	0.015	–0.015	**0.078**	**0.092**	0.017
—New Brunswick	**0.026**	0.008	**0.060**	0.031	**0.290**

Table 4.2 cont.

	Msat–F_{ST}	Msat–R_{ST}	*Lamin* A	*Gapdh*	MtDNA
—Nova Scotia	**0.023**	0.003	**0.065**	**0.100**	**0.403**
—Tromsø	**0.036**	0.030	0.016	0.003	**0.099**
—Soderskar	0.039	0.040	**0.036**	0.024	**0.547**
Southampton Is. vs.					
—Mansel Is.	0.012	0.089	0.044	–0.092	0.165
—Svalbard	0.004	0.008	**0.039**	0.010	**0.043**
—Belcher	**0.029**	**0.039**	**0.104**	**0.039**	0.022
—New Brunswick	**0.030**	–0.004	**0.059**	0.002	**0.192**
—Nova Scotia	**0.026**	0.011	**0.062**	**0.029**	**0.253**
—Tromsø	**0.024**	**0.052**	**0.030**	0.009	**0.060**
—Soderskar	0.033	**0.099**	**0.049**	0.022	**0.476**
Mansel Is. vs.					
—Svalbard	0.004	0.129	0.064	–0.083	**0.272**
—Belcher	0.015	–0.039	–0.067	–0.047	0.152
—New Brunswick	0.027	0.047	**0.176**	–0.078	**0.626**
—Nova Scotia	0.022	0.084	**0.183**	–0.041	**0.851**
—Tromsø	0.031	**0.199**	0.007	–0.084	**0.359**
—Soderskar	0.029	**0.203**	–0.016	–0.074	**0.888**
Svalbard vs.					
—Belcher	**0.029**	0.032	**0.108**	–0.010	**0.132**
—New Brunswick	0.041	0.016	**0.051**	**0.027**	**0.239**
—Nova Scotia	0.028	0.003	**0.044**	**0.035**	**0.300**
—Tromsø	0.014	0.005	0.003	0.000	**0.053**
—Soderskar	0.021	**0.040**	**0.025**	–0.008	**0.333**
Belcher Is. vs.					
—New Brunswick	**0.035**	**0.024**	**0.194**	**0.046**	**0.337**
—Nova Scotia	**0.025**	0.014	**0.197**	**0.037**	**0.485**
—Tromsø	**0.075**	**0.066**	**0.064**	0.025	**0.140**
—Soderskar	**0.071**	**0.063**	**0.054**	0.009	**0.655**
New Brunswick vs.					
—Nova Scotia	0.002	–0.002	–0.004	0.009	**0.077**
—Tromsø	**0.079**	**0.064**	**0.063**	**0.038**	**0.131**
—Soderskar	**0.086**	**0.112**	**0.109**	**0.050**	**0.690**
Nova Scotia vs.					
—Tromsø	**0.065**	**0.039**	**0.057**	**0.082**	**0.143**
—Soderskar	**0.071**	**0.076**	**0.109**	**0.088**	**0.860**
Tromsø vs.					
—Soderskar	0.006	0.007	**0.030**	–0.019	**0.495**

Table 4.3: Hierarchical analysis of molecular variance (AMOVA) of allelic and haplotypic frequencies for populations classified by (1) subspecies and (2) geographic proximity. Significant comparisons are in bold text.

Source of Variation	d.f.	Variance components	% total variation	Φ	*P*-value
Grouped by Subspecies					
Microsatellite – F_{ST}					
Variance among ssp.	3	0.275	7.66	**0.077**	**≤0.001**
Variance among pop. within ssp.	11	0.027	0.75	**0.008**	**≤0.001**
Variance within populations	1415	3.295	91.60	**0.084**	**≤0.001**
Total	1429	3.597	–	–	–
Microsatellite – R_{ST}					
Variance among ssp.	3	6.476	2.62	**0.026**	**≤0.001**
Variance among pop. within ssp.	11	0.702	0.28	**0.003**	**≤0.001**
Variance within populations	1415	240.340	97.10	**0.029**	**≤0.001**
Total	1429	247.572	–	–	–
Lamin A					
Variance among ssp.	3	0.629	5.21	**0.052**	**≤0.001**
Variance among pop. within ssp.	11	0.040	3.27	**0.035**	**≤0.001**
Variance within populations	1151	1.105	91.52	**0.085**	**≤0.001**
Total	1165	1.208	–	–	–
Gapdh					
Variance among ssp.	3	0.099	9.20	**0.092**	**≤0.001**
Variance among pop. within ssp.	11	0.009	0.79	**0.009**	**≤0.001**
Variance within populations	913	0.970	90.01	**0.100**	**≤0.001**
Total	927	1.078	–	–	–
Mitochondrial DNA					
Variance among ssp.	3	0.001	16.60	**0.166**	**≤0.001**
Variance among pop. within ssp.	11	0.002	35.42	**0.425**	**≤0.001**
Variance within populations	435	0.002	47.99	**0.520**	**≤0.001**
Total	449	0.005	–	–	–

Table 4.3 cont.

Source of Variation	d.f.	Variance components	% total variation	Φ	*P*-value
Grouped by Geographic Proximity					
Microsatellite – F_{ST}					
Variance among region	3	0.206	5.78	**0.058**	**≤0.001**
Variance among pop. within region	11	0.063	1.78	**0.019**	**≤0.001**
Variance within populations	1415	3.295	92.45	**0.076**	**≤0.001**
Total	1429	3.564	–	–	–
Microsatellite – R_{ST}					
Variance among region	3	4.719	1.91	**0.019**	**≤0.001**
Variance among pop. within region	11	1.648	0.67	**0.007**	**≤0.001**
Variance within populations	1415	240.340	97.42	**0.026**	**≤0.001**
Total	1429	246.706	–	–	–
Lamin A					
Variance among region	3	0.066	5.44	**0.054**	**≤0.001**
Variance among pop. within region	11	0.035	2.93	**0.031**	**≤0.001**
Variance within populations	1151	1.105	91.63	**0.084**	**≤0.001**
Total	1165	1.206	–	–	–
Gapdh					
Variance among region	3	0.068	6.37	**0.064**	**≤0.001**
Variance among pop. within region	11	0.028	2.63	**0.028**	**≤0.001**
Variance within populations	913	0.971	91.00	**0.090**	**≤0.001**
Total	927	1.066	–	–	–
Mitochondrial DNA					
Variance among region	3	0.001	17.86	**0.179**	**≤0.001**
Variance among pop. within region	11	0.002	34.03	**0.414**	**≤0.001**
Variance within populations	435	0.002	48.10	**0.519**	**≤0.001**
Total	449	0.005	–	–	–

Table 4.4: Proportion of individuals from sampled populations in each of the four clusters inferred from 12 microsatellite loci in STRUCTURE (Pritchard et al. 2000).

Population	Inferred Cluster				*n*
	1	2	3	4	
ALN	0.069	0.591	0.211	0.129	48
YKD	0.039	0.424	0.442	0.095	124
BOD	0.033	0.385	0.483	0.099	100
FLX	0.039	0.411	0.470	0.080	99
KTP	0.031	0.379	0.476	0.114	41
BFN	0.528	0.094	0.111	0.267	15
HDS	0.536	0.062	0.056	0.346	28
SHP	0.617	0.068	0.047	0.269	52
MSL	0.757	0.030	0.026	0.188	3
SVD	0.677	0.036	0.058	0.228	37
BCH	0.344	0.051	0.047	0.557	22
NBW	0.159	0.072	0.049	0.721	40
NVS	0.172	0.062	0.058	0.708	40
TRM	0.867	0.022	0.021	0.090	38
SDK	0.916	0.016	0.016	0.052	27

Table 4.5: Hierarchical analysis of molecular variance of mtDNA haplotype frequencies for populations grouped to test putative source refugia for Common Eider populations breeding in North America and Scandinavia. Significant comparisons are in bold text ($P < 0.01$).

Regions[a]	No. Groups	Φ_{CT}	Φ_{SC}	Φ_{ST}
AC, BD, E, FG	4	**0.259**	**0.367**	**0.531**
A, BCD, E, FG	4	**0.273**	**0.364**	**0.537**
A, BCD, EG, F	4	**0.284**	**0.356**	**0.540**
AB, CDE, FG	3	**0.179**	**0.422**	**0.519**
A, BCDE, FG	3	**0.264**	**0.396**	**0.555**
A, BCD, EFG	3	**0.279**	**0.369**	**0.544**
A, BCDEG, F	3	**0.296**	**0.393**	**0.573**
A, BCDEFG	2	**0.325**	**0.417**	**0.606**
ABC, DEFG	2	**0.232**	**0.413**	**0.549**

[a]Regions; BOD and FLX (A), ALN and YKD (B), KTP (C), SHP, HDS, BCH, MSL, and BFN (D), NVS and NBW (E), SVD and SDK (F), and TRM (G)

Table 4.6: Results of demographic analyses for 12 microsatellite loci under the infinite allele model (IAM), stepwise mutation model (SMM), and two-phased model of mutation (TPM) and sequence data. Parameter estimates θ ($4N_e\mu$ for nuclear DNA, $2N_f\mu$ for mtDNA), exponential growth rate (g) with standard deviation (SD), and time of expansion (τ) calculated from mismatch distributions for each population. Significant comparisons are in bold text.

	ALN	YKD	BOD	FLX	KTP	BFN	HDS	SHP
Msats [a]								
IAM	Eq	**HExc**	**HExc**	**HExc**	Eq	Eq	Eq	Eq
SSM	**HDef**	**HDef**	**HDef**	**HDef**	**HDef**	**HDef**	**HDef**	**HDef**
TPM	Eq	Eq	Eq	Eq	Eq	Eq	Eq	**HDef**
***Lamin* A**								
θ	**0.033**	**0.122**	**0.138**	**0.039**	**0.044**	**0.045**[b]	**0.008**	**0.019**
SD	0.003	0.007	0.012	0.005	0.010	0.022	0.001	0.002
g	**327.0**	**550.8**	**800.5**	**589.9**	**442.6**	**2339.5**	**672.9**	**400.5**
SD	61.9	31.3	46.2	88.5	102.2	355.3	198.7	64.4
Gapdh								
θ	**0.035**	**0.047**	**0.042**	**0.007**	**0.047**	0.004	**0.003**[b]	**0.011**
SD	0.003	0.003	0.004	0.001	0.009	0.002	0.001	0.001
g	**1212.9**	**400.0**	**875.8**	**307.2**[b]	**1778.2**	53.8	-140.3	**283.5**
SD	91.3	50.8	66.6	144.0	182.9	260.5	155.1	101.0
MtDNA								
θ	**0.012**	**0.023**	**0.007**	**0.007**	**0.021**	**0.017**	**0.050**	**0.034**
SD	0.001	0.002	0.001	0.001	0.003	0.005	0.014	0.006
g	**3673.2**	**534.1**	133.9	16.8	**303.4**	**1203.8**	**850.7**	**788.0**
SD	272.7	71.4	123.8	86.0	65.4	230.6	132.2	161.9
τ	0.992	5.709[c]	6.496	7.598	7.877[c]	2.758	3.555	3.863
95% CI	0.074, 1.363	1.559, 11.959	2.832, 10.496	2.950, 11.543	3.921, 15.290	0.443, 4.088	1.099, 7.133	1.082, 5.777

[a] Significant heterozygote deficiency (HDef) indicates population growth and heterozygote excess (Hexc) indicates a population decline, non-significant population estimates at equilibrium (Eq).

[b] Significant to $P < 0.05$, all others $P < 0.003$

[c] Population differs significantly from the sudden expansion model based on SSD statistic but not under Harpending's (1994) raggedness index

Table 4.6 cont.

	MSL	SVD	BCH	NBW	NVS	TRM	SDK
Msats [a]							
IAM	Eq	Eq	Eq	**HExc**	**HExc**	Eq	Eq
SSM	Eq	**HDef**	**HDef**	Eq	Eq	**HDef**	**HDef**
TPM	Eq	Eq	Eq	Eq	Eq	**HDef**	**HDef**
***Lamin* A**							
θ	0.010	**0.031**	**0.018**[b]	**0.530**	**0.100**	**0.025**	**0.009**
SD	0.008	0.005	0.008	0.052	0.010	0.003	0.002
g	373.3	**428.8**	352.3	**1130.7**	**562.2**	**464.0**	437.9
SD	287.9	84.4	272.0	34.8	38.8	63.8	208.5
Gapdh							
θ	100.0	**0.007**	**0.009**	**0.009**	**0.015**	**0.003**[b]	**0.003**[b]
SD	157.0	0.002	0.002	0.001	0.003	0.001	0.001
g	**4269.1**	242.9	**770.3**	**749.9**	**842.3**	-69.4	-127.1
SD	0.1	178.0	208.7	161.1	178.6	171.4	156.8
MtDNA							
θ	8.3	**0.010**	**0.006**[b]	**0.006**	**0.004**	**0.016**	0.008
SD	3.6	0.002	0.002	0.001	0.001	0.005	0.005
g	**6507.1**	260.9	254.9	132.9	102.1	**518.0**[b]	**10000**
SD	298.4	137.3	257.2	120.0	247.6	174.2	2514.1
τ	2.346	2.556	4.115	1.103[c]	3.000	2.689	0.497
95% CI	0.000, 4.888	1.181, 4.111	0.897, 11.396	0.429, 1.697	0.566, 3.984	0.682, 8.814	0.000, 1.091

Table 4.7: Inferred demographic events of the nested clade analysis (only clades with significant D_c/D_n values are shown; Templeton 1998).

Inferred demographic event	Geographic units involved	Clade	Chain of inference
Continuous range expansion	ALN/KTP to FLX	I-2	1-2-11-12-NO
	TRM to BFN and NVS	I-12	1-19-20-2-11-12-NO
Past fragmentation and/or long distance colonization	BFN/BCH/HDS/MSL/SHP to BOD/FLX/YKD and NBW	I-4	1-2-3-5-15-NO
	BOD/FLX/HDS/KTP/SHP/ TRM/YKD to SVD	II-5	1-19-20-2-3-5-15-NO
	All regions	Total	1-2-3-5-15-NO
Restricted gene flow with isolation by distance	Among BFN, BOD, FLX, HDS, KTP, NBW, NVS, SHP, SVD, TRM, and YKD	I-10	1-2-3-4-NO
	Among BCH, BFN, HDS, SDK, SHP, SVD, and TRM	I-15	1-2-3-4-NO
	Among ALN, BOD FLX, KTP, and YKD	II-1	1-2-3-4-NO
	Among ALN, BCH, BFN, BOD, FLX, KTP, HDS, MSL, NBW, SHP, SVD, TRM, and YKD	II-2	1-2-11-17-4-NO
Restricted gene flow with some long distance colonization	BOD/FLX to KTP/HDS/SHP and TRM and YKD	I-21	1-2-3-5-6-7-8-YES
	BFN/BOD/FLX/HDS/KTP/ NBW/NVS/SHP/SVD/TRM/ YKD to BCH	II-3	1-2-3-5-6-7-YES
	BCH/BFN/HDS/SDK/SHP/ SVD/TRM to KTP/NVS/NBW	II-4	1-2-3-5-6-7-YES

Table 4.8: Migration matrix calculated from 12 microsatellite loci, nuclear introns *lamin* A and *gapdh*, and mtDNA control region. Receiving populations and θ ($N_e\mu$ or $N_f\mu$) are in bold text and population pairs with overlapping 95% confidence intervals are shaded in gray.

Population Comparisons [a]	Number of Migrants per Generation (N_em or N_fm) Microsatellites	Introns	MtDNA
Refugia			
North Slope	**0.8615 (0.8165–0.9088)**	**0.0036 (0.0032–0.0041)**	**0.0109 (0.0080–0.0154)**
—Belcher	13.22 (11.87–14.70)	2.18 (1.29–3.54)	0.97 (0.62–3.65)
—New Brunswick	6.91 (6.07–7.86)	0.46 (0.16–1.07)	1.94 (0.55–5.68)
—Svalbard	8.29 (7.34–9.35)	2.41 (1.46–3.86)	0.00 (0.00–0.93)
Belcher Is.	**0.8495 (0.7887–0.9168)**	**0.0070 (0.0061–0.0082)**	**0.0004 (0.0003–0.0006)**
—North Slope	15.22 (13.35–17.35)	1.23 (0.58–2.35)	0.14 (0.08–0.77)
—New Brunswick	13.44 (11.76–15.38)	1.62 (0.84–2.93)	0.55 (0.15–1.74)
—Svalbard	14.96 (13.11–17.07)	0.53 (0.17–1.29)	0.28 (0.04–1.12)
New Brunswick	**0.8556 (0.8093–0.9053)**	**0.0107 (0.0087–0.0134)**	**0.0007 (0.0006–0.0009)**
—North Slope	8.26 (7.28–9.37)	6.10 (3.14–11.34)	0.00 (0.00–0.15)
—Belcher	12.52 (11.19–14.01)	25.25 (16.63–38.63)	0.09 (0.01–0.40)
—Svalbard	11.21 (9.98–12.59)	34.95 (23.65–51.91)	0.00 (0.00–0.15)
Svalbard	**0.2165 (0.2059–0.2278)**	**0.0004 (0.0003–0.0004)**	**0.0040 (0.0028–0.0060)**
—North Slope	4.53 (3.99–5.15)	2.03 (1.27–3.19)	0.00 (0.00–0.81)
—Belcher	6.18 (5.52–6.92)	0.27 (0.09–0.66)	0.40 (0.03–2.18)
—New Brunswick	7.05 (6.30–7.87)	5.54 (3.93–7.80)	3.95 (1.55–9.65)

Table 4.8 cont.

Population Comparisons [a]	Number of Migrants per Generation (N_em or N_fm) Microsatellites	Introns	MtDNA
S. m. v–nigrum			
Aleutians	**0.9373 (0.8759–1.0033)**	**0.0008 (0.0007–0.0020)**	**0.0003 (0.0002–0.0004)**
—YK Delta	17.82 (15.74–20.13)	0.44 (0.26–1.60)	0.00 (0.00–0.14)
—North Slope	16.21 (14.30–18.35)	2.39 (1.81–7.14)	0.15 (0.10–0.56)
—Kent Pen.	17.92 (15.86–20.23)	0.41 (0.24–1.52)	0.00 (0.00–0.14)
YK Delta	**0.5445 (0.5199–0.5708)**	**0.0000 (0.0000–0.0000)**	**0.0021 (0.0016–0.0029)**
—Aleutians	11.14 (10.09–12.29)	0.01 (0.00–0.05)	0.00 (0.00–0.31)
—North Slope	13.83 (12.59–15.20)	0.10 (0.05–0.21)	1.23 (0.46–2.99)
—Kent Pen.	10.91 (9.87–12.05)	1.38 (1.00–1.94)	0.52 (0.13–1.61)
North Slope	**0.5984 (0.5676–0.6314)**	**0.0050 (0.0045–0.0056)**	**0.0052 (0.0040–0.0072)**
—Aleutians	10.79 (9.63–12.09)	5.97 (4.46–7.93)	0.62 (0.15–1.95)
— YK Delta	18.28 (16.55–20.19)	1.03 (0.58–1.73)	1.25 (0.44–3.16)
—Kent Pen.	25.50 (23.26–27.96)	1.03 (0.58–1.74)	0.21 (0.14–1.04)
Kent Pen.	**0.6823 (0.6487–0.7171)**	**0.0061 (0.0045–0.0096)**	**0.0034 (0.0021–0.0057)**
—Aleutians	11.66 (10.48–12.95)	85.90 (53.19–159.22)	0.53 (0.29–3.26)
— YK Delta	15.43 (13.97–17.01)	43.39 (24.97–85.69)	3.17 (0.95–9.84)
—North Slope	21.34 (19.47–23.36)	75.32 (46.07–141.19)	2.74 (0.75–9.04)

Table 4.8 cont.

Population Comparisons [a]	Number of Migrants per Generation (N_em or N_fm) Microsatellites	Introns	MtDNA
Central Canada and Svalbard			
Kent Pen.	**0.9449 (0.8953–0.9990)**	**0.0089 (0.0079–0.0102)**	**0.0024 (0.0016–0.0037)**
—Baffin	15.88 (14.24–17.73)	0.15 (0.01–0.63)	0.90 (0.20–3.18)
—Hudson Straits	13.03 (11.62–14.62)	2.99 (1.78–4.84)	0.60 (0.10–2.47)
—Southampton	11.03 (9.79–12.44)	0.79 (0.30–1.74)	0.00 (0.00–0.63)
—Svalbard	14.79 (13.24–16.53)	0.61 (0.21–1.43)	0.60 (0.10–2.47)
—Belcher	13.53 (12.08–15.16)	0.15 (0.01–0.63)	0.60 (0.10–2.47)
Baffin Is.	**0.9198 (0.8425–1.0053)**	**0.0020 (0.0016–0.0026)**	**0.0007 (0.0004–0.0017)**
—Kent Pen.	21.50 (18.59–24.92)	0.00 (0.00–0.32)	0.00 (0.00–2.43)
—Hudson Straits	30.15 (26.28–34.68)	5.14 (2.93–8.89)	0.78 (0.04–6.56)
—Southampton	17.29 (14.85–20.18)	4.93 (2.79–8.56)	1.56 (0.19–9.53)
—Svalbard	31.81 (27.76–36.56)	5.14 (2.93–8.87)	0.78 (0.04–6.56)
—Belcher	23.20 (20.09–26.85)	2.38 (1.15–4.68)	11.66 (3.83–40.08)
Hudson Straits	**0.5752 (0.5364–0.6181)**	**0.0008 (0.0007–0.0009)**	**0.0076 (0.0062–0.0096)**
—Kent Pen.	11.31 (9.88–12.95)	0.80 (0.36–1.60)	0.34 (0.07–1.13)
—Baffin	20.52 (18.24–23.12)	0.10 (0.01–0.43)	0.68 (0.21–1.76)
—Southampton	9.23 (7.97–10.72)	4.25 (2.80–6.36)	0.51 (0.36–1.46)
—Svalbard	23.00 (20.50–25.84)	6.48 (4.50–9.27)	0.68 (0.21–1.76)
—Belcher	21.25 (18.90–23.92)	1.37 (0.71–2.44)	0.68 (0.21–1.76)

Table 4.8 cont.

Population Comparisons [a]	Number of Migrants per Generation (N_em or N_fm) Microsatellites	Introns	MtDNA
Southampton	**0.2855 (0.2688–0.3035)**	**0.0050 (0.0038–0.0068)**	**0.0100 (0.0071–0.0147)**
—Kent Pen.	5.47 (4.79–6.25)	49.15 (30.51–80.51)	1.63 (0.38–5.45)
—Baffin	8.50 (7.54–9.57)	22.04 (12.33–39.44)	0.00 (0.00–1.08)
—Hudson Straits	5.92 (5.17–6.78)	55.88 (35.13–90.50)	25.05 (13.84–46.43)
—Svalbard	10.99 (9.92–12.31)	0.00 (0.00–1.22)	4.36 (1.63–10.86)
—Belcher	6.79 (5.99–7.70)	2.00 (0.49–6.17)	0.54 (0.04–2.91)
Svalbard	**0.3228 (0.3062–0.3402)**	**0.0105 (0.0092–0.0120)**	**0.0125 (0.0090–0.0180)**
—Kent Pen.	8.75 (7.84–9.76)	1.37 (0.41–3.73)	0.00 (0.00–1.08)
—Baffin	13.21 (11.97–14.55)	14.77 (8.81–24.84)	0.55 (0.04–2.91)
—Hudson Straits	10.13 (9.11–11.25)	2.34 (0.88–5.50)	2.22 (0.62–6.58)
—Southampton	10.65 (9.60–11.80)	2.74 (1.11–6.15)	2.22 (0.62–6.58)
—Belcher	8.55 (7.66–9.52)	4.12 (1.91–8.46)	6.09 (2.56–13.87)
Belcher Is.	**0.6792 (0.6350–0.7275)**	**0.0035 (0.0027–0.0046)**	**0.0056 (0.0032–0.0112)**
—Kent Pen.	14.10 (12.43–15.99)	1.18 (0.52–2.37)	0.00 (0.00–2.80)
—Baffin	17.53 (15.56–19.76)	2.35 (1.28–4.06)	2.07 (0.28–10.97)
—Hudson Straits	21.39 (19.08–24.00)	3.85 (2.35–6.10)	1.03 (0.06–7.55)
—Southampton	10.89 (9.53–12.45)	1.88 (0.96–3.41)	41.37 (17.95–106.19)
—Svalbard	13.03 (11.46–14.81)	1.04 (0.43–2.18)	1.03 (0.06–7.55)

Table 4.8 cont.

Population Comparisons [a]	Number of Migrants per Generation (N_em or N_fm) Microsatellites	Introns	MtDNA
Southern Canadian Populations			
Belcher Is.	**0.9651 (0.8899–1.0385)**	**0.0017 (0.0015–0.0020)**	**0.0194 (0.0012–0.0346)**
—New Brunswick	8.78 (7.53–10.14)	3.27 (2.06–5.09)	8.93 (3.07–25.25)
—Nova Scotia	13.13 (11.41–14.97)	4.65 (3.09–6.94)	4.09 (1.05–14.07)
New Brunswick	**0.4708 (0.4449–0.4989)**	**0.0013 (0.0011–0.0016)**	**0.0015 (0.0012–0.0020)**
—Belcher	7.66 (6.74–8.72)	13.56 (9.06–20.26)	0.41 (0.29–1.11)
—Nova Scotia	19.26 (17.40–21.33)	21.35 (14.93–30.63)	0.21 (0.04–0.71)
Nova Scotia	**0.4161 (0.3948–0.4390)**	**0.0102 (0.0088–0.0119)**	**0.0003 (0.0002–0.0004)**
—Belcher	10.33 (9.26–11.54)	8.21 (5.47–12.14)	0.40 (0.07–9.73)
—New Brunswick	15.95 (14.45–17.62)	13.80 (9.78–19.37)	1.79 (1.12–13.24)
Scandinavia			
Svalbard	**0.3761 (0.3542–0.4002)**	**0.0041 (0.0034–0.0050)**	**0.0067 (0.0041–0.0474)**
—Tromsø	16.76 (15.03–18.68)	9.62 (6.11–14.98)	12.96 (5.23–133.55)
—Soderskar	11.47 (10.13–12.95)	9.38 (5.94–14.66)	11.44 (4.48–120.53)
Tromsø	**1.0265 (0.9599–1.0990)**	**0.0022 (0.0019–0.0026)**	**0.0079 (0.0059–0.0309)**
—Svalbard	24.08 (21.49–27.00)	3.88 (2.39–6.14)	3.68 (1.67–21.92)
—Soderskar	16.14 (14.22–18.28)	10.26 (7.22–14.53)	0.56 (0.37–24.49)
Soderskar	**0.4809 (0.4511–0.5116)**	**0.0103 (0.0086–0.0125)**	**0.0004 (0.0000–0.0006)**
—Svalbard	11.12 (9.81–12.75)	3.31 (1.77–5.91)	0.07 (0.01–0.26)
—Tromsø	9.20 (8.09–10.42)	15.31 (10.44–22.47)	0.00 (0.00–0.00)

[a] Ninety-five percent confidence intervals are in parentheses.

Appendix 4.A: Localities of Common Eiders sampled* in this study.

Somateria mollissima v-nigrum

USA: Alaska, Aleutian Islands, Attu Island 52.938°N, 173.238°E
MRP294, MRP295, UAM13336, UAM13721

USA: Alaska, Aleutian Islands, Agattu Island 52.435°N, 173.576°E
MRP296, MRP297, MRP298, MRP299, MRP306, MRP307, MRP308, MRP309, MRP310, MRP311, MRP312

USA: Alaska, Aleutian Islands, Alaid Island 52.763°N, 173.898°E
DBI2, DBI3, DBI4, DBI5, MRP285, MRP286, MRP287, MRP288, MRP289, MRP290, MRP291, MRP292, MRP293, MRP300, MRP301, MRP302, MRP303, MRP304, MRP305, MRP313, MRP314, MRP315, MRP316, MRP317, MRP318

USA: Alaska, Aleutian Islands, Amchitka Island 51.567°N, 178.878°E
MRP283, MRP284, MRP320, MRP321, MRP322, MRP323, MRP324, MRP325, MRP326

USA: Alaska, Aleutian Islands, Adak Island 51.880°N, 176.658°W
PBAI935

Canada: Nunavut, Kent Peninsula 68.5°N, 107.0°W
COEI-M, DEAD-02, DEAD-03, DEAD-F, EUTH-M, KP1502, KP1505, KP1508, KP1510, KP1512, KP1516, KP1517, KP1518, KP1519, KP1828, KP1829, KP1833, KP1835, KP1836, KP1840, KP4553, KP4596, KP4597, KP4617, KP9816, KP9818, KP9820, KP9823, KP9824, KP9825, KP9827, KP9828, KP9829, KP9830, KP9831, KP9832, KP9833, KP9835, KP9836, KP9837, KP9838

Somateria mollissima borealis

Canada: Nunavut, Southampton Island 64.33°N, 84.667°W
H317, H321, H322, H325, H326, H327, H328, H329, H330, H333, H334, H335, H338, H352, H25052, H25064, H25067, H25069, H25071, H25094, H25095, H25097, H25269, H25276, H91488, H91574, H91575, H91579, H91697, H91698, H91701, H91718, H91723, H91725, H91726, H91727, H91729, H91730, H91736, H91754, H91756, H91981, H95196, H95967, H95969, H95970, H95972, H95974, H95975, H95976, H95987, H95995

Canada: Nunavut, Baffin Island
M801, M803, M804, M805, M806, M808, M809, M810, M811, M812, M813, M814, M815, M816, M817

Canada: Nunavut, Mansel Island 63.417°N, 77.917°W
F45, F47, F49

Canada: Nunavut, Baffin Island, Hudson Straits, Foxe Penninsula 64.1°N, 73.5°W
G33, G34, G37, G38, G39, G41, G42, G43, G49, G50, G51, G56, G57, G59, G60, G61, G66, G68, G72, G73, G74, G75, G76, G77, G79, G80, G82, G85

Norway: Svalbard 78.2°N, 15.5°E
D1, D2, D3, D4, D5, D6, D7, D8, D9, D10, D11, D12, D13, D14, D15, D16, D17, D18, D19, D20, D21, D22, D23, D24, D25, D26, D27, D28, D29, D30, D31, D32, D33, D34, D35, D36, D37

Somateria mollissima sedentaria
Canada: Nunavut, Belcher Islands 56.183°N, 79.250°W
T11, T12, T13, T14, T15, T16, T17, T18, T19, T20, TB01, TB02, TB03, TB04, TF01, TF02, TF03, TF04, TM01, TM02, TM03, TM04

Somateria mollissima dresseri
Canada: New Brunswick 45.5°N, 67.0°W
BD#, BNB, B6, B33, B43, B44, B45, B47, B48, B49, B51, B52, B53, B99, B209, B244, B515, B609, B5170, B21627, B25423, B26170, B40194, B40622, B45320, B45509, B48222, B49008, B49028, B49055, B49071, B49073, B49583, B59510, B59513, B59516, B59519, B59521, B59524, B85408

Canada: Nova Scotia 44.716°N, 65.200°W
C1, C10, C14, C15, C17, C20, C21, C24, C30, C32, C34, C37, C39, C42, C45, C46, C50, C52, C53, C54, C55, C56, C57, C60, C61, C62, C63, C64, C66, C67, C68, C69, C70, C71, C73, C77, C80, C81, C83, C116

Somateria mollissima mollissima
Norway: Troms, Tromsø 69.7°N, 18.9°E
E9, E10, E13, E15, E18, E20, E25, E28, E33, E35, E75, E86, E95, E98, E104, E107, E107-2, E117, E118, E123, E129, E137, E138, E139, E142, E148, E150, E153, E154, E162, E167, E175, E188, E191, E192, E195, E200, E202

Finland: Southern Finland, Soderskar 60.25°N, 25.5°E
A510178, A510588, A510658, A510678, A580197, A580297, A580397, A580497, A584297, A584393, A586797, A586897, A588097, A588597, DT12063, DT35166, DX1028, DX2714, DX3241, DX4333, DX4739, DX5559, DX7428, DX35013, DX120319, DX12218, DX324119

**Sample IDs starting with UAM are located at the University of Alaska Museum, Fairbanks, Alaska. Remaining samples are located in non-museum research collections.

Appendix 4.B: Allele size in base pairs for Common Eiders genotyped at 14 microsatellite loci.

ID	Aph02		Aph08		Aph20		Aph23		Cmo9	
KIG1308	110	110	138	140	172	166	214	210	116	116
KIG1321	116	116	138	140	172	166	210	210	120	122
KIG1331	116	110	138	138	172	166	212	210	112	112
KIG1333	116	116	138	140	168	166	212	212	116	116
KIG1336	116	110	138	138	172	172	214	210	112	116
KIG1339	116	110	138	140	172	166	214	210	110	116
KIG1340	116	112	138	138	172	168	210	210	112	116
KIG1341	116	116	138	140	172	166	210	210	116	116
KIG1342	116	116	138	138	166	166	212	210	112	112
KIG1343	–	–	138	140	168	168	216	210	112	116
KIG1344	116	110	138	138	168	168	210	210	112	116
KIG1346	116	112	138	140	168	168	210	210	102	116
KIG1347	116	116	140	140	172	168	212	210	118	122
KIG2253	116	110	140	140	168	164	210	210	–	–
KIG2254	116	110	138	140	172	168	210	210	116	120
KIG2255	116	116	138	140	168	168	214	212	112	116
KIG2261	110	110	138	138	168	166	216	212	116	116
KIG2262	116	110	138	140	178	172	210	210	112	116
KIG2264	110	110	138	138	172	168	212	212	116	116
KIG2265	110	110	138	140	168	166	212	210	112	122
KIG2266	116	116	140	140	172	166	216	212	116	116
KIG2267	116	110	138	140	172	168	212	210	116	120
KIG2271	116	116	138	140	168	166	212	210	112	116
KIG2278	116	112	138	140	168	166	212	212	112	116
KIG2279	116	110	138	140	172	166	210	210	116	116
KIG2280	116	116	140	140	172	172	212	210	112	112
KIG2283	116	110	138	140	172	166	216	212	116	116
KIG2285	116	110	138	140	168	166	214	210	116	116
KIG2293	116	110	138	138	172	166	216	210	116	116
KIG2296	116	110	138	138	166	166	210	208	112	116
KIG2298	116	110	138	140	172	166	212	210	116	116
KIG2299	116	116	140	140	172	172	212	212	112	116
KIG6334	116	116	138	140	172	168	212	212	116	116
KIG6915	116	116	138	140	168	168	212	210	116	116
KIG6927	114	110	138	138	172	166	212	210	116	116
KIG6944	116	112	138	140	172	168	214	212	116	118
KIG6999	110	110	140	140	168	168	212	210	114	116
BS76451	116	110	138	138	172	166	210	208	112	116
BS76452	116	116	138	138	168	168	212	210	116	116

Appendix 4.B cont.

ID	Aph02		Aph08		Aph20		Aph23		Cmo9	
BS76454	116	110	138	140	172	168	216	212	116	122
BS76455	116	110	138	138	172	166	210	210	112	120
BS76456	116	110	138	138	172	168	214	210	116	116
BS76457	116	112	138	138	172	166	216	210	116	112
BS76459	116	116	138	140	172	168	210	210	116	116
BS76460	116	116	138	138	172	166	210	210	116	116
BS76461	116	110	138	138	166	166	212	212	116	116
BS76462	116	110	138	138	168	168	210	210	116	112
BS76463	110	110	138	140	166	166	212	210	122	116
BS76464	116	110	138	140	172	166	214	210	116	112
BS76465	–	–	138	140	168	166	214	212	120	118
BS76466	–	–	138	138	166	166	212	212	116	116
BS76467	116	110	138	138	168	168	218	214	120	116
BS76469	116	110	138	138	172	166	212	210	116	112
BS76470	116	112	138	140	172	168	216	210	116	116
YK54775	116	116	138	140	172	168	210	210	116	112
YK57894	110	110	140	140	172	166	216	210	116	116
YK76112	110	110	138	140	172	168	212	210	116	116
YK76115	110	110	138	140	166	166	210	210	120	116
YK76117	116	116	140	140	172	172	210	210	116	116
YK76119	116	112	138	138	172	166	212	210	116	112
YK76120	116	110	138	138	172	172	216	214	112	112
YK76122	116	116	138	138	172	172	214	212	112	112
YK76124	116	110	138	140	172	172	210	210	116	116
YK76156	116	116	138	138	172	168	210	210	116	114
YK76157	116	110	138	140	172	168	216	212	116	116
YK76158	116	110	138	138	168	166	212	210	116	116
YK76161	112	110	138	138	172	168	214	210	116	112
YK76163	116	114	138	140	168	166	218	210	114	112
YK76164	116	116	138	140	166	166	212	210	122	116
YK76165	110	110	138	140	–	–	210	210	116	116
YK76167	116	114	138	140	166	166	214	210	116	116
YK76168	116	110	138	140	166	166	212	210	116	112
YK76169	116	110	138	138	168	166	218	210	120	116
YK76170	116	114	138	140	168	166	214	210	116	116
YK76171	116	110	138	138	172	168	218	214	116	116
YK76172	114	110	134	140	172	166	210	210	112	112
YK76173	116	112	138	138	168	166	212	212	118	116
YK76178	116	116	138	138	168	168	212	212	122	116
YK76179	116	110	138	140	168	168	212	212	116	116

Appendix 4.B cont.

ID	Aph02		Aph08		Aph20		Aph23		Cmo9	
YK76181	116	116	138	138	172	168	210	210	120	120
YK76182	116	110	138	138	166	166	214	212	112	112
YK76186	116	110	140	140	168	168	212	210	116	112
YK76187	110	110	138	138	168	168	210	210	116	116
YK76193	116	110	138	140	172	168	210	210	116	116
YK76672	110	110	138	138	172	168	212	210	116	116
YK54748	114	110	138	140	166	166	214	212	120	116
YK76151	116	110	138	140	168	166	218	212	116	116
YK76155	116	116	138	138	168	166	214	210	116	112
YK76264	116	110	138	140	172	168	216	210	112	112
YK76268	116	110	140	140	172	172	216	212	116	116
YK76271	116	110	138	138	172	166	212	210	112	112
YK76432	116	114	138	140	172	168	214	210	116	112
YK76123	114	110	138	140	172	172	210	210	116	112
YK76154	116	116	138	138	168	166	214	210	122	116
YK76159	110	110	138	138	172	168	210	210	116	114
YK76162	116	110	138	138	168	166	212	210	116	112
YK76166	116	110	138	140	168	166	210	210	116	116
YK76192	110	110	138	138	168	166	216	210	116	116
YK76194	116	110	138	140	172	168	218	210	116	116
YK76267	116	116	138	140	172	172	210	210	116	116
YK76316	116	110	138	140	168	166	216	214	116	110
YK76420	116	110	138	138	172	172	210	210	116	116
YK76421	110	110	140	140	172	166	212	210	116	112
YK76464	116	110	138	140	172	168	210	210	118	112
YK76425	114	110	138	140	168	166	214	212	116	112
YK76665	116	110	140	140	168	168	210	210	118	112
YK76851	116	110	138	138	168	168	210	210	116	112
YK76854	116	114	138	138	168	168	212	210	122	116
YK76865	110	110	138	140	172	166	218	210	122	116
YK77552	116	116	138	140	172	172	212	210	116	116
YK77560	116	116	138	138	166	166	212	210	118	116
YK77563	110	110	138	140	168	166	216	210	120	116
YK77565	116	116	138	138	168	166	212	210	118	116
YK77566	110	110	138	140	168	168	216	214	112	112
YK77569	110	110	138	138	172	168	210	210	116	116
YK77573	116	116	138	138	172	166	216	210	116	116
YK77576	110	110	138	140	168	166	216	212	118	116
YK77580	116	110	138	140	168	166	212	210	112	112
YK77581	116	110	138	140	172	166	214	210	116	116

Appendix 4.B cont.

ID	Aph02		Aph08		Aph20		Aph23		Cmo9	
YK77582	110	110	138	140	172	168	212	210	116	116
YK77584	116	110	140	140	172	168	212	210	116	116
YK77585	116	110	138	138	168	166	216	212	116	116
COEI–M	110	110	138	140	168	168	214	212	110	112
DEAD–F	110	116	138	140	172	166	212	212	116	120
EUTH–M	116	116	138	138	172	168	212	210	112	116
KP1516	116	116	138	140	166	166	212	210	116	116
KP1517	116	116	138	138	168	166	216	212	116	118
KP1518	110	114	140	140	172	166	216	210	116	118
KP1519	116	116	138	140	178	166	210	210	116	118
KP4596	110	116	138	138	172	166	210	210	112	116
KP4597	116	116	140	140	172	168	214	212	116	116
KP9827	110	116	138	140	172	168	210	210	118	122
KP9828	110	116	138	138	172	172	218	212	112	116
KP9829	116	116	138	140	166	166	210	210	116	116
KP9830	116	116	138	138	166	166	210	210	116	122
KP9831	116	116	138	140	172	168	212	210	112	116
KP9832	112	116	138	140	166	166	212	210	116	116
KP9833	116	110	140	140	172	166	210	210	116	120
KP9835	110	110	138	138	172	166	212	210	116	116
KP9836	116	116	140	138	172	166	212	210	116	116
KP9837	116	116	138	138	168	166	214	212	116	122
KP9838	110	116	138	138	172	166	210	210	112	116
KP1502	110	116	140	140	168	168	210	210	112	116
KP1505	116	116	138	138	168	166	210	210	116	116
KP1508	110	110	140	140	166	166	210	210	116	116
KP1510	116	116	138	140	178	168	212	210	116	118
KP1512	110	116	138	140	168	166	212	210	118	120
KP1828	114	116	138	140	168	168	210	210	116	116
KP1829	116	116	138	138	178	172	214	210	120	122
KP1833	116	116	138	140	168	166	212	210	116	116
KP1835	112	116	138	138	172	172	210	210	112	120
KP1836	110	116	138	138	172	168	212	212	116	116
KP1840	116	116	138	138	168	168	210	210	112	120
KP4553	110	116	140	140	168	166	212	210	116	118
KP4617	110	116	138	140	168	166	212	212	116	118
KP9816	110	112	140	140	172	168	210	210	112	116
KP9818	110	116	138	138	168	166	216	210	114	116
KP9820	116	116	138	138	172	172	216	212	112	116
KP9823	110	110	138	138	168	166	212	210	116	116

Appendix 4.B cont.

ID	Aph02		Aph08		Aph20		Aph23		Cmo9	
KP9824	110	116	138	140	172	172	210	210	116	120
KP9825	116	116	140	140	166	166	216	210	116	118
DEAD–02	110	116	140	140	168	168	212	210	112	116
DEAD–03	116	116	138	138	172	168	212	212	112	116
CAMP1–1	116	112	138	138	172	168	212	212	116	112
NS27252	114	110	138	138	172	172	212	210	118	112
NS27253	114	110	138	140	168	166	212	212	118	112
NS27256	110	110	138	140	172	172	210	210	116	116
NS27260	116	110	138	138	168	166	210	210	116	116
NS27264	116	116	138	138	166	166	216	212	116	112
NS27269	116	110	138	138	172	172	218	210	116	116
NS27270	116	110	138	140	168	166	212	210	116	112
NS27271	116	110	138	138	172	166	212	210	116	116
NS27272	116	116	138	138	172	168	210	210	116	116
NS27273	110	110	138	138	166	166	210	210	122	112
NS27274	116	116	138	140	168	168	218	210	116	116
NS27275	116	110	138	138	172	168	212	210	116	112
NS27276	116	110	138	140	168	166	212	210	116	112
NS27277	114	110	138	140	168	166	216	210	122	116
NS27278	110	110	140	138	172	168	212	210	116	116
NS27279	116	110	138	140	172	168	212	212	116	116
NS27280	110	110	138	138	168	166	210	210	116	112
NS27281	116	116	138	138	168	166	212	210	118	116
NS27282	110	110	138	140	168	166	216	212	116	112
NS27284	116	110	138	140	172	166	214	212	116	114
NS27286	116	116	138	140	168	166	216	216	116	116
NS27291	116	110	138	138	172	172	216	210	112	110
NS27292	114	114	138	140	168	166	214	210	122	116
NS27293	116	116	138	140	172	172	212	210	116	116
NS272xx	116	116	138	140	172	168	212	210	116	116
NS27304	114	110	138	138	172	168	212	212	116	112
NS27305	116	116	138	138	172	168	210	210	116	116
NS27337	110	110	138	138	172	168	216	210	116	116
NS27338	110	110	138	138	168	166	216	210	124	116
NS27339	116	116	138	140	168	168	218	210	120	116
NS27340	116	110	138	140	172	172	210	210	122	112
NS27341	116	110	138	138	168	166	210	210	122	120
NS27342	116	116	138	140	168	166	216	210	116	116
NS27343	116	110	138	138	172	166	212	210	122	116
NS27344	110	110	138	138	172	172	212	210	122	116

Appendix 4.B cont.

ID	Aph02		Aph08		Aph20		Aph23		Cmo9	
NS27345	116	116	138	138	172	172	210	208	116	116
NS27346	116	116	138	140	172	168	212	210	116	116
NS27347	116	116	138	140	172	168	212	210	116	112
NS24348	116	114	138	138	172	172	212	212	116	116
NS27349	116	110	138	138	172	166	214	210	116	116
NS27350	112	110	138	138	172	168	212	212	116	116
NS24351	116	112	138	138	168	166	210	210	118	116
NS27352	116	110	140	140	172	166	218	210	116	116
NS27354	116	114	138	140	168	168	212	210	116	116
NS27417	116	116	138	138	172	166	216	212	120	116
NS27418	116	110	138	140	172	166	210	210	116	112
NS27419	116	116	140	140	172	170	212	210	116	116
NS27420	116	110	138	138	172	166	210	210	116	116
NS27422	116	116	140	140	172	168	212	212	116	112
NS27423	116	110	138	138	168	166	210	210	116	112
NS27424	110	110	138	140	166	166	210	210	118	112
NS27425	116	112	140	142	172	166	214	210	122	112
NS52251	116	110	138	140	172	172	216	210	116	112
NS52252	110	110	138	138	172	166	210	210	122	116
NS52253	116	110	138	140	166	166	212	210	116	112
NS52254	116	116	138	140	172	168	212	212	112	112
NS52255	114	110	138	140	172	168	210	210	116	116
NS52256	110	110	138	138	168	168	212	212	116	112
NS52257	116	110	138	140	172	166	214	212	116	116
NS52258	116	110	138	140	172	166	214	210	124	116
NS52259	116	110	138	140	172	168	212	212	120	112
NS52260	116	116	138	138	172	168	212	210	112	112
NS52261	116	110	138	140	166	166	212	210	116	112
NS52262	116	110	138	140	172	168	216	210	112	112
NS52263	116	116	138	138	172	172	210	208	122	116
NS52264	116	110	138	138	168	168	212	210	124	116
NS52265	116	116	138	140	172	168	210	210	116	116
NS52266	116	110	138	138	172	168	212	210	116	116
NS52267	116	116	138	140	172	166	214	214	116	116
NS52268	110	110	140	140	172	166	212	210	116	116
NS52269	116	116	138	138	172	172	210	210	116	112
NS52270	110	110	138	140	172	168	212	212	112	112
NS52271	116	116	138	138	172	168	210	210	122	118
NS52272	116	116	138	138	172	168	214	210	116	116
NS52273	116	116	140	140	168	168	210	210	116	116

Appendix 4.B cont.

ID	Aph02		Aph08		Aph20		Aph23		Cmo9	
NS52274	116	110	138	140	172	166	212	210	116	116
NS52275	116	116	138	138	168	168	212	210	116	116
NS52276	116	116	138	138	168	168	214	212	116	116
NS52277	116	114	138	140	172	168	212	210	116	112
NS52278	116	110	138	140	172	172	210	210	120	116
NS52279	116	116	138	140	168	166	210	210	112	112
NS52280	116	114	138	138	168	168	210	210	116	116
NS52281	116	116	138	138	172	168	216	210	118	116
NS52282	114	110	140	138	166	164	212	210	118	116
NS52283	116	116	138	138	172	172	210	210	116	112
NS52284	116	114	138	138	172	166	210	210	112	112
NS52285	114	110	138	138	172	172	212	210	112	112
NS52286	116	110	138	138	168	168	210	210	116	112
NS52287	116	116	140	138	172	168	212	210	116	116
NS52288	116	110	140	138	172	168	210	210	116	116
NS52289	116	112	140	138	172	172	210	210	118	116
NS52290	116	116	138	138	172	166	210	210	118	116
NS52291	112	110	138	138	172	172	212	210	116	112
NS76453	116	110	140	138	168	166	212	210	120	116
NS76471	116	116	140	138	168	166	212	212	118	118
NS76472	110	110	140	138	172	166	212	210	122	112
JAR136	116	116	142	138	172	168	210	210	116	112
JAR144	114	110	138	138	172	172	210	210	116	112
JAR204	116	116	140	138	172	168	212	210	122	118
NS27321	116	116	140	138	172	172	212	210	116	116
NS27322	116	116	140	138	172	168	212	210	122	112
NS27323	116	116	140	140	166	164	218	212	116	112
NS27324	116	110	140	138	172	172	216	210	116	116
NS27325	116	116	140	138	172	172	212	210	116	116
NS27401	116	116	140	138	168	166	210	210	118	116
NS27402	110	110	138	138	172	168	216	210	116	116
NS27404	114	112	140	138	184	166	214	208	124	110
NS27405	116	116	138	138	168	164	216	210	120	116
NS27406	110	110	138	138	168	168	212	210	116	116
NS27407	116	112	140	138	172	172	210	210	116	116
NS27441	116	110	138	138	166	166	212	212	114	112
NS27442	110	110	138	138	172	168	210	210	116	112
NS27443	116	110	138	138	172	172	210	210	116	116
NS76478	116	116	138	138	168	166	214	212	116	116
NS76480	116	110	140	138	166	166	210	210	116	116

Appendix 4.B cont.

ID	Aph02		Aph08		Aph20		Aph23		Cmo9	
NS27251	116	110	140	138	172	166	216	210	116	116
NS76482	116	110	140	138	172	168	212	210	122	118
NS76483	116	116	140	140	172	168	212	210	122	116
NS76485	116	110	138	138	168	168	212	210	120	112
NS76487	116	110	140	138	172	168	216	210	120	112
NS76490	116	116	138	138	172	168	214	210	116	112
NS76491	116	116	140	138	172	168	216	212	116	112
NS76492	116	116	138	138	172	172	216	216	118	112
NS76493	112	110	138	138	168	168	216	210	116	116
NS76494	116	116	140	138	172	166	212	212	122	116
NS76495	116	116	138	138	172	172	216	212	116	112
NS76496	116	116	140	138	172	166	210	210	116	116
NS76497	116	116	138	138	166	166	214	210	116	112
NS76498	116	116	140	140	172	168	212	210	116	116
NS76499	116	116	140	138	168	168	216	212	116	112
NS76500	116	116	140	138	168	166	210	210	120	120
NS76551	110	110	138	138	166	166	212	210	116	116
NS76552	116	110	138	138	172	168	214	214	120	120
NS76553	116	110	138	138	172	172	210	210	116	116
NS76555	116	116	140	140	168	168	–	–	116	112
NS76556	116	110	140	138	172	166	216	210	116	116
NS76557	110	110	140	140	172	168	212	210	116	116
NS76558	116	116	138	138	172	168	214	210	116	112
NS76559	116	114	140	138	172	172	216	210	116	116
NS76560	116	110	138	138	172	166	218	212	116	116
NS82101	114	110	138	138	174	168	–	–	116	116
NS82102	116	110	138	138	172	166	212	210	116	116
NS82104	116	112	–	–	172	168	–	–	116	112
NS82106	116	116	138	138	172	168	210	210	116	112
NS82107	116	116	140	138	172	168	212	210	116	112
NS82109	116	110	138	138	172	168	216	212	112	112
NS82112	116	114	140	138	172	172	210	210	116	112
NS82117	110	110	140	138	172	168	210	210	116	112
NS82118	116	116	140	138	166	166	212	210	116	116
NS82119	116	116	140	138	168	168	212	210	116	116
NS82120	116	110	140	138	172	168	212	210	118	116
NS82121	116	116	140	140	168	166	212	208	116	112
NS82122	116	110	140	138	168	168	210	210	118	112
NS82123	116	116	140	138	172	166	210	210	116	116
NS82127	116	116	140	138	172	166	216	210	116	112

Appendix 4.B cont.

ID	Aph02		Aph08		Aph20		Aph23		Cmo9	
NS82129	116	114	140	138	172	166	214	212	116	116
NS82130	116	110	140	138	168	166	212	210	122	116
NS82133	116	116	138	138	168	168	212	210	116	112
NS82134	116	110	140	140	172	168	210	210	116	112
NS82135	116	110	140	140	168	168	216	212	116	112
NS82136	116	110	138	138	168	166	212	212	118	116
NS82137	116	116	140	138	168	164	214	212	116	116
NS82138	116	110	140	138	168	166	210	210	116	112
NS82141	116	110	138	138	168	166	212	210	116	112
NS82143	116	116	140	140	172	166	218	216	116	112
NS82144	114	110	138	138	168	166	210	210	116	116
NS82145	110	110	140	138	168	166	212	210	120	112
NS82150	–	–	140	138	178	166	212	212	118	116
NS82151	116	110	138	138	172	172	212	210	116	112
NS82152	116	116	140	138	172	168	210	210	116	112
NS82153	116	110	138	138	172	166	212	210	116	112
NS82156	116	114	140	138	168	166	216	212	118	114
NS82157	116	110	138	138	168	168	210	210	120	118
NS82158	116	114	140	138	168	168	216	210	116	112
NS82162	116	116	140	138	168	168	214	212	116	112
NS82163	116	110	140	140	172	164	216	210	116	112
NS82164	–	–	138	138	172	168	212	212	116	116
NS82165	116	110	140	138	172	168	210	210	116	112
NS82166	116	112	140	140	172	168	212	210	120	118
NS82167	116	110	138	138	166	166	212	210	116	116
NS82168	116	116	138	138	172	166	216	210	112	112
NS82142	116	116	140	138	172	166	218	210	116	112
NS82146	116	116	138	138	172	168	210	210	118	112
NS82204	116	110	138	138	168	168	210	210	118	116
NS82205	116	110	138	138	166	166	212	210	120	116
NS82207	114	110	140	138	168	168	212	212	116	112
NS82209	116	110	138	138	172	166	212	210	116	112
NS82211	116	110	138	138	172	168	216	212	116	112
NS82154	116	110	140	138	172	168	214	212	116	116
NS82221	116	110	140	138	172	166	210	210	122	116
NS82224	116	116	138	138	168	164	210	210	116	116
NS82225	116	116	138	138	172	168	210	210	116	116
NS82232	110	110	138	138	168	166	212	210	116	116
NS82160	116	110	140	140	172	172	212	212	116	116
NS82234	116	116	138	138	172	166	210	210	116	112

Appendix 4.B cont.

ID	Aph02		Aph08		Aph20		Aph23		Cmo9	
NS82235	116	110	138	138	172	168	212	210	116	116
NS27407	116	112	140	138	172	172	210	210	116	116
NS82237	116	110	140	138	172	168	214	212	122	116
NS82147	–	–	140	138	172	168	214	212	–	–
NS27421	116	110	140	138	166	166	214	210	122	112
NS27268	110	110	140	138	172	168	210	210	116	112
AK132	116	110	138	138	172	168	210	210	116	110
AK134	110	110	138	138	168	168	210	210	116	112
AK135	112	110	140	138	172	168	216	210	116	112
AK136	116	116	140	138	172	168	212	210	116	116
AK137	114	110	140	138	172	172	212	210	116	116
AK138	116	116	138	138	172	172	212	210	120	116
AK139	110	110	138	138	166	166	210	210	116	116
AK140	116	112	140	138	172	168	210	210	116	112
AK142	116	110	138	138	166	166	210	210	116	112
AK143	116	110	140	138	172	168	216	210	116	112
AK150	116	110	140	138	172	168	212	210	116	112
AK151	116	116	138	138	172	172	212	210	120	116
AK240	112	112	140	138	–	–	212	210	116	116
CA1–1	116	112	138	138	172	168	212	212	116	112
CA149	116	116	140	138	168	168	212	210	112	102
CA150	116	116	138	138	172	168	212	210	112	112
CA152	116	110	140	138	172	166	212	210	116	116
CA153	116	110	140	138	172	168	216	212	116	112
CA156	116	116	140	138	168	168	214	214	116	116
CA159	116	110	138	138	172	168	216	210	122	116
CA162	116	110	138	138	168	166	214	210	116	116
CH116	116	110	138	138	172	168	210	210	116	112
CH118	112	110	140	138	172	168	216	210	116	116
CH119	116	116	138	138	166	166	210	210	116	112
CH121	110	110	140	138	172	168	216	216	116	116
CH124	116	110	140	138	172	166	212	210	116	116
CH131	116	116	138	138	168	168	212	210	118	116
CH201	116	110	140	138	172	172	212	210	116	112
CH261	116	110	138	138	172	162	210	210	116	112
DU136	116	116	142	138	172	168	210	210	116	112
DU210	–	–	140	138	172	168	212	212	–	–
DU227	116	116	140	138	168	166	216	216	116	116
EG1	116	116	138	138	172	172	212	210	116	116
EG10–2	114	110	140	138	172	168	210	210	118	116

Appendix 4.B cont.

ID	Aph02		Aph08		Aph20		Aph23		Cmo9	
EG2	116	116	138	138	172	166	214	214	116	116
EG2–2	116	110	140	140	172	166	218	210	116	116
EG3	116	110	138	138	172	166	210	210	116	110
EG3–2	116	116	138	138	172	168	210	210	118	112
EG4	116	110	138	138	172	168	210	210	118	112
EG5	116	114	138	138	172	166	212	210	116	112
EG7	116	116	140	140	168	166	212	210	116	112
EG9	110	110	140	140	172	166	212	210	116	116
EG9–2	116	110	140	138	168	166	210	210	116	114
LO001	116	110	138	138	172	166	210	210	118	116
LO002	116	116	138	138	166	166	212	210	116	114
LO003	116	116	138	138	172	168	212	210	118	116
LO004	116	116	140	138	168	166	214	212	118	112
LO008	116	116	140	138	166	166	212	210	112	112
LO009	110	110	138	138	172	168	210	210	116	112
LO010	116	110	140	140	168	168	212	210	112	112
LO011	116	116	140	138	168	166	212	210	116	116
LO012	116	116	140	140	168	166	212	210	116	112
LO014	116	114	140	140	166	166	212	210	116	112
LO017	116	116	140	140	168	166	212	208	116	112
LO018	116	110	140	140	166	166	214	210	116	116
LO019	110	110	138	138	172	168	210	210	118	116
LO020	116	116	140	140	172	166	210	210	116	114
LO021	116	116	140	138	168	168	212	210	116	116
LO023	116	112	138	138	172	172	216	210	116	116
LO141	116	110	140	140	166	166	212	210	116	116
LO033	116	116	138	138	168	168	212	210	122	112
LO035	116	110	140	138	168	166	210	210	116	112
ST024–2	116	110	140	138	172	168	212	210	118	116
ST024	116	116	138	138	172	168	212	210	116	112
MS224–2	116	116	140	138	172	166	212	212	116	112
MS226–2	110	110	138	138	172	172	212	210	118	116
MS227	116	116	140	138	172	168	210	210	116	116
MS230–5	110	110	140	140	166	166	216	210	116	116
MS231	116	110	138	138	172	172	212	212	124	116
MS233	116	110	140	138	172	166	210	210	116	112
MS235	114	110	140	140	166	166	212	212	116	112
MS262	116	116	138	138	172	162	210	210	116	116
MS303	116	116	140	138	166	166	210	210	116	116
NS100	116	116	140	140	168	168	212	212	118	116

Appendix 4.B cont.

ID	Aph02		Aph08		Aph20		Aph23		Cmo9	
NS202	116	110	140	138	168	166	212	212	116	112
NS203–1	116	114	140	140	172	168	212	210	122	118
NS204	116	116	140	138	172	168	212	210	122	118
NS218	110	110	140	138	166	166	214	210	116	116
NS219	116	116	138	138	166	166	210	210	116	112
NS220	116	116	140	138	172	166	212	210	118	116
NS222	116	116	140	138	172	168	212	210	122	116
NS223	116	110	140	138	172	168	216	210	122	112
PT102	116	114	138	138	172	166	212	212	116	112
PT103	116	116	138	138	172	168	214	212	116	112
PT105	116	110	140	138	168	168	216	212	118	116
PT109	116	110	138	138	172	168	218	210	116	112
PT110	116	110	138	138	172	168	212	210	116	112
PT111	116	110	140	138	166	166	210	210	116	112
PT114	116	116	138	138	172	172	216	214	118	112
PT222	110	110	140	140	174	174	212	206	102	102
PT223	114	110	140	140	170	162	214	210	102	102
PT225	116	110	140	138	172	166	212	210	116	116
PT226	116	110	140	140	172	168	216	210	116	112
SP001	116	114	140	138	166	166	212	212	116	112
SP002	116	112	140	140	172	168	216	210	122	116
SP003	114	110	138	138	172	172	212	210	116	116
SP017–1	116	110	140	138	168	166	212	210	120	112
SP035	116	116	140	138	172	172	212	210	116	116
SP085	116	110	140	138	168	168	214	210	116	116
SP087	116	116	140	138	174	168	214	212	116	112
SP088	116	110	140	138	168	168	212	210	118	116
SP089	116	116	140	138	172	166	210	210	116	116
SP092	116	110	140	138	166	166	210	210	116	116
SP093	116	110	138	138	168	168	216	212	116	112
SP144–2	116	110	138	138	168	166	212	210	116	112
WA031	116	116	138	138	172	168	214	210	116	116
WA127	116	110	140	140	168	166	212	210	116	116
WA128	110	110	140	138	172	172	214	212	118	116
WA129	116	116	140	138	168	168	210	210	112	110
WA130	116	110	138	138	172	172	216	210	116	112
WA131	116	110	140	138	168	166	212	210	122	116
DX1028	–	–	140	140	172	172	214	214	–	–
DX2714	–	–	140	140	172	166	210	210	–	–
DX324119	–	–	140	140	172	172	214	210	–	–

Appendix 4.B cont.

ID	Aph02		Aph08		Aph20		Aph23		Cmo9	
DX3241	–	–	140	140	172	168	214	214	–	–
DX4333	–	–	140	138	172	172	214	210	–	–
DX4739	–	–	140	140	172	172	214	210	–	–
DX5559	–	–	140	140	172	172	214	214	–	–
DX7428	–	–	140	140	172	168	214	210	–	–
DX120319	–	–	140	140	172	172	214	210	–	–
DT12063	–	–	140	138	172	168	214	210	–	–
DX12218	–	–	140	140	172	168	214	210	–	–
DT35166	–	–	140	140	172	172	214	210	–	–
A510178	–	–	140	140	172	172	214	210	–	–
DX35013	–	–	140	140	172	172	214	214	–	–
A510588	–	–	140	140	172	168	214	210	–	–
A510658	–	–	140	140	172	172	214	214	–	–
A510678	–	–	140	140	172	172	214	214	–	–
A588097	–	–	140	138	172	172	214	210	–	–
A580197	–	–	140	138	172	172	214	214	–	–
A580297	–	–	140	140	172	172	214	210	–	–
A580397	–	–	140	140	170	168	214	214	–	–
A580497	–	–	140	140	172	172	214	210	–	–
A584297	–	–	140	140	172	172	214	214	–	–
A584393	–	–	140	140	172	172	214	210	–	–
A586897	–	–	140	140	172	168	214	214	–	–
A588597	–	–	140	140	172	168	214	210	–	–
A586797	–	–	140	140	172	172	214	210	–	–
BD#	–	–	140	138	168	166	214	210	–	–
BNB	–	–	140	140	168	168	210	210	–	–
B6	–	–	140	138	172	172	210	210	–	–
B33	–	–	140	140	172	168	214	212	–	–
B43	–	–	140	138	172	168	210	210	–	–
B44	–	–	140	138	168	166	210	210	–	–
B45	–	–	140	140	170	168	214	210	–	–
B47	–	–	140	138	172	168	210	210	–	–
B48	–	–	140	138	172	168	212	210	–	–
B49	–	–	140	140	172	172	210	210	–	–
B51	–	–	140	140	172	168	210	210	–	–
B52	–	–	138	138	172	172	210	210	–	–
B53	–	–	140	138	172	172	210	210	–	–
B99	–	–	140	138	168	168	210	210	–	–
B209	–	–	140	138	172	166	214	210	–	–
B244	–	–	140	138	172	168	214	210	–	–

Appendix 4.B cont.

ID	Aph02		Aph08		Aph20		Aph23		Cmo9	
B515	–	–	138	138	172	168	210	208	–	–
B609	–	–	140	140	172	168	214	210	–	–
B5170	–	–	140	138	168	168	214	210	–	–
B21627	–	–	140	140	172	172	210	210	–	–
B85408	–	–	140	140	168	166	214	208	–	–
B25423	–	–	138	138	172	166	210	210	–	–
B26170	–	–	140	140	168	166	212	210	–	–
B59510	–	–	138	138	172	168	210	210	–	–
B59513	–	–	140	140	172	168	210	210	–	–
B59516	–	–	140	138	168	168	216	210	–	–
B59519	–	–	140	138	172	168	210	210	–	–
B59521	–	–	140	138	172	168	214	214	–	–
B59524	–	–	140	140	172	168	212	210	–	–
B40194	–	–	140	138	172	172	212	210	–	–
B40622	–	–	138	138	172	168	210	210	–	–
B45509	–	–	140	138	172	168	210	210	–	–
B45320	–	–	140	138	170	168	210	210	–	–
B48222	–	–	140	140	172	172	210	210	–	–
B49008	–	–	140	138	172	168	212	210	–	–
B49028	–	–	140	138	172	172	210	210	–	–
B49055	–	–	138	138	172	168	212	210	–	–
B49071	–	–	140	138	170	168	210	210	–	–
B49073	–	–	140	140	172	168	216	210	–	–
B49583	–	–	140	138	172	168	212	210	–	–
C1	–	–	140	138	168	166	210	210	–	–
C10	–	–	138	138	172	172	210	210	–	–
C14	–	–	140	138	168	168	210	210	–	–
C15	–	–	140	140	172	168	210	210	–	–
C17	–	–	140	140	172	172	214	210	–	–
C20	–	–	138	138	172	168	210	208	–	–
C21	–	–	140	140	172	168	212	210	–	–
C24	–	–	140	138	172	166	210	210	–	–
C30	–	–	140	138	172	168	212	212	–	–
C32	–	–	138	138	172	172	210	210	–	–
C34	–	–	138	138	172	168	210	210	–	–
C37	–	–	140	140	168	168	210	210	–	–
C39	–	–	140	140	172	168	212	210	–	–
C42	–	–	140	140	172	168	210	210	–	–
C45	–	–	140	138	172	172	210	210	–	–
C46	–	–	138	138	166	166	212	208	–	–

Appendix 4.B cont.

ID	Aph02		Aph08		Aph20		Aph23		Cmo9	
C50	–	–	140	140	172	166	210	210	–	–
C52	–	–	140	140	172	166	214	210	–	–
C53	–	–	140	140	172	172	216	208	–	–
C54	–	–	138	138	172	168	210	210	–	–
C55	–	–	140	140	172	168	210	210	–	–
C56	–	–	140	140	172	172	212	210	–	–
C57	–	–	140	140	168	168	214	210	–	–
C60	–	–	140	140	172	168	214	210	–	–
C61	–	–	140	140	172	172	214	212	–	–
C62	–	–	140	138	172	168	212	210	–	–
C63	–	–	140	138	172	168	214	212	–	–
C64	–	–	140	138	172	172	214	210	–	–
C66	–	–	140	138	172	172	210	208	–	–
C67	–	–	140	140	172	172	212	210	–	–
C68	–	–	140	138	172	166	214	212	–	–
C69	–	–	140	138	172	168	214	210	–	–
C70	–	–	140	140	172	166	214	210	–	–
C71	–	–	140	138	172	168	210	210	–	–
C73	–	–	140	140	172	166	210	210	–	–
C77	–	–	140	138	172	168	214	210	–	–
C80	–	–	140	138	172	168	212	210	–	–
C81	–	–	140	138	168	166	210	210	–	–
C83	–	–	140	140	172	172	210	210	–	–
C116	–	–	140	140	172	168	212	210	–	–
D1	–	–	140	140	172	172	214	210	–	–
D2	–	–	140	140	172	164	214	210	–	–
D3	–	–	140	140	168	166	210	210	–	–
D4	–	–	140	138	172	172	214	210	–	–
D5	–	–	140	140	172	172	210	210	–	–
D6	–	–	140	138	172	172	214	210	–	–
D7	–	–	140	140	172	172	214	210	–	–
D8	–	–	140	138	172	168	212	210	–	–
D9	–	–	140	140	172	168	214	210	–	–
D10	–	–	140	140	172	168	214	214	–	–
D11	–	–	140	140	172	172	214	210	–	–
D12	–	–	140	140	172	168	210	210	–	–
D13	–	–	140	140	172	172	210	210	–	–
D14	–	–	140	140	172	168	214	210	–	–
D15	–	–	140	138	172	168	210	210	–	–
D16	–	–	140	140	172	168	210	208	–	–

Appendix 4.B cont.

ID	Aph02		Aph08		Aph20		Aph23		Cmo9	
D17	–	–	140	140	172	172	214	210	–	–
D18	–	–	140	140	172	166	210	210	–	–
D19	–	–	140	140	172	166	214	210	–	–
D20	–	–	140	140	168	168	214	210	–	–
D21	–	–	140	138	172	172	210	210	–	–
D22	–	–	140	140	172	166	210	210	–	–
D23	–	–	140	140	172	168	214	210	–	–
D24	–	–	140	138	172	172	214	210	–	–
D25	–	–	140	140	172	166	214	214	–	–
D26	–	–	140	138	172	166	214	208	–	–
D27	–	–	140	138	168	166	214	210	–	–
D28	–	–	140	138	172	168	214	212	–	–
D29	–	–	140	140	172	166	214	210	–	–
D30	–	–	140	140	172	172	210	210	–	–
D31	–	–	140	140	172	168	214	210	–	–
D32	–	–	140	140	172	168	212	210	–	–
D33	–	–	140	140	172	168	214	210	–	–
D34	–	–	140	138	172	166	214	208	–	–
D35	–	–	140	140	172	172	214	208	–	–
D36	–	–	140	140	172	168	214	210	–	–
D37	–	–	140	138	172	172	214	210	–	–
E9	–	–	140	138	172	168	214	210	–	–
E10	–	–	140	140	172	166	214	210	–	–
E13	–	–	140	138	168	168	214	214	–	–
E15	–	–	140	140	172	172	214	210	–	–
E18	–	–	140	138	172	172	214	214	–	–
E20	–	–	140	140	172	168	214	210	–	–
E25	–	–	140	138	172	172	214	214	–	–
E28	–	–	140	138	172	172	214	210	–	–
E33	–	–	140	140	172	172	214	214	–	–
E35	–	–	140	140	172	172	214	210	–	–
E75	–	–	140	140	168	168	214	214	–	–
E86	–	–	140	138	172	168	214	214	–	–
E95	–	–	140	140	172	168	214	210	–	–
E98	–	–	140	140	172	172	214	214	–	–
E104	–	–	140	138	168	168	214	214	–	–
E107	–	–	140	140	172	172	214	214	–	–
E107–2	–	–	140	140	172	168	214	210	–	–
E117	–	–	140	140	172	166	214	210	–	–
E118	–	–	140	140	168	166	210	210	–	–

Appendix 4.B cont.

ID	Aph02		Aph08		Aph20		Aph23		Cmo9	
E123	–	–	140	140	168	166	214	210	–	–
E129	–	–	140	138	172	172	214	214	–	–
E137	–	–	140	140	172	170	214	214	–	–
E138	–	–	140	140	172	168	214	212	–	–
E139	–	–	140	140	172	172	214	214	–	–
E142	–	–	140	140	172	168	214	214	–	–
E148	–	–	140	138	172	172	214	214	–	–
E150	–	–	140	140	172	168	210	210	–	–
E153	–	–	140	140	172	172	210	210	–	–
E154	–	–	140	140	172	172	214	210	–	–
E162	–	–	140	140	172	166	214	214	–	–
E167	–	–	140	140	172	172	214	210	–	–
E175	–	–	140	140	172	166	214	210	–	–
E188	–	–	140	140	172	166	214	214	–	–
E191	–	–	140	138	172	168	214	214	–	–
E192	–	–	140	140	168	166	210	210	–	–
E195	–	–	140	138	172	166	210	210	–	–
E200	–	–	140	138	172	166	214	210	–	–
E202	–	–	140	140	172	166	214	210	–	–
F45	–	–	140	140	172	172	214	214	–	–
F47	–	–	140	138	172	172	210	210	–	–
F49	–	–	140	140	168	166	210	210	–	–
G33	–	–	140	138	172	168	214	214	–	–
G34	–	–	140	138	170	168	210	210	–	–
G37	–	–	140	138	168	166	214	210	–	–
G38	–	–	140	140	168	168	210	210	–	–
G39	–	–	140	140	172	172	210	210	–	–
G41	–	–	140	140	172	168	210	210	–	–
G42	–	–	140	136	172	168	210	210	–	–
G43	–	–	140	140	172	172	210	210	–	–
G49	–	–	140	140	172	166	210	210	–	–
G50	–	–	140	140	172	172	210	210	–	–
G51	–	–	140	140	172	172	214	210	–	–
G56	–	–	140	140	168	166	210	210	–	–
G57	–	–	140	138	172	168	214	210	–	–
G59	–	–	140	140	172	172	214	208	–	–
G60	–	–	140	140	172	168	212	210	–	–
G61	–	–	140	138	172	168	210	210	–	–
G66	–	–	140	140	172	168	214	208	–	–
G68	–	–	140	140	172	168	214	210	–	–

Appendix 4.B cont.

ID	Aph02		Aph08		Aph20		Aph23		Cmo9	
G72	–	–	140	140	172	168	212	210	–	–
G73	–	–	140	140	172	172	214	210	–	–
G74	–	–	140	138	172	172	214	210	–	–
G75	–	–	140	140	168	168	212	210	–	–
G76	–	–	140	138	172	172	212	212	–	–
G77	–	–	140	140	172	168	214	208	–	–
G79	–	–	140	138	172	166	214	214	–	–
G80	–	–	140	138	172	172	214	212	–	–
G82	–	–	140	140	172	168	210	210	–	–
G85	–	–	142	140	172	172	210	210	–	–
H317	–	–	140	138	172	168	210	210	–	–
H321	–	–	140	138	172	172	214	214	–	–
H322	–	–	140	138	172	168	214	210	–	–
H325	–	–	140	140	168	168	214	210	–	–
H326	–	–	140	140	172	172	214	210	–	–
H327	–	–	140	138	172	172	214	210	–	–
H328	–	–	140	138	168	168	210	210	–	–
H329	–	–	140	140	172	168	214	210	–	–
H330	–	–	140	140	168	166	214	210	–	–
H333	–	–	140	140	172	172	214	210	–	–
H334	–	–	140	138	172	164	210	210	–	–
H335	–	–	140	138	168	166	214	210	–	–
H338	–	–	140	140	172	168	212	212	–	–
H352	–	–	140	140	172	172	210	210	–	–
H25052	–	–	140	138	172	172	210	210	–	–
H25064	–	–	140	140	168	166	210	210	–	–
H25067	–	–	140	138	172	168	210	210	–	–
H25069	–	–	140	138	168	166	214	210	–	–
H25071	–	–	140	138	172	168	214	210	–	–
H25094	–	–	140	140	166	166	214	210	–	–
H25095	–	–	140	138	172	168	214	210	–	–
H25097	–	–	140	140	172	172	214	210	–	–
H25269	–	–	140	140	172	166	214	210	–	–
H25276	–	–	140	140	172	166	210	210	–	–
H91488	–	–	140	138	172	172	214	208	–	–
H91574	–	–	140	140	172	166	210	210	–	–
H91575	–	–	140	138	172	172	214	214	–	–
H91579	–	–	140	140	172	168	214	210	–	–
H91697	–	–	138	138	172	172	214	210	–	–
H91698	–	–	140	140	168	166	210	210	–	–

Appendix 4.B cont.

ID	Aph02		Aph08		Aph20		Aph23		Cmo9	
H91701	–	–	140	140	168	168	210	210	–	–
H91718	–	–	140	140	172	172	210	210	–	–
H91723	–	–	140	140	168	168	214	210	–	–
H91725	–	–	140	140	172	172	214	210	–	–
H91726	–	–	140	138	172	168	214	210	–	–
H91727	–	–	140	140	172	168	210	210	–	–
H91729	–	–	140	136	172	168	214	210	–	–
H91730	–	–	140	138	184	174	216	208	–	–
H91736	–	–	140	140	172	168	210	210	–	–
H91754	–	–	138	138	172	168	214	210	–	–
H91756	–	–	140	140	172	172	214	214	–	–
H91981	–	–	140	140	168	168	210	208	–	–
H95196	–	–	140	140	172	168	214	210	–	–
JAR210	–	–	140	138	172	168	212	212	–	–
H95967	–	–	140	140	168	166	216	210	–	–
H95969	–	–	140	138	172	172	214	214	–	–
H95970	–	–	140	138	172	172	210	210	–	–
H95972	–	–	140	140	172	168	214	214	–	–
H95974	–	–	140	140	168	168	214	210	–	–
H95975	–	–	140	140	168	168	214	210	–	–
H95976	–	–	140	140	172	168	214	210	–	–
H95987	–	–	140	140	168	166	214	210	–	–
H95995	–	–	140	138	166	166	214	210	–	–
T11	–	–	140	140	168	168	210	210	–	–
T12	–	–	140	138	172	172	210	210	–	–
T13	–	–	140	140	172	172	214	210	–	–
T14	–	–	140	140	172	168	214	212	–	–
T15	–	–	140	140	168	168	210	210	–	–
T16	–	–	140	140	172	166	210	210	–	–
T17	–	–	140	140	172	172	212	210	–	–
T18	–	–	140	140	172	168	210	210	–	–
T19	–	–	140	138	172	166	214	210	–	–
T20	–	–	140	140	172	172	214	212	–	–
TM01	–	–	140	140	172	172	210	210	–	–
TM02	–	–	140	140	172	168	210	210	–	–
TM03	–	–	140	140	168	168	210	210	–	–
TM04	–	–	140	140	172	172	214	210	–	–
TB01	–	–	140	140	172	172	210	210	–	–
TB02	–	–	140	140	168	166	210	210	–	–
TB03	–	–	140	138	172	172	214	212	–	–

Appendix 4.B cont.

ID	Aph02		Aph08		Aph20		Aph23		Cmo9	
TB04	–	–	140	140	172	172	210	210	–	–
TF01	–	–	140	140	172	168	212	210	–	–
TF02	–	–	140	140	168	166	210	210	–	–
TF03	–	–	140	140	172	168	212	210	–	–
TF04	–	–	140	140	172	172	210	210	–	–
M801	–	–	140	140	172	168	214	210	–	–
M803	–	–	140	140	172	172	210	210	–	–
M804	–	–	140	140	168	166	210	208	–	–
M805	–	–	140	140	172	168	214	210	–	–
M806	–	–	140	140	168	166	210	210	–	–
M809	–	–	140	140	172	166	212	210	–	–
M810	–	–	140	140	172	168	210	210	–	–
M811	–	–	140	140	172	172	210	210	–	–
M812	–	–	140	140	172	168	210	210	–	–
M813	–	–	140	140	172	166	214	210	–	–
M814	–	–	140	140	172	168	210	210	–	–
M815	–	–	140	140	172	168	210	210	–	–
M816	–	–	140	140	172	172	212	210	–	–
M817	–	–	140	138	172	172	214	210	–	–
M808	–	–	140	140	172	166	210	208	–	–
MRP283	–	–	138	138	172	166	212	210	–	–
MRP284	–	–	140	138	168	166	212	210	–	–
MRP285	–	–	140	138	172	166	216	210	–	–
MRP286	–	–	140	138	172	172	212	210	–	–
MRP287	–	–	138	138	172	168	212	210	–	–
MRP288	–	–	140	138	178	168	210	210	–	–
MRP289	–	–	140	138	168	166	210	210	–	–
MRP290	–	–	140	140	172	166	210	210	–	–
MRP291	–	–	138	138	168	168	216	210	–	–
MRP292	–	–	138	138	178	172	212	210	–	–
MRP293	–	–	138	138	168	168	212	212	–	–
MRP294	–	–	140	140	168	166	212	212	–	–
MRP295	–	–	140	140	168	168	214	210	–	–
MRP296	–	–	138	138	168	166	210	210	–	–
MRP297	–	–	138	138	172	168	210	210	–	–
MRP298	–	–	140	138	168	166	212	212	–	–
MRP299	–	–	140	138	172	166	212	210	–	–
MRP300	–	–	140	140	166	166	210	210	–	–
MRP301	–	–	140	140	172	166	218	210	–	–
MRP302	–	–	140	138	168	166	216	210	–	–

Appendix 4.B cont.

ID	Aph02		Aph08		Aph20		Aph23		Cmo9	
MRP303	–	–	140	140	172	168	210	210	–	–
MRP304	–	–	138	138	168	166	212	210	–	–
MRP305	–	–	138	138	168	168	210	210	–	–
MRP306	–	–	140	138	172	172	212	210	–	–
MRP307	–	–	138	138	172	172	218	212	–	–
MRP308	–	–	140	138	168	166	218	210	–	–
MRP309	–	–	138	138	172	166	210	210	–	–
MRP310	–	–	138	138	166	166	210	210	–	–
MRP311	–	–	140	140	172	172	210	210	–	–
MRP312	–	–	140	138	168	166	218	210	–	–
MRP313	–	–	140	140	168	166	210	210	–	–
MRP314	–	–	140	140	166	166	210	210	–	–
MRP315	–	–	138	138	168	166	210	210	–	–
MRP316	–	–	140	138	172	168	218	218	–	–
MRP317	–	–	138	138	166	166	210	210	–	–
MRP318	–	–	140	138	172	166	212	210	–	–
MRP320	–	–	140	138	168	168	212	210	–	–
MRP321	–	–	138	138	172	166	216	210	–	–
MRP322	–	–	138	138	166	166	210	210	–	–
MRP323	–	–	140	138	168	166	216	212	–	–
MRP324	–	–	138	138	168	166	216	212	–	–
MRP325	–	–	138	138	172	166	216	212	–	–
MRP326	–	–	138	138	166	166	212	210	–	–
DBI2	–	–	140	138	178	168	212	210	–	–
DBI3	–	–	140	140	168	166	212	212	–	–
DBI4	–	–	140	140	172	166	216	210	–	–
DBI5	–	–	140	140	168	166	218	210	–	–
PBAI935	–	–	140	140	176	172	210	210	–	–
UAM13336	–	–	140	140	172	166	216	210	–	–
UAM13721	–	–	140	140	172	168	216	210	–	–

Appendix 4.B cont.

ID	Bca1		Bca11		Hhi3		Sfi10		Smo4	
KIG1308	110	110	137	137	110	110	153	147	211	155
KIG1321	112	110	137	137	110	110	155	131	211	171
KIG1331	110	108	139	137	110	110	149	149	187	183
KIG1333	110	110	137	137	112	110	147	145	191	155
KIG1336	110	110	137	137	110	110	149	145	231	207
KIG1339	110	110	143	137	110	110	157	147	231	191
KIG1340	110	110	137	137	110	110	147	143	189	171
KIG1341	112	110	143	137	110	110	159	149	231	171
KIG1342	110	110	139	137	110	110	157	147	219	191
KIG1343	110	110	137	137	110	110	153	147	211	211
KIG1344	110	110	137	137	110	110	147	147	211	199
KIG1346	112	110	137	137	110	110	151	145	223	155
KIG1347	110	110	139	139	112	110	159	147	211	155
KIG2253	110	110	137	137	110	110	141	133	239	207
KIG2254	112	110	137	137	110	110	151	143	187	183
KIG2255	110	110	143	137	110	110	151	149	227	171
KIG2261	110	110	137	137	110	110	151	151	219	155
KIG2262	110	110	137	137	110	110	149	133	215	155
KIG2264	112	110	137	137	110	110	149	141	199	167
KIG2265	110	110	139	137	110	110	157	141	219	195
KIG2266	112	110	143	137	110	110	149	145	207	183
KIG2267	110	110	137	137	112	110	149	147	205	171
KIG2271	112	110	137	137	110	110	153	147	219	191
KIG2278	110	108	139	137	112	112	147	133	183	183
KIG2279	112	110	137	137	110	110	151	143	223	187
KIG2280	112	112	139	137	110	110	159	147	211	191
KIG2283	110	108	137	137	110	110	155	151	231	213
KIG2285	112	108	137	137	110	110	149	149	191	183
KIG2293	110	108	139	137	110	110	149	143	199	195
KIG2296	110	108	137	137	112	110	159	147	217	203
KIG2298	112	110	137	137	110	110	151	145	223	213
KIG2299	112	110	139	137	110	110	147	147	219	207
KIG6334	112	110	137	137	112	110	153	149	215	195
KIG6915	110	110	137	137	110	110	147	141	191	171
KIG6927	112	110	137	137	110	110	153	145	233	217
KIG6944	112	110	139	137	110	110	159	147	211	183
KIG6999	110	110	137	137	110	110	157	147	195	175
BS76451	110	110	137	137	110	110	149	145	207	191
BS76452	112	110	137	137	110	110	149	145	191	155
BS76454	112	110	137	137	110	110	141	133	215	215

Appendix 4.B cont.

ID	Bca1		Bca11		Hhi3		Sfi10		Smo4	
BS76455	112	110	137	137	110	110	149	145	213	203
BS76456	112	112	137	137	110	110	149	149	227	209
BS76457	110	108	139	137	110	110	149	133	207	183
BS76459	110	110	137	137	112	112	149	131	219	211
BS76460	112	108	137	137	110	110	155	133	191	183
BS76461	112	110	137	137	110	110	151	149	179	171
BS76462	112	110	137	137	110	110	157	147	207	199
BS76463	110	110	137	137	112	110	149	147	206	191
BS76464	110	110	137	137	110	110	147	133	187	187
BS76465	112	110	137	137	110	110	153	147	227	187
BS76466	112	108	139	137	110	110	147	133	209	183
BS76467	112	110	137	137	110	110	147	143	209	199
BS76469	112	112	147	137	110	110	155	149	211	209
BS76470	110	110	147	137	110	110	157	153	211	207
YK54775	110	110	139	137	110	110	149	147	191	183
YK57894	112	110	137	137	110	110	149	147	219	207
YK76112	110	110	137	137	110	110	151	147	191	175
YK76115	110	110	137	137	112	110	161	131	239	207
YK76117	110	110	143	137	110	110	149	131	211	183
YK76119	–	–	137	137	110	110	147	145	197	191
YK76120	112	108	137	137	110	110	133	131	195	163
YK76122	112	110	147	137	110	110	157	147	199	193
YK76124	110	108	139	137	110	110	153	147	219	183
YK76156	110	110	139	137	110	110	141	133	207	207
YK76157	110	110	143	137	110	110	149	149	219	195
YK76158	110	110	137	137	110	110	149	147	199	167
YK76161	112	110	137	137	110	110	147	147	215	179
YK76163	112	110	139	137	110	110	159	151	215	211
YK76164	112	110	137	137	110	110	151	131	227	207
YK76165	–	–	137	137	110	110	149	133	195	171
YK76167	112	110	139	137	110	110	149	147	215	193
YK76168	–	–	139	137	110	110	151	149	223	211
YK76169	112	110	137	137	110	110	157	135	233	211
YK76170	110	110	143	143	110	110	149	149	259	219
YK76171	110	110	139	137	110	110	153	151	211	209
YK76172	110	110	145	145	110	110	155	149	201	161
YK76173	108	108	139	137	112	112	141	131	191	157
YK76174	110	108	137	137	110	110	159	149	191	171
YK76177	112	110	143	137	110	110	185	149	199	171
YK76178	112	110	137	137	112	110	143	141	235	203

Appendix 4.B cont.

ID	Bca1		Bca11		Hhi3		Sfi10		Smo4	
YK76179	–	–	137	137	110	110	–	–	183	183
YK76181	112	110	137	137	110	110	157	131	187	187
YK76182	112	112	139	137	110	110	133	133	211	183
YK76186	–	–	137	137	110	110	135	133	231	203
YK76187	110	108	137	137	110	110	147	135	199	187
YK76193	110	110	139	137	110	110	147	141	211	207
YK76672	110	108	137	137	110	110	141	131	221	183
YK54748	110	110	137	137	112	110	161	149	239	193
YK76151	112	110	137	137	110	110	157	141	211	183
YK76155	110	110	137	137	110	110	149	149	203	195
YK76264	112	110	143	137	110	110	147	147	227	219
YK76268	110	110	137	137	110	110	149	149	219	213
YK76271	112	108	137	137	112	112	155	143	231	191
YK76432	112	110	137	137	110	110	155	149	239	203
YK76123	110	110	147	137	110	110	155	147	219	203
YK76154	112	110	137	137	112	110	143	141	235	203
YK76159	110	108	137	137	112	112	131	131	219	173
YK76162	110	110	139	137	110	110	143	131	209	203
YK76166	110	110	137	137	112	112	157	149	207	197
YK76192	112	110	143	137	110	110	149	147	189	177
YK76194	110	110	137	137	110	110	149	147	191	191
YK76267	110	110	139	137	112	112	157	149	239	207
YK76316	110	110	139	137	110	110	151	147	207	197
YK76420	110	108	139	137	110	110	159	153	195	155
YK76421	–	–	139	137	112	110	149	145	219	191
YK76464	112	110	137	137	110	110	151	147	211	207
YK76425	112	112	139	137	110	110	159	149	199	193
YK76665	112	110	139	137	110	110	149	149	203	195
YK76851	112	110	137	137	110	110	149	133	227	191
YK76854	110	110	137	137	110	110	147	147	203	191
YK76865	110	110	137	137	110	110	149	147	191	179
YK77552	112	112	139	139	110	110	147	147	203	183
YK77560	112	110	137	137	110	110	147	147	187	187
YK77563	112	112	139	137	110	110	149	147	227	189
YK77565	110	110	139	137	110	110	157	157	207	199
YK77566	110	110	137	137	110	110	143	133	207	201
YK77569	110	108	137	137	110	110	149	149	213	157
YK77573	110	110	137	137	110	110	151	149	207	207
YK77576	112	110	137	137	110	110	147	133	193	187
YK77580	–	–	139	137	110	110	–	–	183	167

Appendix 4.B cont.

ID	Bca1		Bca11		Hhi3		Sfi10		Smo4	
YK77581	110	110	137	137	110	110	149	147	–	–
YK77582	110	110	137	137	110	110	145	133	203	195
YK77584	110	110	139	137	110	110	149	149	219	157
YK77585	110	110	137	137	110	110	167	149	237	199
COEI–M	–	–	137	137	112	112	155	151	187	155
DEAD–F	110	110	137	137	110	110	157	149	187	183
EUTH–M	110	110	147	139	110	110	149	145	219	191
KP1516	110	110	137	137	110	110	153	149	239	207
KP1517	110	110	139	137	110	110	151	147	193	155
KP1518	110	108	137	137	110	110	159	159	219	201
KP1519	112	110	137	137	112	112	141	133	207	183
KP4596	110	108	137	137	110	110	147	133	231	191
KP4597	110	110	137	137	112	112	147	141	207	199
KP9827	110	110	137	137	112	112	151	149	207	199
KP9828	112	112	137	137	110	110	153	143	205	205
KP9829	112	108	137	137	110	110	155	131	207	167
KP9830	112	110	139	139	110	110	133	133	217	187
KP9831	110	110	139	139	110	110	147	143	203	183
KP9832	112	110	147	139	–	–	147	133	187	183
KP9833	110	110	137	137	110	110	149	149	203	187
KP9835	112	108	139	137	110	110	147	133	207	195
KP9836	110	110	137	137	112	112	145	145	195	187
KP9837	110	110	139	139	110	110	149	133	211	193
KP9838	110	110	139	137	110	110	159	149	215	191
KP1502	110	110	137	137	110	110	155	149	247	191
KP1505	110	110	137	137	110	110	153	149	251	199
KP1508	110	108	137	137	110	110	149	147	241	187
KP1510	112	110	137	137	110	110	133	131	227	207
KP1512	110	110	143	137	110	110	155	151	203	193
KP1828	110	110	139	137	110	110	151	151	197	157
KP1829	110	110	147	139	112	112	149	143	219	195
KP1833	112	110	143	137	110	110	151	147	207	183
KP1835	110	108	137	137	112	110	151	141	219	215
KP1836	110	110	137	137	110	110	161	147	207	195
KP1840	110	110	137	137	112	112	155	147	215	193
KP4553	110	110	137	137	110	110	147	143	201	157
KP4617	112	110	137	137	110	110	149	133	223	203
KP9816	112	110	137	137	110	110	149	145	187	175
KP9818	112	110	143	137	110	110	159	131	211	203
KP9820	112	108	139	137	110	110	149	147	203	157

Appendix 4.B cont.

ID	Bca1		Bca11		Hhi3		Sfi10		Smo4	
KP9823	110	110	137	137	110	110	143	133	187	183
KP9824	110	110	137	137	110	110	153	145	231	183
KP9825	112	112	141	137	110	110	149	133	249	241
DEAD–02	110	110	137	137	110	110	145	145	203	197
DEAD–03	110	110	137	137	110	110	149	145	217	183
CAMP1–1	110	110	139	137	110	110	145	133	191	187
NS27252	112	110	143	137	110	110	147	145	195	187
NS27253	112	110	137	137	110	110	133	131	247	211
NS27256	110	110	137	137	110	110	157	149	223	205
NS27260	110	110	137	137	110	110	149	147	191	183
NS27264	112	108	137	137	110	110	159	149	219	219
NS27269	110	110	139	137	110	110	151	145	183	155
NS27270	110	110	143	137	110	110	157	149	219	219
NS27271	112	110	139	139	112	110	149	147	205	187
NS27272	110	108	139	137	110	110	147	147	211	211
NS27273	112	112	139	137	110	110	133	133	231	191
NS27274	110	108	137	137	110	110	133	133	219	183
NS27275	112	110	139	137	–	–	153	149	211	207
NS27276	112	110	137	137	110	110	145	143	227	191
NS27277	110	108	137	137	110	110	157	149	191	183
NS27278	110	110	139	137	110	110	161	147	217	207
NS27279	110	110	139	137	112	112	173	153	235	167
NS27280	110	110	139	137	110	110	149	147	187	175
NS27281	110	110	137	137	110	110	149	149	227	219
NS27282	112	110	139	137	110	110	147	143	199	171
NS27284	112	110	137	137	110	110	149	141	215	155
NS27286	110	108	139	137	110	110	151	149	179	175
NS27291	110	110	139	137	110	110	151	147	199	167
NS27292	110	110	137	137	112	110	151	149	223	199
NS27293	110	110	139	137	110	110	147	147	217	197
NS272xx	–	–	137	137	110	110	149	143	241	207
NS27304	110	110	139	137	110	110	161	131	219	217
NS27305	110	110	137	137	110	110	159	133	199	183
NS27337	112	110	137	137	110	110	149	143	223	187
NS27338	110	110	137	137	110	110	157	149	183	171
NS27339	110	108	137	137	110	110	155	147	211	199
NS27340	110	110	137	137	114	112	149	145	203	171
NS27341	110	110	137	137	114	110	143	133	197	163
NS27342	110	110	137	137	110	110	149	147	213	195
NS27343	110	108	143	137	112	110	175	155	211	155

Appendix 4.B cont.

ID	Bca1		Bca11		Hhi3		Sfi10		Smo4	
NS27344	110	108	143	137	112	110	175	147	219	155
NS27345	–	–	139	137	110	110	149	141	213	187
NS27346	110	110	147	137	110	110	163	157	215	183
NS27347	–	–	139	137	110	110	147	131	187	155
NS24348	110	110	137	137	110	110	153	149	183	155
NS27349	112	110	137	137	110	110	145	133	251	239
NS27350	110	110	143	137	112	110	149	149	219	183
NS24351	110	110	137	137	110	110	155	151	211	179
NS27352	110	110	137	137	112	110	147	147	191	159
NS27354	110	110	137	137	110	110	149	149	223	195
NS27417	112	110	139	137	110	110	145	133	213	183
NS27418	110	110	137	137	110	110	149	147	205	183
NS27419	110	110	137	137	110	110	147	133	171	171
NS27420	112	108	137	137	110	110	149	147	217	187
NS27422	110	110	143	137	110	110	153	149	203	155
NS27423	112	110	137	137	110	110	155	147	211	191
NS27424	110	110	137	137	110	110	149	145	223	191
NS27425	112	112	139	137	114	112	159	147	207	155
NS52251	110	108	137	137	110	110	149	131	233	187
NS52252	112	110	139	137	110	110	143	133	231	219
NS52253	110	108	137	137	112	112	147	147	175	155
NS52254	110	110	137	137	112	112	159	149	239	183
NS52255	110	110	139	139	110	110	147	147	191	191
NS52256	110	110	139	137	114	112	153	149	207	155
NS52257	110	110	137	137	110	110	149	133	235	207
NS52258	110	110	137	137	110	110	155	147	213	155
NS52259	110	110	139	137	110	110	181	147	207	187
NS52260	110	110	137	137	110	110	147	133	257	231
NS52261	110	110	143	137	112	110	155	133	215	207
NS52262	110	110	137	137	110	110	151	145	217	187
NS52263	110	110	137	137	110	110	147	131	203	191
NS52264	112	112	137	137	110	110	149	133	201	159
NS52265	112	110	137	137	110	110	147	145	183	167
NS52266	112	110	137	137	110	110	155	155	195	155
NS52267	112	110	137	137	110	110	145	143	207	195
NS52268	108	108	137	137	112	112	149	133	187	179
NS52269	112	108	137	137	110	110	147	133	207	191
NS52270	110	110	139	137	114	114	181	153	207	183
NS52271	112	110	137	137	112	112	149	149	211	191
NS52272	110	110	137	137	110	110	149	133	207	157

Appendix 4.B cont.

ID	Bca1		Bca11		Hhi3		Sfi10		Smo4	
NS52273	112	110	137	137	112	110	147	141	211	187
NS52274	110	110	137	137	110	110	151	147	219	195
NS52275	110	108	137	137	110	110	145	131	219	179
NS52276	110	110	139	137	110	110	–	–	187	183
NS52277	110	110	137	137	110	110	147	141	195	171
NS52278	110	110	143	137	110	110	141	131	219	215
NS52279	110	110	137	137	112	112	151	147	223	215
NS52280	112	110	137	137	110	110	151	149	231	191
NS52281	110	110	137	137	110	110	153	147	187	175
NS52282	110	108	137	137	110	110	159	147	223	155
NS52283	112	110	137	137	112	110	155	147	231	183
NS52284	112	108	137	137	112	110	133	131	235	155
NS52285	112	110	137	137	110	110	143	133	195	171
NS52286	110	110	137	137	112	110	153	149	247	215
NS52287	110	110	137	137	110	110	155	149	243	215
NS52288	112	110	137	137	110	110	155	149	187	187
NS52289	110	110	139	139	110	110	141	133	203	187
NS52290	110	110	137	137	110	110	151	133	223	217
NS52291	110	110	139	137	110	110	147	141	247	211
NS76453	110	110	137	137	110	110	145	143	227	191
NS76471	110	110	137	137	110	110	155	131	203	155
NS76472	112	110	139	139	112	110	157	149	257	207
JAR136	112	110	139	137	110	110	159	151	191	155
JAR144	110	110	137	137	110	110	151	149	171	155
JAR204	110	110	143	137	110	110	153	147	239	155
NS27321	110	108	139	137	110	110	153	133	183	163
NS27322	110	110	139	137	110	110	145	133	223	163
NS27323	–	–	143	137	112	110	149	133	207	191
NS27324	110	110	137	137	110	110	145	131	219	193
NS27325	112	110	141	137	112	112	149	145	207	199
NS27401	112	110	137	137	110	110	151	147	231	203
NS27402	112	108	143	137	110	110	157	147	227	191
NS27404	110	110	145	145	112	110	151	151	235	235
NS27405	110	108	137	137	110	110	147	131	195	195
NS27406	110	110	139	137	110	110	153	141	239	155
NS27407	110	110	141	139	112	110	153	147	231	211
NS27441	110	110	137	137	110	110	149	133	201	195
NS27442	–	–	137	137	112	112	157	143	207	191
NS27443	110	110	137	137	110	110	153	149	205	191
NS76478	110	110	137	137	110	110	147	143	219	203

Appendix 4.B cont.

ID	Bca1		Bca11		Hhi3		Sfi10		Smo4	
NS76480	112	110	139	137	110	110	141	131	183	175
NS27251	110	108	137	137	110	110	151	133	251	191
NS76482	112	108	137	137	112	112	153	149	207	203
NS76483	–	–	137	137	112	112	155	149	209	187
NS76485	110	108	137	137	110	110	149	147	257	191
NS76487	110	108	137	137	110	110	149	149	207	199
NS76490	110	110	139	137	112	112	149	149	217	195
NS76491	110	110	141	137	112	112	157	147	183	167
NS76492	110	110	137	137	112	112	147	145	199	183
NS76493	112	112	139	137	110	110	157	149	207	191
NS76494	110	110	137	137	112	112	157	149	197	167
NS76495	112	108	139	137	112	110	157	155	187	175
NS76496	110	110	139	137	110	110	147	143	213	205
NS76497	110	110	137	137	110	110	149	147	207	155
NS76498	110	110	137	137	110	110	133	131	223	207
NS76499	110	110	139	137	110	110	157	141	189	167
NS76500	–	–	139	137	110	110	149	133	197	195
NS76551	110	110	137	137	110	110	145	141	187	155
NS76552	110	110	139	137	110	110	149	149	201	201
NS76553	110	110	143	137	112	110	153	153	215	191
NS76555	–	–	141	137	110	110	143	143	171	167
NS76556	110	110	141	137	110	110	147	145	187	183
NS76557	110	110	137	137	110	110	151	143	207	207
NS76558	110	110	147	137	110	110	155	145	207	179
NS76559	110	110	137	137	110	110	131	131	211	207
NS76560	110	110	139	139	110	110	149	147	239	195
NS82101	–	–	137	137	110	110	145	141	215	191
NS82102	110	110	143	137	110	110	149	147	195	171
NS82104	–	–	139	139	110	110	149	147	219	193
NS82106	110	110	137	137	110	110	153	133	207	195
NS82107	110	110	137	137	110	110	147	131	219	203
NS82109	112	110	137	137	110	110	155	151	199	165
NS82112	112	110	143	137	112	112	147	131	239	199
NS82117	110	110	139	137	112	110	155	151	239	183
NS82118	–	–	143	137	110	110	149	149	203	195
NS82119	110	110	137	137	110	110	147	143	191	165
NS82120	110	110	143	137	110	110	155	153	239	171
NS82121	–	–	137	137	110	110	149	149	231	157
NS82122	110	108	139	137	110	110	147	131	223	191
NS82123	112	110	137	137	110	110	147	133	191	191

Appendix 4.B cont.

ID	Bca1		Bca11		Hhi3		Sfi10		Smo4	
NS82127	–	–	139	137	110	110	149	147	235	191
NS82129	110	110	137	137	112	112	149	147	223	183
NS82130	–	–	137	137	110	110	143	133	199	171
NS82133	–	–	137	137	110	110	145	131	223	205
NS82134	110	110	137	137	110	110	147	133	243	197
NS82135	110	110	137	137	110	110	159	147	217	207
NS82136	–	–	137	137	112	110	157	133	171	171
NS82137	110	110	137	137	112	110	–	–	191	171
NS82138	–	–	139	137	110	110	157	157	207	193
NS82141	112	110	139	137	110	110	149	143	207	207
NS82143	112	110	141	137	110	110	149	143	187	155
NS82144	112	110	137	137	110	110	153	149	203	195
NS82145	110	110	137	137	110	110	133	133	241	195
NS82150	110	110	147	137	110	110	147	143	213	163
NS82151	110	110	137	137	112	110	147	133	197	183
NS82152	110	108	143	137	110	110	151	143	213	171
NS82153	110	110	137	137	110	110	153	149	223	171
NS82156	112	110	139	137	110	110	157	153	215	187
NS82157	112	110	137	137	110	110	149	131	197	195
NS82158	110	110	137	137	112	112	151	133	247	155
NS82162	110	110	137	137	110	110	149	133	211	187
NS82163	110	108	137	137	110	110	149	149	219	205
NS82164	–	–	137	137	110	110	149	147	187	171
NS82165	110	110	139	137	110	110	149	143	243	205
NS82166	110	110	139	137	110	110	155	147	191	171
NS82167	110	110	139	137	110	110	159	145	203	171
NS82168	112	108	139	137	110	110	155	147	175	163
NS82142	112	110	139	137	110	110	143	131	257	207
NS82146	110	110	139	137	112	110	143	133	219	191
NS82204	112	110	147	137	110	110	149	147	203	191
NS82205	112	110	137	137	110	110	147	133	217	179
NS82207	112	112	139	137	110	110	161	157	217	187
NS82209	110	110	143	137	110	110	149	145	235	187
NS82211	110	110	137	137	110	110	149	147	191	163
NS82154	112	110	139	137	110	110	151	145	215	203
NS82221	112	110	137	137	110	110	149	147	203	191
NS82224	112	112	143	137	110	110	157	147	211	183
NS82225	110	110	139	137	110	110	147	147	203	193
NS82232	–	–	143	137	110	110	149	149	205	195
NS82160	112	110	139	137	110	110	157	157	187	171

Appendix 4.B cont.

ID	Bca1		Bca11		Hhi3		Sfi10		Smo4	
NS82234	112	110	137	137	110	110	149	131	207	195
NS82235	112	110	137	137	110	110	151	141	197	183
NS27407	110	110	141	139	112	110	153	147	231	211
NS82237	112	110	137	137	110	110	149	149	223	207
NS82147	112	110	139	137	110	110	151	145	215	203
NS27421	112	110	137	137	110	110	151	133	199	195
NS27268	110	110	139	137	112	110	155	151	239	183
AK132	110	110	143	139	110	110	157	151	199	183
AK134	114	110	139	137	110	110	149	147	187	175
AK135	110	108	139	137	–	–	149	141	195	187
AK136	112	110	137	137	110	110	149	143	243	207
AK137	112	112	137	137	110	110	143	133	199	195
AK138	110	110	137	137	110	110	145	143	241	199
AK139	112	110	137	137	110	110	149	147	225	183
AK140	110	110	139	139	110	110	149	147	215	211
AK142	112	110	147	139	110	110	157	147	235	207
AK143	112	110	143	137	110	110	155	149	231	187
AK150	112	110	139	137	110	110	159	149	231	187
AK151	110	110	137	137	110	110	159	147	239	199
AK240	112	112	137	137	–	–	149	149	–	–
CA1–1	110	110	139	137	110	110	145	133	191	187
CA149	110	110	137	137	112	110	149	147	199	187
CA150	110	110	137	137	110	110	149	147	187	155
CA152	112	110	137	137	110	110	149	133	199	171
CA153	110	110	137	137	110	110	151	147	219	183
CA156	112	112	143	137	110	110	147	141	191	191
CA159	112	110	139	137	110	110	151	149	211	211
CA162	110	110	143	137	110	110	157	149	211	199
CH116	112	110	137	137	110	110	157	147	191	183
CH118	110	108	139	137	112	110	141	141	211	195
CH119	110	110	139	137	110	110	149	131	187	187
CH121	110	110	139	137	110	110	147	141	219	211
CH124	110	110	143	137	110	110	157	147	219	191
CH131	110	110	137	137	110	110	149	149	227	219
CH201	112	110	139	137	110	110	157	147	197	183
CH261	112	110	137	137	110	110	157	147	207	183
DU136	112	110	139	137	110	110	159	151	191	155
DU210	112	110	139	137	110	110	–	–	217	187
DU227	110	108	137	137	110	110	151	149	195	155
EG1	112	110	139	139	110	110	151	143	191	179

Appendix 4.B cont.

ID	Bca1		Bca11		Hhi3		Sfi10		Smo4	
EG10–2	112	110	137	137	110	110	149	149	237	207
EG2	112	110	137	137	110	110	149	143	211	167
EG2–2	112	110	139	137	110	110	–	–	179	179
EG3	112	110	137	137	110	110	149	133	191	191
EG3–2	110	110	139	137	110	110	143	133	219	191
EG4	110	110	139	137	110	110	147	143	243	221
EG5	112	110	147	137	110	110	149	147	211	203
EG7	110	110	137	137	110	110	149	143	219	191
EG9	112	110	137	137	110	110	147	147	207	175
EG9–2	110	110	139	137	110	110	133	133	205	183
LO001	112	110	137	137	110	110	153	151	231	207
LO002	112	110	137	137	110	110	157	151	187	187
LO003	112	110	139	137	110	110	157	157	239	195
LO004	110	110	137	137	110	110	147	131	241	213
LO008	112	110	137	137	112	110	147	143	247	235
LO009	110	110	137	137	112	110	157	147	191	191
LO010	110	110	137	137	110	110	147	145	215	183
LO011	110	110	139	137	110	110	149	147	195	191
LO012	110	110	137	137	110	110	147	133	195	187
LO014	112	112	137	137	112	110	159	149	207	207
LO017	110	110	137	137	110	110	149	149	231	155
LO018	110	110	137	137	110	110	147	143	183	179
LO019	110	110	139	137	110	110	149	141	207	191
LO020	110	108	143	137	110	110	151	143	213	183
LO021	112	110	139	137	110	110	147	135	187	155
LO023	112	110	139	139	110	110	149	147	193	155
LO141	110	110	137	137	110	110	147	141	183	183
LO033	110	110	137	137	112	110	149	147	211	183
LO035	112	110	139	137	110	110	157	151	223	187
ST024–2	110	110	137	137	110	110	147	147	201	191
ST024	112	110	137	137	110	110	145	131	223	205
MS224–2	110	110	137	137	110	110	145	133	215	171
MS226–2	112	110	137	137	110	110	153	131	227	211
MS227	110	110	137	137	110	110	159	133	207	199
MS230–5	110	108	137	137	110	110	149	141	221	179
MS231	110	108	139	137	110	110	157	149	199	183
MS233	110	110	137	137	110	110	149	147	205	183
MS235	112	110	139	137	110	110	151	147	219	215
MS262	110	110	137	137	110	110	159	133	207	199
MS303	112	110	137	137	110	110	149	147	211	207

Appendix 4.B cont.

ID	Bca1		Bca11		Hhi3		Sfi10		Smo4	
NS100	112	110	143	137	110	110	147	147	219	191
NS202	112	110	137	137	110	110	157	133	199	199
NS203–1	110	110	137	137	110	110	149	145	239	221
NS204	110	110	143	137	110	110	153	147	239	155
NS218	112	110	137	137	110	110	149	147	217	183
NS219	110	110	139	137	110	110	149	131	187	155
NS220	110	110	137	137	110	110	145	131	241	219
NS222	110	110	147	143	112	110	155	147	207	175
NS223	112	110	139	137	110	110	147	133	235	199
PT102	110	110	137	137	110	110	147	133	183	171
PT103	110	110	139	137	110	110	145	133	191	187
PT105	110	110	137	137	110	110	149	147	219	191
PT109	110	110	137	137	110	110	155	145	207	187
PT110	110	110	139	137	110	110	157	151	199	193
PT111	110	108	137	137	110	110	149	131	219	215
PT114	112	110	139	137	110	110	153	143	243	235
PT222	112	112	137	135	110	110	155	129	159	159
PT223	–	–	143	135	110	110	147	145	–	–
PT225	110	110	137	137	110	110	151	147	195	171
PT226	112	110	139	137	110	110	147	133	233	199
SP001	110	110	137	137	110	110	143	133	215	211
SP002	110	110	137	137	110	110	149	145	207	199
SP003	110	110	137	137	110	110	151	131	255	207
SP017–1	108	108	137	137	110	110	151	141	203	157
SP035	112	110	141	137	112	110	149	145	207	199
SP085	112	110	137	137	112	112	147	131	191	155
SP087	110	110	139	137	112	110	153	133	203	191
SP088	110	110	139	137	112	110	149	133	253	211
SP089	110	110	137	137	110	110	149	131	191	171
SP092	112	110	139	137	110	110	141	131	183	175
SP093	110	110	137	137	110	110	149	141	195	195
SP144–2	112	110	139	137	110	110	149	143	207	206
WA031	112	110	137	137	110	110	153	133	223	207
WA127	110	108	137	137	110	110	149	145	191	183
WA128	112	110	137	137	110	110	141	133	197	183
WA129	110	110	137	137	110	110	153	153	211	195
WA130	110	108	137	137	110	110	153	151	231	207
WA131	110	108	137	137	110	110	149	149	183	171
DX1028	110	110	137	137	110	110	149	147	207	195
DX2714	110	110	137	137	110	110	193	149	201	175

Appendix 4.B cont.

ID	Bca1		Bca11		Hhi3		Sfi10		Smo4	
DX324119	110	110	137	137	110	110	175	149	183	171
DX3241	110	110	137	137	110	110	153	149	175	175
DX4333	112	110	137	137	110	110	175	151	175	171
DX4739	110	110	143	137	110	110	175	151	215	207
DX5559	110	110	137	137	110	110	151	149	201	167
DX7428	110	110	137	137	112	110	163	147	203	175
DX120319	110	110	137	137	110	110	151	147	191	175
DT12063	110	110	137	137	110	110	147	147	211	207
DX12218	112	110	147	137	110	110	153	147	217	205
DT35166	110	110	137	137	110	110	175	147	215	167
A510178	110	110	137	137	110	110	147	147	241	203
DX35013	112	110	137	137	110	110	165	147	171	171
A510588	110	110	137	137	110	110	151	151	219	171
A510658	110	110	137	137	110	110	147	147	195	195
A510678	112	110	137	137	112	110	175	151	215	167
A588097	112	110	143	137	110	110	151	151	179	175
A580197	110	110	137	137	110	110	153	151	207	179
A580297	110	110	143	137	110	110	175	151	203	203
A580397	110	110	137	137	110	110	167	147	211	203
A580497	110	110	137	137	110	110	169	147	207	171
A584297	112	110	145	143	110	110	151	147	175	171
A584393	110	110	137	137	110	110	163	151	171	171
A586897	112	110	137	137	110	110	177	149	175	171
A588597	110	110	143	137	110	110	151	147	201	157
A586797	112	110	137	137	1112	110	149	147	175	175
BD#	110	110	137	137	110	110	155	145	211	199
BNB	112	110	137	137	112	110	155	151	211	207
B6	112	110	137	137	110	110	153	143	187	179
B33	110	110	137	137	110	110	155	143	211	195
B43	112	110	137	137	110	110	143	131	191	179
B44	110	110	137	137	110	110	157	151	219	167
B45	110	110	143	137	112	110	151	143	199	195
B47	110	110	137	137	110	110	153	149	193	191
B48	112	110	137	137	110	110	151	131	235	195
B49	110	108	137	137	110	110	157	149	209	207
B51	112	110	137	137	110	110	155	147	207	199
B52	110	110	137	137	110	110	151	141	207	207
B53	110	110	137	137	110	110	149	143	215	195
B99	112	112	137	137	110	110	149	143	199	183
B209	112	108	137	137	112	110	149	131	195	171

Appendix 4.B cont.

ID	Bca1		Bca11		Hhi3		Sfi10		Smo4	
B244	110	110	137	137	110	110	155	147	179	167
B515	110	110	137	137	110	110	151	145	211	179
B609	110	110	137	137	110	110	203	143	215	207
B5170	110	110	137	137	110	110	151	145	211	203
B21627	110	110	137	137	110	110	155	131	207	179
B85408	112	110	137	137	110	110	151	131	211	191
B25423	110	108	137	137	112	112	147	131	203	171
B26170	112	110	137	137	110	110	147	131	219	187
B59510	110	110	137	137	110	110	155	143	219	171
B59513	110	110	137	137	110	110	157	151	219	203
B59516	112	112	137	137	110	110	151	147	207	203
B59519	110	110	137	137	112	110	153	151	235	207
B59521	112	110	137	137	112	112	155	147	207	171
B59524	110	110	137	137	110	110	147	145	231	167
B40194	112	110	137	137	110	110	157	153	219	195
B40622	110	110	137	137	110	110	149	131	207	191
B45509	110	110	143	137	110	110	151	131	203	199
B45320	110	110	137	137	112	110	155	145	209	183
B48222	112	110	137	137	110	110	145	143	207	195
B49008	110	110	137	137	112	110	149	145	231	187
B49028	112	112	137	137	110	110	157	155	211	187
B49055	112	112	137	137	110	110	151	143	179	175
B49071	112	110	137	137	110	110	155	149	227	183
B49073	110	110	137	137	110	110	155	151	215	171
B49583	112	112	137	137	110	110	155	151	227	171
C1	110	110	137	137	110	110	151	145	195	171
C10	112	110	137	137	110	110	149	145	215	191
C14	110	110	137	137	112	112	157	155	207	191
C15	110	110	137	137	110	110	145	145	195	183
C17	110	110	137	137	110	110	155	145	195	187
C20	110	110	137	137	110	110	157	145	207	171
C21	110	108	137	137	110	110	151	147	207	207
C24	110	110	137	137	112	110	155	147	215	207
C30	112	110	137	137	110	110	155	149	203	203
C32	110	110	137	137	110	110	155	131	199	183
C34	110	110	137	137	110	110	143	131	187	183
C37	112	110	137	137	112	110	155	145	193	191
C39	110	110	137	137	110	110	151	145	179	171
C42	112	110	137	137	112	110	147	131	235	171
C45	110	110	137	137	110	110	155	147	211	207

Appendix 4.B cont.

ID	Bca1		Bca11		Hhi3		Sfi10		Smo4	
C46	112	110	137	137	110	110	179	143	191	167
C50	110	110	137	137	110	110	147	147	187	175
C52	112	112	137	137	110	110	147	143	223	205
C53	110	110	137	137	110	110	171	157	209	201
C54	110	110	137	137	110	110	157	155	207	187
C55	112	110	147	137	110	110	153	151	219	187
C56	112	112	137	137	110	110	153	147	215	207
C57	110	110	137	137	110	110	151	131	219	199
C60	112	112	137	137	110	110	147	147	183	183
C61	110	110	137	137	110	110	151	145	213	191
C62	110	110	137	137	112	110	145	131	219	207
C63	112	110	137	137	110	110	151	147	215	203
C64	110	110	137	137	110	110	147	143	195	171
C66	110	110	137	137	110	110	147	131	197	175
C67	110	110	137	137	110	110	171	149	211	195
C68	112	110	137	137	112	110	155	149	215	211
C69	110	110	137	137	110	110	151	145	227	187
C70	110	110	137	137	110	110	155	147	205	183
C71	112	112	137	137	112	110	171	169	207	193
C73	110	110	137	135	112	110	151	147	195	171
C77	112	110	137	137	110	110	153	151	187	171
C80	110	110	137	137	110	110	145	143	209	191
C81	112	110	137	137	110	110	169	145	211	199
C83	112	110	137	137	110	110	151	147	211	171
C116	112	110	137	137	110	110	155	151	195	191
D1	110	110	137	137	110	110	151	151	203	179
D2	112	110	137	137	110	110	149	143	175	175
D3	110	110	137	137	110	110	147	147	211	205
D4	110	110	137	137	110	110	151	147	179	171
D5	110	110	137	137	110	110	155	145	219	197
D6	110	110	137	137	110	110	149	143	215	171
D7	110	110	137	137	112	110	149	145	211	179
D8	110	110	137	137	110	110	153	149	207	179
D9	112	110	137	137	110	110	147	147	219	175
D10	110	110	137	137	110	110	149	147	229	201
D11	110	110	137	137	110	110	153	151	179	171
D12	110	110	137	137	110	110	149	139	175	171
D13	110	110	137	137	110	110	149	147	175	175
D14	112	112	137	137	110	110	151	147	215	207
D15	110	110	137	137	110	110	151	147	205	171

Appendix 4.B cont.

ID	Bca1		Bca11		Hhi3		Sfi10		Smo4	
D16	110	110	137	137	110	110	159	147	203	199
D17	110	110	137	137	110	110	151	147	187	175
D18	110	110	137	137	110	110	151	149	175	171
D19	112	110	137	137	110	110	149	147	207	207
D20	110	110	143	137	110	110	153	145	207	207
D21	110	110	137	137	112	110	149	131	187	183
D22	110	110	137	137	110	110	149	149	215	191
D23	110	110	137	137	110	110	155	147	195	179
D24	110	110	143	137	110	110	151	149	197	155
D25	110	110	137	137	112	110	161	145	197	191
D26	110	110	137	137	110	110	147	147	215	205
D27	110	110	137	137	110	110	181	149	195	195
D28	110	110	137	137	110	110	173	167	211	171
D29	110	110	137	137	110	110	149	147	203	167
D30	110	110	137	137	110	110	149	147	207	175
D31	110	110	137	137	110	110	145	145	207	175
D32	110	110	137	137	110	110	157	149	219	207
D33	110	110	137	137	112	110	157	153	235	211
D34	110	110	137	137	110	110	147	147	215	205
D35	110	110	137	137	112	110	153	147	207	167
D36	110	110	137	137	112	110	149	145	175	175
D37	110	110	137	137	110	110	155	151	253	205
E9	110	110	137	137	110	110	169	147	175	175
E10	110	110	137	137	110	110	147	147	179	167
E13	110	110	137	137	110	110	147	147	215	211
E15	110	110	137	137	110	110	157	149	179	171
E18	110	110	137	137	110	110	151	147	175	175
E20	110	110	143	143	110	110	201	153	223	175
E25	110	110	137	137	110	110	149	147	179	171
E28	110	110	137	137	110	110	151	147	207	171
E33	110	110	137	137	110	110	151	149	175	175
E35	110	110	143	137	110	110	149	147	211	171
E75	110	110	137	137	110	110	161	147	179	167
E86	110	110	137	137	112	110	149	147	171	171
E95	110	110	143	137	110	110	153	147	213	179
E98	110	110	137	137	110	110	149	147	175	171
E104	110	110	137	137	110	110	151	147	175	171
E107	110	110	143	137	110	110	167	151	215	211
E107–2	110	110	143	137	110	110	151	147	207	179
E117	110	110	137	137	110	110	147	147	215	207

Appendix 4.B cont.

ID	Bca1		Bca11		Hhi3		Sfi10		Smo4	
E118	110	110	137	137	110	110	151	149	175	171
E123	110	110	137	137	110	110	169	149	179	175
E129	110	110	137	137	110	110	151	147	219	215
E137	110	110	137	137	110	110	151	149	203	167
E138	110	110	137	137	110	110	151	147	207	199
E139	110	110	137	137	110	110	153	149	211	179
E142	110	110	137	137	110	110	151	147	175	175
E148	110	110	137	137	110	110	157	149	215	215
E150	110	110	137	137	112	110	163	149	195	171
E153	112	110	137	137	110	110	145	133	217	171
E154	110	110	137	137	110	110	163	151	199	171
E162	112	110	137	137	110	110	169	149	211	207
E167	110	110	137	137	110	110	147	147	209	171
E175	110	110	137	137	110	110	149	147	211	195
E188	110	110	137	137	110	110	151	151	249	171
E191	110	110	137	137	112	110	149	149	203	175
E192	110	110	137	137	110	110	147	147	179	175
E195	110	110	137	137	110	110	149	147	219	211
E200	110	110	137	137	110	110	149	147	215	171
E202	110	110	137	137	112	110	147	147	219	179
F45	110	110	137	137	110	110	153	147	217	213
F47	112	110	137	137	110	110	175	147	211	199
F49	112	110	143	137	110	110	151	149	211	203
G33	112	110	137	137	110	110	147	145	179	175
G34	110	110	137	137	110	110	153	147	219	203
G37	112	110	137	137	110	110	151	147	197	171
G38	110	110	137	137	110	110	149	145	201	187
G39	112	110	143	137	110	110	153	153	215	171
G41	110	110	137	137	110	110	149	149	225	195
G42	112	110	137	137	110	110	153	149	211	187
G43	110	110	137	137	110	110	147	145	175	171
G49	110	110	137	137	110	110	157	131	207	205
G50	110	110	137	137	110	110	165	147	205	171
G51	112	110	137	137	110	110	147	133	219	215
G56	110	110	143	137	110	110	151	147	215	203
G57	110	110	137	137	110	110	151	147	215	171
G59	110	110	137	137	112	110	149	147	213	195
G60	110	110	143	137	110	110	149	149	203	175
G61	110	110	137	137	110	110	149	143	211	195
G66	110	110	137	137	110	110	149	147	227	179

Appendix 4.B cont.

ID	Bca1		Bca11		Hhi3		Sfi10		Smo4	
G68	112	110	143	137	112	110	147	143	207	175
G72	110	110	143	137	112	110	151	149	209	171
G73	110	110	137	137	110	110	147	131	211	209
G74	110	110	137	137	112	110	151	147	219	183
G75	110	110	137	137	110	110	149	147	213	187
G76	112	110	137	137	112	110	161	151	219	201
G77	110	110	137	137	110	110	149	131	227	205
G79	110	110	143	137	110	110	151	147	215	175
G80	110	110	137	137	110	110	149	149	211	187
G82	110	110	137	137	110	110	147	131	197	171
G85	112	110	137	137	112	112	163	133	195	179
H317	110	110	137	137	110	110	151	151	201	171
H321	112	110	137	137	110	110	149	147	207	187
H322	110	110	137	137	110	110	147	131	203	171
H325	110	108	147	137	110	110	215	149	195	187
H326	110	110	137	137	110	110	153	149	209	199
H327	110	108	137	137	110	110	153	151	207	205
H328	110	110	137	137	112	110	155	147	219	175
H329	110	110	139	137	110	110	151	151	223	171
H330	110	110	137	137	110	110	169	149	201	175
H333	110	110	137	137	110	110	163	157	207	203
H334	110	110	137	137	110	110	147	131	175	175
H335	110	110	137	137	110	110	149	147	187	171
H338	110	110	139	137	110	110	151	147	215	203
H352	110	110	137	137	110	110	149	147	207	191
H25052	110	110	137	137	112	110	151	149	211	171
H25064	110	110	137	137	110	110	153	153	171	171
H25067	112	110	137	137	110	110	149	145	199	155
H25069	112	110	143	137	110	110	147	147	183	167
H25071	110	110	137	137	110	110	151	149	175	171
H25094	110	110	143	137	110	110	161	143	227	211
H25095	110	110	137	137	110	110	151	143	207	195
H25097	110	110	137	137	110	110	149	145	211	175
H25269	110	110	137	137	112	110	151	151	195	175
H25276	110	108	137	137	112	110	151	147	241	175
H91488	110	110	139	137	110	110	149	149	203	171
H91574	110	110	139	137	110	110	147	143	207	207
H91575	110	110	137	137	110	110	149	141	229	159
H91579	110	110	137	137	110	110	167	149	219	167
H91697	110	110	137	137	110	110	149	143	209	207

Appendix 4.B cont.

ID	Bca1		Bca11		Hhi3		Sfi10		Smo4	
H91698	110	110	137	137	110	110	151	149	215	175
H91701	110	110	137	137	112	110	151	147	215	175
H91718	110	110	137	137	112	110	151	147	207	187
H91723	110	108	137	137	110	110	151	145	203	175
H91725	110	110	137	137	112	112	151	149	191	171
H91726	112	110	147	137	110	110	147	145	215	195
H91727	110	110	137	137	110	110	149	147	205	175
H91729	110	110	137	137	110	110	149	147	219	195
H91730	112	110	145	143	110	110	157	147	223	213
H91736	110	110	137	137	110	110	151	147	179	175
H91754	110	110	143	137	110	110	155	151	203	179
H91756	110	110	137	137	110	110	163	147	201	197
H91981	110	110	147	143	110	110	153	151	203	167
H95196	110	110	137	137	112	110	151	147	215	215
JAR210	112	110	139	137	110	110	–	–	217	187
H95967	110	110	143	137	110	110	149	149	183	175
H95969	112	110	137	137	110	110	155	149	175	171
H95970	110	110	137	137	110	110	175	149	219	175
H95972	112	110	137	137	110	110	149	147	211	189
H95974	110	110	143	137	110	110	161	141	191	187
H95975	110	110	137	137	110	110	149	131	203	195
H95976	110	110	137	137	112	110	151	151	223	193
H95987	110	110	137	137	110	110	149	147	195	179
H95995	110	110	137	137	110	110	149	147	195	167
T11	112	110	137	137	112	112	213	131	207	197
T12	110	110	137	137	112	112	151	147	219	201
T13	110	110	137	137	110	110	149	147	211	179
T14	112	110	137	137	112	110	153	147	215	167
T15	110	110	137	137	110	110	175	151	199	179
T16	112	110	137	137	112	110	149	147	187	179
T17	114	110	137	137	110	110	151	131	221	183
T18	110	110	137	137	110	110	211	151	197	171
T19	110	108	137	137	110	110	147	145	219	207
T20	112	110	137	137	110	110	151	149	207	207
TM01	110	110	137	137	112	112	149	147	203	197
TM02	110	108	137	137	110	110	149	147	225	207
TM03	110	110	137	137	110	110	151	147	209	195
TM04	110	110	137	137	112	110	147	147	219	207
TB01	110	110	143	137	110	110	153	147	215	179
TB02	112	110	137	137	110	110	149	145	207	171

Appendix 4.B cont.

ID	Bca1		Bca11		Hhi3		Sfi10		Smo4	
TB03	110	110	137	137	110	110	155	147	227	197
TB04	112	110	137	137	110	110	213	151	221	183
TF01	112	110	137	137	110	110	153	147	211	183
TF02	110	110	137	137	110	110	147	147	203	193
TF03	110	110	137	137	110	110	151	151	179	179
TF04	110	110	137	137	110	110	231	147	219	207
M801	110	110	137	137	110	110	147	147	207	191
M803	110	110	137	137	110	110	193	147	215	167
M804	110	110	137	137	110	110	151	149	191	183
M805	112	110	137	137	112	112	151	147	203	175
M806	110	110	137	137	110	110	155	151	211	179
M809	110	110	137	137	110	110	151	149	231	203
M810	110	110	137	137	110	110	149	149	211	187
M811	110	110	137	137	110	110	155	151	195	171
M812	110	110	137	137	110	110	147	147	211	201
M813	110	108	137	137	110	110	179	147	199	195
M814	110	110	137	137	110	110	181	151	211	183
M815	110	110	137	137	112	110	157	151	233	215
M816	110	110	137	137	112	110	159	147	199	167
M817	110	110	137	137	110	110	151	147	207	191
M808	110	110	137	137	110	110	151	149	231	155
MRP283	110	110	137	137	110	110	147	147	187	183
MRP284	110	110	139	137	110	110	149	147	205	175
MRP285	110	110	137	137	112	112	153	147	211	187
MRP286	110	108	137	137	110	110	147	141	215	195
MRP287	110	110	137	137	110	110	147	147	219	187
MRP288	110	110	139	137	110	110	151	147	211	207
MRP289	110	110	137	137	110	110	149	147	215	191
MRP290	112	110	137	137	110	110	155	147	215	191
MRP291	112	110	137	137	110	110	153	147	235	183
MRP292	110	110	139	139	110	110	143	135	207	175
MRP293	110	110	137	137	110	110	135	135	207	187
MRP294	110	110	137	137	110	110	147	145	219	215
MRP295	110	110	147	137	110	110	135	135	215	205
MRP296	–	–	137	137	110	110	155	147	175	175
MRP297	110	110	147	137	110	110	147	141	231	187
MRP298	110	110	137	137	110	110	147	131	191	187
MRP299	112	110	137	137	110	110	147	147	199	199
MRP300	112	110	137	137	110	110	147	145	187	187
MRP301	110	110	139	137	110	110	147	135	199	187

Appendix 4.B cont.

ID	Bca1		Bca11		Hhi3		Sfi10		Smo4	
MRP302	110	110	137	137	110	110	151	147	193	163
MRP303	110	110	139	139	110	110	145	145	207	193
MRP304	110	110	137	137	110	110	155	147	175	175
MRP305	110	110	137	137	110	110	147	147	193	179
MRP306	110	110	139	137	110	110	147	145	211	183
MRP307	112	110	147	139	110	110	147	141	183	183
MRP308	112	110	139	137	110	110	149	145	211	209
MRP309	110	110	137	137	110	110	147	141	187	183
MRP310	110	110	147	137	110	110	147	141	187	175
MRP311	110	110	137	137	110	110	155	135	193	193
MRP312	112	110	137	137	110	110	149	147	211	187
MRP313	110	108	137	137	110	110	147	145	211	207
MRP314	112	110	137	137	110	110	147	147	211	205
MRP315	110	110	139	137	110	110	149	147	215	191
MRP316	112	110	137	137	110	110	147	145	207	175
MRP317	110	110	137	137	110	110	147	145	219	205
MRP318	110	110	137	137	110	110	153	147	213	203
MRP320	112	110	137	137	110	110	149	147	187	183
MRP321	110	110	137	137	110	110	147	141	213	207
MRP322	110	110	137	137	110	110	147	147	215	175
MRP323	112	110	137	137	110	110	149	145	191	187
MRP324	110	108	139	137	110	110	149	143	187	155
MRP325	112	110	137	137	112	110	147	147	187	187
MRP326	112	110	137	137	110	110	147	147	187	179
DBI2	112	110	137	137	110	110	149	147	195	175
DBI3	110	110	137	137	110	110	149	147	187	183
DBI4	110	110	139	137	110	110	149	147	197	187
DBI5	110	110	139	137	110	110	149	147	213	213
PBAI935	112	110	145	145	110	110	151	149	219	183
UAM13336	110	110	139	137	110	110	149	147	197	187
UAM13721	110	108	137	137	110	110	147	147	233	211

Appendix 4.B cont.

ID	Smo7		Smo8		Smo10		Smo12	
KIG1308	203	203	119	119	137	137	110	110
KIG1321	207	203	119	119	139	137	106	104
KIG1331	203	203	119	119	143	137	117	108
KIG1333	203	203	119	123	137	133	110	104
KIG1336	207	203	119	119	137	137	111	104
KIG1339	203	197	119	125	139	137	104	104
KIG1340	203	203	115	119	137	127	106	104
KIG1341	207	203	119	123	141	137	104	104
KIG1342	207	203	119	119	137	133	115	106
KIG1343	203	203	115	119	141	131	111	104
KIG1344	203	203	115	119	141	133	117	104
KIG1346	203	197	115	119	137	135	113	104
KIG1347	203	203	119	123	141	137	106	104
KIG2253	203	203	119	119	145	127	110	108
KIG2254	203	197	115	119	139	137	104	104
KIG2255	203	203	119	119	137	127	106	104
KIG2261	207	203	115	121	137	137	106	104
KIG2262	203	203	119	119	137	127	111	106
KIG2264	207	207	115	119	137	137	106	106
KIG2265	203	197	119	119	141	141	117	112
KIG2266	203	203	117	119	135	133	109	106
KIG2267	203	197	119	115	141	117	106	106
KIG2271	203	197	115	119	153	137	105	104
KIG2278	203	203	119	119	137	131	117	110
KIG2279	203	197	115	119	137	127	108	104
KIG2280	203	203	119	119	141	139	104	104
KIG2283	203	203	119	123	133	129	106	105
KIG2285	203	203	115	119	137	137	117	113
KIG2293	207	203	119	119	133	131	114	108
KIG2296	203	203	115	119	133	131	117	104
KIG2298	203	197	119	119	137	135	105	105
KIG2299	203	203	115	119	141	137	106	104
KIG6334	203	203	121	123	137	137	108	108
KIG6915	207	203	115	115	137	127	107	104
KIG6927	207	203	119	119	137	137	104	104
KIG6944	203	203	119	123	139	137	110	110
KIG6999	203	203	115	119	139	127	106	104
BS76451	203	203	119	123	139	137	117	106
BS76452	207	203	115	119	141	137	108	108
BS76454	207	203	119	119	145	141	114	106

Appendix 4.B cont.

ID	Smo7		Smo8		Smo10		Smo12	
BS76455	203	197	115	119	137	131	110	106
BS76456	207	203	119	123	137	137	104	104
BS76457	207	197	115	123	127	127	104	104
BS76459	207	203	119	119	139	137	111	110
BS76460	207	203	115	115	141	127	106	104
BS76461	203	203	121	121	137	135	106	104
BS76462	203	197	119	119	137	137	108	104
BS76463	203	203	115	115	137	135	105	105
BS76464	203	203	119	121	137	135	104	104
BS76465	207	203	115	119	141	137	106	104
BS76466	207	207	115	119	127	127	104	104
BS76467	207	203	115	119	137	127	108	104
BS76469	207	197	119	119	139	137	106	106
BS76470	203	203	119	123	135	125	115	104
YK54775	203	203	115	119	151	145	111	104
YK57894	203	203	119	119	141	137	114	106
YK76112	207	203	115	119	141	139	111	104
YK76115	203	203	115	123	137	131	114	104
YK76117	207	203	119	123	141	137	110	104
YK76119	203	197	119	121	137	137	104	104
YK76120	203	203	119	123	139	127	110	104
YK76122	203	203	119	123	137	127	106	104
YK76124	203	203	115	121	137	137	108	106
YK76156	203	203	119	119	137	137	117	108
YK76157	203	203	119	119	139	127	115	108
YK76158	207	197	119	123	139	137	108	104
YK76161	203	203	119	121	145	143	104	104
YK76163	203	203	119	123	155	139	108	104
YK76164	203	203	119	123	139	137	104	104
YK76165	–	–	119	119	139	127	110	104
YK76167	203	203	119	123	137	127	115	104
YK76168	203	203	115	119	139	127	111	104
YK76169	207	203	119	119	139	137	104	100
YK76170	203	203	115	119	139	127	104	104
YK76171	203	203	119	123	141	137	104	104
YK76172	205	203	115	115	117	117	106	106
YK76173	203	197	119	119	143	137	106	104
YK76174	203	203	115	119	137	137	107	104
YK76177	203	203	115	119	143	127	110	104
YK76178	203	203	119	123	137	123	110	109

Appendix 4.B cont.

ID	Smo7		Smo8		Smo10		Smo12	
YK76179	203	197	115	115	145	139	104	104
YK76181	–	–	119	119	139	137	107	104
YK76182	207	203	115	123	137	137	106	104
YK76186	203	203	119	119	137	127	106	106
YK76187	207	203	119	119	147	127	108	104
YK76193	203	203	123	115	137	131	109	104
YK76672	207	203	123	119	141	131	104	104
YK54748	203	203	123	115	137	131	114	104
YK76151	207	203	119	115	137	137	109	104
YK76155	203	203	119	115	137	135	104	104
YK76264	203	203	119	119	137	133	109	106
YK76268	203	203	119	119	141	127	106	104
YK76271	203	197	119	115	137	135	114	105
YK76432	203	203	123	119	137	127	106	106
YK76123	203	203	119	119	139	137	114	104
YK76154	203	203	119	119	137	123	104	104
YK76159	203	203	123	119	145	131	114	114
YK76162	203	203	115	115	137	133	104	104
YK76166	203	203	117	115	137	137	117	104
YK76192	203	203	123	119	139	135	105	105
YK76194	203	203	119	115	141	135	105	104
YK76267	207	203	123	119	141	141	106	104
YK76316	207	203	119	119	143	135	104	104
YK76420	203	203	119	119	137	133	104	104
YK76421	207	203	119	115	139	127	106	104
YK76464	207	207	119	119	137	135	104	104
YK76425	207	203	123	115	137	127	115	110
YK76665	203	198	119	119	137	137	110	106
YK76851	203	197	119	119	137	135	111	111
YK76854	207	203	119	115	139	137	109	105
YK76865	203	203	119	119	137	137	104	104
YK77552	203	203	115	115	137	137	110	104
YK77560	203	203	119	119	139	137	106	104
YK77563	203	203	119	119	137	133	106	104
YK77565	207	203	123	119	141	133	110	106
YK77566	203	203	123	115	137	137	106	106
YK77569	207	203	119	119	131	127	104	104
YK77573	207	203	119	115	143	137	105	104
YK77576	203	197	119	115	137	131	110	104
YK77580	207	203	123	119	137	127	105	104

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ID	Smo7		Smo8		Smo10		Smo12	
YK77581	203	203	119	119	143	139	110	104
YK77582	203	197	119	119	139	137	104	104
YK77584	207	203	127	119	141	137	106	106
YK77585	203	197	123	123	139	139	110	104
COEI–M	207	203	119	115	137	133	–	–
DEAD–F	197	197	119	115	137	137	104	104
EUTH–M	203	197	115	115	137	127	104	104
KP1516	203	197	119	119	141	135	110	104
KP1517	203	198	119	115	141	137	104	104
KP1518	203	203	119	115	137	137	104	104
KP1519	203	203	119	115	137	137	114	104
KP4596	207	203	119	115	139	139	110	108
KP4597	207	203	119	119	141	137	110	106
KP9827	203	197	115	115	137	137	104	104
KP9828	203	203	119	115	135	135	104	104
KP9829	203	203	115	115	143	137	104	104
KP9830	203	197	119	115	137	137	104	104
KP9831	203	203	119	119	141	137	106	104
KP9832	203	197	119	119	137	137	106	104
KP9833	207	203	119	119	145	137	104	104
KP9835	203	203	119	115	131	127	110	106
KP9836	203	203	119	115	137	137	114	106
KP9837	203	203	119	119	139	137	112	104
KP9838	203	203	119	115	141	133	114	104
KP1502	207	203	121	115	141	137	106	104
KP1505	197	197	119	115	137	129	112	104
KP1508	207	203	119	119	131	127	108	104
KP1510	203	203	119	115	137	119	117	114
KP1512	203	203	121	119	139	135	110	106
KP1828	207	203	123	119	139	135	106	104
KP1829	207	203	115	115	139	139	106	106
KP1833	207	207	119	119	139	137	110	110
KP1835	207	207	115	115	137	137	104	104
KP1836	203	203	119	115	137	135	111	104
KP1840	203	203	123	115	139	137	108	104
KP4553	203	203	123	119	137	137	116	109
KP4617	203	203	123	119	137	135	111	104
KP9816	203	203	119	115	139	137	108	104
KP9818	207	203	119	119	137	137	114	104
KP9820	207	203	123	119	141	137	104	104

Appendix 4.B cont.

ID	Smo7		Smo8		Smo10		Smo12	
KP9823	207	203	119	119	137	127	114	108
KP9824	203	197	119	115	137	129	107	104
KP9825	207	203	119	115	137	131	108	104
DEAD–02	207	203	125	115	137	135	113	104
DEAD–03	203	203	119	119	141	137	112	106
CAMP1–1	203	203	119	115	141	139	108	106
NS27252	203	203	119	115	147	135	104	104
NS27253	203	198	119	119	137	131	106	106
NS27256	203	203	123	119	137	127	106	104
NS27260	207	203	119	115	137	137	105	104
NS27264	203	203	119	119	137	135	104	104
NS27269	203	203	119	119	141	137	110	104
NS27270	207	203	119	115	133	127	106	106
NS27271	203	203	119	119	141	133	110	108
NS27272	203	203	119	115	137	137	110	106
NS27273	203	203	123	115	137	133	114	111
NS27274	203	203	123	119	145	137	104	104
NS27275	207	203	119	119	141	137	106	104
NS27276	203	203	127	119	137	137	104	104
NS27277	203	203	119	119	137	131	108	104
NS27278	207	203	123	115	141	137	106	104
NS27279	203	203	119	119	137	137	114	110
NS27280	203	203	123	115	137	127	110	104
NS27281	207	198	119	115	137	131	104	104
NS27282	207	203	119	115	143	139	114	111
NS27284	203	203	119	115	141	141	110	104
NS27286	203	198	119	119	137	127	104	104
NS27291	203	203	119	119	139	127	117	111
NS27292	203	203	119	115	137	127	109	106
NS27293	207	203	117	115	137	129	106	104
NS272xx	203	203	119	115	137	127	117	111
NS27304	207	203	115	115	139	137	106	106
NS27305	203	203	119	115	137	131	110	104
NS27337	203	203	123	119	137	137	110	104
NS27338	203	203	115	115	137	135	104	104
NS27339	203	203	123	119	137	137	117	104
NS27340	207	203	123	115	139	137	104	104
NS27341	203	203	121	119	137	135	110	104
NS27342	203	203	119	119	137	133	104	104
NS27343	203	203	123	119	139	137	104	104

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ID	Smo7		Smo8		Smo10		Smo12	
NS27344	203	203	127	123	139	137	104	104
NS27345	203	203	119	119	137	127	104	104
NS27346	203	203	115	115	143	137	110	106
NS27347	203	203	119	119	137	135	111	104
NS24348	207	207	115	115	153	133	108	104
NS27349	203	203	119	115	141	137	108	104
NS27350	207	207	119	115	139	135	115	106
NS24351	207	203	119	119	135	135	111	110
NS27352	203	203	119	119	141	127	104	104
NS27354	203	203	121	119	133	131	117	104
NS27417	203	203	119	119	–	–	116	104
NS27418	207	203	119	115	137	135	117	110
NS27419	203	203	123	119	137	137	108	106
NS27420	207	203	115	115	137	133	106	104
NS27422	203	203	121	119	131	127	104	104
NS27423	207	207	119	119	139	137	114	104
NS27424	203	203	119	115	137	137	117	104
NS27425	203	203	119	115	141	139	117	104
NS52251	203	203	119	119	139	137	111	104
NS52252	203	203	123	119	137	133	114	104
NS52253	203	203	123	115	137	127	104	104
NS52254	203	203	123	119	153	137	106	106
NS52255	203	203	119	115	141	137	117	108
NS52256	207	197	119	119	139	133	117	110
NS52257	203	203	123	115	137	137	106	104
NS52258	203	203	119	119	135	129	110	107
NS52259	203	197	123	115	139	139	106	106
NS52260	203	203	123	119	141	127	104	104
NS52261	207	203	123	121	135	127	110	104
NS52262	198	197	119	115	141	137	109	106
NS52263	207	203	119	115	137	137	116	104
NS52264	203	203	123	119	137	127	106	106
NS52265	203	197	123	119	139	137	106	104
NS52266	207	203	119	119	137	137	104	104
NS52267	203	198	125	115	141	123	104	104
NS52268	203	203	121	119	141	135	107	107
NS52269	203	203	119	119	139	137	106	106
NS52270	203	197	119	115	139	137	110	106
NS52271	203	203	119	115	137	137	110	104
NS52272	203	203	123	119	157	131	105	104

Appendix 4.B cont.

ID	Smo7		Smo8		Smo10		Smo12	
NS52273	207	203	119	119	137	137	104	104
NS52274	203	203	119	115	137	133	108	106
NS52275	203	203	119	115	137	135	104	104
NS52276	207	203	119	119	137	135	106	104
NS52277	203	203	119	119	137	137	110	106
NS52278	203	203	119	119	137	127	104	104
NS52279	203	203	123	115	137	127	111	106
NS52280	203	203	119	115	149	135	108	105
NS52281	207	197	119	115	135	127	111	104
NS52282	203	203	123	115	143	129	104	104
NS52283	203	203	119	117	145	127	108	104
NS52284	203	203	119	119	137	137	104	104
NS52285	203	203	119	115	149	137	106	104
NS52286	203	197	119	119	137	135	104	104
NS52287	207	203	119	119	139	135	–	–
NS52288	203	203	123	119	137	137	110	106
NS52289	203	203	119	115	139	127	107	106
NS52290	203	203	119	115	143	141	104	104
NS52291	203	203	119	119	141	137	106	104
NS76453	203	203	127	119	137	137	104	104
NS76471	203	198	119	117	143	137	110	106
NS76472	203	198	115	115	139	137	115	106
JAR136	203	203	119	115	139	137	117	105
JAR144	207	203	123	119	139	127	104	104
JAR204	207	197	123	119	139	127	114	106
NS27321	203	197	119	119	137	127	117	104
NS27322	203	203	119	115	163	141	104	104
NS27323	207	198	119	115	137	135	117	104
NS27324	207	203	119	115	137	137	108	104
NS27325	203	203	119	119	137	127	104	104
NS27401	207	203	125	115	137	137	106	106
NS27402	207	203	119	119	141	137	117	104
NS27404	207	203	119	119	137	133	110	100
NS27405	203	203	119	119	141	137	111	106
NS27406	203	203	123	119	139	137	108	104
NS27407	207	203	123	119	145	137	104	104
NS27441	203	203	123	119	137	127	109	106
NS27442	203	203	123	119	139	137	117	117
NS27443	203	203	119	115	137	137	106	104
NS76478	207	203	123	119	137	131	104	104

Appendix 4.B cont.

ID	Smo7		Smo8		Smo10		Smo12	
NS76480	203	203	123	119	137	131	115	106
NS27251	203	203	119	119	139	137	106	106
NS76482	203	203	119	119	127	127	106	104
NS76483	203	197	119	119	137	137	–	–
NS76485	203	197	119	115	139	137	106	104
NS76487	207	203	123	119	137	137	115	104
NS76490	203	203	119	115	139	137	110	104
NS76491	207	203	119	119	141	131	107	104
NS76492	203	203	123	119	137	131	104	104
NS76493	207	203	123	121	137	133	110	110
NS76494	207	203	119	119	141	127	115	110
NS76495	203	203	125	119	139	137	108	106
NS76496	203	203	119	119	139	137	115	115
NS76497	207	197	123	121	137	131	104	104
NS76498	203	203	123	119	141	131	106	104
NS76499	203	203	119	119	141	129	104	104
NS76500	203	203	119	115	131	131	117	108
NS76551	203	197	123	119	139	139	111	107
NS76552	203	203	119	115	131	119	113	104
NS76553	203	203	123	115	139	137	114	113
NS76555	203	203	119	115	139	137	104	104
NS76556	207	197	119	119	141	139	107	104
NS76557	207	203	123	119	139	135	107	104
NS76558	207	203	119	119	139	137	104	104
NS76559	203	197	123	115	137	127	110	106
NS76560	203	197	119	119	137	137	106	104
NS82101	203	203	121	119	–	–	106	104
NS82102	203	203	119	119	143	137	111	111
NS82104	203	203	119	119	139	131	–	–
NS82106	203	203	123	115	137	133	115	104
NS82107	203	197	119	115	133	129	106	106
NS82109	203	203	123	119	137	135	104	104
NS82112	207	197	119	119	137	137	104	104
NS82117	203	203	119	119	137	133	106	104
NS82118	203	203	119	115	143	137	104	104
NS82119	203	203	115	115	141	137	106	104
NS82120	203	203	119	119	137	131	110	104
NS82121	203	203	119	115	137	135	104	104
NS82122	203	197	115	115	137	137	106	105
NS82123	207	203	119	119	157	141	117	104

Appendix 4.B cont.

ID	Smo7		Smo8		Smo10		Smo12	
NS82127	203	203	123	119	–	–	110	104
NS82129	207	197	119	119	141	139	108	104
NS82130	207	198	119	119	141	137	104	104
NS82133	–	–	119	119	141	137	106	104
NS82134	203	197	121	119	137	135	117	110
NS82135	203	203	123	115	139	139	110	106
NS82136	203	203	119	119	141	129	104	104
NS82137	203	197	123	123	133	127	117	110
NS82138	203	203	119	115	137	131	115	104
NS82141	203	203	123	119	137	133	117	104
NS82143	207	203	123	119	141	137	110	104
NS82144	203	203	123	119	137	133	106	104
NS82145	203	203	121	119	137	137	117	104
NS82150	207	203	119	115	137	135	116	104
NS82151	207	203	123	119	141	137	115	104
NS82152	197	197	119	115	139	139	107	104
NS82153	203	197	123	119	141	141	104	104
NS82156	203	203	123	115	135	123	106	104
NS82157	203	203	123	119	137	137	111	106
NS82158	203	203	119	119	137	137	106	104
NS82162	207	197	119	119	137	137	110	104
NS82163	203	203	119	115	139	139	104	104
NS82164	207	203	119	115	137	127	106	104
NS82165	203	203	119	115	127	127	114	106
NS82166	203	203	125	119	137	137	106	104
NS82167	203	197	119	119	137	133	106	104
NS82168	203	203	125	119	137	135	108	104
NS82142	203	203	119	115	137	137	111	106
NS82146	207	203	119	119	137	133	104	104
NS82204	207	203	121	119	133	131	110	104
NS82205	197	197	119	119	131	127	106	106
NS82207	207	207	115	115	141	139	106	104
NS82209	203	203	125	119	141	137	111	106
NS82211	207	207	119	119	135	131	107	104
NS82154	203	203	119	119	137	135	110	109
NS82221	207	203	119	119	141	137	117	115
NS82224	203	203	119	119	139	139	116	104
NS82225	207	207	119	115	137	131	117	104
NS82232	207	203	123	115	141	139	104	104
NS82160	203	203	119	119	137	135	111	104

Appendix 4.B cont.

ID	Smo7		Smo8		Smo10		Smo12	
NS82234	203	203	119	115	143	133	108	104
NS82235	203	203	123	123	137	137	104	104
NS27407	207	203	123	119	145	137	104	104
NS82237	205	203	123	119	137	129	111	104
NS82147	203	203	119	119	137	135	110	109
NS27421	203	203	123	115	139	137	117	106
NS27268	203	203	119	119	137	133	106	104
AK132	203	203	119	119	139	137	117	104
AK134	203	203	123	115	137	127	110	104
AK135	203	203	119	115	139	131	104	104
AK136	203	203	119	115	137	127	117	111
AK137	203	203	123	119	149	141	105	104
AK138	203	203	119	115	141	133	104	104
AK139	203	203	119	115	139	137	108	104
AK140	207	203	123	119	137	137	110	106
AK142	203	203	123	119	137	137	115	108
AK143	203	203	121	115	137	127	115	104
AK150	203	203	121	119	137	127	115	104
AK151	203	203	119	115	141	133	104	104
AK240	–	–	119	119	141	141	105	104
CA1–1	203	203	119	115	141	139	108	106
CA149	207	207	119	115	137	131	108	104
CA150	207	197	121	115	137	137	108	104
CA152	203	203	119	119	139	137	104	104
CA153	207	203	119	119	137	137	106	104
CA156	203	197	119	115	137	127	104	104
CA159	203	203	127	119	139	137	104	104
CA162	203	203	123	119	137	137	104	104
CH116	207	203	119	119	145	137	114	111
CH118	203	203	119	115	139	131	104	104
CH119	207	203	119	117	141	137	106	105
CH121	203	203	115	115	139	139	108	104
CH124	207	203	119	119	137	133	117	106
CH131	207	198	119	115	137	131	104	104
CH201	207	203	119	119	145	137	114	104
CH261	207	203	119	119	145	137	114	104
DU136	203	203	119	115	139	137	117	105
DU210	207	207	115	115	141	139	106	104
DU227	203	203	119	119	141	133	106	104
EG1	203	197	119	115	137	137	110	104

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ID	Smo7		Smo8		Smo10		Smo12	
EG10–2	207	203	125	119	139	137	110	110
EG2	203	203	123	115	137	137	117	104
EG2–2	–	–	–	–	–	–	109	104
EG3	–	–	119	119	163	141	104	104
EG3–2	207	203	119	119	137	133	104	104
EG4	207	203	119	119	139	135	116	104
EG5	207	203	123	119	137	137	110	104
EG7	–	–	123	115	137	137	110	104
EG9	203	203	123	123	137	133	105	104
EG9–2	203	203	115	115	135	123	106	104
LO001	207	203	123	119	143	135	110	104
LO002	203	203	123	119	137	123	110	106
LO003	207	203	123	121	139	137	107	104
LO004	207	203	119	115	141	137	104	104
LO008	203	203	119	115	137	137	106	104
LO009	203	203	121	119	137	135	110	106
LO010	203	203	123	115	137	137	104	104
LO011	203	203	119	119	137	131	115	104
LO012	203	203	123	119	135	131	110	106
LO014	203	203	115	115	141	139	110	104
LO017	203	203	119	115	137	135	117	104
LO018	203	203	123	119	137	127	114	107
LO019	203	203	119	119	163	161	104	104
LO020	203	197	119	115	139	133	104	104
LO021	203	203	119	119	139	137	104	104
LO023	203	203	119	115	139	127	114	104
LO141	203	203	123	119	137	127	104	104
LO033	203	203	123	123	137	137	110	105
LO035	203	203	123	115	137	123	110	106
ST024–2	203	203	123	119	141	121	104	104
ST024	–	–	119	119	141	137	105	104
MS224–2	–	–	123	119	137	135	117	104
MS226–2	203	203	119	119	137	137	104	104
MS227	203	203	119	115	139	131	110	104
MS230–5	203	203	119	119	137	115	114	104
MS231	203	203	119	115	143	141	106	104
MS233	203	203	119	115	137	135	117	110
MS235	203	203	119	119	137	137	106	105
MS262	203	203	119	115	139	131	110	104
MS303	213	203	123	121	139	137	117	104

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ID	Smo7		Smo8		Smo10		Smo12	
NS100	207	203	119	119	141	135	106	104
NS202	207	203	119	115	137	137	115	106
NS203–1	203	203	119	119	137	127	114	106
NS204	207	197	123	119	139	127	114	106
NS218	207	203	121	115	147	137	108	106
NS219	207	203	119	117	141	137	106	105
NS220	203	197	125	123	141	137	106	104
NS222	203	203	123	115	139	127	106	105
NS223	207	203	123	115	143	143	114	114
PT102	203	198	119	115	137	137	114	104
PT103	203	203	119	115	141	139	108	106
PT105	207	203	115	115	139	139	111	104
PT109	203	203	119	115	135	135	117	107
PT110	203	203	119	119	137	137	117	108
PT111	203	203	123	119	139	137	111	107
PT114	207	203	123	115	137	131	117	106
PT222	205	203	119	115	139	127	104	101
PT223	207	203	119	119	127	125	104	101
PT225	207	203	119	119	137	133	108	104
PT226	207	203	123	115	–	–	114	104
SP001	203	197	119	115	141	115	104	104
SP002	207	197	119	119	145	137	105	104
SP003	207	197	119	119	137	127	106	104
SP017–1	203	197	123	119	141	141	117	104
SP035	203	203	119	119	137	127	105	104
SP085	203	203	119	119	137	131	116	104
SP087	203	203	119	119	137	133	105	104
SP088	–	–	119	119	133	115	108	106
SP089	203	203	123	119	137	135	115	106
SP092	203	203	123	119	137	131	116	106
SP093	203	203	119	115	137	137	104	104
SP144–2	203	203	123	119	137	133	117	104
WA031	203	203	119	119	137	131	115	104
WA127	203	203	119	115	137	135	110	104
WA128	207	203	119	115	137	133	106	104
WA129	203	203	119	115	139	137	115	104
WA130	207	203	123	119	137	135	110	104
WA131	203	203	123	115	137	137	104	104
DX1028	207	203	119	119	137	131	119	104
DX2714	207	203	119	119	159	147	107	106

Appendix 4.B cont.

ID	Smo7		Smo8		Smo10		Smo12	
DX324119	203	203	119	119	149	131	106	106
DX3241	203	203	119	119	141	131	117	106
DX4333	207	207	119	119	141	137	106	106
DX4739	207	203	119	119	133	131	107	106
DX5559	207	203	119	115	139	133	110	106
DX7428	207	203	115	115	137	133	106	104
DX120319	207	203	119	119	133	133	110	106
DT12063	207	203	119	119	157	131	106	106
DX12218	207	203	119	115	135	131	106	106
DT35166	203	203	119	119	133	131	106	106
A510178	207	207	119	119	141	139	117	106
DX35013	207	203	119	115	139	131	110	110
A510588	203	203	115	115	133	131	117	106
A510658	203	203	119	119	133	133	106	106
A510678	207	203	119	109	131	131	106	106
A588097	207	207	119	119	–	–	106	105
A580197	207	203	119	119	141	131	106	106
A580297	203	203	119	115	157	131	106	106
A580397	207	203	119	119	145	131	106	104
A580497	207	203	119	119	135	133	106	106
A584297	207	203	119	115	145	135	107	106
A584393	203	203	119	119	153	131	110	106
A586897	–	–	119	119	137	131	118	106
A588597	207	203	119	119	157	131	106	106
A586797	203	203	119	115	147	133	107	106
BD#	203	203	119	119	137	131	120	107
BNB	207	207	119	119	137	131	106	106
B6	207	203	123	119	137	133	106	106
B33	207	203	119	119	141	141	106	106
B43	207	203	119	119	137	137	104	104
B44	207	203	121	119	145	143	106	104
B45	207	203	119	117	137	123	109	106
B47	207	203	125	119	151	137	106	104
B48	203	203	119	119	131	131	106	104
B49	207	203	121	115	133	123	106	106
B51	207	207	119	119	145	139	106	104
B52	207	203	119	115	143	123	106	104
B53	203	203	119	119	139	131	106	104
B99	207	207	125	119	141	131	106	106
B209	203	203	119	119	135	133	106	104

Appendix 4.B cont.

ID	Smo7		Smo8		Smo10		Smo12	
B244	203	203	125	121	153	137	106	104
B515	207	203	119	119	149	137	106	104
B609	207	207	119	119	145	137	106	104
B5170	203	203	119	119	135	133	106	106
B21627	203	203	119	119	131	123	106	106
B85408	207	203	121	119	151	147	107	106
B25423	207	207	119	119	131	131	106	106
B26170	203	203	119	119	153	133	106	104
B59510	203	203	125	121	135	135	107	106
B59513	207	207	125	119	141	133	109	106
B59516	203	203	119	119	133	123	106	104
B59519	203	203	119	119	143	133	106	104
B59521	207	203	125	119	155	139	107	104
B59524	207	207	123	119	131	123	106	106
B40194	203	203	125	119	159	135	106	106
B40622	207	203	119	119	143	131	106	106
B45509	207	203	121	119	133	123	106	106
B45320	207	203	119	119	145	123	108	106
B48222	207	203	125	119	143	131	106	106
B49008	203	203	125	119	131	131	106	106
B49028	207	203	125	119	147	123	106	106
B49055	207	203	119	115	151	143	106	104
B49071	203	203	125	121	131	131	106	104
B49073	203	203	119	119	177	131	107	106
B49583	207	203	125	119	137	131	107	106
C1	207	203	119	119	145	123	106	106
C10	207	203	125	119	141	123	106	106
C14	207	203	119	119	137	137	106	106
C15	203	203	119	115	147	141	107	104
C17	207	203	119	119	147	135	107	106
C20	203	203	121	119	137	137	106	104
C21	207	203	125	119	137	131	106	104
C24	207	207	119	115	157	133	106	104
C30	207	203	125	119	139	135	107	106
C32	203	203	129	115	169	133	107	106
C34	203	203	119	115	155	133	106	106
C37	203	203	119	119	165	145	106	106
C39	203	203	119	119	145	123	106	106
C42	203	203	119	119	133	123	106	104
C45	203	203	125	119	131	123	106	104

Appendix 4.B cont.

ID	Smo7		Smo8		Smo10		Smo12	
C46	207	207	119	119	139	131	107	106
C50	203	203	119	115	143	123	106	106
C52	203	203	119	119	151	137	109	106
C53	203	203	119	119	143	143	106	106
C54	207	207	121	115	133	133	106	104
C55	203	203	119	119	145	123	117	106
C56	203	203	119	119	143	137	106	104
C57	203	203	119	115	137	121	107	106
C60	207	203	125	119	161	143	106	106
C61	207	207	121	119	133	131	106	104
C62	203	203	121	119	143	139	107	106
C63	203	203	121	119	153	127	104	104
C64	203	203	121	119	133	133	106	106
C66	207	203	121	121	141	139	106	106
C67	203	203	121	119	137	137	109	106
C68	203	203	119	119	165	141	106	104
C69	207	203	119	119	133	123	106	106
C70	207	203	119	115	137	137	114	106
C71	203	203	125	115	137	137	107	106
C73	203	203	125	119	165	163	106	104
C77	207	203	119	119	159	125	106	106
C80	203	203	125	119	137	135	106	104
C81	207	207	121	119	137	131	106	106
C83	203	203	121	119	145	137	106	106
C116	203	203	119	119	153	131	109	106
D1	207	203	119	119	147	131	106	106
D2	203	203	119	115	147	129	106	106
D3	203	203	119	119	131	131	106	106
D4	207	203	119	115	137	137	106	106
D5	203	203	121	119	137	135	106	104
D6	207	203	119	115	131	131	106	104
D7	207	203	119	115	149	131	106	104
D8	207	203	121	119	131	131	106	104
D9	203	203	119	119	141	131	106	106
D10	207	203	119	115	133	123	106	104
D11	207	203	119	115	151	131	110	105
D12	207	203	119	119	135	123	106	106
D13	203	203	119	119	141	141	106	106
D14	203	203	119	119	131	127	106	106
D15	203	203	119	119	153	145	114	107

Appendix 4.B cont.

ID	Smo7		Smo8		Smo10		Smo12	
D16	203	203	119	115	133	131	106	104
D17	207	203	119	115	155	131	106	106
D18	207	203	119	115	131	131	105	104
D19	207	203	119	115	149	141	111	106
D20	203	203	119	115	145	131	106	106
D21	207	203	119	119	139	139	106	104
D22	207	203	119	119	149	123	107	104
D23	207	203	119	115	135	133	106	106
D24	207	203	119	119	141	133	107	106
D25	203	203	119	119	169	123	106	104
D26	207	203	119	119	147	131	107	106
D27	203	203	119	115	141	141	104	104
D28	207	203	119	115	143	131	106	106
D29	203	203	119	115	135	131	106	106
D30	207	203	119	115	137	135	106	104
D31	207	203	115	115	131	131	106	106
D32	207	207	119	115	141	135	106	106
D33	207	203	117	115	143	133	106	106
D34	207	203	119	119	147	131	107	106
D35	207	203	119	115	141	137	106	106
D36	207	203	119	119	149	125	106	106
D37	203	203	119	119	141	131	106	104
E9	203	203	119	119	149	133	106	104
E10	207	203	119	119	141	137	106	104
E13	203	203	115	115	131	131	106	106
E15	203	203	119	119	135	131	106	106
E18	207	207	119	115	145	131	106	104
E20	203	203	119	119	133	131	106	104
E25	207	207	119	119	151	131	107	106
E28	207	203	123	119	163	133	106	106
E33	207	203	119	119	133	131	117	106
E35	207	203	119	119	143	131	106	104
E75	203	203	119	119	139	133	106	106
E86	203	203	119	119	135	135	106	106
E95	203	203	119	119	139	135	106	106
E98	207	203	119	115	135	133	106	106
E104	203	203	119	115	137	131	106	106
E107	207	203	125	123	133	131	106	106
E107–2	203	203	119	119	143	139	106	106
E117	203	203	119	115	149	133	106	106

Appendix 4.B cont.

ID	Smo7		Smo8		Smo10		Smo12	
E118	203	203	119	119	135	131	107	106
E123	207	203	123	119	141	135	106	104
E129	207	207	119	119	145	133	106	106
E137	207	203	119	119	139	131	106	106
E138	207	203	125	119	131	131	106	106
E139	207	203	115	115	151	131	120	106
E142	207	203	119	115	137	133	106	106
E148	203	203	123	119	153	133	106	106
E150	207	203	119	119	151	133	107	106
E153	207	203	119	119	143	131	106	106
E154	207	203	119	119	137	131	106	105
E162	207	207	119	119	143	131	106	106
E167	207	203	119	119	137	131	106	106
E175	207	203	119	119	151	133	106	104
E188	207	203	119	119	131	131	106	104
E191	207	203	119	115	133	131	106	102
E192	207	203	121	119	147	131	106	106
E195	203	203	119	119	143	135	106	106
E200	203	203	119	119	137	135	107	106
E202	203	203	119	115	137	131	106	106
F45	207	203	123	115	149	131	106	106
F47	203	203	117	115	159	131	106	106
F49	207	203	119	119	131	131	106	106
G33	207	203	119	115	133	123	105	104
G34	203	203	125	119	137	131	106	104
G37	203	203	121	119	145	131	111	106
G38	207	207	119	119	155	135	105	104
G39	203	203	115	115	139	135	111	104
G41	207	203	119	115	131	123	106	106
G42	207	203	115	115	131	123	107	104
G43	207	203	125	119	137	133	106	104
G49	207	203	119	117	131	131	107	106
G50	207	203	119	119	139	137	107	106
G51	207	203	119	119	143	131	120	120
G56	203	203	119	117	147	135	106	106
G57	203	203	119	115	135	131	106	106
G59	207	203	125	119	135	131	105	104
G60	207	203	119	115	149	127	106	104
G61	203	203	119	119	133	131	106	104
G66	203	203	119	115	131	131	104	104

Appendix 4.B cont.

ID	Smo7		Smo8		Smo10		Smo12	
G68	207	203	119	119	131	131	120	104
G72	207	203	125	119	139	129	108	106
G73	207	203	119	119	151	131	106	104
G74	207	203	119	115	133	131	106	104
G75	207	203	119	119	133	123	104	104
G76	203	203	119	119	137	135	106	106
G77	207	203	119	119	143	131	108	106
G79	207	207	119	119	147	131	105	104
G80	203	203	119	119	131	131	117	104
G82	207	203	121	115	141	139	106	106
G85	203	203	119	119	149	131	106	106
H317	203	203	119	119	139	137	106	104
H321	207	203	119	119	145	137	107	105
H322	203	203	119	119	131	131	106	105
H325	207	203	115	115	155	135	105	104
H326	207	203	119	115	139	131	106	106
H327	207	203	119	119	141	133	106	106
H328	203	203	119	119	137	133	106	104
H329	207	207	119	119	165	135	106	104
H330	203	203	119	119	147	139	117	104
H333	207	207	119	115	151	131	109	106
H334	207	203	117	115	141	137	106	106
H335	203	203	119	119	135	135	106	105
H338	203	203	119	119	143	131	106	106
H352	203	203	119	119	137	133	109	106
H25052	203	203	119	115	137	131	106	104
H25064	207	207	119	119	135	135	104	104
H25067	203	198	119	115	139	133	106	104
H25069	203	203	125	119	145	143	106	105
H25071	207	203	119	119	137	131	106	106
H25094	207	207	119	117	139	131	105	104
H25095	207	203	119	115	143	131	106	105
H25097	207	203	121	115	137	131	106	105
H25269	207	203	119	115	135	131	107	107
H25276	207	203	115	115	131	131	106	106
H91488	207	207	119	119	141	137	105	105
H91574	203	203	119	115	133	131	106	106
H91575	198	197	119	119	135	131	106	106
H91579	203	203	119	115	141	123	105	104
H91697	207	203	119	115	137	123	106	106

Appendix 4.B cont.

ID	Smo7		Smo8		Smo10		Smo12	
H91698	207	203	119	115	133	127	106	104
H91701	207	203	119	119	133	131	106	105
H91718	207	203	121	119	131	127	106	105
H91723	203	203	119	119	137	131	107	106
H91725	203	203	119	115	145	135	107	106
H91726	207	203	119	119	145	135	106	106
H91727	207	203	119	119	149	135	105	104
H91729	203	203	119	115	131	131	106	105
H91730	205	203	115	115	145	141	110	106
H91736	203	203	119	119	135	131	118	105
H91754	203	203	119	119	137	131	106	106
H91756	207	203	119	117	147	141	106	105
H91981	203	203	119	119	131	131	109	106
H95196	207	203	125	119	135	123	106	104
JAR210	207	207	115	115	141	139	106	104
H95967	207	203	121	119	131	123	106	105
H95969	203	203	119	115	135	131	106	106
H95970	203	203	119	119	135	131	106	104
H95972	207	207	119	115	143	139	106	104
H95974	207	203	119	115	135	131	107	106
H95975	203	203	119	119	143	131	109	106
H95976	203	203	119	119	143	137	106	104
H95987	207	203	119	119	147	137	107	106
H95995	203	203	119	115	135	131	106	105
T11	207	207	119	111	159	137	107	106
T12	207	207	127	115	155	131	106	104
T13	207	203	119	115	137	123	107	107
T14	203	203	119	117	137	133	106	104
T15	207	203	119	115	149	137	106	104
T16	207	203	119	119	137	135	105	104
T17	207	207	125	119	141	131	106	106
T18	207	207	119	115	137	131	106	106
T19	203	203	119	119	147	143	117	104
T20	203	203	119	115	147	127	106	106
TM01	207	203	115	115	143	123	106	105
TM02	207	203	125	125	137	131	104	104
TM03	203	203	115	115	137	137	106	106
TM04	203	203	119	117	137	137	106	106
TB01	207	207	117	115	137	131	117	106
TB02	207	203	125	119	153	137	106	106

Appendix 4.B cont.

ID	Smo7		Smo8		Smo10		Smo12	
TB03	207	203	125	119	137	131	106	104
TB04	207	203	117	117	151	137	117	107
TF01	203	203	119	119	137	131	107	104
TF02	207	203	121	119	155	149	109	106
TF03	203	203	121	119	151	145	107	106
TF04	207	203	119	119	153	137	104	104
M801	207	203	119	119	143	131	117	105
M803	207	203	119	119	149	131	106	106
M804	203	203	119	115	139	135	106	104
M805	207	197	119	119	137	131	115	106
M806	207	207	119	119	143	131	106	105
M809	203	203	119	119	131	131	104	104
M810	207	203	125	115	141	139	106	105
M811	207	203	119	119	131	131	117	105
M812	203	203	119	119	143	135	106	104
M813	203	203	121	119	137	131	106	104
M814	203	203	125	115	149	141	104	104
M815	203	203	119	115	149	131	106	106
M816	207	207	121	119	133	127	110	104
M817	207	203	119	119	131	131	106	104
M808	203	203	123	119	147	119	105	105
MRP283	207	203	119	119	143	137	106	106
MRP284	203	203	119	119	137	135	111	106
MRP285	203	197	119	119	133	131	106	104
MRP286	207	203	119	115	137	133	111	104
MRP287	203	203	119	119	145	131	106	104
MRP288	203	203	119	115	143	139	104	104
MRP289	207	203	119	119	141	139	110	106
MRP290	203	197	115	115	137	131	106	104
MRP291	203	203	119	119	141	137	106	104
MRP292	207	203	119	119	139	133	106	104
MRP293	203	203	119	119	161	131	116	104
MRP294	203	203	119	115	137	137	104	104
MRP295	203	203	123	119	137	113	106	104
MRP296	203	197	123	119	137	133	106	104
MRP297	203	198	123	119	137	137	106	104
MRP298	203	203	119	119	137	137	110	106
MRP299	203	203	119	115	141	141	106	104
MRP300	203	203	119	119	137	133	106	104
MRP301	207	198	123	119	145	143	106	104

Appendix 4.B cont.

ID	Smo7		Smo8		Smo10		Smo12	
MRP302	207	203	123	119	163	137	104	104
MRP303	203	197	119	115	141	133	106	106
MRP304	203	203	119	119	141	131	106	104
MRP305	203	203	119	115	141	139	106	104
MRP306	203	197	119	119	145	131	104	104
MRP307	203	203	119	115	141	135	106	104
MRP308	203	198	123	119	127	113	106	104
MRP309	203	203	123	119	137	135	106	104
MRP310	207	203	119	119	141	137	106	104
MRP311	203	203	119	119	137	137	106	106
MRP312	203	203	119	119	137	127	106	104
MRP313	203	203	119	119	137	133	106	104
MRP314	203	203	119	119	141	129	117	106
MRP315	203	203	119	115	133	113	104	104
MRP316	203	198	119	119	133	133	106	106
MRP317	207	203	119	115	141	141	108	104
MRP318	203	203	119	119	137	131	106	104
MRP320	203	203	119	119	137	131	117	104
MRP321	203	203	119	119	151	137	106	104
MRP322	203	203	119	119	137	133	106	104
MRP323	203	197	123	119	151	137	106	104
MRP324	207	203	119	115	137	131	106	104
MRP325	207	203	119	119	133	131	104	104
MRP326	203	203	123	123	145	135	104	104
DBI2	203	203	123	115	141	137	106	104
DBI3	203	203	123	119	131	113	106	104
DBI4	203	203	119	119	133	133	106	104
DBI5	203	203	119	119	141	139	106	106
PBAI935	207	207	115	115	117	117	106	106
UAM13336	203	197	119	119	133	133	106	104
UAM13721	203	203	119	119	139	137	106	104

Appendix 4.C: Variable positions and frequency (*n*) of nuclear intron *lamin* A alleles reconstructed in PHASE in Common Eiders.

	7112599111111111111222 8995080115666777792 77 467507814595023	*n*
Allele01	GGACCCCCTGTACGCCGCCTGA	23
Allele02	GGACCCCCTGTACGTCGCCTGA	1
Allele03	GGACCCCCCGTACGCCGCCTGA	76
Allele04	GGACCCCCCGTACGCCGCCTAG	2
Allele05	GGACCCCCCGTACGCCGCCCGA	1
Allele06	GGACCCCCCGTACGCCGCTTGA	4
Allele07	GGACCCCCCGTACGCCACCTGA	6
Allele08	GGACCCCCCGTACGTCGTCTGA	5
Allele09	GGACCCCCCGTCCGCCGCCTGA	5
Allele10	GGACCCCCCGTCCGCCGCCCGA	2
Allele11	GGACCCCCCGTCCGCCGCTTGA	6
Allele12	GGACCCCCCGTCCGCCGCTTAG	1
Allele13	GGACCCCCCGTCCGCTGCCTGA	1
Allele14	GGACCCCCCGTCCGTCGTCTGA	1
Allele15	GGACCCCCCGTCCGTCGTCTAG	2
Allele16	GGACCCCCCGGACGCCGCCTGA	1
Allele17	GGACCCGCTGTACGCCGCCTGA	5
Allele18	GGACCCGCTGTACGCCGCCCGA	2
Allele19	GGACCCGCTGTCCGCCGCCTGA	9
Allele20	GGACCCGCCGTACGCCGCCTGA	30
Allele21	GGACCCGCCGTACGCCGCCCGA	1
Allele22	GGACCCGCCGTACGCCACCTGA	2
Allele23	GGACCCGCCGTACGTCGCCTGA	3
Allele24	GGACCCGCCGTCCGCCGCCTGA	207
Allele25	GGACCCGCCGTCCGCCGCCTAG	4
Allele26	GGACCCGCCGTCCGCCGCCCGA	177
Allele27	GGACCCGCCGTCCGCCGCCCAG	1
Allele28	GGACCCGCCGTCCGCCGCTTGA	29
Allele29	GGACCCGCCGTCCGCCGTCTGA	24
Allele30	GGACCCGCCGTCCGCCACCTGA	2
Allele31	GGACCCGCCGTCCGCTGCCTGA	4
Allele32	GGACCCGCCGTCCGCTGTCTGA	128
Allele33	GGACCCGCCGTCCGCTGTCTAG	5
Allele34	GGACCCGCCGTCCGTCGCCTGA	37
Allele35	GGACCCGCCGTCCGTCGCCTAG	1
Allele36	GGACCCGCCGTCCGTCGCCCGA	3
Allele37	GGACCCGCCGTCCGTCGTCTGA	195
Allele38	GGACCCGCCGTCCGTCGTCTAG	6
Allele39	GGACCCGCCGTCCGTCGTCCGA	1

Appendix 4.C cont.

	7112599111111111111222 8995080115666777792774675078145950 23	*n*
Allele40	GGACCCGCCGTCCGTCGTCCAG	1
Allele41	GGACCCGCCGTCCGTTGTCTGA	3
Allele42	GGACCCGCCGTCCACTGCCTGA	1
Allele43	GGACCCGCCGTCCACTGTCTGA	10
Allele44	GGACCCGCCGTCTGCCGCCCGA	1
Allele45	GGACCCGCCATCCGCCGCCTGA	11
Allele46	GGACCCGTCGTCCGCCGCCTGA	1
Allele47	GGACCCGTCGTCCGCCGCCCGA	4
Allele48	GGACCCGTCGTCCGCCGCTTGA	1
Allele49	GGACCGGCCGTACGCCGCCTGA	3
Allele50	GGACCGGCCGTCCGCCGCCTGA	11
Allele51	GGACCGGCCGTCCGCCGCCCGA	5
Allele52	GGACCGGCCGTCCGCTGTCTGA	3
Allele53	GGACCGGCCGTCCGTCGCCTGA	10
Allele54	GGACCGGCCGTCCGTCGTCTGA	3
Allele55	GGACCGGCCATCCGCCGCCTGA	1
Allele56	GGACTCCCCGTCCGCCGCCTGA	2
Allele57	GGACTCGCTGTCCGCCGCCTGA	3
Allele58	GGACTCGCCGTCCGCCGCCTGA	41
Allele59	GGACTCGCCGTCCGCCGCCCGA	1
Allele60	GGACTCGCCGTCCGCCGCTTGA	19
Allele61	GGATCCCCCGTACGCCGCCTGA	1
Allele62	GGATCCGCCGTCCGCCGCCTGA	1
Allele63	GAGCCCGCCGTCCGCCGCCCGA	1
Allele64	GAGCCCGCCGTCCGCTGTCTGA	1
Allele65	GAGCCCGCCATCCGCCGCCTGA	2
Allele66	CGACCCCCCGTACGCCGCCTGA	1
Allele67	CGACCCGCCGTCCGCCGCCTGA	13
Allele68	CGACCCGCCGTCCGCCGCCTAG	1
Allele69	CGACCCGCCGTCCGCCGCCCGA	6
Allele70	CGACCCGCCGTCCGTCGTCTGA	3

Appendix 4.D: Variable positions and frequency (*n*) of nuclear intron *gapdh* alleles reconstructed in PHASE in Common Eiders.

	12444811111111222233 62389312234467893557 56 4296565062228768	*n*
Allele01	CCCCGGCCAGCGAAGGCGAGAG	5
Allele02	CCCCGGCCAGCGAAGGCGAGGG	1
Allele03	CCCCGGCCAGCGAAGGCGGGAG	86
Allele04	CCCCGGCCAGCGAACACGGGAG	9
Allele05	CCCCGGCCAGCGGAGGCGAGAG	134
Allele06	CCCCGGCCAGCGGAGGCGGGAG	11
Allele07	CCCCGGCCAGCGGAGGTGAGAG	1
Allele08	CCCCGGCCAGCGGAGGTGGGAG	1
Allele09	CCCCGGCCAGCGGGGGCGAGAG	10
Allele10	CCCCGGCCAGCGGGGGTGAGAG	4
Allele11	CCCCGGCCAGTGAAGGCGAGAG	5
Allele12	CCCCGGCCAGTGAAGGCGGGAG	18
Allele13	CCCCGGCCAGTGAAGGCGGAAG	1
Allele14	CCCCGGCCAACGAAGGCGGGAG	1
Allele15	CCCCGGCCAACGGAGGCGAGAG	2
Allele16	CCCCGGCCAACGGAGGCGGGAG	5
Allele17	CCCCGGCCAACGGAGGCGGGAA	1
Allele18	CCCCGGCCAACGGGGGCGAGAG	82
Allele19	CCCCGGCCAACGGGGGCGGGAG	10
Allele20	CCCCGGCCAACGGGGGCGGAAG	1
Allele21	CCCCGGCCAACGGGGGCAAGAG	1
Allele22	CCCCGGCCGGCGAAGGCGAGAG	1
Allele23	CCCCGGCCGGCGGAGGCGAGAG	258
Allele24	CCCCGGCCGGCGGAGGCGGGAG	73
Allele25	CCCCGGCCGGCGGAGGCGGAAG	5
Allele26	CCCCGGCCGGCGGAGGTGAGAG	3
Allele27	CCCCGGCCGGCGGGGGCGGGAG	15
Allele28	CCCCGGCCGGCGGGGGCGGAAG	1
Allele29	CCCCGGCCGGCAGAGGCGAGAG	2
Allele30	CCCCGGCTAGCGGAGGCGAGAG	3
Allele31	CCCCGGCTAGCGGAGGTGAGAG	132
Allele32	CCCCGGCTAGCGGAGGTGAGGG	7
Allele33	CCCCGGCTAGCGGAGGTGGGAG	3
Allele34	CCCCGGCTAGCGGGGGTGAGAG	1
Allele35	CCCCGGGCAGCGGAGGCGAGAG	1
Allele36	CCCCGACTAGCGGAGGTGAGAG	1
Allele37	CCCCAGCCAGCGAACACGAGAG	5
Allele38	CCCCAGCCAGCGAACACGGGAG	18
Allele39	CCCTGGCCAGCGGAGGTGAGAG	1

Appendix 4.D cont.

	12444811111111222233 62389312234467893557 56 4296565062228768	*n*
Allele40	CCCTGGCCGGCGGAGGCGAGAG	3
Allele41	CCTCGGCCAGCGGGGGTGAGAG	1
Allele42	CCTCGGCTAGCGGAGGTGAGAG	2
Allele43	CTCCGGCCAGCGGAGGCGAGAG	3
Allele44	ACCCGGCCAGCGAAGGCGGGAG	1
Allele45	ACCCGGCCAGCGGAGGCGAGAG	8
Allele46	ACCCGGCCAACGGGGGCGAGAG	2
Allele47	ACCCGGCCGGCGGAGGCGAGAG	6
Allele48	ACCCGGCCGGCGGAGGCGGGAG	1

Appendix 4.E: Variable positions and frequency (*n*) for each mtDNA control region haplotype from Common Eiders (dots indicate missing data; – indicates deletion).

	3411223334456666777788111111333445555 12121562485789145906011167247685555 31562445385589	*n*
Hap01	....AACGCGGTCCCACCTAGTGCATCTTTCCGTCT	57
Hap02	....AACGCGGTCCCACCTAGTGCATCTTTCCGTTT	2
Hap03	....AACGCGGTCCCATCTAGTGCATCTTTCCGTCT	17
Hap04	....GACGCGATCCCACCCAGAGCATCTTTTCGTTT	109
Hap05	....GACGCGATCCCACCCAGAGCGTCTTTCCGTTT	14
Hap06	....GACGCGGTCCCACCCAGAGCATCTTTCCGTTT	7
Hap07	....GACGCGGTCCCGCCCAGAGCATCTTTCCGTCT	12
Hap08	....GACGCGGTCCCGCCCGGAACATTTTTCCGTCT	3
Hap09	....GACGCGGTCCCGCCCGGAACATTTTTCTGTCT	1
Hap10	....GACGCGGTCCCGCCCGGAGCATCCTTCCGTCT	3
Hap11	GTTTGACGCGGTCCCGCCCGGAGCATCTTTCCGTCT	27
Hap12	....AACACGGTCCCACCTAGTGCATCTTTCCGTCT	2
Hap13	....GACGCGGTCCCACCCAGAGCATCCTTCCGTCT	11
Hap14	....GACGCGGTCCCACCTAGAGCAGCTTTCCGTCT	1
Hap15	....GACGCGGTCCCGCCCGAAGCATCTTTCCGTCT	1
Hap16	....GGCGCGATCCCACCCAGAGCATCTTTTCGTTT	5
Hap17	GTTTGACGCGGTCCCGCCCGGAGCATTTTTCCGTCT	12
Hap18	GTTTGACGCGGTCCTACCCGGAGCATTTTTCCGTCT	2
Hap19	GTTTGACGCGGTCCTGCCCGGAGCATTTTTCCGTCT	25
Hap20	....AACGCAGTCCCACCTAGTGCATCTTTCCGTCT	10
Hap21	....GACGCGATCCCACCCAGAGCATCCTTTCGTTT	5
Hap22	....GACGCGATTCCACCCAGAGCGTCTTTCCGTTT	5
Hap23	....AACGCGATTCCACCCAGAGCGTCTTTCCGTTT	1
Hap24	....GGCGCGATCCCACCCAGAGCATCCTTTCGTTT	1
Hap25	ACTTGACGTGATCCCACCCAGAGCGTCTTTCCGTTT	21
Hap26	ATTTGACGTGATCCCACCCAGAGCGTCTTTCCGTTT	2
Hap27	ACTTGACGTGATCCCACCCAGAGTGTCTTTCCGTTT	1
Hap28	ACTTGACGTGATCCCACCCAGAGCATCTTTCCGTTT	1
Hap29	ACTTGACGCGATCCCACCCAGAGTATCTTTTCGTTT	10
Hap30	....GACGCGATCCCACCCAGAGCATCTTTTCATTT	5
Hap31	....GACGCAGTCCCACCCAGAGCATCTTTCCGTCT	3
Hap32	....GACGTGATCCCACCCAGAGTATCTTTTCGTTT	1
Hap33	ATTTGACACGATCCCACCCAGAGCATCTTTTCGTTC	1
Hap34	ACTTAACGCGATCCCACCCAGAGCGTCTTTCCGTTT	1
Hap35	....GACGCGATCCCACCCAGAGCATCTTTCCGTTT	5
Hap36	ACTTGATGCGATCCCACCCAGAGCATCTTTCCGTTT	1
Hap37	....AACACGGTCCCACCTAGTGCATCCTTCCATCT	2
Hap38	....GACGCGGTCCCACCCAGAGCATCTTTTCGTTT	3
Hap39	....AACGCGGTCCCACCTAGTGCATTTTTCCGTCT	1

Appendix 4.E cont.

	3411223334456666777788111111333445551212156248578914590601116724768555 31562445385589	*n*
Hap40	....GACACGATCCCACCCAGAGCATCTTTCCGTTT	2
Hap41	....GACGCGATCCCACCCAGAGCATCTTTTCGTCT	1
Hap42	....GACACGATCCCACCCAGAGCATCTTTTCGTTT	6
Hap43	ACTTGACGCGATCCCACCCAGAGCGTCTTCCCGGTT	1
Hap44	....GACGCGATCCCACCCAGAGCGTCTTCCCGTTT	4
Hap45	....AACGCGGTCCCACCCAGTGCATCTTTCCGTCT	1
Hap46	....GACGCGATCCCACCCAGAGCGTCTTTCCGTCT	1
Hap47	....GACGCGATTCCACCCAGAGCATCTTTCCGTTT	2
Hap48	ACTTGACGCGGTCCCACCCAGAGCATTCTTCCGTCT	1
Hap49	ACTTGACGCGGTCCCACCCAGAGCATCTTTCCGTCT	26
Hap50	ACTTGACGCGGTTCCACCCAGAGCATCTTTCCGTCT	2
Hap51	GCCTGACGCGGTCCCACCCAGAGCATCTTTCCGTCT	1
Hap52	ACTTGACGCGATCCCACCTAGAGCATCTTTCCGTTT	1
Hap53	ACCTGACGCGGTCCCACCCAGAGCATCTTTCCGTCT	1
Hap54	ACTTGACGCGGTCCCACCCAGAGTATCTTTCCGTCT	1
Hap55	ACTTGACGCGACCCCACCCAGAGCGTCTTTCCGTTT	3
Hap56	ACTTGACGCGGTCACACTCAGAGCATCTTTCCGTCT	1
Hap57	ACTTGACGCGATCCCACCCAGAGCATCTTTCCATTC	2
Hap58	ACTTGACGCGATCCCACCCAGAGCATCTCTTCGTTT	1
Hap59	ACTTGACGCGATCCCACCCGGAGCATCTTTTCGTTT	1
Hap60	ACTCGACGCG-TCCCACCCAGAGCATCTTTCCGTTT	1
Hap61	ACTTGACACGATCCCACCCAGAGCATCCTTTCGTTT	1
Hap62	ACTTGACGCGATCCCACCCAGAGCATCTTCCCGTTT	1
Hap63	ACTTGACGCGGTCCCACCTAGAGCATCTTTCCGTCT	1
Hap64	ACTTGGCGCGATCCCACCCAGAGCATCTTCTCGTTT	2

CHAPTER 5

DETECTION OF SEX-LINKAGE IN "AUTOSOMAL" MICROSATELLITE LOCUS AND ITS IMPLICATION ON AN ESTIMATOR OF POPULATION DIFFERENTIATION[1]

Summary — Ten currently listed autosomal microsatellite loci were developed for the common eider *(Somateria mollissima)*. However, within and among species genotyping of the *Smo*1 microsatellite locus revealed that no heterozygotes were present in females, and males had observed heterozygosities ranging from 0–83%. This pattern of heterozygotes only present in male individuals is consistent with sex-linkage on the *Z* - chromosome. Therefore, we conclude that microsatellite locus *Smo*1 is sex-linked and occurs on the *Z*-chromosome. We suggest that microsatellite loci be classified as "autosomal" only if pedigree information or sex-specific genotype pattern has explicitly disproved sex-linked inheritance. If improperly analyzed, sex-linked loci may bias estimators of genetic structure (F_{ST}).

[1]Sonsthagen, S.A., J.R. Gust, G.K. Sage, S.L. Talbot, R.Tiedemann, and K.G. McCracken. Detection of sex-linkage in "autosomal" microsatellite locus and its implication on an estimator of population differentiation. Prepared for submission to *Animal Conservation*.

Introduction

Ten polymorphic microsatellite loci were developed in the common eider (*Somateria mollissima*; Paulus & Tiedemann 2003). These were the first microsatellite loci developed for common eiders and have enabled biologists to explore additional avenues of research previously restricted by unavailability of polymorphic microsatellite loci. When reporting newly developed microsatellite loci, autosomal inheritance is sometimes assumed, unless explicitly disproved. However, when microsatellite loci (i) show only a few alleles and (ii) only single parent-offspring comparisons are evaluated, even a sex-linked locus might yield an inheritance pattern that is equally compatible with autosomal inheritance. While sex-linked loci enable researchers to ask questions about sex-biased dispersal (Scribner *et al.* 2001) and estimate paternity (Walker *et al.* 2005), incorrectly analyzing a sex-linked locus assumed to be autosomal may bias estimators of subdivision because of differences in rates of evolution, effective population sizes, and mode of inheritance among sex-linked and autosomal markers (Hedrick & Parker 1997; Wilson *et al.* 2002). Here, we investigate microsatellite locus (*Smo*1) of the common eider, previously assumed to be autosomal and assess the effect of analyzing a sex-linked locus as autosomal.

Methods

Blood, feather, or tissue samples were collected from black brant (*Branta bernicla nigricans*), Canada goose (*B. canadensis*), emperor goose (*Anser canagica*), greater white-fronted goose (*A. albifrons*), harlequin duck (*Histrionicus histrionicus*), long-tailed

duck (*Clangula hyemalis*), black scoter (*Melanitta nigra*), surf scoter (*M. perspicillata*), white-winged scoter (*M. fusca*), king eider (*S. spectabilis*), spectacled eider (*S. fischeri*), Steller's eider (*Polysticta stelleri*), and common eider during mark-recapture or monitoring efforts for various field studies. Samples were archived at the U. S. Geological Survey Molecular Ecology Laboratory, Anchorage, AK. Genomic DNAs were extracted using the "salting out" protocol of Medrano *et al.* (1990), with modifications described in Sonsthagen *et al.* (2004) or a QIAGEN DNeasy Tissue Kit (QIAGEN, Valencia, CA). Genomic DNA extractions were quantified using fluorometry and diluted to 50 ng/μL working solutions. The sex of each individual was determined in the field, and sexes were verified in the laboratory using the sex-linked locus *CHD* (Griffiths *et al.* 1998).

Three hundred seventy individuals including males (n = 3–54) and females (n = 2–95) from each of the 13 species were genotyped at *Smo*1 (Table 1). In addition, two populations of common eider (*S. m. v- nigrum*) were genotyped at 12 autosomal microsatellite loci; *Aph*08, *Aph*20, *Aph*23 (Maak *et al.*, 2003), *Bca*μ1, *Bca*μ11, *Hhi*μ3 (Buchholz *et al.* 1998), *Sfi*μ10 (Libants *et al.* unpubl. data), *Smo*4, *Smo*7, *Smo*08, *Smo*10, and *Smo*12 (Paulus & Tiedemann 2003). Microsatellite loci were amplified using the polymerase chain reaction (PCR) and fluorescently-labeled PCR products were electrophoresed following protocols described in Sonsthagen et al. (2004) for tailed primers and (*Aph*08, *Aph*20, *Aph*23, *Smo*4, *Smo*7, *Smo*08, *Smo*10, and *Smo*12), Pearce *et al.* (2005) for direct-labeled primers (*Bca*μ1, *Bca*μ11, *Hhi*μ3, and *Sfi*μ10). For quality

control purposes, 10% of the samples were re-amplified and genotyped for the 12 autosomal microsatellite loci in duplicate.

Microsatellite loci were tested for Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium in GENEPOP 3.1 (Raymond & Rousset 1995). Summary statistics, allelic frequencies, and expected and observed heterozygosities, also were calculated in GENEPOP. Pairwise F_{ST} and global F-statistics were calculated in FSTAT 2.9.3 (Goudet 1995; 2001) to assess population subdivision among sampled sites.

Results

In the cross-species comparison, no female heterozygotes were present and observed heterozygosities ranged from 0–83% in males (Table 1). Within common eider, three subspecies were screened, and the same pattern was observed as in the cross-species comparison, with no heterozygotes observed in females and 43–67% observed heterozygosity in males (Table 1). This lack of heterozygotes observed within females, across 13 species of waterfowl, is consistent with sex-linkage on the *Z*-chromosome.

To illustrate potential biases in estimates of genetic differentiation when analyzing sex-linked microsatellites with autosomal loci, we calculated pairwise F_{ST} values using 12 autosomal loci for two populations of common eiders *S. m. v-nigrum* (n_1 = 14, n_2 = 31) in FSTAT 2.9.3 (Goudet 1995; 2001). Both populations were composed of approximately equal numbers of males and females, and we repeated the analyses with and without including *Smo*1 and not assuming HWE. For common eiders, significant pairwise F_{ST} values increased from 0.039 to 0.092 with the addition of *Smo*1 (*Smo*1 F_{ST} =

0.600; F_{ST} = 0–0.229 for the other loci). When only female common eiders (n_1 = 7, n_2 = 13) were included, we observed a similar increase in significant pairwise F_{ST} values from 0.041 to 0.135 (*Smo*1 F_{ST} = 0.796; F_{ST} = 0–0.179 for the other loci). When only male common eiders (n_1 = 7, n_2 = 18) were analyzed, we observed an increase in pairwise F_{ST} values from 0.040 (not significant) to 0.062 (significant; *Smo*1 F_{ST} = 0.367; F_{ST} = 0–0.322 for the other loci). Therefore, *Smo*1 appears to be biasing estimates of genetic differentiation in common eiders.

Discussion

Paulus and Tiedemann (2003) detected only three alleles at locus *Smo*1. With so few alleles and a limited data set, a *Z*-specific locus can easily produce an inheritance pattern that does not conflict with allele frequencies predicted by Mendelian inheritance. For example, for a pair consisting of a hemizygous female with allele A and a heterozygous male with alleles AB, female offspring will be hemizygous A or B and male offspring will be homozygous AA or heterozygous AB. However, if a tested parent-offspring sample does not contain a female with genotype B, the data set does not conflict with Mendelian inheritance and females will be erroneously assumed to be homozygous AA.

Sex-linkage may be detected by testing for HWE because sex-linked loci are generally not in HWE when individuals from both sexes have been genotyped because females are scored as homozygotes. Depending upon the design and requirements of the study, the discovery of sex-linkage in a suite of microsatellite loci thought to be

autosomal may require the removal of these loci from population genetic analyses, or the need to replace the locus with an autosomal locus. Although sex-linked loci are interesting because of their relevance in assessing differences between sexes, the problem is that most software programs are unable to account for different types of heritability and, therefore, sex-linked loci need to be analyzed separately from autosomal loci.

In some cases, sex-linkage may not be immediately detected. For example, if studies only include samples from the homogametic sex (i.e. *ZZ* or *XX*), or only a few samples from the heterogametic sex (i.e. *WZ* or *XY*), such as aggregations of largely non-breeding subadult male birds and failed female breeders, loci occurring on the *Z* or *X* chromosomes may not be detected using simple tests for HWE because once samples from the heterogametic sex is removed, these loci are in HWE (e.g. Keller & Largiadér 2002; Thuman *et al.* 2002; Frentiu *et al.* 2003; Dawson *et al.* 2005).

Detection and use of sex-linked microsatellites has recently increased in frequency in literature (*e.g.* Jones *et al.* 1998; Lanctot *et al.* 1999; Stein *et al.* 2002; Beck *et al.* 2003; Gilbey *et al.* 2004; Yeung *et al.* 2004; Gautschi & Koller 2005; Li *et al.* 2005; McRae *et al.* 2005; Perry *et al.* 2005; Van't Hof *et al.* 2005). Though the value of sex-linked loci to detect sex-biased dispersal (Scribner *et al.* 2001) and estimate paternity (Walker *et al.* 2005) has been demonstrated, these markers may bias estimates of genetic differentiation for population genetic studies, as seen above, potentially due to differences in rates of evolution, effective population sizes, and mode of inheritance among sex-linked and autosomal markers if not analyzed appropriately (Hedrick & Parker 1997; Wilson *et al.* 2002). Proper identification of discrete populations,

management units, and evolutionary significant units has been a central concern for conservation geneticists (Moritz 1994; Crandall *et al.* 2000). However, these biases in estimates of genetic structuring could lead to the misidentification of population subunits. Additionally, if researchers do not know that loci are sex-linked, analyses that could have been run and inferences made are not done, resulting in the missed opportunities to ask specific questions about sex-biased dispersal. Due to these differences, potential biases in estimators of genetic structure (F_{ST}), and incorrect analytical methods, we suggest that researchers should classify microsatellite loci as "autosomal" only, if pedigree information or sex-specific genotype pattern have explicitly disproved a sex-linked inheritance.

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Table 5.1. Percent heterozygotes (het.) and homozygotes (hom.) observed in males and females across thirteen species of waterfowl genotyped at microsatellite locus *Smo*1.

Species	**Males**			**Females**		
	% Het.	% Hom.	*n*	% Het.	% Hom.	*n*
Black Brant	17	83	6	0	100	6
Canada Goose	50	50	6	0	100	6
Emperor Goose	17	83	6	0	100	6
Greater White-fronted Goose	33	67	6	0	100	6
Harlequin Duck	80	20	5	0	100	3
Long-tailed Duck	77	23	22	0	100	95
Black Scoter	0	100	6	0	100	6
Surf Scoter	43	57	7	0	100	2
White-winged Scoter	50	50	4	0	100	5
King Eider	83	17	6	0	100	6
Spectacled Eider	17	83	6	0	100	6
Steller's Eider	67	33	3	0	100	7
Common Eider						
– *S.m. v-nigrum*	43	57	54	0	100	47
– *S.m. mollissima*	67	33	6	0	100	6
– *S.m. borealis*	46	54	13	0	100	7
Overall Species	47	53	156	0	100	214

CONCLUSIONS

Discordance was observed at all marker types at varying spatial scales. Estimates of spatial genetic differentiation assayed from mtDNA, in general, were higher than estimates calculated using nuclear markers. Differences between marker types can be attributed to the breeding biology of Common Eiders, such that female eiders exhibit high natal and breeding philopatry, whereas males may disperse long distances between breeding seasons. Alternatively, comparatively higher levels of population subdivision observed at mtDNA relative to nuclear DNA could also be attributed to lineage sorting. MtDNA has a lower effective population size relative to nuclear DNA. Therefore, when mutation rate and selection are held constant, genetic drift has a larger effect on mtDNA than nuclear DNA (Avise 2004), translating in higher estimates of population subdivision (F_{ST}). The effects of lineage sorting and sex-biased differences in philopatry on spatial genetic subdivision are not mutually exclusive and both may be playing a role in the degree of population structure observed. Given differences in the degree of philopatry in Common Eiders between the sexes and congruence in results among loci, differences in estimates of population subdivision may be more attributable to male dispersal and high natal and breeding philopatry in females rather than incomplete lineage sorting.

Common Eiders nesting on the coastal barrier islands in the Beaufort Sea are nesting in closer proximity to more genetically related individuals, creating clusters of non-random associations among individuals. We cannot exclude the possibility that Common Eiders are nesting in close proximity to kin because of extreme natal philopatry rather than preferentially nesting close to more genetically related females. Long-term

demographic data coupled with molecular techniques are needed to determine if the pattern of fine-scale genetic structuring observed in Beaufort Sea Common Eiders is because of extreme natal philopatry or female kin association.

Spatial genetic structuring observed for Common Eiders breeding in the Beaufort Sea and YKD at such microgeographic scales is noteworthy, especially when compared to other arctic breeding waterfowl. Studies of King Eiders (*S. spectabilis*; Pearce et al. 2004) and Harlequin Ducks (*Histrionicus histrionicus*; Lanctot et al. 1999) did not detect significant levels of population genetic structure among sampled sites for microsatellite genotype or mtDNA sequence data. Steller's Eider (*Polysticta stelleri*) breeding in Alaska and Russia exhibited low levels of population differentiation among sites at microsatellite loci (Pearce et al. 2005). Higher levels of population genetic subdivision were observed among breeding populations of Spectacled Eiders (*S. fisheri*; Scribner et al. 2001) for mtDNA and Canada Geese (*Branta canadensis*; Scribner et al. 2003) for mtDNA and nuclear microsatellite loci. However, these studies were conducted at much larger spatial scales relative to our study.

Concordance of proposed glacial refugia utilized by Common Eiders with other arctic species indicates that arctic and subarctic refugia northwest and southeast of the ice-sheets, respectively, were important reservoirs of genetic diversity during the Pleistocene. Southern refugia appear to have served as the main source populations for postglacial colonization of Canada, southern Alaska, and Scandinavia as proposed for other vertebrates (Flagstad and Røed 2003, Scribner et al. 2003). Data suggest a stepwise postglacial colonization of North America and Scandinavia by Common Eiders with

some bouts of long distance dispersal. Restricted gene flow expanding out from proposed refugia is supported by the increase in genetic differentiation with distance (Kimura and Weiss 1964). In contrast to Common Eiders restricted to southern refugia, eiders residing in Beringia (and those on the North Slope of Alaska in particular) contributed little to colonizing deglaciated regions and remain genetically differentiated from Canadian and Scandinavian populations. Minimal colonization from the Beringian refugium is particularly evident in that geographically close populations (Aleutian Islands, Kent Peninsula, and YK Delta) share few haplotypes with North Slope Common Eiders and appear to be more genetically similar to central and eastern Canadian populations. Genetic discordance among populations residing in Beringia and other refugia has been maintained through evolutionary time despite contemporary gene flow among populations through male dispersal.

This study was the first, to our knowledge, to use nuclear introns in assessing inter-population variation in allelic frequencies at microgeographic scales. Introns pose new challenges to phylogenetic and population genetic analysis, such as, recombination impeding gene tree reconstruction (Hare 2001), and selective sweeps potentially confounding gene flow estimates (Storz et al. 2004). However, nuclear intron variation can provide valuable insight on historic processes influencing population demography. Advancements in analytical tools have enabled researchers to address issues of recombination (e.g., PHASE) and selective sweeps (DetSel, Vitalis et al. 2003; Fu's *Fs*, Tajima's *D* in ARLEQUIN) to use these types of markers for population genetic analyses.

These data provide further evidence for the need to use multiple marker types with different modes of inheritance to assess levels of spatial genetic subdivision at varying hierarchical scales. As seen in Common Eiders, nuclear and mtDNA markers show varying levels of genetic partitioning among breeding sites. High levels of natal and breeding philopatry observed in waterfowl do have predictable effects on population genetic structure, and these results confirm that genetic discordance can occur on relatively small spatial scales relative to known dispersal distances and capabilities. Researchers characterizing populations using genetic techniques could under- or over-estimate the degree of population genetic differentiation if estimates are based on a single marker type. Therefore, not utilizing molecular markers with varying modes of inheritance could mislead researchers characterizing genetic variation within populations.

Finally, microsatellite locus *Smo*1 is sex-linked and occurs on the *Z* chromosome. If sex-linkage is not detected and analyzed with autosomal loci, biases in estimates of genetic differentiation for population genetic studies may occur, potentially due to differences in rates of evolution, effective population sizes, and mode of inheritance among sex-linked and autosomal markers (Hedrick & Parker 1997; Wilson *et al.* 2002). Because of these differences, potential biases in estimators of genetic structure (F_{ST}), and incorrect analytical methods, we suggest that researchers should classify microsatellite loci as "autosomal" only, if pedigree information or sex-specific genotype pattern have explicitly disproved a sex-linked inheritance.

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