Animal Conservation

Animal Conservation. Print ISSN 1367-9430

Competing hypotheses for the etiology of cryptorchidism in Sitka black-tailed deer: an evaluation of evolutionary alternatives

E. K. Latch[1], R. P. Amann[2], J. P. Jacobson[3] & O. E. Rhodes, Jr.[1]

1 Department of Forestry and Natural Resources, Purdue University, West Lafayette, IN, USA
2 Animal Reproduction and Biotechnology Laboratory, Colorado State University, Fort Collins, CO, USA
3 Arctic Rivers Guide & Booking Service, Kodiak, AK, USA

Keywords
black-tailed deer; cryptorchidism; environmental contaminant; genetic drift; inbreeding; Kodiak Island; *Odocoileus hemionus*; Prince of Wales Island.

Correspondence
Emily Latch. Current address: Smithsonian's National Zoological Park, Genetics Laboratory, MRC 5513, 3001 Connecticut Ave., NW, Washington, DC 20008, USA. Tel: 202 633 4113; Fax: 202 673 4648
Email: latche@si.edu

Received 26 November 2007; accepted 18 February 2008

doi:10.1111/j.1469-1795.2008.00174.x

Abstract

On the Aliulik Peninsula (AP) of Kodiak Island, Alaska, 70% of male Sitka black-tailed deer (SBTD; *Odocoileus hemionus sitkensis*) are bilaterally cryptorchid (both testes fail to descend; male is sterile). Both genetic and environmental factors have been proposed as possible causes of this problem. We investigated the possibility that population genetic processes (isolation, inbreeding and genetic drift) have contributed to an increased frequency of cryptorchidism in this population. Overall, SBTD on major islands throughout Alaska have unusually low levels of genetic diversity, though we identified a likely glacial refugium on Prince of Wales Island in the Alexander Archipelago. Within the Kodiak Archipelago, deer on the AP did not exhibit the patterns of genetic isolation, inbreeding and drift that would be expected if cryptorchidism in this population was the result of a founder mutation(s). Instead, our data favor exposure to environmental contaminants as a likely alternative mechanism causing high prevalence of cryptorchidism on the AP.

Introduction

Sitka black-tailed deer (SBTD) *Odocoileus hemionus sitkensis* living on the Kodiak Archipelago, in Alaska, USA, apparently originated from 25 animals distributed among three transplants from the Alexander Archipelago in south-east Alaska. Known transplants were to Long Island, just east of Kodiak Island; 14 deer from Baranof Island in 1924 and two deer from Prince of Wales Island in 1930. Nine deer from Kupreanof Island were released on the north end of Kodiak Island in 1934 (Smith, 1979; Van Daele, 2005; Fig. 1). Eventually SBTD migrated to nearby Afognak, Uganik, Raspberry and other islands and dispersed throughout the Kodiak Archipelago (Smith, 1979). However, SBTD apparently were not present on the southern end of Kodiak Island until the 1970s, becoming established on the Aliulik Peninsula (AP) in the early 1980s (L. J. Van Daele, pers. comm.). In 2004, an estimated 60 000 SBTD inhabited the Archipelago (Van Daele, 2005), with 2400 of these occupying the AP based on recent hunting data.

Over the last few years, there has been an increase in the number of reports describing SBTD with abnormal antlers and malformed genitalia on the Kodiak Archipelago. Reports of abnormal males were heavily concentrated at the southern end of Kodiak Island (Van Daele, 2001), and Bubenik *et al.* (2001) confirmed the presence of cryptorchidism (one or both testes not in scrotum) in males on the AP. Detailed analysis of a sample of harvested SBTD (Veeramachaneni, Amann & Jacobson, 2006) revealed extremely high rates of cryptorchidism in AP SBTD (76% of 134 males) as compared with other portions of the Archipelago (12% of 65 males; data include the 2005 hunting season).

Prevalence of cryptorchidism on the AP is in marked contrast to levels reported for presumably non-inbred populations of other vertebrates, which typically are $<5\%$ (Amann & Veeramachaneni, 2007). When high levels of cryptorchidism have been described, they generally have been centered on demonstrably inbred populations [e.g. up to 90% of male Florida panthers *Puma concolor coryi* (Roelke, Martenson & O'Brien, 1993)] or localized hotspots of chemical discharge. Further, among cryptorchid males, a unilateral phenotype (only one undescended testis) characterizes $>75\%$ of affected males for common mammals

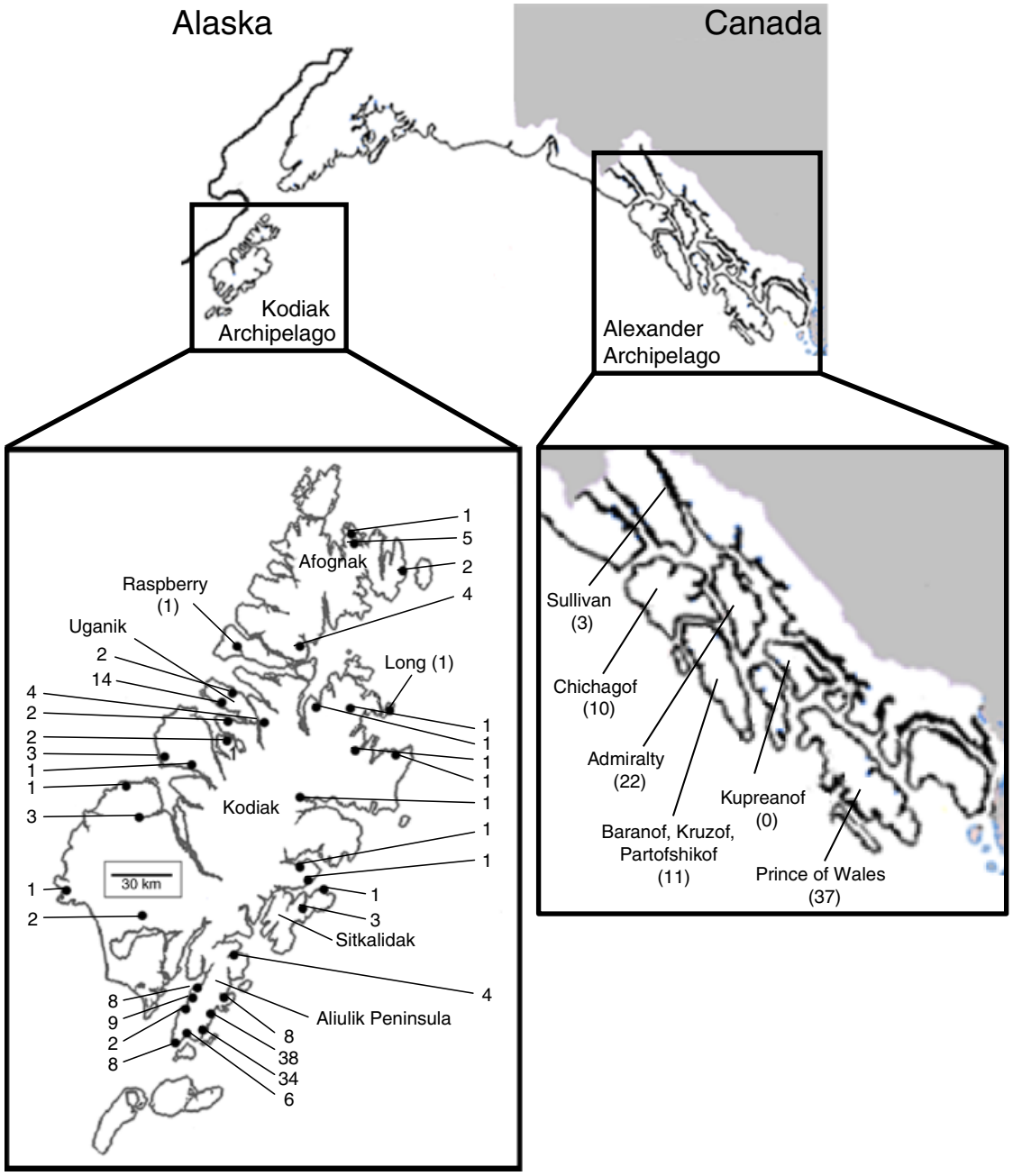

Figure 1 Geographic distribution of samples collected from Sitka black-tailed deer *Odocoileus hemionus sitkensis* in Alaska. For the Alexander Archipelago (right insert), samples were grouped by island of harvest. For the Kodiak Archipelago (left insert), specific harvest locations were within ~5 km of designated landing site. Number of samples per island or harvest location is shown. The Aliulik Peninsula is designated.

(Amann & Veeramachaneni, 2007). In SBTD on the AP, only 7% of cryptorchid males exhibit the unilateral phenotype (the other 93% are bilaterally cryptorchid and thus reproductively sterile; Veeramachaneni *et al.*, 2006). Cryptorchid SBTD on the AP also are unique in that many cryptorchid deer have abnormal antlers (i.e. sharp tips, atypical shape, retention of velvet; Bubenik *et al.*, 2001; Veeramachaneni *et al.*, 2006). Although cryptorchidism (e.g. Leader-Williams, 1979; Hoy *et al.*, 2002) and antler deformities (Robinette & Jones, 1959; Hicks & Rachlow, 2006) have been reported for several cervid species, co-occurrence of these two defects is anomalous.

Wildlife biologists in Alaska assumed the problem of cryptorchid SBTD on the AP was due to inbreeding (Lewis, 1991). Here, we refer to inbreeding as that process which occurs purely because of restricted population size and

resulting genetic drift (Keller & Waller, 2002). Indeed, inbreeding was identified as the ultimate cause of cryptorchidism (as well as a suite of other morphological deformities) in the Florida panther (Roelke *et al.*, 1993). A multi-gene recessive basis for cryptorchidism has been suggested (e.g. in dogs; Nielen, Janss & Knol, 2001), which presupposes at least one abnormal originator. The AP likely was colonized by a few founders. Thus a founder effect involving a mutation(s) early during establishment, which caused unilateral cryptorchidism in males (bilateral cryptorchids are sterile), followed by generations of isolation from other SBTD populations and genetic drift could have resulted in a very high frequency of the mutation(s) in this population. Rather than a classic mutation, the problem could result from an epigenetic modification of gene expression, possibly induced by an environmental agent and transmitted via the germ cells (Anway *et al.*, 2005; Crews & McLachlan, 2006; Gore, Heindel & Zoeller, 2006). With either initial mechanism, it is theoretically possible that such a scenario might rapidly result in the observed incidence of cryptorchidism. Signatures of such a scenario would include genetic similarity and high levels of relatedness among individual deer on the AP, plus evidence of isolation and genetic drift that likely would have resulted in a divergence in gene frequencies of AP SBTD relative to those of SBTD elsewhere on the Kodiak Archipelago.

Alternatively, the developmental anomalies observed in SBTD on the AP could be caused by localized exposure to an unidentified environmental agent. Countless man-made compounds have been introduced into the environment, many of which are known to alter fetal development, including testis descent (Gray, 1998; Gray *et al.*, 2001; Vidaeff & Sever, 2005; Gore *et al.*, 2006; Sharpe, 2006). In SBTD on the AP, exposure to environmental contaminants has been suggested as a single factor that could lead to the multiple, co-occurring abnormalities observed (Veeramachaneni *et al.*, 2006). Veeramachaneni *et al.* (2006) also downplayed genetics as a possible explanation, but did not have data at hand to adequately address the issue.

Our primary goal was to test the hypothesis that there is a genetic signature of genetic isolation, inbreeding and drift in the SBTD population on the AP that is associated with the incidence of cryptorchidism. We compared genetic diversity at neutral markers representing the nuclear and mitochondrial genome between SBTD on the AP and those found throughout the remainder of the Kodiak Archipelago, as well as between 'populations' of SBTD from the AP demonstrating cryptorchidism and those not displaying the condition. Similarities in genetic characteristics among these populations would refute the hypothesis that population genetic processes are maintaining a high prevalence of cryptorchidism in SBTD from the AP, a necessary step toward uncovering the etiology of the condition. A secondary goal was to incorporate a more comprehensive characterization of the evolutionary trajectory of Kodiak Island deer in relation to its original source, the Alexander Archipelago. This is of particular concern given the very small number of individuals used to found the Kodiak Archipelago SBTD population.

Methods

Sample collection

Tissue ($n = 196$) or hair ($n = 72$) samples were collected from 270 legally harvested SBTD at known locations in Alaska (Fig. 1) from 1999 to 2005. Hair samples were placed into individual envelopes, or small pieces of tissue were placed into cryovials containing desiccant beads. As soon as practical, samples were stored at $-20\,^{\circ}C$. For males harvested on the Kodiak Archipelago, locations of the testes in the body were recorded. Testis location was unknown for males harvested in the Alexander Archipelago.

Laboratory methods

We extracted DNA from all samples using a modified ammonium acetate protocol, then electrophoresed the DNA through an agarose gel to assess quality and quantity and diluted the DNA to $\sim 10\,ng\,\mu L^{-1}$ in TLE buffer (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA). We amplified 11 nuclear DNA (nDNA) microsatellite loci for each sample in 10 μL multiplex reactions using the primers and conditions outlined in Table 1. Thermocycler conditions were: a 2-min initial denaturation step at 94 °C; 30 cycles of 30 s at 94 °C, 30 s at the annealing temperature (Table 1) and 2 min at 72 °C; followed by a 60 °C soak for 45 min. Allele sizes were determined by electrophoresis in an ABI 377 DNA sequencer following the manufacturer's instructions (ABI, Foster City, CA, USA). Ten duplicate hair samples were included in the sample set, revealed to the laboratory technician only after data collection was complete. Duplicate multilocus genotypes obtained for these 10 samples were identical. We assessed overall genotyping repeatability by re-amplifying and re-genotyping ~11% of the genotypes (when samples with an incomplete genotype were re-amplified in a multiplex reaction to obtain the complete multilocus genotype). We observed only two instances of mismatches between an original and a verification genotype, both of which were attributed to allelic dropout in hair samples (at different loci) and were corrected. We culled six samples from the dataset, because quality of the DNA precluded obtaining more than five reliable genotypes. Three loci (Odh_C, Odh_G, Odh_K) were found to be monomorphic in all populations and were excluded from calculations. Thus, our final microsatellite dataset included 254 SBTD genotyped at eight polymorphic loci. The percentage of genotypes missing from the final dataset was 2% (40 missing from 2032 total genotypes).

We amplified a 730 bp product of the mitochondrial DNA (mtDNA) control region for all samples using the primers Odh-dloopF 5′-GAGCAACCAATCTCCCT GAG-3′ and Odh-dloopR 5′-GTGTGAGCATGGGCT GATTA-3′. Ten microliter amplification reactions contained 10 ng genomic DNA, 5 pmol each primer, 0.2 mM

Table 1 Locus-specific primers and reaction conditions for microsatellite analysis

Multiplex	Locus	GenBank #	Primer sequence	Primer (pmol)
A	Odh_E	AF102251[a]	F: CAGGGCAGTGTATCAGTGAGG	1
			R: CCAGCCTTCCTGGACTAGAG	
	Odh_K	AF102242[a]	F: GCAGGAAGGAGGAGACAGTA	2
			R: GCTGGTTCGTTATCATTTAGC	
	Odh_C	AF102250[a]	F: TTCATCCATCTTGCCATTCA	7
			R: TTGGAGGCATGTAGGAAAGG	
	BM848	G18477[b]	F: TGGTTGGAAGGAAAACTTGG	8
			R: CCTCTGCTCCTCAAGACAC	
B	C273	AF102246[c]	F: AGGGAAACCTCTGTTCAGGA	5
			R: ACCAAGCAAAATGCCTTACA	
	Odh_P	AF102240[a]	F: TTTCACTGTTTTCTCCTTCAGA	5
			R: TGCCCAATCAGATGTTGTAG	
	Odh_N	AF102244[a]	F: GCAACCAATAGGATAGGTCG	5
			R: GCTGGATGGAACTGAAAGTC	
	Odh_G	AF102253[a]	F: ACCCTATGGTCACAGCAACA	6
			R: CTCTGGGCATCCATGAAGTAG	
C	Odh_O	AF102245[a]	F: ACGAGGTTCAGTGGTTCC	8
			R: CAGGGCATAGTTTCCAAA	
	RT24	U90746[d]	F: TGTATCCATCTGGAAGATTTCAG	7
			R: CAGTTTAACCAGTCCTCTGTG	
	T40	AY514438[c]	F: GTTGGGCTTATGAATCAGG	5
			R: GCACATCTTTGGACCTCTC	

Annealing temperatures were 60 °C for multiplex A, 59 °C for multiplex B, and 58 °C for multiplex C. For each locus, concentrations of forward and reverse primers were the same. The 10 μL PCRs contained 10 ng genomic DNA, 1–8 pmol each primer, 0.2 mM dNTP, and 0.75 U of Taq DNA polymerase in 2 × buffer (20 mM KCl, 20 mM $(NH_4)_2SO_4$, 40 mM Tris-HCl, 4 mM $MgSO_4$, 0.2% Triton X-100).

[a]Originally described in mule deer *Odocoileus hemionus* in Jones, Levine & Banks (2000).

[b]Originally described in cattle *Bos taurus* in Bishop *et al.* (1994).

[c]Originally described in elk *Cervus elaphus* in Meredith *et al.* (2005).

[d]Originally described in caribou *Rangifer tarandus* in Wilson *et al.* (1997).

each dNTP and 0.75 U of Taq DNA polymerase in 1 × reaction buffer. Thermocycler conditions were: 2-min initial denaturation step at 94 °C; 30 cycles of 30 s at 94 °C, 30 s at 61 °C and 30 s at 72 °C; followed by final extension at 72 °C for 5 min. Reactions were cleaned using a low sodium precipitation protocol (Latch & Rhodes, 2005). Approximately 50 ng of cleaned PCR product was used in a 10 μL sequencing reaction containing 3 pmol of either forward or reverse primer and 0.5 μL Big Dye version 3.1 in 1 × ABI buffer. Thermocycler conditions followed the manufacturer's recommendations, except that 60 cycles were used to strengthen sequence signal. Sequenced products were cleaned as above, and were electrophoresed through capillaries in an ABI 3730 DNA sequencer. Sequences were compiled and edited using Sequencher 4.1. We sequenced 20% of the dataset (including at least one individual of each haplotype) in both the forward and reverse directions to verify the accuracy of the sequences. Additionally, the 10 blind duplicate hair samples yielded identical sequences. We performed BLAST searches for each unique sequence obtained from SBTD, and all matched most closely to other reported *Odocoileus hemionus* sequences. We culled eight samples from the dataset for which we could not obtain reliable sequence data, and trimmed all sequences to the same length, yielding 582 bases of mtDNA sequence from 252 SBTD. A representative sequence for each unique haplotype was deposited in GenBank (accession numbers EF672488–EF672501).

Statistical methods

Genetic diversity and structure of SBTD in Alaska

For the entire dataset of Alaska SBTD and for each subpopulation (Alexander Archipelago and Kodiak Archipelago), we tested for a deficiency of heterozygotes relative to Hardy–Weinberg expectations for each locus and globally using F_{IS} as estimated in SPAGEDI (version 1.2 d; Hardy & Vekemans, 2002), determining significance using a permutation test (two-sided test; 20 000 permutations of genotypes among individuals). Owing to the large number of comparisons involved in locus-specific tests for Hardy–Weinberg equilibrium and estimates of F_{IS}, we used a false discovery rate control method (Benjamini & Yekutieli, 2001) to obtain an experiment-wide alpha level (α_{EW}) before assessing significance.

We estimated levels of microsatellite diversity within each subpopulation by calculating observed (H_O) and expected (H_E) heterozygosities, the number of alleles per locus (A), and the number (A_U) and average frequency of unique alleles using GDA (version 1.1; Lewis & Zaykin, 1999). We adjusted the average number of alleles per locus for

variation in subpopulation sample size by calculating a rarefacted allelic richness (A_R) in FSTAT (version 2.9.3; Goudet, 1995). To assess the power of this suite of microsatellite loci, we calculated an unbiased multilocus probability of identity for each subpopulation (Paetkau *et al.*, 1998). Additionally, we estimated levels of genetic differentiation between the two subpopulations by calculating F_{ST} in SPAGEDI. We carried out a one-tailed test for significance in F_{ST} estimates by comparing observed values to those obtained by permuting individuals among populations 20 000 times.

We estimated levels of mtDNA diversity within each subpopulation by calculating the number (H) and diversity (H_D) of haplotypes in DNASP (version 4.10; Rozas *et al.*, 2003). We estimated levels of genetic differentiation among subpopulations by calculating F_{ST} values in SPAGEDI, assessing significance as described above. We constructed a haplotype network representing a set of statistically parsimonious ($P \geq 0.95$) connections between haplotypes using TCS (version 1.21; Clement, Posada & Crandall, 2000) which implements the algorithms described in Templeton, Crandall & Sing (1992). We treated gaps as missing data for haplotype network construction. The haplotype network was nested by hand, following the procedure described in Crandall (1996).

Sometimes, populations defined *a priori* based on geography do not correspond to genetically defined populations. Thus, we also used the Bayesian clustering software STRUCTURE to infer the number of genetically distinct clusters (subgroups) within the entire set of Alaskan SBTD and to assign individual samples to these clusters (version 2.1; Pritchard, Stephens & Donnelly, 2000; Falush, Stephens & Pritchard, 2003). We performed five runs at each value of K (the number of subpopulations), from $K = 1$ to $K = 8$. Each run consisted of 500 000 replicates of the Markov chain following a burn-in of 100 000 replicates. We used the admixture model, and allowed the allele frequencies to be correlated among subpopulations. All other parameters were set to default values (Pritchard & Wen, 2003). Once the true K is reached, likelihoods for larger K's plateau and variance among runs increases (Pritchard & Wen, 2003). Therefore, we used the highest value of ΔK (the second-order rate of change in K) as a point estimate of K (Evanno, Regnaut & Goudet, 2005). To assign each individual to the most appropriate cluster, we performed a final run (1 000 000 burn-in and 1 000 000 replicates) at the inferred K.

Genetic characterization of SBTD from the Alexander Archipelago

For the entire set of samples collected from the Alexander Archipelago, we performed a STRUCTURE analysis as described above to detect any additional substructuring within this region. For the two islands with the largest sample sizes (Admiralty and Prince of Wales Islands), we also estimated levels of genetic diversity and genetic differentiation using the methods outlined above.

Genetic characterization of SBTD on the AP

We sought to characterize genetic diversity on the Kodiak Archipelago and to characterize genetic differences between SBTD harvested from the AP and those harvested from the remainder of the Archipelago outside the AP (non-AP). Within the AP, we tested for genetic differentiation between cryptorchid and non-cryptorchid deer. We estimated levels of genetic diversity and differentiation between these groups (AP/non-AP, cryptorchid/non-cryptorchid) as described above. Additionally, we used STRUCTURE to identify any genetic substructuring within the sample for the entire Kodiak Archipelago, as described above.

To estimate the level of inbreeding (in terms of a deficiency of heterozygotes relative to Hardy–Weinberg expectations) within each of the four groups (AP, non-AP, cryptorchid, non-cryptorchid), we calculated F_{IS} values using SPAGEDI. In addition, we estimated relatedness values for each of the four groups, using the method of Queller & Goodnight (1989) as implemented in SPAGEDI. Standard errors were calculated for estimates of relatedness by jackknifing over loci. Because we were interested in the relative relatedness of these deer (not in absolute relatedness values), we used allele frequencies estimated from the entire Kodiak Archipelago SBTD population as reference frequencies. To look for signatures of genetic drift, we performed a simple mode shift test for bottlenecks on both AP and non-AP deer (Luikart *et al.*, 1998). This test detects the distortion in the distribution of allele frequencies in recently bottlenecked populations from many rare alleles to fewer, more common alleles (Luikart *et al.*, 1998). Although this test is qualitative, it has been shown to effectively detect bottlenecks, particularly in translocated wildlife populations (Mock, Latch & Rhodes, 2004).

Results

Overall genetic diversity and structure in SBTD in Alaska

Both nuclear and mitochondrial diversity were extremely low in Alaskan SBTD, regardless of locale, compared with those observed in other mule deer populations. Based on data for all 11 microsatellite loci, expected heterozygosity was 0.32 in our total dataset of 254 SBTD compared with 0.65 for mainland black-tailed deer populations in British Columbia analyzed by identical methods ($n = 65$; E. K. Latch & O. E. Rhodes, unpubl. data). Furthermore, allelic richness (estimated after adjusting for sample size and averaged across the 11 loci) was nearly three times higher in mainland British Columbia ($A_R = 7.7$; E. K. Latch & O. E. Rhodes, unpubl. data) than in SBTD from Alaska ($A_R = 2.7$). For mtDNA, we found only 14 haplotypes ($H_D = 0.58$) for 252 SBTD in Alaska (Table 3) compared with 28 haplotypes and $H_D = 0.95$ for a sample of only 65 deer from mainland British Columbia (E. K. Latch & O. E. Rhodes, unpubl. data). However, despite the relative lack of genetic variability within SBTD from Alaska as compared

Table 2 Population genetic data from analysis of microsatellites in SBTD

	n	HWE (# loci)	A (A_R)	A_U (average frequency)	H_E	H_O	F_{IS} (*P*)	$1/P_{ID}$	F_{ST} (*P*)
Total	254	3	3.75	N/A	0.446	0.374	0.1617 (0.0000)	2762.4	N/A
Kodiak Archipelago	175	5	2.63 (2.22)	2 (0.0058)	0.435	0.403	0.0753 (0.0012)	2061.9	0.1456 (0.0000)
Alexander Archipelago	79	7	3.5	9 (0.0406)	0.370	0.310	0.1646 (0.0000)	1133.8	
Alexander Archipelago									
Prince of Wales	35	8	3.25 (3.06)	12 (0.0953)	0.455	0.401	0.1201 (0.0077)	13333.3	0.1530 (0.0000)
Admiralty	21	5[a]	1.75	0	0.171	0.173	−0.0078 (0.5913)	281.2	
Kodiak Archipelago									
Aliulik Peninsula (AP)	113	8	2.25	0	0.439	0.411	0.0646 (0.0219)	2127.7	0.0166 (0.0024)
Non-AP	62	5	2.63 (2.62)	3 (0.0192)	0.419	0.387	0.0768 (0.0323)	1897.5	
AP									
Cryptorchid	74	0	2.25 (2.25)	0	0.445	0.412	0.0750 (0.0293)	2277.9	0.0028 (0.2482)
Non-cryptorchid	39	0	2.25	0	0.426	0.409	0.0400 (0.2360)	2178.6	

Statistics provided include sample size (*n*), number of loci conforming to Hardy–Weinberg expectations (HWE; out of eight polymorphic loci), number of alleles per locus (*A*), allelic richness (A_R), the number (A_U) and average frequency of unique alleles, expected (H_E) and observed (H_O) heterozygosities, deviation from random mating within a population (F_{IS}), the inverse of the unbiased probability of identity averaged over loci ($1/P_{ID}$), and genetic differentiation among populations (F_{ST}) with *P*-values.

[a]In this population there were only five polymorphic loci.

with other SBTD, our suite of eight polymorphic microsatellite loci afforded us a reasonably low P_{ID} of 0.00036 for the total sample (one in 2762 individuals will have the same multilocus genotype; Table 2).

For Alaskan SBTD, based on our set of eight loci, we found a marked deviation from random mating ($F_{IS} = 0.16$, $P < 0.0001$, $\alpha_{EW} = 0.0184$), suggesting genetic substructuring within the entire SBTD sample. STRUCTURE identified two major genetic groups within this dataset, corresponding to the Kodiak Archipelago and Alexander Archipelago subpopulations. Most individuals were assigned to a genetic cluster consistent with where they were sampled (86% of deer harvested on Kodiak Archipelago were assigned to one cluster, and 89% of deer harvested in the Alexander Archipelago were assigned to the other cluster). Further, the haplotype network showed separation between haplotypes found in SBTD on the Kodiak Archipelago and those detected in SBTD from the islands of the Alexander Archipelago (Fig. 2). Finally, estimates of F_{ST} confirmed genetic differentiation ($P < 0.0001$) between SBTD sampled in the Kodiak Archipelago versus those sampled in the Alexander Archipelago [$F_{ST(nDNA)} = 0.15$; $F_{ST(mtDNA)} = 0.65$; Tables 2 and 3].

Deer in the Alexander Archipelago exhibited greater genetic diversity than did deer on the Kodiak Archipelago, based both on microsatellite allelic richness and the number of unique alleles (Table 2) and on the number and diversity of mitochondrial haplotypes within populations (Table 3). Note that our estimate of unique alleles did not correct for the fact that we sampled 2.2 times more deer from the Kodiak Archipelago than from the Alexander Archipelago.

SBTD in the Alexander Archipelago

Population genetic analyses considered only the more intensively sampled Admiralty and Prince of Wales Islands, and revealed that deer on the two islands were profoundly different. STRUCTURE identified two clusters, and 100% of individuals were assigned to the island where they were sampled. Traditional F_{ST} estimates supported the distinctiveness of deer from these two islands [$F_{ST(nDNA)} = 0.15$; $F_{ST(mtDNA)} = 0.34$; Tables 2 and 3]. Although no individual microsatellite locus was found to be out of Hardy–Weinberg equilibrium on Prince of Wales Island, we observed a significant overall deficiency of heterozygotes ($F_{IS} = 0.12$; Table 2). We did not find such a deviation on Admiralty Island ($F_{IS} = -0.008$; Table 2).

SBTD from the Alexander Archipelago were characterized by a closely related suite of haplotypes, with the notable exception of SBTD on Prince of Wales Island (Fig. 2). In Prince of Wales Island SBTD, we found evidence of substantially higher genetic variability than was observed for any other population in the entire dataset, both in terms of nDNA and mtDNA (Table 2). Deer on Prince of Wales Island had a unique and diverse lineage of haplotypes (Fig. 2; i.e. haplotype clades 1–5 and 1–6). Six of the seven mtDNA haplotypes found on Prince of Wales Island were absent from the other islands of the Alexander Archipelago (Fig. 2), and nearly half of the 26 microsatellite alleles we detected were unique to deer on Prince of Wales Island (Table 2). Elsewhere in the Alexander Archipelago (i.e. Admiralty, Chichagof and Baranof Islands), deer were found to be genetically similar; 38 of 46 individuals possessed the same mtDNA haplotype. Intriguingly, this haplotype was not found on Prince of Wales Island or the Kodiak Archipelago.

SBTD in Kodiak Archipelago

For SBTD in the Kodiak Archipelago, STRUCTURE resolved two subpopulations, though genetic clustering of individuals only weakly corresponded to geographic

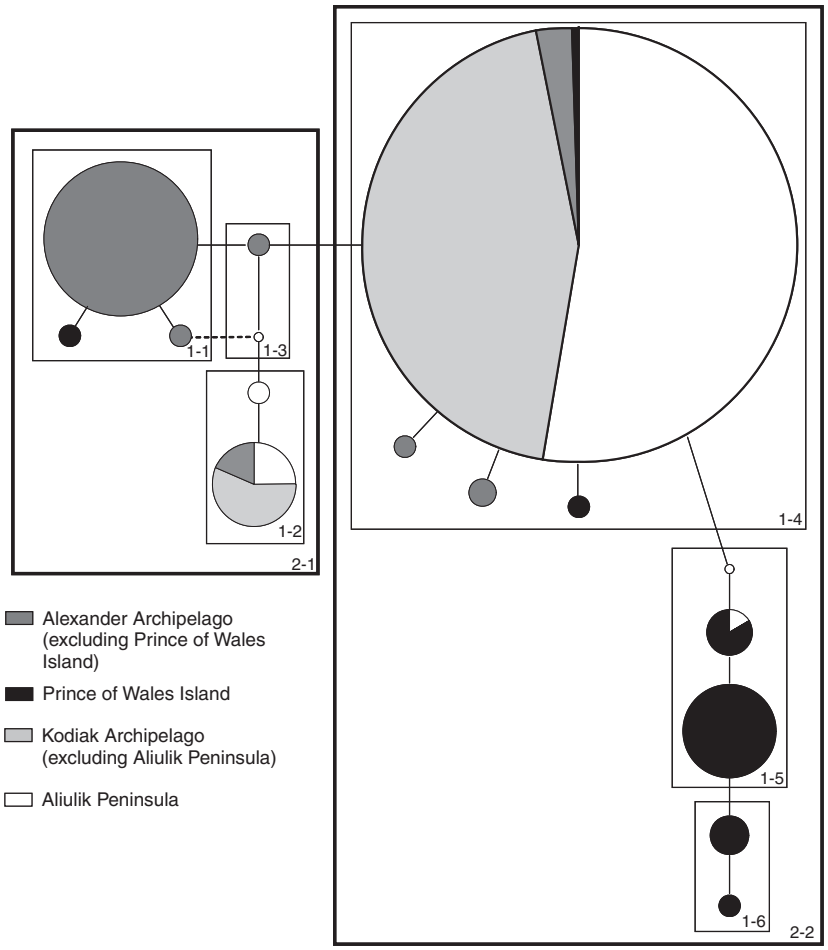

Figure 2 Nested design of haplotype network derived from 252 unique control region sequences for Sitka black-tailed deer *Odocoileus hemionus sitkensis* in Alaska. Each circle represents one haplotype, with sizes proportional to the number of individuals exhibiting that haplotype. A solid line connecting two haplotypes represents a single mutational step, and open circles represent missing or unsampled haplotypes. Thin and thick lined boxes represent one-step and two-step clades, respectively. The dashed line represents an unresolved connection.

Table 3 Population genetic data from analysis of mitochondrial control region sequences in SBTD

	n	*H*	H_U (average frequency)	H_D (SD)	F_{ST} (*P*)
Total	252	14	N/A	0.580 (0.033)	N/A
Kodiak Archipelago	169	5	2 (0.0060)	0.167 (0.038)	0.6493 (0.0000)
Alexander Archipelago	83	12	9 (0.1014)	0.714 (0.042)	
Alexander Archipelago					
Prince of Wales	37	7	7 (0.1429)	0.671 (0.074)	0.3408 (0.0000)
Admiralty	22	3	3 (0.3182)	0.177 (0.106)	
Kodiak Archipelago					
Aliulik Peninsula (AP)	109	4	1 (0.0091)	0.138 (0.044)	0.0194 (0.1398)
Non-AP	60	3	1 (0.0175)	0.223 (0.068)	
AP					
Cryptorchid	71	3	1 (0.0141)	0.083 (0.045)	0.0188 (0.1851)
Non-cryptorchid	38	3	0	0.235 (0.084)	

Statistics provided include sample size (*n*), number of haplotypes (*H*), number (H_U) and average frequency of unique haplotypes, haplotype diversity (H_D) with standard deviation (SD), and genetic differentiation among populations (F_{ST}) with *P*-value.

sampling location; one of the two clusters included 69% of the AP samples (72% of cryptorchid samples) and 52% of the non-AP samples. Similarly, genetic differentiation was low between deer sampled on the AP and those from the rest of the Kodiak Archipelago [non-AP; $F_{ST(nDNA)} = 0.02$; $F_{ST(mtDNA)} = 0.02$; Tables 2 and 3]. Despite the fact that the non-AP sample included deer from across the entire Kodiak Archipelago, whereas the AP sample was from a limited geographic area (Fig. 1), the genetic diversity within these two groups was very similar. Of the few microsatellite

alleles and mitochondrial haplotypes that were unique to one group or to the other, all were present at very low frequencies (Tables 2 and 3).

Both the AP and non-AP subpopulations were characterized by low and similar levels of genetic diversity and a slight deviation from Hardy–Weinberg expectations [$F_{IS(AP)} = 0.0646$; $F_{IS(non-AP)} = 0.0768$; Table 2]. The deviation from random mating was not much greater in the AP subpopulation than in the non-AP subpopulation, contrary to what would be expected if AP deer experienced greater inbreeding. Likewise, level of relatedness among individual deer on the AP ($r = -0.0126$, SE = 0.0216) was not significantly different from that estimated among non-AP deer ($r = 0.0385$, SE = 0.0443). We considered that although the magnitude of the deviations from random mating observed for AP and non-AP SBTD were similar, a deviation from random mating in non-AP deer could have been influenced by the relatively large geographic area included in the non-AP sample (Wahlund effect). Therefore, we calculated F_{IS} values for four localized sampling locations within the Archipelago [Uganik Island ($n = 16$), Western Kodiak Island ($n = 20$), Eastern Kodiak Island ($n = 11$) and Afognak Island ($n = 12$); data not shown]. Although sample sizes were relatively small, each F_{IS} value was very similar to that for the overall non-AP calculation, and also to that for the AP. However, both the AP and non-AP populations showed evidence of a genetic bottleneck. In both AP and non-AP deer, the mode of the allele frequency distribution was shifted from the lowest allele frequency category (0–0.1; as expected in a non-bottlenecked population) to a higher allele frequency category (0.2–0.3 category in AP deer, 0.1–0.2 category in non-AP deer). The shift in the mode of the allele frequency distribution was slightly more pronounced in AP deer than in non-AP deer (median allele frequencies were 0.404 and 0.274, respectively).

On the AP, we did not detect any measurable genetic difference between cryptorchid and non-cryptorchid deer based on nuclear microsatellite loci (Table 2). Estimates of genetic diversity and deviation from random mating were similar for cryptorchid and non-cryptorchid deer on the AP. Likewise, relatedness estimates were not different between cryptorchid ($r = -0.0236$, SE = 0.0348) and non-cryptorchid ($r = 0.0228$, SE = 0.0289) deer. These trends did not change upon exclusion of unilateral cryptorchid deer. Haplotype diversity, on the other hand, was markedly lower in cryptorchid deer than in non-cryptorchid deer (Table 3).

Discussion

Genetic diversity of SBTD in Alaska relative to other mammals

The level of genetic variation found in SBTD in Alaska was far lower than values observed in natural populations that do not have a history of isolation and inbreeding. The low levels of nuclear genetic diversity in SBTD are similar to values observed in mammalian populations that are considered to be threatened by low genetic diversity or have undergone sharp declines in numbers; for example, a population of Rocky Mountain bighorn sheep *Ovis canadensis* founded from 12 individuals ($H_E = 0.43$; Forbes *et al.*, 1995); the cheetah *Acinonyx jubatus*, thought to have experienced an ancient genetic bottleneck ($H_E = 0.39$; Menotti-Raymond & O'Brien, 1995); an insular population of koalas *Phascolarctos cinereus* reestablished from 18 adults ($H_E = 0.33$; Houlden *et al.*, 1996); and a population of Tule elk in California founded from as few as two individuals ($H_E = 0.22$; Williams, Lundrigan & Rhodes, 2004). Although direct comparisons of H_E values among species cannot be made, it is clear that the values we observed in SBTD are quite low.

Declines of high profile species such as the Florida panther (Roelke *et al.*, 1993) and cheetah (O'Brien, 1994) have been attributed to low genetic variation. However, effects of inbreeding usually are evident during or shortly after the initial loss of genetic variation, when deleterious alleles become exposed in homozygous form (Keller & Waller, 2002). In the case of SBTD in Alaska, low genetic variability in native Alexander Archipelago deer suggests that the loss of variation in SBTD occurred before the founding of the Kodiak Archipelago population; logically sufficient time for purging of deleterious alleles.

Although SBTD in Alaska exhibited extraordinarily low genetic diversity, it is not evident whether there have been negative fitness consequences associated specifically with the loss of genetic diversity in deer from this region. SBTD are thriving on the extensive network of semi-isolated islands in and throughout both archipelagos. By extrapolation from numbers of SBTD killed by hunters (Straugh, Converse & White, 2004; Van Daele, 2005), there may be 15 000–30 000 deer on each of the four major islands we sampled in the Alexander Archipelago. In the Kodiak Archipelago, the deer population is estimated to have peaked at >100 000 animals by the mid-1980s (Van Daele, 2005), just 50 years after the introduction of only 25 animals. Following a few winters with high mortality in the 1990s, especially 1998–1999, the population dropped in size but rebounded to *c.* 60 000 animals by 2004 (Van Daele, 2005). The fitness effects of slight inbreeding are sometimes difficult to detect unless outbreeding occurs, particularly in situations for which predation, disease or other demographic pressures are low (Keller & Waller, 2002). Unfortunately, decreased fitness in the AP deer population relative to other populations, in terms of lower birth weight, higher mortality, etc. has not been assessed.

Population success in the face of low genetic variability is not unique. For example, the Kodiak brown bear population *Ursus actos middendorffi*, which presumably was founded around the end of the Wisconsin glaciation and isolated for the last 10 000 years, has an average heterozygosity of 26% (eight microsatellite loci; Paetkau *et al.*, 1998). Although this is dramatically lower than other North American brown bear populations, including small, isolated populations near the southern end of the current species range, Kodiak bears have some of the highest productivity rates ever observed in this species (Paetkau *et al.*, 1998).

SBTD in the Alexander Archipelago

Movement of deer between islands in the Alexander Archipelago (particularly neighboring Admiralty, Baranof and Chichagof Islands) is likely, given that a single haplotype is predominant on all three islands. However, SBTD from none of these islands share haplotypes with deer on Prince of Wales Island, suggesting that SBTD on Prince of Wales Island have been isolated from those on other islands in the Alexander Archipelago for an extended time.

The Prince of Wales Island complex (including Prince of Wales Island, surrounding southern outer islands such as Heceta, Suemez and Dall, and possibly extending to British Columbia's Queen Charlotte Islands to the south) has been identified as an important center of endemism, particularly with respect to mammalian biodiversity (MacDonald & Cook, 1996; Cook, Dawson & MacDonald, 2006). The Prince of Wales Island complex supports a large number of endemic mammalian lineages including those within Keen's mouse *Peromyscus keeni* (Lucid & Cook, 2004), northern flying squirrel *Glaucomys sabrinus* (Bidlack & Cook, 2002), ermine *Mustela erminea haidarum* (Fleming & Cook, 2002), and possibly gray wolf *Canis lupus* (Weckworth *et al.*, 2005; alternative colonization patterns, F. Hailer, pers. comm.). Geologic records indicate that much of the Prince of Wales Island complex likely remained uncovered by ice during the Wisconsin glaciation (Carrara *et al.*, 2003), thus representing a potential refugium. The restricted distribution of the unique SBTD lineage found on Prince of Wales Island and its level of divergence from other lineages in the Alexander Archipelago suggest that this population may be derived from relicts that persisted in a refugium on the Prince of Wales Island complex. Remains of SBTD dating to the time of the Wisconsin glaciation have been identified from caves within the Prince of Wales Island complex (Heaton, Talbot & Shields, 1996). Alternatively, SBTD may have recolonized the Prince of Wales Island complex following deglaciation. In contrast to the rest the Alexander Archipelago, the Prince of Wales Island complex appears to have been recolonized by migration along the North Pacific coast from the south, a route that would have been available very soon after deglaciation (Cook *et al.*, 2006). Since then, animals on this island complex have been nearly, if not completely isolated from other populations throughout the Archipelago, indicating strong, persistent barriers to dispersal.

Given the unique evolutionary history and contemporary demographic independence of SBTD (and other taxa) on the Prince of Wales Island complex, comprehensive plans should be developed to manage these populations, as they cannot be expected to easily recruit immigrants from neighboring mainland (or other island) populations. Furthermore, large increases in human settlement (and associated impacts including habitat conversion and fragmentation, exploitation, development and exotic species introduction) have been particularly intense on Prince of Wales Island, due in part to increased road access across the island (Cook *et al.*, 2006). In addition, climate warming is predicted to substantially increase extinction risk for populations with limited ability to disperse (Thomas *et al.*, 2004), such as those within the Prince of Wales Island complex. These threats further highlight the need for continued monitoring of this biologically unique and diverse region.

On Admiralty Island, genetic diversity is extraordinarily low, yet there is no evidence for any deviation from random mating. Furthermore, although deer on Admiralty Island are almost completely differentiated from deer on Prince of Wales Island, they are very similar to deer found on other islands in the Alexander Archipelago (i.e. Baranof and Chichagof Islands), in terms of the distribution of both microsatellite alleles and mitochondrial haplotypes (Fig. 2). Low genetic diversity on Admiralty Island suggests initial colonization by a few individuals or a recent severe bottleneck. The lack of divergence of microsatellite alleles between Admiralty Island and other islands within the Alexander Archipelago due to genetic drift (excluding Prince of Wales), could either be the result of regular migration among islands (with similar allele frequency distributions) or an overall deficiency of males on the island, leading to more equal reproductive success among males. Although the latter is unlikely (K. Hundertmark, pers. comm.), either option would yield maximum representation of all microsatellite alleles in subsequent generations and would minimize the effects of genetic drift.

SBTD in Kodiak Archipelago

We had assumed that SBTD on the Kodiak Archipelago would represent a subset of the genetic diversity found in the Alexander Archipelago, especially in respect to mtDNA, given that the population was initiated with only 25 individuals around 70–80 years ago. This was not the case. We noted substantial genetic divergence between SBTD in the two regions and very few alleles (or haplotypes) shared between deer from the two regions. The predominant mtDNA haplotype on the Kodiak Archipelago was found in only one SBTD on Prince of Wales Island, from which only two deer were moved in 1930. Dramatic shifts in haplotype frequencies associated with translocated populations have been noted for other species, such as pronghorn *Antilocapra americana* (Stephen *et al.*, 2005), and are attributed to differential reproductive success among female lineages. Additionally, undocumented translocations into the Kodiak Archipelago, immigration from areas adjacent to the Archipelago (unlikely given the large distance between the Archipelago and Alaska mainland) or rapid adaptation to unique environmental conditions on the Archipelago all could be contributing to rapid divergence in haplotype frequencies between the Kodiak and Alexander Archipelago populations. However, the data at hand do not allow us to reject one or more of these possibilities.

SBTD on the AP and remainder of Kodiak Archipelago

For SBTD outside the AP, there was evidence of a founder event resulting from the establishment of a population from relatively few individuals. This was evidenced by a mode shift in the distribution of microsatellite allele frequencies and is consistent with the documented transplant of 25 animals in 1924–1934. As initial colonizers to the AP moved in from the north in the late 1970s, they likely underwent yet another founder event. As expected, we observed a more pronounced shift in the mode of the allele frequency distribution for the AP population than for the non-AP population. This is consistent with a modest secondary bottleneck during colonization of the AP. Despite evidence for a small founding population size, our data do not provide strong evidence for isolation and genetic drift within the AP deer population. The apparently rapid rate of increase in size of the AP population, combined with a lack of substantial genetic divergence from the rest of the Kodiak Archipelago, makes it unlikely that initial colonizers to the peninsula were immediately isolated. Rather, it is likely that immigrants from the Kodiak Archipelago continuously supplemented the AP population over time. Estimates of levels of inbreeding and inter-individual relatedness in AP deer also are similar to those of SBTD found outside the AP, further supporting the idea that deer on the AP do not comprise an isolated population suffering from inbreeding or an accelerated rate of genetic drift.

We found no evidence to support the concept that SBTD on the AP exist as an isolated population experiencing inbreeding. However, there is an important caveat. The overall lack of genetic variability exhibited by SBTD on the Kodiak Archipelago, and modest genetic differentiation between AP and non-AP deer, limits the utility of genetic methods for deriving accurate estimates of migration rates between these two populations. Radiotelemetry could directly document movements of individuals, but probably many animals would have to be collared to ensure monitoring of deer actually moving in or out of the AP.

Over 68% of male SBTD on the AP are bilateral cryptorchids, 7% are unilateral cryptorchids and nearly 25% are non-cryptorchid (updated from Veeramachaneni *et al.*, 2006); thus, only ~32% of the male SBTD on the AP are potentially fertile. Despite a low proportion of potentially fertile males, population growth rates and levels of neutral genetic diversity remain high relative to non-AP populations unafflicted by cryptorchidism. This is not necessarily surprising, given the reproductive behavior of deer (few males may impregnate many females).

We initially proposed that the high incidence of cryptorchidism detected in SBTD in the AP could be a consequence of mutation(s) in one or a few animals, reinforced by inbreeding (due to minimal immigration of additional SBTD) and genetic drift. The lack of detectable differences in gene frequencies and levels of heterozygosity between AP and non-AP deer, or between cryptorchid deer and non-cryptorchid deer, does not support population genetic processes as a mechanism by which this deleterious condition is being maintained in the AP population. Only a few immigrants into the AP, participating as breeding females or males, would be needed to quickly curtail fixation of a multigenic recessive trait such as cryptorchidism. It is possible that rapid expansion of the AP population contributed to the preservation of the deleterious mutation(s) by effectively relaxing selection against them. Thus, population expansion could be impeding the development of local genetic structure due to drift while giving the impression of high gene flow among populations. However, when we consider the presence of antler defects in 70% of bilaterally cryptorchid deer on the AP (Veeramachaneni *et al.*, 2006), concurrent mutations in several genes would have to be simultaneously driven to fixation for both abnormalities to occur.

A second possible mechanism by which a high incidence of cryptorchidism might be maintained is via antagonistic effects between the sexes. We observed reduced haplotype diversity within the cryptorchid group relative to the unaffected group in the same environment. Maternal inheritance of mtDNA (and therefore the lack of mtDNA transmission in males) could create an asymmetric pattern between the sexes for selection on mitochondrial genes by preventing selection on mtDNA haplotypes with male-specific phenotypes (Rand, Haney & Fry, 2004). Thus, mutations with deleterious effects in males can persist in populations, provided they are effectively neutral in females. It is possible that certain mtDNA haplotypes could be correlated with cryptorchidism and maintained at high frequency in the population of cryptorchid deer on the AP in the absence of selection against them. Targeted study of specific functional genes and their control sequences might reveal whether there are underlying genetic differences in autosomal genes between cryptorchid and non-cryptorchid deer that were undetected with our set of neutral markers. Furthermore, a specific investigation of mtDNA haplotypes and transmission patterns in both males and females may be warranted to examine the potential influence of antagonistic effects.

Genetic evidence presented herein suggests that, in contrast to other species such as the Florida panther (O'Brien, 1994), inbreeding (at least on a genome-wide scale) probably is not solely responsible for concentrating cryptorchidism in SBTD on the AP. Hence, these findings cannot support inbreeding as an alternative to the hypothesis favored by Veeramachaneni *et al.* (2006) for the high incidence of cryptorchidism; namely, continuing exposure of pregnant females and their fetuses to an environmental contaminant.

Veeramachaneni *et al.* (2006) suggested that an estrogenic endocrine disruptor, with the potential to mimic or inhibit hormones, could modify testicular and antlerogenesis in SBTD. In their analysis of AP SBTD (including many of the samples used herein), Veeramachaneni *et al.* (2006) noted that hypospadias, a long-known symptom of decreased androgen action during the development of the reproductive system, was absent, and that testicular lesions were proliferative and dysplastic (typical of estrogenic

stimulation) and not regressive (typical of antiandrogens). Animals may be chronically exposed to these contaminants; however, testicular and antler deformities may occur if animals are exposed during a window narrower than 25–40% of gestation, when testicular and antlerogenesis occur in deer (Lincoln, 1973; Veeramachaneni *et al.*, 2006).

To explain the high levels of cryptorchidism in SBTD localized on the AP, an environmental contaminant would necessarily be either (1) more localized on the AP than elsewhere in the region; (2) widespread, but more heavily impact a genetically vulnerable AP population (genotype × environment interaction); (3) widespread, but with a delivery system targeting deer on the AP. Localized presence of an environmental endocrine disruptor seems unlikely, particularly given the low levels of anthropogenic disturbance in this area and the complete lack of reports detailing localized pollution in the area. Furthermore, there are no descriptions of malformations for other species on the AP, though directed inquiries into abnormalities for other vertebrate taxa have not been made.

A widespread environmental contaminant seems a plausible explanation for the abnormalities observed in AP SBTD, but whether such a contaminant is affecting AP deer because of a particular genetic vulnerability within the population or as a consequence of the geographic location of the AP is unknown. Based on neutral genetic markers, AP deer are not genetically differentiated from non-AP deer, nor are cryptorchid deer genetically differentiated from non-cryptorchid deer. However, genetic differences in unstudied functional or regulatory genes may trigger cryptorchidism under certain environmental conditions. This type of genotype × environment interaction appears to be the mechanism for a high prevalence (8.1%) of beak deformities documented in black-capped chickadees *Poecile atricapillus* from southcentral Alaska (Handel *et al.*, 2006). In this case, harsh environmental conditions and nutritional deficiencies during winter may trigger development of beak deformities among chickadees exposed *in ovo* to low levels of PCBs (Handel *et al.*, 2006).

Alternatively, an environmental contaminant may be geographically targeted in its delivery to SBTD inhabiting the AP. Contaminated fog, persisting in the low lying AP, has been suggested as a possible vehicle for targeted delivery of contaminants to AP deer (Veeramachaneni *et al.*, 2006). Contaminated deer browse, deposited preferentially on AP beaches due to reduced carrying capacity of the Alaska Coastal Current and the Alaska Stream as they converge on the AP, could also be responsible for elevated exposure to contaminants in AP deer (Veeramachaneni *et al.*, 2006). If kelp and other materials deposited on shore were contaminated, exposure would reach a peak during the winter, when heavy snows force deer onto beaches to browse.

Persistence of SBTD on the AP may depend on our ability to characterize the etiology of reproductive abnormalities in the vast majority of male deer. Although we have not documented any adverse population-level effects of cryptorchidism in the AP population, environmental contaminants can have a profound impact on the evolutionary trajectory of a species (Bickham *et al.*, 2000). It may be that sufficient time has not yet passed for these effects to be realized, yet their potential impacts cannot be ignored. For now, exclusive management of the AP deer population, by supplementation with stock from outside the AP, regulation of hunting pressure or facilitating movement of SBTD between the AP and elsewhere on Kodiak Island, is unlikely to mitigate the incidence of testicular and antler malformations in AP deer. However, introductions of deer from outside the AP could provide strong evidence for a non-genetic causality if such an introduction did not mitigate the deformity rate. To minimize the effects of a putative endocrine disruptor, conservation strategies should be developed that take into account the types of contaminant(s) that are present in the environment, mode of delivery of these contaminants to AP deer, and specific reproductive targets of these contaminants in adult and fetal deer of both sexes.

Acknowledgements

We thank J.R. Heffelfinger and hunters in south-east Alaska who kindly responded to our requests to provide samples. Analyses by D. Paetkau, via contract from Colorado State University to Wildlife Genetics International, provided impetus for these detailed analyses. D.N.R. Veeramachaneni, a principal in the project at Colorado State University, obtained partial funding for genetic analyses of SBTD. Mr. J.R Heffelfinger was the force behind the North American Deer Project, and helped to obtain samples and funding for this project. J.A. Fike provided skilled technical assistance. We thank D. Paetkau, D.N.R. Veeramachaneni and K. Hundertmark for helpful comments on an early draft of the paper. Funding was provided by Colorado State University (ARBL Morphological Services and CVMBS Research Council), NIH grant 1R21ES014607-01A1 and Purdue University (including contributions to the North American Deer Project from the Boone & Crockett Club, Pope and Young Club, Safari Club International and National Fish and Wildlife Foundation).

References

Amann, R.P. & Veeramachaneni, D.N.R. (2007). Cryptorchidism in common eutherian mammals. *Reproduction* **133**, 541–561.

Anway, M.D., Cupp, A.S., Uzumcu, M. & Skinner, M.K. (2005). Epigenetic transgenerational actions of endocrine disruptors and male fertility. *Science* **308**, 1466–1469.

Benjamini, Y. & Yekutieli, D. (2001). The control of the false discovery rate in multiple testing under dependency. *Ann. Stat.* **29**, 1165–1188.

Bickham, J.W., Sandhu, S., Hebert, P.D.N., Chikhi, L. & Athwal, R. (2000). Effects of chemical contaminants on genetic diversity in natural populations: implications for biomonitoring and ecotoxicology. *Mutat. Res. – Rev. Mutat.* **463**, 33–51.

Bidlack, A.L. & Cook, J.A. (2002). A nuclear perspective on endemism in northern flying squirrels (*Glaucomys sabrinus*) of the Alexander Archipelago, Alaska. *Conserv. Genet.* **3**, 247–259.

Bishop, M.D., Kappes, S.M. & Keele, J.W., Stone, R.T., Sunden, S.L.F., Hawkins, G.A., Toldo, S.S., Fries, R., Grosz, M.D., Yoo, J. & Beattie, C.W. (1994). A genetic linkage map for cattle. *Genetics* **136**, 619–639.

Bubenik, G.A., Jacobson, J.P., Schams, K.D. & Barto, L. (2001). Cryptorchidism, hypogonadism and antler malformation in black-tailed deer (*Odocoileus hemionus sitkensis*) of Kodiak Island. *Z. Jagdw.* **47**, 241–252.

Carrara, P., Ager, T.A., Baichtal, J.F. & VanSistine, D.P. (2003). Map of glacial limits and possible refugia in the souther Alexander Archipelago, Alaska, during the Late Wisconsin Glaciation. US Misc. Field Studies Map MF-2424: 1–13 (+map).

Clement, M., Posada, D. & Crandall, K. (2000). TCS: a computer program to estimate gene genealogies. *Mol. Ecol.* **9**, 1657–1660.

Cook, J.A., Dawson, N.G. & MacDonald, S.O. (2006). Conservation of highly fragmented systems: the north temperate Alexander Archipelago. *Biol. Conserv.* **133**, 1–15.

Crandall, K.A. (1996). Multiple interspecies transmissions of human and simian T-cell leukemia/lymphoma virus type I sequences. *Mol. Biol. Evol.* **13**, 115–131.

Crews, D. & McLachlan, J.A. (2006). Epigenetics, evolution, endocrine disruption, health, and disease. *Endocrinology* **147**, S4–S10.

Evanno, G., Regnaut, S. & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol. Ecol.* **14**, 2611–2620.

Falush, D., Stephens, M. & Pritchard, J.K. (2003). Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics* **164**, 1567–1587.

Fleming, M.A. & Cook, J.A. (2002). Phylogeography of endemic ermine (*Mustela erminea*) in southeast Alaska. *Mol. Ecol.* **11**, 795–808.

Forbes, S.H., Hogg, J.T., Buchanan, F.C., Crawford, A.M. & Allendorf, F.W. (1995). Microsatellite evolution in congeneric mammals – domestic and bighorn sheep. *Mol. Biol. Evol.* **12**, 1106–1113.

Gore, A.C., Heindel, J.J. & Zoeller, R.T. (2006). Endocrine disruption for endocrinologists (and others). *Endocrinology* **147**, S1–S3.

Goudet, J. (1995). FSTAT version 1.2: a computer program to calculate *F*-statistics. *J. Hered.* **86**, 485–486.

Gray, L.E. (1998). Xenoendocrine disrupters: laboratory studies on male reproductive effects. *Toxicol. Lett.* **103**, 331–335.

Gray, L.E., Ostby, J. & Furr, J., Wolf, C.J., Lambright, C., Parks, L., Veeramachaneni, D.N., Wilson, V., Price, M., Hotchkiss, A., Orlando, E. & Guilette, L. (2001). Effects of environmental antiandrogens on reproductive development in experimental animals. *Hum. Reprod. Update* **7**, 248–264.

Handel, C.M., Pajot, L.M. & Matsuoka, S.M., Trust, K.A., Stotts, J.M., Terenzi, J. & Talbot, S.L. (2006). *Potential role of environmental contaminants in the pathology of beak deformities among black-capped chickadees in southcentral Alaska*. Final Report for USGS Project 1130-7F22, USGS Alaska Science Center, Anchorage, AK.

Hardy, O.J. & Vekemans, X. (2002). SPAGeDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. *Mol. Ecol. Notes* **2**, 618–620.

Heaton, T.H., Talbot, S.L. & Shields, G.F. (1996). An Ice Age refugium of large mammals in the Alexander Archipelago, southeastern Alaska. *Quat. Res.* **46**, 186–192.

Hicks, J.F. & Rachlow, J.L. (2006). Is there a genetic basis for antler and pedicle malformations in reintroduced elk in northern Arizona? *Southwest. Nat.* **51**, 276–282.

Houlden, B.A., England, P.R., Taylor, A.C., Greville, W.D. & Sherwin, W.B. (1996). Low genetic variability of the koala *Phascolarctos cinereus* in south-eastern Australia following a severe population bottleneck. *Mol. Ecol.* **5**, 269–281.

Hoy, J.A., Hoy, R., Seba, D. & Kerstetter, T.H. (2002). Genital abnormalities in white-tailed deer (*Odocoileus virginianus*) in west-central Montana: pesticide exposure as a possible cause. *J. Environ. Biol.* **23**, 189–197.

Jones, K.C., Levine, K.F. & Banks, J.D. (2000). DNA-based genetic markers in black-tailed and mule deer for forensic applications. *Calif. Fish Game* **86**, 115–126.

Keller, L.F. & Waller, D.M. (2002). Inbreeding effects in wild populations. *Trends Ecol. Evol.* **17**, 230–241.

Latch, E.K. & Rhodes, O.E. (2005). The effects of gene flow and population isolation on genetic structure of reintroduced wild turkey populations: are genetic signatures of source populations retained? *Conserv. Genet.* **6**, 981–997.

Leader-Williams, N. (1979). Abnormal testes in reindeer, *Rangifer tarandus*. *J. Reprod. Fert.* **57**, 127–130.

Lewis, J.P. (1991). *Assessment of the Exxon-Valdez oil spill on the Sitka black-tailed deer in Prince William Sound and the Kodiak Archipelago: Terrestrial study number 1*. Anchorage: Alaska Department of Fish and Game.

Lewis, P.O. & Zaykin, D. (1999). Genetic Data Analysis: computer program for the analysis of allelic data, version 1.1. Free program distributed by the authors over the internet from the GDA homepage at http://hydrodictyon.eeb.uconn.edu/people/plewis/software.php

Lincoln, G.A. (1973). Appearance of antler pedicles in early foetal life in red deer. *J. Embryol. Exp. Morphol.* **29**, 431–437.

Lucid, M. & Cook, J.A. (2004). Phylogeography of Keen's mouse (*Peromyscus keeni*) in a naturally fragmented landscape. *J. Mammal.* **85**, 1149–1159.

Luikart, G., Allendorf, F.W., Cornuet, J.M. & Sherwin, W.B. (1998). Distortion of allele frequency distributions provides

a test for recent population bottlenecks. *J. Hered.* **89**, 238–247.

MacDonald, S.O. & Cook, J.A. (1996). The land mammal fauna of southeast Alaska. *Can. Field Nat.* **110**, 571–598.

Menotti-Raymond, M. & O'Brien, S.J. (1995). Hypervariable genomic variation to reconstruct the natural history of populations – lessons from the big cats. *Electrophoresis* **16**, 1771–1774.

Meredith, E.P., Rodzen, J.A., Levine, K.F. & Banks, J.D. (2005). Characterization of an additional 14 microsatellite loci in California elk (*Cervus elaphus*) for use in forensic and population applications. *Conserv. Genet.* **6**, 151–153.

Mock, K.E., Latch, E.K. & Rhodes, O.E. (2004). Assessing losses of genetic diversity due to translocation: long-term case histories in Merriam's turkey (*Meleagris gallopavo merriami*). *Conserv. Genet.* **5**, 631–645.

Nielen, A.L., Janss, L.L. & Knol, B.W. (2001). Heritability estimates for diseases, coat color, body weight, and height in a birth cohort of boxers. *Am. J. Vet. Res.* **62**, 1198–1206.

O'Brien, S.J. (1994). Genetic and phylogenetic analyses of endangered species. *Annu. Rev. Genet.* **28**, 467–489.

Paetkau, D., Waits, L., Clarkson, P.L., Craighead, L., Vyse, E., Ward, R. & Strobeck, C. (1998). Variation in genetic diversity across the range of North American brown bears. *Conserv. Biol.* **12**, 418–429.

Pritchard, J.K., Stephens, M. & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics* **155**, 945–959.

Pritchard, J.K. & Wen, W. (2003). Documentation for STRUCTURE software: Version 2. Available from http://pritch.bsd.uchicago.edu.

Queller, D.C. & Goodnight, K.F. (1989). Estimating relatedness using genetic markers. *Evolution* **43**, 258–275.

Rand, D.M., Haney, R.A. & Fry, A.J. (2004). Cytonuclear coevolution: the genomics of cooperation. *Trends Ecol. Evol.* **19**, 645–653.

Robinette, W.L. & Jones, D.A. (1959). Antler anomalies of mule deer. *J. Mammal.* **40**, 96–108.

Roelke, M.E., Martenson, J.S. & O'Brien, S.J. (1993). The consequences of demographic reduction and genetic depletion in the endangered Florida panther. *Curr. Biol.* **3**, 340–350.

Rozas, J., Sánchez-DelBarrio, J.C., Messeguer, X. & Rozas, R. (2003). DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics* **19**, 2496–2497.

Sharpe, R.M. (2006). Pathways of endocrine disruption during male sexual differentiation and masculinisation. *Best Pract. Res. Clin. Endocrinol. Metab.* **20**, 91–110.

Smith, R.B. (1979). History and current status of Sitka black-tailed deer in the Kodiak Archipelago. In *Sitka black-tailed deer, proceedings of a conference*: 184–195. Wallmo, O.C. & Schoen, S.W. (Eds). Juneau: Alaska Department of Fish and Game.

Stephen, C.L., Whittaker, D.G., Gillis, G., Cox, L.L. & Rhodes, O.E. (2005). Genetic consequences of reintroductions: an example from pronghorn antelope in Oregon. *J. Wildl. Mgmt.* **69**, 1463–1474.

Straugh, T.B., Converse, P. & White, K. (2004). *2003 deer hunter survey summary statistics; southeast Alaska August 2003–January 2004 hunting season*. Juneau: Alaska Department of Fish and Game.

Templeton, A.R., Crandall, A.K. & Sing, C.F. (1992). A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. *Genetics* **132**, 619–633.

Thomas, C.D., Cameron, A., Green, R.E., Bakkenes, M., Beaumont, L.J., Collingham, Y.C., Erasmus, B.F.N., de Siqueira, M.F., Grainger, A., Hannah, L., Hughes, L., Huntley, B., van Jaarsveld, A.S., Midgley, G.F., Miles, L., Ortega-Huerta, M.A., Peterson, A.T., Phillips, O.L. & Williams, S.E. (2004). Extinction risk from climate change. *Nature* **427**, 145–148.

Van Daele, L.J. (2001). Unit 8 deer management report. In *Deer management report of survey and inventory activities 1 July 1998–30 June 2000*: 93–100. Hicks, M.V. (Ed.). Juneau: Alaska Department of Fish and Game.

Van Daele, L.J. (2005). Unit 8 deer management report. In *Deer management report of survey and inventory activities 1 July 2002–30 June 2004*: 109–125. Brown, C. (Ed.). Juneau: Alaska Department of Fish and Game.

Veeramachaneni, D.N.R., Amann, R.P. & Jacobson, J.P. (2006). Testis and antler dysgenesis in Sitka black-tailed deer on Kodiak Island, Alaska: Sequela of environmental endocrine disruption. *Environ. Health Perspect.* **114** (Suppl. 1; Ecology Special Issue): 51–59.

Vidaeff, A.C. & Sever, L.E. (2005). In utero exposure to environmental estrogens and male reproductive health: a systematic review of biological and epidemiologic evidence. *Reprod. Toxicol.* **20**, 5–20.

Weckworth, B., Talbot, S., Sage, G., Person, D. & Cook, J. (2005). A signal for independent coastal and continental histories for North American wolves. *Mol. Ecol.* **14**, 917–931.

Williams, C.L., Lundrigan, B. & Rhodes, O.E. (2004). Microsatellite DNA variation in tule elk. *J. Wildl. Mgmt.* **68**, 109–119.

Wilson, G.A., Strobeck, C., Wu, L. & Coffin, J.W. (1997). Characterization of microsatellite loci in caribou *Rangifer tarandus*, and their use in other artiodactyls. *Mol. Ecol.* **6**, 697–699.