Estimating deer colonization rates to offshore islands of Haida Gwaii using microsatellite markers

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Abstract

Sitka black-tailed deer *Odocoileus hemionus sitkensis* were introduced to Haida Gwaii (Queen Charlotte Islands, British Columbia) in the late 1800s, after which they greatly increased in numbers and dispersed throughout the archipelago. In an attempt to reduce the major ecological impact of these introductions, deer populations were culled from two of the most remote of the islands, Reef Island and SGang Gwaay. We estimate here the colonization/dispersal rates of deer to each of these islands from the adjacent source islands, Louise and Moresby, through analysis of 10 microsatellite DNA markers in 141 deer. Eight of the loci surveyed had two alleles, whereas two loci had three alleles. Allelic diversity and allele frequencies were similar between SGang Gwaay and Moresby Island ($F_{st} = 0.1$, $P < 0.01$), between Reef and Louise islands ($F_{st} = 0.072$, $P < 0.01$), and between SGang Gwaay and Reef Island ($F_{st} = 0.079$, $P < 0.01$). N_em values derived from F_{st} values suggest that there are 2.23 migrants per generation between Moresby Island and SGang Gwaay and 3.22 migrants per generation between Louise Island and Reef Island, or approximately one individual per year. These high dispersal rates will reduce the efficacy of deer removal programs.

1. Introduction

One of the most widely distributed exotic species on Haida Gwaii (Queen Charlotte Islands, British Columbia) is the Sitka black-tailed deer *Odocoileus hemionus sitkensis*, which was introduced in the late 1800s, after which the species greatly increased in number and dispersed throughout much of the archipelago (Foster 1965; Carl and Guiget 1972). Two of the remote islands in the southern regions of the archipelago, Reef Island and SGang Gwaay, which contain deer, were chosen for a deer removal experiment and to evaluate the subsequent rate and extent of ecosystem recovery. Reef Island, located 6 km to the southeast of Louise Island, is one of the most distant of the small islands of the archipelago, while SGang Gwaay is located 2 km off the southern tip of Moresby Island but is separated by strong tidal currents. Deer dispersed to these islands by swimming, but the timing of their original colonization is currently unknown, although it probably occurred within the first five decades of their initial introduction to the large islands (Vila and Martin this volume). While mule deer *Odocoileus hemionus* are known to swim up to 25 km between islands in the Great Lakes, the remoteness of the oceanic islands and high current regime, usually with extensive surface wave action, suggest that there may have been only a single or a low number of colonization events.

In this paper, we take a genetic approach to estimating colonization/migration rates of deer to Reef Island and SGang Gwaay from the adjacent source islands, Louise and Moresby. Ten microsatellite DNA genetic markers were surveyed in samples from each of the locales and analyzed to determine to what degree the ocean distance separating the islands presents a barrier to migration.

2. Methods

As a result of culling during 1998 to 2000, tissue samples were obtained from 150 black-tailed deer from multiple sites on Haida Gwaii. Sixty deer were taken from Reef Island and 38 deer from SGang Gwaay. This was thought to comprise the majority (>90%) of the deer population on each island. Thirty-three deer were sampled on Louise Island, the most probable source population for migration of deer to Reef Island, and 19 deer were sampled on the south end of Moresby Island, the most probable source of animals colonizing SGang Gwaay.

2.1 Extraction of DNA, polymerase chain reaction, and electrophoretic conditions

Crude DNA extracts were prepared from liver tissue according to the method of Nelson et al. (1998). Each 25-µL polymerase chain reaction (PCR) required 1 µL of crude extract. We collected genotypic data for 10 microsatellite DNA loci. All loci are listed along with PCR annealing temperatures and gel electrophoresis times in Table 1. An MJ PTC-100 thermal cycler (MJ Research, Watertown, Massachusetts) was used to carry out PCR in 96-well microtitre plates; each reaction of 25 µL contained 10 pmol (0.4 µM) of each primer, 80 µM of each nucleotide, 20 mM Tris pH 8.8, 2 mM $MgSO_4$, 10 mM KCl, 0.1% Triton X-100, 10 mM $(NH_4)SO_4$, and 0.1 mg/mL bovine serum albumin. After a 3-minute incubation at 94°C, PCR mixtures were held at 80°C while 1 unit of Taq DNA polymerase was added, following which

Table 1
Annealing temperature and electrophoresis run time for deer microsatellite DNA loci

Locus	Annealing temperature (°C)	Run time (hours)	Reference
ILSTS001	50	15	Kemp et al. 1995
Cervid1	50	18	DeWoody et al. 1995
OarJMP12	50	15	Lumsden et al. 1996
ILSTS52	52	18	Kemp et al. 1995
ILSTS65	52	15	Kemp et al. 1995
T193	55	18	Jones et al. 2002
DeerC273	55	15	Jones et al. 2002
DeerC89	50	15	Jones et al. 2000
DeerT7	48	18	Jones et al. 2000
DeerT32	57	18	Jones et al. 2000

temperature cycling was initiated. Both the denaturation and extension PCR steps were for 30 seconds at 94°C and 72°C, respectively. Following amplification, 3 µL of 10X loading dye (50 mM EDTA pH 8.0, 30% glycerol, 0.25% bromphenol blue) was added to each reaction, and 10 µL of this solution was loaded per gel electrophoresis lane.

Microsatellite alleles were size-fractionated on nondenaturing polyacrylamide gels 17 cm wide by 14.5 cm long, containing acrylamide to bis-acrylamide in a 19:1 ratio to a total acrylamide concentration of 10%. Gels contained 2X TAE buffer (Sambrook et al. 1989), as did the running buffer. Each gel included three 20 base pair marker (GenSura Labs Inc., Del Mar, California) lanes to create a molecular size grid and 24 individual deer samples. Gels were stained with 0.5 µg/mL ethidium bromide in water and visualized with ultraviolet light. Digital images of gels were obtained with an Eagle Eye system (Stratagene Corp., San Diego, California). Gels were manually scored using Intelligent Quantifier software (Millipore Corp., Bedford, Massachusetts).

2.2 Data analysis

Each sample was tested for departures from Hardy-Weinberg genotypic proportions with the exact test of Guo and Thompson (1992) using GENEPOP version 3.1 (Raymond and Rousset 1997); probability values were corrected with the sequential Bonferroni technique (Holm 1979; Rice 1989) with the initial significance level taken to be 0.05/number of loci (10). Similarly, testing of genetic homogeneity between samples was carried out as described by Raymond and Rousset (1995); this was accomplished with GENEPOP, and probability values were adjusted with the sequential Bonferroni technique with the initial significance level taken to be 0.05/number of loci (10).

F-statistics (Wright 1951) were computed according to Weir and Cockerham (1984) with GENETIX version 4.02, and the number of migrants exchanged per generation (N_em) was also estimated using GENETIX based on the approximation that $N_em = (1 - F_{st}) / 4F_{st}$ (Wright 1969); the significance of F_{st} values was tested by performing 500 permutations.

3. Results

We obtained data regarding genetic heterogeneity at 10 polymorphic microsatellite DNA loci from 141 deer representing four different locales. We calculated whether there were departures from Hardy-Weinberg for each locus within samples from each locality. Of the 40 comparisons possible, only one showed a statistically significant ($P < 0.005$) departure from Hardy-Weinberg, the *ILSTS65* locus at Reef Island. Tests of genetic homogeneity of samples showed that the 1999 and 2000 samples from Louise Island were not significantly different, nor were the 1998 and 1999 samples from SGang Gwaay, so these samples were pooled to increase sample size in further analysis. All locus-by-locus tests of allele frequency homogeneity between Moresby and Louise islands indicated that these two samples were not statistically different, and these were also pooled in further analysis; this pooled sample is referred to as "source."

Eight of the loci surveyed had two alleles, whereas two loci (*T32*, *ILSTS65*) had three alleles (Table 2). Two alleles were observed at locus *C89* in both the source population and Reef Island, whereas only a single allele was observed at this locus in the SGang Gwaay samples. Similarly, at locus *T273*, two alleles were found in the source population and on SGang Gwaay, whereas only a single allele was observed at this locus in the Reef Island samples. No alleles were absent in any of the other samples at any of the other loci. Locus-by-locus comparisons of allele frequencies between SGang Gwaay and the source population and between Reef Island and the source population indicate that these samples are not drawn from the same gene pool, suggesting population subdivision.

Further insight into the pattern of population subdivision between the locales sampled was gained by computing *F*-statistics (Table 3). There was a significant difference in genetic structure between SGang Gwaay and its source ($F_{st} = 0.1$, $P < 0.01$) and between Reef Island and the source ($F_{st} = 0.072$, $P < 0.01$). There were also significant differences between SGang Gwaay and Reef Island ($F_{st} = 0.079$, $P < 0.01$). The N_em values derived from these F_{st} values suggest that 2.23 migrants per generation are exchanged between SGang Gwaay and the source population, while 3.22 migrants are exchanged per generation between Reef Island and the source population.

4. Discussion

We have used microsatellite alleles to estimate deer colonization events for offshore islands of Haida Gwaii. Our data are inconsistent with a single colonization event but rather suggest high levels of dispersal, approximately 2–3 deer per generation among the islands. As generation time in black-tailed deer on Haida Gwaii is 2 or 3 years, this suggests a persistent dispersal of about one deer per year to both Reef Island and SGang Gwaay. As Reef Island represents one of the most remote islands in the archipelago, there will probably be even higher migration rates among the more numerous, more proximate islands in the archipelago.

It is possible that our results may have overestimated migration rates among the islands. This is because the method used to calculate migration does not

Table 2
Allele frequencies for all microsatellite loci in Sitka black-tailed deer samples from Haida Gwaii

Locus	Allele	Source	SGang Gwaay	Reef
DeerT7	1	0.24	0.316	0.07
	2	0.76	0.684	0.93
	N[a]	50	38	50
ILSTS65	1	0.212	0.132	0.108
	2	0.692	0.513	0.608
	3	0.096	0.355	0.284
	N	52	38	51
DeerC89	1	0.279	0	0.13
	2	0.721	1	0.87
	N	52	38	50
DeerT273	1	0.167	0.027	0
	2	0.833	0.973	1
	N	51	37	51
OarJMP1	1	0.288	0.486	0.45
	2	0.712	0.514	0.55
	N	51	37	50
LSTS001	1	0.5	0.139	0.255
	2	0.5	0.861	0.745
	N	51	36	49
DeerT32	1	0.038	0.081	0.19
	2	0.798	0.446	0.61
	3	0.163	0.473	0.2
	N	52	37	50
T193	1	0.346	0.197	0.48
	2	0.654	0.803	0.52
	N	52	38	49
ILSTS52	1	0.298	0.368	0.373
	2	0.702	0.632	0.627
	N	52	38	51
Cervid 1	1	0.817	0.855	0.51
	2	0.183	0.145	0.49
	N	52	28	51

[a] *N* = number of animals successfully analyzed for each locus.

Table 3
Pairwise F_{st} and N_em between Sitka black-tailed deer source populations from Moresby/Louise islands and offshore islands (Reef and SGang Gwaay) on Haida Gwaii

	F_{st}[a]	
	SGang Gwaay	Reef
Source (Moresby/Louise islands)	0.1	0.072
SGang Gwaay		0.079
	N_em	
	SGang Gwaay	Reef
Source (Moresby/Louise islands)	2.23	3.22
SGang Gwaay		2.92

[a] All F_{st} values are significant ($P < 0.01$).

distinguish between ongoing genetic exchange and historical association between locales. Similarity in allele frequencies can result from a single recent colonization event, with only a small amount of divergence due to genetic drift. Currently, we cannot exclude this possibility, but suspect that it is unlikely. At SGang Gwaay, where we estimate about two migrants per generation or one per year based on genetic data, direct empirical observations by a Haida resident on the island (Captain Gold, pers. commun.) show 10 individual dispersal events over a 30-year period (1970–2000), or about 1 every 3 years. This count will be highly conservative, as the observational period represents only a small proportion of the total time. Consequently, the number of deer swimming to the islands will be substantially higher; as a result, our estimates of one per year are probably conservative rather than overestimated. The high estimated migration rate between SGang Gwaay and Reef Island (Table 3), which seems improbable given the geographical separation of these two islands, is probably due to ongoing gene flow from the common source population (Moresby/Louise islands), rather than actual direct exchange of genetic material through migration between SGang Gwaay and Reef Island.

Several conservation implications emerge from our genetic results. Firstly, deer dispersal events, even among the more distant of the offshore islands, appear to be common. If true, single-event deer removal programs will be ineffective as an ecological tool. A single new female colonist and a single male can potentially generate a population near carrying capacity within a decade on each of these islands. Consequently, these islands with deer removal would require yearly assessment for deer occurrence and removal. Secondly, while empirical estimates of the original colonization of individual islands by deer obtained by dendrochronological analyses (Vila and Martin this volume, and references therein) suggest that most islands were already heavily impacted around the mid-20th century, our genetic data on dispersal are consistent with an initial colonization shortly after the late-19th- and early-20th-century introduction on Graham and Moresby islands.

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