Microsatellite DNA markers for the study of population structure in the New Zealand fur seal *Arctocephalus forsteri*

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Bruce C. Robertson and Neil J. Gemmell

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Bruce C. Robertson and Neil J. Gemmell

School of Biological Sciences, University of Canterbury,
Private Bag 4800, Christchurch, New Zealand
email: bruce.robertson@canterbury.ac.nz

ABSTRACT

New Zealand trawl fisheries have accounted for an estimated 10 000 deaths of New Zealand fur seal (*Arctocephalus forsteri*) between 1990 and 2000, with over half occurring in the West Coast Hoki fishery. To assess the impact of bycatch on separate colonies, the effects of commercial fishing bycatch and environmentally driven fluctuations need to be disassociated by identifying the original breeding colony of individual fur seals subject to incidental bycatch. Here we have identified 23 polymorphic loci from a battery of 31 seal-specific microsatellite markers. Using eight of these loci, we have examined the genetic variability and relationships among seven *A. forsteri* breeding colonies around New Zealand. Colonies showed only low levels of genetic differentiation (all F_{ST} values <0.035), which is consistent with moderate levels of gene flow and an expanding population. Despite this limited genetic differentiation, assignment testing resulted in c. 42% of individuals being assigned to their colony of origin and c. 70% of individuals assigned to their region (West Coast versus East Coast). This study represents an essential first step towards the long-term goal of determining the breeding colony provenance of *A. forsteri* killed as fisheries bycatch.

Keywords: genetics, microsatellites, population structure, bycatch, New Zealand fur seals, *Arctocephalus forsteri*, West Coast, New Zealand

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1. Introduction

Trawl fisheries in New Zealand waters are estimated to have accounted for over 10 000 New Zealand fur seal (*Arctocephalus forsteri*) deaths between 1990 and 2000 (Baird 1994, 1999, 2001a, 2001b; Manly et al. 1999). Almost half of these fatalities have occurred in the South Island's West Coast Hoki fishery. Modelling of population dynamic data collected from three West Coast colonies since 1990 show that environmental fluctuations (e.g. El Niño; Bradshaw et al. 2000a) render these populations extremely vulnerable to the impact of incidental fisheries bycatch (H. Best and Starfield unpubl. data). To characterize the impact of bycatch on separate colonies, the effects of commercial fishing bycatch and environmentally driven fluctuations need to be disassociated. This can only be done by identifying the original breeding colony of individual fur seals subject to incidental bycatch.

A. forsteri are philopatric. Females return to their natal site each year to breed, while slightly fewer males return to their natal sites (Reidman 1990). Tagging of pups at natal sites can provide a useful monitoring tool, but requires years of intensive tagging to return useful data (Bradshaw et al. 2000b), as the age of sexual maturity is 4 years in females and 10–12 years in males (King 1990) and tag loss is common. Recent advances in molecular genetics have provided a valuable means of identifying population structure, especially with regard to defining units of conservation, management and evolutionary significance (see Moritz 1994). This genetic approach has uncovered some surprising patterns of genetic population structuring: (e.g. Worthington Wilmer et al. 1994). Concomitant advances in the statistical analysis of molecular data (Luikart & England 1999) now enable individuals to be assigned to their natal area with high certainty (Cornuet et al. 1999; Primmer et al. 2000).

Previous genetic studies of *A. forsteri* population structure (Lento et al. 1994, 1997) suggested high genetic diversity in the population as a whole, with limited gene flow between broad geographic regions. Wynen et al. (2001) reconfirmed the findings of Lento et al. (1994, 1997). All studies examined mitochondrial DNA, but failed to find any colony-specific markers with which to examine the impact of fisheries bycatch on specific colonies. Wynen (2001) examined genetic variation at 10 microsatellite loci among 16 *A. forsteri* from subantarctic Macquarie Island. Moderate levels of genetic diversity were noted, suggesting microsatellite markers should be informative in investigating population structure of *A. forsteri*.

Microsatellite markers (highly polymorphic 2–5 base pair repeat sequences) are the primary molecular marker employed for studies comparing the differentiation of human and other populations (Bruford & Wayne 1993). A range of microsatellite markers are available for pinnipeds and many display cross-species application (Coltman et al. 1996; Gemmell et al. 1997) and have been used successfully for population studies on other seal species (Gemmell et al. 2001; Kretzmann et al. 2001; Trujillo et al. 2004).

In this study, we have identified 8 pinniped microsatellite markers from a battery of 31 primer sets that are informative for *A. forsteri*. These markers

were used to examine the genetic variability and relationships among *A. forsteri* breeding colonies around New Zealand, an essential first step towards the long-term goal of determining the colony provenance of *A. forsteri* killed as fisheries bycatch.

2. Methods

2.1 SAMPLING

Arctocephalus forsteri are oceanic and capable of significant dispersal, which presents the possibility of seasonal variation in the genetic composition of populations. To avoid this potentially confounding variable we sampled pups of the year, which are the direct representatives of their natal site for a given year, for our genetic study. Genetic samples were obtained for 305 *A. forsteri* pups at 7 breeding colonies (Fig. 1) by removing a skin sample from the interdigital margin of the foreflipper using piglet ear-notch pliers (Majluf & Goebel 1992).

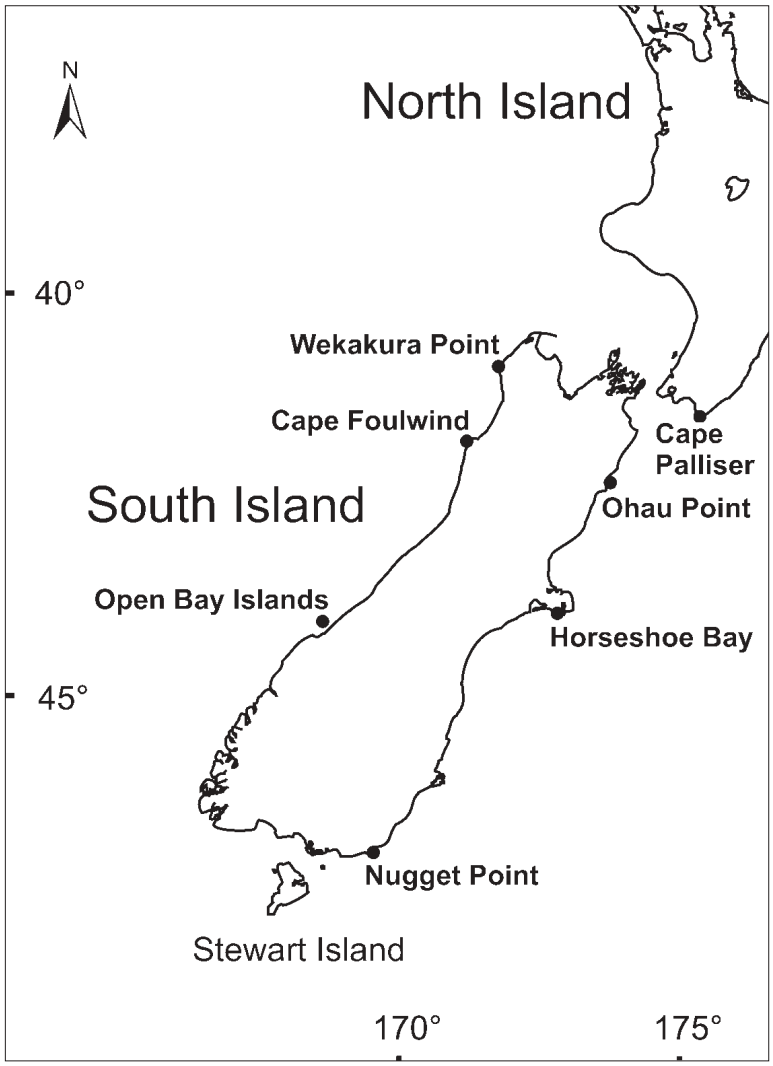

Figure 1. Locations of the seven breeding colonies sampled in the current study of *A. forsteri* population structure.

2.2 IDENTIFICATION OF INFORMATIVE PINNIPED MICROSATELLITE MARKERS

As part of the current study and a previous investigation (Gemmell et al. 1997), we screened genetic variation in fur seals using a battery of pinniped primers to identify informative microsatellite loci. Using up to 10 individuals, a total of 31 pinniped-specific microsatellite markers were screened for genetic variation in *A. forsteri* (Table 1). All trials used the protocol described in Gemmell et al. (1997).

2.2.1 Microsatellite genotyping

Whole genomic DNA was extracted from all samples using c. 1 mm^3 of tissue and a 5% chelex protocol (adapted from Walsh et al. 1991).

All amplification of microsatellite markers was achieved by polymerase chain reaction (PCR) and direct incorporation of αP^{33}-dCTP. The polymerase chain

TABLE 1. GENETIC DIVERSITY IN *A. forsteri* AT 31 PINNIPED-SPECIFIC MICROSATELLITE LOCI. The eight **embolded** loci were used in the present analysis.

LOCUS	N	ALLELE SIZE (bp)	NO. ALLELES
Aa4	7	216	1
Pv3	7	117	1
Pv9	7	166–180	7
Pv10	7	131	1
Pv11	**7**	**156–174**	**6**
Pv16	7	114–126	6
Pv17	7	157–167	5
Hgdii	7	N/A	
Hg0	7	149–153	3
Hg1.3	7	252–260	4
Hg1.4	7	195–205	6
Hg3.6	7	92–104	7
Hg3.7	7	372–410	4
Hg4.2	**7**	**138–186**	**11**
Hg6.1	7	145–153	3
Hg6.3	**7**	**234–248**	**5**
Hg8.9	7	250	1
Hg8.10	**7**	**170–204**	**10**
M11a	**7**	**144–168**	**5**
BG	7	250–251	1
Hl15	5	146	1
Hl16	5	149–189	7
Hl20	5	107–119	3
Lw5	5	159	1
Lw10	5	116–132	5
Lw18	5	109–111	2
Lc5	**5**	**157–163**	**4**
Lc18	5	293–301	5
Lc28	**5**	**134–156**	**5**
G1A	5	189–207	6
PvcA	**10**	**144–170**	**11**

N/A = no amplification; bp = base pairs.

reaction was carried out in 10 µL reactions containing c. 50 ng of template DNA, 0.5 pmol of each primer, 5 nmol each of dATP, dGTP, and dTTP, 0.5 nmol of dCTP, 0.1 µCi of αP^{33}–dCTP, 50 mM KCl, 10 mM Tris-HCl at pH 9.0, 1.5 mM $MgCl_2$ and 0.1 unit of *Taq* polymerase (ROCHE NZ, Auckland). The thermal cycling parameters were an initial 2-min denaturation step at 94°C, followed by: 5 cycles, each of 94°C/15 s, first primer specific annealing temperature (see Table 2) /20 s and 72°C/25 s; and then 30 cycles each of 94°C/15 s, second primer specific annealing temperature (see Table 2) /20 s and 72°C/25 s. Following amplification, PCR products were size-fractionated on 6% denaturing PAGE gels and exposed to Kodak Biomax MR autoradiography film (Radiographic Supplies, Christchurch). The size of amplified fragments was scored manually against a sequencing reaction (forward M13 primer and pBSMB plasmid).

2.2.2 Data analysis

Allele number, allele frequency, observed heterozygosity (H_o) and inbreeding coefficient (F_{IS}) were calculated for each locus in all colonies using GENEPOP 3.1 (Raymond & Rousset 1995). The F_{IS} value provides an indication of population inbreeding when consistently large across multiple loci within a colony; and of null alleles (alleles that do not amplify due to mutations in the PCR primer sites) when large values occur at a single locus within a colony Pemberton et al. (1995). Genetic diversity was summarized for each population using the average number of alleles per locus and average observed heterozygosity. We tested for deficiency of heterozygotes, under the assumption of a Hardy-Weinberg (HW) equilibrium, for all loci using randomisation tests implemented in GENEPOP 3.1. Departures from this equilibrium would be expected if sub-structure was present in the form of localized heterozygote deficiencies which is suggestive of localized inbreeding.

We investigated genetic population structure among the seven colonies of fur seals using three approaches. First, we compared allele frequency distributions in both populations using exact probability tests in GENEPOP 3.1, where the explicit assumption is that significant differences in allele frequency distributions are indicative of reproductively isolated populations. Second, we calculated a pair-wise estimate of the fixation index F_{ST} (θ; Weir & Cockerham 1984) as a measure of genetic differentiation over subpopulations (Hedrick 1999) and tested this value for significant departure from zero using permutation procedures in FSTAT (Goudet 2001). Third, we examined the assignment of individuals to their colony and region (i.e. East Coast versus West Coast) in which the likelihood of their genotype is highest using two individual assignment tests: a Bayesian-based approach (Rannala & Mountain 1997), which combines prior beliefs about the probability of a hypothesis with the likelihood of the hypothesis; and assignment based on reference population allele frequencies (Paetkau et al. 1995). Assignment testing was done in GENECLASS v.1.0.02 using observed allele frequencies and the 'leave one out' option (Cornuet et al.1999).

3. Results

We identified a total of 23 polymorphic loci from a total of 31 pinniped-specific microsatellite loci tested (see Table 1; Gemmell et al. 1997). Fifteen of these markers had greater than five alleles, which is a level of polymorphism comparable to that found in other otariid seals (Gemmell et al. 1997). We chose the eight loci that showed the best combination of polymorphism and ease of amplification and scoring (Gemmell et al. 1997) to investigate *A. forsteri* genetic population structure.

The eight loci showed considerable polymorphism across the seven colonies with a mean of 18.5 alleles per locus (range, 7–25; Table 2). At the colony level, all loci displayed polymorphism, with an average of 12.02 alleles per locus (total range, 5–22) and an average H_O of 0.70 (Table 3). We found no evidence of genotypic disequilibrium using a global test for each pair of loci across all seven colonies (Fishers exact tests, df = 14, all $P > 0.2$) indicating independent assortment of all loci.

A global test of HW proportions (all loci and all colonies) revealed significant heterozygote deficiency compared to expected values in the New Zealand population of fur seals (Markov chain method, $P < 0.0001$), suggesting the presence of population sub-structuring at the level of the colony. We also noted departure from HW proportions when examining all loci within a colony (all colonies, $P < 0.001$). Departure from HW proportions at the colony level is suggestive of non-random mating (inbreeding), selection or the presence of null alleles. Inbreeding, as measured by F_{IS}, had a mean of 0.16 ($P < 0.05$) across all seven colonies. When examining each locus within a colony and correcting for

TABLE 2. PRIMER SEQUENCES, FIRST/SECOND ANNEALING TEMPERATURES, ALLELE SIZES, AND ALLELE NUMBER FOR EACH OF THE EIGHT PINNIPED MICROSATELLITE LOCI USED IN THIS STUDY.

LOCUS	PRIMER SEQUENCE (5'→3')	ANNEALING TEMP. (°C)	SIZE (bp)*	NO. ALLELES	REFERENCE
Hg4.2	F: AATCGAAATGCTGAGCCTCC R: TGATTTGACTTCCCTTCCCTG	54.0/58.0	132–188	25	Allen et al. (1995)
Hg6.3	F: CAGGGGACCTGAGTGCTTATG R: GACCCAGCATCAGAACTCAAG	51.5/53.5	220–248	14	Allen et al. (1995)
Hg8.10	F: AATTCTGAAGCAGCCCAAG R: GAATTCTTTTCTAGCATAGGTTG	54.0/58.0	166–212	24	Allen et al. (1995)
Lc5	F: TCATATAATACCCACCTCTGTAAG R: TGCCTCGGTGATGAAAAACT	48.0/52.0	158–174	7	Davis et al. (2002)
Lc28	F: ATCTTCAGGCTTTCTTCT R: TTCACGGACTCAAATAAT	48.0/52.0	135–159	13	Davis et al. (2002)
M11a	F: TGTTTCCCAGTTTTACCA R: TACATTCACAAGGCTCAA	43.4/47.4	139–181	20	Hoelzel et al. (2001)
PvcA	F: GAGTATACCTCCATACTACAC R: AGTTGTTCTCCTGACCCAAG	48.7/52.8	144–170	21	Coltman et al. (1996)
Pv11	F: GTGCTGGTGAATTAGCCCATTATAAG R: CAGAGTAAGCACCCAAGGAGCAG	43.4/47.4	151–193	24	Goodman (1998)

* bp = base pairs.

TABLE 3. GENETIC DIVERSITY AT EIGHT MICROSATELLITE LOCI FOR THE SEVEN COLONIES OF *A. forsteri* (n = 305 pups).

REGION	WEST COAST									EAST COAST											
COLONY	OPEN BAY ISLANDS			CAPE FOULWIND			WEKAKURA POINT			CAPE PALLISER			OHAU POINT			HORSESHOE BAY			NUGGET POINT		
N =	82			54			24			41			53			26			25		
Locus	A_N	H_O	F_{IS}	A_N	H_O	F_{IS}	A_N	H_O	F_{IS}	A_N	H_O	F_{IS}	A_N	H_O	F_{IS}	A_N	H_O	F_{IS}	A_N	H_O	F_{IS}
Hg4.2	19	0.82	0.07	15	0.65*	0.23*	9	0.47	0.34	17	0.83	0.06	16	0.72	0.14	10	0.72	0.11	13	0.70*	0.23
Hg6.3	12	0.65*	0.27*	8	0.67	0.20	10	0.61	0.30	12	0.69	0.20	11	0.63*	0.28*	7	0.19*	0.74*	7	0.78*	-0.06
Hg8.10	22	0.85*	0.08	20	0.87	0.07	15	0.88	0.06	19	0.68*	0.27*	20	0.65*	0.29*	13	0.88	0.04	17	0.56*	0.40*
Lc5	6	0.80	-0.03	7	0.81	-0.02	5	0.70	0.10	5	0.66	0.12	6	0.74	0.01	5	0.80	-0.10	6	0.79	-0.02
Lc28	13	0.79	0.10	11	0.69	0.20*	9	0.70	0.19	9	0.83	0.03	12	0.82	0.06	10	0.69	0.20	10	0.84	0.04
M11a	19	0.65	0.23*	17	0.83	0.01	12	0.81	0	16	0.63*	0.26*	14	0.65	0.19	11	0.88	-0.05	10	0.71	0.18
PvcA	12	0.66*	0.25*	10	0.45*	0.48*	12	0.67	0.17	13	0.66	0.25	12	0.72*	0.18	8	0.50*	0.41*	12	0.65*	0.22
Pv11	19	0.66	0.19	14	0.68	0.19	10	0.46	0.34	13	0.68*	0.12	14	0.63	0.09	11	0.65	0.21	8	0.71	-0.06
Mean	15.3	0.74	0.15*	12.8	0.71	0.17*	10.3	0.66	0.19*	13	0.71	0.16*	13.1	0.70	0.16*	9.4	0.66	0.20*	10.3	0.72	0.12*
SEM	1.9	0.03	0.04	1.6	0.05	0.06	1.0	0.05	0.05	1.6	0.03	0.03	1.4	0.02	0.04	0.9	0.08	0.10	1.3	0.09	0.17

* $P < 0.05$, after sequential Bonferroni correction.

A_N = allele number; H_O = observed heterozygosity; F_{IS} = inbreeding coefficient.

multiple tests, six loci were not in HW proportions in various colonies (Table 3). A general lack of concordance in heterozygosity deficiency among colonies and the observation that F_{IS} values for these loci/colony combinations were not consistently large or significantly different from zero (Table 3), suggests that the heterozygote deficiency is unlikely to be the result of the presence of null alleles. However, in the case of PvcA, five of the seven colonies displayed heterozygote deficiency and moderate inbreeding coefficients, which might indicate null alleles at this locus.

Population differentiation based on patterns of allele frequencies was noted in 35% of 147 pairwise colony comparisons for all eight loci (Table 4). Of the West Coast colonies, Cape Foulwind showed the greatest number of loci with significant genic differentiation (Table 4). Horseshoe Bay differed from all other populations for at least some loci, while Nugget Point showed the most genic differences in the East Coast (Table 4).

TABLE 4. NUMBER OF MICROSATELLITE LOCI (n = 8 loci) SHOWING SIGNIFICANT* GENIC DIFFERENTIATION IN 147 PAIRWISE COMPARISONS OF EACH FUR SEAL COLONY.

COLONY	WP	CF	OBI	CP	OP	HBAY	NP
Wekakura Point (WP)		2	0	2	1	1	4
Cape Foulwind (CF)			2	5	6	2	6
Open Bay Islands (OBI)				0	0	3	5
Cape Palliser (CP)					0	2	0
Ohau Point (OP)						5	1
Horseshoe Bay (HBAY)							5
Nugget Point (NP)							

* $P < 0.05$, after sequential Bonferroni correction.

Fixation indices indicate significant, but limited, differentiation among colonies. Pairwise comparison of F_{ST} values between colonies are suggestive of 'an isolation by distance pattern of differentiation' (see Slatkin 1993), in that Nugget Point shows the greatest differentiation with Wekakura Point, Cape Foulwind and the Open Bay Islands (Table 5). Interestingly, Horseshoe Bay shows significant levels of population differentiation from five out of six other colonies, including the closest colonies of Ohau Point (0.029) and Nugget Point (0.035).

The relatively low levels of population differentiation are suggestive of gene flow between colonies. We assessed gene flow between the colonies using the 'private alleles' method of Slatkin (1985). Based on this method, a total of six individuals are exchanged between populations per generation. F_{ST} values are typically used to estimate N_m (the number of migrants per generation), as we have done in Table 5, but these must be interpreted with caution, because recently isolated populations are unlikely to be at equilibrium, a key assumption of this approach (Hartl 1987). Overall, higher levels of gene flow were estimated using this approach (Table 5) than for the 'private alleles' methods.

The observed genetic structure of the New Zealand fur seal population allowed a mean of 42.3% of individuals to be correctly assigned to their colony of origin

TABLE 5. PAIRWISE VALUES OF F_{ST} (above the diagonal) AND MIGRATION RATES (N_m: italics below the diagonal) BETWEEN ALL PAIRS OF POPULATIONS OF *A. forsteri*.

COLONY	WP	CF	OBI	CP	OP	HBAY	NP
Wekakura Point (WP)		0.011	0.019*	0.015	0.014*	0.019	0.026*
Cape Foulwind (CF)	*21.6*		0.006*	0.014*	0.018*	0.010*	0.027*
Open Bay Islands (OBI)	*12.9*	*44.8*		0.005	0.007	0.026*	0.020*
Cape Palliser (CP)	*16.8*	*18.0*	*49.8*		0.009	0.033*	0.011
Ohau Point (OP)	*17.3*	*13.5*	*34.5*	*29.0*		0.029*	0.010
Horseshoe Bay (HBAY)	*12.9*	*26.0*	*9.3*	*7.3*	*8.2*		0.035*
Nugget Point (NP)	*9.3*	*9.1*	*12.6*	*23.1*	*24.8*	*7.0*	

* $P < 0.05$, after sequential Bonferroni correction.

and 69.3% of individuals to be assigned to their correct region. Correct assignment of individual fur seals to their colony/region of origin was more successful using a likelihood-based approach (mean of 42.3% to colony and 69.3% to region) compared with assignments based on probability (mean of 28.5% to colony and 61.3% to region). However, the success of assignment testing varied with region, colony and assignment approach (Table 6). For example, Bayesian probability tests resulted in the greatest mean number of individuals correctly assigned to their region of origin for the West Coast (81.3% of individuals), while for East Coast, frequency likelihood tests were superior (75.3% individuals). In simulation studies, Bayesian assignment has been shown to outperform frequency based assignment tests (Cornuet et al. 1999).

TABLE 6. PERCENTAGE OF INDIVIDUAL FUR SEALS CORRECTLY ASSIGNED TO THEIR COLONY OF ORIGIN AND REGION OF ORIGIN (i.e. East or West Coast) USING BAYESIAN AND FREQUENCY ASSIGNMENT TESTS.

SOURCE POPULATION	n	BAYESIAN				FREQUENCY			
		LIKELIHOOD*		PROBABILITY†		LIKELIHOOD*		PROBABILITY†	
		COLONY	REGION	COLONY	REGION	COLONY	REGION	COLONY	REGION
West Coast									
Wekakura Point	24	38	71	21	71	42	75	21	79
Cape Foulwind	54	46	78	46	93	35	57	43	87
Open Bay Islands	82	38	59	62	80	28	56	48	72
West Coast mean		40.6	69.3	43.0	81.3	35.0	62.6	37.3	79.3
East Coast									
Cape Palliser	41	32	63	29	39	24	66	29	44
Ohau Point	53	43	64	17	34	42	70	28	49
Horseshoe Bay	26	50	54	23	27	62	69	12	30
Nugget Point	25	36	92	8	68	76	96	12	84
East Coast mean		40.3	68.3	19.3	42.0	51.0	75.3	20.3	51.8
Mean for all sites		**40.4**	**68.7**	**29.4**	**58.9**	**44.1**	**69.9**	**27.6**	**63.6**

* Likelihood refers to likelihood-based assignment.

† Probability refers to assignment based on the highest probability of a fur seal's genotype likelihood being within the assignment criteria distribution of the reference colonies based on 10 000 simulated individuals (see Cornuet et al. 1999 for details).

4. Discussion

Initial screening of pinniped-specific microsatellite loci revealed that *A. forsteri* displays similar levels of genetic diversity at these nuclear markers as observed in other otariid species (Gemmell et al. 1997). Most of these species, like *A. forsteri* (Bradshaw et al. 2000b), have been through dramatic population bottlenecks due to human exploitation. Population bottlenecks typically result in the loss of genetic variation by sub sampling of alleles in the population (Hartl 1987). The level of genetic diversity in *A. forsteri*, suggests that sufficient allelic diversity remains in this species for microsatellite markers to be informative in genetic analysis of population structure. The hypervariable nature of microsatellite markers frequently identifies genetic variability where little has previously been documented, but this is not always the case in severely bottlenecked populations (e.g. kakapo, *Strigops habroptilus* Robertson et al. 2000; Hawaiian monk seals, *Monachus schauinslandi*, Kretzmann et al. 2001).

At the broad geographic scale, analysis of genetic variation around the South Island of New Zealand uncovered considerable allelic diversity. Population genetic statistics indicated that this variation was not homogenous. Moderate levels of inbreeding, as estimated by the inbreeding coefficient and consistent departure from HW proportions for all loci, all point to strong population substructuring in fur seals, quite possibly at the colony level. Contrary to this expectation, breeding colonies were not strongly differentiated. We noted only low levels of genetic differentiation, which is consistent with homogenizing gene flow between colonies.

Numbers of fur seals in the New Zealand region are increasing (Bradshaw et al. 2000c) and sites previously used as haul-outs have become breeding colonies in the last 30 years. Range expansion and population growth must occur via migration and subsequent philopatric recruitment. *A. forsteri* are capable of significant dispersal (King 1990). However, in a study of an expanding population of fur seals, Bradshaw et al. (2000c) concluded that colony proliferation occurs via a 'spill-over' effect whereby young individuals chose to breed near established breeders. Female fur seals show strong philopatry, as do males, albeit to a lesser extent (Miller 1971; King 1990). Given this mode of colonization we would expect genetic differentiation to conform to an 'isolation by distance pattern', where geographically distance colonies show greater levels of differentiation due to lower levels of gene flow (see Slatkin 1993). Our analysis found some suggestion of this pattern, but it was confounded by strong genetic differentiation of some neigbouring colonies (i.e. Horseshoe Bay, Ohau Point, and Nugget Point). Dispersal over larger distances undoubtedly occurs in fur seals, as evidenced from tagging studies. Pups tagged at both Tonga Island and Cape Palliser regularly appear at Ohau Point during the breeding season (Laura Boren pers. comm.).

The Horseshoe Bay colony, on Banks Peninsula, is quite distinct genetically and might have been founded from a source separate from other East Coast colonies. Lento et al. (1997) noted two distinct clades of fur seals based on

mitochondrial DNA variation and hypothesized this structure might be the result of range recolonisation by an Australian clade and a New Zealand subantarctic clade. If this is true, the Horseshoe Bay colony might contain remnants of the subantarctic clade, which is now slowly being obscured by gene flow from neighbouring colonies. Examination of allelic variability in the New Zealand subantarctic might prove informative in investigating this interesting hypothesis.

Although many of the fur seal breeding colonies in our study were founded recently by migration and are probably influenced by ongoing gene flow, the statistical tools we employed were able to resolve relationships, despite small differences in allele frequencies between colonies. For the West Coast colonies, up to 41% of individuals could be assigned to their colony-of-origin and almost 70% of individuals to their region, based on significant genic differentiation at a maximum of two of the eight microsatellite loci. This finding suggests that greater resolution of relationships and assignment of individuals to colonies/ regions could be achieved by examining more molecular microsatellite loci, specifically to uncover greater genic differences. Simulation studies of the success of assignment testing indicate that success is related to the number of informative loci examined, while number of individuals examined has little affect on success over 30 individuals (Cornuet et al. 1999). With greater than 10 loci, successful assignment to the population of origin reaches to 100%. Based on this, we conclude that the addition of another four informative loci might increase the success of assignment testing to c. 70% for assignment to correct colony, and to 100% for assignment to correct region.

Our study has identified a further 15 microsatellite loci that appear to be good candidates (based on allelic variability) for future use in population genetic analysis. A greater number of loci would not only increase the success of assignment testing, but would also provide a comprehensive genetic database for the New Zealand fur seal. Such a database would act as a reference collection for the routine identification of individual fur seals subject to incidental bycatch in trawl fisheries to their original breeding colony. Such data would enable the effects of commercial fishing to be disassociated from environmental perturbations.

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