Diversity of microsatellite loci in New Zealand otariids: A pilot study

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Introduction

The otariids are a group which exhibit varied genetic diversity despite similar life parameters and ecology and a common demographic history of exploitation. New Zealand otariids are also subject to current impact by fisheries operations and tourism. Previous surveys of mitochondrial DNA diversity in three Southern Hemisphere species reveal significant population structuring and suggest that conservation management units may be defined with similar confirmatory surveys of nuclear genetic markers such as microsatellite loci.

The Hooker's sea lion and New Zealand fur seal have similar life parameters and ecology and a common demographic history of exploitation (as summarised by Lento 1995) and represent two contrasting examples of genetic diversity (as measured by mitochondrial DNA analysis; Lento et al. 1994 and 1996). The Hooker's sea lion exhibits a significantly reduced level of mtDNA diversity while the New Zealand fur seal exhibits a surprisingly large amount of mtDNA diversity. The Hooker's sea lion and Australian sea lion compete for the status of the most rare of the five living sea lion species, while other otariid populations, including the New Zealand fur seal, continue an appreciable recovery in size and distribution. In addition, these two species continue to be subject to fisheries-related mortality in Australia and New Zealand (Mattlin 1994; Cawthorn and Gibson 1994). A study comparing microsatellite diversity of these two species would be valuable in increasing the current resolution of population structure and estimates of genetic diversity in these species. These results may also contribute information relevant to management directives by providing a means to determine the provenance of bycatch animals.

Microsatellites are an abundant class of tandem repeats of 2-5 nucleotides widely dispersed in the nuclear genome (Tautz and Renz 1984). They are highly polymorphic in many species due to slippage during DNA replication (Levinson and Gutman 1987). They are inherited according to Mendelian laws and often conserved in closely related species, allowing novel genomes to be examined using PCR primers developed for other species (Coltman et al. 1996). Polymorphic microsatellite loci are assayable using the powerful polymerase chain reaction (PCR) and can be resolved and scored to single nucleotide differences by standard electrophoresis techniques. Because of these useful features, microsatellite loci are fast becoming the molecular tool of choice to address parentage, male reproductive success, genetic variation, population sub-structure, migration, and species identity (Bruford and Wayne 1993; Paetkau et al. 1995). Microsatellites are the logical choice for continuing to resolve apparently significant population structure among these species as indicated by mitochondrial DNA surveys.

Progress to date

A suite of ten microsatellite loci primers have been developed by our collaborators at Cambridge University for use in pinniped species, which are closely related to the New Zealand species of interest. These primers needed to be tested for their ability to cross-react with DNA from the New Zealand species. Polymerase chain reaction (PCR) amplification reactions for three of these primers, Aa4 f/r, Hg4.2 a/b, and Hg1.3 a/b, have been tested and prove successful for use in these species. Amplification reactions were then optimised for the highest stringency and yield of product. PCR reactions were then repeated using fluorescently labelled reagents necessary for analysis on the ABI 373 GeneScanner.

Thirty individuals each from the Hooker's sea lion (8 from Dunda I, 8 from Enderby I, and 14 from Figure of Eight I) and New Zealand fur seal (15 from West Coast rookeries, and 15 from East Coast rookeries plus The Snares Island) populations and five individuals from three Australian sea lion populations in Bass Straight have been successfully amplified using each of these three microsatellite primer pairs. Amplification reactions were visualised first by electrophoresis on agarose gels. One locus, Hg 1.3 a/b, shows products of varying size across each of the three species, indicating that it is a variable locus in these species. Amplification products from the other two loci do not appear variable on an agarose gel, but an agarose gels is only able to resolve differences in product sizes of at least 20 base pairs. Most microsatellite variation is on the order of 1-8 base pairs.

Attempts to analyse the reactions on the ABI GeneScanner were delayed due to a broken laser on it. The total amount of fluorescently labelled product must be calibrated to achieve an appropriate signal strength on the GeneScanner for quantitative analysis. To date, 120 animals with a combination of the three primer pairs have been analysed on it. Table 1 shows the number of each species and primer. The signal strength for these GeneScan runs is too low to quantify the microsatellite alleles present in these samples. An adjustment in the total amount of DNA submitted to the GeneScanner is required in order to do this quantification. These preparations are under way and, when complete, the data may be tested statistically to estimate their geographic distribution among the sampled populations.

Table 1 - Number of animals analysed for microsatellite variation by species and primer

Primer	NZ fur seal	Hooker's sea lion	Australian sea lion
Hg4.2 a/b	19	21	
Hg1.3 a/b	20	18	
Aa4 f/r	18	20	4

Conclusions

In the absence of appropriate signal strength for quantification, conclusions are limited to the following:

PrimerAa4 does not appear to be variable in Hooker's sea lions and would be uninformative for population characterisation. This locus is somewhat variable in the New Zealand fur seal, but may be variable enough to contribute further resolution of the population structure beyond what is already known. However, this primer is reported to be one of the least variable loci in other pinniped and otariid species. By itself this locus would not provide much useful information, but may augment data from other microsatellite loci. Both primers Hg 1.3 and Hg 4.2 appear to have some appreciable variation and will probably be very useful in measuring microsatellite variability in both populations. Too few individuals from the Australian sea lion population have been tested to make this assessment for this species.

At this still early stage, it has been shown that all primers developed in other species tested so far do cross-react with these three species. Amplification conditions yield strong highly specific products that are amenable to fluorescent analysis on the ABI GeneScanner. At least two primers tested so far reveal some degree of variability in two of these species.

We may conclude that microsatellite variation is extensive enough to warrant further application of these techniques to these populations. It is our recommendation to proceed with the next stage in this pilot study which is to design a suite of fluorescently labelled microsatellite primers that will facilitate optimisation of analytical signal on the GeneScanner. Such a suite of primers will also increase throughput capacity in order to survey much larger numbers of animals and will decrease the cost per animal/per locus for GeneScan runs.

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