Analysis of Mitochondrial Control Region DNA Variation in New Zealand's Brushtail Possums (*Trichosurus vulpecula*)

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ERRATUM

Page 16, line 15 should read: around 0.8 – 1.4 kb long in vertebrates (Sbisa *et al* 1997).

Page 21, lines 20 and 21 should read: and other mammals

Page 22, line 22 should read: Although heteroplasmy has been recorded (Bermingham *et al.* 1986, Cassane *et al* 1997, Fumagalli *et al* 1996, Wilkinson and Chapman 1991) it is relatively rare (Avise *et al.* 1987).

Page 27, line 8 should read: where they occur in low proportions (Kerle *et al* 1991).

Page 43, line 8 should read: Of those six, five were observed in possums of both colours. The exception to this is haplotype 2, which was detected in grey possums only.

Page 65, line 2 should read: the control region is very A + T rich

Page 68, lines 3 and 4 should read: Gels were poured between glass plates, pre-chilled to 4°C, and run vertically.

Page 68, line 18 should read: used to confirm sequence differences by first amplifying the individual using Tv5'F and Tv5'R and then performing a sequencing reaction with the appropriate primer.

ADDED REFERENCES

Casane, D.; Dennebouy, N; de Rochambeua, H.; Mounolou, J.C. and Monnerot, M. 1997. Nonneutral evolution of tandem repeats in the mitochondrial DNA control region of Lagomorphs. *Molecular Biology and Evolution* **14**: 779-789.

Fumagalli, L.; Taberlet, P.; Favre, L. and Hausser, J. 1996. Origin and evolution of homologous repeated sequences in the mitochondrial DNA control region of shrews. *Molecular Biology and Evolution* **13**: 31-46.

Wilkinson, G. S. and Chapman, A. M. 1991. Length and sequence variation in evening bat D-loop mtDNA. *Genetics* **128**: 607-617.

Abstract

Brushtail possums (*Trichosurus vulpecula*) were first introduced from Australia to New Zealand in 1858 to establish a fur industry. Currently numbering more than 65 million, they are recognised as the most important mammalian pest in New Zealand, because of the environmental and agricultural damage they cause. Possums act as a wildlife reservoir of bovine tuberculosis (Tb) and, as such, threaten New Zealand's multi-million dollar beef and dairy industry. Eliminating bovine Tb in livestock requires removal of contact with infected possums. This is mainly achieved through the intensive poisoning of areas of known wildlife Tb infection and the establishment around them of zones of low possum density (known as buffer zones) adjacent to at-risk farmland. Not only does this result in lower possum density, and thus fewer dispersing possums, but may also affect the movement patterns of possums.

Measurement of gene frequency differences between populations associated with a buffer zone would allow a qualitative estimate of the effect of buffer zones on limiting possum movement. The mitochondrial DNA (mtDNA) control region is an effective marker for detecting intraspecific genetic structure because it has a high mutation rate, lack of recombination and uniparental mode of inheritance.

An extensive survey of brushtail possum mtDNA control region variation in New Zealand was conducted to quantify levels of variation and thus assess the utility of the mtDNA control region as a marker for detecting genetic differentiation between possum populations. Nine haplotypes were found among 70 possums from throughout New Zealand. Most of the variation (six haplotypes) was concentrated in the North Island, and the most widespread haplotype (occurring in all four islands surveyed) was also the most common - found in 67% of possums surveyed.

The technique of single stranded conformation polymorphism (SSCP) was developed for the brushtail possum so that a quick, cost-effective and sensitive method for surveying mtDNA control region variation in large numbers of individuals was available. This assay

was applied to screen the variation in possums separated by small spatial scales associated with two buffer zones in the South Island. A total of 234 possums were screened, with 98.7% found to possess the same haplotype. The other 1.3%, all from one location, possessed a second haplotype. The extremely low levels of variation makes it highly unlikely that surveys of variation in mtDNA will be able to detect an effect of buffer zones on possum movement, at least in the South Island. Areas of higher variation, such as certain parts on the North Island, would be better candidates for testing the effect of barriers such as buffer zones on genetic differentiation between possum populations.

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This thesis would not have been possible without the numerous possum tissue samples collected from throughout New Zealand and from Australia. A huge thank you goes to everyone involved in collecting these samples. I would especially like to thank Chris Bee for co-ordinating collection of possums in the South Island buffer zones.

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Abbreviations and Symbols

S.I. (Système Internationale (d'Unités)) notation is adhered to throughout this thesis. Abbreviations used in this thesis are as follows:

A	adenine
AHB	Animal Health Board
bp, kb	base pairs, kilobase pairs
C	cysteine
CSB	conserved sequence block
°C	degrees Celsius
DNA	deoxyribonucleic acid
dNTP	deoxynucleoside triphosphate
D-loop	displacement loop
EtBr	ethidium bromide
ETAS	extended termination associated sequences
g	gravity
G	guanine
H	heterozygosity
MHC	major histocompatibility complex
MP	maximum parsimony
µl, ml, l	microlitre, millilitre, litre
mm, cm, m, km	millimetres, centimetres, metres, kilometres
mtDNA	mitochondrial DNA
M	moles per litre
ng, mg, kg	nanogram, milligram, kilogram
NJ	neighbour-joining
$	New Zealand dollars
nt	nucleotide
pM, µM, mM, M	picomolar, micromolar, millimolar, molar
PCR	polymerase chain reaction

®	registered
RFLP	restriction fragment length polymorphism
SSCP	single stranded conformation polymorphism
1080	sodium monofluoroacetate
SD	standard deviation
TAS	termination associated sequence
T	thymine
™	trademark
tRNA, rRNA	transfer RNA, ribosomal RNA
Tb	bovine tuberculosis
U	unit (of enzyme)
UV	ultraviolet light
VNTR	variable number of tandem repeats
VRA	Vector Risk Area
V	volts
W	watt

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