Sex chromosome microsatellite markers from an Australian marsupial: development, application and evolution

Anna Jayne MacDonald
Bachelor of Science (Honours)

Institute for Applied Ecology
Division of Health, Design and Science
University of Canberra, ACT, 2601, Australia

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Form B

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Abstract

Microsatellites are simple repetitive DNA sequences that are used as genetic markers throughout the biological sciences. The high levels of variation observed at microsatellite loci contribute to their utility in studies at the population and individual levels. This variation is a consequence of mutations that change the length of microsatellite repeat tracts. Current understanding suggests that most mutations are caused by polymerase slippage during DNA replication and lead to changes of a single repeat unit in length, but some changes involving multiple repeats can also occur. Despite this simplistic overview, there is evidence for considerable heterogeneity in mutation processes between species, loci and alleles. Such complex patterns suggest that other mechanisms, including those associated with DNA recombination, are also involved in the generation of microsatellite mutations. Understanding which mutational mechanisms are responsible for variation at microsatellite markers is essential to enable accurate data interpretation in genotyping projects, as many commonly used statistics assume specific mutation models.

I developed microsatellite markers specific to the X and Y chromosomes and an autosome in the tammar wallaby, *Macropus eugenii*, and investigated their evolutionary properties using two approaches: indirectly, as inferred from population data, and directly, from observation of mutation events. First, I found that allelic richness increased with repeat length and that two popular mutation models, the stepwise mutation model and the infinite allele model, were poor at predicting the number of alleles per locus, particularly when gene diversity was high. These results suggest that neither model can account for all mutations at tammar wallaby microsatellites and hint at the involvement of more complex mechanisms than replication slippage. I also determined levels of variation at each locus in two tammar wallaby populations. I found that allelic richness was highest for chromosome 2, intermediate for the X chromosome and lowest for the Y chromosome in both populations. Thus, allelic richness varied between chromosomes in the manner predicted by their relative exposure to recombination, although these results may also be explained by the relative effective population sizes of the chromosomes studied. Second, I used small-pool PCR from sperm DNA to observe *de novo* mutation events at three of the most polymorphic autosomal markers. To determine the reliability of my observations I developed and applied strict criteria for scoring alleles and mutations at microsatellite loci. I observed mutations at all three

markers, with rate variation between loci. Single step mutations could not be distinguished because of the limitations of the approach, but 24 multi-step mutations, involving changes of up to 35 repeat units, were recorded. Many of these mutations involved changes that could not be explained by the gain or loss of whole repeat units. These results imply that a large number of mutations at tammar wallaby microsatellites are caused by mechanisms other than replication slippage and are consistent with a role for recombination in the mutation process.

Taken as a whole, my results provide evidence for complex mutation processes at tammar wallaby microsatellites. I conclude that careful characterisation of microsatellite mutation properties should be conducted on a case-by-case basis to determine the most appropriate mutation models and analysis tools for each locus. In addition, my work has provided a set of chromosome-specific markers for use in macropod genetic studies, which includes the first marsupial Y chromosome microsatellites. Sex chromosome microsatellites open a new range of possibilities for population studies, as they provide opportunities to investigate gene flow in a male context, to complement data from autosomal and maternally-inherited mitochondrial markers.

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This would not be the thesis it is without the advice, help and encouragement of a great many people. To list all of those who have assisted me in some way would be impossible, but I will try my best. You should all know that I will be eternally grateful for the support you have given me. I couldn't have done this PhD without you.

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In the early days of my PhD, I carried out PC2 work in the CCMI lab at the University of Canberra, then under Jenelle Kyd. Members of that lab, including Nancy Fisher, Claire Batum and Donna Easton, were all generous with their time and, when needed, their restriction enzymes. Towards the end of my PhD, when contamination avoidance became extremely important, Dennis McNevin was kind enough to provide access to the forensic lab's PCR setup area.

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Publications associated with this thesis

This thesis includes publications for which I am the senior but not the sole author. I took the lead in this research in that I designed the research, undertook the laboratory work, analysed the data and wrote the manuscripts. I was, however, assisted by my co-authors.

The publications associated with this thesis are as follows:

Chapter 2

MacDonald AJ, Sankovic N, Sarre SD, FitzSimmons NN, Wakefield, MJ, Graves JAM and Zenger, KR (2006) Y chromosome microsatellite markers identified from the tammar wallaby (*Macropus eugenii*) and their amplification in three other macropod species. *Molecular Ecology Notes* **6**, 1202-1204.

Chapter 3

MacDonald AJ, Sarre SD, FitzSimmons NN, Graves JAM (2007) Chromosome-specific microsatellites from the tammar wallaby X chromosome and chromosome 2. *Molecular Ecology Notes* **7**, 1063-1066.

Chapter 4

To be submitted for publication as: MacDonald AJ, Sarre SD and FitzSimmons NN. Microsatellite evolution in marsupial sex chromosomes: which model fits?

Chapter 5

To be submitted for publication as: MacDonald AJ, Sarre SD, FitzSimmons NN and Aitken, NA. Characterising microsatellite mutations using small-pool PCR.

Chapter 6

MacDonald AJ, Sarre SD, FitzSimmons NN. Sex chromosome microsatellites: new tools for macropod population ecology. Submitted for publication in the Proceedings of the Australian Mammal Society Macropod Symposium held in Melbourne, July 2006.