Marsupial Genetics Studied in Tammar, Parma and Black-Striped Wallabies:
Mapping, Conservation and Evolution

by

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SUMMARY

This thesis reports work on the breeding and genetic typing of several species of wallaby. The work was initiated with two main aims in mind. The first was to develop a marsupial species for use as an organism for genetic work, in the first instance genetic mapping. The second was to investigate genetic variability within and between populations of these wallabies, with consideration of conservation priorities and taxonomic relationships. A third aim, to examine MHC variability was added as data on this group of loci became available during the course of the investigation.

Development of an efficient system for generating a linkage map of a marsupial genome through recombination has been difficult in the past largely due to lack of detectable genetic variability within the study species. Two different systems were examined for their potential usefulness. One was an interspecies cross between the parma wallaby and its nearest relative the black-striped wallaby. The other was a cross between two different island populations of tammar wallabies, one from Kangaroo Island (KI) in South Australia and the other from Garden Island (GI) in Western Australia.

Despite reports by others of hybridisation between parmas and black-stripes, no F1 hybrids were produced in either direction during the period of this research. Hence, this interspecies cross was not useful for linkage mapping.

The between sub-species cross was however highly successful. F1 hybrids were produced between KI and GI tammars and both males and females were backcrossed to the parental strains. Approximately 200 testcross progeny were generated for mapping studies over a three year period. Large quantities of DNA and several different tissues have been stored from each progeny allowing numerous investigations to be carried out in the future.

KI and GI tammar wallabies were typed for 54 genetic markers. These data were used to compare the levels of heterozygosity within and between the two
populations. This was done with a view to examining the effects of isolation on small island populations of macropodids. KI is one of Australia's largest offshore islands (450,000 ha) whereas GI is approximately 430 times smaller (1054 ha). Surprisingly, the level of genetic variation in GI tammars is not significantly different to that seen in the much larger KI population.

Of the 54 markers used 19 showed large gene frequency or fixed differences between the two populations. Of these, 15 were used to type backcross progeny for the mapping study. Seven of these belonged to three linkage groups, two autosomal and one X-linked. Identification of large gene frequency or fixed differences between KI and GI tammars was substantially more efficient using DNA probes rather than allozyme and serum protein markers. Seventy percent (n=22) of DNA probes detected fixed differences compared with 13% of biochemical markers (n=32).

As both male and female F1 hybrids are fertile it was possible to compare male and female recombination rates. Previous work has shown that females have severely reduced recombination rates compared with males in two distantly related marsupial species, an American didelphid (*Monodelphis domestica*) and an Australian dasyurid (*Sminthopsis crassicaudata*). In striking contrast, female recombination rates are similar or significantly greater than male recombination rates in the tammar wallaby. More data are now needed from different marsupial species and additional linkage groups in order to understand the evolutionary implications of this finding.

As a result of investigating the level of genetic diversity between parma and black-striped wallabies, data were obtained which allowed several conservation issues regarding the parma wallaby to be addressed. These data were used to assess the suitability of New Zealand derived parma wallabies for reintroduction programs to the Australian mainland. Reservations as to their suitability for such programs included the possibility of small founder numbers which might have resulted in low levels of genetic diversity and the possibility of introgression with black-striped wallabies. Data generated indicated that New Zealand derived parmas do not have a restricted genetic base. No hybridisation with black-striped wallabies could be
detected. Re-examination of the literature suggests that black-stripes may never have been introduced to New Zealand. These findings support the suitability of New Zealand derived parmas for reintroduction programs.

An unexpected and intriguing finding was the level of genetic variability at MHC class II loci in tammars. These data showed that there is significantly reduced polymorphism at these loci in the tammar compared with eutherian species. The most striking difference between eutherians and marsupials is in their modes of reproduction. It is tempting to speculate that different levels of genetic variability at MHC class II loci are a reflection of this, although it is difficult to propose a mechanism with the current state of knowledge.
DECLARATION

I declare that this thesis contains no material which has been accepted or submitted for the award of any other degree or diploma in any University, and that to the best of my knowledge and belief, it contains no material previously published or written by another person, except where due reference is given.

L. M. McKenzie
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### ABBREVIATIONS AND COMMON NAMES USED

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>AI</td>
<td>Abrolhos Island group</td>
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<tr>
<td>APD</td>
<td>Average percent difference</td>
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<tr>
<td>BE</td>
<td>&quot;Breeding Efficiency&quot; defined as the number of births per potential oestrous cycle</td>
</tr>
<tr>
<td>E</td>
<td>Prefix indicating &quot;eugenii&quot;</td>
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<tr>
<td>GI</td>
<td>Garden Island</td>
</tr>
<tr>
<td>HA</td>
<td>Average heterozygosity</td>
</tr>
<tr>
<td>KI</td>
<td>Kangaroo Island</td>
</tr>
<tr>
<td>KI×(KI×GI)</td>
<td>Backcross animal with a KI mother and an F1 father whose mother was KI and father was GI</td>
</tr>
<tr>
<td>MAPD</td>
<td>Mean average percent difference</td>
</tr>
<tr>
<td>MHC</td>
<td>Major Histocompatibility Complex</td>
</tr>
<tr>
<td>MI</td>
<td>Middle Island</td>
</tr>
<tr>
<td>N&lt;sub&gt;e&lt;/sub&gt;</td>
<td>Effective population number</td>
</tr>
<tr>
<td>P</td>
<td>Perup</td>
</tr>
<tr>
<td>RFLP</td>
<td>Restriction fragment length polymorphism</td>
</tr>
<tr>
<td>SA</td>
<td>South Australia</td>
</tr>
<tr>
<td>T</td>
<td>Prefix indicating &quot;tammar&quot;</td>
</tr>
<tr>
<td>WA</td>
<td>Western Australia</td>
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Gene symbols follow the human genetics convention HGM 11, 1991

The species which are the subject of this thesis are:

- *Macropus eugenii*  the tammar wallaby
- *Macropus parma*  the parma wallaby
- *Macropus dorsalis*  the black-striped wallaby

Their common names are used throughout
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