DECLARATION

Except where specific reference is made to other sources, the work presented in this thesis is the work of the author. It has not been submitted, in whole or in part for any other degree.

Amir Mohammadi

14/07/2011
ACKNOWLEDGEMENT

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ABSTRACT

As a representative of Australian marsupials, the recently sequenced genome of a model kangaroo, the tammar wallaby (*Macropus eugenii*) provides unique opportunities to understand the organization and evolution of the genome in marsupials, and in Class Mammalia in general. Comparisons with the fully sequenced genome of the Brazilian short-tailed opossum *Monodelphis domestica* allow me to compare the genomes of American and Australian marsupials, which have been evolving separately in different environments for the last 70 million years. The general aims of this thesis were to examine the extent to which part of the genome has been conserved in marsupials and in therian mammals, as well as to explore the organization and evolution of the largest gene family in mammals, whose members code for olfactory receptors.

As part of the KanGo’s (ARC Centre of Excellence for Kangaroo Genomics) task of establishing a map of the tammar genome, I undertook the comparative mapping of the long arm of chromosome 6 in the tammar wallaby. Firstly I identified segments conserved between opossum and human that I expected from chromosome painting to lie in this region, and isolated large insert clones from a tammar BAC library that contained conserved genes that defined these regions. Then I established their locations and order on the long arm of chromosome 6 in tammar wallaby. I found that there are only few rearrangements between tammar wallaby and the opossum in this part of the genome. However, the genomic parts orthologous to tammar wallaby 6q reside on several chromosomes in human, dog, and chicken, suggesting that the fusion occurred in the marsupial ancestors and remained conserved during marsupial evolution.

I then developed a strategy to explore the olfactory receptor gene (ORG) family in the tammar wallaby. Sequences corresponding to ORGs were extracted from the first assembly of the tammar wallaby genome and sequences classified into families and subfamilies. BACs bearing conserved mammalian ORG clusters were isolated and physically mapped in tammar wallaby. Comparison with the opossum OR repertoire revealed that these two distantly related marsupials share a very similar ORG superfamily. Conserved features include the total numbers of genes, families, and
subfamilies, gene distribution across the families and subfamilies, patterns of expansions and contractions in families and subfamilies and genomic location of major ORG clusters.

I then examined in detail the genomic organization of a highly conserved ORG cluster that lies near the MHC locus in several mammals. I made a BAC contig over the entire chromosome region. I found that this cluster is conserved in tammar wallaby and carries almost the same genes as in the opossum. Preliminary analysis of platypus ORGs dates the origin of this cluster back to the common ancestor of therian and monotreme mammals more than 166 million years ago, and provides examples of both conservation and adaptation of some genes in this cluster.

My general conclusion is that the two distantly related marsupial species have retained very similar genomes since their divergence 70 million years ago. This conservation is reflected both at the level of genome arrangement, and at the organization and evolution of gene families. This conservation is in marked contrast to the variability observed between eutherian groups, both in gross gene arrangement and in the constitution of the ORG family, suggesting that marsupial genomes have been evolving more slowly than other mammals, possibly due to some unique features of their physiology and way of life.
# LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Meaning</th>
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<tbody>
<tr>
<td><strong>GABBR1</strong></td>
<td>Gamma-Aminobutyric Acid (GABA) B Receptor, 1</td>
</tr>
<tr>
<td><strong>HEXDC</strong></td>
<td>Hexosaminidase (glycosyl hydrolase family 20, catalytic domain) Containing</td>
</tr>
<tr>
<td><strong>hsp70</strong></td>
<td>Heat Shock Protein 70</td>
</tr>
<tr>
<td><strong>MOG</strong></td>
<td>Myelin Oligodendrocyte Glycoprotein</td>
</tr>
<tr>
<td><strong>NARF</strong></td>
<td>Nuclear Prelamin A Recognition Factor</td>
</tr>
<tr>
<td><strong>P2RX3</strong></td>
<td>Purinergic Receptor P2X, Ligand-Gated Ion Channel, 3</td>
</tr>
<tr>
<td><strong>PRNP</strong></td>
<td>Prion Protein</td>
</tr>
<tr>
<td><strong>PTPRJ</strong></td>
<td>Protein Tyrosine Phosphatase, Receptor Type, J</td>
</tr>
<tr>
<td><strong>RBMX</strong></td>
<td>RNA Binding Motif Protein, X-linked</td>
</tr>
<tr>
<td><strong>RFX</strong></td>
<td>Regulatory Factor X</td>
</tr>
<tr>
<td><strong>SRZ</strong></td>
<td>Seven-Pass Receptor Family Z</td>
</tr>
<tr>
<td><strong>UBD</strong></td>
<td>Ubiquitin D</td>
</tr>
<tr>
<td><strong>WDR45L</strong></td>
<td>WD Repeat Domain 45 Like</td>
</tr>
<tr>
<td><strong>ZFY</strong></td>
<td>Zinc Finger Protein, Y-linked</td>
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