

## Chapter 10

### Final discussion and conclusions

This thesis has resulted in the identification of 34 MHC-linked olfactory receptor genes in the human MHC extended class I region. These OR genes are located in 2 clusters (the ‘major’ and ‘minor’ cluster) within the MHC extended class I region which contains a number of other gene clusters, including the histones, zinc finger proteins and tRNAs. Both the major and the minor OR clusters are found in LINE-rich, Alu-poor isochores that can be defined across the extended class I region.

A further 56 MHC-linked ORs were identified in the mouse genome. A number of features of both the mouse and human OR genes were investigated. This included their phylogeny, the conservation of structural features within these proteins, and local duplications involved in the creation of new members of this very large gene family. Analysis of the human and mouse MHC-linked OR gene clusters has revealed the syntenic relationships between these genes, allowing the state of the ancestral MHC-linked OR cluster to be considered. Syntenic relationships between OR genes have also revealed putative functional sequences that have been conserved over evolutionary time.

The regulation and expression of the MHC-linked ORs was investigated. MHC-linked ORs were found to be expressed both within and outside the olfactory epithelium. Factors involved in regulating this expression were considered but the variety of approaches only served to suggest that these genes have a mechanism of control not yet observed in any other human multigene family. The polymorphism study of these genes, meanwhile, suggested that the overall level of

polymorphism within the MHC-linked ORs is fairly low, although the functional repertoire may vary between individuals as some genes appear to have both functional and non-functional alleles.

A comparison of the MHC-linked ORs against other ORs in the human genome was performed. This involved constructing an OR database which suggested that the majority of the diversity had been generated through local duplications rather than exchanges of genetic material between chromosomes. The phylogenetic tree constructed from this data led to the proposal of a 'framework MHC' that included OR genes alongside MHC genes.

Finally, 5 pheromone receptor pseudogenes were identified in the human extended MHC class I region. Putative orthologs of these genes were located in the syntenic region on chromosome 13 in mouse: the majority of these mouse VR genes were pseudogenes, although there were a number of functional loci.

Future work on the MHC-linked OR genes could follow a number of lines:

- Alternative splicing within these genes could be investigated further: a survey across the genome would reveal whether alternative splicing is restricted to MHC-linked ORs or whether it is a genome-wide phenomenon.
- The characterisation of clusters of OR genes in more detail across the genome may give more insight into the regulatory control of these genes: if there are small 'control regions', similar to that observed for the MHC-linked ORs, these may be detectable across clusters.

- Syntenic regions could be investigated in more detail: if these regions are not exons, they may be important in controlling gene expression so they could be assayed for promoter activity.
- Data from the polymorphism study, OR pseudogenes and OR hypervariable regions could be combined to provide an integrated picture of selective forces acting upon OR genes: matrices for OR-specific phylogenetic trees would allow the ancestral relationships of these genes to be investigated further.
- The existence of a ‘framework MHC’ containing OR genes representative of the 3 groups identified in Chapter 8 could be investigated in an ancient species such as lamprey or hagfish.
- Further work on the pheromone receptor genes in mouse or another species could be performed to investigate the relationship between the 2 olfactory organs in more detail.