Evolution of Mammalian Sex Chromosomes and Sex Determination Genes: Insights from Monotremes

A thesis submitted for the degree of Doctor of Philosophy

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Abstract

Genetic sex determination systems are generally based on the presence of differentiated sex chromosomes. Birds have a ZZ/ZW sex chromosome system in which males are ZZ and females ZW, whereas mammals have an XX/XY system with males being XY and females XX. Monotremes have an extraordinary sex chromosome system that consists of multiple sex chromosomes: 5X5Y in platypus and 5X4Y in echidna. Intriguingly, the monotreme sex chromosomes show extensive homology to the bird ZW and not to the therian XY. However, sex determination in monotremes is still a mystery; the Y-specific *Sry* gene that triggers male sex determination in therian mammals is absent and so far very few genes have been identified on Y chromosomes in monotremes. To gain more insights into the gene content of Y-chromosomes and to identify potential sex determination genes in the platypus a collaborative large scale transcriptomic approach led to the identification of new male specific genes including the anti-Muellerian hormone AMH that I mapped to Y_5, this makes *Amhy* an exciting new candidate for sex determination in monotremes.

Platypus chromosome 6 is largely homologous to the therian X and therefore it represents the therian proto sex chromosome. In addition, this autosome features a large heteromorphic nucleolus organizer region (NOR) and associates with the sex chromosomes during male meiosis (Casey and Daish personal communication). I investigated chromosome 6 heteromorphism in both sexes and found a number of sex-specific characteristics related to the extent of the NOR heteromorphism, DNA methylation, silver staining patterns and interestingly, meiotic segregation bias. This

raises the possibility that chromosome 6 may have commenced differentiation prior to monotreme therian divergence.

These results led me to investigate the chromosome 6 borne gene *Sox3*, from which *Sry* evolved in therian mammals. This revealed a platypus male-specific *Sox3* allele, which differs from the alleles observed also in females on the length of one of the *Sox3* polyalanine tracts. This raises the possibility that *Sox3* may be working differently in males and females.

We have used our unique knowledge of monotreme sex chromosomes to determine the sex of captively bred echidnas. I used a PCR based genetic sexing technique that utilizes DNA from small hair samples and primers that amplify male-specific genes. Interestingly, I found that seven out of eight echidnas born in captivity were females. Furthermore, I found a *Sox3* deletion in the only male echidna born in captivity. This gives us the unique opportunity to investigate the sexual development of an animal in which this gene is naturally deleted providing an exceptional situation in which to study monotreme sex determination. Furthermore, this sexing technique has the potential of being applied in the wild to investigate sex ratio in natural populations of monotremes, including the critically endangered long-beaked echidna.

Thesis Declaration

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