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

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Table of Contents

Abstract ............................................................................................................................................. 1
Thesis Declaration ............................................................................................................................. 3
Acknowledgments ............................................................................................................................. 4
CHAPTER 1: Introduction .................................................................................................................. 5
  Chapter overview .......................................................................................................................... 5
  Introduction .................................................................................................................................. 6
    Sex chromosome evolution in vertebrates .................................................................................... 8
    Sex chromosomes evolved from a pair of autosomes ................................................................. 9
    The therian XY sex chromosome system .................................................................................... 14
    The prototherian multiple sex chromosome system ................................................................. 17
  Molecular aspects of mammalian sex determination ................................................................. 20
    Sex determination genes in therian mammals ............................................................................ 20
    Sex determination in monotremes .............................................................................................. 26
    Sex ratios in captive animal populations ................................................................................... 28
  Aims of the Project ....................................................................................................................... 30
  Summary ....................................................................................................................................... 31
References ......................................................................................................................................... 34

CHAPTER 2: Prediction of Y-linked genes in the platypus reveals Amhy as the most likely sex-determining candidate gene ................................................................................................. 48
  Chapter overview ......................................................................................................................... 48
  Statement of authorship ............................................................................................................... 49
  Publication: .................................................................................................................................. 50

CHAPTER 3: Non-random meiotic segregation of the therian protosex chromosome in platypus may provide insights into differentiation of sex chromosomes in mammals .................................................................................................................. 51
  Chapter overview ........................................................................................................................ 51
  Statement of Authorship .............................................................................................................. 52
  Manuscript in preparation: .......................................................................................................... 53
  Deborah Toledo-Flores, R. Daniel Kortschak, Frank Grützner. Non-random segregation of the therian proto-sex chromosome at platypus male meiosis. .................................................. 53

CHAPTER 4: Identification and characterization of a male-specific change in Sox3 in the platypus ................................................................................................................................. 89
  Chapter overview ........................................................................................................................ 89
  Identification of a male-specific Sox3 allele in the platypus might suggest a role in male sex determination ....................................................................................................................... 90

CHAPTER 5: Investigating sex bias in captive bred echidnas ....................................................... 117
  Chapter overview ........................................................................................................................ 117
  Statement of authorship .............................................................................................................. 118
  Manuscript in preparation: .......................................................................................................... 119
  Deborah Toledo-Flores, Wan Xian Kang, Arthur Ferguson, Belinda Turner, Enkhjargal Tsend-Ayush, Shu Ly Lim, Frank Grützner. Genetic Sexing Reveals Female Bias in Echidnas Born in Captivity .................................................. 119
  Statement of authorship .............................................................................................................. 144
  Letter in preparation: ............................................................................................................... 145
Deborah Toledo-Flores, Frank Grützner. Identification of a Sox3 deletion in a captive-bred echidna. ......................................................................................................................145

CHAPTER 6: Significance and future directions ..............................................152
  Chapter overview ..............................................................................................152
  Significance and future directions ......................................................................153

Amendments........................................................................................................156
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 Y₅, 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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Deborah Fernanda Toledo-Flores

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