

Abstract

Even though environmental sex determination (ESD) was described almost 60 years ago, our understanding of its mechanisms and evolution remains fragmentary. In ESD, there is no consistent genetic difference between males and females; instead, the sex of an individual depends on environmental stimuli. This contrasts with genotypic sex determination (GSD), where specific genetic factors determine sex. Recent advances in molecular tools have significantly impacted sex determination research. First, the identification of sex chromosomes has revealed GSD in many amniote lineages, leading to the reclassification of many species previously thought to exhibit ESD. This indicates that the prevalence of ESD among reptiles was previously overestimated. Second, molecular tools have enabled transcriptomic studies of ESD during the key phases of their embryonic development. They identified potential players, including epigenetic modifiers (like demethylase *kdmb6b*) and calcium signalling, particularly in the turtle model species *Trachemys scripta*. These findings led to the proposal of an overarching model that combines calcium signalling and oxidative stress, highlighting the role of temperature-sensitive ion channels. Additionally, other mechanisms, such as stress and related hormones and the impact of germ cell numbers on gonad development, have been explored. In Chapter 1, we reviewed current knowledge and proposed a hypothesis about the ancestral state of sex determination in amniotes. We emphasise the role of stress and that ESD shares some similarities with sequential hermaphrodites, which leads us to formulate a hypothesis about the role of heterochronic shifts in the evolution of sex determination in vertebrates. In Chapters 2 and 3 of this thesis, we explored the diversity and variability in ESD among geckos. We present data for five previously unstudied gecko species and show how the reaction norms can differ from traditional models. In Chapter 4, we examine the relationship between the germ cell numbers and gonad development in the turtle *Trachemys scripta*. Despite significant progress in the past decade, many questions remain. For example, the highly female-biased sex ratios at any incubation temperature as in *Pachydactylus tigrinus* challenges some traditional models of ESD and importance of balanced sex ratios in general. Also, the yet unexplored molecular mechanisms in squamate reptiles prevent robust conclusions about ESD homology in amniotes. Clearly, more manipulative experiments are needed to explore causal relationships between specific genes, hormones, and other signalling molecules. Future studies should focus on a broader range of species, especially among squamate reptiles, to elucidate the link between environmental stimuli and sex-determining pathways.