Summary

Because of their relatively discrete nature, chemicals are used in sexual communication and they can be shaped by evolution to become species-, sex- or even individual- specific. By combining different techniques - behavioural assays, transcriptomics, proteomics and phylogenetics, we here tune in this very private communication channel to better understand a language that we, as humans, cannot easily perceive. We took newts (Amphibia, Caudata, Salamandridae) as our model organisms, animals whose sexual communication for long intrigued researchers, who conducted numerous behavioural experiments to try to decipher chemical communication by observing their behaviour. In this thesis we begin our journey in a similar fashion by studying female chemical cues and how they influence the males. We show that female chemical cues induce courtship behaviour in the male and allow him to identify a female of his own species, even without seeing her (**Chapter 1**). We believe that our novel behavioural approach will be inspiring to researchers studying chemical communication, not only in newts, but also in other taxonomic groups, and allow us to eventually identify the molecules involved. Our focus was then shifted to the unusual technique of the male in which he manages to inseminate the female without touching her (**Chapter 2**). We show that the sex pheromones that he wafts to the female with his tail under water largely do the trick and can function even without the male being present, but also that they do not have an effect on female representatives of a sister genus. Our next step was to identify such a potent, but discrete chemical signal. Using combined proteomic and transcriptomic analyses, we did not find one molecule, but identified an array of proteins, termed Sodefrin Precursor-like Factors (SPF) that allow the male to transfer his sperm into the female body while walking away from her (**Chapter 3**). Furthermore, our phylogenetic analyses estimated that these proteins, specifically the two major clades to which they group, diverged from each other after a duplication event of their corresponding genes already in stem salamanders (~300 million years ago) and likely complemented each other as sex pheromones all the way to contemporary newts. This also implied that all extant salamander families might use SPF proteins as sex pheromones. The intriguing diversity and specificity of SPF proteins in a single male newt led us to further investigate how such protein blends can become specific. In **Chapter 4** we analyse the male SPF content of two species of newts in which females do not react to sex

pheromones of males of the other species. We show that this is largely achieved by sequence divergence of the similar set of SPF proteins, rather than shifts recruiting SPF proteins from other clades as sex pheromones, that were also detected in male, but marginally expressed. However, shifts in sex pheromone content in salamanders have already been demonstrated and these marginally expressed SPF transcripts might still see an opportunity for their corresponding proteins to get recognised by females and aid the male to safely transfer them to the next generation. The research presented in this thesis shows that newts, with their unusual courtship display, can teach us a lot about the language of chemicals. We see the identification of female semiochemicals and exploration into the SPF world of salamanders, beyond the currently investigated families of Salamandridae and Plethodontidae, as natural evolution of the work presented in **Chapters 1 & 3**. We also see using two-female experiments to further investigate the emergence of specificity of SPF proteins, by examining their content and effect in more closely related, congeneric taxa of newts based on **Chapters 2 & 4**. Altogether, this thesis provides a vehicle ready to take you further onto the evolutionary roller coaster of chemical communication and associated reproductive modes in salamanders.