Chemical communication plays a key role in the unique reproduction and complex courtship rituals of many salamanders. By integrating multiple scales of biological organization (genome, transcriptome and proteome) with behavioural experiments, we explored the underwater chemistry between male and female salamanders during courtship, and report on the evolution of the most widespread salamander sex pheromone system, the mechanisms involved in attaining species-specificity, and the recruitment of new molecules into existing pheromone blends.

In this thesis, we first identify a series of male protein pheromones—termed Sodefrin Precursor-like Factors (SPFs)—that elicit female mating responses in the European, aquatically reproducing palmate newt (Lissotriton helveticus, Salamandridae) and show that courting males of the commonly used amphibian model organism, the Mexican axolotl (Ambystoma mexicanum, Ambystomatidae), expose females to SPFs as well. These findings expand the ubiquity of SPF pheromones in salamanders far beyond what was previously known. Moreover, phylogenetic and molecular dating analyses suggest that SPF pheromones already served a sex pheromone function in the earliest salamanders (~300 million years ago) and continued exerting this function in distinct salamander lineages ever since. Being the oldest and most widespread salamander sex pheromone system, SPF pheromone blends have diverged in many different ways, often up to a point where they only elicit females of their own species. To explore how species-specificity might evolve, we compared pheromone use in two closely related newt species (Ichthyosaura alpestris and L. helveticus) whose male pheromones fail in eliciting females of the other species. Despite the enormous diversity available in SPF gene copies, we reveal that both species essentially use the same SPF proteins and show that gradual sequence divergence established species-specificity. In the last part of this thesis, we focus on a newly identified newt sex pheromone, termed persuasin. We show that persuasin was recently recruited in the shadow of the ancient SPF pheromone system through duplication and neofunctionalization, and eventually reinforced the SPF pheromone system.

In conclusion, this thesis gives an insight in the dynamic evolution of an ancient salamander sex pheromone system whose complexity is shaped by many gene duplications, sequence diversification, co-option of new molecules and, above all, sexual selection. Our study will allow future work to chart evolutionary patterns on pheromone use on an even wider multi-species scale, study the relationship with diverse reproductive strategies and elucidate the mechanisms behind the increasing level of signal complexity in some salamander lineages.