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**Author:** Vonk, Freek Jacobus  
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Chapter 1: Introduction

Snakes are limbless predators that have evolved two different ways of incapacitating prey: suffocation and envenomation. The evolution of these life-history strategies lie in close relation with the evolution of body-elongation and limblessness. Taken together, these adaptations underlie the massive radiation of snakes which occurred after the K-T boundary and extinction of the dinosaurs, presumably because multiple niches became available and many of their predators got extinct. In this thesis, I examine the evolution of these adaptations. A general question to address would be: what underlies these adaptations in snakes and how do they correspond with their ecology? In addition to these evolutionary questions, I also provide a summary in chapter one on the current status of using snake venom toxins in drug discovery and design, because the evolution of venom is closely related to the evolution of a serpentine body form and fangs, but also to the question of “why” snake venom is so interesting for the pharmacological prospector. The high potency and specificity of snake venom toxins is generated by millions of years of evolution and strong selective pressures, so it is my believe that the different chapters in my thesis are tightly bound together. It is believed that it was Aristotle who first suggested – on the basis of symptoms observed in snakebite victims – that snakes may also be used to cure certain diseases (it was not yet known that venom was responsible for the observed effects). And in 1835 venom was already (although unsuccessfully) used to treat rabies. This field of “bioprospecting” has accelerated especially in the last decade, mainly because the development of many new sequencing and screening techniques. In chapter two, we go into the development and evolution of the different snake venom-conducting fangs. This has been a matter of great controversy among scientists for the last century and many different hypotheses have been proposed. This was particularly because the two groups of snakes with fangs in the front of their upper jaw (Elapidae and Viperidae), have only relatively recently in the 90s been shown not to form a monophyletic group, hinting towards an independent origin and evolution of front fangs. By using modern techniques such as in situ hybridization of snake embryos, and careful histological analysis we were able to reconstruct the evolutionary history and make a solid hypothesis for
the origin and evolution of fangs. In the third chapter of this thesis we look at the evolution of body-elongation and deregionalization in snakes, since that presumably has also been a major driver of venom evolution by providing the ability for snakes to coil up and act as coiled spring that can strike and envenomate prey at a distance. Since *Hox* determine the basic structure of an embryo we have performed many *in situ* hybridisations in snake embryos and carefully compared the expression profiles of different *Hox* genes. For the fourth chapter of this thesis we sequenced a draft genome of the largest venomous snake in the world, the king cobra (*Ophiophagus hannah*) and annotated many of its venom genes. We wanted to find out whether we could find evidence of gene duplication and / or gene modification of physiological genes for use in the venom gland. The birth-and-death model of gene evolution is the canonical framework for venom evolution, but was never actually thoroughly studies due to lack of genomic resources. Our genomic sequences let us study the king cobra venom genes in relation to its ‘normal’ physiological genes. In the fifth chapter, we have set out to develop a new efficient and rapid method of analysing snake venom mixtures and performing a biological assay at the same time. We used the acetylcholine binding protein (AChBP) as biological target, because acetylcholine receptors are also often involved in neurological disorders, such as Alzheimer’s and Myasthenia gravis. In the last chapter of this thesis, I discuss this work and try to put it into broader perspective. The field of evolutionary genomics, molecular evolution as well as bioprospecting is huge and broad. However, I strongly believe that working on the edge of different scientific disciplines will provide you with a better understanding of nature and allows you to answer more questions.