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SHORT COMMUNICATION

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Snake fangs from the Lower Miocene of Germany: evolutionary stability of perfect weapons

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Abstract There is a general consensus that most of today's nonvenomous snakes are descendants of venomous snakes that lost their venomous capabilities secondarily. This implies that the evolutionary history of venomous snakes and their venom apparatus should be older than the current evidence from the fossil record. We compared some of the oldest-known fossil snake fangs from the Lower Miocene of Germany with those of modern viperids and elapids and found their morphology to be indistinguishable from the modern forms. The primary function of recent elapid and viperid snake fangs is to facilitate the extremely rapid, stab-like application of highly toxic venoms. Our findings therefore indicate that the other components of the venom-delivery system of Early Miocene vipers and elapids were also highly developed, and that these snakes used their venom in the same way as their modern relatives. Thus, the fossil record supports the view that snakes used their venoms to rapidly subdue prey long before the mid-Tertiary onset of the global environmental changes that seem to have supported the successful radiation of venomous snakes.

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Introduction

Among living vertebrates, snakes have the most diverse, efficient, and sophisticated venom-delivery systems (Jackson 2003), consisting of large mucous–serous venom glands (Kochva 1987), extensive associated muscles allowing for highly regulated high-pressure ejection of venom (Young et al. 2000), and long tubular fangs facilitating deep injection into prey or enemies (Jackson 2002, 2003). All venomous snakes belong to the Colubroidea, which comprises the vast majority of living snakes, and three separate lineages, the Atractaspididae, Elapidae, and Viperidae, which have independently evolved a venom-delivery system. The front-fanged members of these three lineages have traditionally been referred to as venomous snakes from a clinical point of view, because they have either caused human morbidity and mortality, or they are at least thought to be capable of doing so. They are also venomous snakes in a biological sense, because they use their oral gland secretions to subdue prey (Kardong 2002). Most of the remaining modern colubroids are not considered venomous clinically, although a few have caused severe human envenomation and even fatalities (Kuch and Mebs 2002; Sawai et al. 2002). While many snakes need their body musculature not only for locomotion but also for prey constriction, the capability of envenoming prey enabled colubroids to uncouple their feeding and musculoskeletal systems. This allowed for the evolution of more rapid locomotion and thus a very different ecology, which probably evolved in concert with the evolution of grassland habitats during the Tertiary (Savitzky 1980).

Recent studies (Fry et al. 2003; Jackson 2003; Vidal 2002) support the hypothesis that the presence of supralabial serous secretory cells and differentiated maxillary dentition is a synapomorphy of the Colubroidea, followed in the course of their evolutionary diversification by repeated reduction or loss in those groups that have traditionally been called "nonvenomous colubrids" (Savitzky 1980; Underwood and Kochva 1993). A wide range of possible biological functions has been proposed for the oral gland secretions of colubroids, but evaluating their biological role only from a pharmacological and physiological perspective may not be sufficient in a functional or evolutionary context (Kardong 1996, 2002). In fact, while the molecular arsenal of early colubroids can be inferred (Fry and Wüster 2004), this is not true for the state of their venom-delivery systems. Although the fossil record of snakes is poor, skull bones and vertebrae resembling modern viperid and elapid snakes have been found at several sites in Eurasia and North America (Holman 2000; Rage 1984, Rage and Augé 1993; Szyndlar and Rage 2002, suggesting that such snakes were widely distributed by the beginning of the Miocene (23 million years before present) and had evolved much earlier. Here, we report on new finds of exquisitely preserved fossil venom fangs in Germany from the Lower Miocene and discuss the functional and toxinological implications.

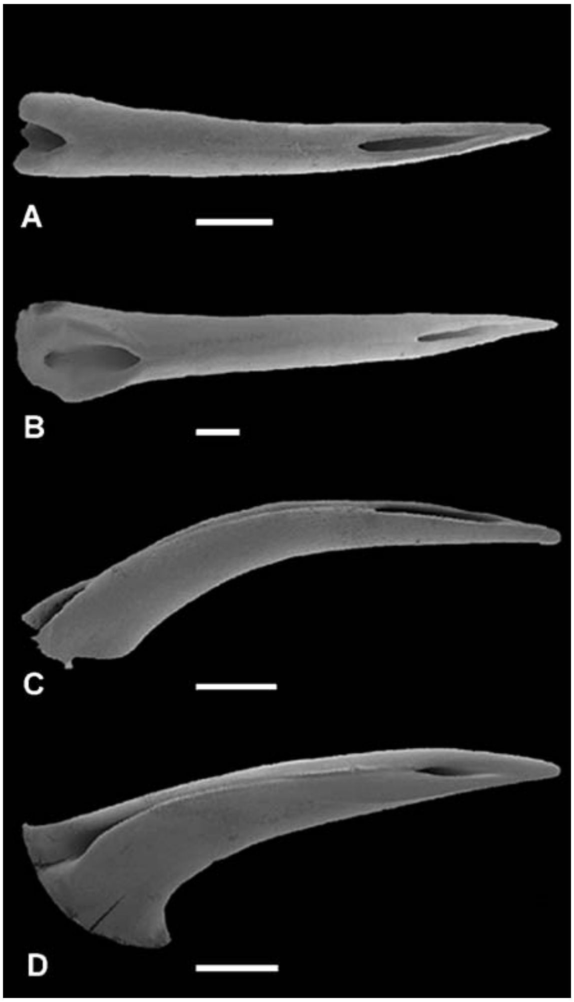

Fig. 1 a–d Scanning electron micrographs of fossil and extant venomous snake fangs. Fangs are pointing to the right, *scale bars* equal to 0.5 mm. The fangs of a fossil (**a**) and an extant viper (**b**), *V. ammodytes*, (both fangs in anterior view) show typical viperid features such as the elongated, slender venom discharge orifice and the presence of an anterior blade ridge on the apex. The morphology of the more strongly curved fossil elapid fang (**c**) is similar to the fangs of modern elapid snakes (**d**), *B. sindanus*, (both fangs in medial view) in having a suture along the anterior surface that extends from the basal end of the venom discharge orifice to the basal end of the tooth

Materials and methods

We recovered a total of 33 tubular snake fangs from the *inflata*-beds of the Oppenheim/Nierstein quarry (Mainz Basin, Rhineland-Palatinate, Germany), which are Early Miocene (Aquitanian) in age and can be assigned to MN2 of the European land mammal levels (Mein 1989). The fossil material is housed at the Institut für Geowissenschaften der Universität Mainz, Germany. After cleaning and drying, the fossil fangs and those from extant viperid and elapid taxa were glued to an aluminum holder, sputtered with gold, and examined using a Hitachi Model S-4,500 scanning electron microscope at an accelerating voltage of 5 kV (cold-field emission electron source).

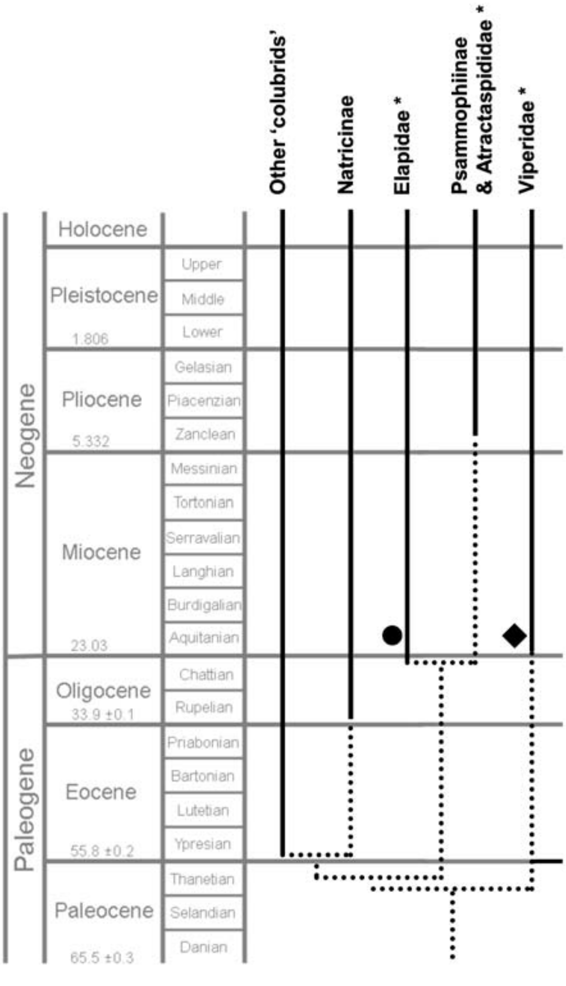

Fig. 2 Stratigraphic occurrence of viperids, elapids, and colubrid-grade snakes in the Cenozoic as currently known; only the clades with a pre-Pleistocene fossil record are shown. The *circle* and the *diamond* indicate the age of the elapid and viperid venom fangs described in this paper. The clades that possess a front-fanged venom-delivery system are marked by an *asterisk*

Results

The majority type among the fossil material is a hollow, tubular fang (Fig. 1a). It is similar to the fangs of New World pitvipers (*Bothrops*, *Crotalus* spp.) and virtually identical to the fang of a modern European viper (sand viper, *Vipera ammodytes*; Fig. 1b) in details such as fang length and curvature, length and shape of the venom discharge orifice, and in having sharp, prominent anterior blade ridges. The second fang type (Fig. 1c), which we refer to an early elapid species, is similar to the fangs of modern elapid snakes such as cobras (*Naja* spp.) or kraits (*Bungarus sindanus*; Fig. 1d) in being more strongly curved and having a suture along the anterior surface that extends from the basal end of the venom discharge orifice to the basal end of the tooth.

Discussion

Although the presence of snake fangs in Lower Miocene sediments is not entirely new (Kinkelin 1892), it is the exquisite preservation of the Oppenheim material that shows convincingly that the fangs of Miocene venomous snakes not only can be referred to modern clades but also are virtually indistinguishable from modern fangs, thereby indicating that the venom-delivery system as a whole had already evolved to modern-day standards by the beginning of the Neogene.

The similarity between the second fang type and the fangs of modern elapids is also interesting, because this would represent the oldest record of elapid snakes in Europe (Szyndlar and Böhme 1993). The vertebrae among the material indeed suggest the presence of nonviperid colubroids, because they are morphologically closest to natricine and non-*Naja* elapid vertebrae as described by Ivanov (Ivanov 2000).

Because of their highly efficient venom apparatus, Elapidae and Viperidae were long believed to be the most advanced venomous snakes. However, both morphological (Scanlon et al. 2003) and recent molecular studies on the higher level phylogeny of snakes place viperid snakes basal to the remaining colubroid snakes, with the possible exception of the Pareatidae and Xenodermatidae (Slowinski and Lawson 2002; Vidal and Hedges 2002, 2004). Our findings suggest that in viperid and elapid snakes, fangs underwent only minimal, if any changes in the past 23 million years, thus dating the evolutionary processes that shaped their venom-delivery systems long before the dramatic radiation of colubroids in the Miocene. Despite the fact that the fossil record of snakes is poor, this interpretation gains support from several fossil finds of colubrid-grade snakes in North America and Asia during the Eocene (Parmley and Holman 2003; Rage et al. 1992, 2003). This early record of colubrid-grade snakes is important, because it implies not only that the divergence from basal taxa such as vipers occurred much earlier than the Oligocene/Miocene transition (Fig. 2) but also provides fossil evidence for an ancient evolutionary history of snake venoms and toxins.

In extant elapid, viperid, and some atractaspidid snakes, grooved or tubular fangs are part of a highly developed venom-delivery system. This comprises also a specialized venom gland producing a complex mixture of toxic and enzymatically active proteins, which is perfectly suited to subdue, kill, and help digest prey, and which is also used for defense. These snake venom components had been recruited from multiple protein families like phospholipases A_2 and proteases or had been modified from existing salivary proteins (Fry 2005; Fujimi et al. 2002; Kochva 1987, Kochva et al. 1983). They became toxic proteins like neurotoxins, cytotoxins, inhibitors, hemorrhagins, or clotting enzymes and may have coevolved with a refined venom-delivery system, particularly with the development of the injection device, the fangs.

Multiple functions have been proposed for oral gland secretions of colubroid snakes, the most obvious in extant elapids and viperids is to cause rapid prey death (Kardong 2002). The apparent structural identity of the fossil fangs with those of modern species implies also functional identity. This includes the ability to deliver an extremely rapid stab-like strike, after which the released prey is searched for and swallowed when dead, or to immobilize prey while holding on to it. Both strategies require venoms that are toxic enough to subdue prey quickly to increase the chance of recovering the envenomed animal or to reduce injury from retaliating prey. Apparently both function and structure of the venom-delivery systems of elapid and viperid snakes had been brought close to perfection at latest by the middle Tertiary, while more recent evolution of these functional entities has likely been restricted to the diversification of venom toxins (Ménez et al. 1992).

The present evidence suggests that it is unlikely that the evolution of snake venoms was a result of the global environmental changes starting in the late Paleogene and continuing, in intervals, down to the present day, because the record of colubrid-grade snakes is older (Fig. 2), and the evolution of the most efficient venom-delivery systems was already completed at the Oligocene–Miocene transition. In fact, already by the Early Cenozoic, elapid and viperid snakes must have killed their prey much like their modern relatives.

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References

Fry BG (2005) From genome to “venome”: molecular origin and evolution of the snake venom proteome inferred from phylogenetic analysis of toxin sequences and related body proteins. Genome Res 15:403–420

Fry BG, Wüster W (2004) Assembling an arsenal: origin and evolution of the snake venom proteome inferred from phylogenetic analysis of toxin sequences. Mol Biol Evol 21:870–883

Fry BG, Lumsden NG, Wüster W, Wickramaratna JC, Hodgson WC, Kini RM (2003) Isolation of a neurotoxin (alpha-colubritoxin) from a nonvenomous colubrid: evidence for early origin of venom in snakes. J Mol Evol 57:446–452

Fujimi TJ, Kariya Y, Tsuchiya T, Tamiya T (2002) Nucleotide sequence of phospholipase A_2 gene expressed in snake pancreas reveals the molecular evolution of toxic phospholipase A_2 genes. Gene 292:225–231

Holman JA (2000) Fossil snakes of North America. Indiana University Press, Bloomington

Ivanov M (2000) Snakes of the lower/middle Miocene transition at Vieux Collonges (Rhône, France), with comments on the colonisation of western Europe by colubroids. Geodiversitas 22:559–588

Jackson K (2002) How tubular venom-conducting fangs are formed. J Morphol 252:291–297

Jackson K (2003) The evolution of venom-delivery systems in snakes. Zool J Linn Soc 137:337–354

Kardong KV (1996) Snake toxins and venoms: an evolutionary perspective. Herpetologica 52:36–46

Kardong KV (2002) Colubrid snakes and Duvernoy's "venom" glands. J Toxicol Toxin Rev 21:1–19

Kinkelin F (1892) Ein fossiler Giftzahn. Zool Anz 15:93–94

Kochva E (1987) The origin of snakes and the evolution of the venom apparatus. Toxicon 25:65–106

Kochva E, Nakar O, Ovadia M (1983) Venom toxins: plausible evolution from digestive enzymes. Am Zool 23:427–430

Kuch U, Mebs D (2002) Envenomations by colubrid snakes in Africa, Europe, and the Middle East. J Toxicol Toxin Rev 21: 159–179

Mein P (1989) Updating of MN Zones. In: Lindsay EH, Fahlbusch V, Mein P (eds) European Neogene Mammal Chronology. Plenum, New York, pp 73–90

Ménez A, Bontems F, Roumestand C, Gilquin B, Toma F (1992) Structural basis for functional diversity of animal toxins. Proc R Soc Edinb B 99:83–103

Parmley D, Holman JA (2003) *Nebraskophis* Holman from the late Eocene of Georgia (USA), the oldest known North American colubrid snake. Acta Zool Cracov 46:1–8

Rage J-C (1984) Handbuch der Paläoherpetologie, Part 11. Serpentes. Gustav Fischer, Stuttgart

Rage J-C, Augé M (1993) Squamates from the Cainozoic of the western part of Europe. A review. Rev Paléobiol vol spéc 7:199–216

Rage J-C, Buffetaut E, Buffetaut-Tong H, Chaimanee Y, Ducrocq S, Jaeger JJ, Suteethorn V (1992) A colubrid snake in the late Eocene of Thailand: the oldest known Colubridae (Reptilia, Serpentes). CR Acad Sci (II) 314:1085–1089

Rage J-C, Bajpai S, Thewissen JGM, Tiwari BN (2003) Early Eocene snakes from Kutch, Western India, with a review of the Palaeophiidae. Geodiversitas 25:695–716

Savitzky AH (1980) The role of venom-delivery strategies in snake evolution. Evolution 34:1194–1204

Sawai Y, Honma M, Kawamura Y, Saki A, Hatsuse M (2002) *Rhabdophis tigrinus* in Japan: pathogenesis of envenomation and production of antivenom. J Toxicol Toxin Rev 21:181–201

Scanlon JD, Lee MSY, Archer M (2003) Mid-Tertiary elapid snakes (Squamata, Colubroidea) from Riversleigh, northern Australia: early steps in a continent-wide adaptive radiation. Geobios 36:573–601

Slowinski JB, Lawson R (2002) Snake phylogeny: evidence from nuclear and mitochondrial genes. Mol Phylogenet Evol 24:194–202

Szyndlar Z, Böhme W (1993) Die fossilen Schlangen Deutschlands: Geschichte der Faunen und ihrer Erforschung. Mertensiella 3:381–431

Szyndlar Z, Rage J-C (2002) Fossil record of the true vipers. In: Schuett GW, Höggren M, Douglas ME, Greene HW (eds) Biology of the vipers. Eagle Mountain, Eagle Mountain, pp 419–444

Underwood G, Kochva E (1993) On the affinities of the burrowing asps *Atractaspis* (Serpentes: Atractaspididae). Zool J Linn Soc 107:3–64

Vidal N (2002) Colubroid systematics: evidence for an early appearance of the venom apparatus followed by extensive evolutionary tinkering. J Toxicol Toxin Rev 21:21–41

Vidal N, Hedges SB (2002) Higher-level relationships of caenophidian snakes inferred from four nuclear and mitochondrial genes. C R Biol 325:987–995

Vidal N, Hedges SB (2004) Molecular evidence for a terrestrial origin of snakes. Proc R Soc Lond B (Suppl) 271:226–229

Young BA, Zahn K, Blair M, Lalor J (2000) Functional subdivision of the venom gland musculature and the regulation of venom expulsion in rattlesnakes. J Morphol 246:249–259