How Tubular Venom-Conducting Fangs Are Formed

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ABSTRACT   Elapids, viperids, and some other groups of colubroid snakes have tubular fangs for the conduction of venom into their prey. The literature describing the development of venom-conducting fangs provides two contradictory accounts of fang development. Some studies claim that the venom canal forms by the infolding of a deep groove along the surface of the tooth to produce an enclosed canal. In other works the tubular fang is said to form by the deposition of material from tip to base, so that the canal develops without any folding. This study was undertaken to examine fang development and to account for the disagreement in the literature by determining whether fang formation varies among groups of venomous snakes and whether it differs between embryos and adults. Adult and embryonic representatives of elapids and viperids were examined. All fangs examined, elapid and viperid, embryos and adults, were found to develop into their tubular shape by the addition of material to the basal end of the tooth rather than by the folding inward of an ungrooved tooth to form a tubular fang. In some cases, the first fang that develops in embryonic snakes differs morphologically from all those formed subsequently. J. Morphol. 252:291–297, 2002.   © 2002 Wiley-Liss, Inc.

KEY WORDS: Serpentes; Elapidae; Viperidae; fangs; teeth; dentition; development; venom

Although several vertebrate groups possess some sort of venom system, it is among the snakes that the venom-delivery system has reached its highest degree of sophistication. Of the approximately 2,700 species of living snakes, approximately 450 are considered venomous. All venomous species are within the large and diverse clade Colubroidea and venom-delivery systems are thought to have evolved independently several times within this group (Cadle, 1988; Knight and Mindell, 1994).

The fangs of the main groups of venomous snakes, Elapidae, Viperidae, and Atractaspididae (*sensu stricto*: Pough et al., 2001), share an elaborate, morphologically specialized structure used to conduct venom into their prey. This structure has been described in detail for *Naja* (Elapidae) by Bogert (1943), and for *Crotalus* (Viperidae) by Klauber (1939, 1956). In all groups, the fang consists of an elongated tooth with a closed canal passing through its interior for the conduction of venom. This canal opens basally as an entrance orifice that connects with the venom duct. The canal opens apically as a discharge orifice. In elapids and atractaspidids the entrance orifice and the discharge orifice are joined by a visible suture along the anterior surface of the fang. In viperids the anterior surface of the fang is generally smooth.

A survey of the literature reveals disagreement regarding the embryonic formation of the venom-conducting fang. Researchers can be divided into two camps based on their hypotheses about how fangs develop, with each camp apparently unaware of the other's work. The most widespread idea about how fangs form is termed here the "infolding" hypothesis (Fig. 1.1). Proponents of this hypothesis maintain that the venom-conducting fang begins as a plain, conical tooth, which develops a groove along its anterior surface. This groove deepens, forming the venom canal. The sides of the groove come together and meet, forming a closed canal, leaving a "suture" in elapids and atractaspidids, but smoothed over in the case of viperids. This scenario is most commonly encountered in general works and in review articles (e.g., Edmund, 1969; Haas, 1980; Cogger, 1992; Shea et al., 1993), but it is also encountered in studies of fang development that use histological techniques to illustrate the formation of the fang (e.g., Tomes, 1875; Kochva, 1963; Lake and Trevor-Jones, 1987, 1995). In these studies, the fang is visualized in cross sections through the shaft. Cross sections of a series of replacement fangs are shown, starting with the plain, circular cross section of the first fang in the series, proceeding to a cross-section shaped like a crescent, representing a grooved fang, and finally finishing with a fully formed, tubular fang, appearing in cross section like a circle with a smaller circle within it, representing the now fully enclosed venom canal.

The other hypothesis is termed here the "brick chimney" hypothesis (Fig. 1.2). The term "brick chimney" comes from a description of fang formation

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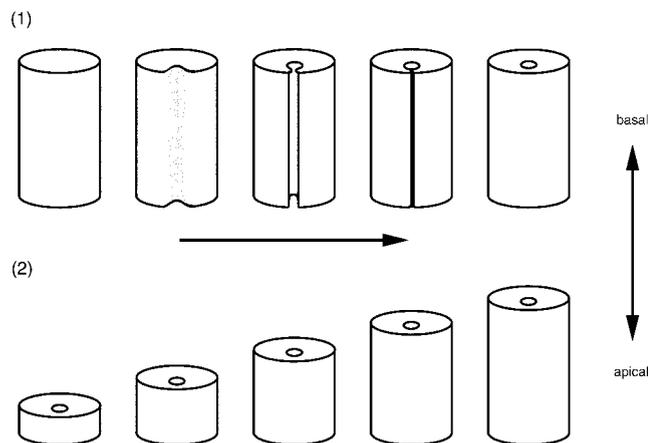
TWO WAYS TO FORM A TUBULAR FANG

Fig. 1. Two conflicting hypotheses to explain the formation of the venom canal in the shaft of the fang: (**1**) the "infolding" hypothesis, in which a cylinder with a tubular canal through it is created by the inward folding of the outside surface of the tooth, and gradual closing over of the groove thus formed, and (**2**) the "brick chimney" hypothesis, in which a cylinder with a tubular canal through it is produced by gradually adding material on top of a hollow cylinder.

by Charles M. Bogert (*in litt* in 1988). In this scenario no bending or infolding of the tooth surface takes place. Instead, mineralized tissue is gradually deposited starting at the apical tip and proceeding basally. As mineralization continues basally, the venom canal is formed, like building a hollow chimney, from apical to basal (Klauber, 1939, 1956; Bogert, 1943). These conclusions are based on the study of series of replacement fangs dissected from adult snakes and examined using light microscopy.

In this study, I evaluated these two hypotheses ("infolding" vs. "brick chimney") using scanning electron microscopy (SEM) of series of developing fangs. SEM was used because this technique allows a three-dimensional view of the developing fang. I obtained an ontogenetic series of developing fangs and described their overall morphology, noting the presence or absence of grooves, hollow venom canal, entrance orifice and discharge orifice, and measured the discharge orifice and overall length of the tooth. I first examined replacement series of fangs in adult snakes of different taxa to assess the possibility that fang development varies widely across taxa, a potential reason for disagreement in the literature. My study included representatives of the two largest groups of venomous snakes, Elapidae and Viperidae. I then examined replacement series from embryos, including the first fang to be formed in the developing snake, to determine whether embryonic fangs and adult replacement fangs develop in the same way.

MATERIALS AND METHODS

Materials examined for interspecific comparisons included *Naja melanoleuca* (Elapidae; MCZ 53501), *Bothrops lansbergii* (Viperidae; MCZ 181484), and *Bitis arietans* (Viperidae; MCZ 14947). Embryonic materials examined represented approximately Zehr (1962) stages 35–37 and included *Bitis arietans* (UMA r-0687, UMA r-0693) and *Naja kaouthia* (MCZ A-24388). All materials were prepared by dissecting out the functional fang and its replacement series on one side. The fangs and replacement fangs were allowed to dry and then were mounted on a stub, sputter-coated with gold, and examined using a JEOL 6400 scanning electron microscope at an acceleration voltage of 15 kV. In addition, some cleared and stained specimens of stage 35–37 *Crotalus horridus* (Viperidae) (AHS L-353, AHS L-354, AHS L-639) were examined using light microscopy. Those specimens were prepared by standard alcian-alizarin procedures.

RESULTS
Adult Fang Series

Elapid. In the *Naja melanoleuca* examined, the replacement series consists of a functional fang, approximately 3.5 mm in length, ankylosed to the maxilla, and four replacement fangs ranging in size from 0.5–3.3 mm (Fig. 2). On the functioning fang and the three largest replacement fangs, a complete discharge orifice is present on the anterior surface, at the apical end of the fang. The discharge orifice is the same size, 0.8 mm in length, on all fangs. Along the anterior surface of the fang is a suture that extends from the basal end of the discharge orifice to the basal end of the tooth. Because the discharge orifice is of equal length in all fangs of the series, the length of the suture increases as the length of the developing tooth increases. The functional fang is 0.2 mm longer than the largest replacement fang and in the proximalmost 0.2 mm the suture on the functioning fang opens out into an entrance orifice. Only the functional fang, which is ankylosed to the maxilla, has an entrance orifice.

Viperid. The *Bitis arietans* examined was a young individual (exact age not known). It was selected because the teeth were small enough to fit in the SEM. There were six fangs in the replacement series, ranging in total length from 1.1–4.3 mm (Fig. 3). The two largest fangs were ankylosed to the maxilla, the first one firmly and the second one less securely. All fangs had discharge orifices. In the two smallest fangs the discharge orifices were open at the basal end, so the length of the discharge orifice could not be measured. The discharge orifices of the remaining fangs decreased in size with increasing development of the fang, with the discharge orifice of the third fang in the series measuring 0.9 mm in length and ranging downward in the other fangs to
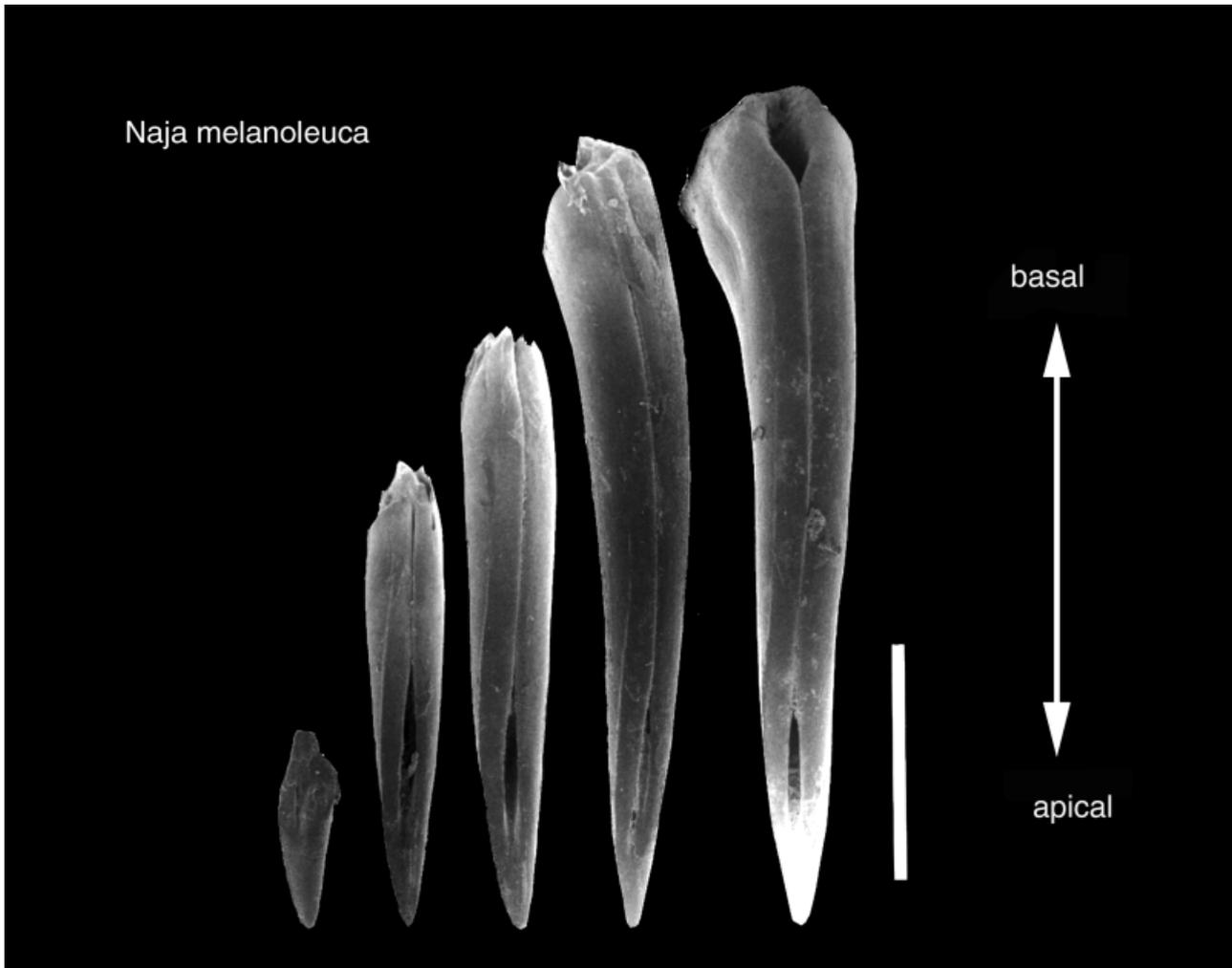
Fig. 2. Functional fang and replacement series from *Naja melanoleuca* (MCZ 53501), arranged from left to right in order of increasing development, viewed from the anterior side. The oldest fang (furthest to the right) is ankylosed to the maxilla, while the replacement fangs (the four teeth to the left of it) are loose in the surrounding tissues. Scale bar = 1 mm.

the final, ankylosed, fang, in which the length of the discharge orifice was 0.4 mm.

Embryonic Fang Series

Two series of fangs were examined from embryos of *Bitis arietans* at developmental stages corresponding approximately to Zehr stages 35 and 37. Stage 37 immediately precedes hatching and stage 35 is somewhat earlier. The stage 35 embryo had three teeth in its replacement series, ranging in total length from 0.4–0.9 mm. The longest was not ankylosed to the maxilla at its base. Each tooth had an open groove along its anterior surface and in the two largest teeth the sides of the groove met at the basal end of the groove, forming a discharge orifice (Fig. 4). In the stage 37 embryo there were six teeth in the replacement series, ranging in length from 1–3.6 mm (Fig. 5). The first two teeth were ankylo-

sed to the maxilla and the second tooth, but not the first, had an entrance orifice. It should be noted that the longest tooth was not the furthest along in the series, but was the second furthest along. Each tooth in the series had a discharge orifice approximately 0.8 mm in length, with the exception of two teeth. The first exception was the smallest tooth in the series, which was so short that the basal end of the discharge orifice was open. The second exception was the first tooth in the series, which was ankylosed to the maxilla, and was soon to be replaced by the second tooth. At 3.2 mm in length it was slightly shorter than the second tooth and the shaft was straighter. It had no entrance orifice, venom canal, or discharge orifice. Instead, it had one long groove extending along the anterior surface of the fang. Thus, the first fang was morphologically distinct from all subsequent fangs.
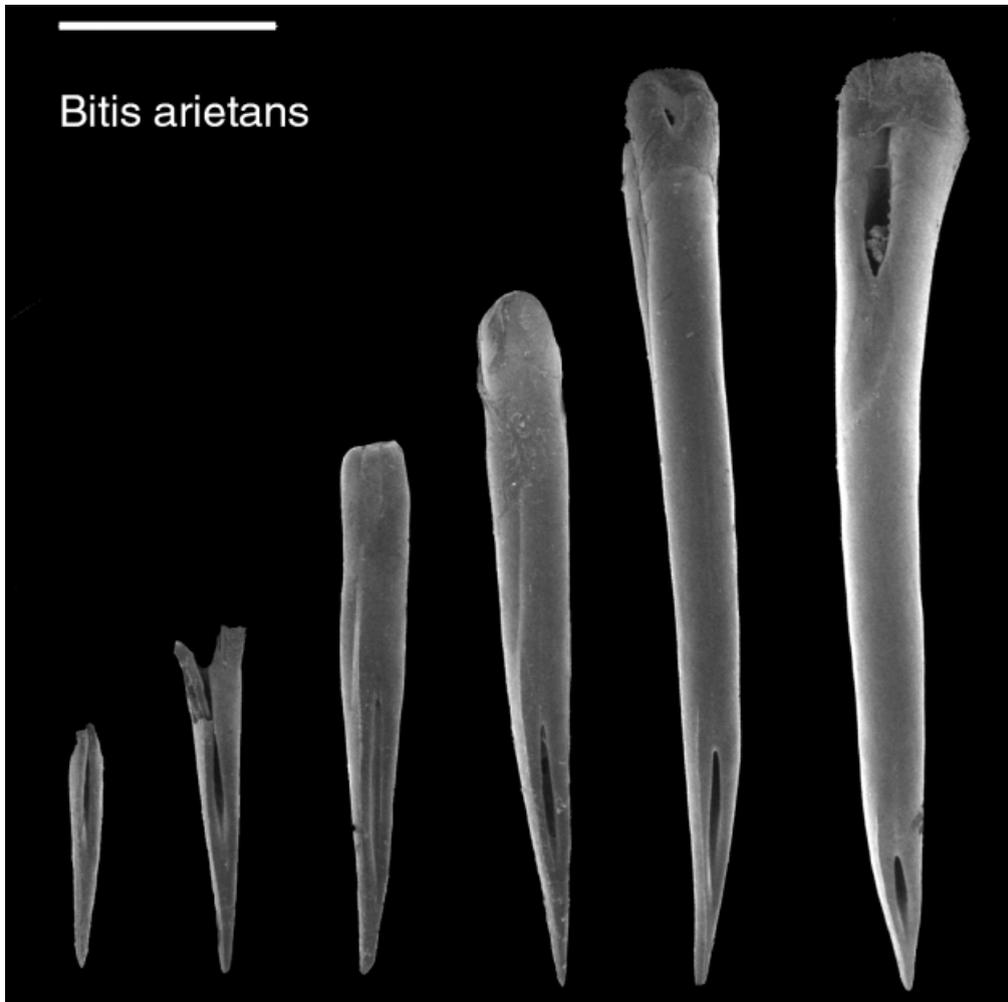
Fig. 3. Functional fang and replacement series from *Bitis arietans* (MCZ 14947), arranged from left to right in order of increasing development, viewed from the anterior side. The oldest two fangs (furthest to the right) are ankylosed to the maxilla. Scale bar = 1 mm.

In addition, fangs were examined in a *Naja kaouthia* embryo at approximately Zehr stage 37. There were three fangs in the series, one fang ankylosed in place on the maxilla and two replacement fangs (Fig. 6). The first fang in the series (the one ankylosed to the maxilla) was 1.5 mm in length. This fang had no venom canal, entrance orifice, or discharge orifice. Instead, a groove extended along the anterior surface of the tooth. The two replacement fangs, measuring 1.6 mm and 1.3 mm, respectively, each had a closed venom canal extending along the anterior surface of the tooth, opening into a discharge orifice at the apical end of the tooth.

The functional fang was examined in cleared and stained embryos of *Crotalus horridus.* In all cases the functional fang resembled a tubular adult fang except that a suture was present along the anterior surface of the tooth. Other fangs in the series could not be examined because of the manner of preparation.

DISCUSSION

In snakes, teeth are replaced continuously throughout life. At any given time there is, for each tooth ankylosed to a dentigerous bone (dentary, palatine, pterygoid, or maxilla), an ontogenetic series of developing teeth in the surrounding tissue. These replacement teeth are not attached to the dentigerous bone during their development, but when they replace the functional tooth their basal end establishes an attachment to the rim of the shallow alveolus.

Adult Fang Series
Elapid. The micrographs of the replacement fang series from *Naja melanoleuca* (Fig. 2) strikingly resemble Bogert's (1943) line drawings of replacement fang series from *N. melanoleuca,* which were intended to illustrate fang development by the "brick
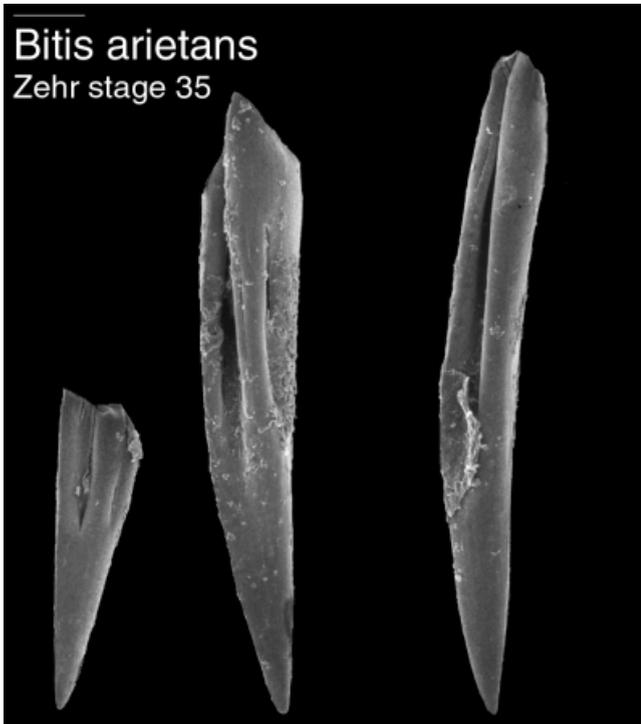
Fig. 4. Fang series from *Bitis arietans* (UMA r-0693), Zehr stage 35, arranged from left to right in order of increasing development, viewed from the anterior side. None of the fangs is ankylosed to the maxilla. Scale bar = 100 μm.

chimney" process. In the fang series illustrated here, as in Bogert's article, the discharge orifice is the same size in replacement fangs that differ from one another in total length. This illustrates the formation of the functioning fang by gradual accretion to the fang from the distal tip to the proximal end. There is no sign of any infolding of an open groove to form the venom canal. This is consistent with the "brick chimney" hypothesis of fang development. If the venom canal were formed by the folding inward of a groove along the tooth surface, the discharge orifice would diminish in size as the sides of the groove came together.

Viperid. The fangs of *Bitis arietans* were examined to determine whether viperids and elapids differed in their mode of fang development. In *B. arietans*, the discharge orifice appears to be closing gradually and decreasing in size as development of the fang progresses. This is different from *Naja melanoleuca* and could be interpreted as evidence of formation of the venom canal by infolding. However, two other factors more likely account for the viperid condition. First, relative to the fang of the elapid, the fang of the viperid is long and curved. This curvature causes a foreshortening effect; the longer and more curved the fang is, the shorter the discharge orifice appears as viewed in anterior aspect. Thus, some of the apparent shortening of the discharge orifice in the developing *B. arietans* fang may be an
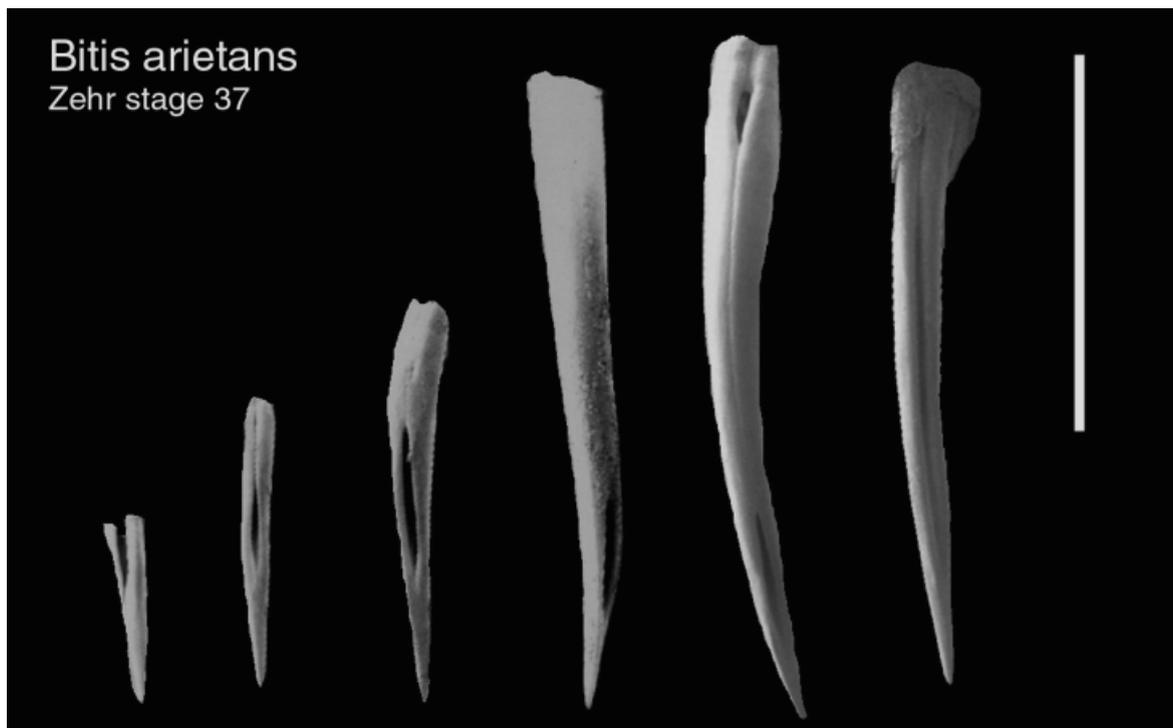

Fig. 5. Fang series from *Bitis arietans* (UMA r-0687), Zehr stage 37, arranged from left to right in order of increasing development, viewed from the anterior side. The oldest two fangs (furthest to the right) are ankylosed to the maxilla. Scale bar = 2 mm.

Fig. 6.   Fang series from *Naja kaouthia* (MCZ A-24388), Zehr stage 37, arranged from left to right in order of increasing development, viewed from the anterior side. The oldest fang (furthest to the right) is ankylosed to the maxilla. Scale bar = 100 μm.

optical illusion, resulting from the orientation in which the fang was mounted on the SEM stub. Second, the specimen of *B. arietans* examined was a young individual, presumably growing quickly. Therefore, the smallest replacement fang was eventually going to be part of a larger tooth than the fang currently in place on the maxilla It might thus be expected to have a proportionately larger discharge orifice. With these factors considered, the development of the fangs in *B. arietans* seems no different from their development in *N. melanoleuca*.

Embryonic Fang Series

Because teeth are replaced continuously in snakes, previous studies have been based on replacement series of fangs from adult snakes. However, the first fang develops in the embryo. Embryonic material was studied here to determine whether embryonic fangs differ in their mode of development from the replacement fangs of adults.

The embryos of *Bitis arietans* and *Naja kaouthia* reveal that fangs in the embryo form in the "brick chimney" manner, like the replacement fangs of adult snakes. This held true for all the fangs in the embryo, including the first fang to form. However, the first fang that forms in the embryonic venomous snake turns out, in some embryonic series, to be morphologically different from all subsequent fangs. In the embryos of both *N. kaouthia* and *B. arietans*, the first fang to form had an open groove along its

anterior surface. It also lacked both the closed venom canal and the associated discharge orifice and entrance orifice characteristic of tubular fangs. The embryonic *Crotalus horridus* examined did not have a fang of this type. The significance of this morphologically distinct first fang to the biology of embryonic and neonatal venomous snakes warrants further investigation, as does the nature of the developmental mechanisms that distinguish the formation of the first fang from that of all subsequent fangs. The origin of tubular front fangs has been proposed by many authors (e.g., Anthony, 1955; McDowell 1968, 1986; Kardong, 1982; Jackson and Fritts, 1995) to be from grooved posterior fangs and it is possible that the grooved first fang of the embryonic snake represents a plesiomorphic condition retained from an opisthoglyphous ancestor.

Origin of the Infolding Hypothesis

The results of this study indicate that tubular fangs in elapids and viperids form according to the "brick chimney" manner (Bogert, 1943; Klauber, 1939, 1956). Why then, has the "infolding" hypothesis not been abandoned? Proponents of the "infolding" hypothesis fall into two groups. One group studied fang development, using histological cross sections of developing fangs (e.g., Tomes, 1875; Kochva, 1963; Lake and Trevor-Jones, 1987, 1995). However, cross sections taken at different points along the shaft of the fang can give the appearance of a series of replacement fangs forming a tubular fang by infolding. For example, the first fang in the series may be sectioned at mid-shaft, giving the appearance of a fully formed tubular fang, whereas the second tooth (also a tubular fang) may be sectioned at the level of the discharge orifice, giving the impression of a grooved fang in the progress of forming a venom canal. The other group of proponents of the "infolding" hypothesis are general works and review articles (e.g., Edmund, 1969; Cogger, 1992; Shea et al., 1993), in which the venom canal of tubular fangs is described as forming by infolding of a grooved tooth.

Also, it should be noted that, although the formation of mineralized teeth and fangs have been shown here and in some earlier studies (Klauber, 1939, 1956; Bogert, 1943) to form according to the "brick chimney" pattern, it is possible that "infolding" may play some role in fang formation at an earlier developmental stage. For example, the soft tissue scaffolding on which mineralized teeth form could be formed through "infolding," even though the mineralized teeth themselves are not.

Implications for the Evolution of the Venom-Delivery System

The front-fanged condition, including the presence of tubular fangs, is thought to have evolved indepen-

dently several times in snakes (Cadle, 1988; Knight and Mindell, 1994). Accordingly, elapids and viperids would have derived tubular fangs independently of one another. This study finds a fundamental similarity in the developmental morphology of elapid and viperid fangs. Bogert (1943) also found such a similarity, interpreting it as evidence of shared common ancestry, and postulating that all venomous snakes arose from a colubrid common ancestor. Further, he proposed that the viperids arose from an elapid ancestor. Although viperids and elapids are now thought to have evolved the front-fanged condition independently, the similarities of structure and development of their fangs are remarkable and it may be that the potential to develop fangs of this type is a synapomorphy of Colubroidea, which has been switched on independently in colubroid groups possessing front-fanged venom systems. The same selective pressures may have led to the origin of different front-fanged groups (Savitzky, 1980). Although tubular (proteroglyph and solenoglyph) fangs have been derived independently in different groups of venomous snakes (e.g., Elapidae, Viperidae, and Atractaspididae), they may have evolved following similar pathways, as postulated by Kardong (1979): natural selection may have favored tooth morphologies that most efficiently conveyed venom into a prey item, such as opisthoglyph (grooved) fangs over aglyph (ungrooved) fangs, opisthoglyph fangs with deep grooves over those with shallow grooves, and fangs with closed canals over those with open grooves. In this way, it could be said that hollow fangs *evolved* following an "infolding" sequence, even though they do not *develop* that way. Thus, similar selective pressures may independently have produced morphologically similar tubular fangs in different groups of venomous snakes.

ACKNOWLEDGMENTS

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