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Evolution of venom across extant and extinct eulipotyphlans

L'évolution du venin chez les eulipotyphles modernes et éteints

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ABSTRACT

Orally delivered venom in animals is found in distantly related invertebrate and vertebrate taxa, but is relatively rare in overall abundance. The trait would appear to be highly adaptive for prey capture and defence, and has been suggested to be a key innovation that led to the diversification of the venomous snakes. In extant mammals, oral venom is only found in the Eulipotyphla (which includes solenodons, shrews, moles and hedgehogs), and is only known to be present in four species. The phylogenetic distribution of venom across extant mammals suggests that venom evolved independently three times in the Eulipotyphla. In extant shrews, grooved teeth are not associated with venomousness; only the solenodon has both grooved lower incisors and salivary venom. Given these data, recent inferences of widespread venomous abilities in extinct eulipotyphlans on the basis of grooved teeth are not justified.

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R É S U M É

Le venin administré avec les dents est un caractère relativement rare chez les vertébrés. Le caractère semble être hautement adaptatif pour la capture de proie ou la défense ; il a été considéré comme une innovation majeure qui a conduit à la diversification des serpents venimeux. Chez les mammifères modernes, le venin oral n'est connu que chez quatre espèces d'Eulipotyphla (qui comprend les solénodontes ou almiqis, les musaraignes, les taupes et les hérissons). La distribution phylogénétique de venin chez les mammifères modernes suggère que l'acquisition de venin a évolué de façon indépendante, trois fois, chez les eulipotyphles. Chez les musaraignes, les dents rainurées ne sont pas associées à la présence de venin, et seul la solénodonte comporte à la fois des dents cannelées et de la salive venimeuse. Compte tenu de ces données, les inférences récentes de capacités venimeuses chez des eulipotyphles éteints sur la base de la présence de dents cannelées ne sont pas justifiées.

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1. Introduction

In animals, venom is found in a wide variety of taxa, including: spiders, wasps, scorpions, octopuses,

amphibians, teleosts, snakes, lizards and a few mammals (Büchler et al., 1968). The ability to subdue prey with venom and defend against predators would appear to be a highly adaptive trait, and thus it is puzzling that venom and venom delivery systems are not more common among animals. The delivery of venom from modified salivary glands has two components – the secretion of modified

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salivary proteins (the venom) and the delivery apparatus (the teeth). The manufacture of venom proteins and the architecture of the associated dental structures are complex, and have been extensively documented elsewhere (Fry et al., 2010; Ligabue-Braun et al., 2012). Venom delivered through hollow teeth is a highly specialized weapon nearly completely restricted to the toxiciferan reptiles (Fry et al., 2006); only the solenodon, an extant eulipotyphlan mammal, also possesses a duct-like tooth as well as salivary venom. Most other venomous vertebrates (e.g. the short-tailed shrew and the Komodo dragon) have sharp teeth for puncturing, after which venomous saliva bathes the wound produced by the puncturing teeth.

The discovery that several species of varanid lizard produce salivary venom (Fry et al., 2006) led to a transformation in how researchers think about the evolutionary history of venom. Fry et al. (2006) proposed the name Toxicofera for the common ancestor of anguimorph and iguanian lizards and snakes, as they believe the origin of venom to predate the split between those clades. This suggests that the capacity to develop venomous saliva may be more widespread than previously believed; in fact, Dufton (1992) and Hurum et al. (2006) posited that venomousness might have been a primitive trait for Mammalia.

Recently, several papers have suggested that venom may have been more widely distributed across mammals in the past, partly based on descriptions of fossil mammals with teeth hypothesized to be adapted for venom delivery (e.g. Cuenca-Bescós and Rofes, 2007; Fox and Scott, 2005; Furió et al., 2010; Hurum et al., 2006; Peigné et al., 2009; Rofes and Cuenca-Bescós, 2009; Turvey, 2010). Since orally delivered venom in extant mammals is restricted to only a few eulipotyphlans (*Blarina brevicauda*, *Neomys fodiens*, *Neomys anomalus*, and *Solenodon paradoxus*), the proposed widespread prevalence of oral venom delivery systems in extinct mammals begs the question: if venom was widespread across fossil mammals, and if it is an adaptive trait, why was it lost in most living mammals? Possible explanations include that venom may be costly to produce, venom may not be an adaptive trait, or purportedly “venomous” fossil mammals were not actually venomous. When phylogeny, current function and comparative anatomy are assessed, it is clear that venom delivery is not the ancestral condition for mammals, and it is therefore highly unlikely that venom was widespread in fossil mammals. In fact, there is no strong evidence for an inference of venom in any fossil mammal.

2. Venom in extant mammals

Venom is rare across mammals. The most famous example of venom in mammals is the male platypus (*Ornithorhynchus anatinus*), which the animal delivers from a spur on the calcaneus. Since the tarsal spur is a sexually dimorphic trait, it likely functions for mate defence in the platypus (Ligabue-Braun et al., 2012). The slow loris (genus *Nycticebus*) secretes a protein from the brachial gland near its elbow, which, when mixed with saliva and injected via a bite, produces anaphylaxis in a victim. Lorises apply their brachial secretions to the fur around their head, and appear to use them primarily as warning signals to conspecifics

(Hagey et al., 2007). The toxin seems to be a secondary function of the exudate, and presumably affects only susceptible species like humans (Hagey et al., 2007).

In extant eutherian mammals, the only order containing members with venomous saliva is the Eulipotyphla (hedgehogs, shrews, moles & solenodons) – this order contains about 452 species (Wilson and Reeder, 2005). Within Eulipotyphla, only four species have been shown to be venomous: the Hispaniolan solenodon (*Solenodon paradoxus*) [the Cuban solenodon (*S. cubanus*) is possibly also venomous, although it has not been tested] and three of 376 species of shrews (Soricidae) (*Blarina brevicauda*, *Neomys fodiens* and *N. anomalus*) (Dufton, 1992; Pucek, 1968). There is some evidence that other species of *Blarina* (*B. carolinensis* and *B. hylophaga*) and the Canarian shrew (*Crociodura canariensis*) (Lopez Jurado and Mateo, 1996) may be venomous; prey bitten by these animals reacts in a similar fashion to prey bitten by known venomous animals, but their saliva has not been tested experimentally (Lopez Jurado and Mateo, 1996). Many other species are as yet untested (including the American shrew, *Sorex cinereus*, and the European mole, *Talpa europaea*) (Dufton, 1992; Ligabue-Braun et al., 2012). Nussbaum and Maser (1969) observed the water shrew *Sorex palustris* immobilize a large vertebrate prey by grasping it by the head and interpreted this as evidence of venom; however, Pearson (1956) reported no significant toxicity in *S. palustris* saliva.

However, a number of species of eulipotyphlans and rodents have been tested and found to lack venom. These include: the European hedgehog (*Erinaceus europaeus*) (Mebs, 1999), the common mole (*Scalopus aquaticus*), the white-footed mouse (*Peromyscus leucopus*), meadow mouse (*Microtus pennsylvanicus*) (Pearson, 1942), a number of long-tailed shrews (*Sorex fumeus*, *S. pacificus*, *S. palustris*, *S. sinuosus*, *S. trowbridgii*, *S. personatus* and *S. vagrans*), the least shrew (*Cryptotis parva*), the shrew-mole (*Neurotrichus gibbsii*) (Pearson, 1950, 1956), and the greater white toothed shrew (*Crociodura russulla*) (Bernard, 1960). These observations suggest that, while not all species have been evaluated, venom is relatively rare across Eulipotyphla.

Of the 376 species of living shrews, only three are known to be venomous (0.8%). Even if we include species that are reasonably likely to be venomous – *B. carolinensis*, *B. hylophaga*, *Crociodura canariensis* – the proportion of venomous shrew species only increases to 1.6%. If we look at the proportion of all eulipotyphlans that are or may be venomous (including the solenodon), there are four known venomous species out of 452 (or eight if we extend it to the possibly venomous species, including *S. cubanus*). This means that 0.9% (at most 1.8%) of eulipotyphlans are venomous. If we assume that the proportion of modern venomous taxa accurately represents the distribution of venom in extinct species (and there is no good reason to assume that venom was more common in the past), we would predict that a similarly tiny fraction (less than 2%) of extinct species of shrew should be venomous.

Assessing the phylogenetic relationships among venomous shrews might shed light on how the trait evolved. Is it, as some have suggested (e.g. Hurum et al., 2006), a primitive trait that was lost in non-venomous lineages? Or is it an evolutionary novelty in a few eulipotyphlans?

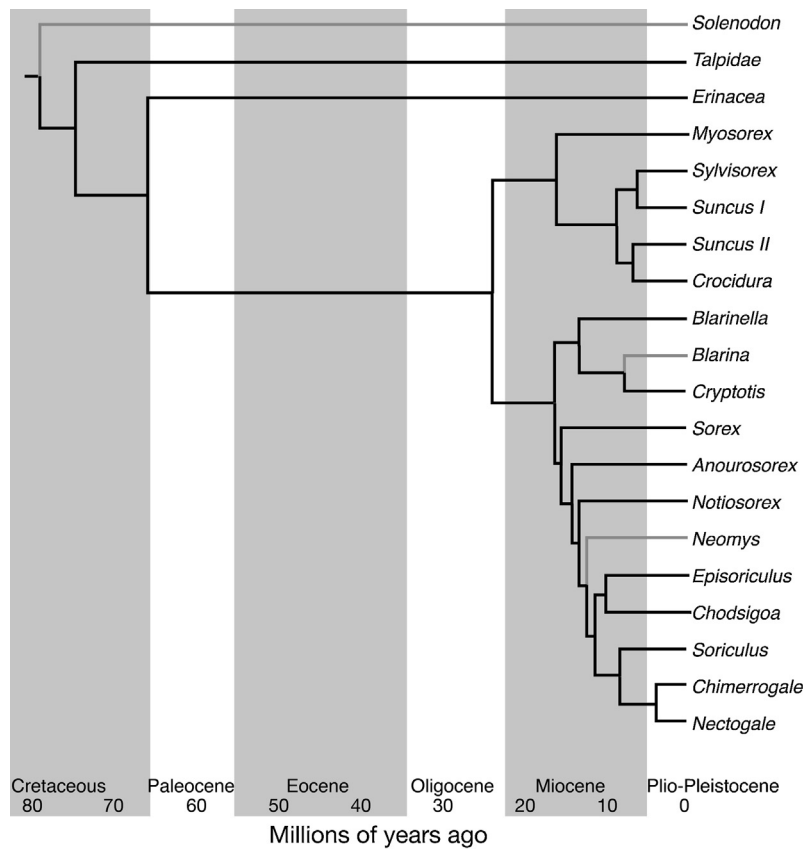


Fig. 1. Phylogenetic relationships in Eulipotyphla. Species with venom shown in grey, species lacking venom shown in black. This graphically illustrates both the rarity of venom among eulipotyphlans, as well as the evolutionary distance between venomous species. Phylogenies from Douady and Douzery (2009); Dubey et al. (2007, 2008); He et al. (2010); Ohdachi et al. (2006). Time calibration dates from Douady and Douzery (2009); Dubey et al. (2007, 2008).

Fig. 1. Relations phylogénétiques chez les Eulipotyphla. Les espèces à venin sont représentées en gris, les espèces sans venin en noir. Cela illustre graphiquement à la fois la rareté du venin au sein des eulipotyphlans et la distance évolutive entre espèces venimeuses. (Phylogénies selon Douady et Douzery, 2009; Dubey et al., 2007, 2008; He et al., 2010; Ohdachi et al., 2006). Échelle de temps selon Douady et Douzery (2009); Dubey et al. (2007, 2008).

If it is a novel trait, how many times did it evolve? To address these questions, I mapped the incidence of venom onto the most recent estimates of eulipotyphlan phylogeny (Fig. 1). Dubey et al. (2007, 2008) used both mtDNA and nuclear DNA to reconstruct relationships among shrews (Soricidae). He et al. (2010) extended this data set by incorporating additional sequences and several new taxa. As their results are congruent with Dubey et al.'s (2007, 2008) tree topology, I constructed a time-calibrated supertree combining the data from all three studies, plus estimates of the placement of basal eulipotyphlans Erinaceae and Solenodontidae (Douady and Douzery, 2009; Roca et al., 2004) (Fig. 1). This topology is consistent with Ohdachi et al.'s (1997) relatively unresolved tree, which also found the monophyletic groups (*Cryptotis*, *Blarina*), (*Episoriculus*, *Nectogale*, *Neomys*) and (*Crocidura*, *Suncus*). An alternate topology by Willows-Munro and Matthee (2011) only included 664 base pairs from the cytochrome *b* gene, and resulted in a different tree topology. Although the Willows-Munro and Matthee (2011) sample was more taxonomically diverse, the larger datasets of Dubey et al. (2007, 2008) and He et al. (2010) include the cytochrome *b*

gene as well as additional mtDNA and nuclear DNA, and is the topology I use here. There is some disagreement over the position of moles (*Talpa*) relative to the Soricomorph clade (*Solenodon*, *Sorex* and *Erinaceus*). In a recent combined morphological and molecular analysis of placental mammals, O'Leary et al. (2013) found *Talpa* to be basal to the Soricomorphs. This would not significantly change the tree topology, as *Solenodon* is still basal to a clade of (*Sorex*, *Erinaceus*).

This phylogeny demonstrates that *Neomys*, *Solenodon* and *Blarina brevicauda* are phylogenetically distant; the *Solenodon* lineage split from the other eulipotyphlans around 80.5 mya (Douady and Douzery, 2009) and the ancestors of *Neomys* and *Blarina* split around 16.5 mya (Dubey et al., 2007) (Fig. 1). If the ancestral condition in Eulipotyphla is to possess venom, with the primitive retention of the trait in *Blarina* and *Neomys* and the loss of venom in other lineages, it would require a minimum of nine convergent losses of venom. However, if venom evolved convergently, it would only require three unique acquisitions. Thus, the most parsimonious explanation of these data is that venom evolved independently in

Solenodon, *Neomys* and *Blarina* from non-venomous ancestors.

The observation that venom evolved multiple times in the eulipotyphlan lineage might suggest that this small mammal group is exapted (sensu Gould and Vrba, 1982) to acquire the ability to secrete venom proteins. The synthesis of venom is complex, so maybe there is a chemical present in the salivary glands of all soricids or eulipotyphlans that allows these taxa to independently acquire venom more easily than in other species. However, the acquisition of venom is rare, occurring in less than 1% of eulipotyphlans, so either it is not particularly adaptive or it is constrained by other factors.

2.1. Venom chemistry and morphology of venom teeth across mammals

The biochemistry, genetic basis and ecological function of oral venom across eulipotyphlans are very poorly understood. Because of the difficulties in collecting large quantities of fresh venom, coupled with the endangered status of *Solenodon*, only the venomous saliva of *Blarina brevicauda* has been well described. Kita et al. (2004, 2005) called the secretion from the submaxillary and sublingual salivary glands of *Blarina* “blarina toxin” (BLTX). BLTX acts to cleave protein bonds in a similar fashion to tissue kallikreins (Kita, 2012; Kita et al., 2004, 2005); the net result is that injection of the toxin causes paralysis, irregular respiration, and convulsions in prey before death (Pearson, 1942, 1956; Pucek, 1959, 1968). Unfortunately, similar studies have not been conducted on the salivary secretions of *Solenodon* and *Neomys*, so we do not yet know whether they have a similar chemical composition. Pucek (1968) and Dufton (1992) noted that *Blarina* venom is the most toxic among venomous eulipotyphlans. The venom of *Neomys fodiens* is about one third as toxic as *Blarina* venom, in *N. anomalus* it is about one sixth as toxic, and *Solenodon* is 1/20 as toxic, based on a minimum lethal dose to mice (Pucek, 1968) (Fig. 1).

In *Solenodon*, *Blarina* and *Neomys*, the venomous saliva is produced by the submaxillary gland and conducted into the mouth via the submaxillary duct in the mandible (Pearson, 1950). There was no effect on experimental animals to secretions from the parotid gland (Ellis and Krayner, 1955; Pearson, 1942; Pucek, 1968); this is in direct contrast to snakes, in which the parotid gland secretes venom delivered through the maxillary fangs (Pearson, 1942). *Solenodons* have a tubular lower incisor that functions like a hypodermic needle to deliver venom. Shrews, on the other hand, deliver venomous saliva to the victim through punctures with their pointed anterior teeth. The teeth of *Blarina* and *Neomys* have no grooves; Pournelle (1968) suggested that the slightly concave inner surface of the lower incisors of those taxa may conduct venom; however, this has not been tested and non-venomous shrew species also have concave lower incisors (e.g. *Cryptotis parva*, *Sorex*, pers. obs.; Churchfield, 1990).

Several recent studies (Folinsbee et al., 2007; Orr et al., 2007) have documented the widespread distribution of grooved teeth across mammals including primates, suids and bats, and noted that grooves are rarely associated with

venom. In fact, in mammals, only solenodons possess both deep channels in their teeth and oral venom. Grooved or channeled teeth are not common in eulipotyphlans, but are present in some. For example, in the common mole (*Scalopus aquaticus*), the lower second incisor is large and has a deep mesial channel (Fig. 2). Pearson (1942) found that saliva extracted from moles using the same methods as for *Blarina* had no effect on experimental animals, therefore this channel in moles does not function to deliver venom.

2.2. Venom function in extant eulipotyphlans

There are several possible hypotheses explaining the rarity of venom in mammals:

- it may not be adaptive in mammals – mammals have little need for venom;
- there is some biological constraint on venom production;
- venom is costly to produce and maintain;
- venom is only adaptive in a narrow range of morphology (possibly small body size combined with active life history and high metabolism).

Perhaps it is too costly to secrete and deliver venom, or antivenoms are difficult to produce and intraspecific aggression results in high mortality. Alternatively, maybe there are more living venomous soricids that have not yet been detected. It seems unlikely that venom is widespread yet undetected; a number of species have been tested and found to lack venom, and there are few natural history observations that would suggest that most eulipotyphlans have venom.

The function of shrew venom appears to be predatory – there are documented accounts of *Blarina brevicauda* preying on voles (Eadie, 1952), mice (Eadie, 1948), long tailed shrews (Eadie, 1949) and snakes (O'Reilly, 1949). Tomasi (1978) and Martin (1981) showed that *Blarina* uses its venom to immobilize prey in order to cache it in tunnels for later consumption; the venom renders the prey comatose, which keeps it fresh for a long period. Pournelle (1968) suggested that the proteolytic enzymes in shrew venom may help to initiate digestion of the large amount of protein they consume. Most descriptions of shrews biting vertebrate prey suggest they preferentially aim for the back of the neck and base of the skull, and take multiple, rapid bites (Bernard, 1960; Churchfield, 1990; Eadie, 1949; Haberl, 2002; Tomasi, 1978). Tomasi (1978) found that bites from *Blarina* were usually fatal when inflicted on small prey (< 10 g) or on larger prey when bitten near the head and neck. In fact, shrew bites were only fatal in about 20% of prey weighing over 10 g ($n=40$). Tomasi concluded that “shrew venom is not particularly important for killing rodent prey, and may have other uses.” (1978: 853). In addition, many non-venomous shrews also hoard and cache prey in storage tunnels [e.g. *Sorex araneus* (Churchfield, 1990), *S. cinereus*, *S. arcticus*, *Talpa europaea* (Vander Wall, 1990), *Cryptotis parva* (Formanowicz et al., 1989)]. Hoarding appears to be related to body mass; Rychlik and Jancewicz (2002) found that smaller shrews hoarded relatively more smaller sized prey, while larger

Table 1

Life history characteristics of venomous and some non-venomous eulipotyphlans for which basal metabolic rate (BMR) is known. Data from Churchfield (1990); George et al. (1986); Hayssen and Lacy (1985); Merritt (1995); Morrison et al. (1957); Nowak (1999); Rychlik and Jancewicz (2002); Tomasi (1984); Wilson and Reeder (2005).

Tableau 1

Caractéristiques de l'histoire de vie des eulipotyphlés venimeux et non venimeux pour lesquels le taux métabolique (BMR) est connu. Les données sont de Churchfield (1990); George et al. (1986); Hayssen et Lacy (1985); Merritt (1995); Morrison et al. (1957); Nowak (1999); Rychlik et Jancewicz (2002); Tomasi (1984); Wilson et Reeder (2005).

Species	Body mass (g)	Primary diet	Life history	Daily food consumption (% body mass)	Basal metabolic rate (mL O ₂ /g h)
Venomous eulipotyphlans					
<i>Blarina brevicauda</i>	15–30	Vertebrates, invertebrates, plants	Territorial, hoarding, nocturnal, fossorial	56	3.18
<i>Solenodon paradoxus</i>	1000	Vertebrates, invertebrates, plants	Social, nocturnal	Unknown	Unknown
<i>Neomys fodiens</i>	12–18	Vertebrates, invertebrates	Territorial, hoarding, solitary, aquatic	60	3.22–4.74
<i>Neomys anomalus</i>	12–18	Vertebrates, invertebrates	Territorial, aquatic	Unknown	4.98–5.1
Non-venomous shrews					
<i>Cryptotis</i> (18 sp.)	4–7	Vertebrates, invertebrates, carrion	Hoarding	–	–
<i>Cryptotis parva</i>	3–6	Insects	Social, diurnal/nocturnal	Unknown	7.0
<i>Sorex</i> (68 species)	2–18	Invertebrates, plants	Hoarding	–	–
<i>Sorex palustris</i>	8–18	Insects	Aquatic, solitary, crepuscular, aggressive, hoarding	95	4.84
<i>Sorex araneus</i>	9	Invertebrates	Hoarding	80–90	7.38
<i>Sorex cinereus</i>	2.5–4	Invertebrates, seeds, fungi		158	5.75–8.92
<i>Sorex minutus</i>	4	Invertebrates		125	7.04–9.62
<i>Sorex vagrans</i>	5.2	Vertebrates, invertebrates, plants	Solitary	Unknown	3.79
<i>Notiosorex</i> (1 sp.)	3–5	Invertebrates, carrion	Desert-adapted, social	–	3.27
<i>Crocidura</i> (158 sp.)	From 3–13 to 37–65	Vertebrates, invertebrates	Aggressive, experience torpor	–	–
<i>Crocidura russula</i>	11	Invertebrates, carrion	Monogamous, semi-social, diurnal/nocturnal	48	2.45–3.7
<i>Crocidura suaveolens</i>	7.5	Invertebrates, carrion	Cathemeral	48	2.81
<i>Crocidura leucodon</i>	6–13	Invertebrates, carrion		70	2.55
<i>Crocidura occidentalis</i>	33	Invertebrates, carrion		Unknown	1.34
<i>Suncus</i> (15 sp.)	From 2 to 105.6	Insects		–	–
<i>Suncus murinus</i>	50–100	Insects		Unknown	1.66
<i>Suncus etruscus</i>	2	Insects		Unknown	3.6–5.75
Other eulipotyphlans					
<i>Scalopus aquaticus</i>	58.5	Invertebrates, vegetation	Cathemeral	Unknown	1.655
<i>Erinaceus europaea</i>	1200	Invertebrates, vertebrates		Unknown	0.396

shrews hoarded fewer prey of larger size. Hoarding appears to be restricted to shrews; captive solenodons were not observed to hoard or cache food (Eisenberg and Gould, 1966).

Body size alone is not correlated with the presence of venom in eulipotyphla (Table 1). Eulipotyphlans with oral venom range in mass from 12 g (*Neomys*) to 1 kg (*Solenodon*). In between these sizes, there are many closely related animals that do not possess venom. If we focus only on shrews, the venomous taxa (*Blarina* and *Neomys*) are of average shrew size (15–30 g and 12–18 g, respectively). Most shrews are not venomous, even those that hunt small prey and store it in burrows (e.g. *Sorex* and *Cryptotis*). In addition, non-venomous shrew clades are incredibly evolutionarily successful despite their lack of venom – there are 158 species of *Crocidura*, 68 species of *Sorex* and 18 species of *Cryptotis* (Nowak, 1999), none of which appear to be venomous.

Venomous shrews do not consume significantly more food on a daily basis than non-venomous shrews; *Blarina* consumes about 56% of its mass daily, and *Neomys* consumes about 60% (Table 1). In captive conditions, however, *Neomys* was observed to eat twice its weight in food in one day (Pournelle, 1968). In contrast, *Sorex cinereus* and *Sorex minutus* consume 158% and 125% of their body mass per day, respectively (Churchfield, 1990).

Smaller mammals have relatively higher basal metabolic rates (BMRs) and require relatively more food per gram of body mass than larger animals. Shrews have an even higher metabolic rate than predicted, relative to other similarly sized small mammals relative to body mass (Churchfield, 1990) and require a high caloric intake (Dufton, 1992); within shrews, crocidurines have a relatively lower BMR and can enter torpor, while soricines do not enter torpor and maintain a higher BMR (Taylor, 1998). This is likely related to ecology; soricines live in seasonal

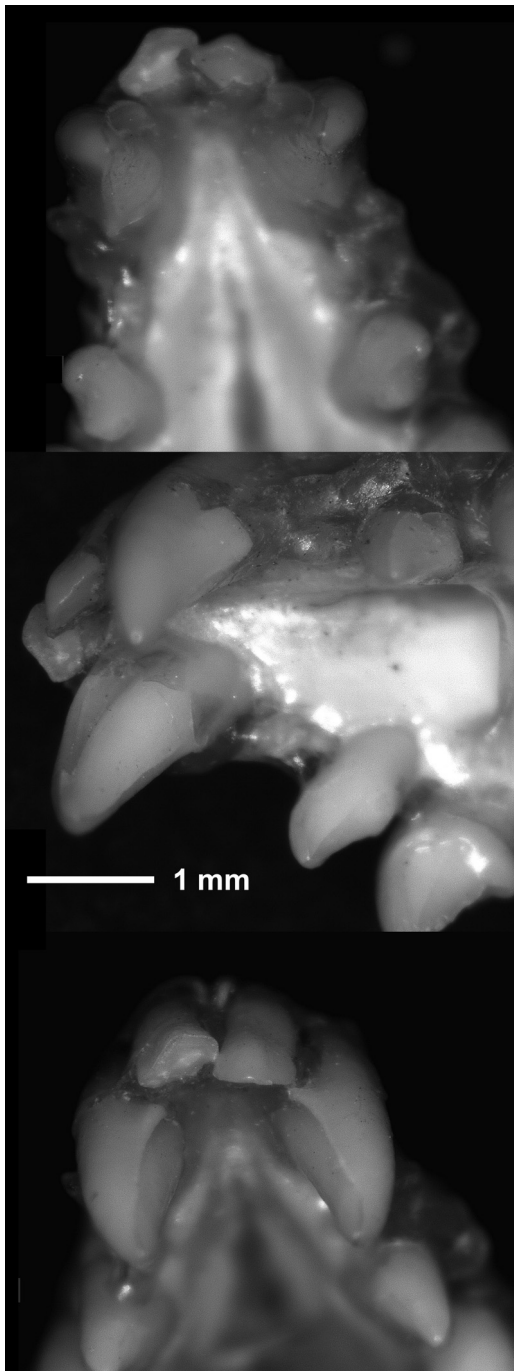


Fig. 2. Anterior portion of the dentary of the eastern mole (*Scalopus aquaticus*) showing deeply grooved mesial surface of the second incisors. This groove runs along the entire surface of each tooth along the anterior lingual corner. The groove is variable in depth among individuals. Moles are not venomous.

Fig. 2. Portion antérieure du dentaire de la taupe orientale (*Scalopus aquaticus*) montrant la surface mésiale des secondes incisives profondément cannelée. Cette cannelure court sur toute la surface de chaque dent, le long du coin antérieur de la langue. La profondeur de la cannelure varie en fonction des individus. Les taupes ne sont pas venimeuses.

environments, so they rely on an energetically expensive strategy, while crocidurines live in warm or unpredictable environments (Dubey et al., 2007; Genoud, 1988). Venomous shrews have a relatively high basal metabolic rate relative to body mass [(3.18 mL O₂/g h in *Blarina* and 3.22 to 5.1 in *Neomys*) (Table 1) (Churchfield, 1990; Genoud, 1988)]. The basal metabolic rate of *Solenodon* is unknown.

Using *ggplot2* (Wickham, 2009) in R (R Core Team, 2012), I plotted body mass relative to BMR in the 18 species for which BMR is known (Table 1, Fig. 3). As expected (see Churchfield, 1990; Genoud, 1988), there is a significant negative relationship between body mass and BMR (slope = -1.74 ± 0.25 , $t = -6.9$, $P < 0.00001$, adjusted $r^2 = 0.73$). However, this relationship may be influenced by the close evolutionary relationships among these taxa; BMR and mass may be inherited through descent rather than causally correlated. To test this, I calculated phylogenetic independent contrasts using *ape* version 3.0–8 in R (Paradis et al., 2004). This relationship is still significant, though less so, even when controlling for phylogeny ($t = -3.22$, $P = 0.0058$).

To test whether venomous species differ significantly from non-venomous species with respect to either BMR or body mass, I calculated phylogenetic independent contrasts on log transformed mass and BMR of the 18 species for which BMR is known. When controlling for phylogeny, there is no significant difference between venomous and non-venomous taxa with respect to either body mass ($t = 1.78$, $P = 0.095$) or BMR ($t = -0.169$, $P = 0.87$). These data suggest that there may be other factors influencing the maintenance of venom in these species, other than the need to maintain a large body mass and high BMR. However, the relatively small sample size (three venomous species) may also explain the lack of a significant relationship.

An observation that argues against the hypothesis that venom functions to allow eulipotyphlans to cache more prey is that *Solenodon* is also venomous and does not hoard or cache food (Eisenberg and Gould, 1966). *Solenodon*, like soricine shrews and unlike crocidurine shrews, does not exhibit a period of seasonal torpor – they are active year-round (Eisenberg and Gould, 1966). They may have a higher than average metabolic rate in order to sustain this continual activity; however, their BMR is not known. *Solenodons* may employ venom for different reasons than shrews – *solenodons* do not seem to be immune to their own venom (Rabb, 1959), and perhaps they experience high intraspecific competition. Assessing the BMR of *Solenodon* would contribute to understanding the relationship between venom, BMR and body mass. There are a number of large eulipotyphlans for which BMR is not known (e.g. *Scutisorex* 30–90 g, *Feroculus* 35 g, *Nectogale* 25–45 g) – assessing their BMR and whether or not they are venomous would further test the relationship between BMR, body size and venom.

As in mammals, metabolic rate in snakes is likely not correlated to venomousness. McCue (2007) showed that two non-venomous (*Python regius*, ball python and *Elaphe obsoleta*, ratsnake) and one venomous (*Crotalus atrox*, western diamondback rattlesnake) species significantly reduced their metabolic rate during periods of starvation. The venomous rattlesnakes showed the greatest

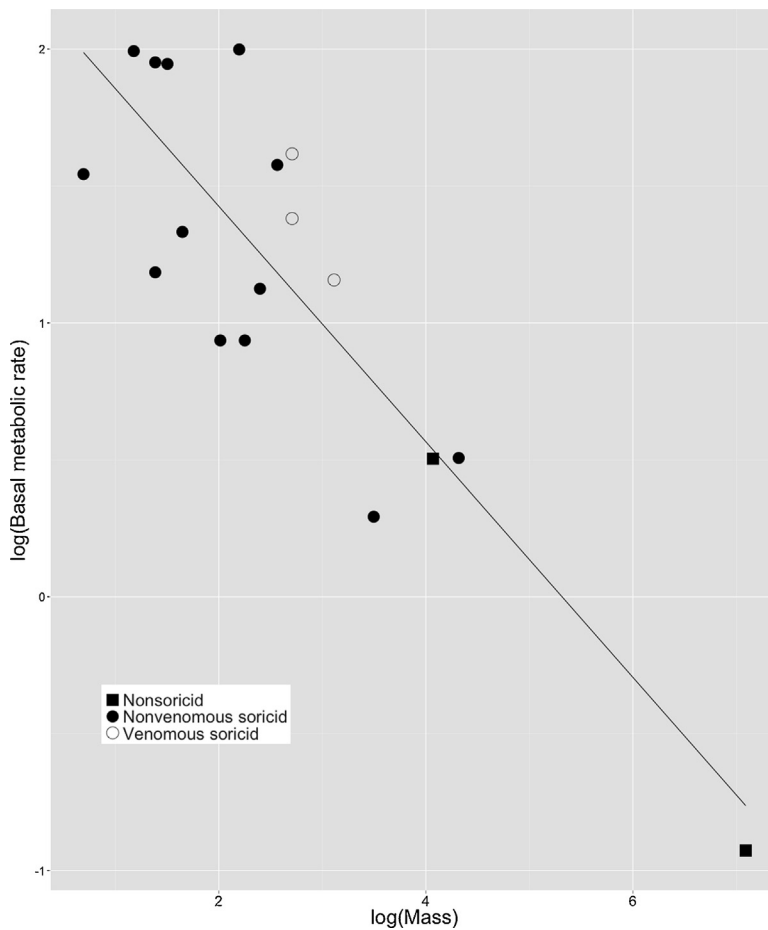


Fig. 3. Relationship between basal metabolic rate (BMR) and body mass in venomous (open circles) and non-venomous (closed shapes) eulipotyphlans. Soricids are represented by closed circles and Eastern mole (*Scalopus aquaticus*) and European hedgehog (*Erinaceus europaea*) are shown in closed squares. Data from Table 1 and sources cited therein. Where mass and BMR are reported as a range, I used the midpoint. There is a significant negative relationship between BMR and body mass (slope = -1.74 ± 0.25 , $t = -6.9$, $P \leq 0.00001$, adjusted $r^2 = 0.73$). When controlling for phylogeny, there is no significant difference between venomous and non-venomous eulipotyphlans with respect to either body mass or BMR.

Fig. 3. Relation entre le taux métabolique de base (BMR) et la masse corporelle chez les eulipotyphlés venimeux (cercles blancs) et non venimeux (symboles noirs). Les Soricidés sont représentés par des cercles noirs et la taupe orientale (*Scalopus aquaticus*) et la taupe européenne (*Erinaceus europaea*) par ces carrés noirs (pour les données et les sources, voir le Tableau 1). Quand la masse et le taux métabolique de base sont fournis dans une fourchette, l'auteur a pris en compte le point médian. Il y a une corrélation négative significative entre le BMR et la masse corporelle (pente = $-1,74 \pm 0,25$, $t = -6,9$, $p \leq 0,00001$, r^2 ajusté = 0,73). Si l'on contrôle la phylogénie, il apparaît qu'il n'existe pas de différence significative au regard, soit la masse corporelle, soit du BMR entre les eulipotyphlés venimeux et non venimeux.

reduction in metabolic rate at 72% during starvation (compared to 41% in the python and 45% in the ratsnake). However, the resting metabolic rate of the venomous species pre-starvation was not significantly higher than the non-venomous species (McCue, 2007). Phylogeny and life history characteristics such as foraging mode and diet are better predictors of metabolic rate in snakes than is venom.

3. The evidence for venom in fossil mammals

Recently, several fossil eulipotyphlans have been described in which venom has been inferred on the basis of grooved teeth (Cuenca-Bescós and Rofes, 2007; Peigné et al., 2009; Rofes and Cuenca-Bescós, 2009; Turvey, 2010). An inference of venom in fossil mammals can be made based on the following criteria:

- if grooved teeth are used as the basis of an inference of venom, the morphology of the grooves should be similar to other venomous mammals with grooved teeth (i.e. only solenodons);
- because grooves alone are insufficient for inferring venom, venom could be inferred in a fossil taxon if it was phylogenetically bracketed between two or more extant venomous taxa (Witmer, 1995).

However, even if a single fossil species can be shown to be venomous based on these criteria, it does not tell us much about the overall significance of venom in mammal evolution.

To apply the first criterion to soricids, most shrews lack grooved teeth, including the venomous shrews (*Blarina brevicauda* and *Neomys*). Venomous shrews salivate copiously

(Churchfield, 1990; Pucek, 1959), and envenomate their prey by delivering multiple rapid but shallow bites to prey, aiming for the head and neck (Bernard, 1960; Churchfield, 1990; Eadie, 1949; Haberl, 2002; Tomasi, 1978). Some of the lower incisors of *Blarina* and *Neomys* show a slight concavity, as do the teeth of a number of other, non-venomous soricids (Pournelle, 1968); however, this very slight guttering is unlikely to function as a means of delivering venom. Venom entrance into a wound appears to be transferred somewhat haphazardly, unlike the needle-like delivery system of snakes, and it is not clear that the slight concavity in the lower teeth serves any function in delivering venom. Many non-venomous shrews also have slightly concave lower incisors (Churchfield, 1990; Pournelle, 1968).

Because no extant shrew possesses both grooved teeth and venomous saliva, grooves on the teeth of fossil shrews are unlikely to be related to venom delivery. Solenodons have venom delivery teeth – the lower second incisors form tubes or channels that are suggested to act like hypodermic needles (Dufton, 1992; McDowell, 1958) – as well as venomous saliva. Only *Solenodon paradoxus* has been shown to possess venom, although *S. cubanus* has not been tested, and two other species recently went extinct. Therefore, fossil solenodon relatives with channeled lower incisors similar to *S. paradoxus* are likely to share the trait through homology, and could be inferred to have also had venom.

Cuenca-Bescós and Rofes (2007) and Rofes and Cuenca-Bescós (2009) described two species of large shrew from the Pleistocene of Sierra de Atapuerca, Spain – *Beremendia fissidens* and *Dolinasorex glyphodon*. On the basis of a groove on the mesial surface of the lower incisors, they infer that these species were likely to have been venomous. Folinsbee et al. (2007) made the assessment that the inference of venom delivery in *Beremendia* and *Dolinasorex* was better supported than for the pantolestid *Bisonalveus browni* (Fox and Scott, 2005), because the former are members of the Eulipotyphla, which contains venomous members. However, this statement bears closer examination – Eulipotyphla is a large clade containing around 452 species (Douady and Douzery, 2009), 99% of which are not venomous. Since no known living shrews possess both grooved teeth and venom, inferring venom on the basis of a mesial groove on the incisors cannot be supported. In fact, the morphology of the groove in the lower left incisors of these two species looks very similar to the grooved lower second incisor of the eastern mole (*Scalopus aquaticus*) (Fig. 2), which has been shown to lack venom (Pearson, 1942).

When we consider Eulipotyphlan phylogeny (Fig. 1), it becomes apparent that venom is a rare trait that likely evolved convergently at least three times. Given the evolutionary rarity of the trait at present, it is unlikely to have been widely distributed in the past. Rofes and Cuenca-Bescós (2009) perform a phylogenetic analysis of morphological characters including both extinct and extant species. Their tree topology is quite different from that of Dubey et al. (2007, 2008) (Fig. 1); however, like Dubey et al. (2007, 2008) and He et al. (2010), they reconstructed a monophyletic group that includes [*Soriculus* (*Chimarrogale*, *Nectogale*)]. Rofes and Cuenca-Bescós (2009) determined that the extinct genera *Dolinasorex* and *Beremendia* fall

within this monophyletic group. An inference of venom might be warranted, although not on the basis of grooved teeth, if *Dolinasorex* and *Beremendia* were the sister taxa of *Neomys* or *Blarina*. However, they are only distantly related to known venomous shrews, and therefore there is no support for the hypothesis that they inherited venom from a venomous ancestor.

Furió et al. (2010) suggested that *Beremendia* used venom to hoard small coleopteran and gastropod prey, in the same way that living venomous shrews do, to contend with the unpredictable Pleistocene environmental conditions. They claim that in living shrews “[t]here is no doubt that the lower incisor is always the main element involved in this venomous injection, and that the groove acts as a channel to conduct the venom from the salivary glands to the anterior part of the lower incisor. Nevertheless, although the grooves do exist in most of the species (Fig. 5), they are usually difficult to discern due to their shape and/or smaller dimensions.” (2010: 933). Small, shallow concavities in the lower incisors may indeed exist in *Sorex* and *Crocidura* (as shown in Furió et al.’s fig. 5); however, neither *Sorex* nor *Crocidura* possess venomous saliva (Pearson, 1942, 1950, 1956). Both *Blarina* and *Neomys* have a slightly concave lingual face of the lower first incisor, which has been suggested to function to transmit venom (Pournelle, 1968), but this has not been tested. In fact, the shallow concavity on the lingual face of the lower central incisors in *Blarina* and *Neomys* could only possibly function as a location for venom to pool, but it is too shallow, open and small to “act as a channel to conduct venom”. An alternative hypothesis is that the groove serves a mechanical function to resist bending.

The shallow concavities in the extant venomous shrews are very different in morphology from the deeper grooves in *Beremendia*, *Dolinasorex* and *Scalopus*. Both *Beremendia* and *Dolinasorex* were large, estimated to be about 40–45 g and 55–60 g, respectively (Cuenca-Bescós and Rofes, 2007; Rofes and Cuenca-Bescós, 2009). Moles are also larger than most shrews, with a body mass of about 65 to 120 g for the European mole and 40 to 140 g for the Eastern American mole (Nowak, 1999). The difference in the size of the concavity or groove in the lower incisors between small (e.g. *Neomys*, *Blarina*, *Sorex* and *Crocidura*, all of which are less than 30 g) and large eulipotyphlyans (e.g. *Scalopus*, *Talpa*, *Beremendia* and *Dolinasorex*) may simply be a result of allometric scaling. Small shrews tend to have slightly concave lower incisors, and larger animals with larger teeth have correspondingly larger grooves. Because the presence of a groove in the lower teeth is not restricted to venomous taxa, we cannot automatically assume that it functions to deliver venom.

Peigné et al. (2009) describe a large soricoid, *Siamosorex debonisi*, from the Oligocene of Thailand. They suggested on the basis of grooved second incisors and its phylogenetic position as a soricid that *S. debonisi* might have had venomous saliva. Similar to *Beremendia* and *Dolinasorex*, *Siamosorex* is relatively large in size, and therefore a simple allometric relationship can explain the presence of a slightly deeper groove in the teeth. Peigné et al. (2009) qualified their assessment, stating that it is almost equally as likely that *Siamosorex* lacked venom.

Although these extinct shrews have some unique traits, they do not meet either criterion for inferring venom: they do not have grooved teeth that are similar to grooved teeth in venomous animals, nor are they closely phylogenetically related to known venomous taxa. Venom in fossil shrews cannot be inferred from the presence of deeply grooved teeth because no extant shrew possesses both deeply grooved teeth and venom.

Turvey (2010) described the unique dental features in the recently extinct genus of West Indian eulipotyphlan, *Neophontes*, which he inferred to be related to venom delivery. He described two deep grooves the upper canines, which look similar to the deep grooves in the lower incisors of solenodons. As Turvey stated, this would require the convergent evolution of toxic saliva from modified parotid glands, rather than from modified submaxillary glands as in all other orally delivered mammalian venom (Dufton, 1992; Pearson, 1950). The phylogenetic placement of *Nesophontes* is uncertain, but it appears to be either the sister-taxon of *Solenodon*, or to fall deeper within the Eulipotyphla clade as the sister to the soricids (Asher et al., 2005; Roca et al., 2004; Turvey, 2010). However, as there is no known mammal with modified parotid glands that secretes venom, this inference must remain tentative. Interestingly, since these animals are recently extinct, there is the possibility that DNA analysis might reveal genes for venom production.

Unfortunately, modern venomous eulipotyphlans, with the exception of solenodons, give no evidence of their venomous capabilities from their skulls and teeth alone. If *Blarina* or *Neomys* were found in the fossil record, we would never infer that they possessed salivary venom. Conversely, the deeply grooved lower second incisors of living eastern moles (Fig. 2), as well as the canines of baboons and bats (Folinsbee et al., 2007; Orr et al., 2007) could suggest to paleontologists that venom is more widely distributed than it is. This starkly illustrates the difficulty in inferring soft tissue-based traits from the fossil record.

A bias toward reporting purportedly “venomous” mammals may result in their overrepresentation in the published literature. Shrews are small, speciose and exist in restricted endemic home ranges. Many species of fossil shrews are unlikely to be thoroughly described in the literature, unless there is a novel aspect about their biology.

4. Evolution of salivary venom across mammals

The hypothesis that the earliest mammals were venomous has been proposed previously, based on the notion that modern venomous mammals are found in “primitive” clades (i.e. Dufton, 1992; Fox and Scott, 2005; Hurum et al., 2006). Dufton (1992) suggested that all early placental insectivores may have been venomous, with only a few species retaining the trait. He proposed that other eulipotyphlans lost the capacity to produce venom because they were too successful as predators and outcompeted their vertebrate prey. This, he argued, resulted in a shift to an insectivorous diet in early eulipotyphlans, and a subsequent loss of the need for venom in all but a few species. This explanation is unlikely for several reasons: first, venomous shrews (*Blarina* and *Neomys*) are engaged

in stable predator-prey webs with rodents (e.g. *Microtus*) without causing a precipitous decline in these vertebrate prey species (Barbehenn, 1958; Bernard, 1960; Eadie, 1948, 1952); natural history observations do not bear out the prediction that venomous shrews are capable of decimating rodent populations, nor has this phenomenon been observed in nature in other species. Second, because venom is so rare and widely dispersed across eulipotyphlans (Fig. 1), this hypothesis would require the homoplastic loss of venom in at least nine lineages. Finally, venom seems to be incredibly effective for preying on invertebrates, which make up the vast majority of the diet in *Blarina* and *Neomys* (Pournelle, 1968). Therefore, it does not make sense that venom would have fallen into disuse across the majority of eulipotyphlan lineages merely because they switched to invertebrate prey; *Blarina* and *Neomys* use their venom to great advantage on invertebrates as well as vertebrates. The rarity of venom, and the fact that it is found in relatively derived eulipotyphlans, suggests that it evolved convergently rather than being a primitive retention.

But how could such complex chemical neurotoxins evolve repeatedly in distantly related species? Reptile venom evolution has been the subject of much more intensive study than venom in mammals, and may provide some insight into how salivary venoms convergently evolve. Salivary enzymes, including lysozymes and amylase, initiate the digestive process by breaking down food when it enters the oral cavity. Fry et al. (2006) suggested that all snakes and lizards have potentially toxic components in their saliva, and the lineage may have originally been venomous. Most venoms are proteins recruited from molecules that perform different functions; simple chemical changes result in the novel protein acquiring a proteolytic or toxic activity (Fry et al., 2009). For example, the protease BLTX in *Blarina* converged on a nearly identical protein structure to the toxin GTX in the Mexican beaded lizard (*Heloderma horridum*) with very similar effects on tissue (Aminetzach et al., 2009). These observations suggest that venom enzymes evolved independently multiple times across a wide range of taxa from basic salivary enzyme building blocks.

Several studies of mammalian venom demonstrated that there are a wide variety of digestive compounds produced in saliva. Pearson (1950) found in *Sorex* and *Cryptotis parva* granular tissue similar to the venom producing cells in *Blarina* – their saliva had no impact on experimental animals. He also observed that estrous female *B. brevicauda* lacked the granular segment of the submaxillary gland, and suggested that hormones may have an impact on venom production in these animals. Hiramatsu et al. (1980) found that saliva from male mice is highly toxic to guinea-pigs, rats and hamsters, and contained kallikrein-like enzymes. They did not test female mice, but suggest that, based on gross morphology, females lack the serous-like granules that produce the compounds. Vampire bats secrete two classes of anticoagulants: plasminogen activators and inhibitors of proteinases (Basanova et al., 2002; Ligabue-Braun et al., 2012), which are similar to the peptidase S1 toxins found in *Blarina* (Fry et al., 2009). Therefore, the basic morphological and chemical components to produce venom are present across a much wider range of

mammalian species than produce venom, and show a large amount of variation.

We suggested previously (Folinsbee et al., 2007) that to infer venom in an extinct species, it should be closely phylogenetically related to a clade in which venom is present. However, recent studies have shown that the chemical building blocks of venom are relatively widespread, suggesting that venom acquisition may evolve more easily than previously thought (Fry et al., 2009). In mammals, this does not require a retention of the venomous condition (contra Dufton, 1992; Fox and Scott, 2005; Hurum et al., 2006); rather, the morphological features (salivary glands, either parotid or submaxillary) and physiology (secretory proteins that digest large molecules) present in all mammals can be modified through genetic mutations to produce and deliver substances that are neurotoxic to prey.

Venom can potentially serve four functions: defence, an aid to digestion, to assist in oral hygiene, and to assist in prey capture (Arbuckle, 2009). Of these, prey capture is the most likely explanation for the presence of venom in mammals (Martin, 1981; Tomasi, 1978).

Although the relationship between venom, BMR and body mass is not significant, *Blarina* and *Neomys* have both large body mass and high metabolic rates (Fig. 3). There may be selective pressure on a trait like venom, which enables large shrews to collect more prey in order to maintain their mass and high metabolism. Smaller shrews, even with high BMRs, do not need to consume as many calories, and may therefore be capable of storing sufficient food without the need for venom.

Alternatively, there may be a high cost to venom production, which results in selection against the evolution of venom in most animals. Although the basic capability to produce, secrete and deliver digestive enzymes of varying degrees of strength is relatively primitive and widespread across mammals, there may be significant metabolic costs to producing neurotoxic agents in salivary glands. There are no data on the metabolic costs of venom production in mammals, but data from snakes and arachnids suggest that replenishing venom incurs a relatively high metabolic cost (McCue and Mason, 2006; Morganstern and King, 2013; Pintor et al., 2010), and that most venomous taxa limit the amount of venom they expend per bite (Morganstern and King, 2013). This alone could explain the rarity of venom across mammals.

5. Future research

A very small proportion of the 452 extant eulipotyphlan species have been tested for oral venom. This is partly because it is logistically difficult – large samples of fresh saliva are difficult to collect, shrews are difficult to maintain in captivity and salivary proteases degenerate rapidly. Shrews are also small, cryptic, nocturnal and tend to be endemic in small ranges. However, to confirm that venom is relatively rare in eulipotyphlans, many other species should be tested for the presence of salivary venom. In addition, understanding the genetic basis for venom in *Neomys* and *Blarina*, and the chemical differences between their venoms would provide a test of the hypothesis postulated here, that venom evolved independently in both species. If

Neomys has a different toxic compound from BLTX, it would be strong support that venom evolved independently in the two taxa [although, since BLTX-like proteases evolved independently in both *Blarina* and the Mexican beaded lizard (Aminetzach et al., 2009), we might predict a similar toxin to be present in *Neomys*].

In addition, more natural history data on the rate of prey caching in venomous compared to non-venomous eulipotyphlans in the wild would greatly enhance the field. Do *Blarina* and *Neomys* cache significantly greater prey mass than other shrews that hoard prey? These venomous species preferentially focus on large prey (Rychlik, 2009; Rychlik and Jancewicz, 2002); does this allow them to store more food overall? More data on *Solenodon*, including determining its basal metabolic rate, daily food intake, and foraging strategies would add enormously to this topic.

Another productive line of research would be to investigate the cost of venom production in mammals. Studies in snakes found that venom production significantly increased metabolic rate in pitvipers (McCue and Mason, 2006) and death adders (Pintor et al., 2010), and both snakes and arachnids meter their venom carefully (Morganstern and King, 2013). Assessing the metabolic cost of venom production in mammals, and determining how mammals expend venom during predation could fill gaps in our knowledge. A comparative phylogenetic approach, looking at the metabolic costs of predation in sister-pairs of venomous and non-venomous shrews, would clarify the energetic expense of venom production in mammals. If venom is metabolically costly, it would explain why the trait is not more widespread among mammals. If it is not costly, then we need to develop alternative hypotheses: perhaps mammalian teeth are used more intensively for oral processing, so there is a constraint on modifying them to inject venom; without modified venom delivery teeth, venom is less adaptive.

6. Conclusions

Ancestral character state reconstruction of the presence of salivary venom in Eulipotyphla reveals that the trait evolved in parallel in at least three distantly related lineages, and was unlikely to be present in all early mammals. This suggests that the capacity for manufacturing venom in salivary glands may be evolutionarily plastic, while the trait itself is still relatively rare. The presence of venom in described fossil shrews with grooved teeth (Cuenca-Bescós and Rofes, 2007; Furió et al., 2010; Rofes and Cuenca-Bescós, 2009) cannot be substantiated by dental morphology alone, because no living shrew possesses both deeply grooved teeth and salivary venom. The solenodon relative *Nesophontes* (Turvey, 2010) also has dental morphology unlike any living venomous taxon, but its close phylogenetic relationship to solenodons and deeply grooved upper canine make the inference of venom somewhat more likely.

It is possible, as suggested by Fox and Scott (2005), that the poor preservation of small mammals from the Early Cenozoic is the reason for the lack of documentation of widespread oral venom delivery systems. However, it is more plausible that the rarity of oral venom systems found

in fossil mammals is due to the extreme rarity of the phenomenon in nature. The really interesting questions are: since some mammals are capable of secreting proteins that can deter predators and immobilize prey, why is this trait so rare among mammals? If venom is energetically expensive (Morganstern and King, 2013), why is it relatively common in living snakes [~ 450 of ~ 2700 (17%) of snakes are venomous]?

More research into the genetic basis of venom, the chemical differences among mammalian oral venom and the natural history of venomous species would resolve these longstanding questions.

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