Dispatch

Evolution: Fangtastic Venoms Underpin Parasitic Mimicry

Venomous teeth are rare in fishes, which typically utilise spines for defence. A new study reveals the evolutionary origins of fangs and venom in the Nemophini blennies and shows that in contrast to snakes and lizards, the fangs pre-date the venom.

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Venom has evolved multiple times across the tree of life [1] as a response to two evolutionary pressures — facilitating prey capture and as a defence against potential predators. Snakes, spiders, scorpions, cnidarians (jellyfish and anemones), molluscs and centipedes have all independently evolved venoms that incapacitate prey [1,2]. Some groups such as the snakes and the spiders have evolved oral delivery systems (fangs), whereas others have evolved stingers (wasps and scorpions), modified venomous legs (centipedes) or stinging tentacles (cnidarians). In many cases, these venoms are also used for self-defence. Scorpions for example, can inject two different venoms — one for self-defence, which is painful but metabolically cheap and one for prey incapacitation, which is protein rich and metabolically expensive [3]. Other organisms have evolved venoms solely to deter attacks on themselves. For example, many fishes have venomous fin spines or barbs, echinoderms (starfish and sea urchins) have venomous spines, ants have venomous stings or sprays and some moth and butterfly larvae have modified setae (urticating hairs).

Among venom systems that have evolved solely for defensive purposes, oral delivery systems are extremely rare. Of some 30,000 species of ray-finned fish, approximately 3,000 species are considered venomous [2,4], but only a hand-full of these species have evolved venomous bites as opposed to venomous spines. This evolutionary oddity is apparently confined to one small genus (28 species) of fangblennies — the *Meiacanthus* — which appear to use venomous fangs solely as an anti-predator defence (Figure 1) **(Au: OK?)**. A new study by Caswell *et al.* reported in this issue of *Current Biology* provides new insight into how venom, venom delivery systems and associated colour pattern mimicry have evolved across a fascinating group of marine fangblennies (Tribe Nemophini) [5].

In their study, Casewell *et al.* use an impressive array of complementary techniques to better understand the evolution and nature of fangblenny venoms and the evolution of the venom delivery system. To investigate the evolution of the fangs, they visualised fang morphology using micro-computer tomography scanning, stacking microscopy and histology. This demonstrated that only the *Meiacanthus* had anteriorly grooved fangs — which aid venom delivery — and, more importantly, revealed venom glands surrounding the base of the fang. In contrast, closely related fangblennies from other genera had fangs, but no venom glands. They then used a new phylogenetic framework, which they generated in their study, to investigate the timing of these two evolutionary innovations. Their results suggest that fangs evolved at the base of fangblennies, whereas the venom gland evolved at the base of the *Meiacanthus*. Thus, the venom delivery system evolved prior to the evolution of the venom**.**

The evolution of the fangblenny venom system provides a contrasting example to the Toxicofera, a clade of venomous reptiles that includes the snakes, monitor and alligator lizards, iguanas, agamas, chameleons and the gila monster. In this proposed clade, the venoms are hypothesised to have evolved at the base of the clade in the lizards, followed by the evolution of the venom delivery system (fangs) in the snakes. Thus, in the Toxicofera, the venom appears to have evolved before the delivery system (although see [6] for an alternative view of venom evolution in reptiles).

To investigate the nature of the fangblenny venom, Casewell *et al*. used comparative transcriptomics (sequencing expressed mRNA from venomous and non-venomous species) to identify expressed genes likely to be involved in venom production. This revealed three different proteins that were strongly implicated in venom production: group X phospholipases A2 (PLA2) and proenkephalin, which are both found in bee, scorpion and snake venoms [7], and neuropeptide Y, which is a constituent of *Conus* snail venom [8].

Casewell *et al*. then tested the biological activity of the fangblenny venom using a range of *in vitro*, biological and animal-model assays. They identified that in the case of PLAs, the fangblenny venom had similar levels of activity to viper venom in hydrolyzing glycerophospholipids. Prencephalin was found to have opioid activity, displaying significant inhibition of cAMP production as is also seen in scorpion venom [9]. The biological activity of neuropeptide Y was investigated by testing the venom on anaesthetised rats which showed hypotensive activity (a significant decrease in arterial blood pressure).

Finally, to investigate the pain-inducing potential of the venom, they used sub-cutaneous injection of the fangblenny venom into the hind paw of an anaesthetized mouse but observed no evidence of behavioral characteristics consistent with pain. Thus, the venom shows biological activity consistent with other venomous taxa, but does not appear to induce pain — at least in the mouse. It is highly unlikely that the venom evolved to combat mammalian predators (which are not frequently found on coral reefs), but has more likely evolved to act on fish predators which may indeed find the venom painful. Although this was not tested in the Caswell *et al*. study [5], there is some evidence that Groupers (large potential predators of fangblennies) learn to avoid eating *Meiacanthus* [10], suggesting a noxious venom component.

Once organisms have evolved venoms, mimicry rings (groups of species who share colour patterns for predator avoidance) also frequently evolve. Mimicry between different venomous species and venomous and non-venomous species are found widely across the animal kingdoms in taxa as varied as snakes [11], butterflies [12] and fish [13]. Mimicry among unrelated organisms can take many forms: it may reduce predation on co-mimics (either Batesian or Müllerian protective mimicry), increase the foraging opportunities of the mimic (aggressive mimicry) or increase the reproductive fitness of the mimic (reproductive mimicry) [14].

In the fangblenny system, there are a number of species that appear to mimic the venomous *Meicanthus spp.*, and in the Casewell *et al*. study the authors suggest that two different forms of mimicry may be found simultaneously. The first, Batesian mimicry, involves a non-venomous mimic (non *Meiacanthus* sp.) adopting the coloration of a venomous model species (*Meiacanthus* sp.) in order to benefit from

learned predator avoidance of the venomous model. The second involves aggressive mimicry in which the mimic (non-*Meiacanthus* micropredatory blennies) evolves to resemble a model species (*Meiacanthus spp.*) that the prey does not consider to be a threat. Thus, mimics benefit from reduced predation by resembling a venomous model, but may simultaneously gain increased foraging opportunities if the model is not considered to be a threat by the target prey.

When each mimicry form is considered alone, both appear to be parasitic on the *Meiacanthus*. In the Batesian relationship, the model bears the cost of educating predators and the mimic reaps the benefits with the fitness of the mimic highest when the mimic is less common than the model, when the model is highly noxious and when alternative prey are common [15]. In the aggressive mimicry component, if the model suffers from increased intolerance by larger fish species and that interferes with their foraging opportunities [16] this will also be parasitic, with the fitness of the mimic being frequency dependent. In a purely aggressive mimicry system (where there is no Batesian component), in which the non-venomous fangblenny (*Plagiotremus rhinorhynchos*) mimics juvenile bluestreaked cleaner wrasse (*Labroides dimidiatus*), the attack success rate of the mimic was shown to increase as mimics became rarer relative to their model [17].

However, in systems where there are two different selection pressures acting in concert (Batesian and aggressive mimicry), as is found here, the dynamics of the model–mimic system are less clear. For example, aggressive attacks by the mimic during micropredatory foraging may reinforce avoidance of the model–mimic coloration by potential predators [18]. Under this scenario, the model may actually

benefit from the aggressive behaviour of the mimic towards potential predators and the mimetic relationship could potentially be described as Müllerian (where both species are unprofitable) or quasi-Batesian (where both species are unprofitable, but the model is more unprofitable than the mimic). Under quasi-Batesian scenarios, the model–mimic relationship could be either parasitic [19] or mutualistic [20]. To my knowledge there has been no theoretical exploration of the dynamics of such a system, where two different forms of mimicry exert selective pressure simultaneously.

Finally, it is interesting to consider why *Meiacanthus* fangblennies have evolved venoms when the other closely related genera have not. One potential explanation is that many of the non-venomous fangblennies are micropredators — they attack larger fish and bite off scales and tissue. The *Meiacanthus*, in contrast, feed on a varied diet of planktonic and benthic invertebrates [10]. The micropredatory species rely on resembling the *Meiacanthus* to facilitate their attacks on other fishes and therefore exploit both the protection of the venomous model (*Meiacanthus)* to reduce their chances of being eaten and also the non-threatening nature of the model (not micropredatory). Were a micropredatory species to evolve a venomous bite, prey may quickly learn to avoid such species. Thus, micropredatory blennies may be unable to evolve venoms as they would subsequently reduce their fitness.

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Figure 1. *Meiacanthus grammistes* displaying its armoury. (photo courtesy of Martin Klein; www.bluevisions.eu)

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