Evolution of molecular resistance to snake venom α-neurotoxins in vertebrates

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Chapter 2: A Biological Arms Race: Animal Venoms, Resistance and Evolution

This chapter is primarily the work of the candidate. It is submitted for publication as: Jory van Thiel, Muzaffar A. Khan, Roel M. Wouters, Richard J. Harris, Nicholas R. Casewell, Bryan G. Fry, R. Manjunatha Kini, Stephen P. Mackessy, Freek J. Vonk, Wolfgang Wüster, and Michael K. Richardson (2021) *Biological Reviews* (under review). The candidate is joint first author (equal contribution) on that submission.

Abstract

Toxins are poisons of biological origin that cause disruption of physiological processes leading to incapacitation or death. Venoms are mixtures of peptide and protein produced in a venom gland and injected into the tissues of other animals via specialised structures such as fangs or stings. Some of the animals targeted by toxins and venoms have the ability to withstand their effects, a phenomenon known as resistance. At least three types of resistance are seen: (i) predator resistance, exemplified by in North American garter snakes that are resistant to tetrodotoxin in their prey, the rough-skinned newt; (ii) prey resistance, as in the mongooses, which are resistant to snake α-neurotoxins; and (iii) autoresistance, where a venomous animal is resistant to its own toxins, as exemplified by the Egyptian cobra. Venomous animals may, in parallel, evolve adaptations to overcome the resistance in their target species (more potent toxins, for instance). This reciprocal adaptation (co-evolution) in the toxic animal and its target species has been characterized as a co-evolutionary 'arms race'. Our main focus in this chapter are the molecular mechanisms of toxin resistance. We review studies on resistance to a wide range of toxin classes. Resistance strategies that we discuss include: modified transmembrane toxin-receptors, ion channels and serum factors (inhibitors). We also briefly consider non-molecular strategies (behavioural, cognitive, anatomical, etc.) for avoiding envenomation in the first place. We conclude that there is a great deal of work to be done on resistance, given the diverse and numerous animal toxins that are known.

Introduction

Biological toxins are poisonous molecules of biological origin, and are produced by animals, plants and many species of microorganisms (Fry, Roelants, Champagne *et al.*, 2009b). They include small molecules, peptides and proteins. Toxins have very high pharmacological potency and typically bind with high affinity to a particular molecular target. And so, when they enter the body of an animal, even in relatively small doses, they activate or disrupt normal physiological processes leading to incapacitation or death.

The physiological effects produced by a toxin depend on its molecular target but may include cell death, neurotoxicity, cardiotoxicity or other effects (Casewell, Wüster, Vonk *et al.*, 2013; Fry, Roelants, Champagne *et al.*, 2009a). Animals may use toxins offensively (to overpower their prey), or defensively (to deter predators or other attackers) (Casewell *et al.*, 2013; Schendel, Rash, Jenner *et al.*, 2019). In some cases, toxins are introduced into the tissue of the target animal by means of hollow teeth (fangs) or stings. In these cases, the injected toxin or toxin mixture is called a 'venom'.

Toxin resistance is the increased ability of an animal to survive the exposure to one or more toxins without being functionally affected. As a result, toxin resistance has evolved in at least three distinct ecological contexts (Figure 1) namely: *predator resistance*, where a predator is resistant to the toxins of its prey (Figure 1A-C); *prey resistance*, where the prey is resistant to the toxins of a predator (Figure 1D); or *autoresistance*, where an animal is resistant to its own toxins (Figure 1E).

In the case of most venomous snakes it is assumed that toxins are used primarily for prey capture. This is suggested by a study on a snake that evolved a habit of living largely on a diet of fish eggs; this species shows evolutionary degeneration of its venom delivery apparatus (venom gland and fangs) (Gopalakrishnakone & Kochva, 1990; Li, Fry & Kini, 2005; McCarthy, 1987). This suggests that the snake had previously used its venom exclusively for capturing living prey and not for defense. By contrast, spitting

cobras are clearly able to use their venom defensively, squirting it into the eyes of an attacker (Kazandjian, Petras, Robinson *et al.*, 2021).

Predator toxins have evolved to bind highly conserved protein targets in the prey (Takacs, Wilhelmsen & Sorota, 2001). The high potency of toxins is presumably due to strong positive selection acting over millions of years (Sunagar & Moran, 2015). One possible driver of this selection could be a need to incapacitate the target animal as quickly and effectively as possible (Casewell *et al.*, 2013; Fry *et al.*, 2009a). This scenario assumes that rapid incapacitation can mean the difference between life and death for the animal using the toxin. Rapid incapacitation of the prey might theoretically be advantageous for two reasons: (i) to prevent the prey from having time to attack the snake (ii) to prevent the prey from escaping. Although these are plausible scenarios, there are objections to them (R. M. Kini, *personal communication*). Often when snakes bite, they only inject a fraction of the total volume of venom available in the gland. Furthermore, their venom sometimes seems to have pharmacological potency far exceeding the apparent need (for example the venom of the Inland taipan (*Oxyuranus microlepidotus*) can kill 250,000 mice) (Broad, Sutherland & Coulter, 1979). This suggests that snakes already possess 'overkill', and so why would there be positive selection for further enhancement of toxin pharmacology? (Barlow, Pook, Harrison *et al.*, 2009; Sasa, 1999). A possible explanation is that a constant enhancement of toxin potency is needed to overcome continuously-evolving prey resistance, as in the 'arms-race' scenario (Duda & Palumbi, 1999a).

Toxins

Animal toxins are typically small molecules found, for example, on the skin surface of some amphibians as secretions. The origin of these toxins is not always known, but in at least some cases, are not synthesised by the toxic animal itself, but by some organism in its diet (such as a plant or microorganism). Several species of amphibians and fishes produce the small

molecule neurotoxins tetrodotoxin (TTX) or bufagenin (Brodie, 1990; Mackessy & Castoe; Ujvari, Casewell, Sunagar *et al.*, 2015). The presence of these toxins serves for protection against predators. Some snakes preying on toxic amphibians have evolved resistance to these toxins (Figure 1 (Brodie, 1990; Geffeney, Fujimoto, Brodie *et al.*, 2005; McGlothlin, Kobiela, Feldman *et al.*, 2016).

Prey and predator interaction against TTX is one of the examples often cited of an evolutionary arms race (Brodie, 1990; Geffeney *et al.*, 2005; McGlothlin *et al.*, 2016). Interestingly, in this example, both prey (*Taricha granulosa*) and predator (*Thamnophis sirtalis*) have developed resistance to TTX (Brodie, 1990; Geffeney *et al.*, 2005; McGlothlin *et al.*, 2016; Toledo, Hanifin, Geffeney *et al.*, 2016; Ujvari *et al.*, 2015; Venkatesh, Lu, Dandona *et al.*, 2005). The physiological action of TTX is to block the function of the sodium channel in neurons (Brodie, Hensel & Johnson, 1974; Kaneko, Matsumoto & Hanyu, 1997b). Bufagenin inhibits the activity of cardiac muscle Na^+/K^+-ATPase (Ujvari *et al.*, 2015). Resistance to bufagenin in some snakes, lizards and mammals is attributed to a mutation in the ATP1a3 gene coding for Na^+/K^+-ATPase (Mohammadi, Savitzky, Lohr *et al.*, 2017c; Ujvari *et al.*, 2015).

Venoms

Venom toxins not only incapacitate or kill the prey, they may also serve the important function of initiating the digestion of its tissues (Berthe, Westhoff & Bleckmann, 2013; Chippaux, Williams & White, 1991; Greene, 1983). The composition of snake venoms shows remarkable species variation (Casewell *et al.*, 2013). One very interesting finding has been that many rattlesnakes, and indeed also the spider *Cupiennius salei*, appear to be able to inject a volume of venom according to the size of the prey (HAYES, 1995; Malli, Kuhn-Nentwig, Imboden *et al.*, 1999; McCue, 2006).

Resistance

The term 'resistance' is used to describe the capacity of animals to endure the venomous effects of a toxin or venom without suffering serious harm (Edmunds, 1974). Resistance among reptiles and mammals, to different snake venoms, has been examined for many years (Allyn, 1937; Calmette, 1895). Toxin resistance is common in those animals who are liable to be frequently exposed to venomous or toxic animals, for example, when there is a predator prey-relationship and their territories overlap geographically (Biardi, Chien & Coss, 2006; Brodie Jr, Ridenhour, Brodie III *et al.*, 2002; Drabeck, Dean & Jansa, 2015; Geffeney *et al.*, 2005). The toxin-producing animal may, in turn, develop countermeasures to overcome prey resistance through adaptive mutation and toxin gene duplication (Benkman, Parchman, Favis *et al.*, 2003; Casewell *et al.*, 2013; Dawkins & Krebs, 1979; Duda & Palumbi, 1999b; Fry, Wüster, Kini *et al.*, 2003).

Animals use a variety of strategies to avoid being adversely effected by venoms or toxins (Khan, Dashevsky, Kerkkamp *et al.*, 2020). For examples of strategies of resistance, see Table 2; and for examples of selected molecular modifications relevant to this review, see Figure 2). These strategies include not only the molecular strategies that are the subject of my thesis, but might also include less obvious things such as the scaly skin on the legs of birds that might provide a physical barrier to envenomation, and the behavioural agility of mammals and birds (Figure 3) that allows them to avoid being bitten in the first place (Khan *et al.*, 2020).

The molecular mechanisms of resistance in the vertebrates against toxins offer a significant insight into the understanding of the evolutionary arms race (Geffeney *et al.*, 2005; Takacs *et al.*, 2001; Toledo *et al.*, 2016; Ujvari *et al.*, 2015; Venkatesh *et al.*, 2005). Moreover, inter-specific competition and a long-time presence of predator and prey in the same geographic area are factors that help drive the arms race (Williams, 2013). Among vertebrates, there are a small number of examples of such an arms race (Barchan, Kachalsky, Neumann *et al.*, 1992a; Barchan, Ovadia, Kochva *et al.*, 1995; Drabeck *et al.*, 2015; McGlothlin *et al.*, 2016; Voss & Jansa, 2012). Our aim

here is to review the literature relevant to toxin resistance in general, and the evolutionary arms race in particular, in the vertebrates.

Serum factors resistance against snake venom toxins

One cause of resistance to snake toxins is the presence of neutralising factors in the serum (Ovadia & Kochva, 1977). Thus, it has been reported that, in many families of the snakes, namely, Viperidae, Crotalidae, Elapidae and Colubridae as well as the hamster (*Mesocricetus aerates*) have humoral factors that neutralize *Vipera palaestinae* venom activity. *V. palaestinae* serum can neutralize its own venom neurotoxic and haemorrhagic activity (Ovadia *et al.*, 1977). Likewise, the serum of rattlesnakes (*Crotalus* sp.) and the Eastern king snake (*Lampropeltis getula*) are able to counteract the anti-haemorrhagic activity of *Crotalus* sp. venom (Moussatché & Perales, 1989).

Mammals such as the Californian beechey ground squirrel (*Spermophilus beecheyi*), and the Douglas ground squirrel (*Spermophilus beecheyi douglasii*) have a plasma protein called snake venom metalloprotease inhibitor (SVMPI) Table 1.

Table 1: Serum resistance factors in mammals.

Serum factor	**Species**	**References**
venom inhibitors (SVMPI)	ground squirrels (resistance to venom metalloprotease of the pacific and black diamond rattlesnake)	(Biardi, Coss & Smith, 2000)
venom inhibitors	Viriginia opossum (resistance to the venom of Brazilian pit vipers, eastern diamondback rattlesnake, timber rattlesnake, cottonmouth, Russell's viper, and monocled cobra)	(Catanese & Kress, 1993; Kilmon Sr, 1976; Moussatché *et al.*, 1989; Werner & Vick, 1977)

This neutralizes the venom metalloprotease activity of the pacific rattlesnake (*Crotalus viridis oreganus*) and the black diamond rattlesnake (*Crotalus oreganus helleri*) (Biardi, Ho, Marcinczyk *et al.*, 2011; Biardi *et al.*,

2006; Biardi *et al.*, 2000). The serum of the rock squirrel (*Spermophilus variegates*) acts specifically against the metalloprotease and haemolytic activity of venoms of *Crotalus* sp. (Biardi & Coss, 2011).

The plasma resistance factors against rattlesnake toxin in squirrels have evolved due to the presence of rattlesnakes in their home ranges. In contrast, squirrels which never encounter rattle snakes in their home ranges have no resistance factors against rattlesnake venom (Biardi *et al.*, 2006). The Virginia opossum (*Didelphis virginiana*) is extremely resistant to the venoms of the monocled cobra (*Naja kaouthia*) and a wide range of *Crotalus* spp. (Catanese *et al.*, 1993; Kilmon Sr, 1976; Moussatché *et al.*, 1989; Werner *et al.*, 1977). This resistance is due to a plasma proteins known as opossum serum α1-proteinase inhibitor (α1-PI) (Catanese *et al.*, 1993; Kilmon Sr, 1976; Moussatché *et al.*, 1989; Werner *et al.*, 1977). In the presence of α1-PI, the plasma protein serpin, a protease inhibitor, remains active and eventually inactivates venom metalloproteinase (Catanese *et al.*, 1993).

Lizards and Birds

Resistance-related mutations have been documented in lizards (clade Toxicofera) that are potentially vulnerable to predation by sympatric, neurotoxic snakes, such as the Central Bearded Dragon (*Pogona vitticeps*; 187–189NYT, 194L) and the Savannah Monitor (*Varanus exanthematicus*; 191G and 195N(Jones, Harris & Fry, 2021; Khan *et al.*, 2020). However, resistance has not been documented in monitor lizards (Varanus spp.) that have been suggested to prey on neurotoxic snakes (Jones, et al. 2021). Several studies hypothesised that morphological adaptations (thick, osteodermic scales) and prey-handling behaviour negated selection pressure for molecular resistance in these lizards(Jones *et al.*, 2021; Youngman, Llinas & Fry, 2021). The evolution of such strategies to avoid envenoming is comparable to what we propose for snake-eating birds (Figure 3). To explain this apparent paradox, we propose that a set of

morphological and behavioural traits in snake-eating birds prevent envenoming in the first place (Figure 3). This could also explain why these birds did not evolve any molecular adaptions, whereas other snake-eating lineages did (e.g., mongoose, honey badger; (Drabeck *et al.*, 2015; Khan *et al.*, 2020).Many birds prey on venomous snakes, including snake specialists such as the Secretary Bird (*Sagittarius serpentarius*), Snake Eagles (*Circaetus* spp.), and Seriemas (family Cariamidea; (Mori, Vyas & Upadhyay, 2017; Portugal, Murn, Sparkes *et al.*, 2016; Redford & Peters, 1986). Birds do not show any known resistance-related modifications associated with α-neurotoxins (Khan *et al.*, 2020). I will discuss lizards in more detail in Chapter 3, and birds in more detail in Chapter 4.

Resistance to snake α-neurotoxins

Snake α-neurotoxins target the highly conserved α-subunit of the nicotinic acetyl choline receptor (nAChR) of the neuromuscular junction (Asher, Lupu-Meiri, Jensen *et al.*, 1998b; Barchan *et al.*, 1995; Fry, Casewell, Wüster *et al.*, 2012; Kularatne & Senanayake, 2014; Takacs *et al.*, 2001). When the toxin binds, it causes paralysis of skeletal muscles (Barchan *et al.*, 1995). Elapid snakes that produce α-neurotoxins are resistant to their own toxins, and are therefore often cited as examples of autoresistance (Takacs *et al.*, 2001; Toledo *et al.*, 2016). Several mammals that attack and eat snakes have also evolved some kind of resistance to cobra venom Table 3 (Drabeck *et al.*, 2015). The resistant animals (cobras and mammals) show convergent evolution of molecular modifications in the α-subunit nAChR (Figure 2; (Drabeck *et al.*, 2015; Neumann, Barchan, Horowitz *et al.*, 1989; Ovadia *et al.*, 1977).

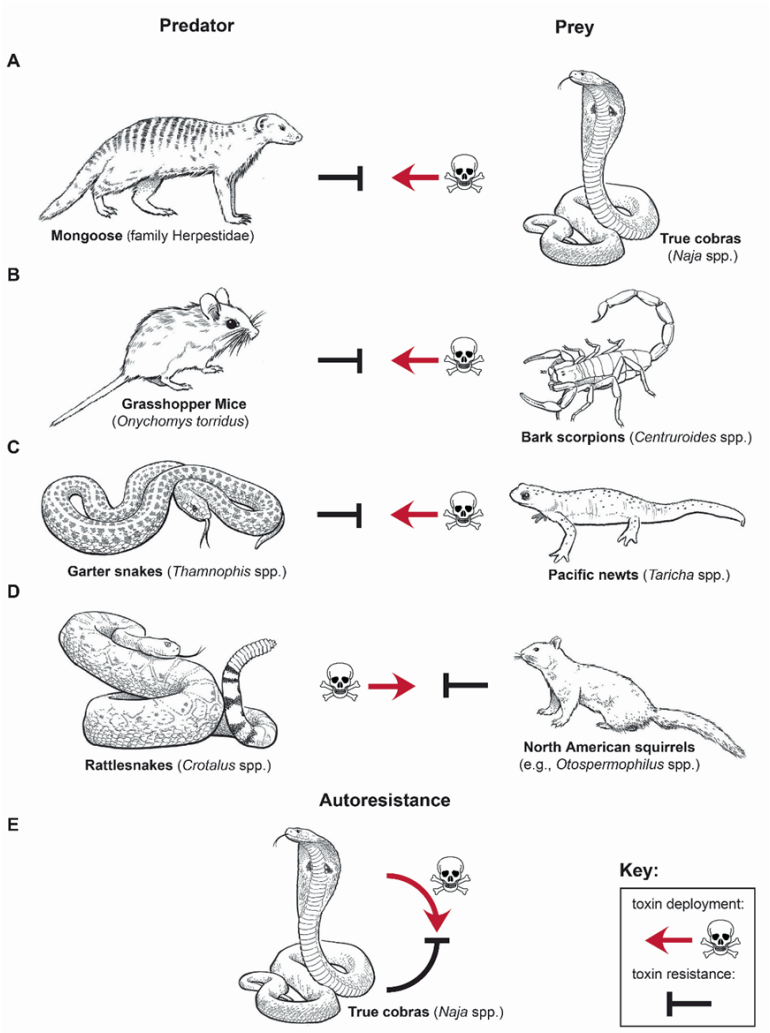

Figure 1. Classic examples of ecological contexts underpinning toxin resistance. A-C. predator resistance, where a predator is resistant to the toxins of its prey. A) the mongoose is known to predate on true cobras. B) The grasshopper mouse preys on bark scorpions. C) Garter snakes prey on toxic newts. D) prey resistance is resistance of a prey species to the toxins of a predator, and is exemplified here by rattlesnakes preying on North American ground squirrels. E) autoresistance, where an animal is resistant to its own toxins. The example shown here is of true cobras that show resistance to cobra α-neurotoxins. Drawings by Sven Ballinger, based on an original idea by Muzaffar Khan, Michael Richardson and Jory van Thiel.

Autoresistance to α-neurotoxin is seen in the Egyptian cobra (*Naja haje*) and is associated with the presence of a glycosylated asparagine (N) at position 189 (the position is numbered according to the human peptide; (Figure 2; (Asher, Lupu-Meiri, Jensen *et al.*, 1998a; Drabeck *et al.*, 2015). In the Egyptian mongoose the same change is seen at position 187 (Drabeck *et al.*, 2015). The European hedgehog the honey badger and the domestic pig all show a change of an aromatic residue to arginine (R) at position 187 (Asher *et al.*, 1998a; Drabeck *et al.*, 2015); this change was not present in a wide range of other mammals examined.

These findings are potentially interesting because of the popular (anecdotal) reports that the mongoose and cobra frequently fight each other; furthermore, the honey badger is reported to eat poisonous snakes (Begg, Begg, Du Toit *et al.*, 2003). Physiological assays have shown that the hedgehog is highly resistant to α-bungarotoxin (α-BTX, which is an α-neurotoxin), and that this is not due to the serum factors (Barchan *et al.*, 1995). It also has the same genetic modification as the honey badger (Barchan *et al.*, 1995; Drabeck *et al.*, 2015). The domestic pig probably also has an additional form or resistance: its tough skin (Table 2; (Drabeck *et al.*, 2015). In one study, α-BTX binding was examined using a site-specific antibody (Kachalsky, Aladjem, Barchan *et al.*, 1993; Mochly-Rosen & Fuchs, 1981). It was found that α-BTX binds to the α-subunit of the mouse nAChR, but does not bind to the mongoose α-subunit (Kachalsky *et al.*, 1993). This was confirmed in further studies which showed that α-BTX did not bind to the α-subunit of the cobra and mongoose nAChR; as mentioned above, these species have evolved a modification in the amino acids at positions 187 and 189 (Table 3; (Asher *et al.*, 1998a; Dellisanti, Yao, Stroud *et al.*, 2007; Kachalsky *et al.*, 1993).

Table 2. Details of strategies in vertebrates for avoiding the adverse effects of venoms or toxin. The strategies include resistance of various types, and various means of avoiding envenomation.

Ecological context	**Strategy**	**Examples**	**References**
autoresistance	target-site modification leading to reduced sensitivity	cobra exposure to its own α-neurotoxin	(Takacs *et al.*, 2001)
	target-site modification leading to reduced sensitivity	newt resistance to tetrodotoxin (TTX)	(Brodie, 1990; Geffeney, Brodie & Ruben, 2002; Kaneko, Matsumoto & Hanyu, 1997a).
	target-site modification leading to reduced sensitivity	puffer fish to bacterial TTX	(Soong & Venkatesh, 2006; Venkatesh *et al.*, 2005)
	target-site modification leading to reduced sensitivity	soft shell clam to bacterial TTX	(Bricelj, Connell, Konoki *et al.*, 2005; Soong *et al.*, 2006; Wiese, D'Agostino, Mihali *et al.*, 2010)
predator resistance	target-site modification leading to reduced sensitivity; also, physical avoidance (thick skin)	domestic pig, resistance to cobra venom	(Drabeck *et al.*, 2015)
	target-site modification leading to reduced sensitivity	garter snake, resistance to newt TTX	(Geffeney *et al.*, 2002)
	target-site modification leading to reduced sensitivity	mongoose, resistance to cobra α-neurotoxin	(Barchan *et al.*, 1992a)

	target-site modification leading to reduced sensitivity	honey badger, resistance to cobra α-neurotoxin	(Drabeck *et al.*, 2015)
	target-site modification leading to reduced sensitivity	African and Asian varanid lizards, resistance to cane toad bufagenins	(Ujvari *et al.*, 2015)
	target-site modification leading to reduced sensitivity	cobra, forest cobra, rhinoceros viper, resistance to cane toad bufagenins	(Ujvari *et al.*, 2015)
	target-site modification leading to reduced sensitivity	European hedgehog and muroid rodents, resistance to cane toad bufagenins	(Ujvari *et al.*, 2015)
	off-target repurposing	grasshopper mice, resistance to bark scorpion venom	(Rowe, Xiao, Rowe *et al.*, 2013b)
	unknown	pallid bat, resistance to bark scorpion venom	(Hopp, Arvidson, Adams *et al.*, 2017)
prey resistance	unknown	African plated lizard, eastern glass lizard and rainbow lizard, resistance to cobra α-neurotoxin and α-bungarotoxin	(Burden, Hartzell & Yoshikami, 1975)
	unknown	*Egernia cunninghami*, *E. striolata* and *E. whitii* and *Ctenotus robustus,* resistance to venom of Australian tiger snake, the eastern brown snake and the death adder	(Minton Jr & Minton, 1981)
behavioural avoidance	physical and behavioural avoidance (strategies to avoid envenomation: scaly or feathered skin; superior intelligence and agility)	snake-eating (ophiophagous) birds , protection against snake envenomation	(Khan *et al.*, 2020)
	aversive behaviour	*Pseudechis porphyriacus* toward cane toad bufagenins	(Phillips & Shine, 2006)
	aversive behaviour	*Heloderma horridum* toward venomous and non-venomous snakes	(Balderas-Valdivia & Ramírez-Bautista, 2005)
Other	Batrachotoxin resistance	Pitohui resistance to batrachotoxins of choresine, monarch butterflies and various plants	(Dumbacher, Menon & Daly, 2009; Dumbacher, Wako, Derrickson *et al.*, 2004).

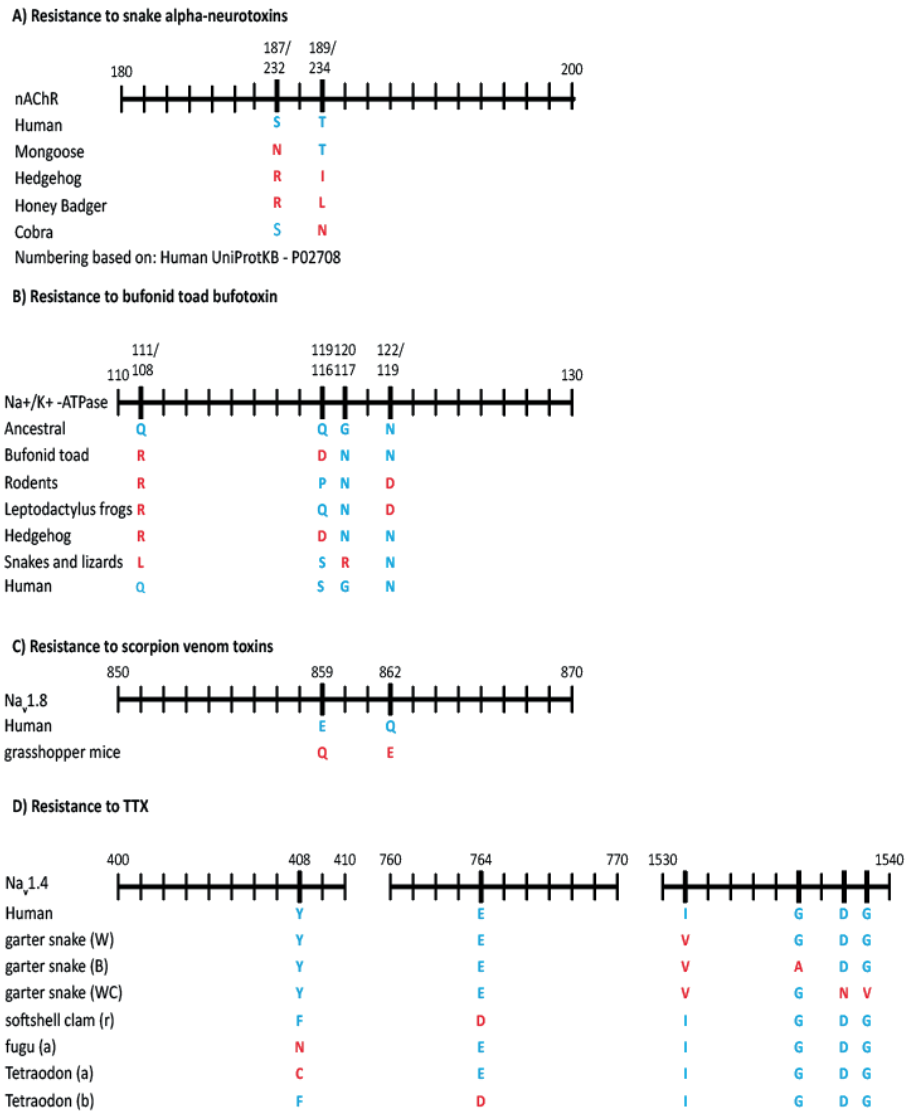

Figure 2. Examples of changes in amino acid sequence related to venom or toxin resistance. Figure by Muzaffar Khan and Harald Kerkkamp; style of presenting sequences is based on (Arbuckle, Rodríguez de la Vega & Casewell, 2017; Drabeck et al., 2015). A) Shows the molecular adaptations leading to resistance to snake alpha neurotoxin. Numbering is based on human acetylcholine receptor subunit alpha (UniProtKB - P02708). B) Shows the molecular adaptations leading to resistance to bufotoxin.

Numbering is based on human Na+/K+ ATPase subunit alpha-3 (UniProt accession number: KB - P13637). C) Shows the molecular adaptations leading to resistance to scorpion venoms. Numbering is based on human sodium channel protein type 10 subunit alpha (UniProtKB - Q9Y5Y9). D) Shows the molecular adaptations leading to resistance to tetrodotoxin. Different *Thamnophis sirtalis* populations are indicated in brackets (W) is Warrenton, (B) is Benton and (WC) is Willow Creek. indicated the numbering is based on human Sodium channel protein type 4 subunit alpha (UniProt accession number: KB - P35499). Key: Blue, amino acid not linked to resistance; Red, amino acid linked to resistance to the toxin. Amino acid coding: F (Phenylalanine), N (Asparagine), W (Tryptophan), R (Arginine), I (Isoleucine), V (Valine), C (cysteine), E (Glutamic acid), D (Aspartic acid), Q (Glutamine), L (Leucine).

Resistance to the venom of the bark scorpion

The bark scorpion (*Centruroides* sp.) is a major prey item for grasshopper mice (*Onychomys torridus*) (Rowe *et al.*, 2013b). The grasshopper mice have evolved analgesic effects against the extremely painful sting of the bark scorpion. Domain II of the grasshopper mouse Na^+ channel (Nav1.8) Figure 3) has either glutamine at position 859 or glutamic acid at position 862, while in the house mouse (*Mus musculus*) and human (*Homo sapiens*) the positions are switched in that they have glutamic acid at position 859 and glutamine at position 862. Therefore, It has been suggested in grasshopper mice that the presence in particular of the negatively-charged glutamic acid at position 862 may underlie the insensitivity to pain (Rowe *et al.*, 2013b).

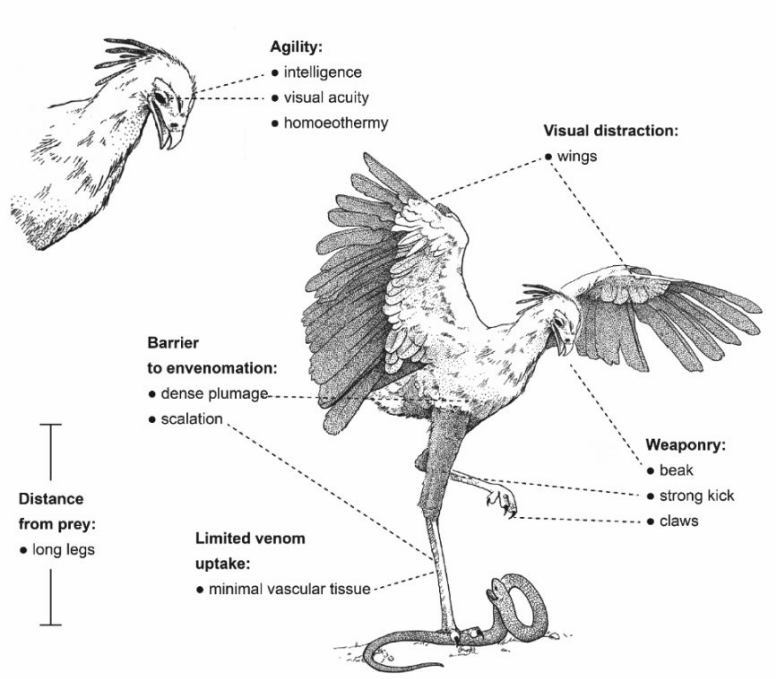

Figure 4. Morphological and behavioural traits proposed to negate selection pressures for evolving molecular resistance in snake-eating birds, such as the Secretary Bird (*Sagittarius serpentarius*). Plumage and leg scales may provide a physical barrier against snakebite. Additionally, bird legs are mainly transmit tendons and lack highly vascular tissue such as skeletal muscle; this may limit the uptake of venom if the bird is bitten. The secretary bird attacks snakes aggressively, directing kicks to the head and neck (Portugal *et al.*, 2016). Its elongated tibiotarsus and tarsometarsus may facilitate a powerful kick (Portugal *et al.*, 2016). Birds of prey, many of which are snake-eaters, have high visual acuity and ambush hunting strategies which may minimise the risk of snakebite (Potier, Lieuvin, Pfaff *et al.*, 2020). The red-legged seriema (*Cariama cristata*) uses its beak to grab the prey behind the neck and then shakes the prey violently so as to fracture its spine (Silva, Nunes, Estrela *et al.*, 2016). Drawings by Sven Ballinger, based on an original idea by Muzaffar Khan, Michael Richardson and Jory van Thiel.

Table 3: Autoresistance sites of α-subunit nAChR in snakes and mammals.

Species	Toxin	Toxin Target (TT)	Species	Amino acid substitutions at TT*
Egyptian cobra (*Naja haje*)	α–neurotoxin	α-subunit (nAChR)	Egyptian cobra (*Naja haje*); Krait (*Bungarus multicinctus*)	F189N
"	α–neurotoxin	α-subunit (nAChR)	Egyptian mongoose (*Herpestes ichneumon*)	W187N

*Key to amino acid substitutions at the target toxin site. F (Phenylalanine) → N (Asparagine), W (Tryptophan) → R (Arginine).

The pallid bat (*Antrozous pallidus*) preys on the bark scorpion (Hopp *et al.*, 2017). Interestingly, during the attack, the bat may be stung a number of times (Hopp *et al.*, 2017). The bat has been observed to attack again with no change of behaviour and without apparent ill-effects from being stung (Hopp *et al.*, 2017). It was shown that the pallid bat does not have the modification of its Na^+ channel seen in the grasshopper mouse Table 4. Therefore, more work is required to identify the mechanism of resistance in this bat (Hopp *et al.*, 2017).

Table 4: Grasshopper and pallid bat pain resistance sites vs. Arizona bark scorpion venom.

Species	Toxin	Toxin Target (TT)	Species	Amino acid substitutions at TT*	References
Arizona bark scorpion (*Centruroides.* Spp)	csev1 (neurotoxin 1)	voltage-gated Na^+ channel Nav1.8 Domain II (DII)	grasshopper mouse (*Onychomys torridus*)	E859Q Q862E	(Rowe *et al.*, 2013b)
"	"	voltage-gated Na^+ channel Nav1.8 Domain II (DII)	pallid bat (*Antrozous pallidus*)	unknown	(Hopp *et al.*, 2017)

*Key to amino acid substitutions at the target toxin site. E (Glutamic acid) → Q (Glutamine), Q (Glutamine) → E (Glutamic acid). The sequence data are show graphically in Figure 5

Resistance to tetrodotoxin (TTX)

Tetrodotoxin (TTX) is a neurotoxic small molecule (Brodie, 1990) that can cause death due to respiratory failure (Brodie, 1968) by binding to Na^+ channels. It is a guanidinium alkaloid. In general, the α-subunit of Na^+ channels is formed from four parallel domains (I-IV) each of which further holds six transmembrane segments designated S1-S6 (Marban, Yamagishi & Tomaselli, 1998). The resistance to toxins is due to amino acid substitutions in one or more domains of the Na^+ channel (Venkatesh *et al.*, 2005). Here, we will discuss resistance of the North American garter snake (*Thamnophis sirtalis*) to the tetrodotoxin of its prey, the rough-skinned newt (*Taricha granulosa*). We will also discuss the resistance of the pufferfish (*Tetraodon nigroviridis*) to its own (food-web derived) TTX (Figure 5).

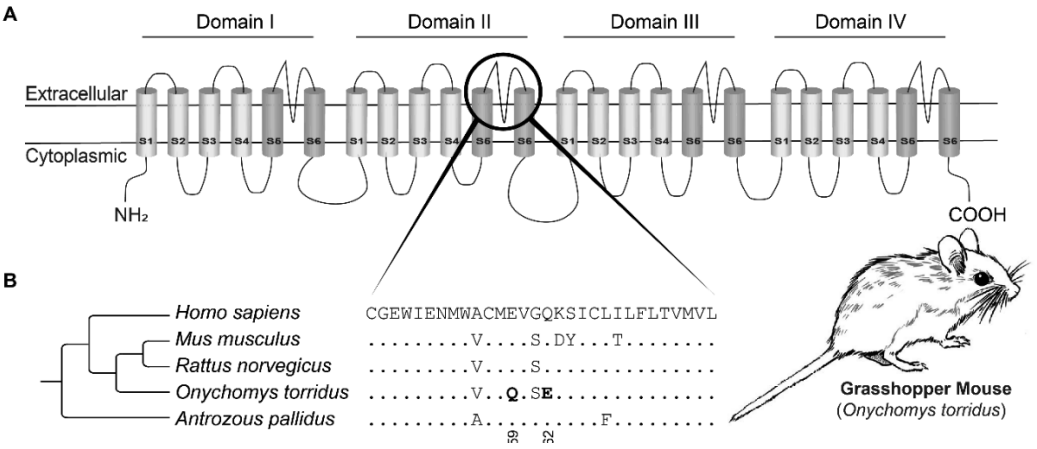

Figure 5. Resistance against pain-inducing scorpion venom in Grasshopper Mouse (*Onychomys torridus*). A. Unfolded protein structure of voltage-gated Na+ channel (Nav 1.8). Black circle indicates the outer pore associated with scorpion-venom binding in the Nav 1.8 channel. Structure was based on (Shen, Zhou, Pan et al., 2017). B. Partial sequence alignment of the outer pore of the α-subunit of domain II of Nav 1.8 channel. The displayed reference amino acid sequence is from humans (*Homo sapiens*) and differences from this sequence are displayed for all other species. Substitutions associated with resistance are highlighted in bold. Tree topology based on TimeTree.org (Kumar, Stecher, Suleski et al., 2017). Drawings by Sven Ballinger, based on an original idea by Jory van Thiel and Muzaffar Khan.

Garter snake

The garter snake has evolved a modification of the domain-IV segments S5-S6 of the $Na_v1.4$ in its skeletal muscles. This modification consists of the replacement of isoleucine by valine in at position 1,561 Table 5; (Brodie, 1990; Feldman, Durso, Hanifin *et al.*, 2015; Geffeney *et al.*, 2002; Geffeney *et al.*, 2005; Venkatesh *et al.*, 2005). Isoleucine is present at this position in the majority of the vertebrates that have been studied (Geffeney *et al.*, 2005). The molecular adaptation that confers resistance in garter snakes to

the newt TTX has presumably evolved because the two species share a common geographical distribution and the newt is a major part of the diet of the garter snake (McGlothlin *et al.*, 2016; Williams, Brodie & Brodie, 2004). The source of the TTX in the newt is a matter of discussion (Hanifin, 2010), but may be bacterial as it is in the pufferfish (see Table 5 (Bane, Lehane, Dikshit *et al.*, 2014; Cardall, Brodie, Brodie *et al.*, 2004). Interestingly, it has been shown that the newt, in captivity, has the capability to produce TTX in its granular skin glands and secrete it onto its dorsal skin surface (Cardall *et al.*, 2004). Physiological assay has shown that the newt resistance to TTX is not humoral-based.

Teleosts

Several marine teleosts have evolved autoresistance to TTX (Venkatesh *et al.*, 2005). Resistance in the fugu (pufferfish; *Fugu pardalis*) is due to the presence of an asparagine in place of phenylalanine at position 401 in domain-I of the $Na_v1.4a$ Table 5 (Venkatesh *et al.*, 2005). The fugu has a cysteine in domain-I, position 401 in place of phenylalanine, and further, in $Na_v1.4b$ due to aspartic acid in place of glutamic acid (domain-II, position 758; (Kaneko *et al.*, 1997a; Soong *et al.*, 2006; Venkatesh *et al.*, 2005; Yotsu-Yamashita, Nishimori, Nitanai *et al.*, 2000).

Saxitoxin

A few species of pufferfish including *Tetraodon fangi* and *T. cutcutia*, have evolved resistance to the chemically-related neurotoxin saxitoxin (Landsberg, Hall, Johannessen *et al.*, 2006; Sato, Kodama, Ogata *et al.*, 1997; Venkatesh *et al.*, 2005). Saxitoxin (STX) is a potent neurotoxin (Schantz, Ghazarossian, Schnoes *et al.*, 1975; Wiese *et al.*, 2010) that is accumulated by several teleosts from eukaryotic dinoflagelates and prokaryotic cyanobacteria in their diet (Bricelj *et al.*, 2005; Wiese *et al.*, 2010; Yotsu-Yamashita, Kim, Dudley *et al.*, 2004b). In addition Zetekitoxin AB, an analog of STX (Yotsu-Yamashita, Kim, Dudley *et al.*, 2004a), has been found in the Panamanian golden frog (*Atelopus zeteki*; (Wiese *et al.*, 2010; Yotsu-

Yamashita *et al.*, 2004a; Yotsu-Yamashita *et al.*, 2004b); its source is unknown. Autoresistance to STX is found in *Tetraodon fangi, T. cutcutia*. It is due to the presence of an asparagine in domain-II of the $Na_v1.4b$ channel (Venkatesh *et al.*, 2005). The soft-shell clam (*Mya arenaria*) contains STX. The clam has evolved resistance to STX due to the presence of aspartic acid at position 758 in place of glutamic acid in domain-II of its neuronal Nav1.4 channel (Soong *et al.*, 2006).

Steroids Toxins

Steroid toxins include several plant toxins such as cardenolides, found in the round-leafed navel-wort (*Cotyledon orbiculata*), kalanchoe pinnata (*Bryophyllum pinnatum*), butterfly weed (*Asclepias tuberosa)*, the oleander (*Nerium oleander*) and the foxglove (*Digitalis purpure*a; (Agrawal, Petschenka, Bingham *et al.*, 2012; Anderson, Schultz, Kellerman *et al.*, 1985; Krenn & Kopp, 1998; Supratman, Fujita, Akiyama *et al.*, 2000).

Bufagenins

Steroid toxins include several plant toxins such as cardenolides, found in the round-leafed navel-wort (*Cotyledon orbiculata*), kalanchoe pinnata (*Bryophyllum pinnatum*), butterfly weed (*Asclepias tuberosa)*, the oleander (*Nerium oleander*) and the foxglove (*Digitalis purpure*a; (Agrawal *et al.*, 2012; Anderson *et al.*, 1985; Krenn *et al.*, 1998; Supratman *et al.*, 2000). Bufagenins are toxic cardiac glycosides chemically related to the cardenolides of plants mentioned above. The cane toad (*Rhinella marina* [*Bufo marinus*]) produces bufagenins in its parotid glands (Phillips *et al.*, 2006). Bufagenins are also found in insects of the families Chrysomelidae and Lampyridae (Van Oycke, Braekman, Daloze *et al.*, 1987). In susceptible predators bufagenins disrupt the activity of Na^+/K^+-ATPase and eventually cause cardiotoxicity (Kamalakkannan, Salim & Capon, 2017; Ujvari *et al.*, 2015). In the cane toad they act as anti-predator defenses (Kamalakkannan *et al.*, 2017; Ujvari *et al.*, 2015).

There is extensive biotransformation of bufagenins in the cane toad by Gram-positive bacteria (*Bacillus* sp.; (Kamalakkannan *et al.*, 2017). The eggs and tadpoles of the cane toad contain bufagenins making them toxic to predators (Shine, 2018). Bufagenins are also present in the adult parotid gland and in the secretion of skin glands (Chen & Kovaříková, 1967). In 1935, the cane toad was released into the sugar cane fields of Australia in the belief that it would control pests (Haynes, 2015; Sabath, Boughton & Easteal, 1981; Shine, 2018). It did not do so, and instead has since become a very troublesome, invasive species. The bufagenins of the cane toad have become a serious threat to Australian wildlife, because they result in the poisoning of many the many Australian native animals that prey on cane toads, and have not evolved any resistance. These predators include snakes, monitor lizards and crocodiles (Phillips, Brown, Greenlees *et al.*, 2007; Shine, 2010). It has been shown that African varanid lizards (*Varanus niloticus, V. albigularis, V. exanthematicus*), Asian varanid lizards (*V. dumerilii, V. bengalensis, V. rudicollis, V.salvator*), the European hedgehog (*Erinaceus europaeus*), and murid rodents (Muridae), that feed on cane toads, have evolved resistance to bufagenin. Further, two species of elapid and viper show resistance (Ujvari *et al.*, 2015).

The resistance in all of these animals is associated with the presence of leucine and arginine in the H1–H2 extracellular domain of the Na^+/K^+-ATPase at positions 111 and 120, respectively (Brodie, 1977; Ujvari *et al.*, 2015). By contrast, the Australian varanid lizard (*Varanus varius*) which is not resistant to bufagenin has a glutamine (Q) at position 111 and glycine (G) at position 120 (Losos & Greene, 1988; Ujvari *et al.*, 2015; Ujvari, Mun, Conigrave *et al.*, 2013) Table 6. In Japan, the Japanese tiger keelback snake (*Rhabdophis tigrinus*) preys on cane toads (Kojima & Mori, 2015). It has a specialised nuchal gland in which bufagenins from the ingested toads are sequestered and then re-used for antipredator defense (Figure 6). In this snake there may also be an endocrine adaptation to the toad toxins. Thus, when the snake ingests a cane toad, its plasma concentration of the stress hormone corticosterone decreases, and that of the mineralocorticoid aldosterone

increases (Mohammadi, French, Neuman-Lee *et al.*, 2017a; Mohammadi, French, Neuman-Lee *et al.*, 2017b). This physiological response is not seen in non-resistant snake species (Mohammadi *et al.*, 2017a).

Table 5. Newt, Fugu, pufferfish and soft shell clams Na+ channel sites of tetrodotoxin resistance.

Species	Toxin	Toxin Target (TT)	Species	Amino acid substitutions at TT*	References
rough-skinned newt (*Taricha granulosa*)	tetrodotoxin (TTX)	skeletal muscle voltage-gated Na+ channel	garter snake (*Thamnophis sirtalis*)	I1561V	(Feldman *et al.*, 2015; Geffeney *et al.*, 2005)
marine bacteria (*Vibrio* sp., *Pseudomonas* sp.) marine actinomycete (*Nocardiopsis dassonvillei*), starfish, gastropods and shrimps.	"	skeletal muscle voltage-gated Na+ channel	fugu (*Fugu pardalis*)	C401N	(Soong *et al.*, 2006; Venkatesh *et al.*, 2005)
"	"	skeletal muscle voltage-gated Na^+ channel	pufferfish (*Tetraodon nigroviridis*)	E758D	(Soong *et al.*, 2006; Venkatesh *et al.*, 2005)
ocean water dinoflagellates and fresh water cyanobacteria	saxitoxin (STX)	Na+ channel (Nav1.4b) domain II	soft-shell clams (*Mya arenaria*)	E945D	(Bricelj *et al.*, 2005; Soong *et al.*, 2006; Wiese *et al.*, 2010)

*Key to amino acid substitutions at the target toxin site. I (Isoleucine) → V (Valine),C (cysteine) → N (Asparagine), E (Glutamic acid) → D (Aspartic acid).

In Australia, the black snake (*Pseudechis porphyriacus*) appears to have evolved both physiological resistance to bufagenin, presumably due to the presence of the cane toad in its geographical range. This resistance is accompanied by behavioural avoidance of the cane toad as potential prey (Phillips *et al.*, 2006). Interestingly, these changes have evolved rapidly in the snake, i.e. in around 23 generations (Phillips *et al.*, 2006). Toxicity testing in Australia shows that the saltwater crocodile (*Crocodylus porosus*) is less susceptible to bufagenin than is the freshwater crocodile *Crocodylus johnstoni* (Smith & Phillips, 2006). Whether the saltwater crocodile has evolved some mechanism of resistance is not known. However, we notice here that the saltwater crocodile has an amino acid leucine (L) at position 111 H1–H2 extracellular domain of the Na^+/K^+-ATPase as do species resistant to bufagenins.

Table 6. Different vertebrates bufotoxins resistance sites.

Species	**Toxin**	**Toxin Target (TT)**	**Species**	**Amino acid substitutions at TT***
bufonids toads (Bufonidae)	bufotoxins	H1–H2 extracellular domain of the Na+/K+ -ATPase	African and Asian varanid lizards, Indian Cobra, forest cobra, puff adder, rhinoceros vipers, European vipers, muroid rodents	Q111L G120R

*Key to amino acid substitutions at the target toxin site Q (Glutamine) → L (Leucine), Q (Glutamine) → R (Arginine).

Batrachotoxins

In 1963, first time the venom was extracted from the skin of the Colombian black-legged poison dart frog (*Phyllobates bicolor*) (Maerki & Witkop, 1963). The name batrachotoxin was given to the major active toxin of this species (Daly, Witkop, Bommer *et al.*, 1965). Batrachotoxins are neurotoxic, lipophilic alkaloids which bind to vertebrate Na^+ channels in nerves and muscles. They have been classified in three highly toxic alkaloids: (i) batrachotoxin; (ii) homobatrachotoxin; and (iii) batrachotoxin A (reviewed in (Daly, 1995; Daly, Brown, Mensah-Dwumah *et al.*, 1978)). It is thought that the poison dart frogs acquire the batrachotoxins from items in their diet, possibly from Melyrid beetles (*Choresine*) (Dumbacher *et al.*, 2004) Figure 6). Batrachotoxins have also been identified on the feathers of certain passerine birds of New Guinea which belong to genus *Pitohui* (Dumbacher, Beehler, Spande *et al.*, 1992). The toxins come from the uropygial glands of these birds and is transferred onto the feathers during preening. The batrachotoxins may help the birds to protect against infections, ectoparasites and potential predators including snakes and birds of prey (Dumbacher *et al.*, 1992; Jacob, 1978; Poulsen, 1994). The batrachotoxins originate from items in the diet of the pitohui birds including beetles of the genus *Choresine*, monarch butterflies (*Danaus plexippus*) and various plants (Figure 6) (Dumbacher *et al.*, 2009; Dumbacher *et al.*, 2004).

Interestingly, as a result of eating these insects *Pitohui* species have developed resistance to homobatrachotoxin (Dumbacher *et al.*, 1992; Dumbacher, Deiner, Thompson *et al.*, 2008). Despite the high concentrations found in these passerine birds, there are no resistance-related modifications in the Na_v channels ($Na_v1.4$ and Na_v 1.5, respectively) which could suggest a comparable strategy as proposed in poison dart frogs (Abderemane-Ali, Rossen, Kobiela *et al.*, 2021).

Resistance and the so-called 'co-evolutionary arms race'

In any predator-prey relationship involving a poisonous or venomous participant, it seems likely that there will be selection for resistance. Given sufficient reciprocal selection in the predator, one can envisage a co-evolutionary arms race, where the prey evolves continuously evolves more effective resistance, and the predator evolves more effective toxins (discussed by (Khan *et al.*, 2020). By 'effective' I mean more potent, and faster acting. The intensity and symmetry of selective forces between prey and predator are highly variable, depending on the importance of the prey species as a resource to the predator, and the importance of the predator as a cause of loss in fitness to the prey. For example, as we discussed above, some animals show a reversal of their resistant genotype in the absence of their toxic counterparts (Khan *et al.*, 2020; Ujvari *et al.*, 2015). This suggests that their might be some fitness cost to maintaining resistance.

Evolutionary theory predicts that toxin resistance is most likely to evolve when the poisonous or venomous opponent exerts strong selection, whether as prey or as predator. In predators of toxic prey, resistance is most likely to evolve when the predator is under strong selection to exploit an abundant but toxic food source. Examples include many reptiles that prey on toxic amphibians (Feldman, Brodie, Brodie *et al.*, 2012; Ujvari *et al.*, 2015); mammalian mesopredators feeding on venomous snakes (Drabeck *et al.*, 2015; Drabeck, Rucavado, Hingst-Zaher *et al.*, 2020) and grasshopper mice eating bark scorpions (Rowe, Xiao, Rowe *et al.*, 2013a). In prey species subject to predation by a venomous predator, prey resistance will most likely evolve if the predator is an important overall cause of mortality, e.g., sea kraits preying on moray eels (Heatwole & Poran, 1995) and rattlesnakes preying on North American squirrels and other rodents (de Wit, 1982; Gibbs, Sanz, Perez *et al.*, 2020; Holding, Biardi & Gibbs, 2016). In the latter example, reciprocal adaptation has been demonstrated, as rattlesnakes match their venom phenotype to the resistance profile of local prey to retain a selective advantage (Holding *et al.*, 2016; Margres, Wray, Hassinger *et al.*, 2017).

Evolutionary theory predicts that resistance is unlikely to evolve when selection pressure is low, for example: i) when predation by a venomous predator is a relatively unimportant selective force for the prey because of scarcity of encounters, ii) a short temporal window of exposure (Marques, Martins, Develey *et al.*, 2012), or iii) when behavioural avoidance of toxic prey is more advantageous than evolving resistance (Brodie Iii, 1993; Portugal *et al.*, 2016; Smith, 1977); see also Figure 3). Finally, it is also possible that resistance is most likely to evolve in situations where incremental increases in resistance confer an increasing selective advantage. Relatively low-level resistance could be adaptive where prey toxicity varies geographically (Feldman *et al.*, 2012). In summary, resistance is seen in many diverse ecological contexts and can be interpreted under a range of evolutionary scenarios. Despite the complex routes towards resistance, a few outcomes are repeatedly seen in unrelated lineages.

Competing selection pressures and convergent evolution

Evolutionary trade-offs usually come with some kind of fitness disadvantage (Blanchard & Moreau, 2017; Brodie Iii & Brodie Jr, 1999; Hague, Toledo, Geffeney *et al.*, 2018). It is important that resistance modifications do not disrupt the physiology of the resistant animal. For example, resistance modifications of the neuromuscular junction, that reduce the binding of snake α–neurotoxins, should not interfere with the physiological binding of the animal's own neurotransmitter (acetyl choline) (Fuchs, Barchan, Kachalsky *et al.*, 1993). Indeed this appears to be the case: multiple substitutions have convergently evolved to reduce snake α-neurotoxin binding, but without compromising the amino acid residues vital for acetylcholine binding (Barchan, Kachalsky, Neumann *et al.*, 1992b; Khan *et al.*, 2020). These observations support the concept of a trade-off between a functional target (e.g., binding site of the endogenous ligand) and the modifications that enhance toxin-resistance.

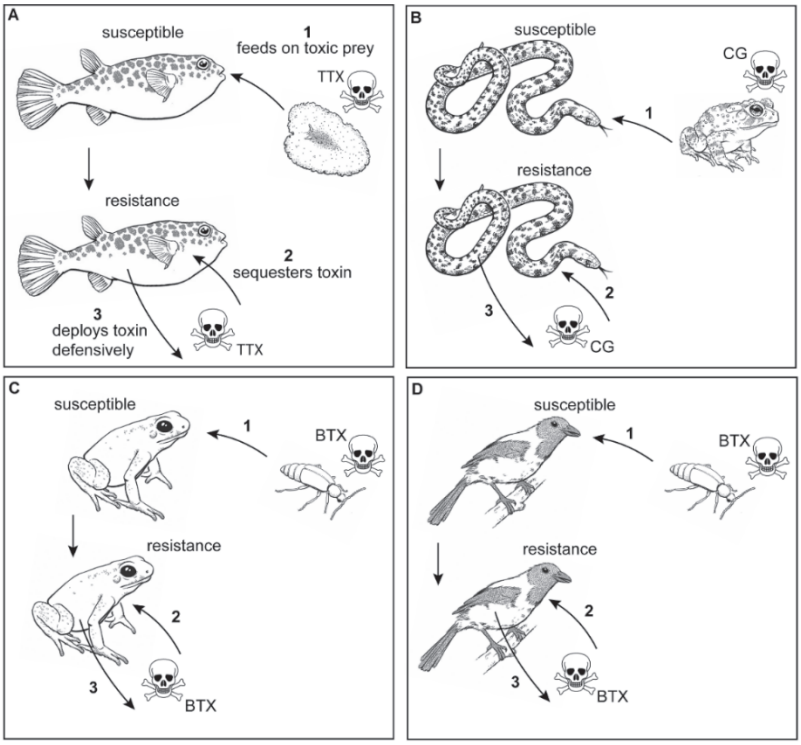

Figure 6. Hypothesised evolutionary arms scenarios for autoresistance in poisonous animals. It is generally assumed that autoresistance is a phenomenon of self-protection. Here, we propose a three-step evolution scenario for the origins of autoresistance: 1) predator resistance is followed by 2) sequestration of the toxin by the predator, and 3) exploitation of the toxin for defence. As this figure indicates, a similar three-step process has been seen in very diverse lineages, suggesting evolutionary convergence. The displayed examples include, A) pufferfish (family Tetraodontidae) feeding on TTX-bearing flatworms, gastropods, and echinoderms, B) keelback snakes (Rhabdophis spp.) feeding on cane toads. C) poison dart frogs (family Dendrobatidae) feeding on toxic arthropods, D) pitohui birds (Pitohui spp.) feeding among others on BTX-bearing melyrid beetles, Key: TTX (tetrodotoxin), CG (cardiac glycosides), BTX (batrachotoxin). Drawings by Sven Ballinger, based on an original idea by Jory van Thiel, Muzaffar Khan and Michael Richardson.

Similar trade-offs may exist in the case of the garter snakes, and their resistance to the tetrodotoxin of newts. Convergent adaptations have been found not only in garter snakes but in multiple distinct colubrid snakes, and

the adaptations are associated with tetrodotoxin resistance. These adaptations have been shown to be mediated by a functional trade-off between ion channel function and tetrodotoxin-insensitivity (Feldman *et al.*, 2012; Lee, Jones, Ahern *et al.*, 2011). A further example of the possible trade-offs involved in toxin resistance is seen in the case of the evolution of cardiac glycoside resistance. This has evolved several times, by means of two or three substitutions (respectively at positions 111, 119, 120 or 122) in the Na^+/K^+-ATPase (Dobler, Dalla, Wagschal *et al.*, 2012; Karageorgi, Groen, Sumbul *et al.*, 2019; Ujvari *et al.*, 2015). In summary, there may be a limited number of amino acid changes that can reduce the binding affinity of toxins, without disrupting the normal physiology of the animal. These same amino acid changes are seen repeatedly in different species under similar selection pressures. We believe that this is a persuasive example of convergent evolution: the arrival at the same solution in independent lineages of animals, in response to similar selection pressures. Only in this way can the animals develop resistance, while maintaining their normal physiology

Origins of autoresistance in poisonous animals

Some animals are resistant to their own toxins, a phenomenon referred to as autoresistance. However, here we argue that this is a much more complicated evolutionary scenario in the case of toxins (e.g., tetrodotoxin, cardiac glycosides, batrachotoxin). The complexity of the issue has already been partially touched upon in previous literature (Santos, Tarvin & O'Connell, 2016; Saporito, Donnelly, Spande *et al.*, 2012). We propose a scenario in which there was a three-step evolution of resistance across phylogenetically distinct poisonous animals: first, (i) predator resistance, followed by (ii) sequestration of the toxin by the predator and finally, (iii) exploitation of the toxin for defence.

Over the course of evolution, predation on a toxic species leads to frequent exposure to a specific toxin or toxins through generalized trophic interactions. In most cases, naïve predators feeding on highly toxic prey (such as TTX-containing newts) are rapidly eliminated, with negative

selection on the wild type thus favouring toxic prey avoidance. However, if variants that are capable of tolerating potent toxins exist in the population, then positive selection should favour the resistant phenotype, as this allows the predator to capitalize on abundant, often underutilized prey species. This then provided an evolutionary selection pressure favouring resistance.

Interestingly, several animals (e.g., poison dart frogs and pufferfish) have been shown to be toxic only after the ingestion of a toxic diet, indicating that the toxins originated exogenously (Noguchi, Arakawa & Takatani, 2006; Saporito, Donnelly, Jain *et al.*, 2007; Yotsu-Yamashita, Gilhen, Russell *et al.*, 2012). Some toxins (e.g., alkaloid or steroidal-based toxins) are not destroyed in the gut, and can thus accumulate in the body. Ultimately this enabled the exploitation of the accumulated toxins for defensive purposes in poisonous animals (as reviewed in (Savitzky, Mori, Hutchinson *et al.*, 2012). Therefore, we hypothesise that autoresistance primarily evolved as predator resistance rather than in its own right.

Conclusions

Toxin resistance provides a fascinating model system for the understanding of convergent evolution. We hope that our review will lead to novel insights into complex evolutionary processes provided by integrating molecular biology, evolution and ecology. Functional constraints on molecular targets explain the convergence of resistant traits that are seen across the animal kingdom. Toxin resistance is an evolved response seen at many trophic levels, underscoring how relatively simple adaptations can bring solutions to complicated problems.

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