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PERSPECTIVE

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Toxic to the touch: The makings of lethal mantles in pitohui birds and poison dart frogs

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How do chemically defended animals resist their own toxins? This intriguing question on the concept of autotoxicity is at the heart of how species interactions evolve. In this issue of Molecular Ecology (Molecular Ecology, 2024, 33), Bodawatta and colleagues report on how Papua New Guinean birds coopted deadly neurotoxins to create lethal mantles that protect against predators and parasites. Combining chemical screening of the plumage of a diverse collection of passerine birds with genome sequencing, the researchers unlocked a deeper understanding of how some birds sequester deadly batrachotoxin (BTX) from their food without poisoning themselves. They identified that birds impervious to BTX bear amino acid substitutions in the toxin-binding site of the voltage-gated sodium channel Nav1.4, whose function is essential for proper contraction and relaxation of vertebrate muscles. Comparative genetic and molecular docking analyses show that several of the substitutions associated with insensitivity to BTX may have become prevalent among toxic birds through positive selection. Intriguingly, poison dart frogs that also co-opted BTX in their lethal mantles were found to harbour similar toxin insensitivity substitutions in their Nav1.4 channels. Taken together, this sets up a powerful model system for studying the mechanisms behind convergent molecular evolution and how it may drive biological diversity.

KEYWORDS
autotoxicity, batrachotoxin, neurotoxins, target-site insensitivity, toxin sponge, voltage-gated sodium channels

In food webs across our planet, many animals find themselves walking a tightrope in the race for survival: eat or be eaten. A mistake may lead to deadly intoxication from chemically defended prey or being eaten alive by predators and parasites (Groen & Whiteman, 2021). The combination of such bottom-up and top-down selection pressures has led to adaptation in numerous animals. Some obtained the capability of co-opting ingested toxins for defence against natural enemies, allowing them to survive and thrive. Iconic examples of toxin co-option are the monarch butterfly, the African crested rat and the tiger keelback snake, which all sequester toxic cardiac glycosides from their diets. The animals accumulate these toxins at their body surface, creating lethal mantles that keep many would-be predators at bay. Intriguingly, the capability to co-opt cardiac glycosides was often facilitated through convergent evolution of amino acid substitutions in the sodium–potassium pump (Na^+/K^+-ATPase). The pump is the molecular target of cardiac glycosides and is a central player in the nervous system. These substitutions in the cardiac glycoside binding site render the Na^+/K^+-ATPase insensitive to the toxins (Groen & Whiteman, 2021). In the case of the monarch butterfly, toxin co-option in turn appears to have spurred further trait evolution in the monarch itself and in other nymphalid butterflies. The monarch evolved warning coloration (aposematism) as a signal to disincentivize predator attack, while the closely related queen butterfly, which is also toxic, and the more distantly related viceroy butterfly, which is not toxic, evolved similar aposematic colour patterns and started engaging in Müllerian and Batesian mimicry, respectively. As such, toxin co-option may be a main driver of generating and maintaining biological diversity. However, observations

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from a wider array of food webs are necessary to determine if this is the case more generally.

In their recent study, Bodawatta et al. (2023) make a major contribution to the study of toxin co-option. They aimed to shed light on the extent of convergent evolution in the sequestration of the neurotoxic steroidal alkaloid BTX in the skin and feathers of Papua New Guinean toxic birds and Neotropical *Phyllobates* poison dart frogs. Although the dietary source of BTX is not well known—the animals most likely acquire it through consuming melyrid beetles (Dumbacher et al., 2004)—the toxin protects these birds and frogs from predators and parasites (Dumbacher, 1999). Building on foundational work by ornithologists including John "Jack" Dumbacher and Jared Diamond, who described the (chemical) ecology of toxic pitohuis (Figure 1), Bodawatta and colleagues cast a wide net and sampled feathers from 143 birds of 47 toxic and non-toxic species in three highland localities in Papua New Guinea to perform chemical and genetic analysis. The chemical analysis of these feathers broadened the extent of a fascinating case of convergently evolved toxin co-option among passerine birds. In addition to the blue-capped ifrit (*Ifrita kowaldi*, Ifritidae) and pitohui species such as the hooded pitohui (*Pitohui dichrous*, Oriolidae), which were known to accumulate BTX, two more bird species, the rufous-naped bellbird *Aleadryas rufinucha* (Oreoicidae) and regent whistler *Pachycephala schlegelii* (Pachycephalidae) were discovered to have lethal mantles through the sequestration of BTX in their plumage.

How do these birds withstand high levels of BTX in their bodies? Successful sequestration strategies require careful handling of toxins after ingestion and xenobiotic detoxification mechanisms play key roles in these (Tarvin et al., 2023). Common mechanisms involve toxin metabolism by the animal or its microbiome, target-site insensitivity, or the existence of alternative targets of toxins in the form of "toxin sponges" (Tarvin et al., 2023). In the case of BTX, previous research on *Phyllobates* poison dart frogs identified amino acid substitutions in the toxin-binding site of the voltage-gated sodium channel Nav1.4. Molecular docking analyses and functional genetic experiments in human cell lines suggest that these substitutions may confer target-site insensitivity in vivo (Márquez et al., 2018; Tarvin et al., 2023, and references therein). However, recent work indicates that, rather than on target-site insensitivity, *Phyllobates* frogs may rely more on a "toxin sponge" in the form of a protein called saxiphilin to prevent BTX autotoxicity (Abderemane-Ali et al., 2021). Bodawatta and co-workers studied what might allow toxic birds to sequester BTX and to which level of biological organization the convergent evolution between toxic birds and poison dart frogs reaches. To avoid any biases toward finding evidence for either potential BTX autoresistance mechanism in toxic birds, they employed whole-genome sequencing of bird specimens and leveraged previously generated reference genome data from passerine birds and poison dart frogs. While the genomes of toxic birds did not reveal any clues for the existence of genes encoding "toxin sponge" proteins like saxiphilin, Bodawatta et al. did identify amino acid substitutions in the BTX-binding site of the birds' Nav1.4 channels. Interestingly, several of these substitutions evolved in parallel between toxic birds or in the same protein domain as substitutions in *Phyllobates* frogs. Furthermore, a subset of substitutions showed evidence of experiencing positive selection. Although unknown mechanisms of BTX resistance might exist, these observations suggest that pitohuis and other toxic birds may rely more on target-site insensitivity than on a "toxin sponge" to prevent BTX autotoxicity. In light of the conflicting findings between studies on the relative importance of different BTX resistance mechanisms in toxin-coopting birds and frogs, future work could focus on gathering in vivo functional genetic data to resolve discrepancies in the literature (Tarvin et al., 2023, and references therein). Ongoing developments in CRISPR/Cas9 technology may enable such tests of the molecular mechanisms that underlie BTX autoresistance in relatively realistic bird and frog model systems. For example, target site insensitivity-conferring mutations might be introduced in the zebra finch *Taeniopygia guttata* as a passerine bird (Gessara et al., 2021) and the African clawed frog *Xenopus laevis* as an anuran (Aslan et al., 2017).

FIGURE 1 A hooded pitohui (*Pitohui dichrous*) at the Yopno, Uruwa and Som rivers (YUS) Conservation Area on the Huon Peninsula (Morobe Province) in Papua New Guinea. Clearly visible is the bird's lethal mantle, made-up of batrachotoxin-containing brown and black feathers. Photo: Benjamin Freeman.

The study by Bodawatta and colleagues paves the way for further explorations of toxin cooption in birds and other animals. Promising clues for toxin cooption are starting to be found among other poisonous plant- and insect-feeding passerines. For example, a close relative of pitohuis, the feathers of the Eurasian golden oriole (*Oriolus oriolus*), are unpalatable to hornets (Groen & Whiteman, 2021, and references therein), whereas feathers of the yellow grosbeak from Central America were toxic in an assay with brine shrimp as test subjects (Andrade-Zuñiga et al., 2018). Interestingly, many of the toxic bird species appear to possess aposematic coloration, combining red, orange, or yellow colours with black pigmentation. It will be interesting to study the drivers and constraints behind this phenotypic convergence. For example, many Papua New Guinean species with these similar plumage colour patterns have never been

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identified as toxic, yet these "birds of a feather" are found in flocks together (Diamond, 1987), which raises the question to which extent toxic and non-toxic birds engage in Müllerian and Batesian mimicry of lethal-mantled models. Ultimately, the work by Bodawatta et al. yields a deeper understanding of the evolution of toxin co-option and its role in the generation and maintenance of biological diversity on our planet.

AUTHOR CONTRIBUTIONS

S.Z. and S.C.G. wrote the article.

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