

Avian chemical defense: Toxic birds not of a feather

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In 1992, Dumbacher *et al.* (1) substantially altered prevailing views of avian physiology, biochemistry, and chemical ecology with their report of the potent neurotoxin homobatrachotoxinin in feathers and other tissues of several species of New Guinean passerine birds of the genus *Pitohui*. Their discovery was significant not only for suggesting a protective mechanism rarely considered for birds (i.e., chemical defense) but for the nature of the compound they discovered, a structurally complex alkaloid that binds Na⁺ channels and depolarizes electrogenic membranes. Alkaloids in tetrapods generally had been thought to be confined to amphibians, whose skins have long been acknowledged as arsenals of these biologically active compounds (2). Indeed, before its discovery in *Pitohui*, homobatrachotoxinin, a member of a family of steroidal alkaloids called batrachotoxinins (BTXs), had been found only in skin secretions of Central and South American poison-dart frogs (Dendrobatidae) of the genus *Phylllobates*. In this issue of PNAS, Dumbacher *et al.* (3) identify in birds the three major alkaloids that occur in these frogs: homobatrachotoxinin, batrachotoxinin, and their synthetic precursor, batrachotoxinin-A.

Birds, in fact, had not escaped notice as bearers of defensive chemicals before Dumbacher *et al.*'s investigations. While collecting and skinning birds in Egypt during 1941, the naturalist H. B. Cott (4) noted that hornets (*Vespa orientalis*) arriving on the scene differentially consumed the avian carcasses that he had discarded (Fig. 1). Stimulated by this observation, Cott conducted an extensive series of experiments on the palatability of bird flesh to hornets and domesticated cats. In addition, he examined the literature and queried field biologists to assess the acceptability of various birds for human consumption. The impression of widespread unpalatability that emerged from these studies inspired Cott to assert that conspicuous plumage might evolve as an aposematic feature signaling the distastefulness of birds to predators. This hypothesis was rekindled by Dumbacher *et al.* (1), who suggested that the distinctive orange and black coloration of the hooded pitohui (*P.*



Fig. 1. Hornets (*Vespa orientalis*) attacking a freshly skinned carcass of a laughing dove (*Streptopelia senegalensis*) (Left) while ignoring that of a pied kingfisher (*Ceryle rudis*). This observation prompted H. B. Cott (4) to undertake an extensive investigation of avian chemical defense. [Reproduced with permission from ref. 4 (Copyright 1947, The Zoological Society of London).]

dichrous), the most toxic of the birds they examined, is aposematic and may be mimicked by the variable pitohui (*P. kirhocephalus*) in parts of its range.

This year, Dumbacher *et al.* (3) document additional toxic species of New Guinean birds and additional BTXs, some of which are newly described from nature. Newly documented among *Pitohui* are a series of BTXs, including four novel alkaloids. All but one of these compounds, batrachotoxinin-A 3'-hydroxypentanoate, occur in the newly featured toxic bird, the blue-capped ifrita (*Ifrita kowaldi*), a monotypic species restricted to high montane rainforests (>1,500 m).

Ifrita, which as adults are approximately 35 g, forages around tree trunks and probes for insects in moss and under branches. It overlaps little, if at all, geographically with the much larger (65–100 g) species of *Pitohui*, which are omnivorous and occur throughout the vegetational column in lower forests down to sea level. The taxonomic position of *Ifrita* is problematic, but systematists at least agree that it belongs to a family other than the Pachycephalidae, which contains *Pitohui*. Thus, these genera, while sharing highly unique natural products, are not closely related or highly sympatric and occupy different niches.

An additional enigma described by Dumbacher *et al.* (3) is the profound differences observed in the concentrations of BTXs among species and populations. Indeed, more comparative data on toxin abundances among populations may be needed to ascertain just what the species-typical toxins are and at what levels they occur. Within the genus *Pitohui*, *P. dichrous* and *P. kirhocephalus* contained the highest amounts of BTXs, although some individuals from some populations were devoid of toxins. Trace or minor amounts of some BTXs were detected in the crested pitohui (*P. cristatus*) and the black pitohui (*P. nigrescens*); however, these compounds were not observed in the few samples obtained from either the rusty pitohui (*P. ferrugineus*) or the whitebellied pitohui (*P. incertus*). Individuals of *Ifrita* ranged from harboring no detectable toxins to containing major quantities of all five of the BTXs that have been detected from this species. One of two specimens of the little shrike-thrush (*Colluricincla megarrhyncha*, Pachycephalidae) possessed trace amounts of only batrachotoxinin-A; several other pachycephalids contained no detectable toxins. The widely varying

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levels of BTXs observed among populations of these birds prompted Dumbacher *et al.* (3) to consider that BTXs are derived from the environment, most likely from the diet.

The emerging picture of avian toxicity, while still early in its development, is similar to that of another group of toxic vertebrates, the dendrobatid frogs. Dendrobatids exhibit great interspecific variation in the concentrations of some alkaloids present in their skin, as do natural populations within a species (5). Variations in toxin levels among frog populations have been attributed to different diets because it has been established that laboratory-reared frogs sequester ingested alkaloids (6) and that compounds identical to those observed in free-ranging frogs occur in potential arthropod prey (7).

The search for BTXs in organisms consumed by birds has yet to indicate an exogenous source. Stomach content studies reveal a variety of arthropods, mostly insects, and occasional fruits, but chemical analyses of these materials fail to reveal the presence of toxins. One can only speculate on sources of avian BTXs if they are not synthesized *de novo*. The occurrence of BTXs in muscle, viscera, and deep regions of the skin argues against these substances being topically applied, i.e., through “anting,” a behavior common in passerines where arthropods, fruits, or other materials are smeared directly onto the plumage (8). Perhaps birds sequester BTXs produced by microorganisms (9) in a way analogous to that in which pufferfish (*Fugu poecilonotus*, Tetrodontidae) may obtain tetrodotoxin, another neurotoxin, from bacteria in their skin (10).

Aside from the toxin origins, aspects of the distribution of BTXs in the different tissues of birds and the mechanisms by which they are disseminated warrant mention. In their initial study of *Pitohui*, Dumbacher *et al.* (1) observed the highest levels of homobatrachotoxinin in the skin and slightly lower levels in the feathers. Although high levels of other BTXs have been confirmed in the epidermis, it is now suspected that the homobatrachotoxinin detected there previously resulted from contamination by down feathers, the small lower shafts of which are difficult to remove completely from the integument (3). The highest levels of BTXs in *Ifrita* and *Pitohui* are observed in the contour feathers of the breast, belly, or legs.

Feathers constitute birds' first line of defense against consumers. Many predators, in fact, such as raptors and carnivores, pluck them from carcasses before commencing to feed. Thus, it is not surprising from a functional standpoint that feathers serve as a repository of defensive chemicals. Dumbacher *et al.* (3) suggest that BTXs might be transferred from feathers onto eggs or nest materials, thus affording protection against nest-raiding vertebrates. In addition, BTX-laden dander or feather pieces shed from the birds may impart these nonvolatile toxins to other organisms—including humans, who may experience respiratory irritation or other untoward reactions to these birds.

The relative involvement of various avian integumentary structures in toxin sequestration (or synthesis) and release needs further clarification. Studies of lipids reveal that different compounds may

be produced in the epidermis (11), feathers (12–14), and uropygial (preen) gland (15), a prominent exocrine organ located at the base of the tail of many birds. Different lipids, or relative proportions of them, occur among feather types as well (12). The separate extractions with methanol or ethanol of the epidermis, feathers, and uropygial gland of New Guinean birds provide preliminary information on the distribution of BTXs (1, 2). Histochemical and radiolabeling techniques, however, which are invaluable in studies of lipogenesis in vertebrate skin, are needed for more definitive results on the genesis of avian skin toxins.

Although having published only two major papers on the chemistry of New Guinean birds, Dumbacher and collaborators clearly have opened a window into a vast realm of unexplored avian biology, raising questions on many levels. The recent demonstration that some ectoparasitic lice from New Guinea avoid the feathers of *Pitohui* or are killed by exposure to them marks a beginning at elucidating the survival value of avian toxins (16). Other consumers and the anticonsumer properties of naturally occurring BTXs need to be tested. A survey of birds for BTXs and related compounds also is called for, particularly in light of the discovery of alkaloids in another brightly colored species, the red warbler (*Ergaticus ruber*) from Mexico (17). Perhaps, as with each of the avian species now established as toxic, the negative chemosensory responses or other sensitivities of humans to certain birds will point to taxa profitably investigated for biologically active natural products (18).

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