Avian toxicity: Batrachotoxins as chemical defense

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Abstract

The knowledge of avian toxicity is relatively new to the science community. Two avian genuses have been found to carry a form of chemical defense, homobatrachotoxin, a potent steroidal alkaloid in the group called batrachotoxins. The first and most well known genus to carry a form of batrachotoxin is the poison-dart frog (*Phyllobates* spp.) which exudes batrachotoxin in a highly concentrated form from glands on its back. Research scientist, John Dumbacher, discovered a similar form of the toxin in the native New Guinea birds of the *Pitohuis* genus. The birds carry homobatrachotoxin in their feathers, skin, and skeletal muscles, presumably for defense against predators and parasites. A second bird was noted by Dumbacher to carry batrachotoxin as well, also a New Guinea species, *Ifrita kowaldi*. John Daly deduced while studying the poison-dart frog that batrachotoxin was not inherited, but sequestered from another source. Dumbacher also found this to be the case with both New Guinea bird genuses. Both of these batrachotoxin carrying avian genuses are, for the most part, geographically and taxonomically separated which suggests independent evolutionary acquisition of the toxin. Recently, another species of bird, *Aethia cristatella,* was found in the sub arctic of the Pacific and Bering Sea to also show an endogenous production of a defensive compound. Although not an alkaloid, the chemical this bird carries is an offensive aldehyde that is a potent invertebrate repellant.

Introduction

Toxicity in birds has been known since antiquity. Over 200 avian species are known to utilize exogenous defensive materials but endogenously toxic bird species are quite rare (Douglas, 2001). According to Bartram and Boland, about thirteen bird species are known or believed to be toxic (2001). In 2001, the crested auklet (*Aethia cristatella*) was the first seabird or colonial bird reported to use chemical defense; the origin of the aldehyde it uses is still unknown (Douglas, 2001). Two of the best known endogenously toxic genuses sequester a class of compounds collectively called batrachotoxins, five species of *Pitohui* and one species of *Ifrita.* In 1992, John Dumbacher astounded the science world with his publication in *Science* outlining his recent identification of homobatrachotoxin, a member of the batrachotoxins (1992). Previously, batrachotoxins were thought to be restricted to neotropical poison-dart frogs (*Phyllobates* genus). The levels of batrachotoxins are greatly varied among species and, especially, among their habitats (Bartram, 2001). Many questions still remain regarding the sequestering of this potent neurotoxin. The origin of the toxin is largely unknown and the exact purpose of the batrachotoxin is debatable. The purpose of this paper is to review recent research regarding avian chemical defense, especially the potent class of compounds known as batrachotoxins.

History

The earliest record of unpalatable avian species is found in the bible. In the *Book of Numbers,* Moses dictates a story of Israelites who were struck by a severe plague when they greedily ate quail (Bartram, 2001). The disease that is the likely culprit of this plague is known as coturnism, defined in Bartram and Boland's paper as "human poisoning after eating European migratory quail (*Coturnix coturnix* L.)" (2001). In the case of coturnism, the toxin is assumed to be exogenous, but according to Bloom and Grivetti, the primary source of the toxin has not been conclusively identified or the mechanism adequately explained (2001).

Avian toxicity is also referred to in American historical accounts. The ruffed grouse (*Bonasa umbellus*) was a sought-after delicacy and in the late 1700's, James Mease, a Philadelphia physician, described "partridge poisoning" in which he blamed the birds' diet of mountain laurel (*Kalmia latifolia*) for the poisoning (Bloom, 2001). Experiments were not performed regarding this hypothesis until the late 1800's in which no conclusive evidence was found to support this theory. Even though laboratory tests seemed to prove that mountain laurel was indeed poisonous, they did not account for the irregular outbreaks of "partridge poisoning". The scientific methods used to study the poisoning were not statistically sound. Often, geographical location, cooking time and temperature, freshness of the meat, and many other variables were not taken into account for a good scientific outcome (Bloom, 2001). According to Bloom and Grivetti, the mechanism of poisoning is still unknown and cases of coturnism seem to have disappeared around the late nineteenth century (2001). This may be due to seasonal hunting restrictions correlating with the time of year the ruffed grouse sequesters the toxins that cause "partridge poisoning".

History provides accounts of recently discovered poisonous birds as well. When John Dumbacher was researching the poisonous *Pitohui* in Papua New Guinea he learned about a book written by a New Zealand anthropologist, Ralph Bulmer, *Birds of My Kalam Country*, in which Bulmer gathered local Kalam tribal wisdom on highland bird species with the help of his Kalam colleague, Ian Saem Majnep. Dumbacher traveled to the southwestern corner of the Mandang Province to discuss the *Pitohui* with Majnep. The Kalam people referred to *Pitohui* as the wobob bird that evoked war-magic spells. The word *wobob* refers to a skin disease that is caused by contact with the bird (Tidwell, 2001). This historical account of *Pitohui* indicates the natives have long known the poisonous qualities the birds possess before westerners had any knowledge of it existing.

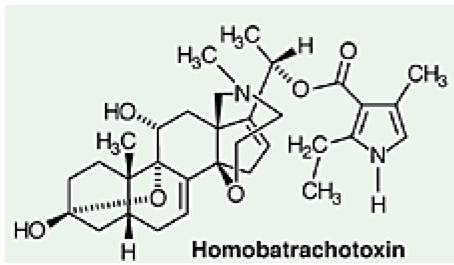
Toxicity and Chemical Nature

The recent discovery of the chemical repellents in the crested auklet (*Aethia cristatella*) is the first report of defensive compounds used by a seabird or colonial bird and a rare example of chemical defense in polar or subpolar marine vertebrates (Douglas, 2001). The chemical compounds responsible for the auklets repellant are saturated and mono-unsaturated short chained aldehydes, but the toxin's origin is unknown. Bartram and Boland discuss other well documented poisonous birds in their paper published at approximately the same time, *Chemistry and Ecology of Toxic Birds*. In some of these bird species the toxin origin is unknown, such as the European migratory quail, the red warbler (*Ergaticus rubber*), the blue-capped ifrita (*Ifrita kowaldi*), and all the species of the *Pitohui* genus (2001). The *Pitohui* and the *Ifrita* are the only birds known to sequester batrachotoxins.

Before Dumbacher stumbled upon the toxicity of *Pitohui* in New Guinea the only known genus to sequester batrachotoxins was *Phyllobates*, neotropical frogs in the family Dendrobatidae (2000). John Daly, researcher at the National Institute of Health (NIH), spent many years studying the unique steroidal alkaloids found in these poison-dart frogs. He found the frogs emitted powerful concentrations of batrachotoxins from glands on their backs, amounts equivalent to a few grains of salt would be lethal to a person (Tidwell, 2001). Without this knowledge, he would have never identified the batrachotoxins in *Pitohui* feathers so readily. Daly's identification was important to Dumbacher's discovery of the potent neurotoxin in *Pitohui* feathers (Tidwell, 2001). When Majnep was discussing the *wobob* bird with Dumbacher, he also gave him information about the *Ifrita* as well and when Dumbacher sent *Ifrita* feathers to NIH they showed nearly identical profiles of batrachotoxin alkaloids as the *Pitohui* feathers (Tidwell, 2001).

The form of batrachotoxin that is found in the *Pitohui* is a highly potent neurotoxin called homobatrachotoxin. When it comes in contact with human skin it causes numbness and burning and provokes sneezing (Bartram, 2001). Bioassays conducted using crude-extracts and mice as subjects indicated the skin and feathers of the *Pitohui* were the most toxic, followed by the much less toxic striated muscle and the least toxic heart-liver, stomach, intestines, and uropygial gland (Dumbacher, 1992). Direct-probe mass spectrometry and TLC with a sensitive colorimetric reagent was used to detect the presence of homobatrachotoxin (Dumbacher, 2000). Dumbacher et. al. used HPLC-CIMS to further analyze skin and feather extracts and discovered several other batrachotoxin alkaloids: Batrachotoxin-A-20R cis-crotonate, Batrachotoxin-A-20R 3'-hydroxypentanoate, Batrachotoxin-A-20R acetate, and Batrachotoxin-A (2000). According to Burtt, "homobatrachotoxin (is) a potent alkaloid that binds sodium channels and depolarizes electrogenic membranes, thereby disabling the nerves and muscles of most vertebrates and invertebrates" (1999). The varying levels of homobatrachotoxin found in the avian species are up to three orders of magnitude lower than in *Phyllobates*

(Bartram, 2001). Dumbacher illustrates this in his paper by stating: "…bioassays of toxicity (illustrated that) a 65-g hooded pitohui (*P. dichrous*) contains 15 to 20 µg of homobatrachotoxin in the skin and another 2 to 3 µg in the feathers…in contrast, the total amount of batrachotoxins in the skin of Columbian poison-dart frogs ranges from 100 µg in two species to 1000 µg in a third" (1992).



According to Dumbacher, levels of toxicity vary amongst species and populations. The hooded pitohui's and the variable pitohui's (*P. kirhocephalus*) feathers contain the highest concentrations of the toxin, while the feathers of the rusty pitohui (*P. ferrugineus*) are considerably less toxic, and the crested pitohui (*P. Cristatus*) and black pitohui (*P. nigrescens*) feather toxins are almost undetectable (Dumbacher, 1999; Weldon, 2000). A second study done by Dumbacher et. al. and published in 2000 showed similar results, except that the rusty pitohui and the white-bellied pitohui (*P. incertus*) were devoid of toxins. The white-bellied pitohui had not been previously studied because of its limited range, but the change in levels amongst the rusty pitohuis as well as the variation found within other *Pitohui* species indicates environmental factors rather than genetics are influencing the toxicity of these birds (Dumbacher, 2000; Weldon, 2000). *Ifrita* ranged as well, from containing major quantities of all five batrachotoxins to storing no detectable toxins at all (Weldon, 2000).

Due to geographical and diet variation as well as size differences, *Pitohui* and *Ifrita* diets do not overlap. Although, according to Majnep (Dumbacher, 2000), ifrtas are entomophagous but may incidentally ingest mosses while foraging for insects. This could be the source of their toxicity. Although, at the time of publication of Dumbacher et. al.'s article on *Ifrita* in 2000, analysis of *Ifrita* stomach content had not been published. *Pitohui*, a larger bird, feeds on large insects, fruits, and possibly small vertebrates (Dumbacher, 2000). According to Weldon, analyses of *Pitohui* stomach contents fail to indicate toxin presence. The presence of toxin in the muscles and skin indicate the toxin isn't topically applied. Thus, he hypothesizes that batrachotoxins may be sequestered from microorganisms (2000). It is interesting to note that frogs of the genus *Phyllobates* that are reared in laboratory situations completely lose their toxicity by the second generation, indicating the inability to synthesis the toxins de novo (Bartram, 2001). When Daly fed laboratory reared frogs small amounts of batrachotoxins the toxin accumulated in their skin, indicating the source of batrachotoxin existed in the frogs' natural insect diet (2003; Tidwell, 2001). The crested auklet's natural diet of zooplankton is also thought to be the source for it's repellant. Similar to the frogs, in captivity, the bird does not possess the same aldehydes it does in the wild (Douglas, 2001).

The specific purpose of batrachotoxins in avian species is debatable, although most agree that it serves as protection in the form of chemical defense and perhaps mimicry is involved.

Homobatrachotoxin as a defense against ectoparasites is the only theory that has been researched to any extent. In 1999, Dumbacher wrote a paper discussing the research he did to identify the effects of homobatrachotoxin on chewing lice (1999). Both choice and life-span experiments were performed on lice with different feather treatments. In the choice experiments, lice showed a statistically significant preference against feeding on the *Pitohui* feathers. In the life span experiments, he found homobatrachotoxin to greatly increase louse mortality (1999). He concluded that the toxins "(1) reduced louse fecundity, (2) reduced louse survival, (3) reduced the influence of lice on host fitness (by delaying maturation, lengthening the life cycle, or suppressing appetite), and (4) favorably effected louse transmission rates by reducing immigration or inducing emigration" (1999).

Crested auklets are also presumed to use their sequestered chemicals as ectoparasite repellants. Aldehyde compounds such as those found emitted by the auklet have powerful citrus odors and act as strong invertebrate repellants. The social nature of crested auklets requires a powerful pest repellant such as these aldehydes to prevent large outbreaks of ectoparasites in auklet colonies (Douglas, 2001).

It has been proposed that the *Pitohui* and *Ifrita* use homobatrachotoxin as defense again larger predators as well. Birds of prey may avoid the smaller birds due to the discomfort caused when coming in contact with the toxic skin and feathers. An argument against this is the bird of prey would not know the smaller bird is unpleasant until it's too late for the small bird. Bold coloration would be effective at signaling the toxins presence, thus alluding to the role of aposematic coloration (Poulsen, 1994). Snakes are another predator the toxin might protect against. Since the toxin is abundant on the breast feathers of the adult *Pitohui* it may prevent snakes from stealing eggs from the nest by transferring the toxin to the egg's surface (Bartram, 2001; Poulsen, 1994).

Dumbacher states the toxins may defend *Pitohui* from human hunters as well. He hypothesizes the contrasting color patterns of *Pitohui* serve as an aposematic signal to visually oriented predators such as humans (2001). These colorations may also be an example of Müllerian mimicry in birds. Since some birds are not as poisonous as others this may be a case for Batesian mimicry as well (Diamond, 1992). One of Dumbacher's more recent publications discusses the phylogenetic evidence for Müllerian mimicry. He used DNA isolation and sequencing of museum skins and phylogeny and ancestral character state reconstruction to yield a phylogram showing the relationships of *Pitohui*. His studies found that "although not conclusive…data are consistant with the hypothesis that Müllerian mimicry helped drive plumage evolution on the Wandammen Peninsula" (2001). The remoteness of the location and the politically sensitive habitat limited research potentials. This, combined with a lack of genetic research and phylogenetic evidence, and the presence of multiple subspecies showing sexual plumage dichromatism, prevents this study from being a direct test of mimicry (Dumbacher, 2001).

Ecological Importance

The bright plumage of both *Ifrita* and *Pitohui* birds help protect them from native New Guineans and maybe other predators as well. Both species build nests roughly 2 – 3 meters from the ground (Dumbacher, 2000). The most toxic part of the birds is in the breast and belly feathers, which may protect their eggs from predators that ingest whole eggs while also repelling ectoparasites that reduce the birds' health.

Natural products research has also greatly benefited form the discovery of these batrachotoxins. The skin of *Phyllobates* has provided many biologically active compounds. Research has produced many unique alkaloids that have a major impact on biomedical research (Daly, 2003). Weldon states: “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.” (2000).

Conclusion

Avian toxicity has been recognizable since ancient times but people are just now beginning to study birds outside the realm associated with human agriculture. Scientists are beginning to focus beyond transmission of human or domestic livestock pathogens (Burtt, 1999) into exciting research that has the potential to be highly chemically and possibly medically important. Many aspects of avian toxicity still need to be explored.

The sources of “partridge poisoning”, the aldehydes used by crested auklets, and batrachotoxins are still unknown. In 2001, Todd Capson, an independent ethnobiologist at the Smithsonian Tropical Research Institute in Panama, headed for New Guinea to search for the *Pitohui* poison and hopefully answer some questions concerning the source of the homobatrachotoxin (Tidwell, 2001).

Also mentioned in the literature reviewed was the necessity for more molecular-based research. Frogs in the genus *Phyllobates* appear to be insensitive to their own toxins due to a genetically controlled modification of their sodium channel protein in the nerves and muscles. Studies need to be done to determine the molecular basis for the resistance of *Pitohui* and *Ifrita* to the homobatrachotoxins they sequester (Bartram, 2001).

The exact reasons birds sequester homobatrachotoxins or other chemical compounds are unknown. Defense is a logical conclusion and there is a lot of room for expansion on this topic. The study of the effects of homobatrachotoxin on feather lice by Dumbacher (referred to earlier) is effective at beginning to illustrate the benefits of the toxin to *Pitohui* but additional research is needed on more detrimental ectoparasites (1999). It is also questionable whether the lice can build immunity to the homobatrachotoxin. If the lice can adapt, can *Pitohui* counter (Burtt, 1999)? Burtt poses a number of questions in his paper *Think Small*. He suggests researching behavior of both the birds and the lice with respects to the toxin levels. Studies regarding the reason certain bird species sequester such potent nerve toxins results in questions regarding mimicry. The need for in depth genetic research and phylogenetic evidence is clear in order to provide a direct test from mimicry.

It is obvious there is still much work to be done before scientists can fully understand the mechanisms, chemistry, and functions of avian chemical defense. This brief review of currently available literature depicts many gaps that have yet to be filled through extensive research.

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