JGP Journal of General Physiology

COMMENTARY

How do batrachotoxin-bearing frogs and birds avoid self intoxication?

Roberto Márquez[1,2a]

Naturally occurring toxins have been linked to human activities since prehistory. From pest control (Dev and Koul, 1997) and weapon enhancement (Posada Arango, 1869; Bisset, 1989) to disease treatment (Petrovska, 2012; Kapoor, 2010) and rituals (Schultes, 1998; Hoffman, 2019), modern humans, and perhaps even Neanderthals (Solecki, 1977), have found endless uses for natural toxins. Throughout the last century, biologists have studied natural toxins and their action mechanisms to address subjects across fields, from systematics, ecology, and evolution (Mebs, 2001; Berenbaum, 1995; Myers and Daly, 1976) to molecular physiology and drug design (Lee, 2021; Keller et al., 1986). An especially informative aspect of toxicity is toxin resistance. Several distantly related species often evolve resistance to the same toxin, for example when predators evolve to resist toxic prey (which must also resist their own toxins), or when multiple species consume the same toxic food items. Such instances of replicated evolution provide excellent opportunities to draw generalities about the evolutionary processes and functional mechanisms underlying toxicity and toxin resistance, as well as the biochemical and physiological properties of toxins themselves. In an earlier issue of the *Journal of General Physiology*, Abderemane-Ali et al. (2021) address the physiological mechanisms through which frogs and birds that secrete the deadly neurotoxin batrachotoxin (BTX) are able to resist its noxious effects. Their results challenge previous ideas on BTX resistance and provide exciting new hypotheses and future directions.

Batrachotoxin is a steroidal alkaloid that binds to voltage-gated sodium channels, reducing their ion selectivity and preventing pore closure, which renders them unable to produce action potentials (Daly et al., 1965; Wang et al., 2006; Warnick et al., 1975). It is found in frogs of the genus *Phyllobates* (Myers et al., 1978), birds in the genera *Pitohui* and *Ifrita* (Dumbacher et al., 1992, 2000), and beetles in the genus *Choresine* (Dumbacher et al., 2004). BTX was discovered independently by the precolonial inhabitants of the Northwestern Andes (present day Colombia), who obtained it from *Phyllobates* frogs to poison their blowgun darts (Posada Arango, 1869; Wassén, 1957), and the native inhabitants of New Guinea, who recognized the similarly noxious and uncomfortable effects of handling and eating BTX-bearing birds and beetles (Majnep and Bulmer, 1977; Dumbacher et al., 2004). Later, in the mid-20th century, chemists were able to isolate BTX from *Phyllobates aurotaenia* skins and characterize its chemical composition (Märki and Witkop, 1963; Tokuyama et al., 1968; Daly et al., 1965). The notoriously high lethality (LD_{50} = 2 µg/kg subcutaneous in mice; Tokuyama et al., 1968) and biomedical potential of BTX motivated a sizable body of work spanning several aspects of BTX chemistry and biology, such as its mode of action on sodium channels (e.g., Green et al., 1987; Warnick et al., 1975; Trainer et al., 1996), or the chemical ecology of BTX-bearing species (e.g., Myers et al., 1978; Dumbacher et al., 2004; Protti-Sánchez et al., 2019).

Although it was established early on that isolated muscles of *Phyllobates terribilis* and *Ph. aurotaenia* resist high concentrations of BTX (Albuquerque et al., 1973; Daly et al., 1980), the mechanisms underlying resistance in BTX-bearing species remain unknown. Work on isolated mammalian and insect sodium channels identified the binding site of BTX at the inner pore of the channel, and showed that amino acid replacements at or near the binding site can result in BTX-resistant channels in vitro (e.g., Wang and Wang, 1999; Wang et al., 2007; Du et al., 2011; Linford et al., 1998). With these results in mind, initial attempts to understand BTX autoresistance in natural populations focused on identifying putative resistance-conferring mutations on muscular voltage-gated sodium channels of *Phyllobates* frogs using reconstructions of sequence evolution and molecular docking simulations. Five candidate mutations at sites involved in BTX binding were identified by initial studies (Tarvin et al., 2016; Márquez et al., 2019), yet only one of them provided BTX resistance to rat sodium channels in follow up electrophysiological experiments (Wang and Wang, 2017). This result, together with the fact that this mutation was found segregating at low frequencies in only one of the five BTX-bearing species of frogs (Márquez et al., 2019), hinted that mutations at target sites alone may not tell the whole story behind BTX resistance in *Phyllobates*.

[1]Department of Ecology and Evolution, University of Chicago, Chicago, IL; [2]Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI.

Correspondence to Roberto Márquez: marquezr@umich.edu.

© 2021 Márquez. This article is distributed under the terms of an Attribution–Noncommercial–Share Alike–No Mirror Sites license for the first six months after the publication date (see http://www.rupress.org/terms/). After six months it is available under a Creative Commons License (Attribution–Noncommercial–Share Alike 4.0 International license, as described at https://creativecommons.org/licenses/by-nc-sa/4.0/).

Abderemane-Ali et al. (2021) conducted BTX resistance assays on voltage-gated sodium channels of *Pitohui uropygialis* and *Ph. terribilis*. Mammalian channels, as well as those of *Dendrobates tinctorius*—a poison frog that does not secrete BTX—were tested as controls. Their results were unexpected; none of the tested channels showed signs of BTX resistance. Adding the putative resistance-conferring mutation identified previously (Tarvin et al., 2016; Wang and Wang, 2017) generated partially resistant bird and mammal channels, albeit at a high functional cost, since mutant channels were harder to open and easier to inactivate. Frog channels with this mutation also suffered conspicuously negative effects, but did not exhibit resistance. Tests on live frogs, however, confirmed that *Ph. terribilis* are resistant to high concentrations of BTX, and revealed that they also resist saxitoxin (STX) and tetrodotoxin (TTX), two other neurotoxic alkaloids that target voltage-gated sodium channels, which are not found in *Phyllobates*. Surprisingly, other poisonous frogs that do not secrete BTX, TTX, or STX (*D. tinctorius* and *Mantella aurantiaca)* were also resistant to all three toxins, and one nonpoisonous frog (*Polypedates leucomystax*) was partially resistant to BTX and resistant to TTX. *Xenopus laevis*, which does not secrete alkaloids, was susceptible to all toxins. These results suggest that perhaps a more generalized mechanism for neurotoxin resistance may have evolved in alkaloid-bearing frogs (but see Albuquerque et al., 1973, who showed that *Ph. aurotaenia* muscles are susceptible to TTX) and provide strong evidence against the idea that sodium channel mutations are central to autoresistance in BTX-bearing vertebrates.

The notion that mutations at BTX target sites are not the main mechanism responsible for BTX resistance in *Phyllobates* and *Pitohui* may initially come as a surprise. However, in retrospect, this scenario may not be the most likely from an evolutionary perspective: although mutations that confer target-site insensitivity are a commonly invoked mechanism for neurotoxin resistance (e.g., Geffeney et al., 2005; Zhen et al., 2012; Martinez-Torres et al., 1999), they are often accompanied by functional costs since they involve meddling with functionally important regions of highly fine-tuned proteins (Hague et al., 2018). This was made evident by the functional costs observed in BTX-resistant bird and mammalian channels generated by Abderemane-Ali et al. (2021). Since BTX is able to modify many of the different voltage-gated sodium channels encoded in vertebrate genomes (e.g., Bosmans et al., 2004; Wang and Wang, 1998; Li et al., 2002; Linford et al., 1998; Wang et al., 2007), self-resistance through target-site insensitivity at multiple channels could potentially result in major functional tradeoffs at the organismal level. In poison frogs, this effect could be further amplified by the fact that most species accumulate dozens of different neurotoxic alkaloids (Daly et al., 2005), many of which target several different ion channels (Santos et al., 2016).

How do *Phyllobates* and *Pithohui* species resist BTX then? A second mechanism of toxin resistance that is frequently observed in nature is preventing toxin molecules from interacting with their targets, for instance through compartmentalization in specialized glands or organelles (e.g., Zhou and Fritz, 1994; Reinhard et al., 1987), or through sequestration by "toxin sponge" molecules that bind toxins to impede target binding. A famous example of the latter is STX resistance in American bullfrogs (*Rana catesbaiana*), where saxiphilin, a protein in the transferrin family, binds STX in the frogs' tissues, protecting voltage-gated sodium channels from intoxication (Mahar et al., 1991; Morabito and Moczydlowski, 1994). Abderemane-Ali et al. (2021) proposed that a similar mechanism involving toxin sponges may underlie BTX resistance in *Phyllobates* and *Pitohui*. As an initial step toward testing this hypothesis, the authors exposed *Ph. terribilis* sodium channels to STX and found that they are readily inactivated by STX, despite live frogs being resistant the toxin. They then showed that exposing the channels to a mixture of saxiphilin and STX or adding saxiphilin after STX exposure resulted in fully functional channels, demonstrating that saxiphilin protects *Ph. terribilis* channels from STX intoxication, likely by outcompeting them for STX binding. Whether proteins with high affinity for BTX and other alkaloids are present in poison frog or poison bird tissues, and whether they produce toxin resistance through a similar mechanism, however, remains unknown.

Although yet to be tested, the toxin sponge hypothesis provides a putatively simpler scenario for the evolution of BTX self-resistance than the fixation of resistant (and potentially costly) mutations at multiple voltage-gated sodium channels. Neither poison frogs nor poison birds are able to synthesize BTX and must instead obtain it from dietary sources and transport it to specialized glands in the skin (Daly et al., 1994; Dumbacher et al., 2009). Throughout this process, BTX comes in contact with multiple tissues (e.g., gut, liver, muscle, skin; see Dumbacher et al., 1992; O'Connell et al., 2021; Prates et al., 2012), and therefore has the opportunity to interact with several sodium channels, each encoded by a different gene. Considering the costs often associated to neurotoxin-resistant sodium channel mutants, it seems unlikely for resistance to evolve through independent mutations at each of these genes, since this could easily result in considerable functional costs at the organismal level. In contrast, a scenario where one or a few transport proteins (e.g., transferrins) acquire the ability to sequester BTX in the gut and prevent it from binding sodium channels as it is transported to the skin, would involve changes at a smaller number of genes, and potentially less drastic functional tradeoffs. Since toxin transport proteins do not play such demanding and physiologically essential roles as ion channels, function-altering mutations should carry a smaller risk of functional costs. Furthermore, since BTX is obtained from dietary sources, resistance must evolve either prior to or in tandem with the ability to sequester and accumulate it. A protein that binds BTX with high affinity would provide a unified physiological mechanism for both sequestration/accumulation and resistance, facilitating the coordinated evolution of these two traits.

Overall, the paper by Abderemane-Ali and colleagues (2021) challenges previously established ideas on the physiological underpinnings of BTX resistance in frogs and birds, and focuses our attention on alternative mechanisms that may facilitate the evolution of this complex trait, opening an exciting avenue of research questions: are there toxin sponge molecules that can bind BTX and/or other alkaloid neurotoxins in addition to STX and TTX? How did they acquire toxin-binding capabilities? Does

evolving resistance to some toxins (via toxin sponges or otherwise) result in resistance to others? If sodium channel mutations do not underlie BTX resistance, what is the role (if any) of the multiple sodium channel mutations that coincide with the evolution of toxicity in poison frogs? Future work aimed at understanding the pharmacokinetics of alkaloid sequestration and accumulation, as well as the evolutionary history of the proteins involved and their functions, will certainly contribute to our understanding of how and why so many different taxa across the tree of life have evolved to sequester and secrete defensive neurotoxins.

Acknowledgments

Christopher J. Lingle served as editor.

The author is grateful to Valeria Ramírez-Castañeda and Valentina Gómez-Bahamón for comments and discussion.

The author's work is supported by the National Science Foundation (award number IOS-1827333) and by the Michigan Society of Fellows at the University of Michigan.

The author declares no competing financial interests.

References

Abderemane-Ali, F., N.D. Rossen, M.E. Kobiela, R.A. Craig, C.E. Garrison, Z. Chen, C.M. Colleran, L.A. O'Connell, J. Du Bois, J.P. Dumbacher, and D.L. Minor. 2021. Evidence that toxin resistance in poison birds and frogs is not rooted in sodium channel mutations and may rely on "toxin sponge" proteins. *J. Gen. Physiol.* 153:e202112872. https://doi.org/10.1085/jgp.202112872

Albuquerque, E.X., J.E. Warnick, F.M. Sansone, and J. Daly 1973 The pharmacology of batrachotoxin. V. A comparative study of membrane properties and the effect of batrachotoxin on sartorius muscles of the frogs *Phyllobates aurotaenia* and *Rana pipiens J. Pharmacol. Exp. Ther.* 184: 315–329

Berenbaum, M.R. 1995. The chemistry of defense: theory and practice. *Proc. Natl. Acad. Sci. USA.* 92:2–8. https://doi.org/10.1073/pnas.92.1.2

Bisset, N.G. 1989. Arrow and dart poisons. *J. Ethnopharmacol.* 25:1–41. https://doi.org/10.1016/0378-8741(89)90043-3

Bosmans, F., C. Maertens, F. Verdonck, and J. Tytgat. 2004. The poison Dart frog's batrachotoxin modulates $Na_v1.8$. *FEBS Lett.* 577:245–248. https://doi.org/10.1016/j.febslet.2004.10.017

Daly, J.W., C.W. Myers, J.E. Warnick, and E.X. Albuquerque. 1980. Levels of batrachotoxin and lack of sensitivity to its action in poison-dart frogs (*Phyllobates*). *Science.* 208:1383–1385. https://doi.org/10.1126/science.6246586

Daly, J.W., S.I. Secunda, H.M. Garraffo, T.F. Spande, A. Wisnieski, and J.F. Cover Jr. 1994. An uptake system for dietary alkaloids in poison frogs (Dendrobatidae). *Toxicon.* 32:657–663. https://doi.org/10.1016/0041-0101(94)90335-2

Daly, J.W., T.F. Spande, and H.M. Garraffo. 2005. Alkaloids from amphibian skin: a tabulation of over eight-hundred compounds. *J. Nat. Prod.* 68: 1556–1575. https://doi.org/10.1021/np0580560

Daly, J.W., B. Witkop, P. Bommer, and K. Biemann. 1965. Batrachotoxin. The active principle of the Colombian arrow poison frog, *Phyllobates bicolor J. Am. Chem. Soc.* 87:124–126. https://doi.org/10.1021/ja01079a026

Dev, S., and O. Koul. 1997. Insecticides of natural origin. Harwood Academic, Amsterdam, the Netherlands

Du, Y., D.P. Garden, L. Wang, B.S. Zhorov, and K. Dong. 2011. Identification of new batrachotoxin-sensing residues in segment IIIS6 of the sodium channel. *J. Biol. Chem.* 286:13151–13160. https://doi.org/10.1074/jbc.M110.208496

Dumbacher, J.P., B.M. Beehler, T.F. Spande, H.M. Garraffo, and J.W. Daly. 1992. Homobatrachotoxin in the genus *Pitohui*: chemical defense in birds? *Science.* 258:799–801. https://doi.org/10.1126/science.1439786

Dumbacher, J.P., G.K. Menon, and J.W. Daly. 2009. Skin as a toxin storage organ in the endemic New Guinean genus *Pitohui Auk.* 126:520–530. https://doi.org/10.1525/auk.2009.08230

Dumbacher, J.P., T.F. Spande, and J.W. Daly. 2000. Batrachotoxin alkaloids from passerine birds: a second toxic bird genus (*Ifrita kowaldi*) from New Guinea. *Proc. Natl. Acad. Sci. USA.* 97:12970–12975. https://doi.org/10.1073/pnas.200346897

Dumbacher, J.P., A. Wako, S.R. Derrickson, A. Samuelson, T.F. Spande, and J.W. Daly. 2004. Melyrid beetles (*Choresine*): a putative source for the batrachotoxin alkaloids found in poison-dart frogs and toxic passerine birds. *Proc. Natl. Acad. Sci. USA.* 101:15857–15860. https://doi.org/10.1073/pnas.0407197101

Geffeney, S.L., E. Fujimoto, E.D. Brodie III, E.D. Brodie Jr., and P.C. Ruben. 2005. Evolutionary diversification of TTX-resistant sodium channels in a predator-prey interaction. *Nature.* 434:759–763. https://doi.org/10.1038/nature03444

Green, W.N., L.B. Weiss, and O.S. Andersen. 1987. Batrachotoxin-modified sodium channels in planar lipid bilayers. Ion permeation and block *J. Gen. Physiol.* 89:841–872. https://doi.org/10.1085/jgp.89.6.841

Hague, M.T.J., G. Toledo, S.L. Geffeney, C.T. Hanifin, E.D. Brodie Jr., and E.D. Brodie III. 2018. Large-effect mutations generate trade-off between predatory and locomotor ability during arms race coevolution with deadly prey. *Evol. Lett.* 2:406–416. https://doi.org/10.1002/evl3.76

Hoffman, M.A. 2019. Entheogens (psychedelic drugs) and the ancient mystery religions. *In* Toxicology in Antiquity. P. Wexler, editor. Elsevier Academic Press, London. 353–362. https://doi.org/10.1016/B978-0-12-815339-0.00025-1

Kapoor, V.K. 2010. Natural toxins and their therapeutic potential. *Indian J. Exp. Biol.* 48:228–237

Keller, B.U., R.P. Hartshorne, J.A. Talvenheimo, W.A. Catterall, and M. Montal. 1986. Sodium channels in planar lipid bilayers. Channel gating kinetics of purified sodium channels modified by batrachotoxin. *J. Gen. Physiol.* 88:1–23. https://doi.org/10.1085/jgp.88.1.1

Lee, G. 2021. Drug Development using natural toxins. *Toxins (Basel).* 13:414. https://doi.org/10.3390/toxins13060414

Li, H.-L., D. Hadid, and D.S. Ragsdale. 2002. The batrachotoxin receptor on the voltage-gated sodium channel is guarded by the channel activation gate. *Mol. Pharmacol.* 61:905–912. https://doi.org/10.1124/mol.61.4.905

Linford, N.J., A.R. Cantrell, Y. Qu, T. Scheuer, and W.A. Catterall. 1998. Interaction of batrachotoxin with the local anesthetic receptor site in transmembrane segment IVS6 of the voltage-gated sodium channel. *Proc. Natl. Acad. Sci. USA.* 95:13947–13952. https://doi.org/10.1073/pnas.95.23.13947

Mahar, J., G.L. Lukács, Y. Li, S. Hall, and E. Moczydlowski. 1991. Pharmacological and biochemical properties of saxiphilin, a soluble saxitoxin-binding protein from the bullfrog (*Rana catesbeiana*). *Toxicon.* 29: 53–71. https://doi.org/10.1016/0041-0101(91)90039-T

Majnep, I.S., and R.N.H. Bulmer. 1977. Mnmon yad Kalam yakt: Birds of may Kalam country. Auckland University Press, Auckland.

Märki, F., and B. Witkop. 1963. The venom of the Colombian arrow poison frog *Phyllobates bicolor Experientia.* 19:329–338. https://doi.org/10.1007/BF02152303

Márquez, R., V. Ramírez-Castañeda, and A. Amézquita. 2019. Does batrachotoxin autoresistance coevolve with toxicity in *Phyllobates* poison-dart frogs? *Evolution.* 73:390–400. https://doi.org/10.1111/evo.13672

Martinez-Torres, D., C. Chevillon, A. Brun-Barale, J.B. Bergé, N. Pasteur, and D. Pauron. 1999. Voltage-dependent Na^+ channels in pyrethroid-resistant *Culex pipiens* L mosquitoes. *Pestic. Sci.* 55:1012–1020. https://doi.org/10.1002/(SICI)1096-9063(199910)55:10<1012::AID-PS39>3.0.CO;2-5

Mebs, D. 2001. Toxicity in animals. Trends in evolution? *Toxicon.* 39:87–96. https://doi.org/10.1016/S0041-0101(00)00155-0

Morabito, M.A., and E. Moczydlowski. 1994. Molecular cloning of bullfrog saxiphilin: a unique relative of the transferrin family that binds saxitoxin. *Proc. Natl. Acad. Sci. USA.* 91:2478–2482. https://doi.org/10.1073/pnas.91.7.2478

Myers, C.W., and J.W. Daly. 1976. Preliminary evaluation of skin toxins and vocalizations in taxonomic and evolutionary studies of poison-dart frogs (Dendrobatidae). *Bull. Am. Mus. Nat. Hist.* 157:173–262. http://hdl.handle.net/2246/622

Myers, C.W., J.W. Daly, and B. Malkin. 1978. A dangerously toxic new frog (*Phyllobates*) used by Emberá Indians of western Colombia, with discussion of blowgun fabrication and dart poisoning. *Bull. Am. Mus. Nat. Hist.* 161:307–366. http://hdl.handle.net/2246/1286

O'Connell, L.A., J.D. O'Connell, J.A. Paulo, S.A. Trauger, S.P. Gygi, and A.W. Murray. LS50: Integrated Science Laboratory Course. 2021. Rapid toxin sequestration modifies poison frog physiology. *J. Exp. Biol.* 224: jeb230342. https://doi.org/10.1242/jeb.230342

Petrovska, B.B. 2012. Historical review of medicinal plants' usage. *Pharmacogn. Rev.* 6:1–5. https://doi.org/10.4103/0973-7847.95849

Posada Arango, A. 1869. Mémoire sur le poison de rainette des sauvages du Chocó présentè à la Sociéte Allemande de Paris. *Soc. Med. All. Par.* 1869: 203–213.

Prates, I., M.M. Antoniazzi, J.M. Sciani, D.C. Pimenta, L.F. Toledo, C.F.B. Haddad, and C. Jared. 2012. Skin glands, poison and mimicry in dendrobatid and leptodactylid amphibians. *J. Morphol.* 273:279–290. https://doi.org/10.1002/jmor.11021

Protti-Sánchez, F., L. Quirós-Guerrero, V. Vásquez, B. Willink, M. Pacheco, E. León, H. Pröhl, and F. Bolaños. 2019. Toxicity and alkaloid profiling of the skin of the Golfo Dulcean poison frog *Phyllobates vittatus* (Dendrobatidae). *J. Chem. Ecol.* 45:914–925. https://doi.org/10.1007/s10886-019-01116-x

Reinhard, J.F. Jr., E.J. Diliberto Jr., O.H. Viveros, and A.J. Daniels. 1987. Subcellular compartmentalization of 1-methyl-4-phenylpyridinium with catecholamines in adrenal medullary chromaffin vesicles may explain the lack of toxicity to adrenal chromaffin cells. *Proc. Natl. Acad. Sci. USA.* 84:8160–8164. https://doi.org/10.1073/pnas.84.22.8160

Santos, J.C., R.D. Tarvin, and L.A. O'Connell. 2016. A Review of chemical defense in poison frogs (Dendrobatidae): Ecology, pharmacokinetics, and autoresistance. *In* Chemical Signals in Vertebrates 13. Springer International Publishing, New York. 305–337. https://doi.org/10.1007/978-3-319-22026-0_21

Schultes, R.E. 1998. Antiquity of the use of new world hallucinogens. *In* Heffter Review of Psychedelic Research. Vol. 1. D.E. Nichols, editor. The Heffter Research Institute, Santa Fe, NM. 1–7.

Solecki, R.S. 1977. The implications of the Shanidar cave Neanderthal Flower Burrial. *Ann. N. Y. Acad. Sci.* 293:114–124. https://doi.org/10.1111/j.1749-6632.1977.tb41808.x

Tarvin, R.D., J.C. Santos, L.A. O'Connell, H.H. Zakon, and D.C. Cannatella. 2016. Convergent substitutions in a sodium channel suggest multiple origins of toxin resistance in poison frogs. *Mol. Biol. Evol.* 33:1068–1080. https://doi.org/10.1093/molbev/msv350

Tokuyama, T., J. Daly, B. Witkop, I.L. Karle, and J. Karle. 1968. The structure of batrachotoxinin A, a novel steroidal alkaloid from the Colombian arrow poison frog, *Phyllobates aurotaenia J. Am. Chem. Soc.* 90:1917–1918. https://doi.org/10.1021/ja01009a052

Trainer, V.L., G.B. Brown, and W.A. Catterall. 1996. Site of covalent labeling by a photoreactive batrachotoxin derivative near transmembrane segment IS6 of the sodium channel α subunit. *J. Biol. Chem.* 271:11261–11267. https://doi.org/10.1074/jbc.271.19.11261

Wang, S.-Y., J. Mitchell, D.B. Tikhonov, B.S. Zhorov, and G.K. Wang. 2006. How batrachotoxin modifies the sodium channel permeation pathway: computer modeling and site-directed mutagenesis. *Mol. Pharmacol.* 69: 788–795. https://doi.org/10.1124/mol.105.018200

Wang, S.-Y., D.B. Tikhonov, J. Mitchell, B.S. Zhorov, and G.K. Wang. 2007. Irreversible block of cardiac mutant Na^+ channels by batrachotoxin. *Channels (Austin).* 1:179–188. https://doi.org/10.4161/chan.4437

Wang, S.-Y., and G.K. Wang. 1999. Batrachotoxin-resistant Na^+ channels derived from point mutations in transmembrane segment D4-S6. *Biophys. J.* 76:3141–3149. https://doi.org/10.1016/S0006-3495(99)77465-5

Wang, S.-Y., and G.K. Wang. 2017. Single rat muscle Na^+ channel mutation confers batrachotoxin autoresistance found in poison-dart frog *Phyllobates terribilis Proc. Natl. Acad. Sci. USA.* 114:10491–10496. https://doi.org/10.1073/pnas.1707873114

Wang, S.Y., and G.K. Wang. 1998. Point mutations in segment I-S6 render voltage-gated Na^+ channels resistant to batrachotoxin. *Proc. Natl. Acad. Sci. USA.* 95:2653–2658. https://doi.org/10.1073/pnas.95.5.2653

Warnick, J.E., E.X. Albuquerque, R. Onur, S.E. Jansson, J. Daly, T. Tokuyama, and B. Witkop. 1975. The pharmacology of batrachotoxin. VII. Structure-activity relationships and the effects of pH. *J. Pharmacol. Exp. Ther.* 193:232–245.

Wassén, S.H. 1957. On dendrobates-frog-poison material among Emperá (Chocó)-speaking Indians in Western Caldas, Colombia. Annals 1955-1956. Gothenburg Ethnographic Museum, Gothenburg.

Zhen, Y., M.L. Aardema, E.M. Medina, M. Schumer, and P. Andolfatto. 2012. Parallel molecular evolution in an herbivore community. *Science.* 337: 1634–1637. https://doi.org/10.1126/science.1226630

Zhou, J., and L. Fritz. 1994. Okadaic acid antibody localizes to chloroplasts in the DSP-toxin-producing dinoflagellates *Prorocentrum lima* and *Prorocentrum maculosum Phycologia.* 33:455–461. https://doi.org/10.2216/i0031-8884-33-6-455.1