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Letter to the Editor

# Replies to Fry et al. (Toxicon 2012, 60/4, 434–448). Part B. Properties and biological roles of squamate oral products: The "venomous lifestyle" and preadaptation

To The Editor:

The toxinology of snake oral secretions has been largely concerned with the chemical and pharmacological characterization of the secretions and relevance to possible medical significance. Less attention has been paid to the relevance of these chemical characters and the contribution or not they may make to the success of the snake in the wild. The absence of such information has made it difficult for functional and evolutionary biologists to transfer this chemical pharmacology per se to understanding the adaptive value, if any, of the biochemical properties alone. The importance of doing so was emphasized long ago, with the important distinction made between a trait or property of an organism and the biological role of that trait (Bock, 1980). As this relates to snake oral secretions, we should distinguish between the properties and biological roles of components in these oral secretions.

For example, the table nearby shows examples of measured "properties" of snake oral secretions usually identified by chemical analysis in the laboratory. The "biological roles" are different and represent contributions to the snake's survival and successful reproduction. They are identified in the wild or under simulated natural conditions in the laboratory.

| Property | ≠ | Biological role |
|---|---|---|
| Color (yellow, white, etc) | | Digestion |
| Specific gravity | | Tranquilization |
| Toxicity | | Lubrication |
| Viscosity | | Antibacterial |
| Volume | | Venomous |
| Etc. | | Etc. |

Discovering the biochemical or pharmacological properties of an oral secretion does not automatically tell us what biological role, if any, they may perform on behalf of the snake's survival. For example, components of human saliva are toxic (property) (Bonilla et al., 1971) but this alone does not allow us to conclude that humans are venomous animals (biological role). To discover the biological role a separate study must be done to directly relate the specific component to how it may enhance the snake's performance in a harsh, threatening, and challenging environment. The failure to distinguish between a property or properties of an oral secretion and the biological role of this oral secretion has created much misinterpretation (Fry, 2005; Fry et al., 2006, 2012) of evolutionary events within snakes.

## 1. Rapid prey death

Toxinology over the years has addressed interests of the discipline serving its own purposes, which centered on the toxicity and medical significance of secretions. This is understandable, but it means that many of the diverse biological roles for snake oral secretions have not been as thoroughly investigated especially the subtle but important differences in oral secretion effects upon events of prey capture and processing. We certainly wish that a well-established terminology had grown up to capture these subtleties, but it has not. That is why we have a clumsy terminology when applied to "venomous" mammals or insects. A noble attempt has been made to re-define venom (Fry et al., 2009), but it is so broad as to be unhelpful in distinguishing the variety of ways chemical compounds, especially proteins, are deployed by animals. We, and certainly others, have considered producing a robust terminology but we decided that such is not advisable until many of the variety of biological roles have been better documented. To do so before then is to clutter the literature with weak meaning terms likely destined to a quick obsolescence. In the meantime, specifically in snakes, "rapid prey death" has been used academically as the interim standard against which to compare the biological effects of snake oral secretions on prey capture (Kardong, 1996). Certainly there are disadvantages (Fry et al., 2012), but

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besides the absence of a serviceable existing terminology, there are reasons at this time to use such a working standard. First, it is easily understood as a biological role. Second, it can be quantified. Third, functional biologists interested in snake evolution easily or at least intuitively understand it. Fourth, it represents the most derived strategy of prey handling in the most derived groups of snakes (cobras, vipers), and is therefore a high metric against which other prey processing secretions can be compared and evaluated. Fifth, the demands of a venom system that produces rapid prey death are correlated to the necessary and specific features of cranial structure (hollow fangs, pressurized venom gland, strike behavior, etc.); therefore it helps us understand the form/function design of venom systems and of non-venom systems in snakes. Sixth, by using "rapid prey death" as the working standard, it helps identify snakes using their oral systems in ways different from vipers and cobras, and this then encourages a broader based examination of snake oral systems rather than settling for the easy route of just calling it all venomous. For example, prey tranquilizing (e.g. Rodríguez-Robles and Thomas, 1992) is not the same thing as producing rapid prey death. These are different predatory strategies with different structural and behavioral features contributing. Comparing prey death rates makes this clear.

## 2. Toxicity ≠ venomous

The property of toxicity alone (see above) cannot tell us whether or not the secretion contributes the biological role of rapid prey death. Concluding such, as has been done in recent literature, only confuses and confounds the study of snake venom systems. It also has had the unfortunate effect of misleading the lay public who might be less familiar with the reasoning behind the sometimes uncritical use of the terms, "toxic" and "venom".

If an oral chemical is to be used to verify a snake's venom system, then deployment of that chemical must reach the following benchmarks:

1) The chemical is injected at levels where its properties make a biological difference in the snake's survival. For example, some toxins in the venom of rattlesnakes are apparently not injected at levels where they directly contribute to prey death; instead they participate in a different biological role of disabling the prey's locomotor system (Weinstein et al., 2010). If the injected chemical does not reach levels where it has a significant effect on prey survival, then whatever its pharmacological properties it is not a venom.

2) The biological role of the suspect chemical must be demonstrated, not just its potent properties. For the example given above, human saliva although it contains toxic chemical compounds these do not provide any assistance in procuring live prey. The toxic properties of human saliva are an epiphenomenon, an incidental byproduct of the chemical without any role to play in survival.

The authors provide several tables listing properties of various proteins that they relate to like molecular species detected in reptile venoms. Certainly these proteins may in derived snakes be recruited into later venom systems. This is an intriguing piece of work that was initiated earlier by these authors. But it again unfortunately only repeats the earlier problem of confusing properties with unsubstantiated biological roles. If some or any of these listed proteins are intended to be part of a "venom system" then the benchmarks listed above must be met by the authors, which again they do not do, leading us to wonder what new insight these lists are to provide.

We share the lament of the authors (Fry et al., 2012, p. 11) that demonstrating the use of toxins in prey subjugation could be an arduous task. But all is not hopeless. We use the same approach as has been done in medical sciences and biological sciences for decades. We pick our "white mouse" of the snake clade of interest. This selected snake species becomes the proxy for the clade, and with cautious interpretation the basic characteristics of the clade can be proposed. That has certainly been done, even if inadvertently, in elapids and vipers by which we assign, cautiously, general characteristics to the full clades. We have used this ourselves in examining *Boiga irregularis* as a surrogate for colubrids with Duvernoy's gland and grooved fangs (Rochelle and Kardong, 1993). Doing so has the further advantage that if colleagues dissent from our experimental results (e.g. Fry et al., 2012) then there is a remedy. They can seek to reproduce our experiments and compare their findings with ours. Reproducibility and experimental verification are integral foundations of the scientific method.

## 3. Multiple functions, multiple roles

Snakes exhibit multiple prey handling techniques, with multiple prey types, with multiple designs of oral morphology and behavior to do so, of which a true venom system (e.g. cobras, vipers) is but one strategy. Other prey handling strategies exist with other roles for oral secretions other that producing rapid prey death (Kardong, 1996, 2002; Weinstein and Kardong, 1994). Some snakes have primarily serous labial glands, others a Duvernoy's gland, others a venom gland. And even within venom glands, there are different types presumably representing different adaptations to specific challenges of prey procurement and packing of the venom gland – limited to the temporal region or in strips along the neck/body (Weinstein et al., 2010). Collapsing all these varied functions to just "venom glands" (Fry et al., 2003) obscures the diverse functional and evolutionary events. It subsumes under one or two categories, biological roles which are in fact quite varied, and therefore obscures the varied selective regimes (Leroi et al., 1994) that may help understand evolution of snake oral glands and the various survival strategies based on these oral glands.

## 4. Co-option

Considering particular chemicals in basal snakes as "toxins" because they are part of a venom system in derived

snakes commits a fundamental mistake in interpretation of evolutionary events. For example, Stephen J. Gould has reminded us (Gould, 2002; Gould and Vrba, 1982) that a feature present in ancestors is often co-opted in descendants into a new context with a new biological role. Think for example of the backbone of fishes (swimming) to the backbone of birds (flight). Certainly that is what is happening within snake oral secretions; chemicals in one role in ancestors are co-opted into new roles (e.g. venom) in descendants. This is why it is important to establish the biological role of a chemical in basal groups and not just in derived venomous snakes. We cannot assign a biological role to a chemical on the basis of guilt by association. Just because a chemical in descendants participates in a true venom system does not mean it participates in a true venom system in ancestors.

It is testimony to the authors' honesty to admit that some "venom systems" as in Iguania have "little or no known functional or ecological importance." But here is the problem: if these venom glands are without function, what are they doing in iguanians? If they have no biological role to perform, why are function-less venom glands not eliminated by a thrifty natural selection? The obvious answer would be because they are doing something else of survival value besides being a venom gland! Eventually some components of these Iguanian oral glands may be phylogenetically co-opted and become incorporated into the venom system of a much later descendant. Would it not be useful to discover the role of the oral glands in iguanians so as to identify the preconditions and biological roles that precede venom systems?

Proteins that end up in the venom systems of advance snakes are likely recruited from diverse ancestral activities such as antimicrobial, blood pressure control, vasoconstriction of cardiac and smooth muscle, and so on. Once a venom gland begins to evolve, gene encoding for proteins in descendants might be duplicated with a copy expressed in this arising venom gland. These proteins would be incorporated into a new context and co-opted into a new biological role.

## 5. Not all snakes are venomous

Venomousness is a lifestyle not just a bag of toxic chemicals. Thus to declare a snake venomous one must demonstrate that in fact the snake lives that lifestyle.

Failure to establish the biological role of the snake oral system, in basal snakes in particular, makes declaring most of the squamate clade a venom clade an unfortunate mistake or at least premature. In the absence of such evidence of biological roles, declaring that the clade is venomous at present is only an entertaining "just so story" (Kipling, 2008) of how the snake got its venom.

## Conflict of interest

None.

## References

Bock, W.J., 1980. The definition and recognition of biological adaptation. American Zoologist 20, 217–227.
Bonilla, C.A., Fiero, M.K., Seifert, W., 1971. Comparative biochemistry and pharmacology of salivary glands. 1. Electrophoretic analysis of the proteins in the secretions from human parotid and reptilian (Duvernoy's) glands. Chromatography 56, 368–372.
Fry, B.G., 2005. From genome to "venome": molecular origin and evolution of the snake venom proteome inferred from phylogenetic analysis of toxin sequences and related body proteins. Genome Research 15, 403–420.
Fry, B.G., Wüster, W., Ryan Ramjan, S.F., Jackson, T., Martelli, P., Kini, R.M., 2003. Analysis of Colubroidea snake venoms by liquid chromatography with mass spectrometry: evolutionary and toxinological implications. Rapid Communications in Mass Spectrometry 17, 2047–2062.
Fry, B.G., Vidal, N., Norman, J.A., Vonk, F.J., Scheib, H., Ramjan, S.F.R., Kuruppu, S., Fung, K., Hedges, S.B., Richardson, M.K., Hodgson, W.C., Ignjatovic, V., Summerhayes, R., Kochva, E., 2006. Early evolution of the venom system in lizards and snakes. Nature 439, 584–588.
Fry, B.G., Roelants, K., Champagne, D.E., Scheib, H., Tyndall, J.D., King, G.F., Nevalainen, T.J., Norman, J.A., Lewis, R.J., Norton, R.S., Renjifo, C., De La Vega, R.C., 2009. The toxicogenomic multiverse: convergent recruitment of proteins into animal venoms. Annual Review Genomics Human Genetics 10, 483–511.
Fry, B.G., Casewell, N.R., Wüster, W., Vidal, N., Young, B., Jackson, N. W.J., 2012. The structural and functional diversification of the Toxicofera reptile venom system. Toxicon. http://dx.doi.org/10.1016/2012.02.013.
Gould, S.J., 2002. The Structure of Evolutionary Theory. Belknap Press, Cambridge. 1464.
Gould, S.I., Vrba, S., 1982. Exaptation-A missing term in the science of form. Paleobiology 8, 4–15.
Kardong, K.V., 1996. Snake toxins and venoms: an evolutionary perspective. Herpetologica 52, 36–46.
Kardong, K.V., 2002. Co1ubrid snakes and Duvernoy's "venom" glands. Journal of Toxicology: Toxin Reviews 21, 1–15.
Kipling, Rudyard, 2008. Just So Stories. NuVision Publ., LLC.
Leroi, A.M., Rose, M.R., Lauder, G.V., 1994. What does the comparative method reveal about adaptation? American Naturalist 143, 381–402.
Rochelle, M., Kardong, K.V., 1993. Constriction vs. envenomation in prey capture by the brown tree snake *Boiga irregularis* (Squamata: Colubridae). Herpetologica 49, 297–300.
Rodríguez-Robles, J.A., Thomas, R., 1992. Venom function in the Puerto Rican racer, *Alsophis portoricensis* (Serpentes: Colubridae). Copeia 1992 (1), 62–68.
Weinstein, S.A., Kardong, K.V., 1994. Properties of Duvernoy's secretions from opisthoglyphous and aglyphous colubrid snakes: a critical review. Toxicon 32, 1161–1185.
Weinstein, S.A., Smith, T.L., Kardong, K.V., 2010. Reptile venom glands: form, function, and future, pp. 65–91. In: Mackessy, S.P. (Ed.), CRC Handbook of Reptile Venoms and Toxins. CRC, Taylor Francis, Boca Raton, p. 521.

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