Chemoecology (2012) 22:159–168
DOI 10.1007/s00049-011-0088-0

CHEMOECOLOGY

REVIEW PAPER

A review of chemical ecology in poison frogs

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Received: 28 July 2011 / Accepted: 19 August 2011 / Published online: 9 September 2011

Abstract Herein we review what is known about the chemical ecology of poison frogs with a focus on dendrobatid poison frogs. While five anuran families are known to have an alkaloid-derived chemical defense, the dendrobatids have been studied in greatest detail and provides chemical ecologists with a complex model system for understanding how chemical defenses operate in real time and may have evolved through evolutionary time. We describe the diversity of alkaloid defenses known from frogs, alkaloid sequestration, biosynthesis and modification, and we review what is known concerning arthropod sources for alkaloids. There is variation in nearly every attribute of the system and we try to describe some of the challenges associated with unraveling the complexities of this model system.

Keywords Ants · Bufonids · Chemical defense · Dendrobatids · Mantellids · *Melanophryniscus* · Oribatid mites · *Pseudophryne* · Sequestration

Sequestered chemical defenses in vertebrates, dedicated to J.W. DALY.

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Introduction

Chemical defenses are widespread among animals and represent complex adaptations for avoiding predators and/or parasites. However, our knowledge of the ecological and evolutionary nature of these adaptations remains incomplete (Berenbaum 1995; Boppre 1990; Dobler 2001). Animals generally synthesize chemical defenses, although some species acquire defenses from external sources such as symbionts or dietary sources (Termonia et al. 2001). Sequestration from diet involves the uptake, accumulation, and storage of defensive chemicals (or their chemical precursors) that were originally present in other organisms (Mebs 2001). In general, the process of sequestration is highly selective, and both active and passive modes of sequestration have been documented (Mebs 2001; Opitz and Müller 2009; Williams et al. 2011 in review). Animals that sequester defensive chemicals are typically dependent on specific dietary sources, which can result in complex ecological interactions and evolutionary relationships among organisms (e.g., Berenbaum and Zangerl 1998; Boppre 1990). The chemical properties and biological occurrence of defensive compounds that mediate trophic interactions among organisms are fundamental to the ecological and evolutionary bases of these systems.

The ability to sequester chemical defenses from dietary sources has evolved convergently in numerous animal lineages (Termonia et al. 2001). Phytophagous arthropods represent the largest and most well-studied group of animals that sequester defensive chemicals (e.g., Nishida 2002; Opitz and Müller 2009), and defense sequestration is also known in flatworms (Kubanek et al. 1995), nudibranchs (Fahey and Garson 2002), amphibians (Saporito et al. 2009a), snakes (Hutchinson et al. 2011), and likely birds (Dumbacher et al. 2004, 2009). Poison frogs

represent the most diverse and well-studied vertebrates that sequester chemicals from diet, and thus provide an interesting system in which to study this trophic-based defensive strategy.

Numerous comprehensive reviews exist on the chemical structures, pharmacology, and biological and geographical distribution of the defensive chemicals present in poison frogs, largely a result of more than 45 years of research on the chemistry and biology of poison frogs by Daly et al. (e.g., 1987, 1999, 2005). Herein we provide a brief review of the major areas of research surrounding the chemical ecology of poison frogs and point out promising new research directions.

Poison frogs

The term 'poison frog' is used to refer to certain members of the four anuran families characterized by an ability to sequester an alkaloid-based chemical defense from dietary arthropods. A fifth family of anurans was recently discovered to also contain alkaloids that are likely sequestered from diet (Rodríguez et al. 2011). Certain alkaloids in poison frogs appear to serve as a chemical defense against potential invertebrate and vertebrate predators (e.g., Brodie and Tumbarello 1978; Fritz et al. 1981; Szelistowski 1985; see Saporito et al. 2007a for additional references), microorganisms (Macfoy et al. 2005), and possibly ectoparasites (Weldon et al. 2006). It should be noted that the term 'poison frog' is not synonymous with the term 'dart-poison frog,' which is properly used only to describe poison frogs of three species in the genus *Phyllobates* (Dendrobatidae) that have been used in dart-poisoning (see Myers et al. 1978). Currently, poison frogs include members of certain genera in the families Dendrobatidae, Bufonidae, Mantellidae, Myobatrachidae, and most recently, Eleutherodactylidae, and occur in both the New and Old Worlds. Alkaloid-containing poison frogs are diurnally active and brightly colored, the latter property being considered to function as an aposematic signal to visual predators (Darst et al. 2006; Osborne 1989; Santos and Grant 2011; Saporito et al. 2007a; Vences et al. 2003). Some bufonids are brightly colored on their ventral surface, which may be exposed to predators when the animals display the "unken-reflex" behavior (Baldo and Basso 2004). Recent evidence suggests that at least some members of the bufonid genus *Melanophryniscus* are diurnal (see Santos and Grant 2011). Dendrobatid frogs are found in Central and South America and represent the most species-rich group of poison frogs, including more than 90 species in eight genera (*Adelphobates*, *Ameerega*, *Dendrobates*, *Epipedobates*, *Minyobates*, *Oophaga*, *Ranitomeya*, and *Phyllobates*; for recent taxonomy, see Grant et al. 2006; Frost 2011). Bufonid poison frogs are found in southern South America and include at least 25 species in the genus *Melanophryniscus* (Frost 2011). The 17 species of mantellid poison frogs are found solely in Madagascar and all are in the genus *Mantella* (Glaw and Vences 2007). Myobatrachid poison frogs are found in Australia and currently consist of 13 species in the genus *Pseudophryne* (Frost 2011). The recently characterized eleutherodactylid poison frogs are found in Cuba and consist of two tiny species in the genus *Eleutherodactylus* (*E. iberia* and *E. orientalis*), which appear to be largely diurnal (Rodríguez et al. 2011). Although a tremendous amount of work has been done in search of biologically active compounds in frogs (including alkaloids; see Daly et al. 1987; Daly et al. 2004), only a fraction of anuran diversity has been examined, and there remain numerous species that have not yet been studied and also species that deserve to be reexamined with more sensitive techniques now available.

Alkaloid diversity in poison frogs

Over the past 40 years, more than 850 lipophilic alkaloids, organized into more than 20 structural classes, have been detected in the skin of poison frogs, a number that apparently reflects the large diversity of alkaloids present in arthropods (Table 1). The majority of these alkaloids have molecular weights of less than 400 atomic mass units. As a result of the large number of alkaloids present in poison frogs, alkaloids have been assigned code names that consist of a bold-faced number for the nominal mass and a bold-faced letter to distinguish alkaloids of the same nominal mass (see Daly et al. 2005). Table 1 summarizes the number of alkaloids present (arranged by structural class), for each of the known four lineages of poison frogs. The majority of alkaloids in poison frogs are 5,8-disubstuted indolizidines, pumiliotoxins, 5,6,8-trisubstituted indolizdines, tricyclics, and 2,5-disubsituted decahydroquionolines, which together represent approximately 64% of all classified alkaloids. The chemical structures for some common poison frog alkaloids are depicted in Fig. 1. Approximately 30% of all alkaloids detected in poison frogs are currently unclassified and will require additional structural characterization. The numerical distribution of alkaloids within each class is similar among most groups of poison frogs. However, the majority of alkaloids present within each class are unique to each anuran group (see Fig. 2). The newly discovered eleutherodactylid poison frogs are not included in our analysis. The pseudophrynamine alkaloids present only in myobatrachids, appear to be synthesized by the frogs (see "Alkaloid sequestration, biosynthesis, and modification in poison frogs"), and therefore relatively few alkaloids are shared between myobatrachids and other poison frogs.

Table 1 The total number of alkaloids identified for each group of poison frogs and arthropod sources arranged by structural class

Alkaloid class Alkaloid sub-class	Carbon	Poison frogs					Arthropod sources			
	Skeleton	Dendrobatids	Mantellids	Bufonids	Myobatrachids	All poison frogs	Mites	Ants	Beetles	Millipedes
5,8-Disubstituted indolizidines	B	51 (20)	52 (25)	7 (1)	0 (0)	77	13	1	0	0
Dehydro-5,8-disubstituted indolizidines	B	12 (7)	24 (20)	1 (0)	0 (0)	32	3	0	0	0
Pumiliotoxins	B	31 (7)	26 (3)	15 (4)	3 (0)	39	5	2	0	0
Allopumiliotoxins	B	18 (12)	11 (5)	2 (0)	2 (0)	23	*	0	0	0
Deoxypumiliotoxins	B	5 (2)	12 (9)	1 (1)	0 (0)	15	1	0	0	0
Dehydrodesmethylpumiliotoxins	B	0 (0)	4 (4)	0 (0)	0 (0)	4	0	0	0	0
Desmethylpumiliotoxins	B	1 (1)	2 (2)	0 (0)	0 (0)	3	0	0	0	0
5,6,8-Trisubstituted indolizidines	B	47 (24)	50 (27)	7 (1)	0 (0)	76	10	0	0	0
Tricyclics	B	31 (21)	39 (29)	10 (6)	0 (0)	66	1	0	2	0
Decahydroquinolines	U	30 (19)	5 (1)	9 (1)	1 (0)	32	0	3	0	0
Decahydroquinoline-dimers	U	5 (4)	2 (1)	0 (0)	0 (0)	6	0	0	0	0
N-Methyldecahydroquinolines	U	5 (5)	0 (0)	0 (0)	0 (0)	5	0	0	0	0
Piperidines	U	26 (23)	6 (3)	0 (0)	0 (0)	29	0	3	0	0
Homopumiliotoxins	B	4 (1)	15 (11)	4 (2)	0 (0)	18	1	0	0	0
Desmethylhomopumiliotoxins	B	1 (0)	4 (3)	0 (0)	0 (0)	4	0	0	0	0
Deoxyhomopumiliotoxins	B	3 (2)	1 (0)	1 (0)	0 (0)	3	0	0	0	0
3,5-Disubstituted pyrrolizidines	U	13 (4)	18 (10)	4 (0)	0 (0)	23	0	4	0	0
3,5-Disubstituted indolizidines	U	15 (7)	13 (5)	3 (1)	0 (0)	21	1	4	0	0
Dehydro-3,5-disubstituted indolizidines	U	0 (0)	2 (2)	0 (0)	0 (0)	2	0	0	0	0
1,4-Disubstituted quinolizidines	B	11 (3)	18 (10)	2 (1)	0 (0)	22	2	0	0	0
Histrionicotoxins	U	16 (16)	0 (0)	0 (0)	0 (0)	16	0	0	0	0
Pseudophrynamines	–	0 (0)	0 (0)	0 (0)	16 (16)	16	0	0	0	0
Pyrrolidines	U	10 (7)	3 (0)	0 (0)	0 (0)	10	2	5	0	0
Cyclopentaquinazolines	B	9 (9)	0 (0)	0 (0)	0 (0)	9	0	0	0	0
Lehmizidines	U	9 (9)	0 (0)	0 (0)	0 (0)	9	0	0	0	0
Spiropyrrolizidines	B	5 (1)	6 (1)	1 (0)	1 (0)	7	1	0	0	6
4,6-Disubstituted quinolizidines	U	2 (1)	3 (2)	2 (2)	0 (0)	6	1	1	0	0
Batrachotoxins	–	6 (6)	0 (0)	0 (0)	0 (0)	6	0	0	0	0
Epibatidines	–	4 (4)	0 (0)	0 (0)	0 (0)	4	0	0	0	0
Pyridinic alkaloids[a]	–	3 (2)	1 (0)	0 (0)	0 (0)	3	0	0	0	0
Gephyrotoxins	U	2 (2)	0 (0)	0 (0)	0 (0)	2	0	0	0	0
Indolic alkaloids[b]	–	2 (2)	0 (0)	0 (0)	0 (0)	2	0	0	0	0
Epiquinamide	U	1 (1)	0 (0)	0 (0)	0 (0)	1	0	0	0	0
Unclassified izidines[c]	–	38 (27)	23 (16)	12 (5)	0 (0)	60	0	0	0	0
Dehydroizidines	–	0 (0)	3 (3)	0 (0)	0 (0)	3	0	0	0	0
Unclassified alkaloids[d]	–	104 (88)	86 (72)	24 (12)	0 (0)	192	6	0	0	0
Other alkaloids[e]	–	3 (1)	4 (2)	0 (0)	0 (0)	5	0	0	0	0
Total number of alkaloids		523 (338)	433 (266)	105 (37)	23 (16)	851	47	24	2	6

Alkaloid classes with branched carbon skeletons are indicated by the letter (B); Alkaloid classes with unbrached carbon skeletons are indicated by the letter (U). The numbers in parenthesis represent the number of unique alkaloids present. The column "All poison frogs" represents the combined number of alkaloids identified for each alkaloid class

[a] Other than the pyridinic epibatidine alkaloids, and including the alkaloids nicotine, pyridylnicotine, and noranabasamine

[b] Other than the indolic pseudophrynamine alkaloids, and including the alkaloids calycanthine and chimonanthine

[c] Alkaloids with an "izidine" structure, that cannot be placed into one of the several izidine classes

[d] Alkaloids that have not been classified to structural class

[e] Includes: adenine, dihydroPTX, *N*-methylpyrrolidine, octahydroquinoline, and tetrahydroquinoline

* Identified in mixed arthropod samples, including mites (Daly et al. 2002)

Data are updated from Daly et al. 2005 and includes, Clark et al. 2005; Mebs et al. 2005; Clark et al. 2006; Saporito et al. 2007b, c; Daly et al. 2007; Daly et al. 2008a, b; Daly et al. 2009; Andriamaharavo et al. 2010; Saporito et al. unpublished data

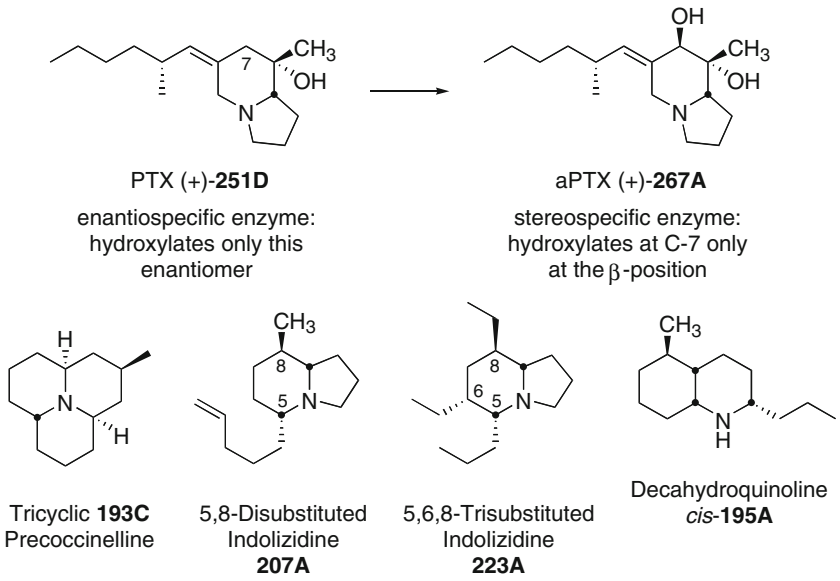

Fig. 1 Representative alkaloids identified from poison frog skin extracts. Figure also illustrates the enantio- and stereo- specific hydroxylation of pumiliotoxin **251D** to allopumiliotoxin PTX **267A**

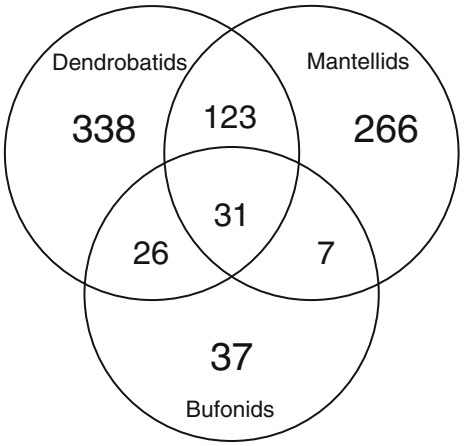

Fig. 2 Venn Diagram of the number of unique and shared alkaloids for dendrobatid, mantellid, and bufonid poison frogs

Most alkaloids in poison frogs have branch-points in their carbon skeleton; the alkaloids containing no branch-points presumably are derived from linear precursors. These differences appear to be related to specific arthropod sources (see "Arthropod sources for alkaloids"). Finally, most research on alkaloids in poison frogs has been conducted on dendrobatids and mantellids, but the diversity of alkaloids known from bufonids (e.g., *Melanophryniscus* sp.) and eleutherodactylids is expected to increase significantly with additional research.

Alkaloid sequestration, biosynthesis, and modification in poison frogs

The lipophilic alkaloids in poison frogs appear to be sequestered from a diet of alkaloid-containing arthropods (see "Arthropod sources for alkaloids"). A history of the observations and research that led to this discovery has been reviewed (see Saporito et al. 2009a) and is summarized briefly here. A series of feeding experiments with dendrobatid frog species in the genera *Adelphobates*, *Dendrobates*, *Epipedobates*, *Oophaga*, and *Phyllobates* established a dietary uptake system for alkaloids of the pyrrolizidine, indolizidine, quinolizidine, decahydroquinoline, histrionicotoxin, pumiliotoxin, and batrachotoxin classes, whereas pyrrolidines and piperidines were accumulated poorly by the frogs (Saporito et al. 2009a). A similar alkaloid uptake system is present in mantellids, and it is likely that bufonid and eleutherodactylid poison frogs also possess an uptake system. The uptake system is absent in dendrobatoid frogs of the genus *Colostethus*, which are not defended by lipophilic skin alkaloids. However, it should be noted that tetrodotoxin and an unknown water-soluble toxin have been detected in two species of *Colostethus* (Daly et al. 1994; Grant 2007), although the definitive source of these presumed defenses is unknown (see Daly 2004). Myobatrachids (*Pseudophryne* sp.) can sequester pumiliotoxins (PTXs) from diet, yet they also produce pseudophrynamine alkaloids (Smith et al. 2002). Consequently, myobatrachids are the only poison frogs known to both sequester and synthesize alkaloids. Furthermore, it appears that sequestration of high levels of PTXs inhibit synthesis of pseudophrynamines, suggesting that species of *Pseudophryne* may be capable of maintaining steady-state levels of chemical defense. Poison frog alkaloids are stored in, and secreted from dermal granular glands (see "Alkaloid autotoxicity in poison frogs"), but small quantities of alkaloids have recently been detected in liver and muscle tissue of certain dendrobatid and bufonid frogs (Saporito, Donnelly, Garraffo, and Spande,

unpublished data; Grant, Colombo, Verrastro, and Saporito, unpublished data). To date, no studies have examined the mechanism(s) by which alkaloids are transported from dietary arthropods to granular glands.

The majority of poison frog alkaloids appear to be sequestered unchanged; however, metabolism of alkaloids obtained from dietary arthropods has been demonstrated in some poison frogs. Some dendrobatids (*Dendrobates* and *Adelphobates*) can hydroxylate over 70% of the dietary pumiliotoxin (+)-**251D** to allopumiliotoxin (+)-**267A** (Daly et al. 2003; Fig. 1). However, these frogs do not hydroxylate the unnatural (−)-enantiomer of pumiliotoxin **251D**. The presence of an enantio- and stereo-selective hydroxylase suggests that *Dendrobates* and *Adelphobates* (and possibly other poison frogs) may convert other PTX alkaloids, such as PTX **307A** to aPTX **323B** or PTX **323A** to aPTX **339A**; however, such conversion have not been demonstrated. Both PTXs and aPTXs have been identified in arthropods (Daly et al. 2002; Saporito et al. 2004, 2007b; Takada et al. 2005), suggesting metabolic hydroxylation may not be the only route by which poison frogs obtain aPTXs. Species in the genus *Pseudophryne* are capable of enzymatic reduction and/or hydroxylation of dietary PTX **307A** into PTXs of molecular weights 309, 323, and 325 (Smith et al. 2002). Although the biological function of modification is not yet understood, allo PTX (+)-**267A**, is approximately five times more toxic to mice by subcutaneous injection than PTX (+)-**251D** (Daly et al. 2003), suggesting the possibility that hydroxylation increases the effectiveness of chemical defense. To date, these are the only documented examples of alkaloid modification in poison frogs. Recently, a new subclass of alkaloids (*N*-methyldecahydroquinolines) was identified in certain *Ameerega* species (Dendrobatidae), and it is possible that these alkaloids are the result of *N*-methylation in the frogs (Daly et al. 2009). Future experiments should be aimed at (1) elucidating the mechanism of alkaloid transport within poison frogs (2) determining if other pumiliotoxins are hydroxylated by the frogs (3) assessing whether there is *N*-methylation of decahydroquinolines in *Ameerega* species, and (4) identifying the potential biological significance of hydroxylations and methylations.

Arthropod sources for alkaloids

A large number of alkaloids have been identified in arthropods (for review, see Braekman et al. 1998), and insofar as is known, poison frog alkaloids are sequestered from a diet of alkaloid-containing mites, ants, beetles, and millipedes (for review, see Saporito et al. 2009a). The only exceptions are the pseudophrynamine alkaloids, which are synthesized by frogs in the genus *Pseudophryne* (see "Alkaloid sequestration, biosynthesis, and modification in poison frogs").

Table 1 lists the known and/or suspected arthropod sources for each major alkaloid class. The majority of alkaloids present in oribatid mites are branched-chained izidines, which represent the largest group of alkaloids present in poison frogs. These izidines include 5,8-disubstituted indolizidines, dehydro-5,8-disubstituted indolizidines, 5,6,8-trisubstituted indolizidines, pumiliotoxins, a deoxypumiliotoxin, a homopumiliotoxin, and 1,4-disubstituted quinolizidines (Saporito et al. 2007b, 2011; Takada et al. 2005). Additional branched-chained izidines have been detected in oribatid mites, but have not been detected in poison frogs to date (Saporito et al. 2007b, 2011). Certain tricyclic alkaloids and one spiropyrrolizidine also have been detected in oribatid mites (Saporito et al. 2007b, 2011; Takada et al. 2005). Furthermore, a number of straight-chain alkaloids originally considered to be of ant origin have since been detected in oribatid mites, including a 3,5-disubstituted indolizidine, a 4,6-disubsituted quinolizidine, and two pyrrolidines (Saporito et al. 2007b). Therefore, it appears that mites are key players in alkaloid sequestration by poison frogs.

The majority of alkaloids present in ants are straight-chain alkaloids, and include 2,5-disubstituted decahydroquinolines, 3,5-disubstituted pyrrolizidines, 3,5-disubstituted indolizidines, 4,6-disubstituted quinolizidines, pyrrolidines, and piperidines, all of which have been identified in myrmicine ants (Saporito et al. 2009a). The straight-chain histrionicotoxins have recently been identified in a myrmicine ant from Central America (personal communication T.H. Jones). It is likely that the straight-chain lehmizidines and gephyrotoxins found in frogs are also derived from myrmicine ants, but have yet to be identified in arthropods. However, a mono-substituted lehmizidine has been detected in an ant of the myrmicine genus *Myrmicaria* (Jones et al. 2007). Two 3,5-disubstituted pyrrolizidines and a pyrrolidine were reported from a formicine ant and a 3,5-disubstituted pyrrolizidine was reported from a ponerine ant (Clark et al. 2006). A few branched-chain alkaloids have been identified in ants, including two pumiliotoxins from Panamanian formicine ants (Saporito et al. 2004) and a 5,8-disubstituted indolizidine from a Malagasy myrmicine ant (Clark et al. 2005).

Precoccinelline and other tricyclics detected in frogs appear to be derived from coccinellid beetles (Daloze et al. 1995), but they have also been reported recently from oribatid mites (Saporito et al. 2007b; Takada et al. 2005). The tricyclics represent a large alkaloid group of varied structural types, which are characterized by having a mass spectrum with many intense peaks and without any alpha-side chains, typical of the coccinelline alkaloids. Spiropyrrolizidine alkaloids in frogs appear to be derived from

siphonotid millipedes (Clark et al. 2005; Saporito et al. 2003), although one such alkaloid was recently reported from an oribatid mite (Saporito et al. 2007b). Batrachotoxin alkaloids from the feathers of birds in the genera *Pitohui* and *Ifrita* from New Guinea have been identified in melyrid beetles (Dumbacher et al. 2004).

It is likely that some poison frog alkaloids result from trophic interactions between plants, arthropods, and the frogs. The indolic plant alkaloids chimonanthine and calycanthine have been detected in the dendrobatid frog *Phyllobates terribilis*, but of chirality opposite to that found in the plant (Tokuyama and Daly 1983), and the plant alkaloid nicotine (or an enantiomer) of unknown chirality has been identified in both dendrobatid (Daly et al. unpublished data) and mantellid frogs (Clark et al. 2005). It is possible in the case of nicotine that an unknown arthropod mediates transfer from plants to frogs, but this is unlikely for chimonanthine or calycanthine, for which the possibility of their synthesis by *P. terribilis* does remain. The alkaloid epibatidine, which has only been detected in certain populations of frogs in the dendrobatid genus *Epipedobates*, shares certain structural features with nicotine and tropane alkaloids and is a remotely possible candidate for sequestration from arthropods that obtained these alkaloids from a plant (see Daly et al. 1999). The alkaloid noranabasamine has been found in three species of *Phyllobates*, and similar compounds, such as anabaseine, are also present in ants and plants (see Daly et al. 1999). Recently, identical alkaloids have been detected in different species of arthropods (see above), suggesting the possibility of complex trophic interactions among arthropods, some of which may also involve microorganisms and/or plants. The extent to which these trophic interactions are involved in the chemical defense of poison frogs is currently unknown, but illustrates some of the complexities involving organisms that sequester chemical defenses.

Most alkaloids present in poison frogs appear to be of oribatid mite and myrmicine ant origin, which is consistent with the natural diet of poison frogs, which are known to be composed largely of mites and ants (e.g., Bonansea and Vaira 2007; Daly et al. 2008a; Valderrama-Vernaza et al. 2009 and references therein). Some dendrobatid frogs have been called "ant-mite specialists" (Caldwell 1996; Donnelly 1991; Simon and Toft 1991). Dietary specialization is common among organisms that sequester chemical defenses from diet, particularly phytophagous arthropods (e.g., Nishida 2002), and has been proposed as an important component in the evolution of sequestered defenses in dendrobatid poison frogs (e.g., Darst et al. 2005; Santos et al. 2003; Santos and Cannatella 2011; Vences et al. 1998).

Identifying dietary sources for alkaloids in poison frogs is a challenge because identical alkaloids are found in different arthropods (Saporito et al. 2009a). Several possibilities present themselves: (1) Are different arthropod groups producing identical alkaloids? (2) Are certain alkaloids being transferred among arthropods? (3) Are certain alkaloids in arthropods the product of dietary sequestration (e.g., from plants, other arthropods)? and/or (4) Are certain alkaloids the products of a microsymbiont? Furthermore, the presence of identical alkaloids in different arthropods suggests that there may be multiple dietary sources for the same alkaloid, adding an extra layer of complexity to the study of sequestered chemical defenses and the evolution of the phenomenon in poison frogs. Oribatid mites contain mainly alkaloids with branched-skeletons, whereas ants contain alkaloids mainly with unbranched-skeletons (Saporito et al. 2009a). The majority of alkaloids in poison frogs have branched-skeletons, suggesting that mites contribute predominately to the alkaloid diversity present in these frogs (see Saporito et al. 2007b). Although the literature suggests that arthropod sources for alkaloids are well known, fewer than 10% of the more than 850 alkaloids known from poison frogs have been identified in arthropods. Furthermore, the majority of frog dietary studies (i.e., analysis of stomach contents) do not identify arthropods below the family level (however, see Clark et al. 2005; Daly et al. 2008a), making determination of dietary sources difficult or impossible. Future studies devoted to the identification of arthropod sources of alkaloids will need to include detailed chemical analyses coupled with studies of frog diet and the availability of arthropods, which will help to solve the interesting questions posed by the poison-frog sequestration system.

Variation in alkaloids among poison frogs

Organisms that sequester chemical defenses generally derive them from dietary sources, and variation in the availability and/or chemical composition of these sources can result in variable chemical defense. Variation in alkaloid composition (a measure of the number, type, and amount) within and among species of poison frogs has been documented for dendrobatids (Daly et al. 1987, 2009; Saporito et al. 2007c), bufonids (Daly et al. 2007, 2008a; Mebs et al. 2005), mantellids (Daly et al. 1996, 2008b; Clark et al. 2006), and myobatrachids (Daly et al. 1990; Smith et al. 2002). The majority of studies described variation at a population level, in which samples of frogs collected from a population were pooled together for chemical analysis (e.g., Daly et al. 1987, 1996, 2008a; Mortari et al. 2004). Recently, studies have focused on alkaloid variation among individuals within populations of dendrobatids (Myers et al. 1995; Saporito et al. 2006), bufonids (Daly et al. 2007; Mebs et al. 2005) and

mantellids (Clark et al. 2006; Daly et al. 2008b). In general, alkaloid composition appears to vary geographically, and close species and/or populations have more similar compositions than do more distant species and/or populations. Populations of the same species differ in alkaloid composition among locations, and some populations of different species have similar alkaloid composition when present in the same location (e.g., Daly et al. 2008b; Myers et al. 1995; Saporito et al. 2006, 2007c). However, there are also examples of differences in alkaloid composition among populations of different species at the same location (e.g., Daly et al. 2008b; Myers et al. 1995). Alkaloid composition changes temporally and seasonal changes in arthropod availability may explain part of this temporal variation (e.g., Daly et al. 2007; Saporito et al. 2006, 2007c). Juvenile poison frogs contain smaller quantities of alkaloids than do adults (e.g., Daly et al. 2002). Recently, differences in alkaloid composition were described between males and females in the dendrobatid *Oophaga pumilio* (Saporito et al. 2009b), and there appear to be similar differences between sexes of certain mantellid frogs (Daly et al. 2008b).

Differences in the availability of alkaloid-containing arthropods appear to be largely responsible for the scale-dependent geographic and temporal variation in alkaloid composition of poison frogs (see Daly et al. 2002, 2008b; Saporito et al. 2007c). Environmental factors that affect the availability and distribution of these arthropods are likely to play a significant role in chemical defenses of poison frogs. Recently, differences in habitat disturbance have been proposed as one mechanism to explain variation in chemical defenses among populations of mantellid frogs (see Clark et al. 2006); however, our current understanding of the relationship between habitat disturbance and defense variation is not well understood and will require additional research (Andriamaharavo et al. 2010).

Alkaloid variation has been reported within the same species of arthropod (see Saporito et al. 2009a), which may also contribute to observed variation in alkaloid composition of poison frogs. Additional factors undoubtedly contribute to alkaloid variation in poison frogs, and some of these include: (1) modification (metabolism) of dietary alkaloids (Daly et al. 2003; Smith et al. 2002); (2) a differential ability or efficiency in sequestering certain alkaloids or alkaloid classes (Daly et al. 2003); and (3) differences in dietary preference or feeding behavior. Finally, alkaloid composition of poison frogs represents a balance between lifetime sequestration and release of alkaloids, and therefore variation is likely to be dependent on the age of the individuals sampled.

Variation in chemical defenses may have important implications for predator–prey interactions. Such variation in certain phytophagous arthropods can result in 'palatability spectra,' in which individual arthropods of the same species differ in their palatability to potential predators (e.g., Bowers and Williams 1995). Palatability spectra can lead to behavioral differences of predators towards prey and thus affect prey survival (e.g., Moranz and Brower 1998). Differences in amounts (and probably composition) of alkaloids in *Oophaga pumilio* were reported to correspond to differences in 'toxicity' among populations from Bocas, Panama (Daly and Myers 1967; Maan and Cummings 2011, *in press*), suggesting that variation in alkaloid composition may lead to differences in frog palatability to natural predators. Whether all poison frogs exhibit such palatability differences are currently unknown, but alkaloid variation, common among all poison frogs, may translate into differences in palatability (also see, Saporito et al. 2007c). Recent studies by Darst et al. (2006) and Darst and Cummings (2006) have demonstrated experimentally that naïve domestic chickens are able to detect differences in alkaloid-based chemical defenses between some frog species in the dendrobatid genus *Ameerega*, suggesting that natural predators may be able to detect differences in frog palatability. At present there is too little information regarding the identity of natural predators, the effectiveness of different classes of alkaloids in defending against these predators, and the ability of these predators to detect differences in chemical defense. Additionally, a further understanding of the link between variable alkaloid defenses and aposematic displays of poison frogs is necessary to better understand the ecology and evolution of natural predator–prey interactions (see Daly and Myers 1967; Maan and Cummings 2011, in press; Summers and Clough 2001; Wang 2011).

Alkaloid autotoxicity in poison frogs

Animals that chemically defend themselves from predation must possess adaptations to circumvent autotoxicity (i.e., self-intoxication). Some of these adaptations include, compartmentalization of defensive compounds into specialized structures (e.g., glands, vacuoles), modifying or metabolizing defenses into a less toxic form (for storage and/or transport; e.g., conversion of pyrrolizidine alkaloids to *N*-oxides), and molecular changes to receptor or ion-channel sites (Daly et al. 1980; Lindigkeit et al. 1997; Mebs 2001). The alkaloids from poison frogs act to disrupt normal ion-channel activity or neurotransmitter-receptor binding in nerve and muscle cells (for review, see Daly et al. 1999), suggesting that poison frogs must contain adaptations to deal with autotoxicity. Alkaloids in poison frogs are stored in dermal granular (poison) glands that are located mainly on the dorsum of the frogs (Delfino et al. 1998; Neuwirth et al. 1979; Saporito et al. 2010), and appear to compartmentalize alkaloids once they are

sequestered. Compartmentalization may reduce autotoxicity, but this has not been examined. The nerve and muscle cells of the dendrobatid poison frogs *Phyllobates aurotaenia* and *P. terribilis* are virtually insensitive to the action of the alkaloid batrachotoxin (known only from species in the genus *Phyllobates*; Daly et al. 1980). However, nerve and muscle cells in *Rana pipiens* (a non-poison frog) and in *Oophaga histrionica* (a dendrobatid poison frog) are highly sensitive to batrachotoxin (cited as unpublished data in Daly et al. 1980). In *P. terribilis*, insensitivity to batrachotoxin appears to be the result of a minor modification to the regulatory site controlling sodium-channel activation and permeability, thus preventing binding by batrachotoxin in these frogs (Daly et al. 1980). It is possible that a similar modification of sodium channels and/or other ion channels accounts for the prevention of autotoxicity in other poison frogs. To date, the only published study to have examined autotoxicity in poison frogs is that of Daly et al. (1980).

Conclusion

Our review of the chemical ecology of poison frogs represents more than 45 years of research, much of which was initiated and carried forward by John W. Daly and his numerous colleagues worldwide. Nevertheless, there still remain many challenges and unanswered questions that will require further study. Although more than 850 alkaloids have been discovered among all groups of poison frogs, only about 75 (ca. 9%) have been identified in a potential dietary source (Table 1). At present the source of approximately 15 structural classes remain unknown, including the well-characterized batrachotoxins and epibatidines, but also a large number of unclassified alkaloids. It appears that the diversity of alkaloids present in poison frogs are a direct reflection of the distribution and diversity of these compounds in arthropods, yet the uptake and transport system that sequesters such alkaloids into skin glands of frogs is virtually unknown. Currently, there are over 250 alkaloids of unknown structural class awaiting isolation and chemical characterization, a number that is expected to grow as new poison frogs species are discovered and studied. Understanding how variation in age, diet, environmental heterogeneity, and arthropod production/sequestration of alkaloids affect the chemical ecology of poison frogs will require efforts on the part of several research teams. John Daly's research questions have opened a variety of doors for future inquiry, and it is our hope that this review will stimulate additional research in this complex and dynamic field and will continue to unravel the complexities of poison frog chemical ecology.

Acknowledgments This paper is dedicated to the memory of our friend and colleague John W. Daly, whose pioneering research founded and continues to inspire the study of poison frog chemical ecology. We would also like to acknowledge his life-long colleague, C.W. Myers (Curator Emeritus, Department of Herpetology, American Museum of Natural History), who collaborated extensively with John over the past 40 years on the study of poison frogs. We thank Tappey H. Jones (Virginia Military Institute), who collaborated with John in the identification and synthesis of ant alkaloids. We also thank Richard L. Hoffman (Virginia Museum of Natural History), John T. Longino (Evergreen State College), and Roy A. Norton (State University of New York, Syracuse) for their assistance in identifying many of the alkaloid-containing arthropods. We are grateful to Alan H. Savitzky and Jenise M. Snyder for providing valuable comments that improved the quality of this manuscript. We would like to thank the Environmental Protection Agency, Florida International University, National Geographic Society, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Organization for Tropical Studies, and Smithsonian Tropical Research Institute for their generous funding. A National Science Foundation Postdoctoral Research Fellowship partially supported R.A.S. This work is derived from a presentation in the symposium "Sequestered Defensive Compounds in Tetrapod Vertebrates: A Symposium in Memory of John W. Daly," held at the Sixth World Congress of Herpetology in Manaus, Brazil, on 21 August 2008 and supported by NSF IOS-0813842.

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