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Sex-related Differences in Alkaloid Chemical Defenses of the Dendrobatid Frog *Oophaga pumilio* from Cayo Nancy, Bocas del Toro, Panama[⊥]

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Poison frogs contain an alkaloid-based chemical defense that is sequestered directly from a diet of alkaloid-containing arthropods. Geographic and temporal variation in alkaloid defense is common in poison frogs, and is generally attributed to differences in the availability of alkaloid-containing arthropods. Variable chemical defense in poison frogs may have important consequences for predator-prey interactions, requiring a full understanding of the factors involved in explaining such variation. In the present study, we examine alkaloid variation in the dendrobatid poison frog *Oophaga pumilio* between males and females on Cayo Nancy (Isla Solarte), located in the Bocas del Toro archipelago of Panama. On average, females contained a significantly larger number and quantity of alkaloids when compared to males. Alkaloid composition varied significantly between males and females, illustrating that chemical defense in this population of *O. pumilio* is sex-dependent. The variation in alkaloids between sexes is attributed to differences in feeding and behavior between males and females. The majority of alkaloids present in the skin of *O. pumilio* appear to be of oribatid mite origin, supporting the importance of these dietary arthropods in the chemical defense of poison frogs.

Animals that sequester chemical defenses from diet are dependent on specific food sources for protection against predation,[1–3] and therefore differences in the chemical composition, availability, or consumption of these dietary sources can result in variable defenses.[4–6] Variation in chemical defense is common among animals that sequester defenses, and can have important consequences for predator-prey interactions.[7–9] The ecological and evolutionary implications of sequestering defenses have been well studied among some phytophagous arthropods,[1,10–11] but are not well understood in vertebrates.

Among vertebrates, the ability to sequester defenses from diet has been proposed in birds of the genera *Pitohui* and *Ifrita* from Papua New Guinea[12] and experimentally demonstrated in the snake *Rhabdophis tigrinus* from Japan[13] and four lineages of poison frogs found worldwide (dendrobatids of Central and South America, bufonids of South America, mantellids of Madagascar, and myobatrachids of Australia[14]). Poison frogs, which represent the best studied group, sequester alkaloid-based chemical defenses from dietary arthropods (including mites,

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ants, beetles, and millipedes[15]). More than 800 alkaloids have been characterized in poison frog skins, reflecting the large diversity of alkaloids present in arthropods.[14] Geographic and temporal variation in alkaloid composition (the number, amount, and type of alkaloids) is common within and among species of poison frogs, and appears to be largely related to the availability of alkaloid-containing arthropods[6,15–18] and differences in the amount and type of alkaloids present in arthropods.[6,19] Furthermore, alkaloids are accumulated over a lifetime and juvenile dendrobatid poison frogs are known to contain smaller quantities of alkaloids than adults,[16,20] suggesting that variation in alkaloid composition is also related to frog age. Differences in alkaloid composition have also been proposed for individuals of different sex.[6,21] Recently, differences in the type and amount of certain alkaloids between males and females of the mantellid poison frog *Mantella bernhardi*, and similar differences in *M. aurantiaca* and *M. milotympanum* have been observed;[17] however, no studies have been specifically conducted to examine differences in alkaloid composition between sexes.

Alkaloid composition has been well documented in the dendrobatid poison frog *Oophaga pumilio*, and more than 30 years of research with this species has led to the detection and identification of more than 230 alkaloids from 21 structural classes,[6] thus providing a model species for which to examine differences in alkaloid defense between males and females. Here, we quantitatively assess differences in the number, amount, and type of alkaloids between males and females of *O. pumilio* from Cayo Nancy (Isla Solarte), Bocas del Toro, Panama.

Results and Discussion

GC-MS analyses of 26 individual *Oophaga pumilio* skin extracts resulted in the detection of 43 alkaloids (including isomers), representing 15 structural classes (Table 1). Most of these alkaloids (67%) have branch points in their carbon skeleton, and are therefore likely derived from oribatid mites.[15,22] However, some of these branched-chain alkaloids have been identified in other arthropods as well (e.g., spiropyrrolizidines **236** and **252A**, also identified in millipedes[23] and pumiliotoxins **307A** and **323A**, also identified in formicine ants),[19] suggesting that in some cases there may be multiple dietary sources for the same alkaloid.[15] Although the chemical structures for izidine **211C** and unclassified **323I** have not yet been determined, they also appear to be derived from oribatid mites.[22] A smaller number of alkaloids (26%) do not have branch points in their carbon skeleton, and likely originate from myrmicine ants.[15,24–25] The occurrence of all alkaloids and isomers that were detected in frog skins of the present study and their likely dietary sources are presented in Table 1.

The most common alkaloids (number of times identified and abundance) detected in the Cayo Nancy frogs were the pumiliotoxins (PTX) **307A**, **307F”**, **307F”’**, and **323A**, allopumiliotoxin (aPTX) **323B**, 5,8-disubstituted indolizidines (5,8-I) **235B”** and **251B**, spiropyrrolizidines (Spiro) **236** and **252A**, and tricyclic (Tri) **205B**, all of which have branched carbon skeletons, and the 2,5-disubstituted decahydroquinolines (DHQ) **195A** and **211A**, 3,5-disubstituted pyrrolizidine *cis*-**223H**, and 4,6-disubstituted quinolizidine (4,6-Q) **237I**, all of which have unbranched carbon skeletons. The structures of these common alkaloids are presented in Figure 1. The presence of some PTX, 5,8-I, and DHQ alkaloids in *Oophaga pumilio* from Cayo Nancy appears to have remained relatively constant over the past 30 years. Saporito et al. (2007)[6] reported large quantities of PTX **307A**, aPTX **323B**, and DHQ **195A** in frogs from Cayo Nancy sampled in 1981 and 1983, and Mebs et al. (2008)[26] reported the presence of 5,8-I **235B”** and DHQ **195A** in frogs sampled in 2006. However, there have been clear differences also in the presence of some alkaloids over time, particularly the presence of Spiro, Tri, 3,5-P, and 4,6-Q alkaloids in the present study, that were not detected in prior studies.[6,26] A change in the presence/absence of alkaloids over time further supports the hypothesis that the availability of alkaloid-containing arthropods changes with time.[6,16,18,27]

Oophaga pumilio contained an average of 14 alkaloids and an average quantity of 259 μg of alkaloid per skin (Table 2). The number (t_{24} = 2.89; P = 0.009; Figure 2) and quantity (t_{24} = 3.60; P = 0.001; Figure 3) of alkaloids differed significantly between males and females, with males containing both a lower number and a lower quantity of alkaloids. On average, males contained 11 alkaloids and 115 μg of alkaloid per skin, whereas females contained 17 alkaloids and 402 μg of alkaloid per skin (Table 2). There was no effect of frog body size (SVL) on the number ($F_{1,23}$ = 1.62; P = 0.216) or quantity ($F_{1,23}$ = 0.854; P = 0.365) of alkaloids detected in *O. pumilio*, suggesting that variation in frog size did not contribute to the differences in alkaloids observed in the present study. It is interesting to note that *O. pumilio* males and females were, on average, approximately the same size (19.7 and 19.6 mm SVL, respectively). Of the 43 alkaloids detected in frog skins from the present study, 33 (77%) alkaloids were detected in males and 42 (98%) alkaloids were detected in females (see Table 1). Males did not contain alkaloids in the histrionicotoxin (HTX), dehydro-5,8-disubstituted indolizidine (D-5,8-I), or izidine (unknown izidines) structural classes, which are represented in the present study by HTX **291A**, D-5,8-I **233E**, and Izidine **211C**, respectively. Females contained at least one alkaloid in all 15 classes seen here, and the only alkaloid present in males and absent in females was Unclass **339F**, which has not been assigned to one of the known structural classes (see Table 1). Overall, 32 (74%) alkaloids were shared between males and females. As a result of the differences in number, quantity, and structural types of alkaloids between sexes, alkaloid composition as depicted in Figure 4 also varied significantly between male and female *O. pumilio* in the present study (Global R = 0.23; P = 0.002). However, the difference in composition was smaller than that reported among populations of *O. pumilio* from an adjacent island, Isla Bastimentos,[18] indicating that differences in alkaloid composition are generally greater between populations than within populations.

The presence of alkaloids in *Oophaga pumilio* (and other poison frogs) is dependent on the sequestration of these compounds from dietary arthropods, including mites, ants, millipedes, and beetles.[15] Therefore, differences in the composition of alkaloids between males and females may be related to differences in diet between sexes. In a study of *O. pumilio* diet from northeastern Costa Rica, Donnelly (1991)[28] reported that female frogs consumed significantly more arthropods (including mites and ants) when compared to males. If similar differences in diet exist in *O. pumilio* from Cayo Nancy, then this could explain the larger amount (number and quantity) of alkaloids observed in females of the present study. Furthermore, Donnelly (1991)[28] suggested that differences in diet between sexes might be related to behavioral differences. Male *O. pumilio* are highly territorial[29–31] and have smaller home ranges than females,[32–33] which may limit the diversity and number of alkaloid-containing arthropods available to them, and result in a lower amount of alkaloids in males. Likewise, the larger home range of females may allow for them to encounter a more diverse array of alkaloid-containing arthropods, resulting in a larger amount of alkaloids sequestered by females. Additionally, it is possible that differences in the amount of alkaloids between sexes could be the result of differences in natural predation rates on males and females. Male *O. pumilio* spend a large portion of the day emitting advertisement calls from elevated perches within their territories to maintain boundaries with other males and to attract females,[29,31,34] a behavior that may increase the exposure of males to natural predators. If this exposure were to result in more predation attempts on males as compared to females, then it is possible that the lower amount of alkaloids in males is due to a potential loss of alkaloids, due to secretion, during predator attacks.

The quantity of alkaloids was positively correlated to the number of alkaloids in males and females combined ($F_{1,24}$ = 36.41; $P \leq 0.001$; R^2 = 0.60; Figure 5), and in general, frogs with a larger number of alkaloids tended to also have larger quantities of alkaloids. This relationship remained the same when males ($F_{1,11}$ = 12.19; P = 0.005; R^2 = 0.53; Figure 5) and females ($F_{1,11}$ = 9.41; P = 0.011; R^2 = 0.46; Figure 5) were analyzed separately, demonstrating that

this relationship is common to both sexes. Saporito et al. (2007)[6] also reported a positive relationship between the number and amount of alkaloids for 53 populations of *O. pumilio* studied throughout Costa Rica and Panama, indicating that this phenomenon is common to this species, and perhaps to all poison frogs. This observation is not a direct consequence of easier detection when a larger amount of alkaloids is present in an extract since some of the additional alkaloids detected are not present in trace amounts.

The large difference in chemical defense observed between males and females of *Oophaga pumilio* may have important implications with regard to predator-prey interactions. On average, female *O. pumilio* contained a larger number (i.e., diversity) and approximately 3.5 times more alkaloid than males, suggesting that females may be better defended against predation. Although it is not currently known whether or not larger amounts of alkaloids in *O. pumilio* will translate into increased avoidance by natural predators, Daly and Myers (1967)[35] reported that differences in the amount of alkaloids among adults correspond to differences in 'toxicity' when extracts were injected sub-cutaneously into laboratory mice. However, not all poison frog alkaloids are considered 'toxic',[20,36–37] and it may be generally more appropriate to consider alkaloids as 'unpalatable' because of their unpleasant and/or bitter taste.[6,38–42] The bitter nature of alkaloids appears to act as a warning of potential toxicity to predators before ingestion,[11,43–44] and it is therefore reasonable to expect that increased amounts of alkaloids in female *O. pumilio* will result in increased predator avoidance. Whether or not natural predators can detect differences in the amount of alkaloids between males and females of *O. pumilio* will require future study. Additional research is clearly needed to understand the complex relationship among arthropod diet, natural predation, and alkaloid-based chemical defense in *O. pumilio*.

Experimental Section

General Experimental Procedures

GC-MS analysis was performed on a Thermo-Electron Polaris-Q instrument coupled to a Focus GC with a 30 m × 0.25 mm i.d. Restek-5MS (Bellefonte, PA, USA) fused silica column. GC separation of alkaloids was achieved using a temperature program from 100 to 280 °C at a rate of 10 °C per minute with He as the carrier gas (flow rate: 1 mL/min.). Each alkaloid fraction was analyzed with both electron impact-mass spectrometry (EI-MS) and chemical ionization-mass spectrometry (CI-MS) with NH_3 as the reagent gas.

Individual alkaloid fractions were prepared from methanol extracts of skin for each specimen of *Oophaga pumilio*. Individual frogs were skinned and the skins were stored in methanol (see below for details). This methanol extract was treated as follows: For each individual, 10 μg of nicotine ((-)-nicotine ≥99%, Sigma-Aldrich, Milwaukee, Wisconsin) in a methanol solution (internal standard) and 50 μL of 1N HCl were added to 1 mL of the original MeOH extract. This combined MeOH extract was concentrated with N_2 to 100 μL, and then diluted with 200 μL of water. This solution was then extracted 4 times, each time with 300 μL of hexane. The HCl fraction was then basified with saturated $NaHCO_3$, followed by extraction 3 times, each time with 300 μL of ethyl acetate. The combined ethyl acetate fractions were dried with anhydrous Na_2SO_4 and evaporated to 100 μL. The wet skins were estimated to weigh 100 mg, and therefore in the final volume of the ethyl acetate alkaloid extract, 1 μL is equivalent to about 1 mg of wet skin.

Identification of individual alkaloids was based on comparison of mass spectrometry properties and GC retention times with those of previously reported anuran alkaloids.[14] Anuran alkaloids have been assigned code names, consisting of a bold-faced number corresponding to the nominal mass and a bold-faced letter to distinguish of alkaloids with the same nominal mass.[14] All alkaloids within each fraction were assessed quantitatively by comparison of the alkaloid

peak area to the peak area of the nicotine internal standard, using the ICIS peak detection function in Xcalibur © version 1.4 SR1 (Thermo Electron Corporation, 1998–2003).

Frog Collections

A total of 26 adult *Oophaga pumilio* (13 males & 13 females; average size: 19.7 mm snout-to-vent length (SVL); size range: 19.0–21.0 mm SVL) were collected from Cayo Nancy (Isla Solarte), Bocas del Toro Province, Panama on August 17, 2005 (GPS coordinates: 09° 19’59.06” N, 82°13’09.18” W). All frogs were collected within a single 45 × 45 m plot. Individual frogs were measured for SVL, euthanized by freezing, skinned, and frog skins were stored in glass vials with Teflon lined caps, containing 4 mL of 100% methanol. Voucher specimens are deposited in the herpetological collection at Florida International University.

Data Analysis

Alkaloid composition is a combined measure of the number, type, and quantity of alkaloids present within an individual frog skin. Non-metric multidimensional scaling (nMDS) was used to graphically visualize patterns of alkaloid composition between males and females. In nMDS plots, the distance between any two points is directly equivalent to the dissimilarity in alkaloid composition between these same two points. A one-way analysis of similarity (ANOSIM) was used to detect differences in alkaloid composition between males and females. See Saporito et al. (2006, 2007)[6,18] and Daly et al. (2008)[17] for additional examples and discussion on the use of nMDS and ANOSIM in the study of poison frog alkaloids. nMDS and ANOSIM results are based on Bray-Curtis dissimilarity matrices. All multivariate statistical analyses were performed using PRIMER-E version 5.

In order to meet the assumptions of normality (i.e., normally distributed data) and homoscedasticity (i.e., equality of variances between variables), all of our raw data was $\log_{10}$ transformed. Differences between males and females in the number and quantity of alkaloids were determined using *t*-tests. To control for differences in the number/quantity of alkaloids in males and females as a function of size (SVL), a one-way analysis of covariance (ANCOVA) with SVL as a covariate was used. The number of alkaloids present in an individual frog skin is a measure of alkaloid diversity, whereas the quantity of alkaloids is a measure of the total amount of alkaloid and may be independent of diversity. Linear regression was used to determine if the quantity of alkaloids is related to the number of alkaloids for males and females. All statistical analyses were performed using SPSS version 17.0 for Mac (SPSS, Inc., Chicago, IL).

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References and Notes

1. Bowers, MD. Insect Chemical Ecology: An Evolutionary Approach. Roitberg, BD.; Isman, MB., editors. London: Chapman & Hall; 1992. p. 216-244.
2. Termonia A, Pasteels JM, Windsor DM, Milinkovitch MC. Proc. R. Soc. Lond. B 2002;269:1–6.

3. Mebs D. Toxicon 2001;39:87–96. [PubMed: 10936625]
4. Bowers MD, Williams EH. Ecol. Entom 1995;20:208–212.
5. Fahey SJ, Garson MJ. J. Chem. Ecol 2002;28:1773–1785. [PubMed: 12449505]
6. Saporito RA, Donnelly MA, Jain P, Garraffo HM, Spande TF, Daly JW. Toxicon 2007;50:757–778. [PubMed: 17706737]
7. Brower LP, Ryerson WN, Coppinger LL, Glazier SC. Science 1968;161:1349–1351. [PubMed: 17831347]
8. Moranz R, Brower LP. J. Chem. Ecol 1998;24:905–932.
9. Fordyce JA. Entomol. Exp. Appl 2001;100:339–346.
10. Rowell-Rahier, M.; Pasteels, JM. Herbivores: Their Interactions with Secondary Plant Metabolites: Ecological and Evolutionary Processes. Rosenthal, GA.; Berenbaum, MR., editors. New York: Academic Press; 1992. p. 243-277.
11. Nishida R. Annu. Rev. Entomol 2002;47:57–92. [PubMed: 11729069]
12. Dumbacher JP, Beehler BM, Spande TF, Garraffo HM. Science 1992;258:799–801. [PubMed: 1439786] Dumbacher JP, Spande TF, Daly JW. Proc. Nat. Acad. Sci. U.S.A 2000;97:12970–12975.Dumbacher JP, Wako A, Derrickson SR, Samuelson A, Spande TF, Daly JW. Proc. Nat. Acad. Sci. U.S.A 2004;101:15857–15860.
13. Hutchinson DA, Mori A, Savitzky AH, Burghardt GM, Wu XG, Meinwald J, Schroeder FC. Proc. Nat. Acad. Sci. U.S.A 2007;104:2265–2270.Hutchinson DA, Savitzky AH, Mori A, Meinwald J, Schroeder FC. Chemoecology 2008;18:181–190.
14. Daly JW, Spande TF, Garraffo HM. J. Nat. Prod 2005;68:1556–1575. [PubMed: 16252926]
15. Saporito RA, Spande TF, Garraffo HM, Donnelly MA. Heterocycles 2009;79:277–297.
16. Daly JW, Kaneko T, Wilham J, Garraffo HM, Spande TF, Espinosa A, Donnelly MA. Proc. Nat. Acad. Sci. U.S.A 2002;99:13996–14001.
17. Daly JW, Garraffo HM, Spande TF, Giddings LA, Saporito RA, Vieites DR, Vences M. J. Chem. Ecol 2008;34:252–279. [PubMed: 18196341] Andriamaharavo NR, Garraffo HM, Spande TF, Giddings L, Saporito RA, Vieites DR, Vences M. unpublished data.
18. Saporito RA, Donnelly MA, Garraffo HM, Spande TF, Daly JW. J. Chem. Ecol 2006;32:795–814. [PubMed: 16718571]
19. Saporito RA, Garraffo HM, Donnelly MA, Edwards AL, Longino JT, Daly JW. Proc. Nat. Acad. Sci. U.S.A 2004;101:8045–8050.
20. Myers CW, Daly JW, Malkin B. Am. Mus. Nov 1978;161:307–366.
21. Myers CW, Daly JW, Garraffo HM, Wisnieski A, Cover JF Jr. Am. Mus. Nov 1995;3144:1–21.
22. Saporito RA, Donnelly MA, Norton RA, Garraffo HM, Spande TF, Daly JW. Proc. Nat. Acad. Sci. U.S.A 2007;104:8885–8890.Saporito RA, Grant T, Colombo P, Norton RA, Andriamaharavo NR, Garraffo HM, Spande TF. unpublished data.
23. Saporito RA, Donnelly MA, Hoffman RL, Garraffo HM, Daly JW. J. Chem. Ecol 2003;29:2781–2786. [PubMed: 14969363]
24. Jones TH, Gorman JST, Snelling RR, Delabie JHC, Blum MS, Garraffo HM, Jain P, Daly JW, Spande TF. J. Chem. Ecol 1999;25:1179–1193.
25. Daly JW, Garraffo HM, Jain P, Spande TF, Snelling RR, Jaramillo C, Rand SA. J. Chem. Ecol 2000;26:73–85.
26. Mebs D, Pogoda W, Batista A, Ponce M, Köhler G, Kauert G. Salamandra 2008;44:241–247.
27. Daly JW, Myers CW, Whittaker N. Toxicon 1987;25:1023–1095. [PubMed: 3321567]
28. Donnelly MA. Copeia 1991;3:723–730.
29. Bunnell P. Copeia 1973;2:277–284.
30. McVey ME, Zahary RG, Perry D, MacDougal J. Copeia 1981;1:1–8.
31. Pröhl H. Amphib-Reptil 1997;18:437–442.
32. Donnelly MA. Oecologia 1989;81:212–218.
33. Pröhl H, Berke O. Oecologia 2001;129:534–542.
34. Pröhl H, Hagemann S, Karsch J, Höbel G. Ethology 2007;113:825–837.
35. Daly JW, Myers CW. Science 1967;156:970–973. [PubMed: 6023266]

36. Daly JW, Brown GB, Mensahdwumah M, Myers CW. Toxicon 1978;16:163–188. [PubMed: 635931]
37. Daly JW, Garraffo HM, Spande TF, Clark VC, Ma JY, Ziffer H, Cover JF Jr. Proc. Nat. Acad. Sci. U.S.A 2003;100:11092–11097.
38. Silverstone PA. Nat. Hist. Mus. L. A 1975;21:1–55.
39. Myers CW, Daly JW. Am. Mus. Nov 1976;157:173–262.
40. Fritz G, Rand SA, Depamphilis CW. Biotropica 1981;13:158–159.
41. Szelistowski WA. Biotropica 1985;17:345–346.
42. Hagman M. Herp. Rev 2006;37:73–74.
43. Brower, LP. The Biology of Butterflies. Vane-Wright, RI.; Ackery, PR., editors. New York: Academic Press; 1984. p. 109-134.
44. Hartmann, T. Herbivores: Their Interactions with Secondary Plant Metabolites: The Chemical Participants. Rosenthal, GA.; Berenbaum, MR., editors. San Diego: Academic Press; 1991. p. 79-121.

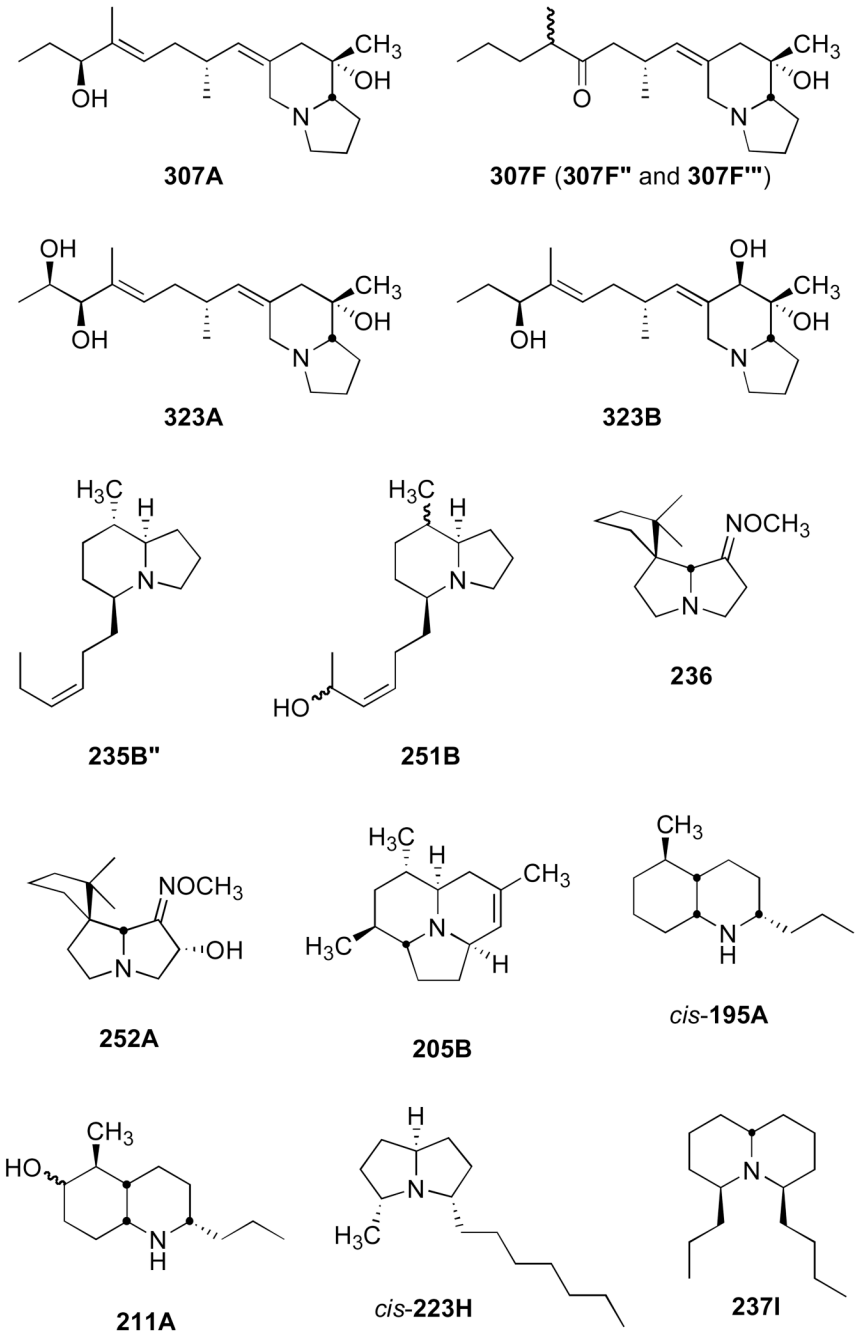

Figure 1.
Structures of the most common alkaloids detected in *Oophaga pumilio* from Cayo Nancy, Bocas del Toro, Panama.

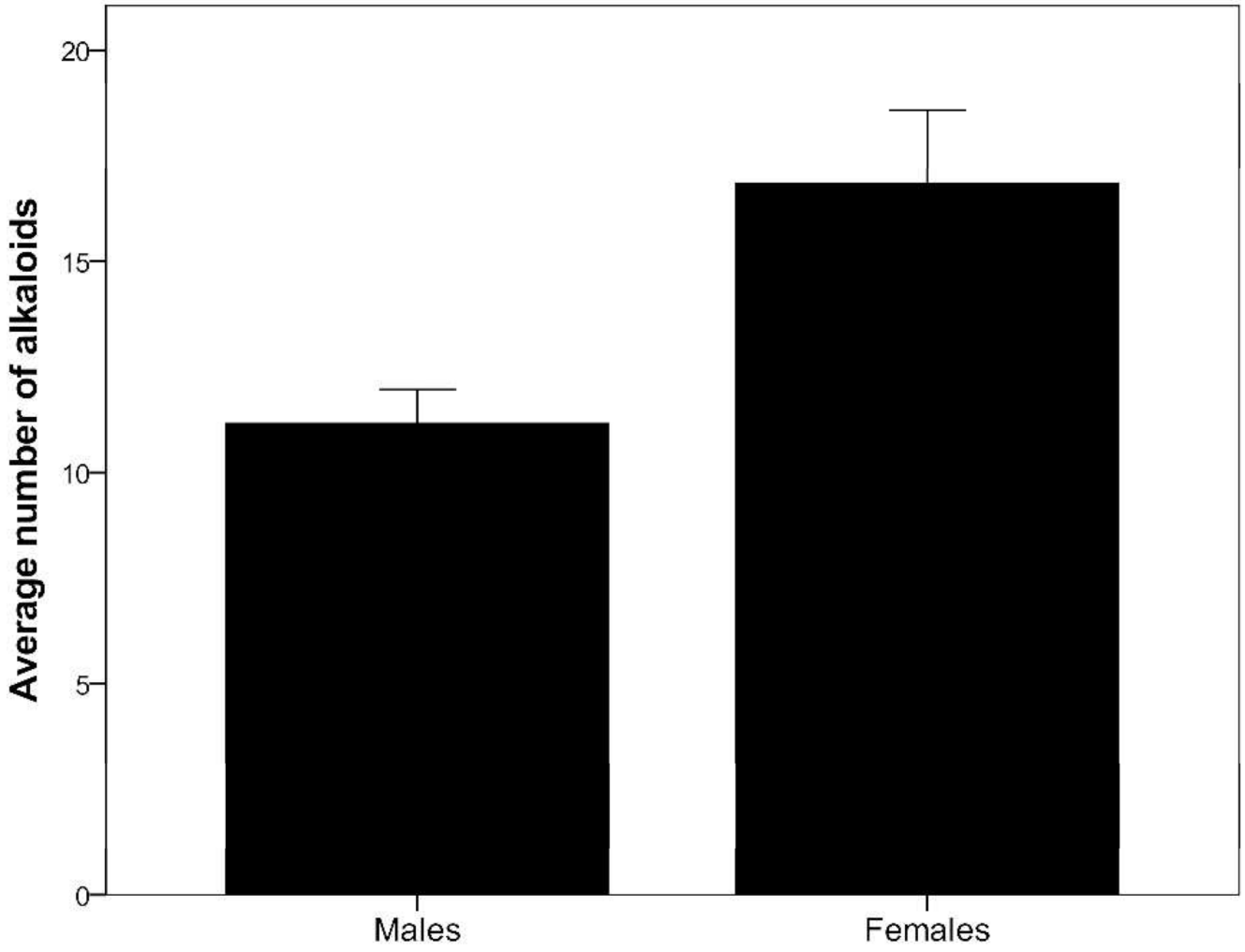

Figure 2.
Number of alkaloids (+/− 1 SE) between males and females of *Oophaga pumilio* from Cayo Nancy, Bocas del Toro, Panama.

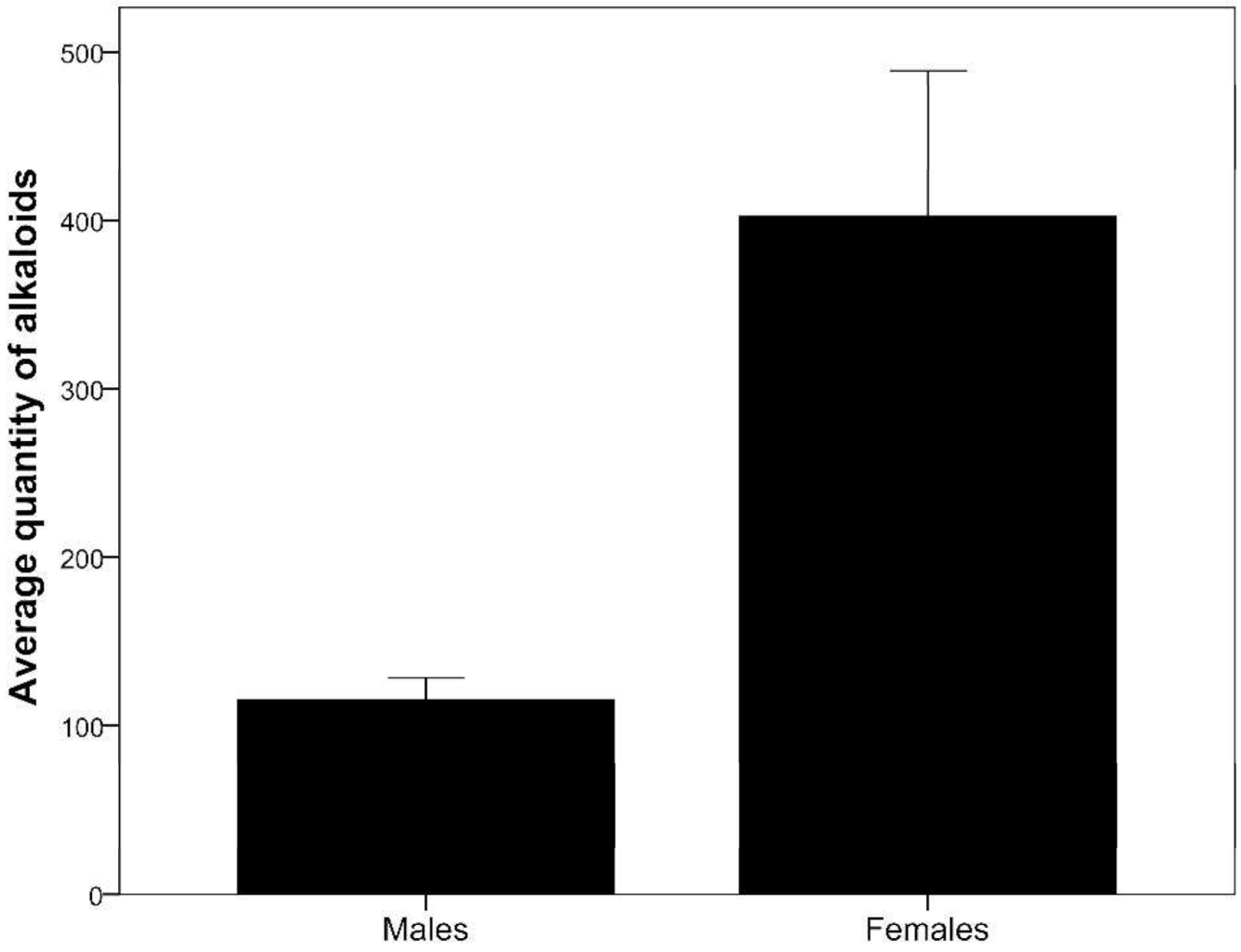

Figure 3.
Average quantity of alkaloids (+/− 1 SE) between males and females of *Oophaga pumilio* from Cayo Nancy, Bocas del Toro, Panama. Quantity of alkaloids is reported as μg per frog skin (range: 43 – 1122 μg per frog skin).

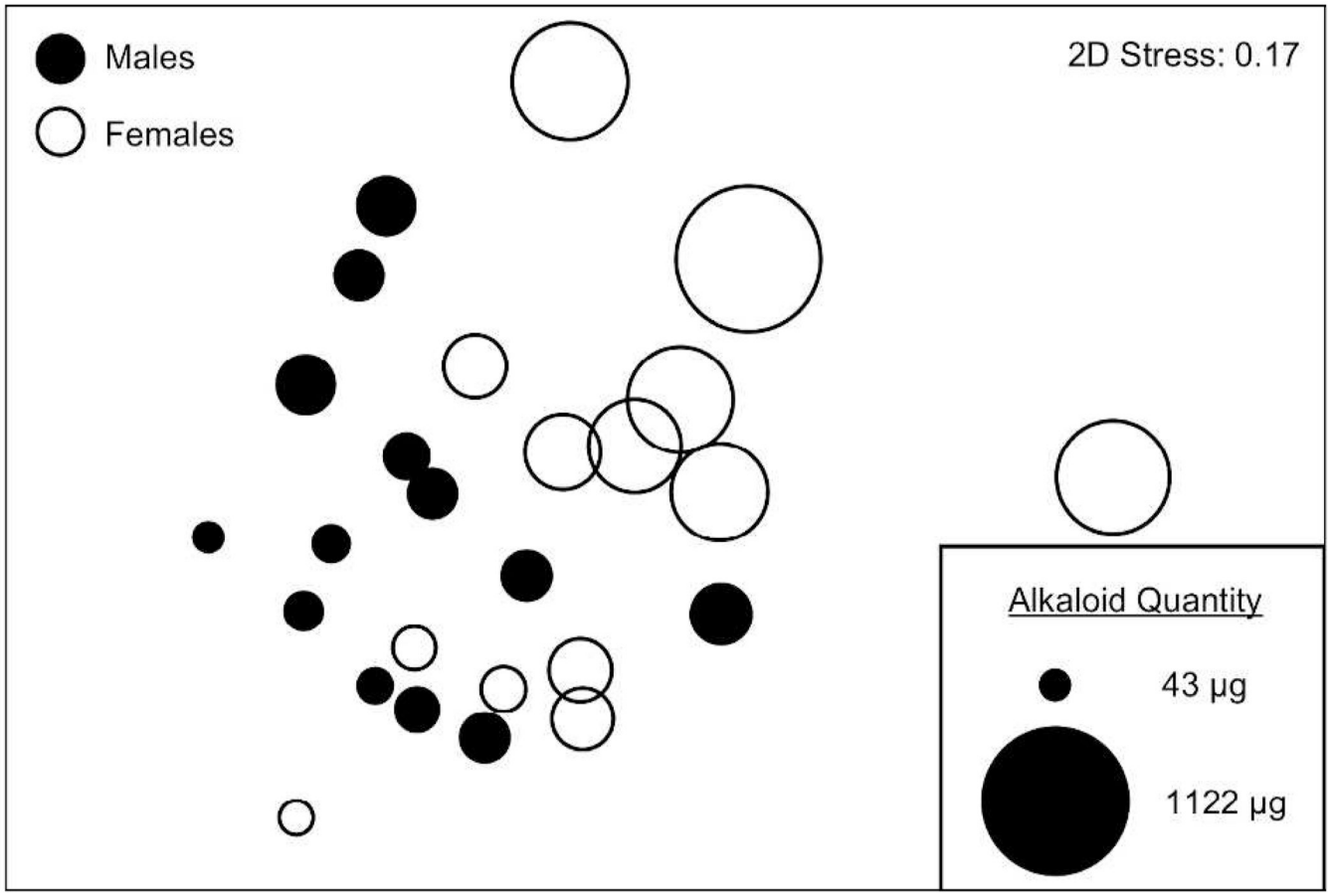

Figure 4.
nMDS plot of alkaloid composition between males and females of *Oophaga pumilio* from Cayo Nancy, Bocas del Toro, Panama. Each circle represents an individual male or female frog, and the distance between symbols represents the difference in alkaloid composition. The diameter of each circle is directly equivalent to the quantity of alkaloids present in that frog (μg per frog skin).

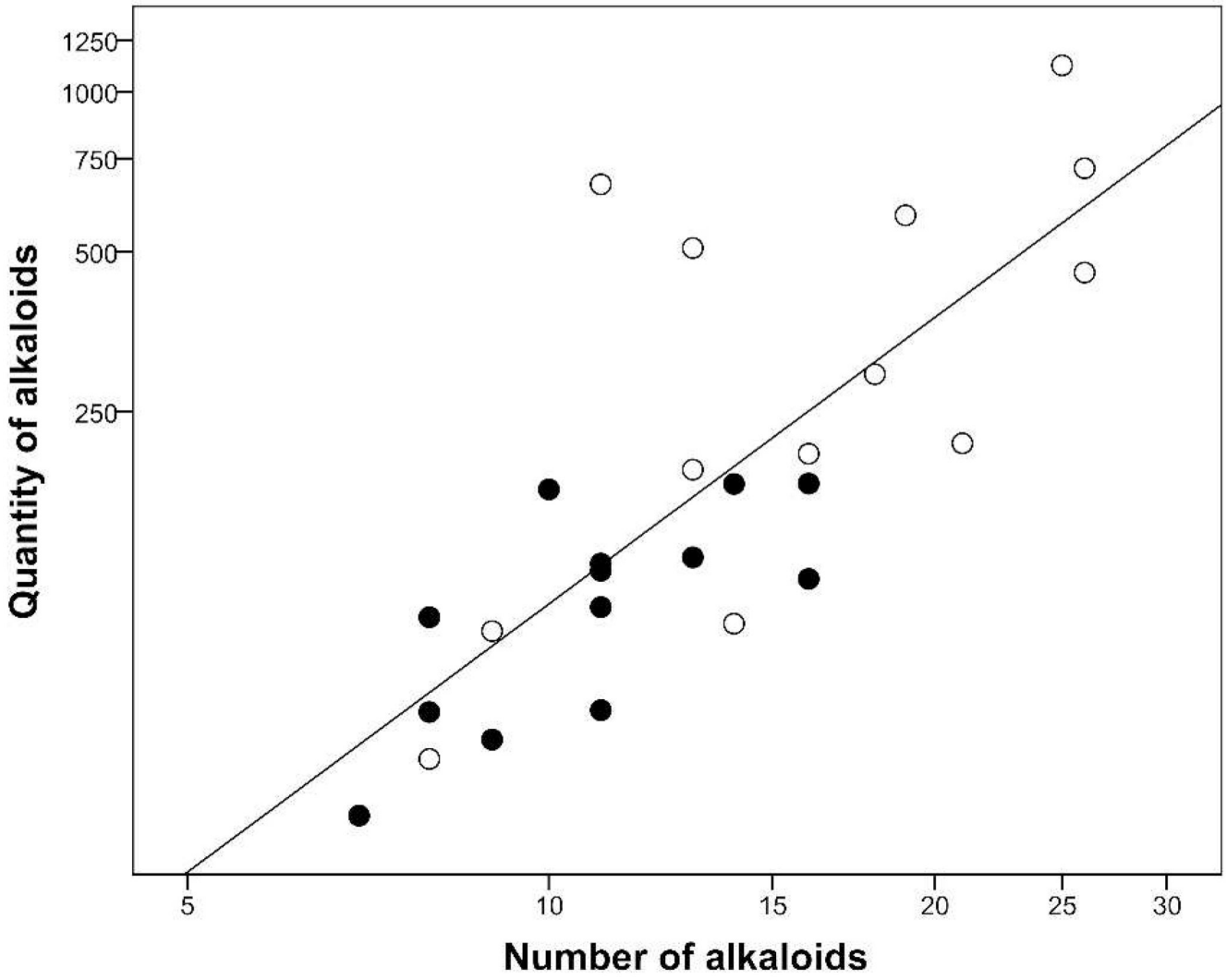

Figure 5.
Relationship between quantity of alkaloids (μg per frog skin) and the number of alkaloids in males and females of *Oophaga pumilio* from Cayo Nancy, Bocas del Toro, Panama. Filled circles indicate males and open circles indicate females. Graph axes are $\log_{10}$-scaled.

Table 1

Alkaloids detected in male and female *Oophaga pumilio* from Cayo Nancy, Bocas del Toro, Panama. Numb. refers to the number of individuals of each sex that contained a specific alkaloid. Quant. refers to the average quantity of alkaloids for individuals of each sex that contained a specific alkaloid. Iso. refers to isomers of an alkaloid. Alkaloid quantities are as follows: +++ > 50 μg per skin; ++ 5–50 μg per skin; + < 5 μg per skin

Structural Class (Likely Arthropod Source)					Structural Class (Likely Arthropod Source)				
Alkaloid	Male		Female		Alkaloid	Male		Female	
	Numb.	Quant.	Numb.	Quant.		Numb.	Quant.	Numb.	Quant.
HTX (Ant)					**5,8-I** (Mite)				
291A	0	–	3	+	**233D**	1	+	10	+
					233D (iso)	0	–	4	+
DHQ (Ant)					**235B"**	12	++	12	++
195A	13	++	13	+++	**251B**	4	++	9	++
211A	6	+	5	++	**251B** (iso)	2	+	8	+
275B	1	+	1	++	**251B** (iso2)	1	+	3	+
					259B	0	–	3	++
3,5-P (Ant)									
cis-**223H**	6	+	3	++	**D-5,8-I** (Mite)				
trans-**223H**	1	+	1	+	**233E**	0	–	3	+
cis-**251K**	1	+	1	++					
trans-**251K**	2	+	1	+	**5,6,8-I** (Mite)				
					223A	0	–	2	++
4,6-Q (Ant)					**231B**	0	–	2	+
237I	8	++	7	+	**253H**	2	+	2	+
					277E	1	+	3	+
Pyr (Ant)									
197B	1	+	1	+	**Spiro** (Millipede/Mite)				
					236	5	++	8	++
Pip (Ant)					**252A**	12	+	11	++
241D	2	+	4	+					
					Tri (Beetle/Mite)				
PTX (Mite/Ant)					**191F**	1	+	2	++
251D	3	+	2	+	**205B**	8	++	12	++

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Structural Class (Likely Arthropod Source)					Structural Class (Likely Arthropod Source)				
Alkaloid	**Male**		**Female**		**Alkaloid**	**Male**		**Female**	
	Numb.	**Quant.**	**Numb.**	**Quant.**		**Numb.**	**Quant.**	**Numb.**	**Quant.**
307A	12	++	13	+++	**223P**	2	+	2	++
307B	1	+	1	+					
307F"	5	+	11	+	**Izidine** (Unknown[a])				
307F"'	5	+	11	++	**211C**[b]	0	–	4	++
309A	5	+	3	++					
321A	0	–	3	+	**Unclass** (Unknown[a])				
323A	4	++	9	+++	**323I**[b]	1	++	2	+
323A (iso)	0	–	2	++	**339F**	3	+	0	–
aPTX (Mite)									
323B	7	++	9	++					
323B (iso)	0	–	4	++					
325A	2	+	1	++					

HTX, histrionicotoxins; DHQ, 2,5-disubstituted decahydroquinolines; 3,5-P, 3,5-disubstituted pyrrolizidines; 4,6-Q, 4,6-disubstituted quinolizidines; Pyr, pyrrolidines; Pip, Piperidines; PTX, pumiliotoxins; aPTX, allopumiliotoxins; 5,8-I, 5,8-disubstituted indolizidines; D-5,8-I, Dehydro-5,8-disubstituted indolizidines; 5,6,8-I, 5,6,8-trisubstituted indolizidines; Spiro, spiropyrrolizidines; Tri, tricyclics; Izidine, izidines; Unclass, unclassified.

[a] A probable dietary origin has not been proposed for Izidine or Unclassified alkaloids.

[b] Identified in an oribatid mite.[22]

Table 2

Sample size, mean number, and mean quantity of alkaloids in *Oophaga pumilio*

	Sample Size	Number of Alkaloids		Quantity of Alkaloids (μg per frog skin)	
	(n)	Mean +/− SE	Range	Mean +/− SE	Range
All Individuals	26	14 +/− 1	7 – 26	259 +/− 51	43 – 1122
Males	13	11 +/− 1	7 – 16	115 +/− 13	43 – 183
Females	13	17 +/− 2	8 – 26	402 +/− 87	55 – 1122

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