Toxicity of Panamanian Poison Frogs (Dendrobates): Some Biological and Chemical Aspects

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John W. Daly

Charles W. Myers

PANAMANIAN FROG

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Abstract. *A small Neotropical frog,* Dendrobates pumilio, *undergoes interpopulational variation in color, degree of toxicity, size, and habits. Differences in body coloration encompass the visible spectrum from red to blue, as well as achromatic black and white. There are wide variations in the degree of toxicity, but these variations are not correlated with supposed warning colors. Extracts of skin yield two toxic compounds characterized as steroidal alkaloids with molecular formulae* $C_{19}H_{33}NO_2$ *and* $C_{19}H_{33}NO_3$. *The rapid rate of divergent evolution among populations of this frog may result from isolation and chance restriction of original heterozygosity, with subsequent selection acting on different and greatly limited mixtures of alleles.*

Many amphibians produce irritating and unpleasant skin secretions that, in some cases at least, provide partial defense against predation; compounds isolated from such amphibians often possess remarkable pharmacological activity (Table 1). Some vividly pigmented frogs in the Neotropical family Dendrobatidae are generally presumed to be poisonous and, hence, to have warning coloration. Certainly, the only dendrobatid heretofore investigated in detail has bright coloration, and also an extremely active poison, batrachotoxin (Table 1). Recent discovery, in northwestern Panama, of an extraordinary populational complex of the genus *Dendrobates* raises some interesting questions related to natural selection and toxicology (*1*).

In the Archipelago of Bocas del Toro and on the adjacent mainland of Almirante Bay and Chiriqui Lagoon, no fewer than 16 populations of small, rotund *Dendrobates* exhibit astonishing geographic variation in color. The basic dorsal color is red, orange, green, olive green, blue, or black; venters are yellow, red, white, or blue; the limbs are frequently black or blackish; the dorsums are unicolored, speckled, boldly spotted with black, or, in one instance, black with whitish longitudinal marks. These frogs differ not only in color and pattern but in size, in habits, and, less noticeably, in their cricket-like voices. Average body length varies from about 17 to 20 mm. Individuals at some localities confine their activity to within several inches of ground level and scurry into leaf litter when pursued; at other places there are distinct arboreal tendencies, with the individuals singing and foraging at heights up to 6 m and more. Even different demes of a single insular population vary in extent of arborealism. That there is an interpopulational shuffling and combining of these various attributes suggests that but a single species is involved. All populations are tentatively allocated to *Dendrobates pumilio* Schmidt, 1858 (*2*); this species exhibits relatively little variation outside of Panama, an area from which it ranges north in the Atlantic lowlands to Nicaragua as a red-bodied frog (*3*). In northwestern Panama, many of the populations occupy similar microhabitats, and it is difficult to think of selective agents that can produce such extreme variations in color.

To learn whether the various populations also differ with respect to toxicity and, if so, to determine if higher degrees of toxicity are associated with the brighter or supposed warning colors, we tested, in the period from January to March 1966, seven populations (representing all basic groundcolors save black) for toxicity. Although intrapopulational variation in color is normally slight, the dorsal color in a population on Bastimentos Island ranges from pale green to red; samples of green and reddish orange frogs from this population were tested separately (Table 2). For each sample 11 or 12 frogs were pithed and immediately skinned; the skins were extracted three times with five volumes of 80 percent buffered methanol (*p*H 7.4). The methanol extracts were concentrated, and portions were assayed for toxicity after subcutaneous injection into 20-g white mice (NIH, general purpose). The time of death as a function of concentration is shown in Fig. 1 for the reddish orange Bastimentos sample. The amount of toxic principle that causes death in 9 minutes was arbitrarily assigned the value of one standard dose. After the mice were injected, they had locomotor difficulty with partial paralysis of the hind limbs. Piloerection, salivation, extensor move-

Table 1. Examples of pharmacologically active compounds from amphibians.

Compound	Genus	Activity	Minimum lethal dose* (mg/kg mouse)
Nitrogenous bases			
Batrachotoxin	*Phyllobates (4, 5, 22)*	Cardio- and neurotoxin	0.002
Samandarine	*Salamandra (6), Pseudophryne (7)*	Centrally active convulsant	0.3
Tetrodotoxin (tarichatoxin)	*Taricha (8)*	Neurotoxin	0.008
Compound A ($C_{19}H_{33}NO_2$)	*Dendrobates*	Nerve-muscle activity	2.5
Compound B ($C_{19}H_{33}NO_3$)	*Dendrobates*	Nerve-muscle activity	1.5
Indole-alkylamines			
Serotonin	*Bufo (9), Leptodactylus (10)*	Vasoconstrictor	300
Dehydrobufotenine	*Bufo (11)*	Convulsant	6
O-Methylbufotenine	*Bufo (12)*	Hallucinogen	75
Phenolic and catechol amines			
Norepinephrine	*Bufo (13)*	Hypertensive agent	5
Candicine	*Leptodactylus (14)*	Cholinergic agent	>10
Leptodactyline	*Leptodactylus (15)*	Cholinergic agent	10
Imidazole-alkylamines			
Histamine	*Leptodactylus (10, 16)*	Local irritant	13,000
Spinaceamine	*Leptodactylus (16)*		
Carnosine	*Eleutherodactylus (17)*		
Bufogenins and bufotoxins			
Bufotalin	*Bufo (18)*	Cardiotoxin	
Bufotoxin	*Bufo (18)*	Cardiotoxin	0.4
Kinins			
Bradykinin	*Rana (19)*	Local irritant	
Physalaemin	*Physalaemus (20)*	Hypotensive agent	
Other kinins	*Ascaphus (19), Phyllomedusa (19)*		
Proteins			
Hemolysins	*Triturus (18)*	Hemolytic agents	0.002

* For comparison, the minimum lethal dose of curare and strychnine is 0.5 mg/kg; that of sodium cyanide is 10 mg/kg. The compounds were administered to mice subcutaneously.

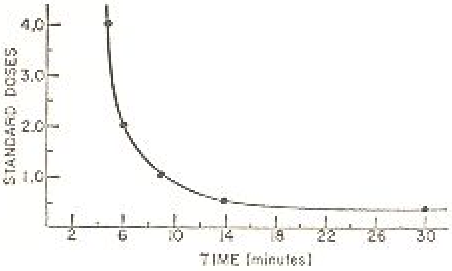

Fig. 1. Assay of toxic principles in skin extract of *Dendrobates pumilio* (reddish orange frogs of Bastimentos Island), showing relationship of dose (subcutaneous in mice) to survival time. Each point is an average of three trials.

ments of the hind limbs, and, finally, clonic convulsions and death occurred. Apparent irritation at the site of injection was observed at the lower dosages, but was not noted at higher doses. Although methanol itself is toxic at sufficiently high dosage, controls that were injected with methanol showed no ill effects.

While there is considerable interpopulational range in toxicity, there is no apparent correlation between toxicity and the color of the frogs (Table 2). Thus, the reddish orange frogs and the green frogs of the Bastimentos population are equally toxic. Dark blue frogs, difficult to see under shaded forest conditions, are considerably more toxic than several samples of conspicuous red frogs. When all populations are considered, there is no obvious correlation of either coloration or toxicity with environmental factors or behavior.

At least two principles are involved in the toxicity, and these were partially purified in the following manner. Concentrated methanol extracts from 20 skins were partitioned between ten volumes of 0.1*N* hydrochloric acid and ten volumes of chloroform. The nonbasic chloroform extract contained pigments and other compounds, including cholesterol, but was not toxic. The aqueous acid was adjusted to *p*H 9 and extracted twice with two volumes of chloroform. Most (70 to 80 percent) of the toxicity was found in this basic chloroform extract. The aqueous layer was not studied further. Figure 2 shows a thin-layer chromatograph of the basic chloroform extracts obtained from populations sampled in this study. The relative toxicities of crude extracts from these same populations (Table 2) are comparable to the amounts of compounds A and B seen in the partially purified extracts (Fig. 2). Compounds A and B, detected through use of iodine vapor (Fig. 2), could also be detected with the alkaloid reagent potassium iodoplatinate.

Further purification of compounds A and B was carried out with column chromatography on neutral alumina. Compound A was eluted with chloroform; compound B was eluted with a mixture of six parts chloroform and one part methanol. Approximately 1.5 mg of each substance was isolated by this method. The estimated minimum lethal dose as judged by subcutaneous injection of the compounds in mice is 2.5 mg/kg for compound A and 1.5 mg/kg for compound B. One standard dose (Fig. 1) corresponds to 100 μg of a mixture of equal parts of compounds A and B. The purified compounds elicited pharmacological effects similar to those caused by crude methanol extracts, except that the salivation and piloerection were now minimal.

The ultraviolet absorption spectra of compounds A and B showed only end absorption, while the characteristics of the infrared spectra precluded the presence of carbonyl groups, double bonds, or the oxazolidine group of the salamandra alkaloids (*6*). The nuclear magnetic resonance (NMR) spectra ($CDCl_3$) showed peaks at 5.5, 4.0, and 2.82 δ (parts per million) and a general profile suggestive of a steroid ring system with two angular methyl groups. The mass spectrum of compound A (Fig. 3) indicated a molecular ion of $C_{19}H_{33}NO_2$ (calculated molecular weight, 307.251; found molecular

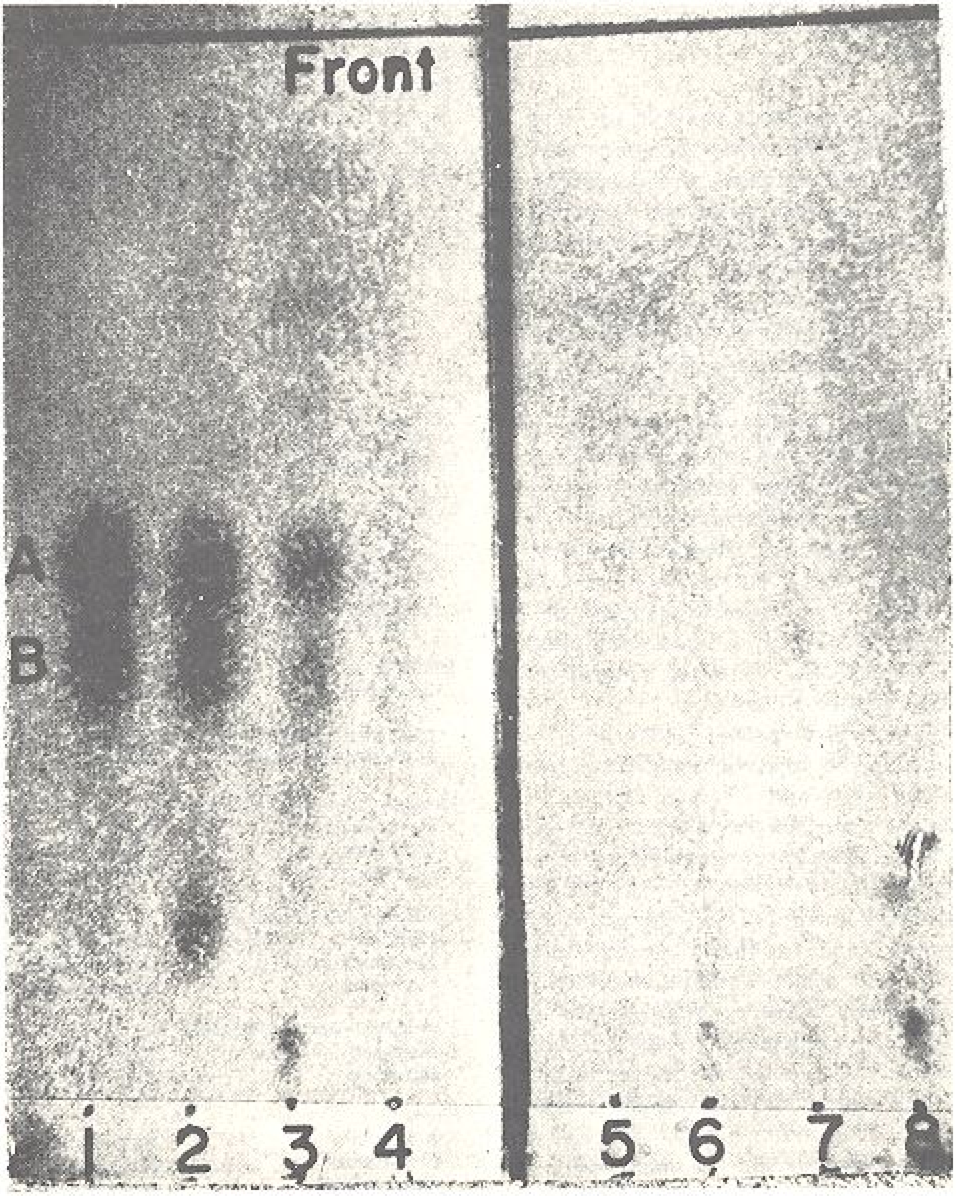

Fig. 2. Thin-layer chromatography (silica gel-G; solvent was a mixture of 50 parts methanol, 5 parts chloroform, and 0.5 part 6*N* ammonium hydroxide; detection with iodine vapor) of compounds A and B in partially purified skin extracts from samples of *Dendrobates pumilio*. The sample designations (1–8) correspond in order with the populations listed in Table 2. Concentrated samples with the equivalent of 5 mg of skin were chromatographed.

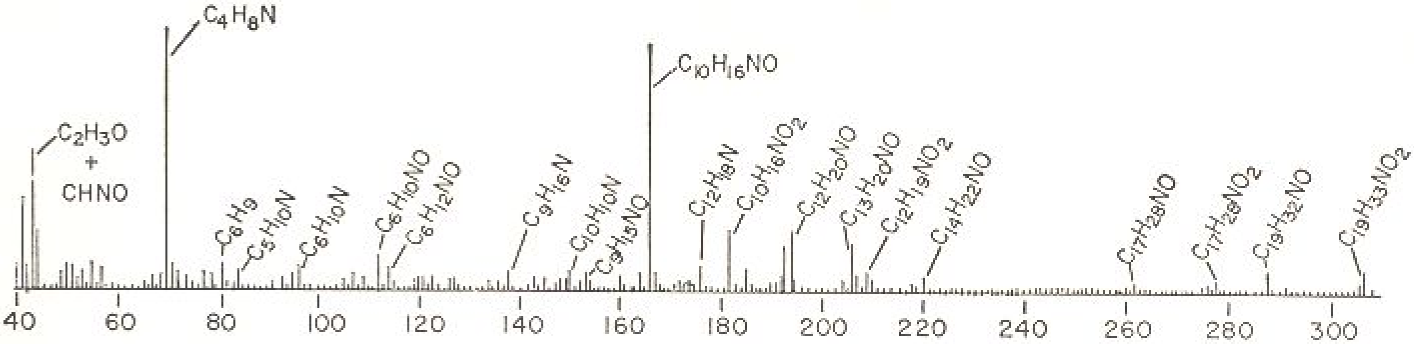

Fig. 3. Mass spectrum of compound A from *Dendrobates pumilio* (AEI-MS-9 mass spectrometer, direct inlet, 70 ev).

weight, 307.251), whereas compound B yielded a similar spectrum with one additional oxygen in the molecular ion $C_{19}H_{33}NO_3$ (calculated molecular weight, 323.246; found molecular weight, 323.244). The nitrogen and one oxygen atom are part of a carbinolamine function as deduced from a CH_2NO fragment and from the facile conversion of both compounds to *O*-methyl ethers with methanolic hydrogen chloride. The NMR spectrum ($CDCl_3$) confirms this carbinolamine grouping with 1 proton at 5.5 δ units. Two protons in the NMR spectra represented by a multiplet at 2.8 δ in conjunction with the large fragment C_2H_3O for both compounds A and B suggests the presence of a $-CH_2-NH-CHOH-CH_2-$ grouping, while a multiplet due to one or two protons (compounds A or B) at 4.0 δ suggests the presence of one or two secondary alcohol groups. Acetylation of compounds A and B afforded neutral *N*-acetyl derivatives with, respectively, 1- and 2-*O*-acetyl groups as shown by their mass spectra.

Compounds A and B seem thus to be related in structure to the toxic alkaloid samandarine ($C_{19}H_{31}NO_2$), first isolated from the European fire salamander and more recently reported in an Australian anuran (Table 1). Alkaloids of the samandarine type are examples of convergent chemical evolution in diverse amphibians, demonstrating that molecular data are not inherently better than any other kind for the classification of animals. Another nitrogenous substance, tetrodotoxin, provides an even better example, as it is more complex and it evolved separately in a family of fishes and in one of salamanders (*8*). The biological function and significance of such toxic compounds are not, however, always easily demonstrated.

The insular populations of *Dendrobates pumilio* probably originated during a postglacial rise in sea level, whereas the equally numerous mainland populations are possibly separated by areas of swamp forest, a habitat not usually occupied. The regional population is thus broken into local units by terrestrial conditions and by straits of open water. This is true of other organisms too (for example, *Phyllobates lugubris*, another dendrobatid), but only in *D. pumilio* have the isolations resulted in extensive change. That random events in the founding and in the density fluctuations of small populations have played a part seems likely; but there are factors which suggest that selection is also in force. On the mainland near Chiriquí Grande, a population of red frogs having either red or blue venters occupies ground slightly elevated from adjacent swamp forest; a population of green frogs having blue or yellow venters occurs in the swamp, where the individuals show a decided tendency towards arborealism. This is the only instance yet discovered where two populations occur virtually together, where a marginal habitat (swamp forest) is utilized by one population, and where the color variation exceeds even that of the frogs of Isla Bastimentos (Table 2, first two samples). The overlap of ventral colors suggests some genetic exchange, but the correlation of two color types with different habitats indicates that natural selection is operating and maintaining the differences. Whether selection works directly on either color or toxicity is not known, but theoretical considerations suggest that it should. Bright hues, at least of diurnal animals, generally only evolve when positive selective values are conferred. That brilliant coloration is rare in the mostly color-blind Mammalia contrasted with the fact that vivid colors are important in many flower-bird, insect-bird, and intraspecific bird coactions convincingly supports this generalization. Most birds are thought to have color vision, and some doubtlessly include small frogs in their diets; in the presence of avian predation it is unlikely that small diurnal frogs would evolve bright coloration unless for the function of advertisement. Thus, a warning role seems the most likely function of bright coloration when it occurs in diurnal frogs, although coloration might also serve for intraspecific recognition in view of evidence (*21*) that some frogs possess color vision. Individuals from each population tested for toxicity were found to secrete an unpleasant tasting, toxic, milky fluid when injured or when pressure was applied on the skin. The substance responsible for the taste was lost in purification, but small quantities of the purified toxic principles cause the human throat to tighten; the substance batrachotoxin from a Colombian dendrobatid (*Phyllobates*) (*4, 5, 22*) is more

Table 2. Relative lethality of skin extracts of frogs from various populations of *Dendrobates pumilio*.

Locality	General description*	Relative lethality†
Isla Bastimentos	Reddish orange; white; spotted	5.2
Isla Bastimentos	Pale green; white; spotted	5.0
Mainland near Isla Split Hill	Dark blue; powder blue; unicolor or speckled	4.2
Cayo Nancy	Bright red; same; unicolor	1.0
Isla San Cristobal	Bright red; same; speckled	<.5
Near Almirante, mainland	Dull red; red; unicolor	<.5
Isla Shepherd	Olive green; yellowish; speckled	<.5
Isla Colón	Green; yellow; spotted	<.2

* In order: Dorsal color; ventral color; presence or absence of dorsal black spotting (large) or speckling (flecks to small spots). † Standard doses contained in methanol extract of 100 mg of wet skin. (Frogs averaged 80 to 100 mg skin per individual frog in the eight samples.)

active and numbs the mouth. The sensitivity of buccal tissue to such secretions should offer these frogs partial protection against certain kinds of predators. Conseqently, within a species that tends toward bright coloration and which is likely the prey of diurnal birds, selection might result in a combination of aposematic coloring and a high level of toxicity. Therefore, the chaotic interpopulational variation of color and toxicity in *D. pumilio* seems at first paradoxical. Some of the hues seem cryptic and others flamboyant, and the various populations appear to be evolving different ways of life. Conceivably, any protective aspects of the toxic compounds might be secondary, with the true physiological function entirely unrelated to toxicity.

With all its intraspecific differences—as in appearance, habits, and chemical composition of skin—*Dendrobates pumilio* is the most variable species of vertebrate known to us. A tentative hypothesis would contain the several interacting factors that must be involved, namely, isolation and small population size, inherent variability, chance, and selection. A map of Atlantic-side Costa Rica and Panama reveals that opportunities for isolation are greater along some of the flooded coastal reaches of the latter country than elsewhere. This partly explains the chaotic variation of *D. pumilio* in northwestern Panama; the variation in Costa Rica (*3*), on the other hand, seems largely clinal in nature. Presumably the predecessor of existing populations had a high mutation rate and harbored tremendous genetic variability, making the frog potentially capable of adjusting to diverse conditions in a forest environment. Either the chance fragmentation of this stock, or the chance founding of new colonies in a geographic mosaic, resulted in small populations each of which possessed a unique and greatly limited mixture of alleles on which selection could operate. There was consequent adaptation of different populations not only to different micro- and macrohabitats, but seemingly even to different ways of avoiding predation.

JOHN W. DALY

National Institute of Arthritis
and Metabolic Diseases,
Bethesda, Maryland

CHARLES W. MYERS

Gorgas Memorial Laboratory,
Apartado 6991, Panamá, R. de P., and
Museum of Natural History,
University of Kansas, Lawrence

References and Notes

1. C. W. Myers (1965), C. W. Myers, J. W. Daly (1966), in papers read at meetings of the Amer. Soc. Ichthyologists and Herpetologists, Lawrence, Kansas, and Miami Beach, Florida, respectively. C.W.M. thanks William E. Duellman, who shared in the discovery of some of the populations in a trip to Archipiélago de Bocas del Toro. Tomás Quintero rendered valuable assistance on each of several trips.
2. O. Schmidt, *Denkschr. Akad. Wiss.* **14**, 250 (14 in reprint), Taf. 2, Fig. 13 (1858). Taxonomic notes: E. R. Dunn, *Occas. Pap. Boston Soc. Nat. Hist.* 5, 393 (1931), put into the synonomy of *pumilio* the name *Dendrobates typographus* Keferstein, 1867, which has been resurrected for Costa Rican populations by E. H. Taylor, *Univ. Kansas Sci. Bull.* **35**, [1] 633 (1952). Taylor had ample reason for believing that the original figure of *D. pumilio* "shows a species that could scarcely be regarded as . . . *typographus*," but discovery of the new Panamanian populations indicates that a single, highly variable species is involved, especially if one allows for a certain, obvious crudity in the execution of Schmidt's illustrations. Also, *Dendrobates galindoi* H. Trapido [*H. Trapido, Fieldiana, Zool., Chicago Nat. Hist. Mus.* **34**, 181 (1933)], type locality Bastimentos Island, is for present purposes placed in the synonomy of *D. pumilio*. These various epithets (*pumilio, typographus, galindoi*) are available for subspecific names, if one wishes to determine for which populations they are applicable and then coin names for every additional population; but a static subspecies concept does not seem to us well suited to the dynamics of the situation.
3. Norman J. Scott, Univ. of Southern California, kindly supplied information on color variation of *D. pumilio* in Costa Rica, on the basis of populational samples from five localities spaced nearly the length of the coastal plain. Individual frogs all have reddish bodies, but there is an interpopulational trend from red to reddish orange and, in a southward direction, a correlated increase in the amount of black spotting on the dorsum. The frogs in northeastern Costa Rica are sparsely dotted with black and have bright blue (rather than black) hind limbs.
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21. W. R. A. Muntz, *J. Neurophysiol.* **25**, 712 (1962) and *Sci. Amer.* **210**, 111 (1964).
22. *Phyllobates aurotaenia* new combination, originally described as a *Dendrobates* by G. A. Boulenger, *Proc. Zool. Soc. London* **1913**, 1029, plate CIV, Fig. 1 (1913), and called *P. bicolor* in recent chemical literature (*4, 5*). J. M. Savage, Univ. of Southern California, examined the type of *aurotaenia* and confirmed our belief that the name belongs with *Phyllobates*.
23. Preparation of this paper was supported in part by NIH grant GM 12020.

COVER

Dendrobates pumilio, originally collected on Bastimentos Island, Panama. The color of the dorsal surface of this population of frogs ranges from pale green to red, with varying degrees of black spotting. The ventral surfaces are usually white, but an occasional frog has blotches of orange. The toe pads allow the frog to climb well, but only certain populations have adopted an arboreal life. The extreme variations in color and markings, habits, and toxicity of skin secretions makes *D. pumilio* one of the most variable species of vertebrates known (average body length, 17 mm.) See page 970. [Frank T. Caporael, NIH]