PRELIMINARY EVALUATION OF SKIN TOXINS AND VOCALIZATIONS IN TAXONOMIC AND EVOLUTIONARY STUDIES OF POISON-DART FROGS (DENDROBATIDAE)

CHARLES W. MYERS AND JOHN W. DALY

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ABSTRACT

Studies in progress reveal at least three novel classes of toxic alkaloids in skin secretions of Neotropical dendrobatid frogs. *Batrachotoxins* are characterized by a steroidal ring structure; those batrachotoxins having a pyrrole carboxylate substituent are among the most toxic of nonprotein poisons. *Pumiliotoxins* are less toxic and poorly known, but pumiliotoxins A and B appear to have a bicyclic ring system with differing side chains; pumiliotoxin C is a simple *cis*-decahydroquinoline. *Histrionicotoxins* have an unusual spiro-ring system and both 4-carbon and 5-carbon side chains, on the cyclohexane and piperidine rings, respectively. Structures are uncertain for other alkaloids of lower molecular weight, but some appear structurally related to pumiliotoxins and others to histrionicotoxins. Methods of study include thin-layer chromatography, gas chromatography, electron impact and chemical ionization mass spectrometry, and quantitative mass spectrometry. Combined gas chromatography-mass spectrometry gives reproducible results for small-sample taxonomic comparisons of frogs containing pumiliotoxins, histrionicotoxins, and similar alkaloids. Limitations of molecular data in taxonomic and evolutionary studies are considered.

Biochemical and other variation are analyzed in *Dendrobates histrionicus*, a rain-forest frog of western Colombia and northwestern Ecuador. Sexual dimorphism is slight, and geographic variation in body size appears correlated with climate. There are geographic differences in relative tibia length and in escape behavior. Interpopulational differences in color and color pattern are extreme, and intrapopulational variation also may be considerable. Most colorations are thought to be aposematic, but highly variable frogs of one population seem cryptically colored. *Dendrobates histrionicus* elaborates histrionicotoxins and lower molecular-weight alkaloids; one population sample had trace amounts of a higher molecular-weight compound, tentatively identified as pumiliotoxin B. Southern populations produce large amounts of histrionicotoxins and lesser amounts of lower weight alkaloids, a situation reversed in most northern populations. Individual populations have 8-10 of 19 alkaloids detected by gas chromatography-mass spectrometry. Alkaloid similarity comparisons show that most populations share more compounds with near than with distant populations; a geographically intermediate population shares as many or more alkaloids with distant as with neighboring populations.

Analysis of geographic variation in skin toxins supports the notion of conspecificity of most *histrionicus*-like frogs, but different spectra of toxins revealed two additional sibling species. These new species resemble *D. histrionicus* in morphology (including absence of omosternum), vocalizations, and type of male aggressiveness; and their color patterns, although distinctive, are approached in the variation of *histrionicus*. *Dendrobates lehmanni*, new species, lacks histrionicotoxins and produces pumiliotoxins and other alkaloids not detected in *histrionicus*; it is a black frog with crossbands of vivid orange or orange-red. *Dendrobates lehmanni* was known for years only from specimens sold in the animal trade, but its habitat is traced to a restricted area of montane forest in the Río Anchicayá drainage of the western Andes, Department of Valle, Colombia. *Dendrobates occultator*, new species, shows greater biochemical resemblance to *histrionicus*, but shared alkaloid similarity values are relatively low, and it produces significant amounts of pumiliotoxins and only two kinds of histrionicotoxins (5-7 kinds in populations of *histrionicus*). *Dendrobates occultator* is a red-backed frog with yellow lateral spots; it occurs in the Pacific lowlands, in the upper Río Saija drainage, Department of Cauca, Colombia, where it is sympatric with a population of *histrionicus*. Both *lehmanni* and *occultator* may have speciated from geographical isolates of *histrionicus*; it is suggested that some character divergence might have occurred after the range of *occultator* was rejoined with the parent species.

Dendrobates viridis, new species, is also described, as it occurs sympatrically with the other two new species and evidently has an extensive range in Pacific-side Colombia, along the western flank of the Andes. It is a miniature, uniformly green frog, whose skin secretions include pumiliotoxins.

Species of *Dendrobates* in Central America and northwestern South America seem characterized by either of two kinds of vocalizations, which correlate with calling behavior and type of aggressiveness. *Buzz calls* are a nearly uniform series of pulses, which are produced too fast for

resolution by the human ear but which can be directly visualized on sound spectrograms made with a wide-band filter. Buzz calls are given by *D. auratus* and *D. minutus* (and *"Phyllobates" espinosai*), species that call relatively infrequently and which seem relatively unaggressive. *Chirp calls* are trains of harsh, poorly modulated notes, in which pulses are produced too fast for resolution on wide-band sound spectrograms. Chirp calls are given by *D. granuliferus*, *D. histrionicus*, *D. lehmanni*, *D. occultator*, *D. pumilio*, and *D. speciosus*—all of which are characterized by nearly incessant calling from perches, and by pronounced male aggressiveness related to territorial defense. Geographic variation in normal and aggressive calls suggests that *D. pumilio* may actually be a composite of two species, but biochemical and other variation have yet to be investigated.

RESUMEN

Estudios en progreso muestran al menos tres clases nuevas de alcaloides tóxicos en secreciones cutáneas de ranas neotropicales dendrobátidas. *Batracotoxinas* están caracterizadas por una estructura cíclica esteroidal; batracotoxinas que poseen un substituyente carbóxilo pirrólico están entre los mas tóxicos de los venenos no-proteínicos. *Pumiliotoxinas* son menos tóxicas y no muy bien conocidas, pero aparentemente pumiliotoxinas A y B poseen un sistema anular bicíclico con diferentes cadenas laterales; pumiliotoxina C es una simple *cis*-decahidroisoquinolina. *Histrionicotoxinas* poseen un sistema espiro anular poco comun y cadenas de 4 y 5 carbonos respectivamente en los anillos de ciclohexano y piperidina. Las estructuras de otros alcaloides de bajo peso molecular son inciertas, sin embargo algunas parecen estar relacionadas a las pumiliotoxinas y otras a las histrionicotoxinas. Los métodos de estudio incluyen cromatografía en capa delgada, cromatografía de gases, espectrometría de masas de impacto electrónico y de ionización química, y espectrometría de masas cuantitativa. La combinación de cromatografía de gases-espectrometría de masas da resultados reproductibles en comparaciones taxonómicas en pequeña escala de ranas que contienen pumiliotoxinas, histrionicotoxinas, y similares alcaloides. Se toma en consideración las limitaciones encontradas en la aplicación de información molecular en estudios taxonómicos y evolucionarios.

Cambios bioquímicos y otras variaciones son analizadas en *Dendrobates histrionicus*, una rana de bosque del occidente de Colombia y noroeste del Ecuador. Dimorfismo sexual es mínimo, y el cambio en el tamaño del cuerpo con cambio geográfico parece estar relacionado con el clima. Hay diferencias geográficas en el tamaño relativo de la tibia y comportamiento defensivo. Diferencias de color y diseño entre grupos son extremas, y variaciones dentro de un mismo grupo pueden ser considerables también. Se piensa que la mayoría de los colores son aposemáticos, sin embargo ranas sumamente variables de un grupo están coloradas para ocultarse. *Dendrobates histrionicus* produce histrionicotoxinas y alcaloides de bajo peso molecular; especímenes de una población mostraron trazas de un compuesto de mayor peso molecular, tentativamente identificado como pumiliotoxina B. Las poblaciones del sur producen cantidades mayores de histrionicotoxinas y cantidades menores de alcaloides de menor peso molecular, una situación que es inversa a la de la mayoría de las poblaciones del norte. Grupos tomados individualmente tienen de 8 a 10 de los 19 alcaloides descubiertos por medio de cromatografía de gases-espectrometría de masas. Comparación de la similaridad de los alcaloides identificados demuestra que la mayoría de los grupos tiene más compuestos en común con grupos cercanos que con grupos distantes; un grupo geográficamente y bioquímicamente intermedio comparte tantos o más alcaloides con grupos distantes como con grupos cercanos.

Análisis del cambio geográfico en las toxinas de la piel sostiene la idea de conespecificidad para la mayoría de las ranas del tipo *histrionicus*, sin embargo diferentes espectros de toxinas mostraron dos especies hermanas. Estas especies nuevas se asemejan a *D. histrionicus* en morfología (incluyendo la ausencia de omoesternón), vocalizaciones, y tipo de agresividad masculina—y la patrón de la coloración, aunque distintivo se aproxima a la encontrada en la variación de *histrionicus*. *Dendrobates lehmanni* (n. sp.) carece de histrionicotoxinas, produce pumiliotoxinas y otros alcaloides no detectados en *histrionicus*; es una rana negra con bandas de color anaranjado brillante o rojo-anaranjado. *Dendrobates lehmanni* fue conocida por años solamente a través de especímenes vendidos en el

mercado, pero su habitat se localiza en una limitada área en la zona del Río Anchicayá en los Andes occidentales, Departamento del Valle, Colombia. *Dendrobates occultator* (n. sp.) tiene una mayor similitud bioquímica a *histrionicus*, sin embargo los valores de similaridad de alcaloides en común son relativamente bajos, y produce cantidades substanciales de pumiliotoxinas y solamente dos tipos de histrionicotoxinas (de 5 a 7 tipos en poblaciones de *histrionicus*). *Dendrobates occultator* es una rana de lomo rojo con manchas laterales amarillas; aparece en las tierras bajas del Pacífico, zona superior del Río Saija, Departamento de Cauca, Colombia, lugar en el cual es simpátrica con una población de *histrionicus*. Tanto *lehmanni* como *occultator* pueden haberse formado como especies a partir de grupos geográficamente aislados de *histrionicus*; se hace la sugerencia de que cierta divergencia en carácter puede haber ocurrido despues de que ambas, *occultator* y la especie progenitora, *histrionicus*, fueron geográficamente reincorporadas.

Dendrobates viridis (n. sp.) también es descrita, aparece simpátricamente con las otras dos especies neuvas y evidentemente abarca un área extensa en la zona colombiana del Pacífico, a lo largo del flanco occidental de los Andes. Es una rana miniatura, uniformemente verde cuyas secreciones cutáneas contienen pumiliotoxinas.

Especies de *Dendrobates* en América Central y zona noroeste de Sudamérica están aparentemente caracterizadas por cualquiera de dos tipos de vocalizaciones, los cuales están relacionados con tipos de llamadas y clases de agresividad. Los *zumbidos* son una serie casi uniforme de pulsos, los cuales son producidos demasiado rápidamente para permitir su resolución por oídos humanos, pero pueden ser directamente visualizados en espectrogramas sónicos hechos con un filtro de banda ancha. Estos zumbidos son emitidos por *D. auratus* y *D. minutus* (y "*Phyllobates*" *espinosai*), especies que emiten llamadas no muy frecuentemente y que parecen ser relativamente no agresivas. Los *chirridos* son series de notas ásperas pobremente moduladas, en las cuales los pulsos son producidos demasiado rápidamente para permitir su resolución en espectrogramas sónicos de banda ancha. Estos chirridos son emitidos por *D. granuliferus*, *D. histrionicus*, *D. lehmanni*, *D. occultator*, *D. pumilio*, y *D. speciosus*—todas las cuales están caracterizadas por su casi incesante llamar desde perchas, y por una pronunciada agresividad masculina la cual se relaciona a la defensa de sus territorios. El cambio geográfico en llamadas normales y de agresiones sugiere que tal vez *D. pumilio* está compuesta de dos especies, sin embargo diferencias bioquímicas y otras variaciones aun tienen que ser investigadas.

INTRODUCTION

Neotropical frogs of the family Dendrobatidae currently are arrayed among three genera—the usually cryptically colored and nontoxic *Colostethus*, and the usually brightly colored and toxic *Phyllobates* and *Dendrobates*. So far as is known, all are diurnal frogs that lay terrestrial eggs, from which situation the tadpoles are carried to water on the back of an attendant parent; at least some species have unfroglike patterns of courtship and other social behavior. The evolution of species within the family appears to be rapid and ongoing. A few species are among the most variable of all vertebrates, with conspecific populations differing remarkably not only in coloration but even in life-style and apparently different strategies for avoiding predation!

Aside from the unusual biological attributes of dendrobatids generally, the poisonous species also are of interest because of their usefulness to man. Thus, a few species in western Colombia have long been used by Chocó Indians of the Emberá and Noanamá groups for poisoning blowgun darts. On the eastern side of the South American continent, there is a widespread belief that a dendrobatid is the causative agent in *tapirage*—the practice of inducing color change in the plumage of live parrots. But, although the role of frogs in *tapirage* is questionable and use of the blowgun is declining, the toxic skin secretions of dendrobatid frogs are fast acquiring an importance in the biomedical sciences. The past several years have witnessed the discovery that at least three distinct classes of toxic alkaloids have evolved in the Dendrobatidae, namely the batrachotoxins, the histrionicotoxins, and the pumiliotoxins. These structurally diverse compounds have striking effects on animal physiology and are thus proving to be useful

tools for the study of nerve, muscle, and cardiac phenomena.

One might expect the taxonomy of dendrobatid frogs to have been well investigated because of their interest to both theoretical and applied science, but few authors have given more than casual attention to this family. The generic limits so usefully defined by Jay M. Savage (1968) for the Central American species prove difficult to maintain when certain groups of South American species are considered, and, incredibly, there remain an appreciable number of undescribed species. In *Dendrobates* alone, for example, we are aware of at least six new species (including three described herein), a substantial increment for a genus of usually conspicuous, diurnal frogs that presently comprise fewer than two dozen recognized species.[1] There are several reasons for this state of dendrobatid taxonomy, including inadequate collecting and the apparent paucity of generically useful characters. Another factor is the sometimes extraordinary intraspecific variation in color and pattern, making it difficult to judge whether a given population represents a distinct species or merely a geographic form of another species.

We believe that the biochemistry of dendrobatid skin secretions can be used in conjunction with other characters in segregating species and in clarifying relationships within the family. To this end we give a brief introduction to our in-progress work on dendrobatid toxins and make a preliminary assessment of their taxonomic potential, with particular attention to the genus *Dendrobates*. Two of the new species described herein are separated from the highly variable *Dendrobates histrionicus* more convincingly by their toxins than by any other character heretofore available.

Many male dendrobatids are noisy little animals, whose insect-like voices contribute noticeably to the daytime sounds of tropical wet forest. Although we did not set out specifically to study dendrobatid vocalizations, enough data have been accumulated to show that the subject is worth pursuing. Consequently, we have added a chapter on vocalizations in which we attempt to put forth some of the generalizations that can be made. For this purpose, the present treatment is limited to species of *Dendrobates* occurring in Central America and northwestern South America.

We think that data are insufficient for the purpose of determining how all the species of dendrobatids are to be grouped into genera. This problem is not addressed in the present paper, although we intend to discuss it elsewhere. Meanwhile, the "genera" are being treated nomenclaturally as "collective groups," thus avoiding type-species concepts and maintaining some measure of stability until intrafamily relationships are better documented (Myers and Daly, 1971). We follow Savage (1968, p. 747) in using the family name Dendrobatidae Cope, rather than an older available name (Phyllobatidae Fitzinger); such usage eventually should be legalized by the International Commission on Zoological Nomenclature, even though Savage seemed to suggest that the matter is settled. Finally, we have coined the name "Poison-Dart Frogs" as a general English term for the members of this family, although many species are nontoxic and only a few have been used for poisoning blowgun darts. The often used name "Poison-Arrow Frogs" is mildly misleading, based perhaps on the Spanish "*flecha*" (an arrow *or* dart), which is usually translated as "arrow." The batrachotoxins have been commonly used as dart poisons, and conceivably might have been applied occasionally to arrows also, but we have been unable to verify the latter either in the field or in the anthropological literature.

[1]Silverstone (1975) recognized 16 species in a revision published after the present manuscript had been completed. We disagree with his reallocation of several nominal species (including the generic type, see Myers and Daly, 1971) from *Dendrobates* to *Phyllobates*. In our judgment, Silverstone has been confused by a few character-states that are primitive in the family and variously maintained in different phyletic lines. Nonetheless, Silverstone has produced an admirably clear and useful monograph, and we have interpolated last-minute references to it in the present paper.

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MUSEUM ABBREVIATIONS

Catalogue numbers of museum specimens and recording tapes are preceded by the following abbreviations:

AMNH, the American Museum of Natural History, New York
KU, University of Kansas Museum of Natural History, Lawrence
USNM, National Museum of Natural History, Smithsonian Institution, Washington, D.C.

THE DENDROBATID TOXINS

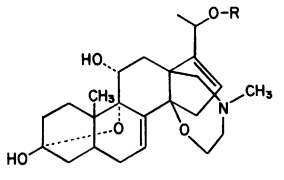
Skin secretions of certain dendrobatid frogs have long been known to contain substances that are poisonous when injected into other animals by blowgun dart or hypodermic needle, but the chemical nature of these substances has become delineated only very recently. The toxins are complex and occur in minute quantities in each frog, forcing the chemist to work on the level of a few milligrams or less. Thus, use of the classical techniques of chemical degradation was precluded and structural elucidation had to wait on the advent and perfection of modern methodology, including mass spectrometry, nuclear magnetic resonance spectroscopy, and X-ray analysis. The early literature was concerned primarily with the pharmacology of crude venom (mostly obtained from blowgun darts) and was reviewed by Märki and Witkop (1963), whose paper initiated a new era of research. Essentially all our knowledge of the chemistry of dendrobatid toxins has been gained within the last decade.

It is now evident that dendrobatid frogs have elaborated not one but several distinctive kinds of skin toxins, some of which contain structural features that are unprecedented in nature. All these toxins, however, are chemically classified as alkaloids—organic, nitrogenous ring compounds that, among other characteristics, are soluble in alcohol and often exhibit marked pharmacological activity.

We currently recognize three classes, or major groups, of toxic alkaloids in the Dendrobatidae. The names of these classes are plural words derived from the names of individual toxins, for example, "batrachotoxins," a class of structurally related steroidal alkaloids that includes batrachotoxin.

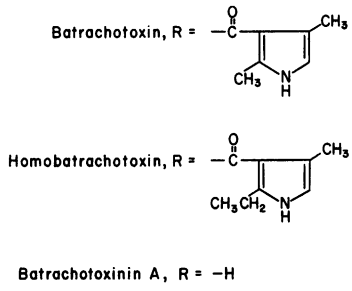
BATRACHOTOXINS

Previous investigation of large samples of skin from *Phyllobates aurotaenia* (Boulenger) led to the isolation and characterization of three steroidal alkaloids with interesting pharmacological properties (Tokuyama, Daly, and Witkop, 1969; Albuquerque, Daly, and Witkop, 1971). These compounds were batrachotoxin, homobatrachotoxin, and batrachotoxinin A. A fourth compound, pseudobatrachotoxin, readily converted to batrachotoxinin A during isolation. The batrachotoxins are differentiated from all other dendrobatid toxins studied by their steroidal ring structure.



The basic steroidal ring system shown above is identical for all three batrachotoxins, which

differ from one another only in the nature of the substituent R, as follows:

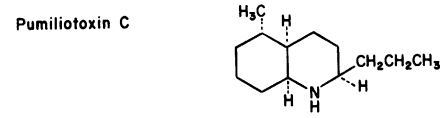


Certain structural features of the batrachotoxins appear unique in nature. The unique features are: (1) the hemiketal 3α, 9α-oxygen bridge, HOC···O···CH; (2) the oxygen and nitrogen-containing heterocyclic ring fused to the steroidal skeleton; (3) the dialkyl pyrrole carboxylate substituents (R) present in batrachotoxin and homobatrachotoxin.

The batrachotoxins also differ from other dendrobatid toxins in their much higher toxicity (table 1), and in their unique pharmacology. The toxicity is primarily due to cardiac effects, wherein depolarization elicited by batrachotoxins results in arrhythmias, fibrillation, and cardiac failure. A similar depolarization occurs in a variety of nerve and muscle preparations via the batrachotoxin-elicited selective increase in cell-membrane permeability to sodium ions. Selective activation of sodium channels by batrachotoxin has provided the pharmacologist with a powerful tool for the study of the function of such membrane channels in conduction of nerve impulses, release of neurotransmitters, and contraction of muscle. The potencies of batrachotoxins are strongly dependent on the presence of the pyrrole carboxylate substituent (R). Thus, batrachotoxin and homobatrachotoxin are much more active than batrachotoxinin A, and, indeed, are among the most toxic substances known to man.

PUMILIOTOXINS

Large samples of skins from *Dendrobates pumilio* Schmidt yielded two major alkaloids, pumiliotoxin A and pumiliotoxin B (Daly and Myers, 1967). A third compound, pumiliotoxin C, was later isolated (Daly, Tokuyama, and Habermehl, 1969) and shown to be a simple *cis*-decahydroquinoline:



Pumiliotoxins A and B are more complex alkaloids whose structures are still being investigated. It was initially thought (Daly and Myers, 1967) that a steroidal ring structure was involved, but this was a misinterpretation and it now appears that both compounds have a bicyclic ring system containing nitrogen and differing only in the terminal portion of a side chain (-$CHOHCH_2CH_3$ in pumiliotoxin A, and -$CHOHCHOHCH_3$ in pumiliotoxin B). The pumiliotoxins are only moderately toxic compared with the steroidal batrachotoxins, but pumiliotoxins A and B are more toxic than the histrionicotoxins (table 1). The pharmacology of the pumiliotoxins has not yet been investigated in any detail. Total synthesis of pumiliotoxin C has been recently reported (Ibuka et al., 1975; Oppolzer, Fröstl, and Weber, 1975; Habermehl, Anders, and Witkop, 1975).

A variety of other alkaloids, apparently related in structure either to pumiliotoxin A and B or to pumiliotoxin C, occur in various species of *Dendrobates*. The structures of these alkaloids, and of other dendrobatid alkaloids apparently related to histrionicotoxins (see below), are under active investigation (Daly et al., MS.).

HISTRIONICOTOXINS

Large samples of skin from *Dendrobates histrionicus* Berthold yielded further bicyclic compounds of unexpectedly novel structure (Daly et al., 1971). Although even less toxic than the pumiliotoxins (table 1), these compounds have extremely interesting pharmacological properties (Albuquerque et al., 1973; Lapa et al., 1975). The histrionicotoxins are characterized by an unusual spiro-ring system (two rings joined by

TABLE 1
Some Pharmacologically Active Alkaloids Isolated from Dendrobatid Frogs

Species of Frog	Alkaloid	Toxicity[a]	Molecular Weight[b]	Empirical Formula[b]	R_f Value[c]	Approximate Amount[d] per Frog (μg)	Approximate Amount[d] per 100 mg. of Wet Skin (μg)
Phyllobates aurotaenia	Batrachotoxin	0.04	538	$C_{31}H_{42}N_2O_6$	0.42	20	10
(1 skin ~ 200 mg.)	Homobatrachotoxin	0.06	552	$C_{32}H_{44}N_2O_6$	0.47	10	5
	Pseudobatrachotoxin	?	399[e]	$C_{24}H_{33}NO_4$[e]	0.44	20	10
	Batrachotoxinin A	20	417	$C_{24}H_{35}NO_5$	0.28	30	15
Dendrobates pumilio	Pumiliotoxin A	50	307	$C_{19}H_{33}NO_2$	0.42	80	90
(1 skin ~ 90 mg.)	Pumiliotoxin B	30	323	$C_{19}H_{33}NO_3$	0.20	120	120
	Pumiliotoxin C	400	195	$C_{13}H_{25}N$	0.28	0-70	0-80
Dendrobates histrionicus	Histrionicotoxin	>100	283	$C_{19}H_{25}NO$	0.52	190	60
(1 skin ~ 300 mg.)	Isodihydrohistrionicotoxin	>100	285	$C_{19}H_{27}NO$	0.44	290	100
	Neodihydrohistrionicotoxin	?	285	$C_{19}H_{27}NO$	0.50	<15	<6
	Allodihydrohistrionicotoxin	?	285	$C_{19}H_{27}NO$	0.44	>15	>6
	Isotetrahydrohistrionicotoxin	?	287	$C_{19}H_{29}NO$	0.48	<10	<4
	Tetrahydrohistrionicotoxin	?	287	$C_{19}H_{29}NO$	0.48	<5	<2
	Octahydrohistrionicotoxin	?	291	$C_{19}H_{33}NO$	0.42	<10	<4
	"HTX-D"	?	287	$C_{19}H_{29}NO$	0.18	40	13
	"H-267"	?	267	$C_{16}H_{29}NO_2$	0.38	<5	<2
	"H-259"	?	259	$C_{17}H_{25}NO$	0.35	<5	<2
	"H-239"	?	239	$C_{15}H_{29}NO_2$	0.15	<5	<2

[a] Approximate minimal lethal dose (subcutaneous) – μg/20 g. mouse.

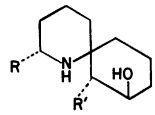
[b] Based on mass spectrometry. High resolution electron impact spectra determined at 70 ev., and chemical ionization spectra determined with isobutane gas.

[c] See page 186.

[d] Based on quantities isolated from large samples of following frog populations: *P. aurotaenia*, Playa de Oro, upper Río San Juan, Chocó, Colombia (Tokuyama, Daly, and Witkop, 1969). *D. pumilio*, Isla Bastimentos, Bocos del Toro, Panama (Daly and Myers, 1967; Daly, Tokuyama, and Habermehl, 1969). *D. histrionicus*, Guayacana, Nariño, Colombia (Daly et al., 1971; Tokuyama et al., 1974).

[e] True molecular ion of this unstable compound was probably not detected; present weight and formula probably those of a fragment (Tokuyama, Daly, and Witkop, 1969).

a single carbon), and by the presence of a 5-carbon side chain as the substituent R on the piperidine ring, and a 4-carbon side chain as the substituent R′ on the cyclohexane ring.



The major compounds isolated were the two spiro-alkaloids, histrionicotoxin and isodihydrohistrionicotoxin. The latter name was rendered "dihydroisohistrionicotoxin" when first published (Daly et al., 1971), but the present spelling is preferable because the compound is an isomer of a dihydro-histrionicotoxin, rather than the converse.

Histrionicotoxin, R = $-CH_2-\overset{H}{\underset{H}{C}}=\overset{H}{\underset{H}{C}}-C\equiv CH$

R′ = $-\overset{H}{\underset{H}{C}}=\overset{H}{\underset{H}{C}}-C\equiv CH$

Isodihydrohistrionicotoxin, R = $-CH_2CH_2-CH=C=CH_2$

R′ = $-\overset{H}{\underset{H}{C}}=\overset{H}{\underset{H}{C}}-C\equiv CH$

A number of minor alkaloids varying only in the nature of the unsaturation of the side chains have also been isolated from this frog (Tokuyama et al., 1974). These include neodihydrohistrionicotoxin (where R = *cis*-CH_2-CH=CH-C≡CH; R′ = *cis*-CH=CHCH=CH_2), isotetrahydrohistrionicotoxin (R = -CH_2CH_2-CH=C=CH_2; R′ = *cis*-CH=CHCH=CH_2), tetrahydrohistrionicotoxin (R = *cis*-CH_2-CH=CHCH=CH_2; R′ = *cis*-CH=CHCH=CH_2), and octahydrohistrionicotoxin (R = -$CH_2CH_2CH_2CH$=CH_2; R′ = -CH_2CH_2CH=CH_2). A third dihydrohistrionicotoxin, referred to as allodihydrohistrionicotoxin, has been recently identified in extracts from *Dendrobates histrionicus* (T. Tokuyama, unpubl. data); tentatively, the side chains in this new compound are R = -CH_2-CH_2-CH_2-C=CH and R′ = *cis*-CH=CHCH≡CH. Another compound, isomeric with tetrahydrohistrionicotoxin, has been referred to as "HTX-D" (Tokuyama et al., 1974); its structure has been only partially elucidated (R = *cis*-CH_2CH=CHCH≡CH), and its mass spectrum is not typical of the histrionicotoxins. Several additional minor alkaloids from *Dendrobates histrionicus* are unnamed and are designated in table 1 by code numbers derived from their molecular weights. These minor compounds are of unknown structure but one appears closely related to the histrionicotoxins. Thus, "H-259" appears to have R = -CH_2CH=CH_2, R′ = *cis*-CH=CHCH≡CH as side chains (Daly et al., MS). Another compound, "H-267," appears related in structure to pumiliotoxin A and B. The term histrionicotoxins is presently used to designate *only* those spiropiperidine alkaloids having both 5-carbon (R) and 4-carbon (R′) side chains.

The histrionicotoxins are the only known alkaloids containing the spiropiperidine ring system. In addition, such acetylenic (-C≡CH) or allenic (-CH=C=CH_2) groups have not been detected in other alkaloids to our knowledge. Certain fatty acids of plants do, however, contain similar functional groups. The pharmacology of the histrionicotoxins is extremely interesting and is strongly dependent on the nature of the R and R′ substituents. Histrionicotoxins antagonize in a unique way the increase in ionic permeability, which couples nerve excitation and release of acetylcholine to a subsequent depolarization and contracture of muscle (Albuquerque et al., 1973; Lapa et al., 1975). Dependent on the nature of the R and R′ substituents, histrionicotoxins may also antagonize the changes in conductances of sodium and/or potassium ions, which are associated with electrical transmission in nerve and muscle cells.

The perhydro-derivative of histrionicotoxin has been recently synthesized (Corey, Arnett, and Widiger, 1975; Aratani et al., 1975). A synthesis of the naturally occurring octahydrohistrionicotoxin also has been reported (Fukuyama et al., 1975).

ANALYSIS OF THE TOXINS FOR TAXONOMIC PURPOSES

Investigation of the foregoing toxins was made possible by the abundance of three species of frogs from which relatively large samples of skins could be obtained. Initial bioassays included tasting[1] the back skins of the frogs in the field, and subcutaneous injection of extracts into laboratory mice. Elucidation of chemical structure was accomplished by the techniques of mass spectrometry, nuclear magnetic resonance, and especially by X-ray crystallography.

The unexpected diversity and complexity of the toxins suggested that they might prove to be valuable indicators of relationship and evolution within the Dendrobatidae. Accordingly, we have extended the initial studies to include a taxonomic survey of as many species as possible. Different methodologies are required for this extended survey because only small numbers of frogs can be obtained in most cases, thus rendering virtually impossible the type of large-scale isolation and quantification previously carried out with large samples of *Phyllobates aurotaenia*, *Dendrobates pumilio*, and *Dendrobates histrionicus*. In addition, certain alkaloids were found to be rather unstable and selectively lost or altered during isolation. Bioassay tests were considered of limited value except to check for the presence of batrachotoxins, which are difficult to isolate in small amounts but easily detected by the high toxicity. Instead, techniques had to be developed for separating and identifying the pumiliotoxin-like and histrionicotoxin-like alkaloids in very small samples. The following methodologies will be discussed in the chronological order in which they were successfully employed in these studies:

1) Thin-layer Chromatography
2) Mass Spectrometry (M. S.)
   a) Electron Impact M. S.
   b) Chemical Ionization M. S.
3) Quantitative M. S.
4) Gas Chromatography (Combined with M. S.)

The gas chromatographic technique has great potential because of its sensitivity and reproducibility. When combined with mass spectrometry, gas chromatography permits identification and quantification of both major and minor constituents in crude skin extracts from dendrobatid frogs.

Crude skin extracts were obtained mostly in the field by placing whole skins in alcohol (Appendix 1), although a few samples from laboratory frogs were also prepared in order to check on the possible effects of diet and stress. Partition of the crude skin extracts yielded an *alkaloid fraction* (Appendix 1), which contained the compounds to be analyzed.

THIN-LAYER CHROMATOGRAPHY

For initial screening, a simple and rapid isolation of the basic alkaloid fraction was obtained by extraction of minced skins with methanol followed by partition between aqueous and organic phases. The alkaloids isolated in this way were redissolved in methanol and could then be separated by thin-layer chromatography (for details, see Appendix 1). Relative proportions could be estimated by visualization of the separated alkaloids as brown "spots" after placing the thin-layer chromatoplate in a chamber containing iodine vapor. Results of a typical chromatography of the methanolic alkaloid fraction corresponding to 10 mg. of skin from *Dendrobates pumilio* and *D. histrionicus* are shown in figure 1. Such extracts from *D. pumilio* (Bastimentos population) and *D. histrionicus* (Guayacana population) have served as reference standards in chromatographic analysis of extracts from other species and populations of *Dendrobates*. The source and history of extracts used for chromatographic analysis in the present paper are given in table 2; note that data can be obtained from very small samples.

[1]In our experience, a frog with a disagreeable taste usually contains a pharmacologically active substance in the skin (often not secreted until the frog has been injured). This field test seems safe enough in the case of *Dendrobates*, but we do not recommend tasting those species of *Phyllobates* (*sensu stricto*) that might contain dangerous amounts of batrachotoxins. Unfortunately there is no *a priori* way of infallibly identifying such species.

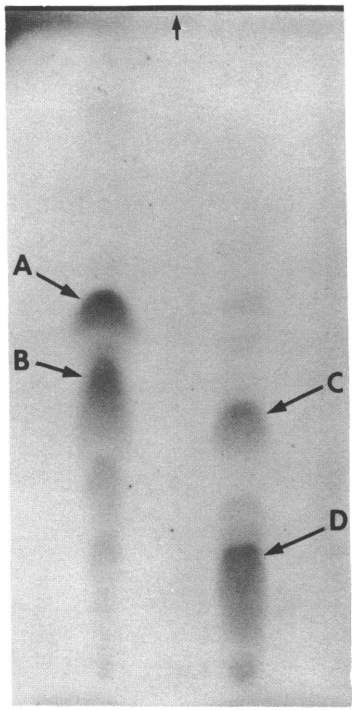


FIG. 1. Representative thin-layer chromatoplate of alkaloids from *Dendrobates histrionicus* (Guayacana population) on left, and *Dendrobates pumilio* (Bastimentos population) on right. A. Histrionicotoxin. B. Isodihydrohistrionicotoxin. C. Pumiliotoxin A. D. Pumiliotoxin B.

Samples of 10 μl of methanolic alkaloids equivalent to amount in 10 mg. wet skin were applied near bottom of chromatoplate, with final position of solvent front marked by dark line across top. Visualization after chromatography by exposure to iodine vapor.

The technique of thin-layer chromatography is useful first of all in separating and identifying individual alkaloids. Secondarily, but importantly, comparison of total alkaloid fractions as resolved in the chromatograms gives a visual picture not only of qualitative but also quantitative differences between species of frogs and, indeed, between populations of the same species. The quantity of an alkaloid on the chromatoplate is roughly proportional to the size of the iodine-visualized spot and the relative depth of its brownish color, whereas the relative extent of migration of the alkaloid in this solvent system—i.e., the R_f (ratio of fronts) value of the spot—is a characteristic physical constant for each compound. An R_f value is obtained by dividing the distance that the center of a spot has traveled from the origin, by the distance that the solvent front has traveled from the origin. More polar alkaloids tend to migrate to a lesser extent than less polar related compounds.

Chromatogram-revealed differences between some species of dendrobatid frogs are seen in figures 1 and 2; intraspecific populational differences also can be seen in figure 2 (samples A-H) and in Daly and Myers (1967, fig. 2). Despite the usefulness of thin-layer chromatography, however, it has certain limitations that should be kept in mind. For one thing, although simple chromatograms (e.g., fig. 1) are easily compared, the more complex chromatograms are harder to compare even when reduced to graphic representation (e.g., fig. 2) in an attempt at simplification by emphasizing only the salient features. Furthermore, the semi-quantitative aspects of chromatograms (i.e., intensity and size of spots) are less easily ascertained than the qualitative aspects and are difficult to discuss objectively. Certain alkaloids, for example, will react with iodine to give a much darker product than other less reactive alkaloids. Minor differences in R_f values must be treated with caution, because R_f values will vary somewhat with different samples of commercial silica gel plates on which the chromatogram is made, and will also depend on the amount of alkaloids applied to the chromatoplate (Appendix 1). Most importantly, it cannot be stressed too strongly that R_f values of different alkaloids may be identical or nearly so (see table 1), and that correspondence of an R_f value to that of a known alkaloid is not in itself sufficient to prove the identity of an unknown compound, nor can it be certain that what appears as a single spot contains only a single alkaloid. Two-dimensional thin-layer chromatography could be expected to give better separation of alkaloids in some cases (e.g., Tokuyama, Daly, and Witkop, 1969, fig. 7), but rechromatography was not thought necessary in the present studies because of success obtained by mass spectral analysis following one-dimensional chromatography. Therefore, thin-layer chromatography has been used mainly for preliminary screening.

TABLE 2

Sources of Methanolic Extracts Used for Some Small-Sample Analyses[a] of Dendrobatid Alkaloids

Species of Frog	Locality	Date of Collection	Number Frogs	Wet Weight of Combined Skins (Grams)
1.[b] *Dendrobates histrionicus*	A.[b] Sta. Cecilia, Colombia	Feb. 1970	12	3.0
	B. Playa de Oro, Colombia	Feb. 1971	8	2.4
	C. Quebrada Vicordó , Colombia	Feb. 1971	3	1.1
	D. Quebrada Docordó (south bank), Colombia	Feb. 1971	8	2.2
	E. Quebrada Guanguí, Colombia	Feb. 1973	10	3.2
	F. Río Guapi, Colombia	Feb. 1973	5	1.4
	G. Guayacana, Colombia	Oct. 1972[c]	10	2.9
	H. Río Baba, Ecuador	Feb. 1974	5	0.9
2. *Dendrobates pumilio*	Isla Bastimentos, Panama	Oct. 1972	10	0.9
3. *Dendrobates lehmanni*, n. sp.	Anchicayá Valley, Colombia[d]	Jan. 1973	8	2.8
4. *Dendrobates occultator*, n. sp.	Quebrada Guanguí and La Brea, Colombia[e]	Feb. 1973	4	0.6
5. *Dendrobates viridis*, n. sp.	Anchicayá Valley, Colombia[d]	Jan. 1973	2	0.06

[a]As follows: (1) Isolation by thin-layer chromatography (fig. 2) and subsequent analysis by chemical ionization and electron impact mass spectrometry. (2) Independent analysis (April-August, 1973, and [for 1-H] March, 1974) of total alkaloid fractions by quantitative chemical ionization mass spectrometry (fig. 3). (3) Gas chromatography-mass spectrometry (figs. 5, 15, 25, 26). Skins were stored in 70-80 percent methanol kept at ambient temperature *ca.* one week-two months and subsequently at -5° C. until fractionation.

[b]Numerals on left side correspond with numbered samples in figures 2 and 3; letters A-H (under *D. histrionicus*) additionally correspond to letters A-H in plate 1 and map 1. See Appendix 3 for fuller citation of *D. histrionicus* localities.

[c]Similar results obtained from extract made at this locality in November, 1971 (fig. 4).

[d]The type locality; map 2.

[e]Three skins from Quebrada Guanguí (map 1), plus one skin from La Brea (= type locality, about 15 km. by river below Quebrada Guanguí).

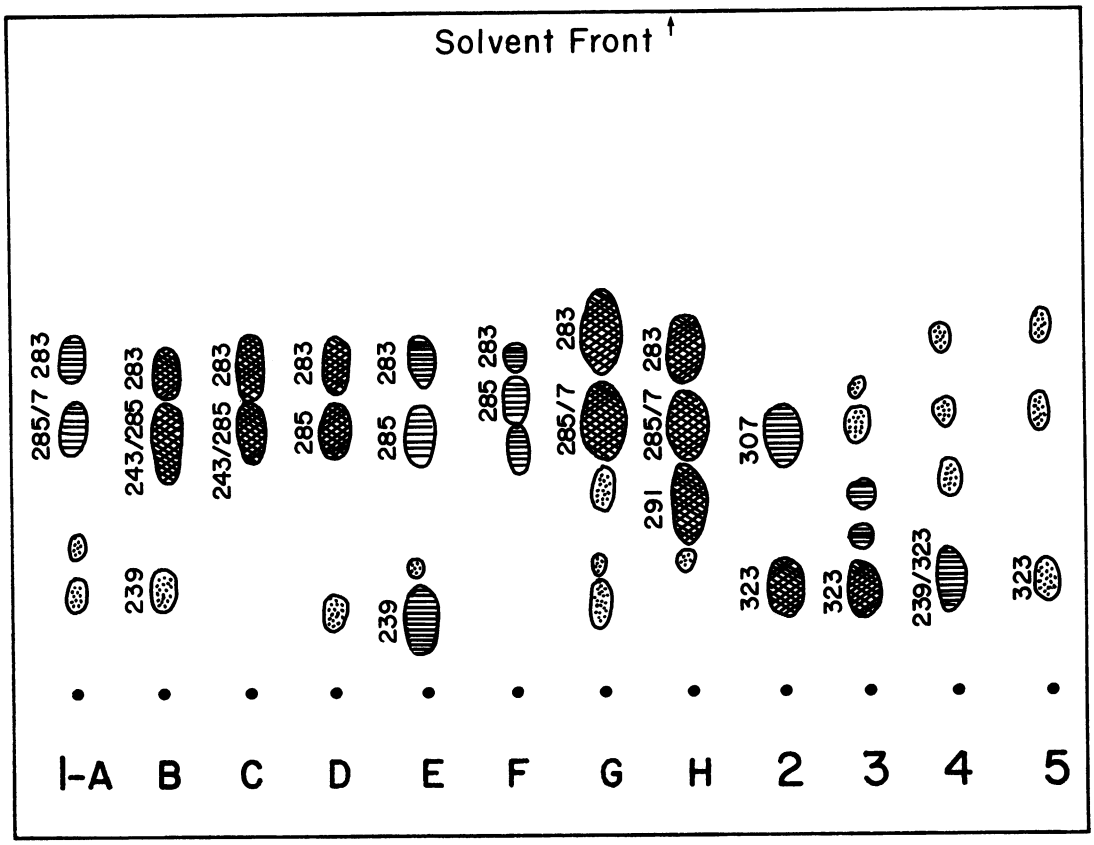


FIG. 2. Thin-layer chromatographic and mass spectral analysis of alkaloids from species of *Dendrobates*: 1 (A-H). *D. histrionicus* (eight populations, with letters A-H corresponding to representative frogs in pl. 1 and localities in map 1). 2. *D. pumilio*. 3. *D. lehmanni*, new species. 4. *D. occultator*, new species. 5. *D. viridis*, new species. (See table 2 for sources.)

Chromatography conducted as in figure 1, which served as reference standard for depicting following spot intensities: Cross-hatched pattern=large amounts of alkaloids. Horizontal lines= moderate amounts. Dots=small amounts. Numbers by the spots are molecular weights, as determined by electron-impact and chemical-ionization mass spectral analyses of alkaloids extracted from spots on thin-layer chromatoplates. Numbers separated by solidus (/) show presence of two compounds in same spot.

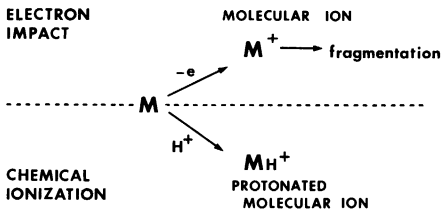
MASS SPECTROMETRY

An electrically neutral molecule, when volatilized and bombarded by electrons in a mass spectrometer, will be converted to a positively charged ion by loss of one electron. This positively charged ion (the molecular ion) corresponds in molecular weight to the original compound. Because of their high energy content, many of the molecular ions undergo extensive breakdown by fairly predictable pathways to positively charged and neutral fragments of lower molecular weight. All the various positively charged fragments, and any of the remaining unfragmented molecular ions, can be separated on the basis of their charge and mass by a combination of electrical and magnetic fields. The proportions of separated ions are quantified with a photomultiplier detector to give a spectrum of masses, which is referred to as a "mass spectrum" and which is uniquely characteristic of each particular organic compound. This technique of

electron impact mass spectrometry is valuable in that major fragmentation pathways provide not only structural insights into the molecule, but also a *detailed* mass spectrum that characterizes the compound. Often, however, fragmentation is so extensive that the molecular ion cannot be detected with certainty.

Complementary to the above is *chemical ionization mass spectrometry*, wherein a gas (methane, isobutane, ammonia) within the mass spectrometer is converted to positively charged molecules by electron bombardment. These positively charged molecules then act as acids that preferentially protonate the compound being analyzed on basic atoms such as nitrogen or oxygen. The result is a relatively stable protonated molecular ion of an alkaloid that undergoes little or no fragmentation. The most common fragmentation that does occur is by loss of water. Chemical ionization mass spectrometry therefore provides the investigator with the molecular weight of an alkaloid but with little additional information. It should be remembered in following discussions, that the molecular weight is obtained by subtracting 1 (hydrogen atom) from the weight of the protonated molecular ion.

The above mass spectral techniques can be simply visualized as follows, where M represents the initially neutral molecule:



After separation of alkaloid components by thin-layer chromatography, the compounds corresponding to the various bands were extracted into ethyl acetate (Appendix 1) and analyzed by mass spectrometry. Combination of the two mass spectral techniques, coupled with the R_f values of thin-layer chromatography, thus provides fairly positive physical characterization of each alkaloid. Even so, it must be stressed that isomeric compounds will have the same molecular ion (hence the same molecular weight and empirical formula), that they frequently will have similar chromatographic properties (R_f values), and that they may have in some cases quite similar mass fragmentation patterns. Therefore, although the above techniques do provide positive identification and semiquantification of many of the dendrobatid alkaloids, minor structural variations such as in isomeric dihydro- and tetrahydrohistrionicotoxins would not be readily detected.[1] In addition, certain alkaloids tend to undergo decomposition during isolation by thin-layer chromatography, rendering this type of analysis quite difficult. Tentative identifications of the molecular ions corresponding to the major alkaloids in different regions of thin-layer chromatoplates are shown in figure 2.

QUANTITATIVE MASS SPECTROMETRY

In order to circumvent the aforesaid problem of alkaloid decomposition, total unresolved alkaloid fractions were quantitatively analyzed by chemical ionization mass spectrometry—that is, without first separating the compounds by thin-layer chromatography.

A small sample of the alkaloid fraction in methanol, corresponding to 1-5 mg. of frog skin and containing less than 100 μg of total alkaloids, was evaporated on the direct inlet probe in a Finnigan 1015 mass spectrometer containing isobutane. The entire sample was volatilized slowly over a temperature range of 80-200°C., during which time the data from repetitive scans (1/sec.) were stored in the computer. As many as 100 spectra were often obtained from a single sample before it was completely volatilized. Complete volatilization of a sample is evident

[1]Analysis of isolated alkaloids by other techniques, such as nuclear magnetic resonance spectroscopy, would distinguish between such isomers and would provide a further physical characterization of the alkaloids found in the various extracts (cf., data in Daly and Myers, 1967; Daly, Tokuyama, and Habermehl, 1969; Tokuyama, Daly, and Witkop, 1969; Daly et al., 1971), but this kind of analysis requires quite pure and relatively large samples of about 3-10 mg. of each alkaloid. The large number of frogs required as a source of these amounts precluded such analyses except in relatively rare instances.

when the photomultiplier detector shows that the total ion current has returned to low levels. Total ion current for each ion was then summated by the computer for the approximately 100 spectra, providing a composite mass spectrum for the entire sample. Because a chemical ionization spectrum of an alkaloid consists predominantly of the protonated molecular ion, the relative proportions of different alkaloids can be estimated from the relative intensity of the various summated protonated molecular ions (plus, in some cases, the ion corresponding to loss of water or some other readily identified fragment). Test analyses with small samples of crude skin extracts were consistent with the relative proportions of alkaloids isolated (by other means) from large samples of *Dendrobates pumilio* (Bastimentos population) and *D. histrionicus* (Guayacana). The results even from very small samples can be presented as readily visualized and easily compared histograms, as in figure 3.

Protonated parent ions of alkaloids have even masses, which are plotted in figure 3 as percentages of the total intensity of all ions of even mass in the range of mass 210-400. Ions with odd-number masses were ignored because these represented nonalkaloid contaminants. Other data on purified alkaloid fractions indicated that the protonated molecular ion of 324 (pumiliotoxin B) readily lost water, giving a peak of nearly equal intensity at mass 306; this latter peak was therefore omitted in figure 3, in which the percentage for mass 324 was calculated from the summed intensity of mass 324 and 306. A similar correction was made for mass 268 ("H-267," table 1), the percentage of which was calculated from the summed intensity of mass 268 and a loss-of-water fragment at mass 250. Other such corrections in figure 3 include the protonated molecular ions at: Mass 220, which gave a significant fragment ion at 178; mass 244, which had a small fragment ion at 202; and masses 308 (pumiliotoxin A) and 324 (pumiliotoxin B, see also above), which both yielded small fragments at 194. Fortunately, the histrionicotoxins (protonated molecular ions at 284, 286, 288, 292) give virtually no fragment ions.

Although the type of data in figure 3 is useful for a preliminary evaluation of alkaloid content in a crude sample, there are problems inherent in such an approach to exact quantitative analysis. First, only compounds that give a similar yield of protonated molecular ions in the mass spectrometer will give comparable ion currents. Within a series of similar alkaloids this condition is probably met fairly well, but more volatile compounds within a series may be vaporized too rapidly during initial heating and give relatively lower yields of ion currents. This was obvious in the present investigation, in which careful, slow initial heating was necessary in order to analyze the more volatile alkaloids (e.g., histrionicotoxin) in the presence of less volatile alkaloids (e.g., the pumiliotoxins); very nonvolatile alkaloids (the batrachotoxins) underwent extensive pyrolysis and fragmentation and could not be satisfactorily analyzed quantitatively by the present technique. Second, *any* fragmentation of the protonated molecular ion will greatly complicate quantitative analysis of the data. Thus, without prior knowledge of the exact quantitative fragmentation of each compound one would not be justified in making corrections such as were done in figure 3. Indeed, masses below 210 were purposely omitted from figure 3, since, with few exceptions (see above), it was uncertain to what extent such ions represented protonated molecular ions or fragment ions. Finally, one must consider the fact that mass spectrometers, particularly the quadrapole type used in the present study, are inherently less sensitive as the mass of the analyzed ion increases; this does not, however, have a major effect over the mass range of 200-400 and has not caused any serious bias in figure 3. Our experience indicates that computer-assisted analysis of a total summated chemical ionization mass spectrum can provide useful preliminary data, in instances where satisfactory separation techniques have not been developed for classes of natural products.

GAS CHROMATOGRAPHY (COMBINED WITH MASS SPECTROMETRY)

Gas chromatographic techniques have proved of great importance in the qualitative and quantitative analysis of a variety of natural products. Application of gas chromatography to analysis of

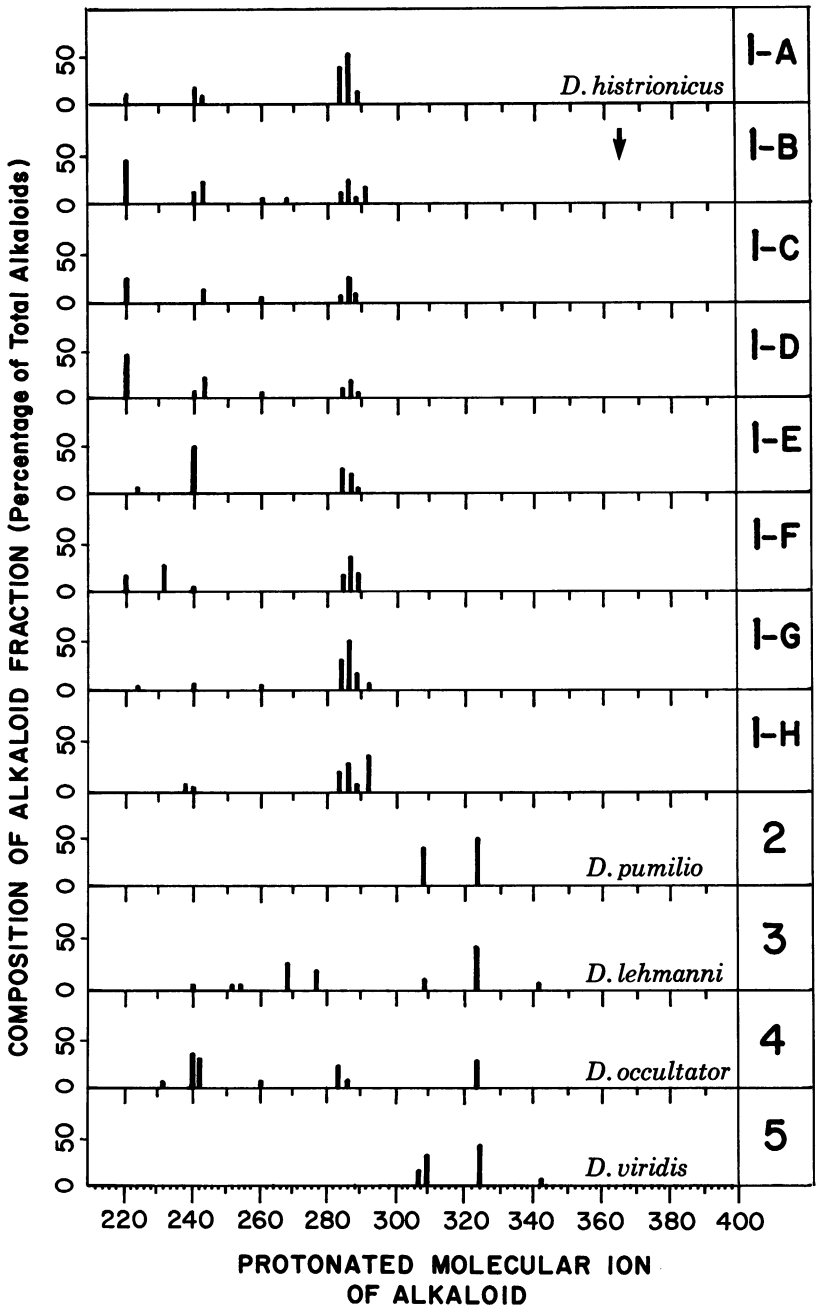


FIG. 3. Quantitative chemical ionization mass spectral analysis of alkaloids in skin extracts from: 1 (A-H). *D. histrionicus* (eight populations, with letters A-H corresponding to representative frogs in pl. 1 and localities in map 1). 2. *D. pumilio*. 3. *D. lehmanni*. 4. *D. occultator*. 5. *D. viridis*. (See table 2.)

Bars show occurrence and relative percentages of alkaloids in each population sample. For each an extract containing less than 100 μg total alkaloids was completely volatilized in a mass spectrometer with isobutane as the proton source. Mass spectra taken repetitively (1/sec.) and computer stored, for final summation to give composite spectrum: percentages then calculated for total protonated molecular ions representing different alkaloids.

dendrobatid alkaloids was initially unsuccessful because of the thermal lability of pumiliotoxin A and B. This problem was solved in 1974 (see Appendix 2 for technical data), and quantitative analysis is now possible for all the alkaloids thus far detected as major components of the extracts from frogs of the genus *Dendrobates*. By combining gas chromatography with chemical ionization mass spectrometric analysis of the effluent gas, we have the most powerful tool yet available for the identification and quantification of the alkaloids in small samples of *Dendrobates* skin extracts. Further studies are necessary to determine whether more complex dendrobatid alkaloids can be analyzed by gas chromatography. It would appear that the batrachotoxins undergo extensive decomposition during the process.

In gas chromatography, the mixture to be analyzed is dissolved in a suitable, volatile organic solvent (methanol in the present investigations), and injected directly onto a heated column that contains a stationary liquid phase (a methylsiloxane polymer in the present study) coated on an inert support, over which is flowing an inert carrier gas. The organic solvent, because of its high volatility, passes directly through the heated column in the gas phase, and emerges into a detection chamber where it is combusted in a hydrogen flame. The ions formed during combustion of the solvent, and those formed during combustion of subsequent emerging compounds, are measured as current and recorded as the detector response. Such a detection system is referred to as a "flame ionization detector." Compounds with high volatility, and/or low solubility in the stationary liquid phase on the column, emerge quickly with the carrier gas, whereas compounds with low volatility and/or high solubility emerge more slowly. Within a series of similar compounds, those of lower molecular weight will emerge first. The degree of response of the flame ionization detector will be directly proportional to the amount of a particular compound emerging from the column, although the relative detector response for two different compounds may differ slightly. In order to identify the compounds separated by gas chromatography, methane is used as the carrier gas and proton source, and the column effluent is passed directly into a mass spectrometer, where the compounds are detected by chemical ionization mass spectrometry rather than by combustion (Appendix 2).

In a typical analysis, 2 μl of methanolic alkaloid fraction, corresponding to 2 mg. skin, is injected onto the gas chromatograph column. Representative chromatograms are shown in figures 4 and 5, which should be read with the following points in mind:

1) The injection point of the alkaloid is shown by the small upward arrow, at 150°C.

2) The first large peak (top omitted) is due to the solvent methanol.

3) Certain low molecular weight alkaloids (mol. wt. < 225) emerge in the latter portion or "tail" of the solvent peak.

4) Immediately after the maximum of the solvent peak had been passed, the column was programmed at 10°C. per minute to 280°C., as shown by the time-temperature scale at the bottom of the chromatogram. The temperature at which a compound emerges from a particular column is fairly reproducible, but will vary slightly with different batches of column packing.

5) Numbers identifying the alkaloids are the weights of the protonated molecular ions (subtract 1 to obtain molecular weight). These ions were identified in separate chromatographic analysis by combined gas chromatography-mass spectrometry.

6) Areas represented by the various peaks are roughly proportional to the total amount of each of the corresponding alkaloids. These areas can be compared most easily by computer programming, or, in the case of simple peaks (e.g., fig. 5), by cutting out and weighing the different peaks.

It is apparent in the gas chromatogram for *Dendrobates histrionicus* (fig. 4) that at least two major compounds are present that are poorly separated by the chromatography. Actually, several minor alkaloid constituents also contribute to this poorly resolved pair of major peaks. Determination of the alkaloids was made by means of combined gas chromatography-mass spectrometry (Appendix 2). After injection of 1-2 μl of a methanolic alkaloid fraction corresponding to 1-2 mg. of skin, repetitive mass spectra (1/sec.) were stored in the computer.

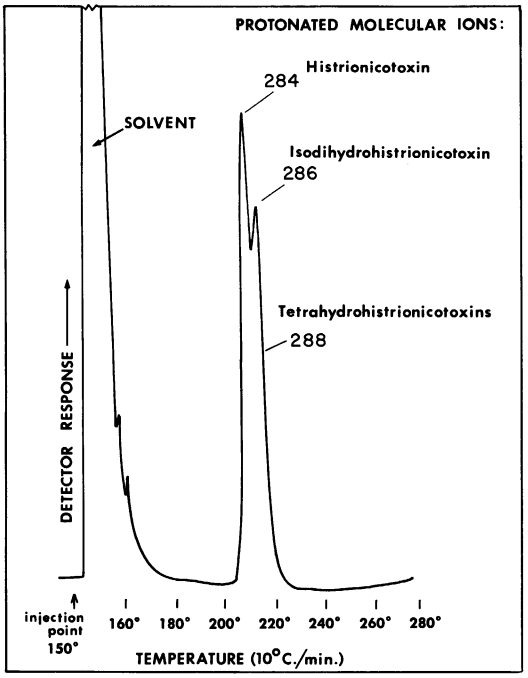


FIG. 4. Gas chromatogram of alkaloids from a population sample of *Dendrobates histrionicus* (10 frogs from Guayacana, Colombia, November, 1971). Chromatography run with 2 μl of methanolic extract containing concentrated alkaloids equivalent to amount in 2 mg. of wet skin. See text for interpretation.

Reproducibility of this technique is seen by comparing this chromatogram with figure 15G, which shows different sample (October, 1972) obtained from same population of frogs.

Subsequent analysis of these data permits designation of various protonated molecular ions corresponding to the peaks seen on flame ionization gas chromatograms. Thus, in figure 4, 284 refers to the protonated ion of histrionicotoxin and 286 to isodihydrohistrionicotoxin, emerging at temperatures of about 210° and 215°C. respectively. The two tetrahydrohistrionicotoxins (288) and HTX-D[1] (288) emerge at temperatures of 213-218°C. and contribute mainly to the right side of the poorly resolved pair of peaks in figure 4; octahydrohistrionicotoxin, another minor constituent, emerges between histrionicotoxin and dihydrohistrionicotoxin at a temperature of about 212°C.; neodihydrohistrionicotoxin and allodihydrohistrionicotoxin emerge at 210° and 211°C., respectively.

The quantitative composition of such poorly resolved peaks has been easily ascertained by using the computer and stored mass spectral scans to provide the total ion current of the various protonated molecular ions. In figure 4, for example, the relative total intensities of masses 284, 286, 288, and 292 were 10:17:6:0.5. Carrying the analysis a step farther, computer-generated gas-chromatograph traces corresponding only to the ion 286 clearly demonstrated the presence of at least three compounds, with a major peak emerging at the temperature ex-

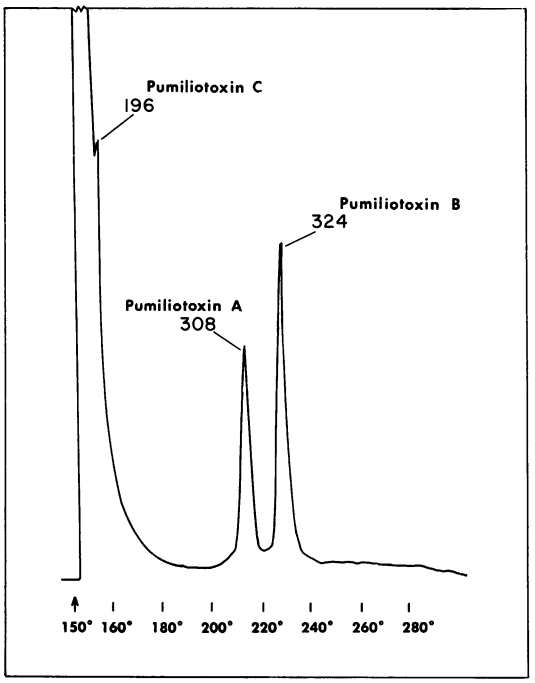


FIG. 5. Gas chromatogram of alkaloids from a population sample of *Dendrobates pumilio* (10 frogs from Isla Bastimentos, Panama, October, 1972). Chromatography run with 2 μl of methanolic extract containing concentrated alkaloids equivalent to amount in 2 mg. of wet skin. Compare with figure 4; see text for interpretation.

[1]The histrionicotoxins and related alkaloids are briefly defined on p. 182 and in table 1.

pected for isodihydrohistrionicotoxin (215°C.), and an earlier minor peak emerging at the expected temperatures for neodihydrohistrionicotoxin and allodihydrohistrionicotoxin (210-211°C.). Similar analyses were performed on all subsequent data obtained from the combined technique of gas chromatography and chemical ionization mass spectrometry.

In the case of *Dendrobates pumilio* (fig. 5), flame ionization gas chromatography shows two fully resolved peaks emerging at about 216° and 230°C. These were shown by mass spectral analysis, and by comparison to standards, to be due exclusively to pumiliotoxin A (308) and pumiliotoxin B (324). Somewhat more fragmentation of these compounds was observed with methane as the protonating gas than had been the case with isobutane (see p. 190). Pumiliotoxin A showed fragment ions at mass 290 and 194, and pumiliotoxin B had fragments at mass 306 and 194; such fragment ions and a correspondence of their emergent temperature profile to that of the parent ion provides additional confirmation of these alkaloids. The extract from *Dendrobates pumilio* also contained small amounts of an alkaloid that emerged only slightly after the solvent (fig. 5). This alkaloid had a protonated molecular ion at mass 196 and fragments at mass 194 and 152. Comparison to a standard confirmed its identity as pumiliotoxin C, a compound that had been detected in widely varying amounts (0-70 μg/frog, table 1) in samples of *Dendrobates pumilio* collected at various times on Isla Bastimentos, Panama. We plan to conduct further studies of intra- and interpopulation variation of the skin toxins of this frog.

Additional examples of analysis by combined gas chromatography-mass spectrometry are shown in figures 15, 25, 26. In each case, the figures represent flame-ionization responses, whereas identification of the peaks was done by the combined technique.

MOLECULAR DATA IN SYSTEMATIC STUDIES

"The taxonomist, ever avid for new data to use in refining or if necessary rebuilding his system, is happy to make use of chemical characters as they become available. At least good taxonomists are; we shall consign the others to outer darkness."

A. Cronquist (1974, p. 31)

Taxonomists (even "good" ones), however, are sometimes inclined to be overly zealous in advocating use of chemical or other "nonmorphological" characters. Cronquist (*loc. cit.*) partly explained why this is so: "The greatest problem is that the data are relatively hard to get.... The natural psychological result of the difficulty of getting the chemical information is to consider that it must be inherently more important than classical morphological features. We all tend to value things by their scarcity or cost." Another reason, perhaps inextricably bound with the above, is an unverified assumption that the "closer" a character is to the genes, the greater is its potential value to systematics. This viewpoint was expressed by Cei, Erspamer, and Roseghini (1967, pp. 328, 329), in one of a series of important papers on biogenic amines and polypeptides in amphibian skin: "It is obvious that each amine, and each amino acid link, is a trait by which we can trace species evolution. Thus, biochemical taxonomy, in its broadest sense, has possibly even a greater validity than traditional taxonomy, based on somatic or osteological structures, size or coloration." Such ebullience remains to be justified. Anyway, evolutionary and taxonomic studies, although closely interrelated, are not strictly synonymous, and, in either case, molecular data present problems that must be recognized and realistically dealt with.

Generally speaking, there are two major approaches to molecular taxonomy and evolution—one approach dealing with genes and/or macromolecular gene products, and the other dealing with so-called micromolecules, or metabolic end products. Recent work in the first field would seem particularly promising to studies of phylogenetic branching and rates of evolution. It has been suggested (Sarich and Wilson, 1967; Kimura, 1968; King and Jukes,

1969; Kimura and Ohta, 1971a, 1971b) that homologous proteins of different species evolve at constant, determinable rates because of selectively neutral mutations that are fixed at constant rates. The tenets of the "evolutionary clock" hypothesis are, however, not well established, as this is a new and active field. The neutrality hypothesis is disputed by some authors (e.g., Ayala, 1974), and others (e.g., Jukes and Holmquist, 1972; Holmquist and Jukes, 1975) have suggested that evolutionary rates of homologous proteins are species-dependent, as well as time-dependent. It has not been clear to some workers that the hypotheses being generated in this field are based on relatively little comparative data and require testing rather than uncritical acceptance (or rejection). Baldwin and Riggs (1974), for example, calculated rates of hemoglobin evolution in *Rana catesbeiana* and *R. esculenta*, and suggested that these living frogs "may have been separated from one another for as long as 150 to 250 $\times$ 10^6 years," but had the authors compared their data with the known history of frogs they might have suspected that something was amiss.[1] Also, there is growing realization that protein evolution and anatomical evolution have proceeded independently, and that measures of "genetic distance" are not well correlated with morphology, which, by necessity, forms the basis of most classifications. Indeed, A. C. Wilson and collaborators are advancing the idea that protein evolution does not provide the primary basis for organismal evolution after all (Wilson, Maxson, and Sarich, 1974; Wilson, Sarich, and Maxson, 1974; King and Wilson, 1975; Prager and Wilson, 1975).

The second major approach to molecular taxonomy and evolution has been longer established and involves studies of micromolecular, secondary metabolites. The dendrobatid alkaloids that are of concern in the present paper represent an example of such metabolites. These compounds theoretically seem less suitable than macromolecules for providing measures of genetic distance (see above), but they are more informative than proteins when it comes to *explaining* the selective basis for such evolutionary happenings as development of conspicuousness in certain frogs. Problems inherent in utilizing micromolecular data in systematic studies are discussed in the following paragraphs. Certain of the remarks also are applicable to macromolecular and other nonmorphological characters, but the main purpose is to put our own data in proper perspective.

Sufficiency of Data. Many kinds of nonmorphological data have been and are being used to elucidate relationships and lineages of particular organisms. Such studies obviously have taxonomic value, but reliable classifications can be broadly based only on *comparative* data that are available for all taxa, or at least most, in the group being classified. Excepting some microorganisms, only comparative morphology normally meets this criterion. Usually it is beyond an investigator's physical or economic means to obtain comparative nonmorphological data on all the species he wishes to classify, unless the taxonomic group is small and geographically restricted. Furthermore, biochemical characters should be assumed to vary intraspecifically unless shown otherwise, giving the investigator an added burden of obtaining adequate samples within and between conspecific populations.

The difficulty or impossibility of obtaining sufficient information usually relegates biochemical data to the secondary role of refining and elaborating on a basically morphological classification. For this reason alone, biochemical data can hardly be said to have more "validity" than morphology. It is recognized, of course, that some instances of sibling (cryptic) species and other fine-grained phenomena cannot be resolved without recourse to nonmorphological characters. We suspect that this may be true in some sections of the Dendrobatidae.

Problems of Detection. It is one thing to determine the presence of some particular micromolecule, but something quite different to conclusively demonstrate its absence. The latter may be practically impossible, and in this regard is in striking contrast to most morphological char-

[1] A date of 150 million years before present would have ancestral bullfrogs living at the same time as some of the oldest-known (Jurassic) frogs, whereas 250 million years B.P. would put them with Upper Palaeozoic proanurans. Even the lesser date is unlikely, although at least conceivable, inasmuch as undisputed ranids are unknown before the Oligocene, less than 50 million years B.P. (e.g., Estes, 1970; Estes and Reig, 1973).

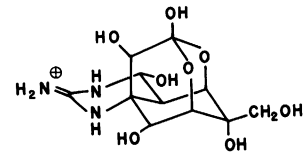
acters. Often, however, there may be good reason for believing that a given constituent is absent—but even so it does not necessarily follow that its genetic basis is absent. Some biosynthetic pathways can be interrupted or switched off, because of physiological or environmental stress for example.

Detection of trace amounts of compounds is dependent partly on technique and partly on sample size, thus relating back to sufficiency of data as discussed above. In the Dendrobatidae, much smaller samples are now required for the chemical detection of trace amounts of histrionicotoxins and pumiliotoxins, because of development of the gas chromatographic techniques. Detection of batrachotoxins must still rely on less sensitive thin-layer chromatographic methodology.

Problems of Homology. Present-day usage of the word "homology" stems primarily from the work of Richard Owen, the nineteenth-century anatomist, who used the term to mean essential correspondence in anatomical structures of different animals. Most evolutionary biologists since Darwin have considered common ancestry to be the explanation of such correspondencies. The detection of homologies is thus a principal morphological method of determining phylogeny. Statements arising from use of this method have been labeled "viciously circular" by some critics, but Hull (1967, p. 188) examined the logic, of the method and the criticism, and seems correct in concluding that, "the superficial appearance of epistemological circularity . . . arises from the method of successive approximation used by evolutionary taxonomists."

Historically, homology is a morphological concept, but workers in nearly every field of comparative biology have adopted the term to express the evolutionary connotations of their data. But, although the anatomist often can elegantly and convincingly demonstrate homology or nonhomology (given sufficient complexity of the structures compared), workers in other fields often lack the wherewithal of doing this unless reference can be made to morphological correlates. This problem has led some authors to so despair of identifying ancestral prototypes (genes for example) that suggestions have been made to define homology only in terms of essential similarity—thus returning (often unknowingly) to a usage similar to Owen's (e.g., see exchange of letters in "Science," by Winter, Walsh, and Neurath, 1968, and Margoliash, 1969). Other authors simply have ignored problems of homology and have unrigorously assumed common ancestry on the basis of similarities in some nonmorphological or submorphological character.[1]

The problem of detecting homology is particularly acute in the case of secondary metabolites, some of which have been independently evolved in obviously unrelated organisms. Take, for instance, tetrodotoxin, a complex, nitrogenous substance of high toxicity (illustration is of the pharmacologically active cationic form):



This compound appears identical in tetraodontid pufferfish and in salamanders of the genus *Taricha* (Mosher et al., 1964), and furthermore has been recently identified as a constituent in

[1]This has been particularly evident to us in the case of some chromosome studies. Similar or dissimilar karyotypes, or even chromosome numbers alone, have led some authors to make uncritical statements about relationships and evolutionary trends at generic and suprageneric levels, with little regard for other data. Bona fide chromosome markers can be invaluable, especially in detecting evolutionary trends in clusters of closely related species, but usefulness of karyotypes varies from group to group, as chromosome similarities are not automatically good evidence of homology. For example, Charles J. Cole has called our attention to the fact that some lizards (*Sceloporus*) have karyotypes more resembling those of some toads (*Bufo*) than those of other congeneric lizards: The chromosome resemblances include identical number, similarity in absolute size, similar size classes (i.e., six large and five small pairs), similar centromere positions, and the absence of recognizable pairs of sex chromosomes (compare fig. 11 in Cole, 1970, with fig. 1 in Cole, Lowe, and Wright, 1968; then compare with fig. 1 in Cole, 1970).

Chromosome studies presumably could become important in the systematics of dendrobatid frogs, but we dismiss the published data as meager and at present inadequate for meaningful interpretation, although Silverstone (1971, p. 262) claimed "chromosomal evidence" in support of his generic concepts.

skins of terrestrial frogs of the genus *Atelopus* (Kim et al., 1975).

Few taxonomists would argue with the use of tetrodotoxin as a taxonomic character in pufferfish, in salamanders, or in frogs, but nearly everyone would object to any claim that these groups are closely related by virtue of identity of their toxins! The implication to be drawn is that chemical data cannot stand alone because, obviously, structural identity or close similarity of even a complex molecule is not proof of homology in an evolutionary sense.

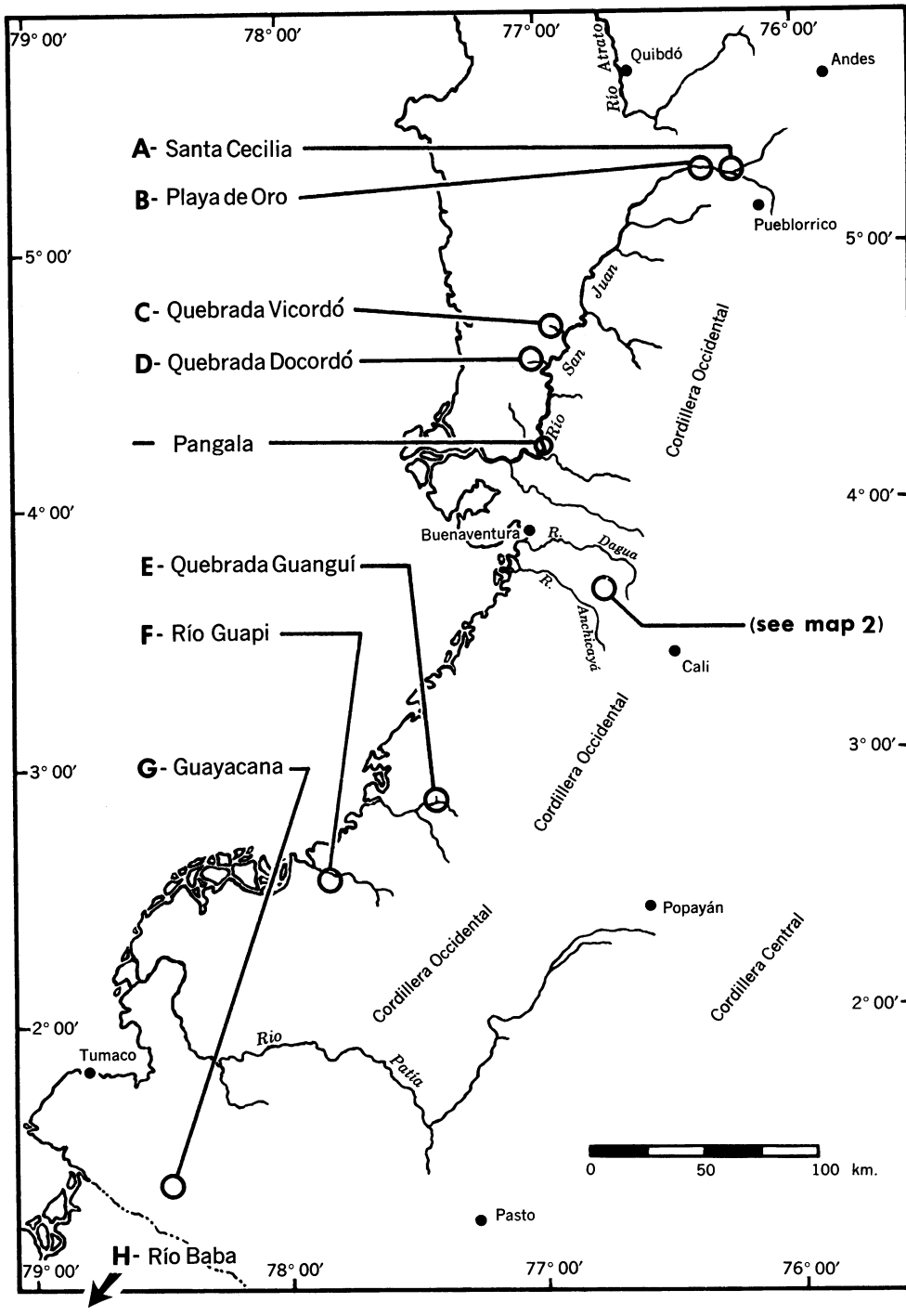
Although the occurrence of identical metabolites in different species is not certain evidence, homology is the most parsimonious view when the species are considered to be related on other grounds, and intuitively seems most likely to be correct. Knowledge of enzyme systems and biosynthetic pathways eventually should either corroborate or cause reinterpretation of hypotheses based on molecular similarities. Nothing is yet known of the biosyntheses of dendrobatid toxins. Administration of radioactive cholesterol, mevalonate, and acetate to several dendrobatid species did not produce radioactivity in the skin toxins, although cholesterol and mevalonate seem likely precursors of the steroidal batrachotoxins and acetate a likely "building block" for formation of histrionicotoxins and pumiliotoxins (Johnson and Daly, 1971).

The preceding paragraphs have outlined what seem to be the principal difficulties in making broad systematic use of the dendrobatid toxins. Sufficiency of data is the most pressing problem, but, if the future is kind, we are optimistic about obtaining adequate comparative data from most of the toxic dendrobatids; approximately 90 alkaloids already have been isolated from some 20 species (Daly et al., MS). Problems of analysis have been largely resolved, and at least the major alkaloids can be detected in small samples and, most importantly, the use of small, pooled samples gives reproducible results (e.g., compare figs. 4 and 15G), even though problems of possible seasonal and individual variation have not been fully addressed. Concerning the question of homology, we shall be parsimonious with our hypotheses and assume that identical alkaloids in different species owe their existence to a common evolutionary event. Some future data, including structural elucidation of pumiliotoxin A and B, hopefully will throw light on probable biosynthetic pathways and allow statements to be made about primitive and derived conditions based on the biochemistry alone. Meanwhile, data already gathered are proving valuable in determining the species limits of some biologically variable and taxonomically vexatious frogs, as will be seen in following sections of this paper.

BIOCHEMICAL AND OTHER VARIATION IN *DENDROBATES HISTRIONICUS* BERTHOLD

Among the most variable of the species of *Dendrobates*, and, indeed, among the most variable of all vertebrates, are the Central American *D. pumilio* and the South American *D. histrionicus*. The former species has been noted to undergo interpopulational and intrapopulational variation in skin chemistry and toxicity, size and morphology, vocalizations, habitats and behavior, and in color and color pattern, with differences in color encompassing "the visible spectrum from red to blue, as well as achromatic black and white" (Daly and Myers, 1967, p. 970; and unpubl. data). Similar statements can be made about *Dendrobates histrionicus*, which, albeit less variable than *pumilio* in color (hue) and morphology, is even more variable in color pattern. Either species appears ideal for our purpose of assessing biochemical variation within a single species of *Dendrobates*. Our investigations of *D. pumilio*, however, are continuing and we eventually hope to treat this species in considerable detail, whereas our more superficial studies of *D. histrionicus* are nearly concluded and can now be summarized.

Dendrobates histrionicus has been difficult to sample adequately because its geographic range covers thousands of square kilometers of rain forest along the Pacific lowlands of western Colombia and northwestern Ecuador. Widely scattered samples were obtained throughout most of the geographic range. The principal populations sampled are shown in map 1, designated by the letters A-H in this map and in the various illustrations. Some additional samples



MAP 1. Western Colombia and northwestern Ecuador, showing localities where samples of skin toxins were obtained from *Dendrobates histrionicus*. Letters A-H correspond to representative frogs pictured in plate 1, and to toxin samples analyzed in figures 2, 3, and 15. As indicated by the arrow, locality H is off the map (approx. 200 km. SSW of loc. G). See Appendix 3 for complete citation of localities.

are listed in table 3, and complete locality data and museum-voucher specimens are given in Appendix 3. Most of these samples, including A-H, were collected by us in the years 1970-1974, but a few (especially at Pangala, table 3) were obtained for the American Museum of Natural History by Borys Malkin, in 1971-1972.

The geographic and intrapopulational variation of *Dendrobates histrionicus* is discussed primarily on the basis of these recently collected samples, which hopefully are sufficient to reveal the general nature of this frog. Additional populations are represented by specimens in various museums (see Silverstone, 1975, pp. 18-26), but study of this material would have contributed nothing to our primary objective of comparing biochemical variation with variation in other characters. The characters to be discussed are:

1) Size and proportions
2) Color and pattern
3) Escape behavior
4) Skin alkaloids

First, however, comments are in order concerning use of the species name and on our disinclination to apply the subspecies concept to *Dendrobates histrionicus.*

NOMENCLATURE AND THE SUBSPECIES CONCEPT

Nomenclature. "Dendrobates histrionicus, nov. Sp." was so named by A. A. Berthold (1845, p. 43), who briefly described a specimen obtained by "Hr Degenhardt während eines längern Aufenthaltes in der Provinz Popayan" (*op. cit.*, p. 38). Berthold ("1847" [1846?]) elaborated on the original description in a second, longer paper,[1] adding coordinates to the locality—"etwa 2°N.B. und 301°L." (*op. cit.*, p. 4). Dunn and Stuart (1951, p. 56), in referring to the "original description" of another Berthold species, pointed out that this archaic method of reckoning longitude placed the locality at [near] the city of Popayán, which they regarded as the type locality of species collected by Degenhardt and named by Berthold. It seems possible, however, that the coordinates were editorially added by Berthold, and that Degenhardt obtained specimens from a variety of places in Popayán "Province." The old colonial "Provincia de Popayán" at one time included nearly all of Pacific-side Colombia. In any case, *Dendrobates histrionicus* does not now, and probably never did, occur at the inland, highland town of Popayán. As presently understood, the range of this species is Pacific northwestern South America, in lowland and foothill rain forest west of the Andes, from the Bahía de Solano and upper Río Atrato region of northwestern Colombia south into northwestern Ecuador (Funkhouser, 1956; Daly et al., 1971; Silverstone, 1975).

The holotype of *Dendrobates histrionicus* is figured by Berthold in his second paper and is here reproduced as figure 6. This illustration is uncolored in the journal version ("1847" [1846?]),[2] but is hand-colored in the one copy of the preprint (1846)[3] that is available to us. The colored plate shows a black frog with very pale red markings, which presumably represent red markings faded by preservative. The type specimen of *D. histrionicus* is therefore rather similar to frogs occurring on the upper Río San Juan in the present-day Department of Risaralda (compare fig. 6 with fig. 7 and pl. 1, fig. A). Lacking Degenhardt's detailed itinerary, however, it seems impossible ever to say with certainty where the holotype came from, since there are potentially thousands of unsampled populations of this variable species. The type locality is best cited simply as "Provinz Popayan (= western Colombia)." We see no need to arbitrarily assign ("restrict") a more specific type locality unless absolutely necessitated by some future biological problem (e.g., sibling species).

[1]This work bears the same title as Berthold's 1845 paper, as it was not unusual for German authors in the nineteenth century to publish two papers on the same collection, and to indicate the same species as being "new" in both places. This has caused no little confusion among recent authors, and Berthold's 1845 species are usually attributed to the 1846 preprint (reprint?) of his "1847" paper; see Literature Cited for bibliographic details. Cochran and Goin (1970) and Silverstone (1975) are among the few recent authors who have correctly allocated the name *histrionicus* in a bibliographic sense, although they seem to have been unaware of Berthold's second, more detailed account.

[2]Copy in library of the Academy of Natural Sciences of Philadelphia.

[3]Copy in Department of Herpetology library, the American Museum of Natural History.

Subspecies. Authors of the older literature (e.g., Boulenger, 1913) were confused by variation in *Dendrobates histrionicus*, and were hampered by scarcity of specimens and lack of knowledge of South American zoogeography. *Dendrobates histrionicus* and its color varieties were considered as subspecies of *D. tinctorius* (Schneider), a distinctive and geographically remote species of the Guayana Shield. This now untenable arrangement was recently perpetuated in an important monograph on Colombian frogs, by authors who showed little interest in geographic ranges (Cochran and Goin, 1970).[1] *Dendrobates histrionicus* was properly treated as a full species by Funkhouser (1956), who added two subspecies names to several names coined by previous authors. Silverstone (1975) placed these various names in synonymy and treated *histrionicus* as a monotypic species, an action with which we are in complete accord, but not because we wish to appear "modern" by rushing to join the anti-subspecies movement.

Recent zoological literature shows an increasing trend away from the practice of applying infraspecific names. Part of this trend, without doubt, is a reaction against the application of subspecies concepts to poorly differentiated populations, or, worse, the frequency with which names have been given to one or a few specimens without prior knowledge of intraspecific variation. Misuse of a taxonomic category, however, is not sufficient to invalidate the category. One of us (Myers, 1974, pp. 13, 14) recently advocated a much reduced use of subspecies, and

[1]These authors only listed undigested locality records and seemed unaware of the importance of such formidable barriers as the Andean mountains. On page 40, for example, Cochran and Goin found it "interesting" that mainland specimens of *Phyllobates boulengeri* are identical with specimens from an adjacent coastal island, "instead of being more similar to *Phyllobates femoralis*, a species associated solely with the mainland." The authors simply failed to realize that *boulengeri* and *femoralis* are lowland species whose ranges are separated by the Andes. Such inattentiveness to zoogeographic clues also led Cochran and Goin to confuse two distinct species with the Amazonian *femoralis*: The Pacific lowland *Phyllobates aurotaenia* (Boulenger) was thus placed in synonymy (p. 40, without examination of specimens), and a possibly undescribed species close to *Dendrobates pictus* went undetected, although represented by specimens from the eastern slope of the inter-Andean Cauca Valley (p. 42, specimens purportedly from Caldas).

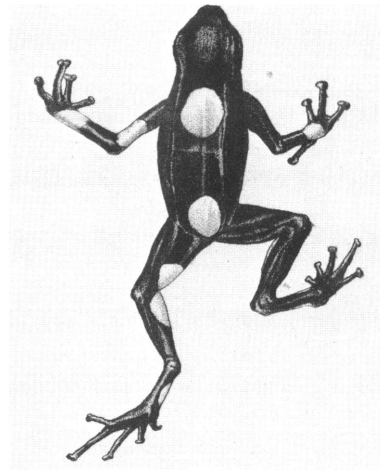


FIG. 6. Holotype of *Dendrobates histrionicus*, from unknown locality in western Colombia. Reproduced same size from Berthold (1846, pl. 1, fig. 8).

suggested criteria by which trinomens might be retained as a biological and nomenclatural convenience, particularly for "geographical units that are sufficiently distinct to be judged either as species or good (easily recognizable) subspecies, depending on the evidence." This statement relates mainly to the useful concept of "megasubspecies" recently discussed by Amadon and Short (In press), who, however, would also continue recognizing minor subspecies. Nonetheless, the subspecies is not an obligate taxonomic category and can be properly ignored by any worker who chooses to do so, but those authors who cavalierly dismiss the concept by implying that it is old-fashioned should re-examine their motives in adopting propaganda techniques. Certainly there are recent papers in which subspecies have been meaningfully applied in biogeographic and evolutionary studies of various kinds of organisms (e.g., from the South American literature: Vanzolini and Williams, 1970; Simpson, 1973; Brown and Benson, 1974; Haffer, 1974).

A definite drift away from subspecies is, however, evident in Neotropical herpetology, especially in lower Central America, where the trend can be dated from the 1960s, when investigators started analyzing intraspecific variation on the basis of reasonably large population samples

(e.g., Daly and Myers, 1967; Savage and Heyer, 1967; Trueb, 1968). It now occurs to us that the trend has perhaps been influenced as much by the animals as by preconceived philosophical convictions. It may be that amphibians and reptiles of this region simply are not often arranged in large, easily recognized geographic units that might *usefully* be called subspecies—but that the species more often show clinal variation, or some other pattern in which different characters do not show concordance. This is a testable hypothesis, and, if true, a historical explanation is likely to be found in the pronounced climatic changes of the Quaternary and in related faunal movements on the Central American land bridge. Our suggestion, then, is that subspecies (especially major ones) may be more applicable to some geographic regions and taxonomic groups than to others, and that workers should bear this in mind before engaging in polemics.

The reason for not recognizing subspecies in *Dendrobates histrionicus* is basically the same as that previously given for *D. pumilio*, namely that "a static subspecies concept does not seem . . . well suited to the dynamics of the situation" (Daly and Myers, 1967, p. 973). By this, we mean that interpopulational variation is largely microgeographic and mosaic and appears capable of rapid change. Spectacular differences, especially in coloration, occur from one local deme to the next, but attempting to give formal names to the countless populations would be an arbitrary, seemingly never-ending task that would contribute little or nothing to an understanding of variational processes.

It should be kept in mind that the following account and illustrations only sample and do not describe variation in *Dendrobates histrionicus*. These frogs have been noticeably different at every locality that we have sampled to date. The full extent of variation is likely to remain inadequately estimated, for such a goal would necessitate a difficult sampling program in many thousand square kilometers of rain forest, only a small part of which is accessible by road or major river.

SIZE AND PROPORTIONS

Two measurements were made to the nearest 0.1 mm., with dial calipers, on each of 483 preserved, adult specimens. Body length was measured from tip of the snout to the cloacal opening; the right tibia (shank) was measured from the heel to the fold of skin on the knee while the leg and foot were flexed. Body lengths (snout-vent) are compared directly, whereas tibia lengths were divided by snout-vent lengths and are compared as ratios. Sexes were distinguished by presence or absence of vocal slits and by dissection of selected specimens. Maturity of the smallest adults was judged from gross examination of the gonads and, in males, also by the presence of well-developed vocal slits. Juveniles of this species were infrequently encountered and are excluded from analysis because of the very small sample sizes.

Body lengths and relative tibia lengths are summarized for 12 samples in table 3. It was assumed ahead of time that sufficiently large samples would reveal statistically significant differences between almost any populations being compared but that biological significance could most safely be inferred from the available samples if there were relatively *large* differences in the characters themselves. Unless clines seemed evident, it was decided that interpopulational differences of less than 2 mm. mean snout-vent length were unlikely to be of biological significance. A significance level of 0.01 was selected prior to analysis, for the few *t*-tests that seemed warranted.

Sexual Dimorphism. The sexes have similar ranges in size, and mean body lengths usually differ by less than 1 mm. within a geographic sample (table 3). However, except in two samples, males do tend to be slightly larger than females. The situation is as follows in the three largest samples from table 3:

LOCALITY	N♂:♀	DIFFERENCE BETWEEN MEANS	*t*	*P*
– East bank, Pangala	115:60	0.82 mm. (♂ larger)	+2.961	< 0.01
G. Guayacana	72:43	0.13 mm. (♂ larger)	+0.562	> 0.05
E. Guanguí	24:32	0.43 mm. (♀ larger)	–1.247	> 0.2

TABLE 3
Adult Body Size and Relative Tibia Length in Twelve Samples of *Dendrobates histrionicus*

Locality[a]	N (♂:♀)	Snout-Vent Length (mm.)[b]		Tibia Length/Snout-Vent Length[b]	
		Males	Females	Males	Females
A. Santa Cecilia	12 (10:2)	30.50±0.28 (29.3-31.8)	30.25 (30.2-30.3)	0.395±0.005 (0.37-0.42)	0.370 (0.36-0.38)
B. Playa de Oro	28 (16:12)	33.98±0.38 (31.7-36.8)	33.97±0.35 (31.2-35.1)	0.382±0.005 (0.35-0.43)	0.375±0.003 (0.36-0.39)
C. Quebrada Vicordó	2 (0:2)	–	34.80 (34.6-35.0)	–	0.370 (0.36-0.38)
D. Quebrada Docordó	10 (8:2)	33.48±0.21 (32.5-34.1)	33.45 (33.3-33.6)	0.380±0.006 (0.36-0.41)	0.380 (0.38-0.38)
– East bank, Pangala	175 (115:60)	34.10±0.16 (28.2-37.8)	33.28±0.24 (27.3-36.0)	0.389±0.002 (0.35-0.44)	0.384±0.002 (0.36-0.43)
– West bank, Pangala	39 (27:12)	33.91±0.25 (31.4-36.8)	33.80±0.55 (29.3-35.9)	0.387±0.002 (0.36-0.41)	0.379±0.004 (0.36-0.40)
E. Quebrada Guanguí	56 (24:32)	33.30±0.34 (27.9-35.6)	33.73±0.18 (33.0-36.2)	0.392±0.003 (0.36-0.41)	0.380±0.002 (0.35-0.41)
F. Río Guapi	10 (6:4)	32.95±0.69 (30.4-34.4)	29.58 (27.0-34.2)	0.375±0.010 (0.35-0.42)	0.395 (0.37-0.41)
G. Guayacana	115 (72:43)	30.83±0.12 (27.3-32.8)	30.70±0.22 (24.6-32.4)	0.376±0.002 (0.33-0.42)	0.372±0.002 (0.34-0.40)
H. Río Baba	19 (15:4)	26.81±0.28 (24.9-28.2)	26.35 (25.4-28.3)	0.366±0.005 (0.34-0.40)	0.370 (0.37-0.37)
– 15 km. S Sto. Domingo	12 (9:3)	27.81±0.27 (26.2-28.8)	28.13 (27.2-28.9)	0.381±0.003 (0.37-0.40)	0.376 (0.37-0.39)
– Estac. Biol. Río Palenque	5 (3:2)	27.07 (25.9-27.7)	27.20 (26.9-27.5)	0.367 (0.36-0.38)	0.365 (0.36-0.37)
Total N and combined ranges	483 (305:178)	24.9-37.8	24.6-36.2	0.33-0.44	0.34-0.43

[a]Localities are listed from north to south; see Appendix 3 for list of specimens and more detailed locality data. The letters A-H correspond to localities on map 1 and representative frogs in pl. 1.

[b]Mean and standard error of mean, followed by range of variation in parentheses.

Practically speaking, there is no difference between the sexes, in spite of demonstrated statistical significance in the largest sample above. There is little variability in snout-vent length, in either male or female samples of the various populations of *Dendrobates histrionicus*; the range in coefficients of variation is 1.80-5.65 for the samples in table 3.

The tendency for male *histrionicus* to be as large as females, or even slightly larger, is of interest because it runs counter to the generality that female frogs are larger than males of the same species. In some groups (e.g., *Eleutherodactylus*), not only are females larger in average size, but the ranges of adult body sizes may even show little or no overlap, a condition obviously correlated with the different reproductive roles of males and females. Natural selection may have favored large size in male *Dendrobates histrionicus* because of the pronounced aggressive behavior, with the larger males being presumably better capable of defending territories and securing mates. This hypothesis might be tested indirectly by comparing sexual dimorphism in other species characterized by chirp calls and pronounced male aggressiveness, against species that presently seem less aggressive (see Vocalizations and Calling Behavior of *Dendrobates*).

Differences between the sexes in mean tibia/snout-vent length are in the range 0.000-0.025 for the samples in table 3. Males have slightly larger ratios (the converse only in samples F and H), but statistical significance was demonstrated only in sample E (Quebrada Guanguí), where $t = 3.272$, $P < 0.001$. The differences are too slight to be of taxonomic consequence, but a relatively longer tibia in males is suggested by the data. The range in coefficients of variation for this character is 2.36-6.40.

To the human eye, male and female *Dendrobates histrionicus* are virtually identical, not only in the two characters discussed above but also in other aspects of habitus that were not subjected to rigorous comparison. Polder (1973, p. 17) suggested that male *histrionicus* may have wider and differently shaped finger discs than those of females, but this does not seem to be so. Measurements were made with an ocular micrometer of disc and finger width of the 16 adult males and 12 adult females that comprise sample B; the greatest width of the disc on the longest (third) finger was measured at a right angle to axis of the finger, and the width of the adjacent (penultimate) phalange was measured from a dorsal aspect. Disc width is 1.4-1.8 mm. in males, 1.4-2.0 mm. in females. Means, standard errors, and ranges of disc width divided by finger width are 1.982±0.028 (1.88-2.25) in males, 2.037±0.047 (1.75-2.28) in females. In both sexes, therefore, the disc is about twice as wide as the adjacent part of the finger. The discs are less noticeably enlarged in juvenile *histrionicus*. Three unsexed juveniles (13.6-15.5 mm. snout-vent) from sample B have the discs only 1.20-1.25 times wider than the adjacent phalange. Possible geographic variation in disc width has not been tested for.

It will be noted from table 3 that males outnumber females in most samples. The reality of these unequal sex ratios has not been field tested, but there doubtless has been some sampling bias, in that males are more conspicuous and likely to be caught because they are audible and tend to call from elevated sites on logs and low vegetation (Silverstone, 1973, p. 297, and personal observ.). Despite the near absence of sexual dimorphism, the small female samples are not combined and it is primarily the males that are discussed in the following sections on interpopulational variation in body size and tibia length.

Geographic Variation in Body Size. Total range in snout-vent length of 305 adult males is 24.9-37.8 mm., with population means ranging from 26.81 to 34.10 mm. (table 3). Sample A on the upper Río San Juan (map 1) is comprised of significantly smaller frogs than sample B, from about 20 airline kilometers lower in the same drainage ($t = 4.852$, $P < 0.001$); there is a 3.48 mm. difference between the means of these two populations, and the ranges barely overlap. But along most of the Río San Juan, *Dendrobates histrionicus* appears quite uniform in average body size. Differences between means are less than 1 mm. (range of means 33.48-34.10 mm.) in the five samples from Playa de Oro to Pangala, an airline distance of nearly 150 km. from the upper to the lower Río San Juan. South of the San

Juan drainage, the remaining populations exhibit a north-south cline in decreasing body size, as follows:

E. Guanguí	33.30 mm.
F. Guapi	32.95
G. Guayacana	30.83
H. Río Baba	26.81

Thus, the largest frogs occur along the Río San Juan, except for the Santa Cecilia population of smaller frogs toward the headwaters of that river. The southern cline terminates in northwestern Ecuador, where *D. histrionicus* is found at its smallest adult body size. This pattern of geographic size variation is correlated with climatic changes, with the largest frogs occurring in the wettest, hottest part of the sampled range, namely in the Río San Juan basin. The Santa Cecilia population of small frogs on the upper San Juan does not seem anomalous in this regard: Santa Cecilia is approached by road through a dry, rain-shadow valley extending northwestward of Pueblorrico (map 1); Santa Cecilia, lower in the same drainage, is in a narrow valley of wet forest, but its proximity to the dry area suggests that it may receive less rainfall than localities in the main part of the San Juan basin. Reliable climatic data are lacking in most of the range of *D. histrionicus*, but there appears to be a decreasing amount of rainfall from the San Juan basin southward, with the relatively driest conditions occurring in northwestern Ecuador. This trend may be illustrated by comparing meteorological data from two Colombian stations within the range of *histrionicus*–Andagoya, Chocó (Río San Juan between localities B and C on map 1), and Guayacana, Nariño (locality G); data are from West (1957, pp. 30, 31).

STATION	AVERAGE ANNUAL RAINFALL	RAINFALL IN DRIEST MONTH
Andagoya	7,028.7 mm.	> 500 mm.
Guayacana	6,542.4 mm.	< 400 mm.

Seasonal variability in precipitation would seem of greater importance than total annual amounts. West (*loc. cit.*) indicated monthly rainfall averages of about 520-650 mm. for Andagoya, and a much greater monthly range of about 375-800 mm. for the relatively drier Guayacana locality (based on records for 33 and six years, respectively). According to West (*op. cit.*), mean monthly temperature shows a remarkably low seasonal range of usually less than 1°C. at most localities, with the hottest part of the western Colombian lowlands corresponding to the Río Atrato-Río San Juan depression. The range in mean temperature between coolest and hottest months is 26.8-27.6°C. for Andagoya, and 24.2-25.3°C. for Guayacana.

Geographic Variation in Relative Tibia Length. The range of means for tibia length/body length, in adult males, is 0.366-0.395 (table 3). The larger frogs have relatively longer tibiae than those of the smaller frogs although the correlation is not perfect. There is little variation in the Río San Juan basin (means, 0.380-0.395). The highest ratio occurs in the Santa Cecilia population of relatively small frogs on the upper Río San Juan, but this mean is not significantly different from that of the larger frogs downstream at Playa de Oro ($t = 1.734$, $P = 0.1$). The second highest mean ratio (0.392) occurs far south of the San Juan basin, at Quebrada Guanguí, but this is not significantly different from the San Juan frogs (e.g., compared with Pangala, east bank: $t = 0.815$, $P > 0.4$). South of Quebrada Guanguí, the frogs have smaller body sizes and also relatively shorter tibiae (e.g., Guanguí compared with Guayacana: $t = 4.213$, $P < 0.001$).

Thus, there appears to be a broad regional differentiation, with the large frogs from Guanguí north having relatively long tibiae (means, 0.380-0.395), and the smaller frogs from Guapi south having relatively short tibiae (0.366-0.381). Comparison of larger samples within the two regions probably would document some degree of mosaic or even clinal variation in tibia length, but based on present samples the interpopulational differences are slight. There is no statistical difference, for example, between the two extreme means in the southern group ($t = 2.210$, $P > 0.02$).

Cochran and Goin (1970, pp. 30, 32), on the basis of few measurements, suggested that tibia length might differ between the "subspecies" of Colombian *Dendrobates tinctorius* (= *histrionicus*). But tibia size in *histrionicus* is not an independent variable, being partly correlated with interpopulational differences in absolute

body size, and, in any case, geographic variation in relative tibia length seems too slight for this character to be of much taxonomic value.

COLOR AND PATTERN

The following descriptions of color and color pattern are based on field notes, color transparencies, and reference to the museum voucher specimens listed in Appendix 3. Most specimens collected were skinned for the biochemical analyses, but, at each locality, efforts were made to preserve sufficient specimens for showing the range of observed variation in color pattern. Because the preserved samples are sometimes small and often are biased so as to include as much variability as possible, statements about relative abundance of particular patterns are based almost solely on notes made in the field. "Typically" colored specimens from localities A-H are shown in color plate 1, and various aspects of variation are demonstrated in the black and white text figures. No sexual dimorphism has been noted in color pattern, except that the ventral throat surfaces of some live specimens (presumed males) are suffused with blackish gray. See Silverstone (1975, pp. 19-23) for an additional account of color pattern variation in *Dendrobates histrionicus.*

A. *Santa Cecilia.* Frogs at this locality have light brown to black bodies (brown in most) and usually a large orange or red-orange spot high on the back (pl. 1, fig. A). The vivid dorsal spot tends to be rimmed in black and usually is round or slightly oval, but it is elongately ovoid in one preserved specimen, and in another is very small, not much larger than the eye. The dorsal marking is absent in one individual, which had in place of the spot only a slight suffusion of red that is not evident in the photograph (fig. 7C). There is a strong tendency for orange bracelets (often broken) to be present on the lower arm, shank, and basal part of the foot, but not on the upper arm or thigh, although some specimens have a spot on the underside or rear of the thigh. Venters are more extensively colored than the dorsums; most specimens have a large orange patch on the throat and a larger one on the belly, and in some individuals these two markings are fused.

The dorsal spot and limb markings in this population are very similar to those of the holo-

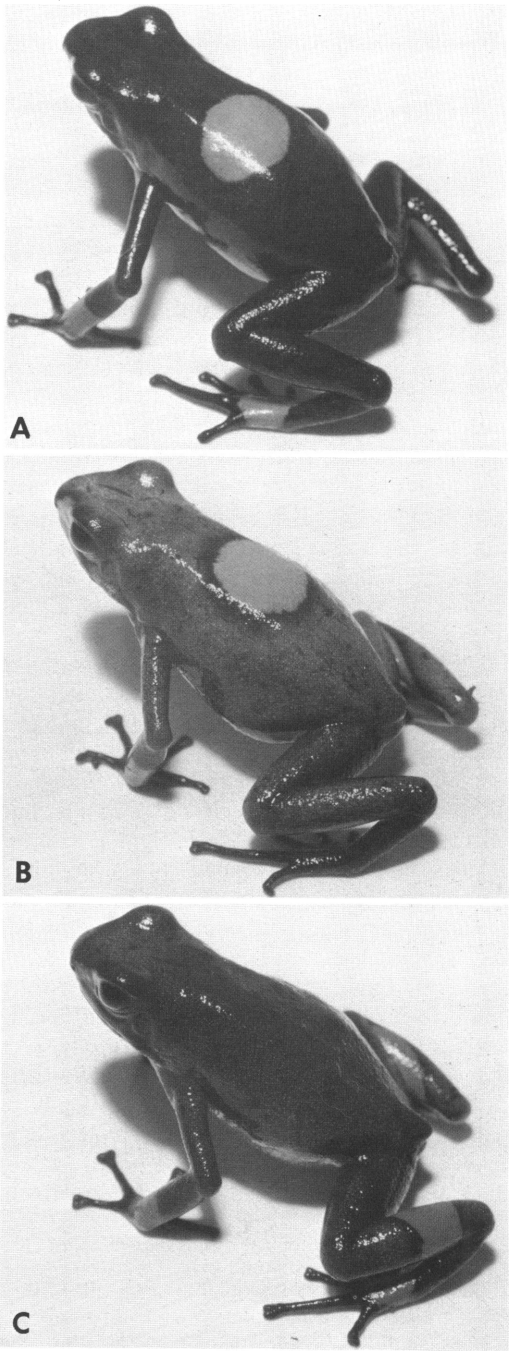


FIG. 7. *Dendrobates histrionicus.* Intrapopulational variation near Santa Cecilia, upper Río San Juan, Colombia (map 1, loc. A). A. AMNH 85161, black with red-orange spot. B. AMNH 85159, brown with orange spot (see also pl. 1, fig. A). C. AMNH 85160, black, spot absent.

type (fig. 6), which, however, has an additional spot in the sacral region and a bracelet above the elbow of the left arm. Polder (1974b, p. 328) published a colored photograph of a similarly patterned frog (locality unknown), which differs from Santa Cecilia frogs in having yellow (instead of orange) markings, including a yellow snout tip. Cochran and Goin (1970, pl. 4 a-c) pictured a preserved specimen which, dorsally and ventrally, matches the common pattern in the Santa Cecilia population, but this specimen (USNM 13977) is purported to have come from "75 miles south of Andagoya," which seems so unlikely that the actual origin of the specimen should be considered as unknown.[1] Silverstone (1975) has likened a slightly different color pattern to Berthold's type specimen, but in these frogs (from the Río Arquí, upper Atrato drainage) the dorsal spot is situated on the head and there are large lateral spots (Silverstone, *op. cit.*, p. 22 ["subpattern 3a"], figs. 13 H, L, and frontisp. 1 [top center, in color]).

B. *Playa de Oro. Dendrobates histrionicus* at this locality is notable for great variability and relative lack of conspicuousness in the color pattern (fig. 8). The basic pattern of most observed individuals consists of large orange or yellow spots and limb bracelets on a background of light or dark brown (plate 1, fig. B), with the markings distributed as follows: (1) A large oval spot on each side of body; (2) one or two smaller spots atop the head, and sometimes one on the back; (3) bracelets or spots on lower and/or upper arm, thigh, shank, and basal part of foot. In pure form and on a dark ground, the aforesaid markings make a conspicuous frog (e.g., fig. 8A), but few such individuals were seen; usually there is a mottling of black pigment that tends to break up the pattern and make it less conspicuous (fig. 8B, C). Another common pattern type is that of a brown frog with darker brown, poorly defined spots or blotches, and a corresponding tendency toward loss of the bright limb bracelets (fig. 8E-G; for color illus. see Daly et al., 1971, fig. G). Extreme and less common types included a nearly uniform brown pattern (fig. 8H), and a strongly reticulate pattern of dark pigment on a pale ground (fig. 8D). Venters tend to be uniform black or blackish brown, with slight, faded orange blotches in a few specimens.

The preceding description of Playa de Oro pattern variation is based on specimens from the proximity of the south bank of the Río San Juan. A small sample from about 1.5 km. inland (south) did not include the common riverbank types of pattern: The flanks of these specimens lack a discrete, large spot, but instead are overall yellowish, with variable amounts of brown markings; the dorsums are brown, with indefinite black smudging and contrasting yellow or pale orange spots or blotches (fig. 9A-C; for color illus. see Daly et al., 1971, pl. 1, fig. E). This kind of pattern seemed to occur near the riverbank only as a rare variant (fig. 8D).

Interdeme variation in color pattern of *Dendrobates histrionicus* may be extensive along the upper Río San Juan, and we have possibly mixed specimens from more than one deme in our "population" sample from the riverbank area. Restrictions to gene flow may occur because of frequent areas of swampy forest, which seem to be usually avoided by *histrionicus*; most specimens were found in relatively well-drained situations on low ridges and along river and stream banks. Only a few specimens were obtained while walking the trail between Santa Cecilia and Playa de Oro (i.e., between localities A and B, map 1). These specimens probably represent several demes, but they most closely resemble the Playa de Oro population in body size (32.3-35.5 mm., N=5) and color pattern (fig. 9D-G).

C. *Quebrada Vicordó.* The few frogs obtained at this locality had large, bright red or orange spots on a blackish brown ground color (pl. 1, fig. C). The four or five dorsal head and body spots are arranged in a median line. There are one or two lateral body spots and another in the tympanic region. There is a bracelet-like spot on the upper and lower segment of each limb and another atop the hind foot. Ventrally, there tends to be an orange spot across the throat and

[1]Whether by airline or river distance, 75 miles (120 km.) south of Andagoya would place the locality somewhere in the lower Río San Juan drainage, near other, more convenient reference points. We are not aware that the collector (M. Latham) traveled on the lower San Juan, but we do know that she stayed at the Andagoya mining camp, on her way from the nearby Condoto landing strip to Playa de Oro. She spent some time at Playa de Oro purchasing specimens, including, apparently, some specimens brought downriver (from the direction of Santa Cecilia) by Indians.

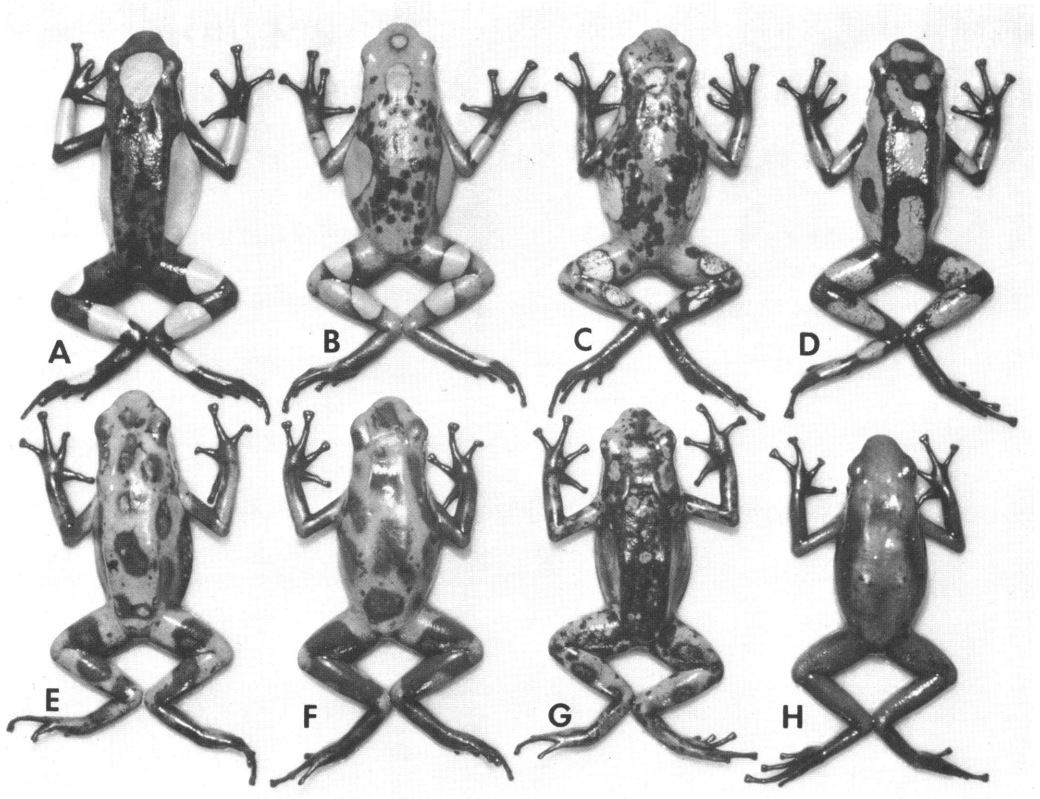


FIG. 8. *Dendrobates histrionicus.* Intrapopulational variation 2 km. above Playa de Oro, south bank upper Río San Juan, Colombia (map 1, loc. B). Top row, left to right, AMNH 86957-86960. Bottom row, AMNH 86961-86964.

one large or several smaller spots on the chest.

D. *Quebrada Docordó.* Frogs at this locality are black (not blackish brown as above), with bright orange or reddish orange spots. Interdeme variation in size and number of the spots is striking, giving quite different aspects to frogs from the north and south sides of the Docordó River, a small tributary of the Río San Juan (map 1 and fig. 10).

Frogs from a low ridge on the north side are rather similar to those described above from Quebrada Vicordó, except that the dorsal spots are more numerous in some individuals and are not arranged in a median line (fig. 10A; for color illus. of another specimen, see Daly et al., 1971, pl. 1, fig. C). There are about three to 20 small to moderate-sized dorsal spots (some or all of which are noticeably larger than the largest spots on specimens from the opposite bank, see below and fig. 10); there is a larger spot or blotch on the side of the body and one or two spots on the lower side of the head. Usually there is a bracelet-like spot on the upper and lower segment of each limb and another atop the hind foot, but these markings do not completely encircle the limbs. Ventral surfaces vary from nearly uniform black to extensively marked with large reddish orange spots, especially under the head and on the chest.

Frogs from a low ridge on the south side of the Docordó River are remarkably different in that the body and upper limb surfaces are densely patterned with small orange spots (pl. 1, fig. D; fig. 10B; also Daly et al., 1971, pl. 1, fig. D). Very few of the dorsal body spots approach the size of the eye or tympanum. There is a tendency for somewhat larger spots on the flanks and dorsal limb surfaces, but there is no

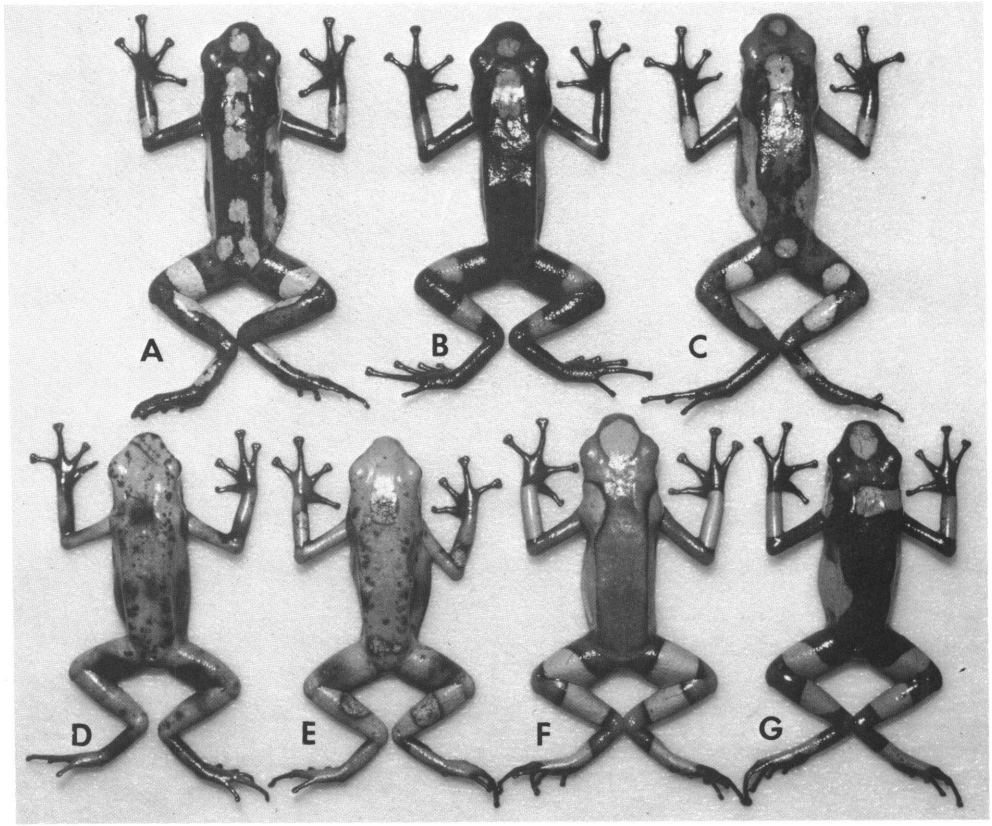


FIG. 9. *Dendrobates histrionicus.* Intrapopulational and microgeographic variation on upper Río San Juan, Colombia.

Top row, left to right, AMNH 85178-85180; these frogs are from locality B (map 1), but were collected about 1.5 km. south (inland) of those shown in figure 8.

Bottom row, AMNH 85186-85189, from trail between Guarato and Playa de Oro (between locs. A and B, map 1).

appearance of bracelet-like markings on the limbs. Field notes indicate that the bright spots are less reddish than in specimens from the opposite bank, but the color differences are slight and are little evident in color transparencies. The ventral surfaces are extensively mottled or spotted with orange, the markings being several times larger than those on the dorsum.

Pangala. Among collections made for the American Museum by Borys Malkin is a large series of *Dendrobates histrionicus* from this locality on the lower Río San Juan (map 1). Most specimens were caught by Chocó Indians, on both the east and west banks of the river, which, because of its width, is presumably a major obstacle to gene flow between populations of the terrestrial *histrionicus.* Malkin noted the riverside origins of more than 200 specimens, which form the basis of this account. The preserved specimens are black, with vivid pinkish or white markings, which were reddish in life. Variation in color pattern is remarkably like that described above for Quebrada Docordó.

Specimens from the east side of the Río San Juan are reminiscent of the population on the north side of Quebrada Docordó, nearly 50 km. to the north (map 1). Many specimens, in fact,

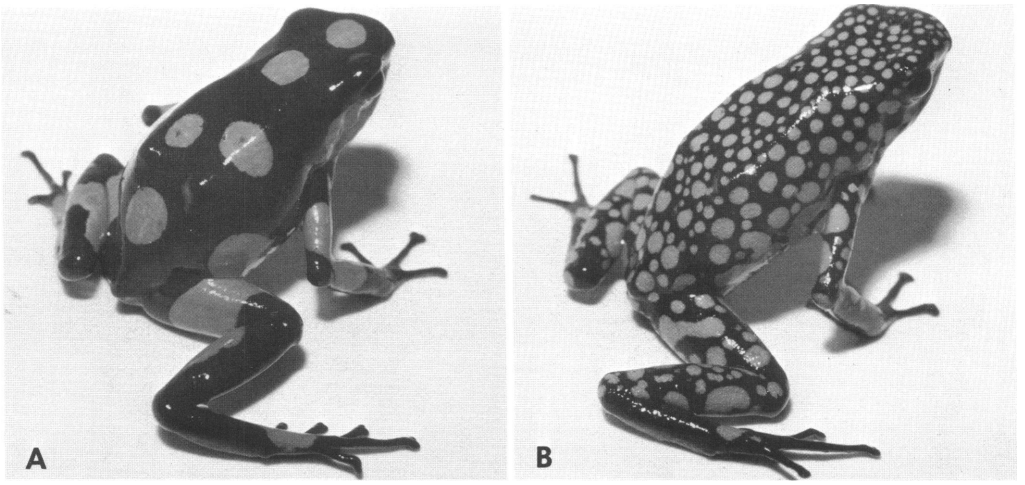


FIG. 10. *Dendrobates histrionicus.* Microgeographic variation at Quebrada Docordó, middle Río San Juan drainage, Colombia (map 1, loc. D). These frogs are representative of two populations separated only by a small river. A. North side Quebrada Docordó, AMNH 86985. B. South side, AMNH 86979 (also pl. 1, fig. D).

are quite similar to the Docordó specimen in figure 10A; pattern variation in this east-bank Pangala sample is, however, much greater, which may be due to genetic influence from different demes as well as to larger sample size. Some individuals have the dorsal spots arranged in a median row, like the specimen in plate 1 (fig. C) from Quebrada Vicordó, whereas other specimens have the body markings strongly coalesced to produce striking and varied reticulated patterns. Most specimens have bracelet-like spots on the upper and lower segment of each limb and atop the hind foot, but these markings also are frequently coalesced, making the limbs of some specimens more white (reddish) than black in dorsal view; the dorsal limb markings tend to partly encircle the limbs, but they rarely form complete bracelets. Venters are extensively spotted or mottled, or, in some specimens, there is a wide, transverse reddish band across the belly, and sometimes another band across the throat.

Pangala specimens from the west side of the Río San Juan are like frogs from the south bank of Quebrada Docordó (see fig. 10B). They are densely patterned with small spots on both body and limbs, which lack bracelet-like markings. There also is good correspondence in ventral pattern. In fact, the resemblance is so great between west-bank Pangala and south-bank Docordó populations that the specimens give the appearance of having been drawn from the same inbreeding population. In most species, such resemblances would lead one to hypothesize a geographical continuity in the color pattern, but such speculation is unwise in the case of *Dendrobates histrionicus*!

E. *Quebrada Guanguí.* Specimens from this locality resemble no other population known to us. Color variation is extraordinary. The frogs are basically yellow, orange, or orange-red—with highly irregular black mottling and scratchlike markings that vary from extensive (usually) to slight. Large yellow spots often occur on the basically orange frogs (e.g., pl. 1, fig. E), and orange suffusions often occur on the basically yellow frogs. The limbs are colored like the bodies, and only rarely (e.g., fig. 10A) might the light areas be compared with the bright bracelets that adorn the limbs of some northern *histrionicus.* The digital discs often are whitish above but not conspicuously so. Ventral surfaces are colored much like the dorsums, but vary from almost unicolor orange to almost solid black, although there often is less black than on the dorsum.

An appreciation of the extent of variation will

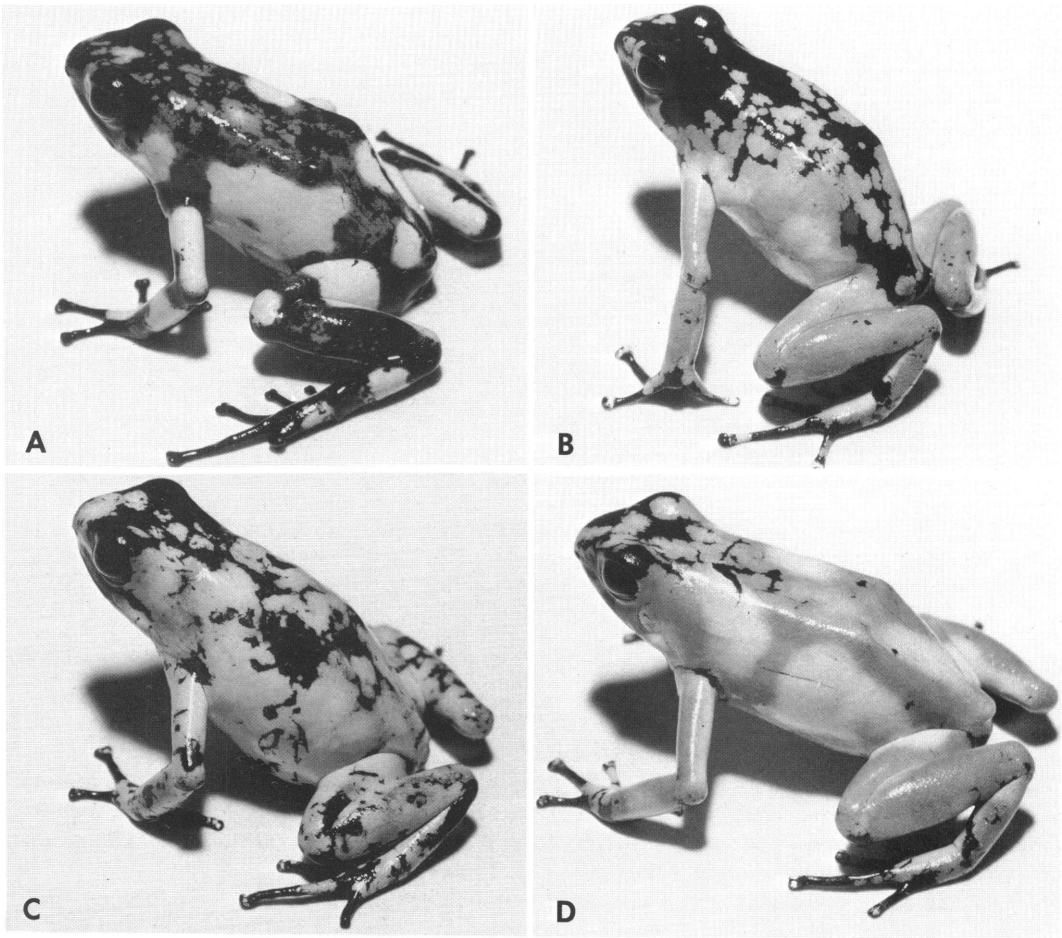


FIG. 11. *Dendrobates histrionicus.* Intrapopulational variation at Quebrada Guanguí, Colombia (map 1, loc. E). A. AMNH 88845. B. AMNH 88843. C. AMNH 88841. D. AMNH 88842.

be gained by comparing the following descriptions with the photographs of specimens A-D in figure 11; the orange and yellow pigmentations may be visualized by first looking at plate 1, figure E: Specimen A is basically golden yellow, except for an extensive orange suffusion that encloses the dorsal black mottling and which also appears in the black area below the left knee. Specimen B is mostly bright orange, with extensive black dorsal mottling and a splotch of pale yellow on the flank and in the axillary region. Specimen C, also heavily black mottled, is basically golden yellow, but with widespread suffusions of very pale orange (seen as slightly darker areas in the photograph). Specimen D, which has very little black, is bright orange with large blotches of golden yellow.

Dendrobates histrionicus was too thinly spread in the Guanguí forest for us to associate color differences with possible interdeme variation; all of the specimens, however, were taken within a few hours walk from our base camp.

F. *Río Guapi.* Frogs at this locality were obtained within several hundred feet of one another and probably represent a single deme. They are basically orange-red, usually with a rather heavy amount of irregular black marbling

(pl. 1, fig. F). The orange tends to give way to a light golden hue on the hands and feet. Ventral surfaces are nearly the same orange hue as the dorsum, but appear brighter because of a reduced amount of black pigmentation.

Geographically, the Guapi sample of *histrionicus* is closest to the Guanguí population, but we were surprised to discover that Guapi frogs most nearly resemble those from Guayacana, far to the south (map 1).

G. *Guayacana.* Several thousand specimens from this locality were processed for skin toxins, and the preserved sample is quite representative. Most of the frogs are basically reddish orange, in a normal range of bright orange to bright red; rare individuals are dull yellow or golden brown. Frequently there are varying amounts of yellow or golden yellow on the limbs, giving some orange frogs a bicolored appearance. All specimens have black markings, although rare individuals are nearly unicolor orange or red, with only slight black flecking (fig. 12B; same specimen pictured in color in Daly et al., 1971, pl. 1, fig. B). Black pigmentation usually shows as a poorly defined reticulum, with scratchlike markings and some pigment clumping being common (pl. 1, fig. G; fig. 12A). Occasionally, the black pigment is extensively coalesced as large blotches, giving one rare variant a distinctive cap (fig. 12C). Ventral surfaces are colored like the dorsum, except that distribution of the black pigment tends to be more irregular.

H. *Río Baba, 5-10 km. SSW Santo Domingo.* Most frogs in this population are dull yellow or orangish yellow, with a gray reticulum (pl. 1, fig. H[1]). The gray reticulum varies from distinct to vague, as may be seen in figure 13. Extreme variants included a few that were basically bright yellow or bright orange, and two rare variants that were orange-red. One of the yellow specimens had the gray reticulum suffused with orange. One of the orange-red variants (fig. 13C) has a vague gray reticulum, but the other specimen (fig. 13D) is more mottled and has a black cap similar to rare variants in some Colombian populations (figs. 11B, 12C). Some individuals

[1]This specimen was orangish yellow in life, but defective film emulsion rendered the transparency orange-red. Partial color correction of figure H was done by dot etching.

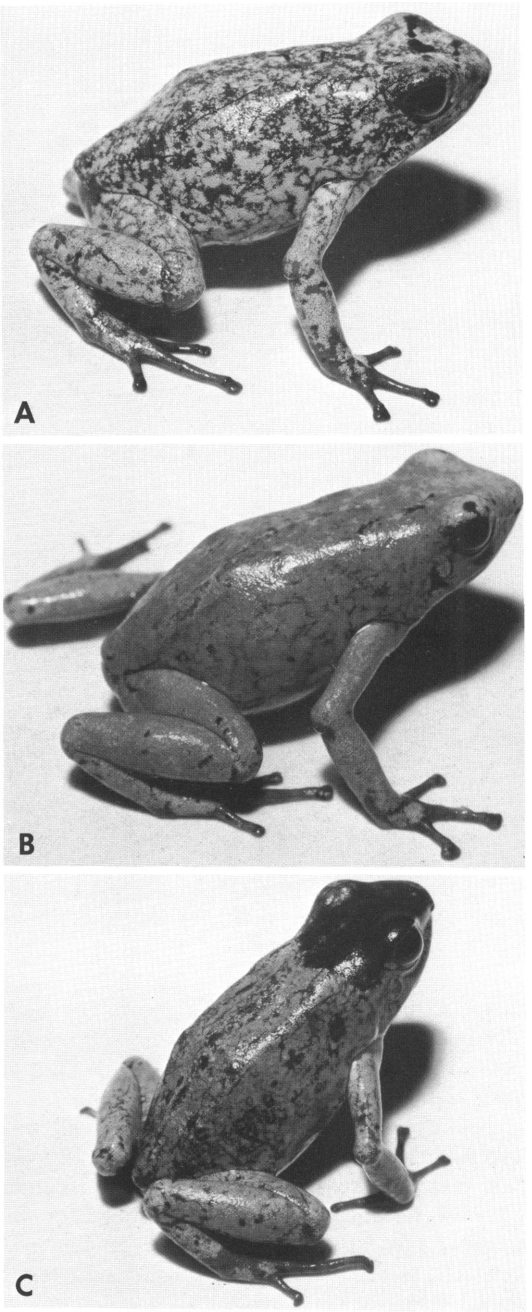


FIG. 12. *Dendrobates histrionicus.* Intrapopulational variation at Guayacana, Colombia (map 1, loc. G). A. AMNH 85049, usual pattern. B. AMNH 85048, rare, nearly unicolored variant. C. AMNH 85050, rare variant with black cap.

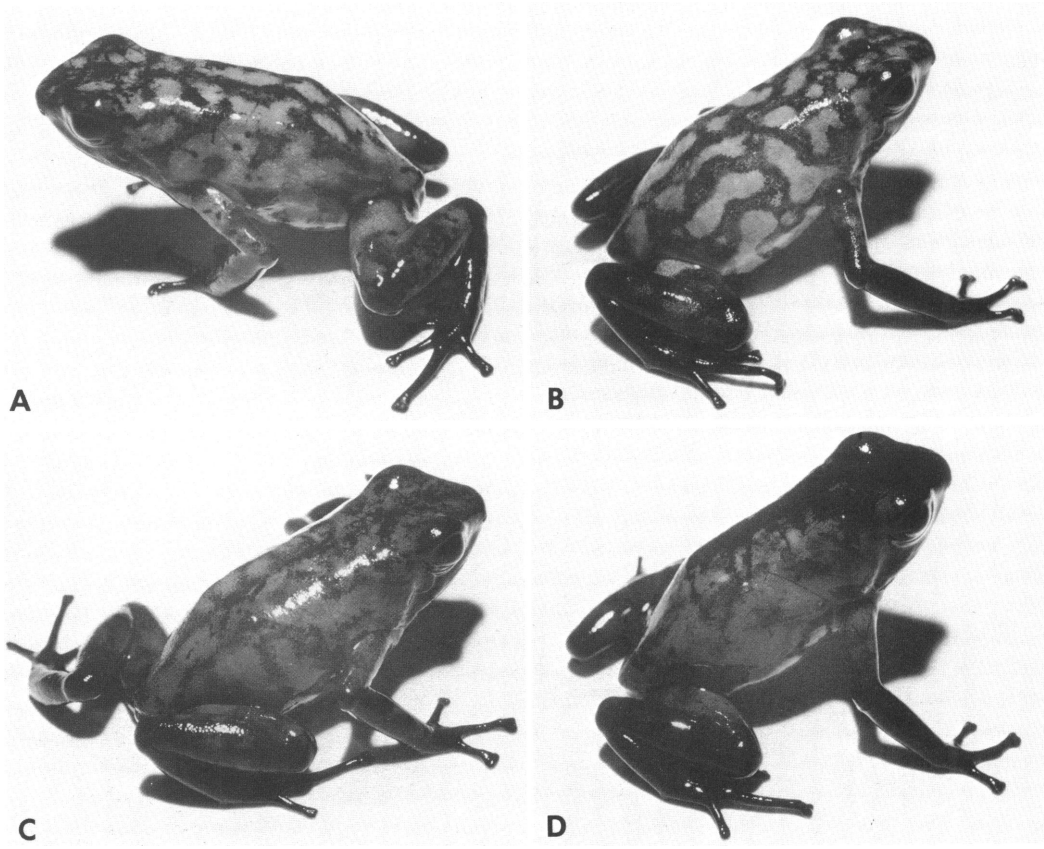


FIG. 13. *Dendrobates histrionicus.* Intrapopulational variation at Río Baba, near Santo Domingo, Ecuador (map 1, loc. H). A. AMNH 89579 (also pl. 1, fig. H). B. AMNH 89581. C. AMNH 89582. D. AMNH 89580.

have all black limbs, but, on most, the body coloring extends at least to the elbow and knee. Ventral surfaces are similar to the dorsum in color and pattern.

The above description, and the small preserved sample, was drawn from a sample of more than 400 specimens. We were unable to designate a precise distance from Santo Domingo for this locality, but all specimens were caught in a restricted area of forest and probably represent a single deme.

15 km. S Santo Domingo. Most individuals in the small sample are some shade of red, varying from orange-red to dull rusty red, these colors usually showing as discrete spots within a gray reticulum (fig. 14B). A few specimens tend toward a uniform reddish color, with the dark reticulum showing only as an indefinite blackish suffusion. The belly is reddish and there are reddish spots on the throat, but otherwise the ventral surfaces, including the chest, are black. One individual also had a scattering of pale copper flecks on the venter.

Two individuals stood out from the above sample, in possessing gray and green coloring that we have not seen elsewhere in *Dendrobates histrionicus.* One of these frogs (fig. 14C) was black, with poorly defined small blotches of *silvery gray* over all dorsal surfaces (including the limbs), these blotches becoming coalesced and larger on the flanks and on the chest and belly; ventral surfaces were otherwise black, except for a few small silvery gray blotches underneath the limbs and sides of the throat. The other frog (fig. 14D)

was *olive-green* dorsally, with an ill-defined black reticulum; ventral surfaces were black, with silvery gray blotches on throat, chest and belly, and some olive-green color along the sides of belly.

This sample of frogs was sent to us by an American dealer, who provided information that they had been collected "about 10 miles south of Santo Domingo de los Colorados, in banana plantation." This population is of interest in providing apparently the first specimens of *Dendrobates histrionicus* with gray and green pigmentation, but we were unsuccessful in trying to rediscover the population in the extensive banana plantations south of Santo Domingo. It is our impression that *histrionicus* avoids banana plantations except in areas adjacent to standing forest, which has been extensively destroyed in the Santo Domingo region.

Estación Biologica Río Palenque. This locality, about 40 km. south-southwest of Santo Domingo, lies near the southern limits of the rain-forest habitat of *Dendrobates histrionicus*; the rain forest gives way to seasonally dry forest in the region of Quevedo, on the road to Guayaquil. *Dendrobates histrionicus* is abundant at the biology station, but only a small sample was collected. The frogs have a variable blackish gray reticulum, with spots of yellowish orange, yellow, or dull orange (fig. 14A); the dorsal color

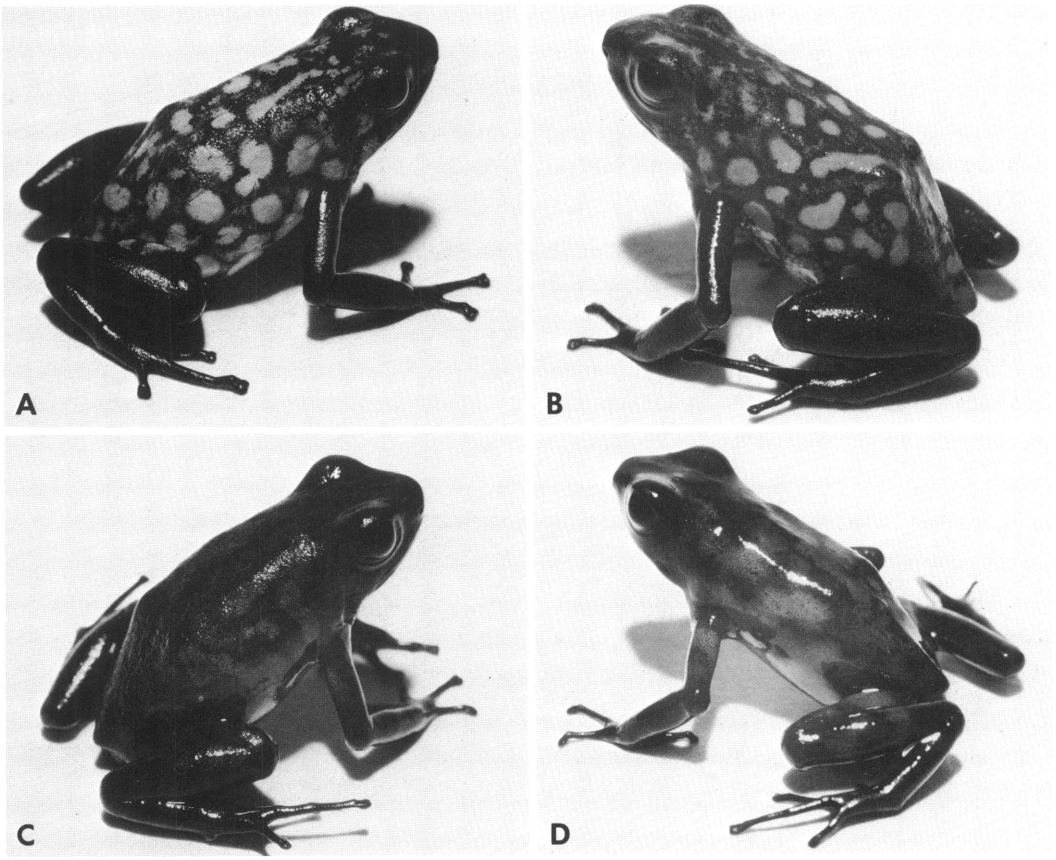


FIG. 14. *Dendrobates histrionicus* from northwestern Ecuador (see also fig. 13). A. Specimen (AMNH 89574) from Estación Biologica Río Palenque, about 40 km. SSW Santo Domingo, near southern terminus of range. B-D. Intrapopulational variation in population about 15 km. S Santo Domingo (B. AMNH 88207. C. AMNH 88211. D. AMNH 88210.)

pattern extends ventrally onto the chest and belly, and, in some individuals, onto the black throat.

ESCAPE BEHAVIOR

We previously (Daly et al., 1971) mentioned the existence of interpopulational differences in escape behavior of *Dendrobates histrionicus*, and take this occasion to elaborate on our impressions. Escape behavior also has been described for a population of *histrionicus* in the Serranía de Baudó, northwestern Colombia, in the only published behavioral study of this species (Silverstone, 1973).

In most populations, *Dendrobates histrionicus* is a conspicuously colored frog that is readily visible to diurnal predators. The contrasting color patterns in some populations (pl. 1) possibly make the frogs recognizable even to certain predators that lack color vision. The possibility that coloration also might serve in intraspecific recognition cannot be ruled out (Daly and Myers, 1967, p. 972), and, indeed, there is some indication for this in *Colostethus*, at least in species that are sexually dimorphic in ventral color (Dole and Durant, 1974). Nonetheless, it is a reasonable hypothesis that the brilliant pigmentation and bold patterns in *Dendrobates* serve as warning coloration that evolved in conjunction with the skin toxins, which are released upon injury and which are distasteful at least to man and to some snakes (but not all, unpubl. observ.). The hypothesis of warning coloration in *Dendrobates histrionicus* is supported by the relatively unhurried escape behavior that we have observed in most populations. In populations A, C, D, E, and H, and probably G (limited observ.), the frogs are relatively bold and can be approached closely before they try to escape. There are two principal methods of escape in these populations, and, although there is overlap, the two strategies seem generally to characterize different populations: (1) In some populations, notably E and D, the frogs attempt to escape simply by hopping away and, in our experience, they seldom seek refuge under debris on the forest floor. (2) In other populations, notably H, the frogs will let a slow-moving intruder approach within a meter or less, but will then drop from their low perches and hide in the litter, under logs, or in bases of trees. Strategy number 2 corresponds with Silverstone's (1973, p. 297) observations in the Serranía de Baudó. Silverstone also perceived that "Individuals in elevated positions never escaped by climbing, but always dropped to the ground to seek refuge." This is true also in our experience, except that frogs in strategy 1 populations tend merely to hop away after reaching the ground.

In contrast to the above, we have noticed that frogs in two populations (B and F) are more secretive. In both cases, the escape behavior may be classified as strategy 2 (hiding), but the tendency is for the frogs to take cover *before* an intruder approaches within grabbing distance. This was most evident at locality F (Río Guapi), where our field notes described the frogs as being "*very* shy (unlike the Guanguí [E] population)." These frogs were calling from low elevations in the usual manner, but when they sensed the approach of a still distant intruder they quit calling and could sometimes be seen leaving their perches with apparent stealth and crawling down into litter or underneath logs. The escape behavior possibly was initiated by auditory as well as visual stimuli (it was difficult to move quietly in parts of this dense forest), and, in any case, some of the frogs seemed to sense our approach from a distance of nearly 10 meters.

Frogs at locality B (Playa de Oro) could be approached more closely, but many individuals still seemed to seek cover more quickly than at some other localities (H, for example), and certainly they were more secretive and harder to catch than in strategy 1 (hop-away) populations. Our unverified impression was that most frogs at Playa de Oro probably also slept under cover, although one was found at night on a leaf several centimeters above ground. Less time was spent in night collecting at locality D (strategy 1 population), where three specimens were found sleeping on leaves about 0.3-0.6 meters aboveground. As previously indicated (see also fig. 8), the color pattern of Playa de Oro *histrionicus* is extremely variable and, in most specimens, not very conspicuous. It is difficult to imagine such variable and disruptive patterns as serving a warning function, and we have suggested (Daly et al., 1971, p. 1872) that natural selection in this population has shifted toward concealing

coloration and correspondingly cryptic behavior.

It should be clear that the above statements are based on general collecting impressions rather than on the quantified data that would be desirable in a serious behavioral study. Nonetheless, whatever the reliability of our generalizations about particular populations, it seems evident that geographic variation in *Dendrobates histrionicus* extends to behavioral differences. Similar interpopulational variation in escape behavior (and also differences in arboreal tendencies) have been noted for Panamanian populations of *Dendrobates pumilio* (*fide* Daly and Myers, 1967, p. 970; and subsequent unpubl. data).

SKIN TOXINS

Comparison of the skin toxins in different populations of *Dendrobates histrionicus* reveals that the populations, as a whole, form a rather coherent group, especially when compared with other species. An initial appreciation of this can be visualized by examination of the histograms in figure 3, where it can be seen that the *histrionicus* populations show a clustering of compounds in the range of protonated molecular ions at 284-292 (molecular weights = 283-291)—these are the histrionicotoxins (table 1), and each population sample contains several kinds, even though the relative proportions differ markedly. This repeated occurrence of histrionicotoxins and the apparent absence of higher molecular-weight alkaloids provide a measure of biochemical similarity among the populations of *D. histrionicus*. The dissimilarity among the *histrionicus* populations is most evident toward the left side of the histograms in figure 3, where considerable variability is seen in the presence or absence of significant amounts of lower weight alkaloids. Of these, the alkaloids with protonated molecular ions at 220, 240, and 244 are most commonly present as major constituents. Few structural data are yet available for the lower molecular-weight alkaloids, but preliminary data suggest that at least some of these compounds have the same basic spiropiperidine ring system as the histrionicotoxins but in certain cases (220, 244) lack the hydroxyl group.

Interpopulational differences in the skin toxins of *Dendrobates histrionicus* can therefore be discussed on the basis of the amounts and kinds of detectable histrionicotoxins and lower molecular-weight alkaloids. For this discussion, we rely chiefly on combined gas chromatographic-mass spectral analysis, which extends and confirms in a more quantitative and reproducible way the data previously attained by various other mass spectral analyses and summarized in figures 2 and 3. The gas chromatographic profiles are grouped in figure 15 and form the basis for an approximation of interpopulational variation in "major" and "minor" alkaloids, as summarized in table 4.

Examination of table 4 reveals that only three compounds (histrionicotoxin, isodihydrohistrionicotoxin, allodihydrohistrionicotoxin) are universally present as constituents of the alkaloid fraction. Each population sample of *Dendrobates histrionicus* contains only 8-10 (mean, 9.2) of the 19 compounds detected in all samples combined. Some compounds show obvious geographic correlation—for example, octahydrohistrionicotoxin, which was detected only in the southern populations. Other compounds (e.g., neodihydrohistrionicotoxin) show no definite geographic trends. It must be remembered, however, that failure to detect a compound does not prove its absence, because detection of trace amounts is dependent on sample size. Therefore, not much reliance can be placed on simple present-or-absent comparisons for *single* alkaloids, nor do we know enough to fully assess the importance of the rough quantitative comparisons ("major" vs. "minor" alkaloids). To avoid some of this kind ot bias, and to search for geographic patterns, we have reduced the data to numerical terms and have compared numbers of alkaloids shared by pairs of populations. An alkaloid similarity value, for a pair of populations, is readily calculated by the formula $100\ C/N_1$, where C represents the number of shared alkaloids and N_1 is the number of alkaloids in the population sample containing the smaller number of compounds. Thus, $100\ C/N_1$ gives the percentage of alkaloids of the N_1 sample also present in the other sample. Zoogeographers will recognize this formula as one of several used in measuring faunal resemblance. It was selected over other similarity coefficients for reasons given by Simpson (1960).

The paired comparisons of numbers of shared

TABLE 4
Interpopulational Variation in Skin Alkaloids of *Dendrobates histrionicus*
(++ = Present as Major Compound; + = Minor Compound)[a]

Alkaloid	Populations[b] A	B	C	D	E	F	G	H	I
HISTRIONICOTOXINS[c]									
1) Histrionicotoxin (284)	++	++	++	++	++	++	++	++	++
2) Isodihydrohistrionicotoxin (286)	++	++	++	++	++	++	++	++	++
3) Neodihydrohistrionicotoxin (286)	–	+	–	–	+	–	+	+	–
4) Allodihydrohistrionicotoxin (286)	+	+	++	+	+	+	++	++	+
5) Isotetrahydrohistrionicotoxin (288)	+	+	+	+	+	+	+	–	–
6) Tetrahydrohistrionicotoxin (288)	+	–	+	+	–	–	+	+	+
7) Octahydrohistrionicotoxin (292)	–	–	–	–	–	+	+	++	+
OTHER ALKALOIDS[d]									
8) HTX-D (288)	+	+	–	–	–	+	+	–	–
9) 220	+	++	++	++	+	–	–	–	–
10) 220	–	–	–	–	–	+	–	+	+
11) 224	–	–	–	–	++	+	+	+	+
12) 232	–	–	–	+	–	++	–	+	+
13) 236	–	–	+	–	–	–	–	–	–
14) 238	–	–	–	–	–	–	–	+	–
15) 240[e]	++	+	–	++	++	–	–	–	–
16) 244	–	++	++	++	–	–	–	–	–
17) 260	–	+	+	+	–	–	–	–	–
18) 284	–	–	–	–	++	–	–	–	–
19) 324[f]	–	–	–	–	+	–	–	–	–
TOTAL PER POPULATION	8	10	9	10	10	9	9	10	8

[a]"Major" and "minor" are arbitrary designations referring to relative proportions of alkaloids within individual population samples. Alkaloids identified by combined gas chromatography-mass spectrometry.

[b]The letters A-H correspond to localities shown in map 1 and to the gas chromatographic traces in figure 15. Letter I designates the Río Palenque Biological Station, NW Ecuador, near the southern limit of the range. See Appendix 3 for full citation of locality data.

[c]These alkaloids emerge as a poorly resolved peak (see p. 192 and fig. 4 for discussion). Numbers in parentheses are the protonated molecular ions.

[d]Other alkaloids are unnamed and are designated only by their protonated molecular ions except in the case of HTX-D (protonated mol. ion 288), which is closely related to the histrionicotoxins and which emerges as part of the same, poorly resolved peak.

[e]Isomeric alkaloids arbitrarily treated as a single compound (see p. 218).

[f]This alkaloid, present in only trace amounts in one population, was tentatively identified as pumiliotoxin B.

alkaloids and the corresponding similarity values are shown in table 5. These figures reveal a tendency for a greater number of compounds to be shared by neighboring populations than by distant populations. For example, sample A has eight alkaloids, of which as many as seven are shared with relatively close populations, and only four are shared with the most distant populations. The similarity values for this north-south series are:

A→88→75→88→75→63→75→50→50

Similarly good or better distance correlations are seen in most other comparisons, population H for example:

50←40←44←50←50←78←78←**H**→100

Only one population (E) shows higher similarity values with distant populations than with its nearest neighbors:

75←70←56←60←**E**→56→67→50→50

There seems to be no valid basis for testing the

significance of such comparisons in a statistical sense, but the method is valuable in detecting patterns that are not obvious in the raw data (table 4) or in the gas chromatographic traces (fig. 15).

The various kinds of comparisons are brought together in the following paragraphs. It should be emphasized that the illustrated gas chromatographic traces were all obtained from extracts prepared in the field, and mostly at the same time of the year (February, with one exception, see table 2). Present evidence is that such traces are reproducible, but that samples taken from frogs maintained in the laboratory will show quantitative but not qualitative changes in the alkaloid fraction (see discussion under population G). Further studies are in progress on the possible influence of stress, diet, and seasonality.

A-D. *Río San Juan Drainage.* As demonstrated above, these four populations include a diversity of color pattern and behavioral types, and, in addition, population A differs also in smaller body size. If our knowledge of *Dendrobates histrionicus* were based *only* on these four populations, it is most probable that three species (A, B, and C-D) would be recognized. But, be that as it may, analysis of the skin toxins is strongly supportive of prior notions of the conspecificity of these diverse populations.

Frogs in the neighboring populations A (Santa Cecilia) and B (Playa de Oro) differ in appearance almost as much as in any two populations in the species' range, but they share seven of a combined total of 11 alkaloids (table 4), for a relatively high 100 C/N_1 value of 88. The gas chromatographic traces (fig. 15A, B) look different mainly because of quantitative differences in compound 220, and because of significant amounts of compound 244 in B and its absence in A. Population A contains one alkaloid not detected in B, and B contains three compounds not in A.

Population B has a gas chromatographic trace more resembling that of C than A, but the alkaloid similarity value is slightly lower (78 for B-C vs. 88 for A-B). Populations C and D show the closest correspondence in coloration and also have similar gas chromatographs (the one for D is based on frogs from the south bank of the Quebrada Docordó, as the north-bank population was not sampled for toxins). However, the dissimilar frogs of populations B and D have very similar gas chromatographic traces, and also a relatively high 100 C/N_1 value (80); these two populations share eight of a possible 12 alkaloids.

To summarize, interpopulational variation in the gas chromatographic traces of the Río San Juan populations is due more to quantitative differences in the alkaloids than to qualitative differences. The four populations collectively

TABLE 5
Relationship of Geographic Proximity to Number of Alkaloids Shared among Populations of *Dendrobates histrionicus*[a]

Population[b]	A	B	C	D	E	F	G	H	I
A	**8**	7	6	7	6	5	6	4	4
B	*88*	**10**	7	8	7	5	6	4	3
C	*75*	*78*	**9**	8	5	4	5	4	4
D	*88*	*80*	*89*	**10**	6	5	5	5	5
E	*75*	*70*	*56*	*60*	**10**	5	6	5	4
F	*63*	*56*	*44*	*56*	*56*	**9**	7	7	7
G	*75*	*67*	*56*	*56*	*67*	*78*	**9**	7	6
H	*50*	*40*	*44*	*50*	*50*	*78*	*78*	**10**	8
I	*50*	*38*	*50*	*63*	*50*	*88*	*75*	*100*	8

[a] Diagonal line of numbers in boldface type shows actual number of alkaloids in each population sample (data from table 4). Numbers in Roman type are those of identical alkaloids shared by any two populations. Numbers in italic type are measures of similarity derived by the formula 100 C/N_1.

[b] The letters A-H correspond to localities shown in map 1 and to the gas chromatographic traces in figure 15. Letter I designates the Río Palenque Biological Station, NW Ecuador, near the southern limit of the range. See Appendix 3 for full citation of locality data.

contain 13 of the 19 *histrionicus* alkaloids listed in table 4, and the individual populations contain 8-10 of these 13 compounds, resulting in high similarity values of 75-89. Especially high similarity values are obtained by comparison of populations A and B and A and D, even though the frogs from these populations look as though they might belong to three different species.

Collectively, the Río San Juan populations differ from all others only in the possession of alkaloids with protonated molecular ions at 244 (major compound) and 260 (minor compound). These two compounds were present in populations B-D but "absent" in all others, including population A. A minor compound with a protonated molecular ion at 236 was detected only in population C, but A, B, and D have no unique compounds not shared with other populations.

The San Juan populations share few compounds with the distant Ecuadorian populations (similarity values 38-63) but an intermediate number with the other Colombian populations E-G (similarity values 44-75). The highest values (75), in the latter cases, are for comparisons of population A with E and G. This apparent reversal of the nearest neighbor effect is, however, not due as much to the absolute number of shared alkaloids as to the relatively small number of total alkaloids in population A (= N_1 in each case).

E. *Quebrada Guanguí.* The major alkaloids in this distinctive population of frogs show protonated molecular ions at 240. At least two and perhaps four isomeric alkaloids—with molecular weights of 239 and unseparated by gas chromatography—are apparently responsible for the protonated ions at 240;[1] these isomeric compounds were detected elsewhere only in the Río San Juan populations A, B, and D. Population E also contained significant amounts of a 224 compound that is shared with all the more southern populations but that is absent in the Río San Juan populations to the north. On the basis of shared alkaloids (tables 4, 5), population E shows no evidence of a nearest neighbor effect, with 100 C/N_1 values as follows:

75←70←56←60←**E**→56→67→50→50

Thus, this geographically intermediate population (map 1) also is rather intermediate in its shared alkaloids.

Population E, however, also seems biochemically unique in possessing minor amounts of two alkaloids not detected in any other population (the only other unique compounds were found in populations C and H, with one each). One of the unique compounds has the same molecular weight as histrionicotoxin (protonated molecular ion, 284) but with an earlier emergent temperature at 191°C. (fig. 15E). Trace amounts of pumiliotoxin B appeared to be present on the basis of mass spectral data, but absolute identification was not possible.

In addition to extracts that we obtained in the field (February, 1973), analysis also was made of extracts from five population-E frogs shipped alive to the American Museum by Borys Malkin (November, 1971). These frogs were subjected to several weeks of arduous conditions of transport, as well as having been collected at a different time of year. In the gas chromatogram (not shown), the proportions of the 240 peak and the later peaks corresponding to histrionicotoxins were significantly reduced, whereas the proportion of the early 284 peak at 191°C. was greatly increased. No qualitative differences were noted, except that the trace compound 324, presumably pumiliotoxin B, was not detected in this small sample.

F. *Río Guapi.* Frogs from the lower drainage

[1]These isomers were arbitrarily treated as a single compound in table 4 and in the similarity comparisons in table 5.

Plate 1. Geographic variation in *Dendrobates histrionicus*, showing representative frogs from populations sampled for skin toxins. Letters A-H correspond to localities in map 1, and to alkaloid samples analyzed in figures 2, 3, and 15. See Appendix 3 for complete citation of localities.

A. Santa Cecilia, AMNH 85159. B. Playa de Oro, AMNH 85172. C. Quebrada Vicordó, AMNH 86994. D. Quebrada Docordó, AMNH 86979. E. Quebrada Guanguí, AMNH 88844. F. Río Guapi, AMNH 88489. G. Guayacana, AMNH 85051. H. Río Baba, AMNH 89579. (Frogs not to scale; range in snout-vent lengths, 27.5-35.0 mm.)

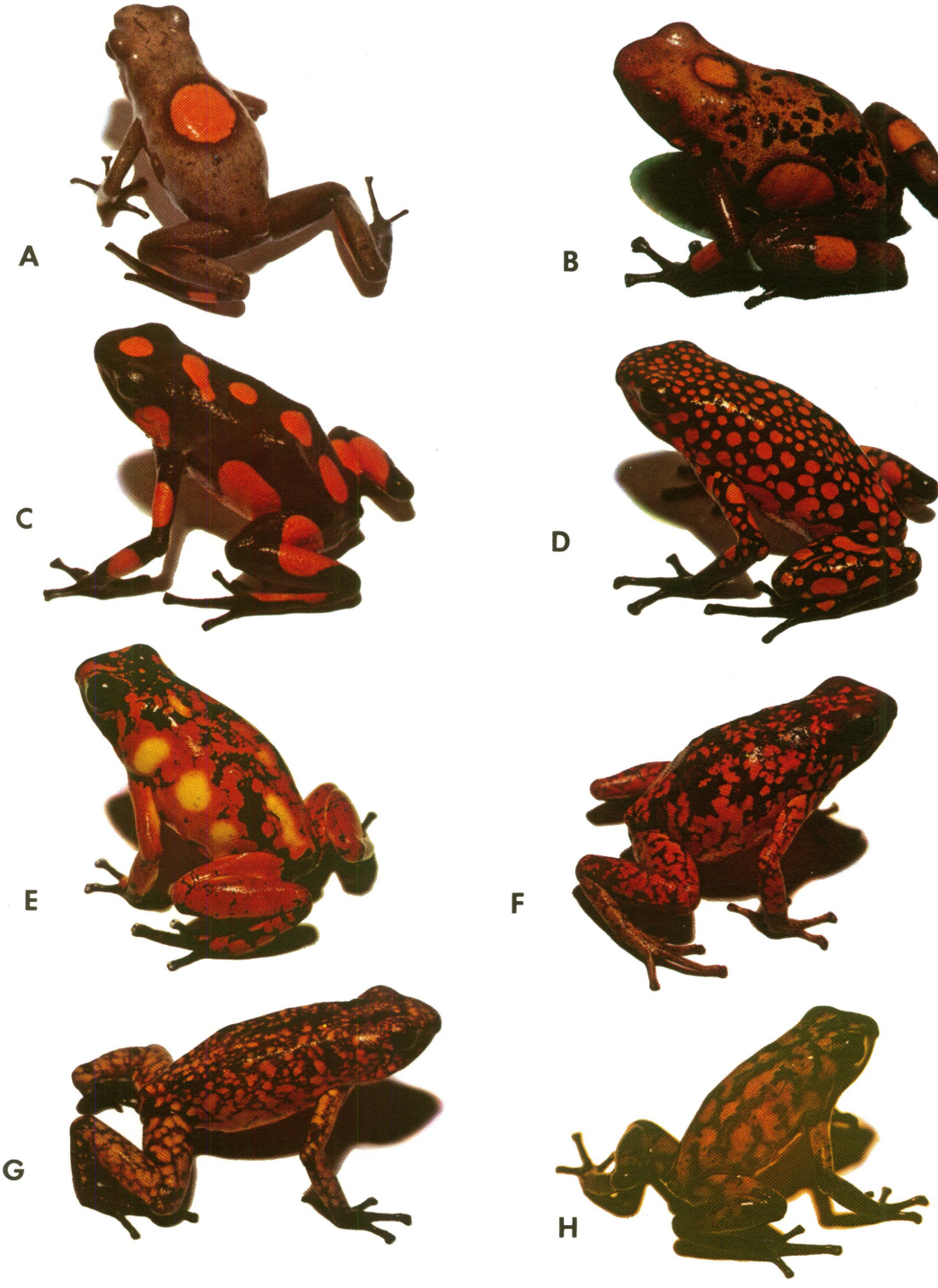
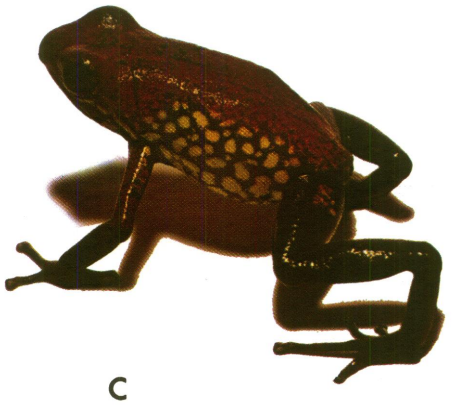

A
B
C
D
E
F
G
H

Plate 2. New species of *Dendrobates* (not to scale). A, B. *Dendrobates lehmanni*: A, AMNH 88153 (holotype, 33.3 mm. snout-vent length); B, AMNH 88154 (paratopotype, 35.0 mm.). C. *Dendrobates occultator*, AMNH 88143 (holotype, 25.0 mm.). D. *Dendrobates viridis*, AMNH 88133 (holotype, 14.7 mm.).

A

B



C

D

of this river contained significant amounts of alkaloids with protonated molecular ions at 220 and 232 (fig. 15F) in addition to the poorly resolved peaks corresponding to histrionicotoxins. On the basis of shared alkaloids (tables 4, 5), the Río Guapi population shows greatest similarity with its southern neighbors, even though the geographically nearest population (E) is to the north:

63←56←44←56←56←**F**→78→78→88

However, the gas chromatographic trace of F is more like those of E and other northern populations in showing a preponderance of lower molecular-weight alkaloids and relatively small amounts of histrionicotoxins (fig. 15). Population F, therefore, shows biochemical intermediateness as does population E, although, unlike the latter, population F does exhibit nearest neighbor tendencies.

G. *Guayacana.* Frogs in this population stand out from other Colombian *histrionicus* in containing very large amounts of histrionicotoxins (fig. 15G). Furthermore, it is the only population studied in which *all* the histrionicotoxins and the related HTX-D were detected by gas chromatographic-mass spectral analysis of the relatively small taxonomic samples (table 4). The lower molecular-weight alkaloids are very minor constituents of the alkaloid fraction in this population. Several lower weight alkaloids were isolated as trace compounds in very large samples of skin extracts (table 1), but were not detected by gas chromatographic-mass spectral analysis of the taxonomic samples. However, one lower weight alkaloid (protonated molecular ion, 224) was detected by the latter methodology but was not separated in the large sample analyses. Shared alkaloid comparisons give 100 C/N_1 similarity values as follows:

75←67←56←56←67←78←**G**→78→75

Thus, population G most closely resembles its nearest neighbors, but similarity is also seen with populations A and B on the upper Río San Juan. However, the resemblance with populations to the south, in Ecuador, is the most striking when the gas chromatographic traces are considered (fig. 15). The Guayacana and Ecuadorian populations contain very large quantities of histrionicotoxins and only very minor amounts of lower weight compounds.

The Guayacana population of *Dendrobates histrionicus* is biochemically the best known. Large samples of skin extracts obtained here provided material for the original structural determinations of the histrionicotoxins by X-ray crystallography and mass spectrometry (Daly et al., 1971; Tokuyama et al., 1974). Several small samples have been analyzed by combined gas chromatography-mass spectrometry and provide evidence on the reproducibility of this method. For example, the gas chromatographic trace in figure 15G is based on combined extract from skins of 10 frogs collected October 17, 1972; this trace is nearly identical with that of figure 4, which is based on extracts from 10 frogs collected November 30, 1971. A virtually identical spectrum (not shown) also was obtained from 10 frogs collected on the latter date, and maintained in plastic bags for two weeks prior to sacrifice. Ten frogs collected at Guayacana on February 24, 1974, were transported to the National Institutes of Health and maintained in a terrarium for six months prior to sacrifice and preparation of extracts. The gas chromatographic profile of alkaloids for this sample (not shown) was quite similar to those of figures 4 and 15G, but the proportion of octahydrohistrionicotoxin had significantly increased and a greater amount of a minor, lower weight alkaloid (protonated molecular ion, 224) was present. Field-prepared extracts from 10 frogs therefore seem to provide reproducible and adequate population samples, although prolonged maintenance of live frogs (or seasonality?) seems to cause quantitative changes in the alkaloid spectrum; see also the last paragraph in the discussion under population E.

H. *Río Baba and Other Ecuadorian Populations.* The gas chromatogram of population H (Río Baba) is quite similar to that of population G. Mass spectral analysis of the peaks showed the main differences to be: 1) octahydrohistrionicotoxin is a major constituent in H but a minor one in G; 2) isotetrahydrohistrionicotoxin and HTX-D are present as minor compounds in G but are "absent" in H; 3) a minor, lower weight alkaloid with a protonated molecular ion at 238 is present in H but has been detected in no other population of *histrionicus.* Population H shows

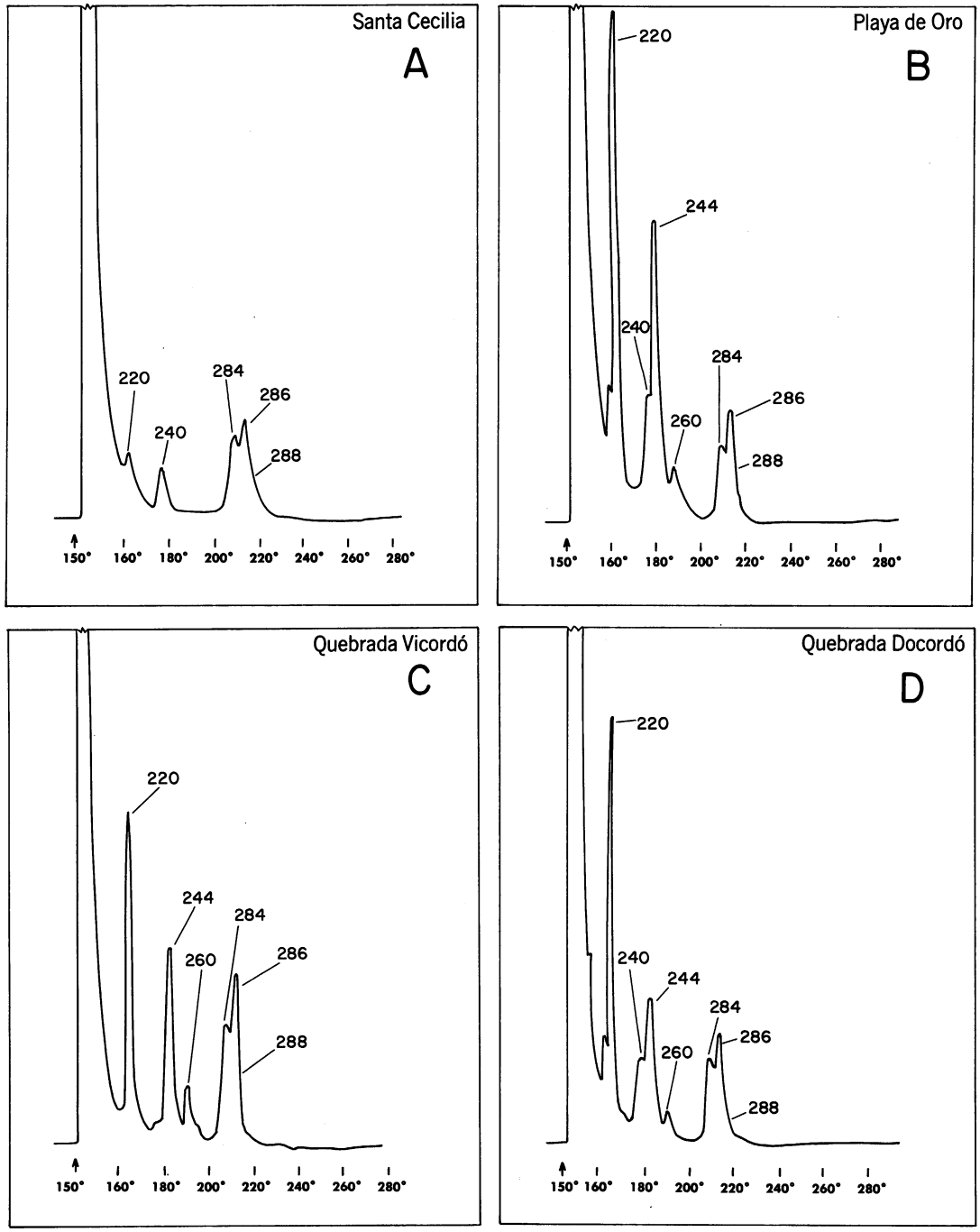


FIG. 15. Gas chromatograms of alkaloids from eight population samples of *Dendrobates histrionicus* (see map 1 and table 2 for sources). For each sample, chromatography was run with 2 μl of methanolic extract containing concentrated alkaloids equivalent to amount contained in 2 mg. of wet skin. Alkaloids are identified by their protonated molecular ions, as determined by combined gas chromatography-mass spectrometry.

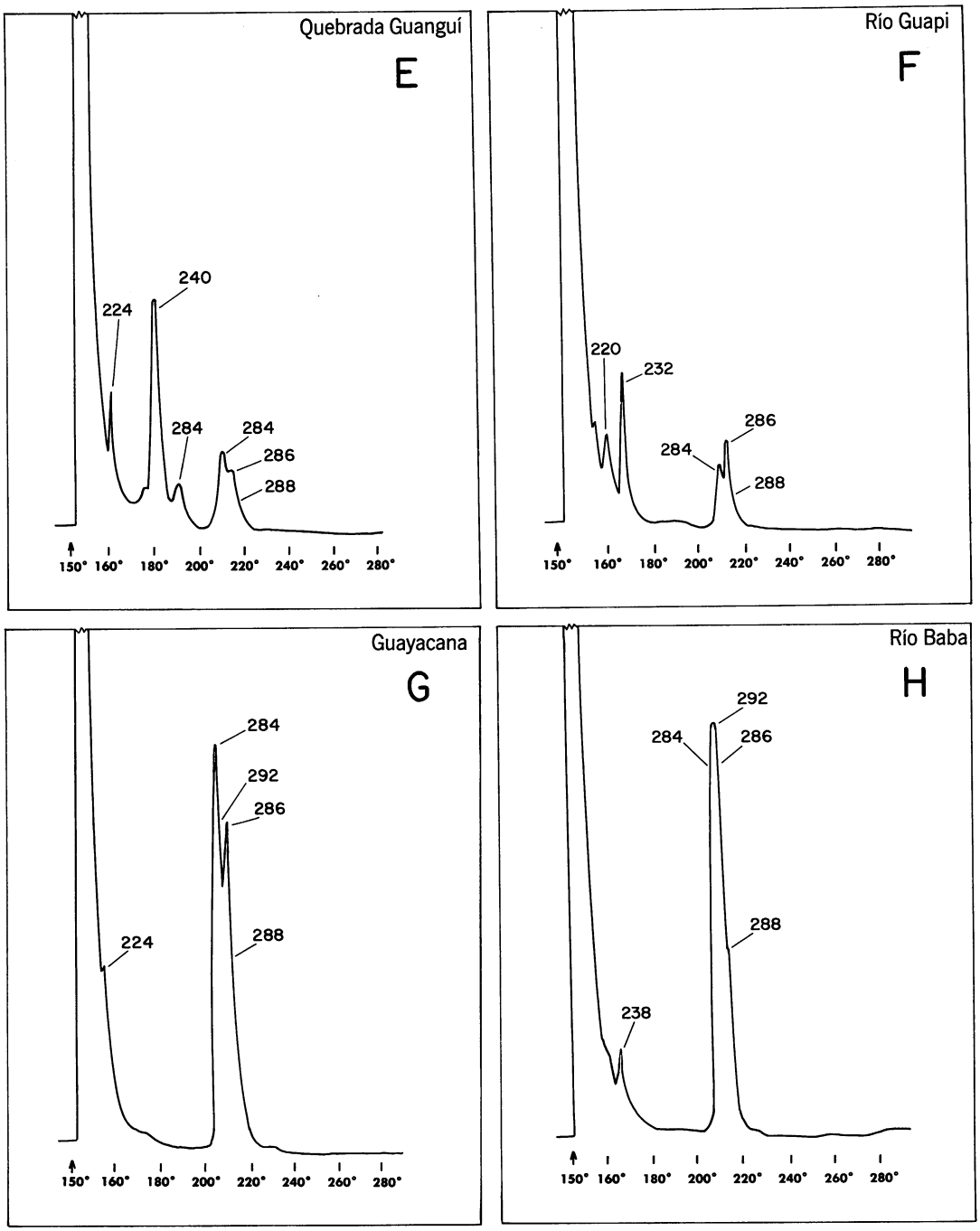


FIG. 15—(*Continued*). Comparison of relative areas under different peaks provides rough quantitative estimation of alkaloids present. Reproducibility is seen by comparing sample G (10 frogs, October, 1972) with figure 4, which shows different sample obtained from same population (10 frogs, November, 1971). See text starting p. 192 for further interpretation.

greatest 100 C/N_1 similarity values with its nearest neighbors, namely F-G and "I" (see following text):

50←40←44←50←50←78←78←**H**→100

A gas chromatogram nearly identical with that of H was obtained (results not shown) with extracts from eight frogs purchased from a dealer and purportedly collected in June, 1973, about 10 miles [15 km.] south of Santo Domingo de los Colorados (i.e., in a locale close to population H [Appendix 3], which we sampled in February, 1974). This population of *Dendrobates histrionicus* included some unusually colored variants, as discussed under Color and Pattern, but none of the gray or green frogs was included in the sample of skin extracts.

In February, 1974, we also obtained a small sample of extracts from five frogs at the Estación Biologica Río Palenque, about 40 km. south-southwest of Santo Domingo de los Colorados, near the southern limits of the rain-forest habitat of *Dendrobates histrionicus.* This sample is designated "I" in tables 4 and 5, but it is half the size of the other taxonomic samples A-H and was analyzed only by combined gas chromatography-mass spectrometry. The gas chromatographic trace of I (not shown) is more like that of the Colombian population G than that of the nearest neighbor population H, except that amounts of histrionicotoxins were less than one-third those of the population G frogs. Octahydrohistrionicotoxin was only a trace constituent as in G, and not a major compound as in H. Small amounts of alkaloids with protonated molecular ions at 220, 224, and 232 were detected in population I; the 220 compound (no. 10 in table 4) also is present in F and H but can reasonably be considered to be absent in the well-known population G. Population I shares 6-8 alkaloids with populations F, G, and H and 3-5 alkaloids with the northern populations A-E. Thus, there is a definite nearest neighbor tendency, as indicated by the 100 C/N_1 similarity values:

50←38←50←63←50←88←75←100←**I**

DISCUSSION AND DETECTION OF SIBLING SPECIES

Summary of Variation. We have briefly surveyed various aspects of variation in *Dendrobates histrionicus*, a species whose range includes thousands of square kilometers of rain forest in the Pacific lowlands of western Colombia and northwestern Ecuador. Morphologically, the frogs appear rather uniform throughout their geographic range, and external sexual dimorphism is practically absent. Males tend to be as large, or slightly larger than females, a trait perhaps related to pronounced male aggressiveness and territoriality in this species (males of most anuran species are smaller than females). Males also tend to have relatively longer tibiae than those of females, but the differences are slight. There is some regional differentiation in tibia length/snout-vent length, in that larger bodied frogs of the northern populations have relatively longer tibiae than the smaller bodied frogs in southwestern Colombia and Ecuador. The most significant interpopulational differences are in snout-vent length, which is correlated with (if not related to) climatic change. The largest frogs occur in the wettest, hottest part of the sampled range, namely in the Río San Juan basin of northwestern Colombia. A population of smaller frogs occurs near the headwaters of the Río San Juan, in forest that presumably receives less rainfall because of its proximity to a rain-shadow dry area, and there is a cline of decreasing body size from southwestern Colombia into northwestern Ecuador.

There are evident interpopulational differences in escape behavior, although behavioral aspects of variation in this species need to be better studied and quantified.

By far the most striking aspect of variation in *Dendrobates histrionicus* is in color and color pattern, as illustrated in color plate 1 and text figures 6-14. Each local population tends to differ markedly from the next, and a greater or lesser degree of intrapopulational variation is also present. Geographic variation in color pattern appears, initially at least, to be of a mosaic nature, although total variation in this species certainly has been inadequately sampled. If only a few of the diverse populations were known, the taxonomist would seem justified in assuming the presence of discrete species on the basis of color and pattern alone. But, because of known variability, the different populations have been almost intuitively considered as conspecific by authors with various backgrounds and training

(Boulenger, 1913; Funkhouser, 1956; Cochran and Goin, 1970; Daly et al., 1971; Silverstone, 1975), even though some accounts have the populations listed as subspecies of the Guayanan *D. tinctorius.*

The conspecificity of most of the *histrionicus*-like populations (with two exceptions) is here supported by analysis of variation in the skin toxins of populations sampled nearly throughout the geographic range. Despite noteworthy interpopulational variation, the populations here considered to be *histrionicus* do show definite biochemical similarity and seem to form a unified whole. Of 19 alkaloids detected by combined gas chromatography-mass spectrometry, 8-10 compounds are found in the individual populations, and only three populations (C, E, and H) contained minor amounts of alkaloids not detected in other populations. Comparison of numbers of shared alkaloids, and calculation of similarity values, revealed that most populations show a nearest neighbor effect, meaning that they tend to share more alkaloids with neighboring populations than with geographically distant ones. The principal exception to the nearest neighbor effect is population E—a geographically intermediate population that seems to form a kind of biochemical link in sharing as many or more alkaloids with distant populations as with its nearest neighbors. The shared alkaloid comparisons, including the universal presence of histrionicotoxin and isodihydrohistrionicotoxin as "major" alkaloids, are the strongest evidence yet available for the genetic continuity of the various populations. It should be emphasized that populations showing extreme diversity in color pattern and, to a lesser extent, in size and behavior, have relatively high 100 C/N_1 values (compare A and B and A and D in pl. 1 and table 5).

Geographic variation in the skin toxins of *Dendrobates histrionicus* is as much quantitative as qualitative, and certain broad, regional tendencies appear evident in the sampled populations. Thus, populations in southern Colombia and Ecuador have very large amounts of histrionicotoxins as their major alkaloids but only trace amounts of the lower molecular-weight alkaloids, some of which appear to be related (in a structural and probably biosynthetic sense) to the histrionicotoxins. The more northern populations also have significant amounts of histrionicotoxins, but, except in population A, there are even larger amounts of the lower weight alkaloids, and often a larger number of such compounds.

Detection of Sibling Species. Analysis of the skin toxins of *histrionicus*-like frogs revealed that two populations apparently represent distinct species, which are formally named on following pages as *Dendrobates lehmanni* and *Dendrobates occultator.* These new species are distinctively colored and easily recognized frogs (pl. 2, figs. A-C), and so the term "sibling species" is not entirely appropriate, but, the point is, many of the populations of *D. histrionicus* are as distinctively colored and their very distinctiveness is a hindrance in judging whether they are in fact conspecific with *histrionicus.* Sibling species usually *look* alike and must often be distinguished by some nonmorphological character.[1] *Dendrobates lehmanni* and *D. occultator*, although visually distinct, look as though they might easily belong to the variational spectrum of *D. histrionicus*, and, like classic sibling species, their specific status is best indicated by a nonmorphological character (skin toxins).

Dendrobates lehmanni and *D. occultator* are similar to *D. histrionicus* in general morphology, including size, with *lehmanni* approaching the size of the largest *histrionicus* and with *occultator* at the lowest extreme. They usually lack the omosternum, the absence of which has been noted elsewhere in the genus only in *D. leucomelas* (*fide* Silverstone, 1975). *Dendrobates lehmanni* and *D. occultator* furthermore resemble *D. histrionicus* in having similar vocalizations and similar calling behavior and male aggressiveness. There are, however, marked differences in the skin toxins, as commented on below.

Eight alkaloids were identified in the skin secretions of *Dendrobates lehmanni.* These compounds are listed below in the same format

[1]The term "cryptic species" is also used in this sense, and some recent authors would restrict sibling species to mean sister species, without reference to degree of distinctiveness. Such a definition of sibling species is etymologically sound but differs from prevailing usage, and it is likely to cause confusion unless authors are careful to define their terms. Sibling species, in the usual sense, seem most likely to be recently evolved sister species, although the possibility of convergence also must be considered.

as table 4, for comparison with the alkaloids of *D. histrionicus*:

PUMILIOTOXINS	
Pumiliotoxin A (308)	+
Pumiliotoxin B (324)	++
OTHER ALKALOIDS	
254	+
268	++
276	++
308	+
308	+
342	+

The *only* similarity is that both *lehmanni* and *histrionicus*-population E have alkaloids with protonated molecular ions at 324. The 324 alkaloid is pumiliotoxin B in *lehmanni*, in which it is a major compound, but there were only minute amounts of the 324 alkaloid in *histrionicus*, and its identification as pumiliotoxin B is not absolute. If we assume that the identification of this alkaloid is correct, the 100 C/N_1 similarity value is only 13 when *lehmanni* is compared with population E, and the value is 0 in comparisons with all other *histrionicus* populations. A compound with a protonated molecular ion at 276 is a major constituent of the alkaloid fraction of *D. lehmanni* (see gas chromatogram, fig. 25), but has so far been detected elsewhere only as a trace compound in *D. auratus* (unpubl. data). *Dendrobates lehmanni*, therefore, is biochemically distinct. It elaborates pumiliotoxins and other high and low weight alkaloids that appear not to occur in *D. histrionicus*, which has a different spectrum of compounds (fig. 3).

Ten alkaloids were identified in the skin secretions of *Dendrobates occultator*, as follows:

PUMILIOTOXINS	
Pumiliotoxin B (324)	++
HISTRIONICOTOXINS	
Histrionicotoxin (284)	++
Isodihydrohistrionicotoxin (286)	++
OTHER ALKALOIDS	
232	++
240	++
240	++
242	+
260	+
266	+
284	++

Of the above, only the two minor alkaloids with protonated molecular ions at 242 and 266 have not been detected also in *D. histrionicus*. The 100 C/N_1 similarity values, for alkaloids shared with *histrionicus* populations A-I, are shown below; for these calculations, the 240 isomers were arbitrarily treated as a single compound (p. 218n), giving N=9 for *occultator*:

(A) 38
↑
(B) 44
↑
(C) 33
↑
(D) 56
↑
occultator → (E) 56
↓
(F) 33
↓
(G) 22
↓
(H) 33
↓
(I) 38

Thus, there are definite biochemical similarities between *D. occultator* and *D. histrionicus*. The greatest similarity values are with populations D and E. Population E is sympatric with *occultator*, and these two populations have rather similar gas chromatograms (compare figs. 15E, 26). The existence of geographical sympatry made it seem obvious that two species were involved, one a new species and the other presumably a population of *D. histrionicus*. We have considered the following facts in deciding to apply the name *histrionicus* to population E frogs and not to the frogs herein named *occultator*:

1) *Dendrobates occultator* elaborates pumiliotoxin B as a major compound (protonated molecular ion 324, figs. 3, 26). This seems a significant departure from *D. histrionicus*, which appears to lack pumiliotoxins except for the tentative identification of trace amounts in population E.

2) Only two histrionicotoxins were detected in the alkaloid fraction of *D. occultator*, compared with five histrionicotoxins in population E and five to seven histrionicotoxins in the other populations of *D. histrionicus*.

3) The paired similarity comparisons are lower

for *occultator* (100 C/N_1 = range of 22-56) than for population E (50-75), when these frogs are compared with *histrionicus* populations A-D and F-I. The total similarity values have a range of 38-100 for the *histrionicus-histrionicus* comparisons (table 5), but six of the nine *occultator-histrionicus* comparisons (22-56) give values below 39, and four have values below 34.

4) The population E frogs have a mean snout-vent length > 33 mm., which appears to fit a geographic cline in *D. histrionicus.* The smaller *D. occultator* (maximum snout-vent < 28 mm.) is comparable in size only with geographically remote populations of Ecuadorian *histrionicus.*

5) Based on limited observation, *D. occultator* seems to be more secretive and more arboreal than *D. histrionicus*, whereas the behavior of population E frogs is more comparable to other populations of *histrionicus.*

Based primarily on the skin toxins, therefore, we conclude that two populations of *histrionicus*-like frogs represent previously unnamed "sibling" species. Present evidence makes this conclusion virtually inescapable in the case of *Dendrobates lehmanni.* The biochemical evidence is also suggestive (albeit weaker) for the specific status of *D. occultator*, and the hypothesis becomes a logical necessity when evidence of sympatry and behavior are considered. Some pertinent comparisons with *D. histrionicus*, and preliminary speculations on evolution, are made under Remarks in the descriptions of the new species.

As mentioned, the new species and *Dendrobates histrionicus* have similar vocalizations and calling behavior. Little comparative data were gathered on this aspect of *histrionicus* and its "siblings," but the similarities prompted us to review available tape recordings of *Dendrobates* calls, and the results are presented in the following section as a prelude and, hopefully, a stimulus to additional bioacoustical and behavioral studies.

VOCALIZATIONS AND CALLING BEHAVIOR OF *DENDROBATES*

The biological importance and taxonomic potential of anuran vocalization is generally appreciated (e.g., Bogert, 1960; Duellman, 1970; Martin, 1972), although serious bioacoustical research has not been carried out for the Dendrobatidae or for many other groups of frogs. A few authors have attempted to analyze aspects of calling behavior of dendrobatids without, however, commenting on the nature of the calls. Verbal descriptions of the calls of several dendrobatid species have been published, but, although useful, such descriptions can be difficult to interpret. For example, from the literature, one might gain the totally false impression that the "5-80 insect-like buzzes" of *Dendrobates granuliferus* more resemble the "soft buzzing sound" of *D. auratus*, rather than the actually similar "tick-tick-tick-tick" series of *D. pumilio* (partial quotations from Goodman, 1971; Dunn, 1941; and Duellman, 1966, respectively). For better comparisons, it obviously is desirable to obtain tape recordings and to make sound spectrograms.

We here attempt the beginnings of an objective study of dendrobatid vocalizations. The results are imperfect, even though our present observations are confined to those species of *Dendrobates* that occur in Central America and extreme northwestern South America. The calls of six species in this region have still to be identified, whereas some others either have not been recorded or else recorded only from frogs calling in plastic bags or terraria. In spite of these limitations, it is possible to separate the known calls into two major types. A given species gives only one type of call, and it seems that a different kind of calling behavior is associated with each of the call types, which are onomatopoetically referred to as "buzz calls" and "chirp calls."

The accompanying audiospectrograms were produced on a model 6061-A "Sona-Graph" (Kay Electric Co.), at narrow or wide bandwidths of 45 or 300 Hertz, with the automatic gain control set at "0" (not off). The preprinted scales, on which the spectrograms are mounted, have been adjusted along the vertical (Hz) axis to correct for temporal variability in the internal calibration signals of the Sona-Graph. Several

different machines (Uher 4000, Report L and S models) and microphones were used in recording calls.

BUZZ CALLS

These are vocalizations in which a uniform series of pulses is produced too fast to be resolved by the human ear, but slow enough to be directly visualized on sound spectrograms made with a wide-band filter (fig. 16). Buzz calls may prove widespread in *Dendrobates* (see below under Other Calls), although only two species are presently known to be characterized by this kind of call, namely *Dendrobates auratus* and *D. minutus*.

Limited observations of frogs in the field and in terraria indicate that *D. auratus* and *D. minutus* call relatively infrequently, and that a particular calling station is not maintained for any great length of time. Field observations of these two species are further complicated by the fact that their buzzes are soft and do not carry far. All of this is in marked contrast to those species of *Dendrobates* that are characterized by the relatively loud and continuously given chirp calls.

Dendrobates auratus (Girard)

Dunn (1941, pp. 89, 90) timed the call of *Dendrobates auratus* at two seconds duration and

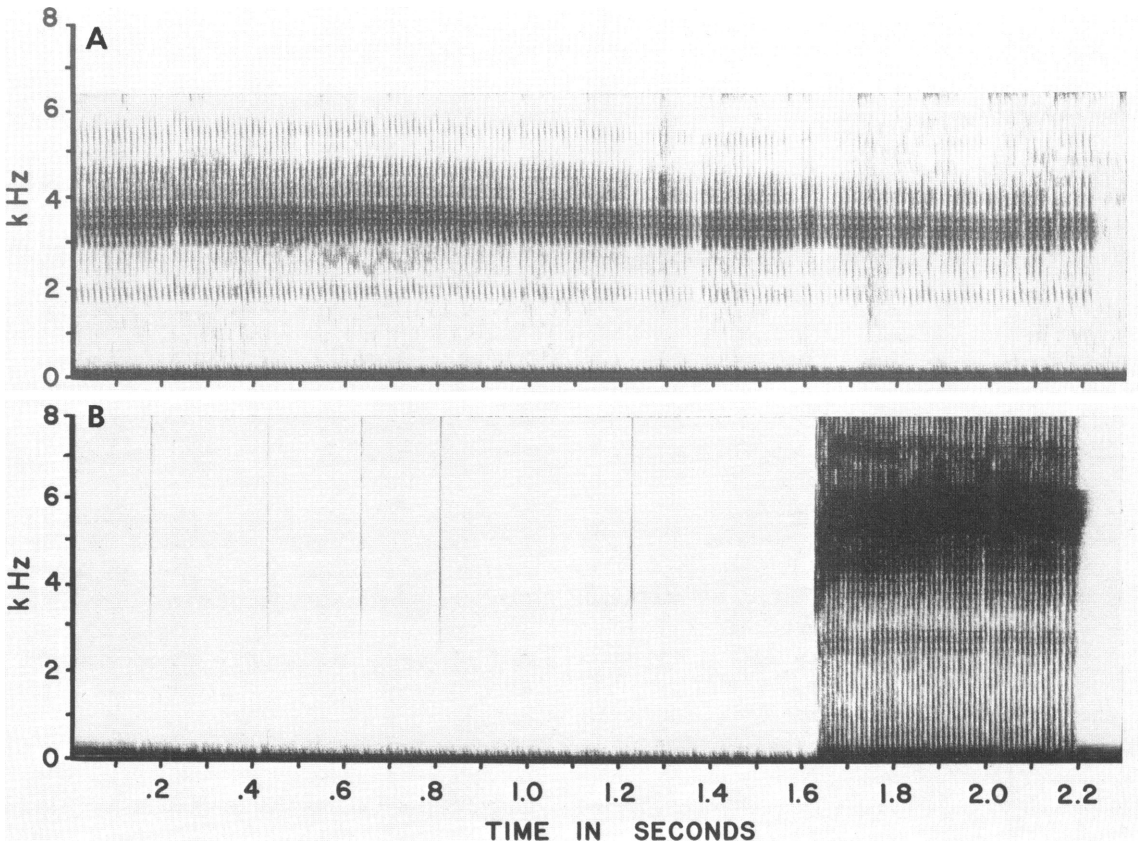


FIG. 16. Buzz calls (wide band, 300-Hz filter). A. *Dendrobates auratus.* Last part of 3.5-second call of specimen from Barro Colorado Island, Panama Canal Zone, April 9, 1968; recording by A. Stanley Rand (copy on AMNH tape 179; temperature not noted). B. *Dendrobates minutus.* Complete call on right, preceded by five "clicks." Locality uncertain (recorded in terrarium containing frogs from central Panama and Colombian Chocó); March, 1970, air temperature 27° C. (AMNH tape 175).

aptly described it as a "low, soft, buzzing sound." A recording of six calls from one individual with a mean of 2.8 seconds duration per call (range, 1.5-4.0 sec.), was provided by A. Stanley Rand; the terminal part of one of these calls is reproduced here as figure 16A. The call starts and ends abruptly. As visualized on the sound spectrogram, the call has a slight wavering quality, although this is not very noticeable to the ear. Maximum energy output is between 3000-4000 Hz, with a dominant frequency at 3500 Hz throughout most of the call, but as high as 4000 Hz in a section made at the start of the figured call; the pulse rate is 75 per second (temperature data lacking). We have occasionally heard this call emitted by frogs in terraria and in the field, but only rarely have we seen a frog call. The call is a nearly uniform, hence nonmusical buzz, and it has a lower dominant frequency than the calls of other Central American *Dendrobates* and therefore is lower in pitch. Savage (1968, p. 767; also paraphrased by Villa, 1972, p. 70) quite differently described the call of *D. auratus* as a "slurred cheez-cheez-cheez *with a fairly high pitch* [compared with *D. granuliferus*] *and somewhat musical quality*, followed by a pause and repeat; usually from three to five notes, then a pause." (Italics ours.) We do not doubt Savage's identification, and presume that he heard a specimen give a series of short buzzes, but italicized aspects of the description may point out the fallibility of the human ear and the difficulty in verbalizing frog calls.

Breder (1946, pp. 408, 425) commented that he did not hear *Dendrobates auratus* call in several months (February-April) of field work in eastern Panama, where he paid particular attention to frog vocalizations. For a short time on March 29, 1965, Myers observed a specimen that was occasionally calling as it moved about on a forested hillside above Miramar, Chiriquí Lagoon, northwestern Panama. Rand recorded his specimen on April 9, 1968, on Barro Colorado Island in central Panama. Dunn (1941, pp. 89, 90) heard specimens calling in July, 1939, and September, 1940, on an island off the coast of Pacific-central Panama. These scattered observations are from climatically dissimilar regions and provide no clue to possible seasonal variation in calling activity.

Dunn (*loc. cit.*) made his observations on calling behavior of *Dendrobates auratus* on Isla Taboga,[1] in the Bay of Panama: "The call was often given from a slight elevation, such as the highest point of a fallen mango leaf. There was no calling station: the males moved constantly, calling occasionally between hops. Some moved 30 to 40 feet while we watched them. A calling male was usually followed by several females, who sometimes actually jumped on him. The male seemed aware of his suite and if pursuit lagged would halt longer, become more vociferous, and finally disappear beneath the leaves, closely followed by one female, the others having paused by the wayside. In one such case after 30 minutes the female emerged and went off. The male came out a few minutes later, called once, and remained for awhile in the vicinity. We cleared around the areas of three such disappearances and went back in the afternoon and examined all three places leaf by leaf, but found nothing. I do not know whether any actual mating took place . . . We caught one 'caller' and one 'follower' and put them into a bag, which we kept damp. Next day we found two eggs together in the bag."

There is no published evidence of territoriality in *Dendrobates auratus*, assuming that all the "followers" mentioned by Dunn (see above) were actually courting females. Certainly there is no obvious territoriality in the dry season congregations, where dozens of frogs occur together under rocks and in hollow logs. Nor have we ever witnessed a "fight" among the thousands of

[1]*Dendrobates auratus* is extraordinarily abundant on Isla Taboga (the type locality), where, in the dry season, hundreds of these frogs congregate in small areas in the few *quebradas* that stay moderately moist. The high population density might in part be due to reduced interaction with other, unknown competitors, as compared with mainland situations, but the density is likely enhanced also by reduced intraspecific competition between the larvae of *auratus*. On the mainland, *auratus* seems normally to carry its tadpoles to small pockets of water in tree trunk holes, but the tadpoles are cannibalistic (personal observ.) and few can coexist in a tiny space with presumably limited nutrients. In this species, therefore, cannibalism may prove to be a regularly occurring and adaptive behavior, rather than the aberration it usually is. Isla Taboga is relatively xeric, but man's activities have produced many artificial sites for tadpole deposition in forest-edge trash heaps (water-containing cans, etc.) and drums of rainwater for cattle. A small can will usually hold but one tadpole, whereas a large drum may contain a dozen or so.

auratus that we have seen in the field. Nonetheless, aggressive behavior among female *auratus* has been described for captive frogs by Senfft (1936) and Polder (1974a), and the matter remains to be investigated under natural conditions.

Dendrobates minutus Shreve

The call of this tiny frog is a short, high-pitched, soft buzz and does not carry far. The call occupies the entire available frequency range (0-8000 Hz) of the standard spectrogram (fig. 16B); sections made from several calls show no single dominant frequency, but rather an emphasized band variably situated between about 5400 and 6400 Hz. Twenty complete calls at constant temperature (27°C.) show a range in duration of 0.2-0.8 second and contain 20-71 pulses with extrapolated rates of 86-125 pulses/second. There is strong, positive correlation between duration of the call and the number of pulses ($r = 0.98, P < 0.001$) but the pulse rate is negatively correlated with duration ($r = -0.67$, $P < 0.01$); in other words, the longer calls are pulsed more slowly than the shorter calls. Four incomplete or broken calls were recorded in addition to 20 normal calls. The broken calls contain one or more gaps between short trains of 1-12 pulses (7+5 pulses, 7+12, 8+3+1, and 5+3+2+1).

About half the recorded calls are preceded and/or followed by one to five single, widely spaced pulses that are heard as "clicks" (e.g., fig. 16B). A click presumably is equivalent to an individual pulse in the buzz call, except in being less intense on the spectrogram and probably in containing less energy. The click sounds are much softer than the buzz and were not actually perceived until playback of the recordings at increased volume. Clicks were detected only in association with a buzz call and presumably were produced by the same frog making the call, although this is not certain.

The calls on which the above description is based were recorded by chance in March, 1970, after specimens of *Dendrobates minutus* were brought to the American Museum from western Colombia (Playa de Oro, Chocó) and central Panama (Cerro Campana, Panamá). The frogs had been placed in a single terrarium before they started calling, so the exact provenance of the specimen(s) recorded is unfortunately not known. The call had not previously been identified in the field, but we subsequently have heard it at localities in central Panama. The short call is infrequently given, which makes field observation difficult; the 24 calls recorded under terrarium conditions take up a total of only about 10.5 seconds in 23 minutes of actual tape time (not including several long periods when the tape recorder was turned off after calling had temporarily ceased). Casual field observations so far lead us to believe that a single calling site is not maintained. Aggressive behavior has not been witnessed.

CHIRP CALLS

As here defined, a *Dendrobates* chirp call is a series of more or less uniform notes that have a wide frequency range and poor modulation, with pulses being produced much too fast to be resolved by the human ear or directly visualized on wide-band spectrograms. Chirp calls are relatively loud and tend to carry for many feet, even in heavy forest; the notes sometimes are uttered singly but more often are given as a continuous train lasting from several seconds to several minutes. The poor modulation of at least certain segments of the individual notes causes the call to have a harsh, nonmusical sound. This sound varies according to the idiosyncrasies of the human ear, as well as to the species and size of frog, but "chirp" is as imitative a word as any for describing individual notes in all the different calls taken together.

The following species of Central American and northwestern South American *Dendrobates* are characterized by chirp calls: *D. granuliferus*, *D. histrionicus*, *D. lehmanni*, *D. occultator*, and *D. pumilio* (and *D. speciosus*, p. 237n). Aggressive behavior (apparently related to territoriality) is well developed in these species, and is sometimes observable when a batch of recently caught specimens are confined in close quarters. There is a tendency to maintain fixed calling sites for relatively long periods.

Dendrobates pumilio is treated first in order to provide a standard of comparison. More recordings are available for this species than for all the others combined.

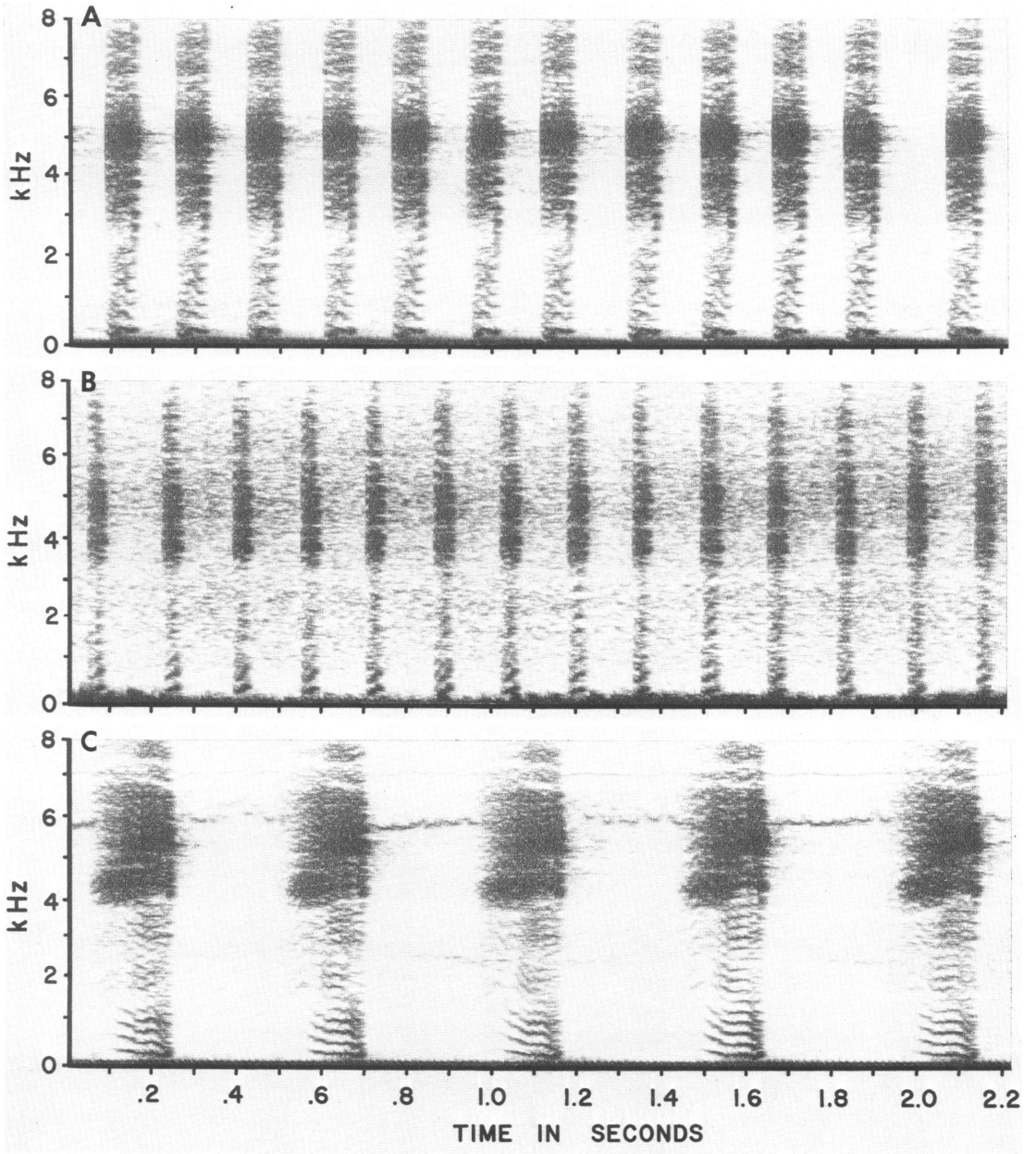


FIG. 17. Intrapopulational and geographic variation in chirp calls of *Dendrobates pumilio* from Panama (narrow band, 45-Hz filter). Recorded in field at following localities: A. Punta Rocosa, Isla Colón, Bocas del Toro Province; March 20, 1965, air temperature 25° C. (KU tape 468). B. Isla Cayo de Agua, Bocas del Toro Province; March 17, 1965, air 29° C. (KU tape 446). C. Mouth of Río Concepción, Veraguas Province; October 23, 1966, air 27°C. (KU tape 669).

Uppermost sound spectrogram (A) is representative of "western" *pumilio* populations from the Bocas del Toro Archipelago and adjacent mainland; second spectrogram (B) shows series of shorter notes produced by occasional frogs in some of the same populations. Lowermost call (C) shows the long and more slowly produced notes that seem to characterize "eastern" *pumilio* populations in coastal Panama east of the Valiente Peninsula.

TABLE 6
Geographic Differentiation in Vocalizations of *Dendrobates pumilio* in Western Panama

Sample	N	Notes/Second[a] Mean±1 S.E.	S.D.	C.V.±1 S.E.	Range
Western Group: Bocas del Toro Archipelago and Chiriquí Lagoon region (March 1965; 24.1-29.1°C.)	51	7.8±0.13	0.95	12.17±1.20	(6-10)
Eastern Group: mainland east of Peninsula Valiente (Oct. 1966; 26.3-27.4°C.)	6	2.8±0.10	0.26	9.28±2.68	(2-3)
Comparison of Means[b]		$t = 12.742, P < 0.001$			

[a] Note repetition rate. Determined from 2.0-second intervals on sound spectrograms, from middles of calls. See figure 18 for correlation with temperature.

[b] Ideally, comparisons should be of mean values at constant temperature, a criterion not met in these data. Nonetheless, a high degree of statistical significance in the first two comparisons is certain, because of the nonoverlapping ranges and the very high *t*- values.

Dendrobates pumilio Schmidt

Approximately 85 calls of this species were recorded by W. E. Duellman and C. W. Myers in 1965-1966, at 14 localities in northwestern Panama, with an additional recording made at Puerto Viejo, Heredia Province, northeastern Costa Rica. Call length was determined from more than 70 recordings by use of a stop watch. Sound spectrograms were made of 2.4 second intervals from the middles of about 60 calls; note repetition rate (notes/sec.), and note duration (in centiseconds, see table 6, note *c*), were determined from the spectrograms. Nearly all specimens were recorded in natural situations, giving apparently "normal" calls (i.e., calls not influenced by another male intruding into the caller's territory); known exceptions are not included in the statistical summations, although a few examples are mentioned in the following text.

Based on the parameters of note repetition rate and note duration, which must be at least partially interdependent, two geographical groupings of Panamanian *Dendrobates pumilio* (*sensu lato*) are immediately evident (table 6). Differences in the aforesaid parameters are nonoverlapping in the samples and are readily visualized by comparing the sound spectrograms of the "western" group (fig. 17A, B) and the "eastern" group (fig. 17C).

The localities for the western group are as follows, with the number of sound spectrograms studied given in parentheses; all localities are in Bocas del Toro Province, Panama:

WESTERN GROUP: REGION OF BOCAS DEL TORO ARCHIPELAGO AND CHIRIQUI LAGOON

5 km. W Almirante (3)
Isla Colón, Punta Rocosa (5)
Isla Bastimentos, near Bastimentos (6)
North end Cayo Nancy (2)
Isla Shepherd (5)
Isla San Cristóbal (3)
Mainland W Isla Split Hill (5)
Isla Split Hill (4)
Isla Popa (5)
Isla Cayo de Agua (5)
Mainland at Chiriquí Grande (5)[1]
Bluefields, north end Peninsula Valiente (5)

These localities include all the populations that we previously sampled for skin toxins (Daly and Myers, 1967), and which Duellman (1967) sampled for gross karyotype studies. The qualitative chemical data, and the chromosomes, are suggestive of a single species, despite astonish-

[1] Two apparently hybridizing populations of *D. pumilio* occur at this locality (Daly and Myers, 1967, p. 972). Because of equipment failure, only the red-backed variety was recorded.

Note Duration (sec.)[c]					Call Length (sec.)[d]				
N	Mean±1 S.E.	S.D.	C.V.±1 S.E.	Range	N	Mean±1 S.E.	S.D.	C.V.±1 S.E.	Range
51	0.055±0.001	0.008	14.54±1.44	(0.04-0.07)	69	24.6±2.07	17.21	69.96±5.96	(3-83)
6	0.142±0.019	0.046	32.39±9.35	(0.10-0.20)	8	14.5±2.16	6.12	42.21±10.55	(4-20)
	$t = -12.730, P < 0.001$					$t = 1.640, P > 0.1$			

[c]Measured from sound spectrograms. We have reservations about the accuracy of these readings (oscillograph readings are preferable), but the numbers nevertheless are expressive of relative time differences in note duration (see fig. 17A-C). Negative correlation with temperature (see text).

[d]Determined from recordings by stop watch. No correlation with temperature (see text).

ing variation in other attributes. This problem remains under study, but the idea of a single, highly variable species is further indicated by similarity in the calls of *pumilio*-like frogs from the above localities. There possibly are interpopulational differences in loudness of the call, related to differences in body size, as suggested by Daly and Myers (1967), but there is little variation in note repetition rate, note duration, and area of emphasized frequency. The call is a train of notes that, at least in the middle of the train, are about 0.04-0.07 second in duration and are given at a rate of 6-10 per second (fig. 17A, B). These are harsh, nonmusical notes that sounded like "tick-tick-tick-tick" to Duellman (1966, p. 218), and "buzz-buzz-buzz" to Savage (1968, p. 767). There is no single dominant frequency to a note, but rather an emphasized band situated between about 4000 to 6000 Hz (figs. 17A, B; 22C, D). Call length is highly variable, ranging from 3 to 83 seconds in the recorded calls; thus, the coefficient of variation is 69.96 for call length, as compared with 12.17 for the note repetition rate and 14.54 for note duration (table 6).

In the parameters studied, we detect no geographic variation within the "western" group of *pumilio* calls. Any interpopulational variation is presumably masked by the variability occurring among calls recorded at single localities and at constant air temperature. On Isla Cayo de Agua, for example, five frogs recorded at 29°C. showed the following variation: call length, 4-70 seconds; note repetition rate, 6.5-10; note duration, 0.04-0.05 second.

Temperature has no apparent effect on call length ($r = 0.08, P > 0.1$, for the 69 western calls in table 6), at least in the range of 24°-29°C. Temperature does, however, influence note repetition rate and note duration. The rate at which notes are produced shows a highly significant positive correlation with temperature (fig. 18; $r = 0.45, P = 0.001$), whereas note duration is negatively correlated ($r = -0.39, P < 0.01$). These comparisons are crude, in that data are combined from a dozen populations, and only air temperatures were recorded (vs. body temperatures, which are difficult to obtain for such small frogs). The low correlation coefficients for note repetition and duration are probably due mainly to extensive variation in values obtained at single localities and temperatures (e.g., see preceding paragraph); conceivably, however, subtle interpopulational differences may have affected the regression data one way or the other. Nonetheless, the data indicate that temperature should not be ignored in bioacoustical studies of frogs in the humid tropics, where environments are not so "stable" as sometimes thought.

Field work in 1966 extended the known range of *pumilio*-like frogs eastward, to the following localities on the north coast of Panama:

EASTERN GROUP: MAINLAND EAST OF PENINSULA VALIENTE

Bocas del Toro, mouth Río Cahuita (2 audiospectrograms)
Veraguas, mouth Río Concepción (4)

The meager call data obtained at the above localities are contrasted with the western group in table 6 and in figures 17 and 18. It is readily apparent that frogs of the eastern group are very different in producing significantly longer notes at a significantly slower rate; ranges of variation do not overlap in note repetition rate or note duration, in the samples of normally calling (i.e., nonaggressive) frogs. Other differences are less evident and may be due in part to artifices of sampling. Relative variability in note repetition rates of the two groups does not seem different, nor can statistical significance be attached to the quite disparate coefficients of variation for note duration (table 6). The small eastern sample does have a greater absolute-time spread (0.11 sec.) in note duration values than the large western sample (0.04 sec.), but the relative magnitudes of the individual spreads are similar (×2.0 and 1.8, respectively). Based on available data, the eastern group differs in having the note repetition rate negatively correlated with temperature, but the correlation is not statistically significant (fig. 18). The eastern group has a shorter mean call length, and less variability, than the western group; biological significance seems intuitively possible in this case, although no statistical significance can presently be attached to the call-length differences (table 6).

We have not yet determined the toxic alkaloids of the eastern group of "*pumilio*," but the call differences alone lead one to wonder if the eastern frogs are really conspecific with the western group. Geographic variation in these frogs is extraordinarily complex and is still under study. One intriguing line of investigation will be to determine if there are geographically correlated differences in the calling behavior of aggressive frogs. That this might be the case is suggested by two aggression calls plotted in figure 18: A fresh-caught specimen from Río Concepción (eastern group) and another from Isla Bastimentos (western group) were separately recorded under essentially identical conditions of temperature and confinement, namely in plastic bags with other frogs from the same localities, which induced aggressive contact ("wrestling")[1] and different calling rates. The Río Concepción individual called two to three times as fast as other recorded individuals from the eastern populations; the individual notes in the aggressive call measured 0.11 second on a spectrogram, which is within the normal limits of other eastern frogs (table 6), but the note repetition rate approaches that of normally calling western frogs (fig. 18). The aggressive Isla Bastimentos frog, however, called at a *slower* rate than normally calling frogs of its group; note duration (0.05 sec.) was within normal limits for a western-group frog, but, taking temperature into account, the note repetition rate is somewhat closer to that of normally calling frogs of the eastern group (fig. 18). The aforesaid observations suggest that eastern and western "*pumilio*" behave in exactly opposite ways when changing from a normal to an aggressive call, vocalizing at an increased rate in the former group and at a decreased rate in the latter.

A slowing of the aggressive call, in the western group of Panamanian *pumilio*, is in accord with Bunnell (1973), who conducted an interesting set of experiments on the territorial vocalizations of this species in Costa Rica. Bunnell recorded normal calls ("bouts"[2]) and played the calls back to the same individuals, which thus acted as their own controls; when the speaker was placed close enough, the frog became stimulated by its own call, as evidenced by its approaching the speaker and calling at a slower rate and at decreased volume. One may reasonably conclude that such behavior serves as a warning when the territory of one frog is being invaded by another calling male.

We do not dispute the general thrust of Bunnell's data nor her conclusions, but we do have reservations about the degree of accuracy in her raw data. Bunnell (1973, p. 280; *in litt.*, Oct.

[1]Aggressive grappling between male *Dendrobates pumilio*, on Isla Bastimentos, is described and figured under the synonymous name *D. galindoi* by Duellman (1966).

[2]Bunnell's terminology differs from ours in the following respects: Her "bout" is what we term a call (i.e., an unbroken train of notes), whereas her "call" is what we define as a single note within a call; "rate of calling" is notes ("calls") per second.

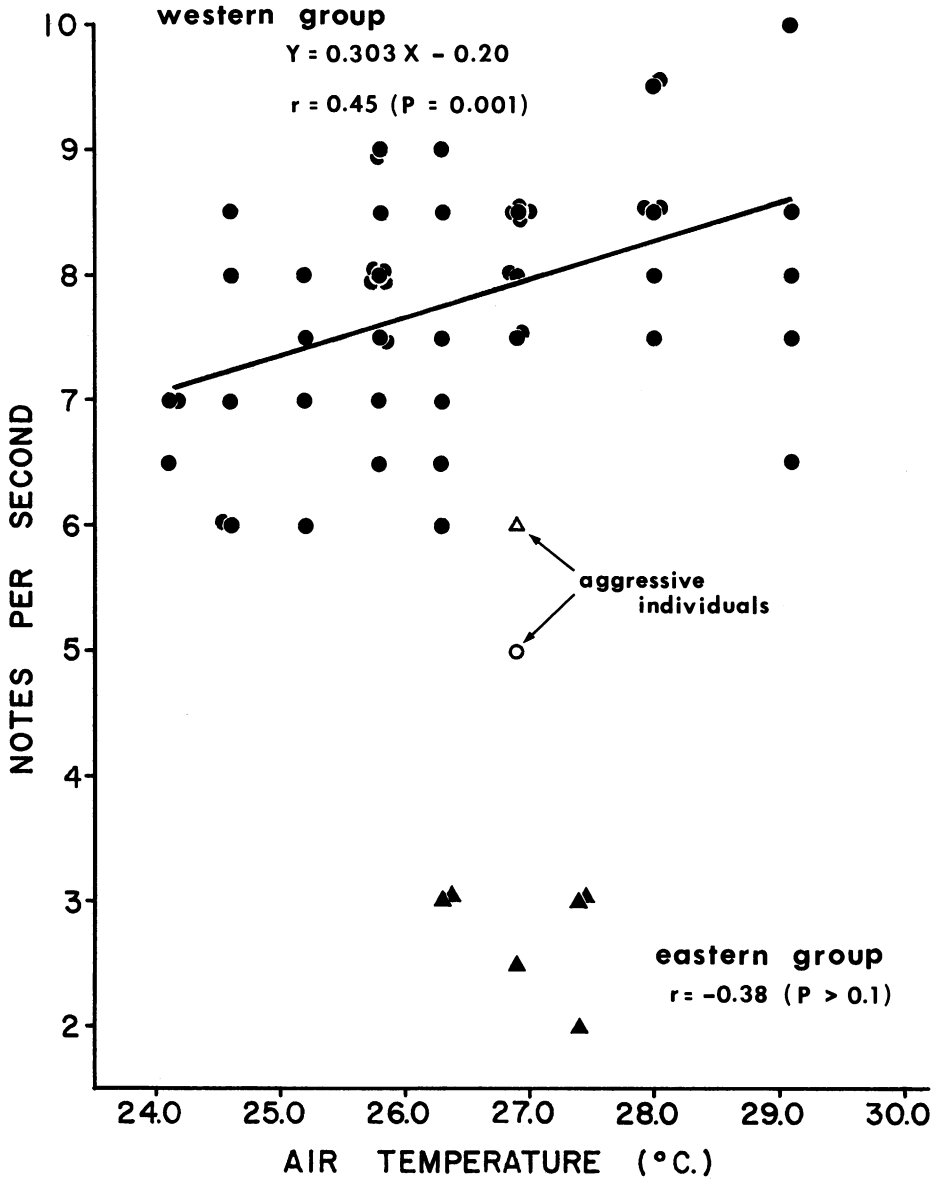


FIG. 18. Geographic variation in note repetition rate, and relationship to temperature and behavior, in Panamanian *Dendrobates pumilio*. Solid symbols represent 57 normally calling frogs for which data are summarized in table 6; open symbols represent two aggressive individuals confined with other frogs in plastic bags (see page 232). Line of best fit calculated for normally calling "western" frogs by method of least squares.

18, 1974) was hampered in having but one recorder, which was used to tape only the stimulus call. Subsequent data (in her table 1) were obtained directly from the subject frog "by use of a stopwatch and counting." Except by playing back a tape at one-half speed, we were unable to count the notes in a 32-seconds *pumilio* call that was recorded in the vicinity of

Bunnell's study area. Bunnell gave mean note repetition rates of 3.9-4.6 notes/second for normal calls, and 2.3-3.7 notes/second after stimulation by playback of a normal call. Although obtained at low morning temperatures (21-23°C.), these values still seem low compared with our one Costa Rican recording (6.1 notes/sec. at 25.2°C.), in which rate of calling fits well with data plotted for western-group Panamanian frogs (fig. 18), although differing in other respects as discussed below. Bunnell's data and conclusions could usefully be confirmed or modified by study of actual recordings made at comparable temperatures.

Bunnell's (*op. cit.*) study was conducted at La Selva, near Puerto Viejo, Heredia Province, in northern Costa Rica. As indicated above, we have available a single call of *Dendrobates pumilio* from Puerto Viejo, recorded at a temperature of 25.2°C. (fig. 19). The call is 32 seconds in length and, when played back at one-half speed, contains 195 countable notes, for a rate of 6.1 notes per second (6.5 notes/sec. on 2-sec. interval of sound spectrogram); note duration is 0.08 second. This call differs from the western group of Panamanian calls (table 6) in slightly longer duration of individual notes, and, especially, in having a pronounced break in the area of emphasized frequency, with twin peaks at about 3000 and 4000 Hz (see fig. 19). The emphasized frequency is also lower than in the Panamanian calls, in which the greatest energy output occurs above 4000 Hz (compare sections in figs. 19 and 22). Still, the Costa Rican call is much more similar to the calls of the western group of Panamanian *pumilio* than to the more geographically remote eastern group, especially in note repetition rate and note duration.

Dendrobates granuliferus Taylor

Before taking any serious interest in dendrobatid vocalizations, we casually noted in the field that the call of *Dendrobates granuliferus* sounds rather like that of *D. pumilio*, a resemblance also remarked on by Savage (1968, p. 768). Savage (*loc. cit.*) and Goodman (1971, p. 367) similarly described the call as a series of insect-like buzzes, whereas we hear the notes as harsh "chirps." Two available recordings consist of short sequences of notes uttered in a terrarium at an air temperature of 23.5°C., which probably falls within the lower part of the temperature range of naturally calling individuals. Several sound spectrograms made from these recordings show well-defined notes that vary in duration from 0.10-0.19 second (0.12-0.19 in fig. 20), with a region of emphasized frequency at about 4000

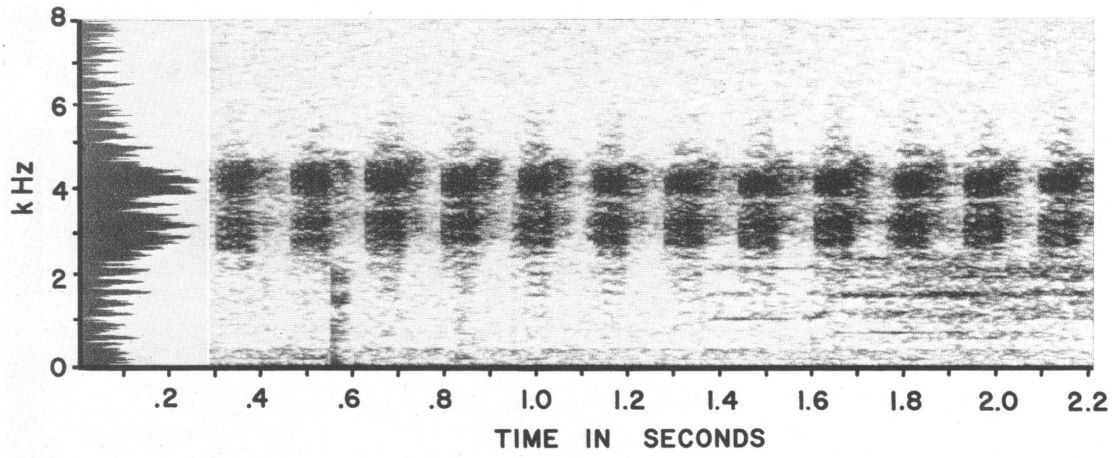


FIG. 19. Chirp call of *Dendrobates pumilio* from Puerto Viejo, Heredia Province, Costa Rica; August 1, 1965, air temperature 25.2° C. (narrow band, 45-Hz filter; KU tape 426). Section is of sixth note from left. Notice break in emphasized frequency at about 3600 Hz, and compare with calls of Panamian *D. pumilio* in figure 17 (sections in fig. 22).

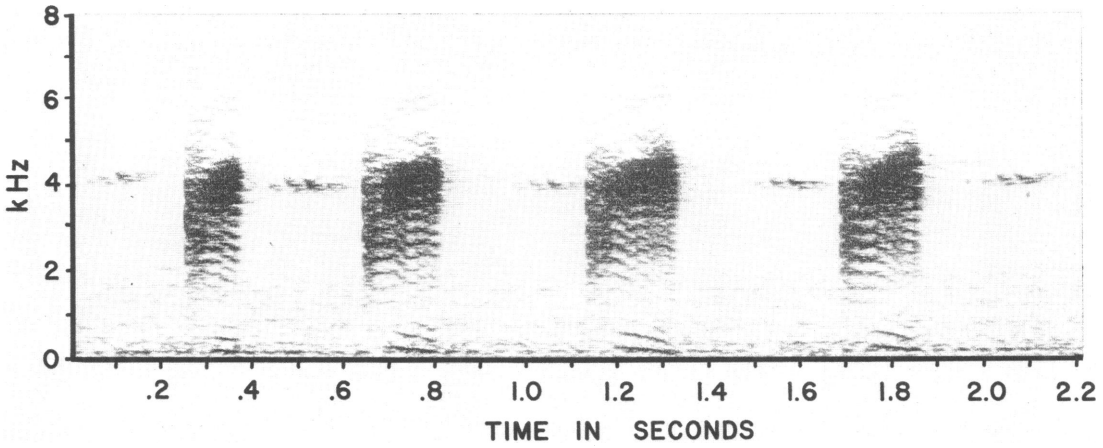


FIG. 20. Series of chirp notes of *Dendrobates granuliferus* from 4.5 km. W. Rincón de Osa, Puntarenas Province, Costa Rica (narrow band, 45-Hz filter). Recorded in terrarium, August, 1966, air temperature 23.5° C. (KU tape 673). Notice varying duration of individual notes.

Hz (figs. 20, 22F); in each case, the repetition rate is two notes per second. In note duration and note repetition rate, these sound spectrograms resemble those of the eastern group of Panamanian *pumilio* (compare fig. 20 with fig. 17C), but, in the relatively low frequency, the resemblance is to the call of a Costa Rican *pumilio* (compare figs. 20 and 22F with fig. 19). The *granuliferus* calls are unique in the variable duration of individual notes within the same sequence.

The above characteristics need to be confirmed by recordings made in the field. Judging from a paper by Goodman, however, the low note repetition rate of *Dendrobates granuliferus* may occur under natural conditions as well as in captivity. Goodman (1971, fig. 2) indicated an average call rate of one note per second in the calling sequence of an individual male that appeared to be responding to the activity of a nearby female; unfortunately, Goodman did not state whether he used a stopwatch or merely estimated elapsed time while counting notes. Goodman (*op. cit.*, pp. 367, 368) stated that normal calls contain five to 80 notes, but he indicated one exceptionally long call that consisted of more than 240 notes (*ca.* 4 min. duration according to his fig. 2). Call length, therefore, is perhaps even more variable than in the western group of Panamanian *D. pumilio*.

Fragmentary observations by Goodman (1971) and Crump (1972) suggest a territorial function of the call of *Dendrobates granuliferus*, as well as a female-attracting function. Goodman (*op. cit.*, p. 368) observed grappling between two males that continued to utter calls, "which to my ear had the pitch of the normal call but with greatly decreased frequency." Thus, *D. granuliferus* may resemble some populations of *D. pumilio* in having a decreased note repetition rate in the aggression call.

Dendrobates histrionicus Berthold

The call is an often long train of harsh chirps that are loud and relatively low pitched. The sound spectrogram of *D. histrionicus* (fig. 21A) is from a recording of an individual confined with other frogs in a plastic bag; thus there might be an aggressive element in the call, as in note repetition rate for example. Silverstone (1973, p. 297) stated that the notes are given at a rate of about three per second, whereas the rate is six per second in figure 21A. Silverstone's (*loc. cit.*) observations were made in the Serranía de Baudó, extreme northwestern Colombia, and are worth quoting in full: "The call of *D. histrionicus* is a single hoarse note similar to the sound of a small saw on wood, repeated about three times per second. When emitted singly, the call-

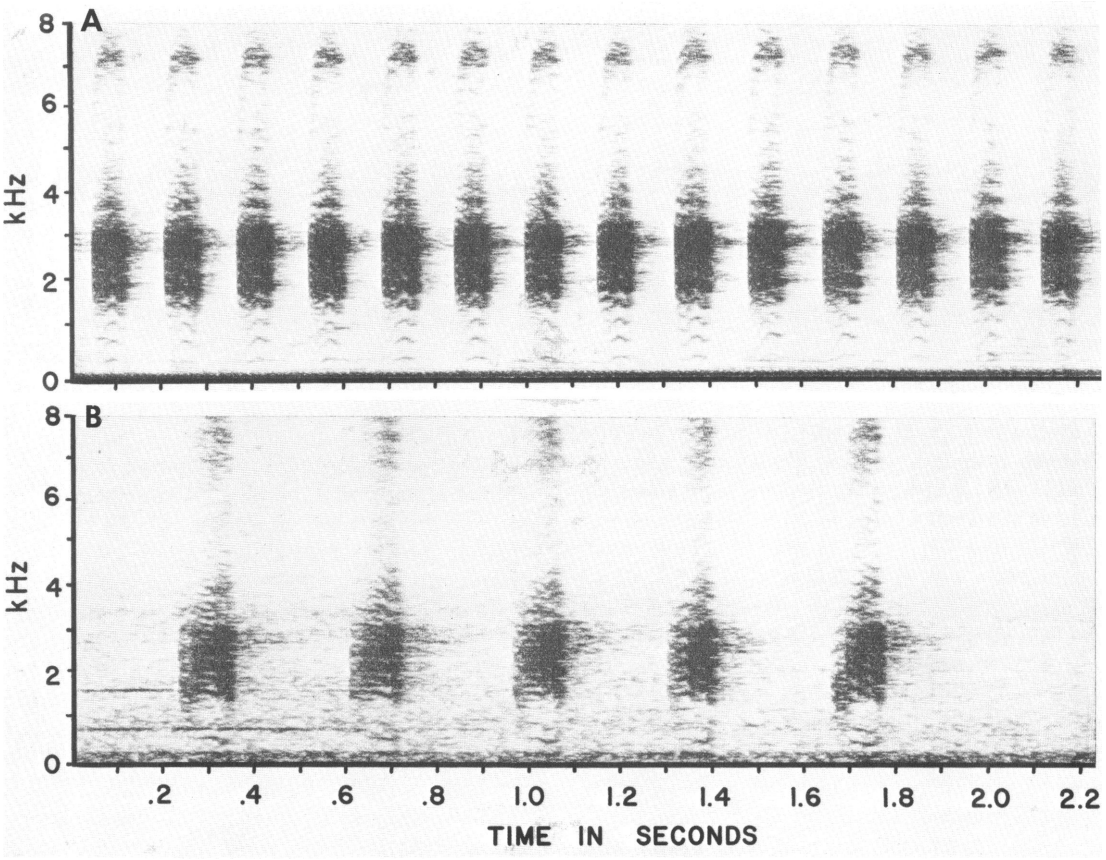


FIG. 21. Chirp calls (narrow band, 45-Hz filter). A. *Dendrobates histrionicus.* Portion of call of frog confined in plastic bag at Guayacana, Dept. Nariño, Colombia; January 29, 1970, air temperature 27° C. (specimen one of AMNH 85048-85154). B. *Dendrobates lehmanni*, new species. Portion of call of specimen presumably from type locality (purchased in Cali market); frog confined in plastic bag, air temperature 28° C. (Both from AMNH tape 176.)

note is reminiscent of the quack of a duck. The call-notes were emitted continuously for as long as 4 min, 40 s. Calls were emitted individually, not in a chorus of frogs." Funkhouser (1956, p. 75) likened the call to the sound made "by a wood borer in dead wood."

Dendrobates histrionicus has a rather long range along the Pacific lowlands of western Colombia and northwestern Ecuador. Although it seems likely that detailed study might reveal some geographic variation in vocalizations, the call is at least grossly similar to our ears throughout the range. Individuals of this species call frequently and its presence at a locality can be easily ascertained by a few minutes of walking and listening.

Dendrobates lehmanni, new species

The call of this new species is similar to that of *Dendrobates histrionicus.* The sound spectrograms presented here (fig. 21A, B) show differences in note repetition rate and note duration, but no significance can be attached to these without some knowledge of intraspecific variation. The calls of both species are similarly low pitched, with an area of emphasized frequency in the range of about 1500-3500 Hz (fig. 22A, B).

Dendrobates occultator, new species

The call of this secretive species is also similar to that of *Dendrobates histrionicus.* No record-

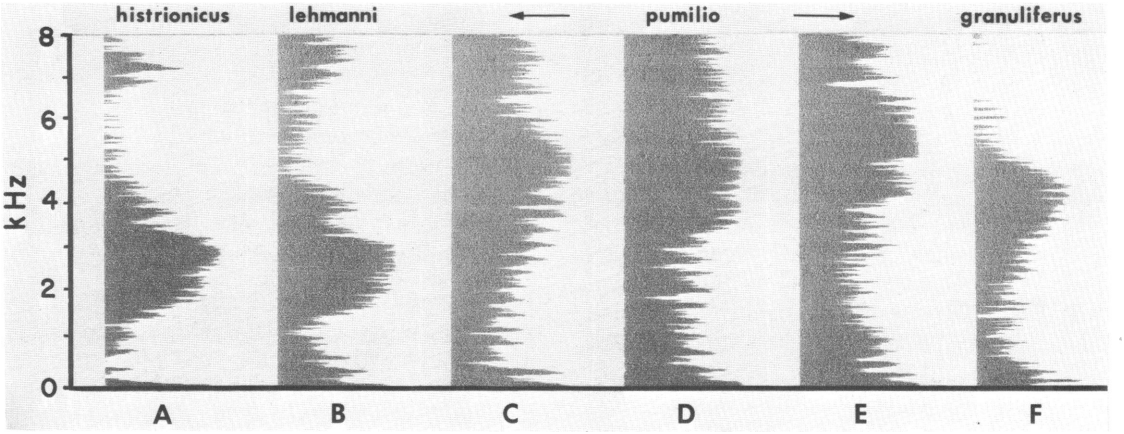


FIG. 22. Chirp calls of *Dendrobates*. Representative sections of individual notes, showing interspecific differences in the regions of dominant or emphasized frequencies (i.e., those portions of sections extending farthest right). A. *D. histrionicus* (section from middle of note 4 in fig. 21A). B. *D. lehmanni* (note 3 from fig. 21B). C, D, E. All Panamanian *D. pumilio* (notes 1, 7, and 3, from fig. 17A-C, respectively). F. *D. granuliferus* (note 3 from fig. 20).

ings are available. We did not differentiate between the calls of *occultator* and *histrionicus* in the field, although both species may not have been heard calling simultaneously (see p. 254).

OTHER CALLS

The foregoing discussion of dendrobatid vocalization has been limited to species of *Dendrobates* occurring in Central America and northwestern South America. Within this region, we have not yet visited the habitats of *D. altobueyensis*, *D. opisthomelas*, or *D. speciosus*,[1] but presume that the latter, at least, has a chirp call as do its morphologically and geographically close relatives (*pumilio* and *granuliferus*). We have had field experience with *D. fulguritus*, *D. truncatus*, *D. viridis*, and with a small undescribed species from western Colombia, but we have not seen these species calling nor have we heard chirp calls that might be attributed to them. These last four species may have buzz calls, which tend to be infrequently given and which therefore are not easily detected (see p. 252 regarding *D. viridis*). Another species probably having a buzz call is the Guayanan *D. azureus*, the "croak" of which was described by Polder (1974a, p. 188) as "a very soft, low-pitched sound, lasting ± 2 sec." (translated from the Dutch). Buzz calls may well prove more common and widespread in the genus than now known.

However, buzz calls and chirp calls are not the only vocalizations produced by species of *Dendrobates*. Some other species, in the Guayanan and Amazonian regions, produce a variety of more musical whistling notes, reminiscent of the calls of some *Colostethus* and *Phyllobates*. A number of additional dendrobatid species have been recorded, but several critical species remain to be studied in the field and we prefer to withhold discussion of the other types of calls until another time.

TAXONOMIC AND OTHER IMPLICATIONS

Complicated and diverse kinds of courtship and reproductive behavior are present in each nominal genus of dendrobatid frogs, namely *Colostethus* (e.g., Dole and Durant, 1974), *Dendrobates* (e.g., Silverstone, 1973), and *Phyllobates* (personal observ.). Territorial behavior is widespread in the family, and some form of aggressive behavior seems to be characteristic of many, perhaps most, species. It is not yet advisable to generalize too broadly concerning the behavioral patterns of dendrobatids, both because of a paucity of data and the complexity

[1]Note added in proof: Recent field work has verified that *D. speciosus* has a chirp call similar to "western" *D. pumilio*.

of their social interactions.[1] Nonetheless, it appears that types of vocalization may be strongly tied in with the general life-styles of dendrobatid frogs. At least this seems to be so in the case of the northern species of *Dendrobates* that we have studied in the present report. To summarize:

1) *Chirp calls* are given by forest-inhabiting *Dendrobates* that call nearly continuously, usually from elevated perches or even arboreal situations. These species are strongly territorial, and aggressive "wrestling" behavior between males is characteristic. In some (if not all) species the note repetition rate is altered in response to the proximity of a potential rival. [Sources: present paper; Bunnell (1973); Crump (1972); Daly and Myers (1967); Duellman (1966); Goodman (1971); Silverstone (1973).]

2) *Buzz calls* are given by the forest-inhabiting *Dendrobates auratus* and *D. minutus* that call infrequently and, so far as known, only from the ground or very low perches (although arboreal tendencies may otherwise be present). These species appear to be nonterritorial, or at least less strongly so, and aggressive behavior, if present, is less frequent and perhaps limited to special circumstances. Possibly the call serves primarily as a mate-attracting function, with little if any territorial component. [Sources: present paper; Dunn (1941); Senfft (1936).]

Given the above correlations between type of vocalization and behavior, can any inferences be drawn concerning evolutionary relationships? It is reasonable to expect that closely related frogs often will have similar calls and behavior, especially if the species have allopatric distributions, and all the northern *Dendrobates* that have a chirp call and highly developed territoriality may in fact be closely related. On morphological and geographic grounds they at least form two seemingly natural clusters of species, as follows:

pumilio-granuliferus-speciosus

Size small to medium; omosternum present; dorsum unicolor or dotted, rarely spotted; Nicaragua to western Panama.

histrionicus-lehmanni-occultator

Size medium to large; omosternum usually absent; dorsum blotched, banded, or with large spots, rarely unicolor; Pacific versant of Colombia and northwestern Ecuador.

Both pumiliotoxins and histrionicotoxins occur in the above species clusters, either separately or as mixtures within species, thus supporting the notion of close relationship suggested by behavior and vocalizations. Also, we would add that chirp calls, as herein defined, are unknown in *Phyllobates* and in the various species of *Colostethus* with which we are familiar. Nonetheless, the chirp calls and associated behavior can presently provide only supporting evidence of relationship between the above groups; in no substantial way do these characters "prove" the presumed relationships. The reason for this is the frequent convergence of such traits, and the inherent difficulty of homologizing either behavior or sounds in the absence or ignorance of morphological correlates—a point well made by Atz (1970) regarding behavior, and Martin (1972) regarding vocalization—and the same limitation presently applies to the skin toxins, as we have shown elsewhere in the present paper.

The danger in assuming relationship on the basis of vocalization is particularly well illustrated by the buzz call. The two species of *Dendrobates* (*auratus* and *minutus*) definitely known to produce this type of call are quite dissimilar in size and coloration. The skin toxins do not contradict the possibility of close relationship, as both species produce a mixture of pumiliotoxin B and lower molecular-weight alkaloids, but *minutus* is the smallest species in the genus and has a striped pattern, whereas *auratus* is a large species with a blotched or spotted pattern. Furthermore, we have also discovered a buzz call in "*Phyllobates*" *espinosai* Funkhouser, an Ecuadoran dendrobatid that has not been, and seemingly should not be, considered as congeneric with *Dendrobates*. The small *espinosai* produces a buzz (fig. 23) intermediate in length between the call of *Dendrobates minutus* and

[1]For example, several authors (Senfft, 1936; Crump, 1972; Polder, 1974a) have noted the extraordinary absence of amplexus during dendrobatid matings. Even so, this does not mean that amplexant behavior has been entirely lost, for in some *Dendrobates* (e.g., Polder, 1974a) and *Phyllobates* (personal observ.) such behavior has been incorporated as part of the aggressive repertory!

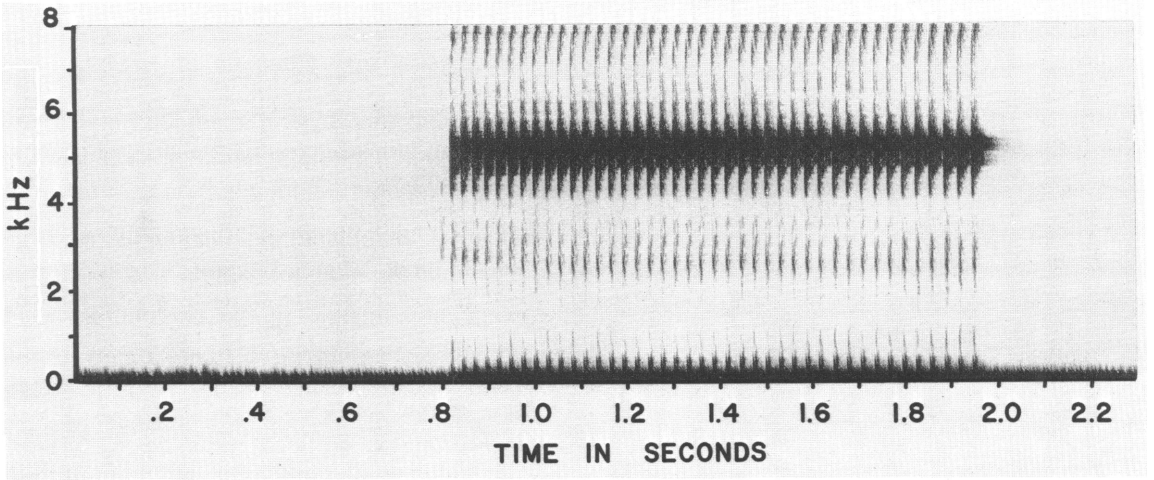


FIG. 23. Buzz call of "*Phyllobates*" *espinosai* from 1-2 km. NW Tandapi, Pichincha Province, Ecuador (wide band, 300-Hz filter). Recorded in terrarium, November, 1974, air temperature 22.8° C. (AMNH tape 179; specimen one of AMNH 90332-90338).

This call sounds much like buzz calls of *Dendrobates auratus* and *D. minutus*, and structural similarities of calls can be visualized by comparing with figure 16. Such correspondency, however, is not necessarily proof of relationship, although similar behavioral traits seem indicated (see text).

that of *D. auratus*, and it resembles these species behaviorally in being a terrestrial forest frog that calls relatively infrequently or perhaps seasonally.[1] Our assumption, at present, is that similar selection pressures have resulted in similar calls and life-styles in these diverse dendrobatids. Neither vocalizations nor molecular data should be viewed as unequivocal criteria of "close" relationship, unless morphological contradictions are otherwise explainable.

DESCRIPTIONS OF NEW SPECIES

Although dendrobatids in general are relatively small frogs, there is nonetheless considerable size variation within the family, with size being a useful and frequently diagnostic character. Of the three species being described as new, one is among the smallest of dendrobatids, one is medium-sized, and one is large. We arbitrarily define size classes of dendrobatids as follows (mean snout-vent length of sexually mature frogs, for given populations):

Large	> 30 mm. (> 40 mm. = very large)
Medium	20-30 mm.
Small	< 20 mm. (< 16 mm. = very small)

The above size classes are similar to those given by Savage (1968) for Central American dendrobatids, but, partly for ease of remembrance, it is convenient to make the classes nonoverlapping and to round off his figures to the 20 mm. and 30 mm. limits. Savage's assignments to size classes apparently were based on measurements of individuals throughout the geographic range of a species, whereas we prefer to use averages based on defined populations in order to allow for pronounced geographic variation in some species. Thus, *Dendrobates histrionicus* is a medium-sized

[1] *Phyllobates espinosai* was heard calling commonly at Tandapi, Ecuador, on November 13-14, 1974, but none was heard calling the previous February in nearby populations south of Santo Domingo de los Colorados. In contrast, species characterized by chirp calls seem to call daily throughout the year.

to large species when all populations are considered, and *D. pumilio* varies from small to medium-sized. Many dendrobatids, however, will fall within a single class regardless of interpopulational variation. Differences between the sexes usually are not great enough to cause an overlap in the arbitrary size classes. With some exceptions, the sexes of dendrobatids are not well differentiated by external characters. Savage's (1968, p. 748) statement that females of the larger species "average several mm longer than males" is not applicable to the two large South American frogs discussed in the present paper, namely *Dendrobates histrionicus* (table 3) and *D. lehmanni* (below), in which males tend to be slightly larger than females.

The first species described below has been known to us since 1968, and color photographs of it have appeared in several popular and semi-technical magazines. However, all previous specimens have originated with animal dealers, and only recently have we traced the source of these frogs to a restricted area of montane forest in the western Andes. We name this strikingly colored species in memory of a friend and colleague who immensely aided our field work in Colombia.

Dendrobates lehmanni, new species
Plate 2, figures A, B; text figures 2, 3, 21B, 22B, 24, 25; map 2

Dendrobates histrionicus, not of Berthold: Hoogmoed, 1971, p. 1 (color plate). Polder, 1974a, p. 190 (black and white photograph). Silverstone, 1975, part: pp. 19 (fig. 13 O, drawing of body pattern), 23.

Dendrobates tinctorius histrionicus, not of Berthold: Nieuwenhuizen, 1972 (black and white photograph).

Holotype. AMNH 88153 (field no. CWM 11474), an adult female obtained by John W. Daly and Charles W. Myers on January 28 or 29, 1973, in montane forest approximately 13 km. west of Dagua (town), 850-1200 meters elevation on south-facing versant of upper Río Anchicayá drainage, Department of Valle, Colombia (map 2).

Paratypes. Fifty-seven specimens, as follows: AMNH 88154-88199, 88227-88234 (cleared and stained), KU 152918-152920, with same collecting data as holotype.

Etymology. Named for the late F. Carlos Lehmann Valencia—biologist, conservationist, and founder of natural history museums in his native Colombia.[1]

Definition and Diagnosis. A large *Dendrobates*, to about 35.5 mm. snout to vent; black with two vivid orange or orange-red crossbands (occasionally broken) on nape and sacral regions, orange bracelets on limbs, and conspicuously white digit tips. Teeth absent; omosternum usually absent; first finger slightly shorter than second; tarsal tubercle present; skin secretions including pumiliotoxins, lacking histrionicotoxins.

The unique color pattern immediately distinguishes *Dendrobates lehmanni* from all other dendrobatids, including all known populations of *D. histrionicus*. Different skin chemistry indicates *lehmanni* as a species distinct from the less toxic *histrionicus*, which is geographically variable in color pattern but which always contains significant amounts of histrionicotoxins and usually lacks pumiliotoxins (see p. 224).

Description. (Fifty specimens plus eight cleared and stained.) Size large, to 35.3 mm. snout-vent length, with males in the sample averaging a bit larger than females: 26 adult males 31.3-35.3 mm., mean 33.33±0.20; 24 adult females 31.3–34.3 mm., mean 32.80±0.16. [Difference between means statistically not significant, $t = 1.863$, $P < 0.10 > 0.05$; but slightly larger average size of males possibly a natural phenomenon rather than sample bias, inasmuch as six of eight individuals measuring 34.0 mm. or greater are males, including the five largest (34.5-35.3 mm.).] Vocal slits present in males.

In life, vividly marked with bright orange or orange-red bands on a black ground, or occasionally (several specimens) with light orange bands or rarely (one individual) with very faded orange bands approaching yellow. Tips of all or most digits conspicuously bluish white. Iris dark brown, with pupil distinguishable from iris in average light. Bright orangish markings distributed as follows: (1) Around end of snout, this area confluent or not with a spot underneath

[1]Dr. Lehmann died in Cali on August 15, 1974. He also is commemorated in the name *Colostethus lehmanni* Silverstone. Despite the uncertain generic partitioning of dendrobatid frogs (Myers and Daly, 1971), we express confidence that these two very different species will be maintained in separate genera.

chin. (2) A transverse band behind head, occasionally broken dorsally, often expanded laterally under eye and above forelimb; sometimes complete under throat, but often broken or absent ventrally or extending forward along mouth to chin. (3) A transverse band on rear of body, occasionally broken dorsally or laterally; ventrally, usually chevron-shaped or otherwise extended anteriorly (thus crossing middle of venter) but often broken, sometimes represented by large isolated spot on chest and belly. (4) A broad band on upper part of lower arm, usually including elbow, often incomplete ventrally. (5) On hind limb, usually a band on thigh above knee and another on lower part of shank, these markings usually incomplete ventrally; also, many specimens with a small band or dorsal blotch on hind foot. Dorsal black interspace wider than either nape or sacral band. Occasional specimens with vague splotches of orangish brown in the black ground color, especially on venter, rarely forming a faint median spot on back (fig. 24D). In preservative, black with gray or grayish white bands.

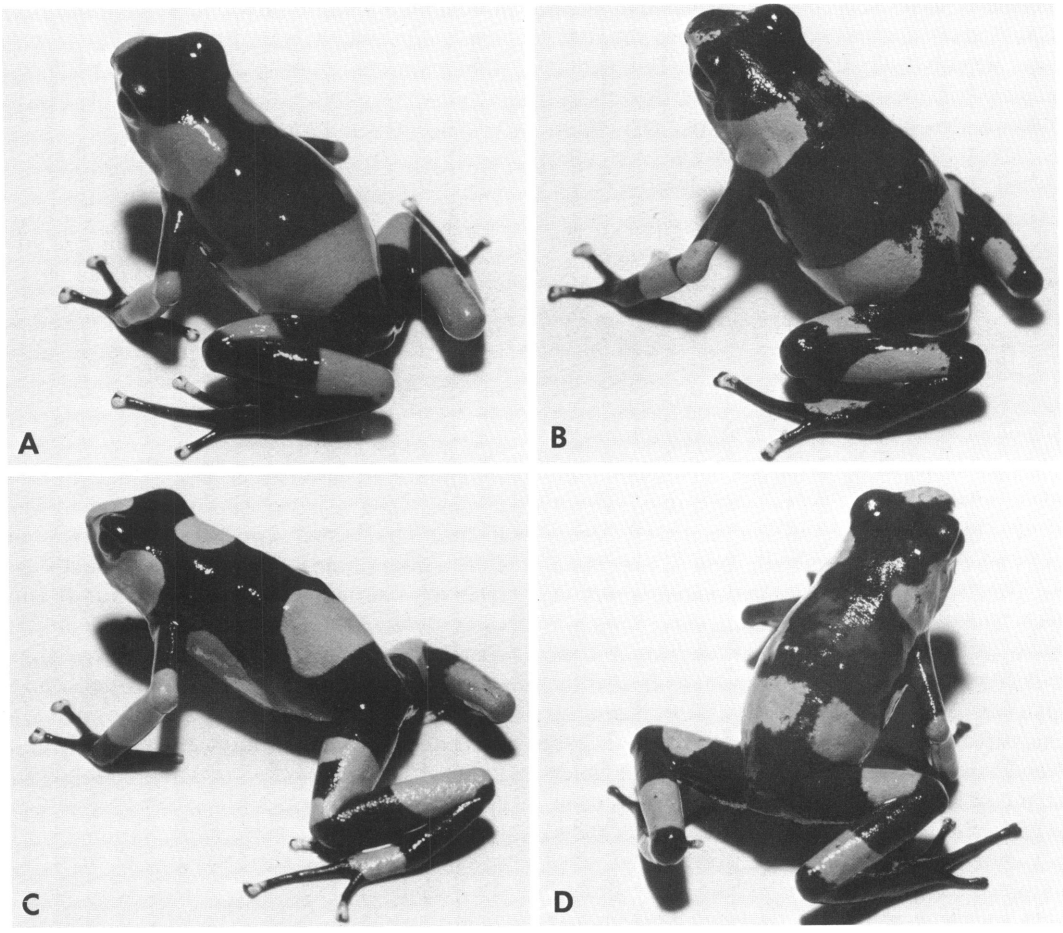


FIG. 24. Color pattern variation in *Dendrobates lehmanni*, new species. All paratypes, as follows: A. AMNH 88156. B. AMNH 88157. C. AMNH 88155. D. AMNH 88158. These specimens exhibit nearly entire observed range of variation in broken nape and sacral crossbands; although type series is somewhat biased toward such variants, they are uncommon in nature (see text, and compare color pl. 2). Note also variation in limb bracelets and in degree of white coloring on tips of digits.

Skin appearing virtually smooth in most individuals, although under magnification tending to be somewhat pitted in places (especially atop head of some individuals) and weakly rugose; often very weakly granular on thigh and under throat and, rarely, on venter. Head considerably to slightly narrower than body, with widest part between outer edges of upper eyelids; eyes prominent; diameter of orbit less than snout length, rarely almost equal. Snout short, sloping; truncate in dorsal or ventral aspect, obtuse in lateral profile. Naris directed posterolaterally, both nares visible in ventral or head-on view but not visible from above; nares much closer to tip of snout than to eye. Canthus rostralis rounded, loreal region slightly concave. Tympanum rounded or, occasionally, a vertical oval; indistinct posteriorly and dorsally, varying in relative size from less than one-half to more than one-half diameter of eye (sexual dimorphism, if present, not marked).

Relative lengths of appressed fingers $3 > 4 > 2 > 1$, each terminating in expanded disc; disc of first finger smallest, little wider than finger, those of third and fourth fingers nearly twice finger width; first finger long, its tip reaching or nearly reaching disc of second finger. A large medial tubercle at base of palm, usually a small inner metacarpel tubercle, and one or two undivided subarticular tubercles on fingers (one each on fingers 1 and 2, two each on fingers 3 and 4); tubercles low, with rounded surfaces. Hind limbs of moderate length, heel of appressed limb usually just reaching eye, falling short in some individuals; tibia/snout-vent length = 0.38-0.43. Relative lengths of appressed toes $4 > 3 > 5 > 2 > 1$, each terminating in slightly expanded disc. Tarsal ridge very short, forming a usually obliquely elongated and keel-like tubercle on inner side of tarsus; distinct inner and outer metatarsal tubercles; first and second toe each with a basal subarticular tubercle, and a few weaker subarticular tubercles usually discernible on other toes. Palms and soles fleshy. Digits of hands and feet flattened on bottom; lacking webbing, supernumerary tubercles, or lateral fringe (if desiccated, ventrolateral edge of digit might be misinterpreted as an unscalloped fringe).

Omosternum present in two frogs, absent or unossified in six (AMNH 88227-88234, cleared and stained). Flesh blackish. Teeth absent.

Measurements of Holotype (in Mm.). Holotype not dissected, determined to be female because it lacks vocal slits, and presumed to be mature because of its size. Length from snout to vent 33.3; tibia length from heel to fold of skin on knee 13.4; greatest width of body 15.0; greatest head width (between outer edges upper eyelids) 9.9; head length from tip of snout to angle of jaws 8.5; tip of snout to center of naris 1.5; center of naris to edge of eye 2.7; diameter of orbit 3.7; horizontal diameter of tympanum (indistinct) <2.0; distance between centers of nares 4.0; length from proximal edge of palmar tubercle to tip of longest (3rd) finger 9.5; width of disc of third finger 1.7; width of finger (penultimate phalanx) below disc 0.9.

Voice. A *histrionicus*-like chirp call (p. 236, fig. 21B).

Skin Toxins. Methanolic extracts of skins contain at least eight alkaloids, including relatively large amounts of pumiliotoxin B, small amounts of pumiliotoxin A, and moderate amounts of two unnamed compounds with molecular weights of 267 and 275. There are trace amounts of four other alkaloids having molecular weights of 253, 307, 307, and 341. Histrionicotoxins appear to be absent.

Thin-layer chromatography had revealed five alkaloids (fig. 2). Isolation of these and identification by mass spectrometry proved difficult, since some of the compounds were relatively unstable, but pumiliotoxin B (molecular wt. 323) was identified. Quantitative chemical ionization mass spectrometry (fig. 3) of the total alkaloid fraction provided evidence for significant amounts of alkaloids with protonated molecular ions at mass 324 (pumiliotoxin B), 268, 276, and 308 (pumiliotoxin A or an isomer), as well as trace amounts, or fragments (e.g., at mass 240), of several other compounds. Gas chromatographic-mass spectral analysis (fig. 25) confirmed the four major alkaloids, and two trace compounds with protonated molecular ions at 254 and 342. This technique additionally revealed two other compounds (besides pumiliotoxin A) that show protonated molecular ions at mass 308.

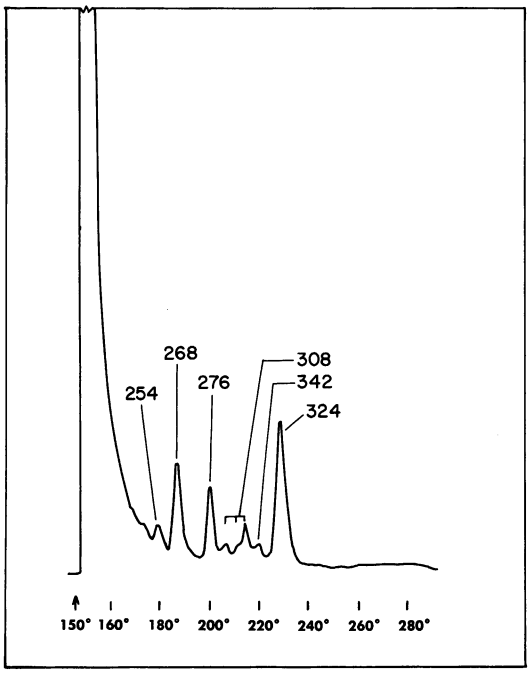


FIG. 25. Gas chromatogram of alkaloids from population sample of *Dendrobates lehmanni*, new species (eight frogs from type locality, January, 1973). Chromatography run with 2 μl of methanolic extract containing concentrated alkaloids equivalent to amount in 2 mg. of wet skin.

At least eight alkaloids are present, including pumiliotoxin B (324), pumiliotoxin A (308), and two other compounds with protonated molecular ions at mass 308. The compound with an ion at mass 276 has not been found in significant amounts in any other species.

Remarks. A total of 182 specimens of *Dendrobates lehmanni* was obtained at the type locality on January 28-29, 1973, of which 50 individuals were preserved entire and the remainder skinned for extraction of the toxic alkaloids. Eight of the skinned carcasses were cleared and stained and are designated as paratypes. The other carcasses were preserved but are not catalogued; in a sense, these remains might be considered paratypic by virtue of the data from their skin toxins, but they do not otherwise contribute to the preceding description. In order to obtain as complete a variational range in color pattern as possible, most of the relatively rare "variants" were picked out of the total sample and preserved. Hence, the type series is a biased sample in that 15 (30%) of the 50 frogs have one or both of the orange crossbands broken dorsally or laterally (see fig. 24); the actual frequency of such individuals in the natural population is probably no more than about 10-15 percent.

Before January, 1973, *Dendrobates lehmanni* was known to us only from specimens purchased in the Cali street market and New York pet shops, and, indirectly, through photographs either sent by colleagues or published in the following sources: Anon. (1971), Grzimek (1970), Hoogmoed (1971), Nieuwenhuizen (1972), and Toner (1972). The frogs in the published photographs presumably were all obtained through the animal trade and were either unidentified or listed under the name *histrionicus*. Silverstone (1975, p. 23) also considered market specimens of these distinctively colored frogs to represent a population of *Dendrobates histrionicus*, certainly not an unjustified conclusion considering the remarkable geographic variation of *D. histrionicus* (pl. 1). Silverstone (*loc. cit.*) considered absence of an omosternum and "the same breeding call" as showing that only one species is involved; nonetheless, the newly described *D. occultator* also has a similar call and lacks an omosternum, whereas some *lehmanni* do have an omosternum, thus reducing the value of these characters in defining *histrionicus*.

The fundamentally different spectrum of skin alkaloids makes it extremely difficult to accept *Dendrobates lehmanni* as a population of *histrionicus* (see p. 223). Not only does *lehmanni* differ in having relatively high molecular-weight alkaloids, notably the pumiliotoxins, it also lacks histrionicotoxins, which appear always to be detectable in *D. histrionicus*.

Dendrobates lehmanni has embarked on an evolutionary course different from that of *D. histrionicus*, judging from the aforesaid biochemical differences, the crossbanded color pattern of *lehmanni*, and the habitat differences (montane vs. lower elevation forest). Such differences however, might not necessarily preclude a close relationship between these two species. Despite the biochemical similarity among populations of *histrionicus*, there is also pronounced

interpopulational variation (see Biochemical and Other Variation in *D. histrionicus*). Because of the striking differences in skin toxins that exist between and within species, we are considering the hypothesis that populations of some species have the potential for making substantial shifts in the alkaloids produced, possibly even to the extent of elaborating one major class of compounds (e.g., pumiliotoxins) and dropping another (e.g., histrionicotoxins).[1] If we allow for this possiblity, *Dendrobates lehmanni* may have speciated as a disjunct population of ancestral *histrionicus* on the western flank of the Cordillera Occidental. Under these circumstances, there would not necessarily be selection for a change in the calling behavior or in the call, if indeed the vocalizations are actually as similar as they now appear on the basis of very limited data (p. 236 and fig. 21A, B).

The unique color pattern of *Dendrobates lehmanni* is at least theoretically derivable from variation contained in extant populations of *D. histrionicus*. Known populations of *histrionicus* are characterized by a spotted, blotched, or nearly unicolor pattern, but the bright limb bracelets of *lehmanni* have their counterpart in some populations of *histrionicus*, and it is not difficult to imagine the development of a crossbanded pattern in certain of these populations (e.g., pl. 1); seemingly transitional patterns, in the form of partial crossbanding, have in fact been demonstrated in the variation of two northern populations of *histrionicus* (Silverstone, 1975, fig. 13L, N [fig. 13O = *lehmanni*]).

In *Dendrobates occultator* and some populations of *D. histrionicus*, the digit tips are slightly whitish, although the white tends to be confined to the ultimate phalanx (disc) and is not conspicuous as in *D. lehmanni*. Captive *lehmanni* frequently were seen to twitch and flutter the tips of their white digits. Such digit fluttering was brief, lasting no more than a few seconds at a time, but the behavior was sufficiently conspicuous to evoke comment from various persons looking at the terrarium-confined frogs. This behavior has also been seen in some other dendrobatids, but is more evident in *lehmanni* because of the noticeably pale digits.

[1]It will not be feasible to speculate on biosynthetic pathways until we learn more about the structures of pumiliotoxin-like molecules. Such a major shift in skin alkaloids would, however, seem to bespeak a change in evolutionary direction, presumably under strong selective forces.

Dendrobates occultator, new species
Plate 2, figure C; text figures 2, 3, 26; map 1 (loc. E)

Holotype. AMNH 88143 (field no. CWM 11965), an adult male obtained by John W. Daly and Charles W. Myers February 10-12, 1972, at La Brea, 50 meters elevation, on the Río Patia (= upper tributary of Río Saija), at an estimated 15 km. by river below mouth of Quebrada Guanguí, Department of Cauca, Colombia.

Paratypes. Thirteen specimens, as follows: AMNH 88144, 88145, KU 152922, same collecting data as holotype. AMNH 85978, 85979, 88146-88152 [88148, 88149, cleared and stained], KU 152923, Quebrada Guanguí, about 0.5 km. above its junction with Río Patia, 100-200 meters elevation, in upper Río Saija drainage, Department of Cauca, Colombia.

Etymology. The specific epithet, a Latin noun in apposition, means "a hider," in reference to the species' secretive habits.

Definition and Diagnosis. A medium-sized red *Dendrobates* with conspicuously yellow-spotted sides, attaining a snout-vent length of about 27-28 mm. Teeth absent; omosternum absent; first finger slightly shorter than second; tarsal tubercle present; skin secretions including pumiliotoxin B and histrionicotoxins.

The yellow-spotted sides below a red dorsum distinguishes *Dendrobates occultator* from all other dendrobatids. Its sympatric occurrence with a population of *D. histrionicus*, combined with the presence of significant amounts of pumiliotoxin B, suggests *occultator* as a species distinct from the variable *histrionicus*.

Description. (Fourteen specimens.) Medium size, to 27.1 mm. snout to vent: one subadult male (vocal slits small) 20.8 mm.; 11 adult males 24.4-27.1 mm., mean 25.99±0.26; two adult females (enlarged ova in smallest) 26.1, 26.8 mm. Vocal slits present in males.

In life, a deep red dorsally with variable scratchlike black markings; sides of body densely marked with small golden yellow spots on a

black ground. Red color of dorsum extending in variable amounts onto upper parts of limbs, which otherwise are black; hands and feet black except digit tips tending to be white on dorsal side of discs. Undersides black, with golden yellow spots or mottling extending from sides of body across venter and onto throat and chin; the bright ventral markings tending to be confluent and larger than on sides, this tendency reaching an extreme in holotype, whose chest and belly were almost completely golden yellow save for a few small, scratchlike black markings. Two individuals with a few tiny ventral blotches of pale blue additional to the yellow markings. Iris dark, virtually no contrast between it and pupil in normal light. In preservative, a few specimens uniformly black, others with pale gray lateral and ventral spots.

Skin appearing virtually smooth, or somewhat pitted and rugose under magnification. Head narrower than, to about same width as, body with widest part between outer edges of upper eyelids; eyes prominent, diameter of orbit somewhat less than snout length. Snout short, sloping; truncate in dorsal and ventral aspect, obtuse in lateral profile. Naris directed posterolaterally, both nares visible in ventral or head-on view but not visible from above; nares much closer to tip of snout than to eye. Canthus rostralis rounded; vertical loreal region flat to slightly concave. Tympanum rounded or a vertical oval, indistinct posteriorly and dorsally, roughly one-half of eye diameter.

Relative lengths of appressed fingers $3 > 4 > 2 > 1$, each terminating in expanded disc; disc of first finger smallest, those of third and fourth fingers nearly twice finger width; first finger long, its tip reaching or nearly reaching disc of second finger. A large medial tubercle at base of palm, a smaller and less prominent inner metacarpel tubercle, and one or two prominent subarticular tubercles on fingers (one each on fingers 1 and 2, two each on fingers 3 and 4). Hind limbs of moderate length, heel of appressed limb reaching eye or just falling short; tibia/snout-vent length = 0.38-0.43. Relative lengths of toes $4 > 3 > 5 > 2 > 1$, each terminating in slightly expanded disc. Tarsal ridge very short, forming an obliquely elongated keel-like tubercle on inner side of tarsus; elongated inner and rounded outer metatarsal tubercles distinct; one to three often indistinct subarticular tubercles (one each on toes 1 and 2, two each on 3 and 5, three on 4). Palms and soles fleshy. Digits of hands and feet flattened on bottom; lacking webbing, supernumerary tubercles, or lateral fringe (if desiccated, ventrolateral edge of digit might be misinterpreted as an unscalloped fringe).

Omosternum absent (AMNH 88148 and 88149, cleared and stained). Flesh blackish. Teeth absent.

Measurements of Holotype (in Mm.). The undissected holotype is an adult male as evidenced by well-developed vocal slits and the fact that it was calling when found. Length from snout to vent 25.0; tibia length from heel to fold of skin on knee 9.7; greatest width of body 9.5; greatest head width (between outer edges upper eyelids) 7.7; head length from tip of snout to angle of jaws 7.4; tip of snout to center of naris 0.8; center of naris to edge of eye 2.1; diameter of orbit 3.2; horizontal diameter of tympanum (posteriorly indistinct) > 1.5; distance between centers of nares 3.2; length from proximal edge of palmar tubercle to tip of longest (3rd) finger 7.0; width of disc of third finger 1.1; width of finger (penultimate phalanx) below disc 0.6.

Voice. A *histrionicus*-like chirp call (pp. 236, 254).

Skin Toxins. Methanolic extracts from four skins (an unpreserved topotype and three paratypes, AMNH 88148-88150) contained at least 10 alkaloids, including significant amounts of pumiliotoxin B and lesser amounts of histrionicotoxin and isodihydrohistrionicotoxin. The other alkaloids present are unnamed compounds with molecular weights of 231, 239 (two or perhaps four isomeric compounds), 241, 259, 265, and 283. None of these alkaloids appears unique to this frog.

Thin-layer chromatography had indicated the presence of at least four alkaloids, with the major constituent having a relatively low R_f value (fig. 2). Isolation and identification by mass spectrometry were rendered difficult by the limited sample size, the complexity of the alkaloid mixture, and the lability of certain compounds. However, pumiliotoxin B (molecular wt. 323) and the alkaloids with a molecular weight

of 239 were identified as constituents of the major "spot" (fig. 2); the isomeric compounds (molecular wt. 239) yield major fragments at masses 196, 182, 180, and 166. Quantitative chemical ionization mass spectrometry of the total alkaloid fraction provided evidence for major protonated molecular ions at mass 240, 242, 284, and 324 (fig. 3). Combined gas chromatographic-mass spectral analysis revealed

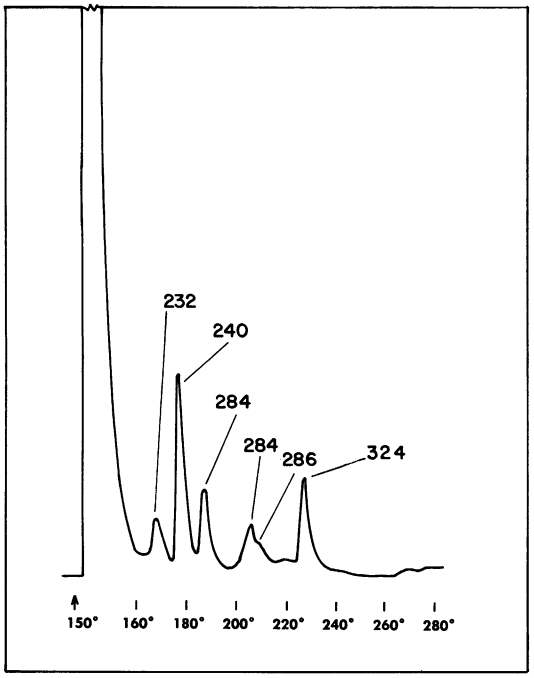


FIG. 26. Gas chromatogram of alkaloids from a mixed sample of *Dendrobates occultator*, new species (four skins, see table 2, note *e*). Chromatography run with 2 μl of methanolic extract containing concentrated alkaloids equivalent to amount in 2 mg. of wet skin.

At least 10 alkaloids are present, including major amounts of isomeric compounds with protonated molecular ions at mass 240, compounds with ions at 232 and 284, histrionicotoxin (284), isodihydrohistrionicotoxin (286), and pumiliotoxin B (324). Peak labeled 240 also contained minor amount of an alkaloid with an ion at 242, whereas peak labeled 284 also contained minor amounts of alkaloids with ions at 260 and 266.

the presence of at least 10 alkaloids (fig. 26): Pumiliotoxin B (protonated molecular ion, 324) was identified, as were histrionicotoxin (284) and isodihydrohistrionicotoxin (286). Another compound with a protonated molecular ion at 284 (additional to histrionicotoxin) was present, as were the isomeric compounds with protonated ions at 240, and other compounds with protonated ions at 232, 242, 260, and 266.

Remarks. The first two specimens of *Dendrobates occultator* were obtained in 1971 by Borys Malkin, as part of a collection made at Quebrada Guanguí for the American Museum of Natural History. Two species of *histrionicus*-like *Dendrobates* were included in Malkin's collection, namely a large species (> 30 mm.) and two specimens of a medium-sized species (< 28 mm.). Additional material obtained by us in 1973, and subsequent analysis of the skin toxins, suggested that the larger frogs represented a distinctive and heretofore unknown population of *D. histrionicus* (pl. 1, fig. E; fig. 11) and that the smaller frogs were a new species, herein named *D. occultator.*

Dendrobates occultator (mean s-v length about 26 mm.) is a much smaller species than the sympatric population of *D. histrionicus* (about 33.5 mm.) and, on the average, is smaller than any known population of *histrionicus* (table 3). Nonetheless, size differences alone are not sufficient to separate the two species, and without knowledge of their skin toxins and sympatric occurrence, we almost certainly would have decided that *occultator* represented nothing more than another interesting geographic variety of *histrionicus.* Comparison of the skin toxins permits us to assign names to the sympatric populations with reasonable confidence (see p. 224) and to conclude that the coloration of *occultator* is indeed diagnostic of a distinct species. This conclusion is supported by the differences in size and behavior (see below and p. 254).

In habitus and size, *Dendrobates occultator* most closely resembles the nominal *D. histrionicus sylvaticus* Funkhouser (1956) from northwestern Ecuador (pl. 1, fig. H; figs. 13, 14). We have not found characters that would reliably separate poorly preserved specimens (i.e., those having lost color pattern) of these forms,

although *sylvaticus* attains a slightly larger size and has relatively shorter limbs than *occultator*:

SNOUT-VENT LENGTH (MM.)	TIBIA LENGTH/ SNOUT-VENT
D. h. sylvaticus (N=15♂)	
26.81±0.28 (24.9-28.2)	0.366±0.005 (0.34-0.40)
D. occultator (N=11♂)[a]	
25.99±0.26 (24.4-27.1)	0.396±0.006 (0.38-0.43)
Comparison of Means	
$t = 2.085, P = 0.05$	$t = -3.754, P = 0.01$

[a]For tibia length/snout-vent, N = 8.

The similarities are sufficiently close for us to have considered the possibility that *sylvaticus* itself might be a full species, with a northern population (*occultator*) that overlaps the geographic range of *D. histrionicus*. However, analysis of skin toxins from samples of Ecuadoran *sylvaticus* show that it presently is best treated as the southernmost population of *histrionicus* (see p. 219)—even though significantly smaller than Colombian *histrionicus*—and, in any case, that it is specifically distinct from *Dendrobates occultator*.

Nonetheless, *Dendrobates occultator* does seem closely related to *D. histrionicus*. Both species lack an omosternum and have similar kinds of calls and aggressive behavior, nor is it difficult to visualize the coloration of *occultator* as having arisen from some population of the variable *histrionicus*. *Dendrobates occultator* differs biochemically from *histrionicus*, particularly in containing pumiliotoxin B as a major compound and in a reduced number (2 vs. 5-7) of histrionicotoxins, as well as in alkaloid-similarity comparisons (p. 224). But there are also similarities in skin chemistry that should not be discounted. *Dendrobates occultator* does contain significant amounts of histrionicotoxin and isodihydrohistrionicotoxin, and it is most interesting that the gas chromatographic trace of *occultator* (fig. 26) most closely resembles that of the *sympatric* population of *histrionicus* (fig. 15E). Both of these sympatric populations appear to have the same set of isomeric compounds (protonated molecular ions at 240) as a major constituent of the alkaloid fraction, and both populations have smaller amounts of a compound with a protonated ion at 284—with histrionicotoxin (also 284) and isodihydrohistrionicotoxin (286) appearing later in the gas chromatographic traces. There is even evidence for trace amounts of pumiliotoxin B in the Quebrada Guanquí population of *histrionicus* (see p. 218), whereas pumiliotoxins have not been detected in the other populations of this species.

We suspect that *Dendrobates occultator* diverged as an isolated population of *D. histrionicus*, and that their geographic ranges were rejoined after speciation had been completed. The biochemical similarities suggest that *occultator* is more closely related to the sympatric *histrionicus* than to any other population, despite the similarity in size with Ecuadoran *histrionicus*. If this hypothesis is correct, natural selection may have reinforced certain differences *after* the ranges had been rejoined, which offers a possible explanation as to why *histrionicus* from Quebrada Guanguí differ so strikingly in color from all other dendrobatids (p. 209). Therefore, in the case of *Dendrobates occultator* and sympatric *D. histrionicus*, the pronounced differences in coloration, size, and habits (p. 254) may be an instance of character divergence between competing species.

Dendrobates viridis, new species
Plate 2, figure D; text figures 2, 3; maps 1 (loc. E), 2

Holotype. AMNH 88133 (field no. CWM 11523), an adult female obtained by John W. Daly and Charles W. Myers on January 28 or 29, 1973, in montane forest approximately 13 km. west of Dagua (town), 850-1200 meters elevation on south-facing versant of upper Río Anchicayá drainage, Department of Valle, Colombia (map 2).

Paratypes. Ten specimens as follows: AMNH 88134-88140 [88139, cleared and stained], KU 152921, with same collecting data as holotype. AMNH 88141, 88142, from Quebrada Guanguí, about 0.5 km. above its junction with Río Patia, 100-200 meters elevation, in upper Río Saija drainage, Department of Cauca, Colombia.

Etymology. The specific epithet is a Latin adjective meaning "green," in reference to the diagnostic coloration.

Definition and Diagnosis. A very small, green *Dendrobates* attaining a snout-vent length of about 15.5 mm. Teeth absent; omosternum present; first finger shorter than second; tarsal tubercle absent; skin secretions including pumiliotoxin B.

The overall green coloration, without markings, distinguishes *Dendrobates viridis* from all other known species of dendrobatids. See Remarks for comparison with *D. altobueyensis*.

Description. (Eleven specimens.) Size very small, the largest specimen an adult female 15.2 mm. snout to vent (enlarged ova present; another dissected female of 14.7 mm. also with enlarged ova); largest male 14.0 mm. Vocal slits present in males.

In life, holotype and topoparatypes metallic green over all dorsal surfaces, including tops of hands and feet; all ventral surfaces are slightly lighter and more metallic green than dorsum, except that palms and soles are black. (Inspection with a ×3.3 lens showed that skin is black around bases of dorsal granules, thus causing green of dorsum to appear a few shades darker than green of venter.) Slight geographic variation in color indicated by the two paratypes from Quebrada Guanguí, in which lips and venters were yellowish green (color otherwise resembling specimens from type locality). A recently transformed juvenile (AMNH 88137, 6.9 mm. snout to vent) was more metallic green dorsally than adults, and all its ventral surfaces were uniformly black rather than green; a larger juvenile (AMNH 88138, 10.1 mm. snout to vent) had the adult coloration. Eye black, no contrast between iris and pupil. Color in preservative black dorsally, grayish black ventrally.

Skin moderately granular, especially on hind legs and venter, becoming weakly granular to nearly smooth on backs. Head almost as wide as body or distinctly narrower in more rotund specimens (e.g., see Measurements of Holotype), with widest part between outer edges of upper eyelids; eyes relatively large and protuberant, about equal to length of snout. Snout short, sloping; truncate in dorsal or ventral aspect, rounded in lateral profile. Naris directed ventrolaterally, both nares visible in ventral or head-on view but not visible from above; nares much closer to tip of snout than to eye. Canthus rostralis rounded; loreal region with slight inward slope toward lip, very slightly concave in some individuals. Tympanum a posteriorly inclined oval, less than one-half area of eye, indistinct in most individuals, especially posterodorsally where tympanic ring is not evident.

Relative lengths of appressed fingers $3 > 4 > 2 > 1$, all except first with distinct discs expanded less than twice finger width; first finger distinct but short (about one-half length of appressed second finger) with vestigial, unexpanded disc. A large medial tubercle at base of palm, a smaller, inner metacarpal tubercle, and one or two subarticular tubercles on fingers (one each on fingers 1 and 2, one or two on fingers 3 and 4); all tubercles low, with rounded surfaces, and rather inconspicuous. Hind limbs of moderate length, heel of appressed limb reaching eye or falling short; tibia/snout-vent length = 0.38-0.42. Relative lengths of appressed toes $4 > 3 > 5 > 2 > 1$; first a nub with vestigial, unexpanded disc, other toes with slightly expanded discs. Tarsal ridge absent or weak, no tarsal tubercle; small, rounded inner and outer metatarsal tubercles; a few specimens with hint of a subarticular tubercle at bases of first few toes. Palms and soles fleshy. Digits of hands and feet flattened on bottom; lacking webbing, lateral fringe, or supernumerary tubercles.

Omosternum present (in AMNH 88139, a cleared and stained female). Flesh blackish. Teeth absent.

Measurements of Holotype (in Mm.). Although not dissected, the holotype is evidently a female because it lacks vocal slits and evidently mature because an identical-sized paratype contained enlarged ova. Length from snout to vent 14.7; tibia length from heel to fold of skin on knee 5.6; greatest width of body 7.1; greatest head width (between outer edges upper eyelids) 4.8; head length from tip of snout to angle of jaws 3.3; tip of snout to center of naris 0.3; center of naris to edge of eye 1.3; diameter of orbit 1.8; horizontal diameter of tympanum 0.8; distance between centers of nares 2.0; length

from proximal edge of palmar tubercle to tip of longest (3rd) finger 3.2; width of disc of third finger 0.6; width of finger (penultimate phalanx) below disc 0.4.

Voice. Unknown, possibly a buzz call similar to *Dendrobates minutus* (see p. 252).

Skin Toxins. Methanolic extracts from skins of two paratypes (AMNH 88139, 88140) contained small amounts of at least four alkaloids, including pumiliotoxin B and probably pumiliotoxin A. Histrionicotoxins were not detected.

All the extremely small sample was used in an attempt to determine the three apparent alkaloids detected by thin-layer chromatography (fig. 2). Only pumiliotoxin B was positively identified. Quantitative chemical ionization mass spectrometry (fig. 3) indicated the presence of pumiliotoxin B (324), probably pumiliotoxin A (308), and a compound with a protonated molecular ion at mass 310. Trace amounts of a protonated ion at 342 were detected. No sample remained for gas chromatographic analysis.

Remarks. When the first specimens of *Dendrobates viridis* were found, in montane forest of the Anchicayá Valley, we assumed that the new species probably had a restricted geographic range—perhaps equivalent to the apparently small area occupied by the sympatric *D. lehmanni.* This hypothesis was abandoned a few weeks later, when two additional specimens of *viridis* were obtained approximately 120 km. to the southwest, in lowland rain forest at the Quebrada Guanguí. These widely separated localities (map 1) indicate that *D. viridis* occupies a fairly extensive range along the western flank of the Cordillera Occidental, between 100 and 1200 meters elevation. Forest at the higher elevations seems to represent a better environment for *viridis* than the lowland rain forest, possibly because of competition from other small dendrobatids in the latter habitat (see Field Notes on the New Species).

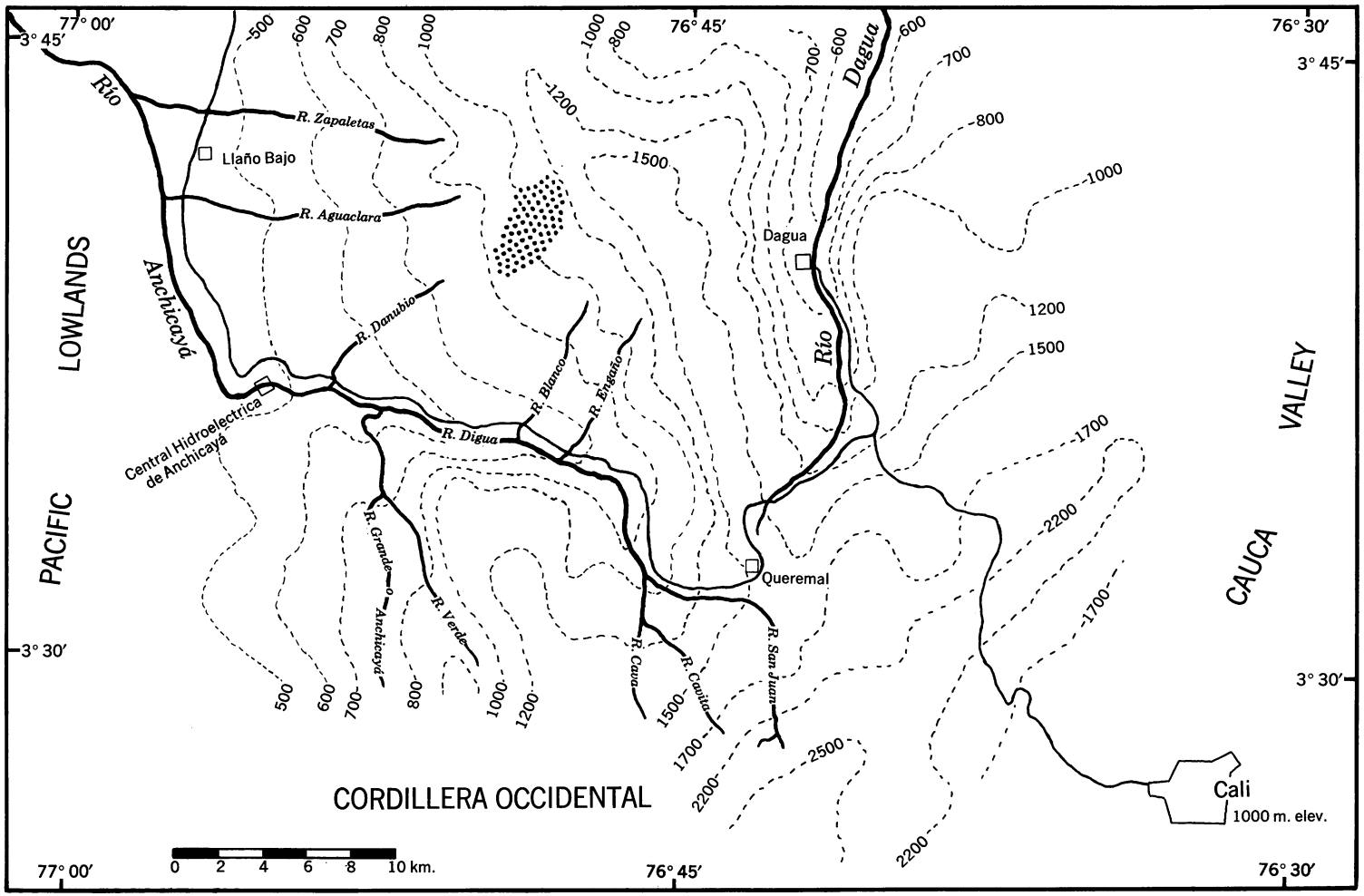
The relationships of *Dendrobates viridis* are presently obscure, but it might best be compared with the recently described *D. altobueyensis* Silverstone (1975, p. 27), a small frog from the Serranía de Baudó of northwestern Colombia. We have not seen specimens of *altobueyensis*, but, judged from the description, it differs from *viridis* mainly in being "entirely yellow or metallic gold, sometimes with greenish tinge [and] small black spots," and in being slightly larger (adults 15.5-17.0 mm. vs. 14.0-15.2 mm. in *viridis*). Judging from the illustration of *altobueyenis* (Silverstone, *op. cit.*, frontisp. 1), that species also differs from *viridis* in having a larger hand, including a notably longer first finger (in *viridis*, this digit is only about half the length of the appressed second finger).

FIELD NOTES ON THE NEW SPECIES

Specimens of the three new species were collected early in 1973, on our fifth trip to western Colombia. On January 28, we traveled with a few packers north from the old Cali-Buenaventura road to an overnight campsite on the upper slopes of the Anchicayá Valley, in the Cordillera Occidental about 40 km. northwest of Cali (map 2). This brief trip ended a long search for the native habitat of *Dendrobates lehmanni* (see p. 240) and also resulted in the discovery of *D. viridis.*

After celebrating the new discoveries, we flew from Cali southwest to the Pacific coastal town of Guapi, and traveled by boat northward along the coast and then up the Río Saija and its tributary, the Río Patia,[1] nearly to the foot of the western Andes. We worked in the upper Saija drainage February 6-22, spending most time in the vicinity of an Emberá (or Emperá) Chocó village on the Quebrada Guanguí (map 1). Specimens of the new *Dendrobates occultator*, and two additional specimens of *D. viridis*, were obtained. In all, nine species of dendrobatid frogs are represented in our collection of more than

[1]Shown on some maps as "Río Patía del Norte," but local residents accent the first syllable of "Patia," which should not be confused with a major river (Río Patía) to the south.



MAP 2. Segment of Cordillera Occidental of western Colombia between Cali and Buenaventura (cf. map 1). Stippled pattern marks shared type locality of *Dendrobates lehmanni* and *Dendrobates viridis*, on south-facing versant of upper Anchicayá Valley. See figures 27 and 28 for habitats. (Adapted from 1:200,000 map *Departamento del Valle del Cauca*, Dirección Nacional de Estadística, República de Colombia. 1953.)

100 species of amphibians and reptiles from this rich faunal area; the collection is supplemented by a smaller one made in 1971 by Borys Malkin, who obtained the first two specimens of *D. occultator* (see p. 246).

Dendrobates lehmanni

This large, conspicuously colored species is known only from its type locality—shown by the stippled pattern in map 2—an area of montane forest at 850-1200 meters elevation on the south-facing versant of the upper Anchicayá Valley. Circumstantial evidence suggests that the geographic range of *D. lehmanni* may, in fact, be almost as restricted as shown on the map: we heard no specimens calling in patches of forest between 700 and 950 meters elevation along the Río Engaño, only about 8 km. southeast of the type locality; furthermore, persons living on the upper Engaño assured us, in 1972, that the frogs do not occur there but only at a place 2½-3 hours away by trail, toward the general area where we finally collected them in 1973. The range of the species may well extend from the type locality several kilometers north and northeast toward the Pacific coastal plain, and possibly a short distance eastward to the top of the Anchicayá-Dagua divide, beyond which the species is not expected due to lack of suitable forest in the rain-shadow valley of the upper Río Dagua. Even in the Anchicayá Valley itself, *D. lehmanni* may occur only on the northern slopes; Mr. Steven Hilty (personal commun.) did not see it in the year he spent in ornithological research on the tributary Río Verde, in forest of equivalent elevation about 20-30 km. south of the type locality (map 2).

The forest has been almost completely cleared below the type locality, and it seems impossible to say whether *Dendrobates lehmanni* ever occurred much below 800 meters elevation, on slopes that are now grassy and sun-exposed. Even the upper montane forest where we collected the frogs is being rapidly cleared for pasture (figs. 27, 28A). The forest remaining is of moderate height, evergreen, and extremely dense (fig. 28B), with many saplings and a scattering of tall palms having slender boles. Buttressed trees and those with stilt roots are much less common than in lowland rain forest; spiny palms are rare. The forest tends to be scrubby and especially dense on the steeper inclines. Many tree trunks bear thick, wet moss; bromeliads are not uncommon near ground level, and these and other epiphytes are abundant higher up on the trunks. The reddish brown soil is resistant to becoming muddy in the forest because of the myriad of ground-level roots and rootlets, but this condition changes noticeably after the forest has been cleared. The area is drained by rocky, clearwater streams.

Dendrobates lehmanni is abundant at the type locality, where it was found by day on the ground, and on logs and in vegetation to heights in excess of 2 meters aboveground. A few individuals were found in a partially cleared new pasture that was bounded by forest on three sides (fig. 28A), but the great majority of frogs were scattered through the adjacent forest, where they were not concentrated to any obvious extent. It took five persons (including our guide and packers), collecting two half-days, to accumulate 182 specimens. Although the frogs are conspicuous and make little effort to hide, they are not easily approached or caught because of the precipitous slopes and dense nature of the forest; many escaped or could not be reached. The ongoing destruction of the forest seems much more of a threat to this species than the relative inefficiency of man's collecting, even though several hundreds of specimens have been collected annually for the animal trade, for at least several years in succession.

Males were calling in the forest, but no tadpoles or juvenile frogs were found during our short stay. The tiny *Dendrobates viridis* was the only other dendrobatid found at this locaity.

Dendrobates viridis

This diminutive species shares its type locality with *Dendrobates lehmanni*, and the habitat is described above. *Dendrobates viridis* seemingly is less abundant than *lehmanni*, and certainly much less noticeable due to its small size and dark green coloration, which combine to make these little frogs inconspicuous in the dim light of the forest. All our specimens were found on the ground by day. Two individuals were in the

FIG. 27. Montane forest being cleared for pasture at type locality (map 2) of *Dendrobates lehmanni* and *Dendrobates viridis*. Arrow denotes upper edge of partly cleared pasture, 950 m. elevation. Although some specimens of both species were found in the new clearing, most were in surrounding forest between 850 and 1200 m. elevation. View is looking due north from an 800 m. hilltop (January 29, 1973).

partially cleared new pasture bordered on three sides by forest (fig. 28A). The remaining seven specimens from the type locality were hopping about on ground litter inside the dense forest; the forest-floor habitat of several individuals is shown in figure 28B.

A few hours before the first specimen of *Dendrobates viridis* was collected, one of us heard a short buzzlike noise emanating from the forest floor and thought it belonged to the wide-ranging *Dendrobates minutus*, but none was found and it is conceivable that the call was that of *viridis*.

Two specimens of *Dendrobates viridis* also were obtained in the upper Río Saija drainage at the Quebrada Guanguí (map 1), about 120 km. southwest of the type locality. One specimen was brought in by an Indian, and the other was caught in leaf litter on a forested hillside. The habitat at Quebrada Guanguí is lowland rain forest (100-200 m. elev.) of quite different aspect than the montane forest (850-1200 m.) at the type locality, and *D. viridis* was much less common in the lowland forest. Although the two of us collected nine specimens in parts of two days at the type locality, only a single specimen was caught in 16 days at the Quebrada Guanguí, where many hours were spent searching the forest floor for small frogs. The rarity of *D. viridis* in the lowland rain forest is possibly due, at least in part, to competition from other small frogs, which appear more numerous in species and numbers than in the montane forest. For example, whereas only two dendrobatids (*D. lehmanni*, *D. viridis*) were obtained in the higher elevation forest, nine species were taken in the lowland forest, including four species of *Dendrobates*, two *Colostethus*, one large *Phyllobates*, and two smaller "*Phyllobates*" [*auctorum*] spp. Two of the *Dendrobates* (*histrionicus*, *occul-*

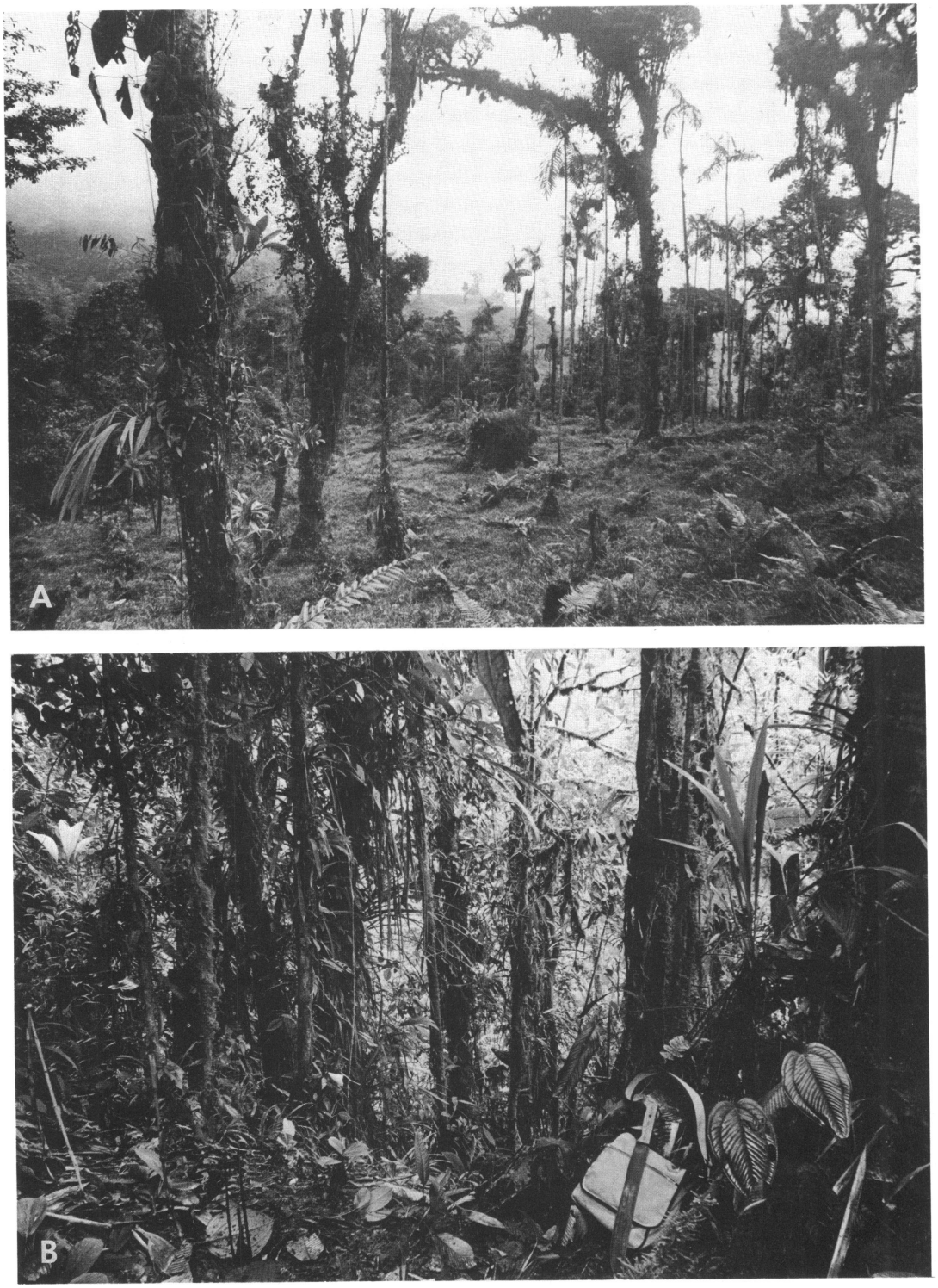


FIG. 28. Habitats of *Dendrobates lehmanni* and *Dendrobates viridis* at type locality (January 29, 1973). A. Partly cleared, new pasture bordered on three sides by forest, 900 m. elevation (below arrow in fig. 27). B. Montane evergreen forest, 1000 m. elevation (above arrow in fig. 27).

tator) are much larger than *viridis*, but the wide-ranging *D. minutus* is similar in size and occupies the same microhabitat (leaf litter) as *viridis*.

Dendrobates occultator

Only 14 specimens are known, all from the upper Río Saija drainage. Ten specimens from the Quebrada Guanguí were brought into camp by Indians. We collected the remaining four specimens in the forest at "La Brea," the site of a rural schoolhouse on a river-front bluff roughly 15 km., by river, below the mouth of the Quebrada Guanguí. The entire region is in lowland tropical rain forest, but the La Brea forest is atypical in growing on peculiarly wet, almost swampy, low ridgetops. The ground on these low ridges was saturated and there were some little pools of standing water; the ridge and ravine forest was comprised mostly of small trees and was dense and brushy, these characteristics probably being partly due to the cutting of larger trees by resident humans.

The first specimen of *Dendrobates occultator* obtained at La Brea was about 1.5 meters aboveground, on the trunk of a fallen tree. Two males (AMNH 88144, KU 152922) were wrestling on the ground, in the sort of aggressive combat that seems widespread in *Dendrobates*. The male holotype was traced by its *histrionicus*-like call; this specimen was about 3 meters aboveground, completely concealed under a dense tangle of vines on the side of a tree. Several other frogs, presumably of this species, were heard calling from similar situations but they could not be found. These few observations on calling sites indicate that *D. occultator* is more arboreal and more secretive than *D. histrionicus*, which usually calls from more exposed perches closer to the ground. Such habits, and the *histrionicus*-like nature of the call, are possibly the reasons why we did not earlier find *occultator* at the Quebrada Guanguí, where we knew it to be present but searched in vain for more than two weeks. *Dendrobates occultator* and *D. histrionicus* occur at least macrosympatrically, but, whereas *histrionicus* was common at the Quebrada Guanguí, we did not find it during our short stay at La Brea, where the only dendrobatids found with *occultator* were *Dendrobates minutus* (sight record) and a large *Phyllobates*.

APPENDIX 1: THIN-LAYER CHROMATOGRAPHY

Frogs were skinned in the field (a quick procedure, after which the animals were killed immediately by dropping into formalin). The skins were accumulated in plastic or glass bottles containing 70-100 percent methanol for transport to the laboratory, where the skins and extracts were stored at -5°C. until use. Prior to fractionation, the skins were finely minced and macerated, the methanol decanted, and the skins extracted and macerated with a fresh portion of methanol. At least 20 ml. of methanol was used per 3-4 g. of skin (roughly 3-4 g. of wet skin is obtained from 10 adult *Dendrobates histrionicus* or 40 adult *D. pumilio*). The two methanol extracts were combined and diluted with an equal volume of distilled water and extracted two times with equivalent volumes of chloroform. Basic chloroform-soluble alkaloids were then extracted from the chloroform into 0.1 N HCl (extract twice with two 10 ml. portions of 0.1 N HCl for each 20 ml. of combined chloroform extracts). The combined 0.1 N HCl solutions were basified (pH 9) with 1 N aqueous ammonia, followed by re-extraction of the alkaloids into chloroform (extract twice with two 20 ml. portions of chloroform for each 10 ml. of aqueous solution); these combined chloroform extracts were dried for 15 minutes over anhydrous sodium sulfate, and evaporated to dryness *in vacuo*. The residue, herein referred to as the *alkaloid fraction*, was redissolved in methanol so that 10 μl corresponded to 10 mg. of the original wet weight of frog skins.

A sample of 10 μl of methanolic alkaloids was applied as a small spot to a commercial (Analtech) silica gel GF plate (250 μ thick), followed by development of the plate with a 9:1 mixture of chloroform and methanol. After drying the plate, it was placed in a chamber containing iodine vapor to allow detection of alkaloids as yellow-brown spots on a light background. For isolation of alkaloids corresponding to specific iodine-positive spots, other plates were streaked with 100-200 μl of methanolic alkaloids and developed and dried. Most of the chromatoplate was covered tightly with another glass plate.

Thus, the bands corresponding to alkaloids were visualized after exposure to iodine vapor only in the uncovered portion of the plate. The silica gel corresponding to iodine-positive regions was then scraped from the unreacted (covered) portion of the chromatoplate; this material was placed in small columns and the alkaloids eluted with a few ml. of ethyl acetate or a 1:1 mixture of chloroform and methanol. After concentration of the solvent, part of the isolated alkaloid fractions was checked for homogeneity by further thin-layer chromatography and the remainder analyzed by mass spectrometry.

APPENDIX 2: GAS CHROMATOGRAPHY (COMBINED WITH MASS SPECTROMETRY)

The instrument used was a Finnigan 9500 gas chromatograph with a flame ionization detector. The column packing consisted of 1.5 percent OV-1 (a methylsiloxane polymer) on Chromosorb G AW-DMCS (80-100 mesh) in a glass column (U-shaped 5 ft. by 2 mm. i.d.). After initial packing, columns were silanized by an injection of 40 μl of 5 percent dimethyldichlorosilane in toluene. In a typical analysis, 2 μl of methanol containing the concentrated alkaloid fraction equivalent to 2 mg. of wet skin was injected directly on the column (injection port temperature 280°C., column temperature 150°C., detector temperature 300°C.). Immediately after the maximum of the solvent peak had passed, the column temperature was programmed at 10° per minute, to 280°C. Nitrogen was used as the carrier gas with a flow rate of 20-25 cc. per minute.

For combination gas chromatography-mass spectrometry, the same column was placed in a Finnigan 1015 mass spectrometer. Methane was the carrier gas, but other conditions were identical. A separator to remove the methane is not necessary for chemical ionization m. s. because the methane now serves as the proton source (instead of isobutane, see Quantitative Mass Spectrometry in text). Dependent upon the amount of compounds present in the methanolic alkaloid fraction, a 0.5-3 μl aliquot was injected. Mass spectra were collected at a rate of one per second throughout the temperature programming, and stored in the computer (Finnigan MS data system 6000) for subsequent analysis.

More recently, a further characterization and often unambiguous identification of alkaloids was obtained by the combination of gas chromatography and electron impact m. s. (data not shown in present paper). The instrument used was a LKB-3000 mass spectrometer, with helium as the carrier gas and operated at 70 ev.

APPENDIX 3: *DENDROBATES HISTRIONICUS* BERTHOLD, 1845

Following is a list of museum specimens that represent populations mentioned in the variational study, including voucher specimens for populations from which samples of skin toxins were obtained. Letters A-H correspond to representative frogs pictured in color plate 1, and to toxin samples analyzed in figures 2, 3, and 15; localities A-H, plus the unlettered Pangala locality, are indicated in map 1.

A. Colombia: Risaralda: about 7 km. airline SE Santa Cecilia, nr. junc. Río Tatamá with upper Río San Juan, 400-800 m. (AMNH 85159-85170). Frogs found in hillside forest, on ground or on bases of tree trunks or low shrubs no higher than about 15 cm. above ground.

– Colombia: Chocó: trail between Guarato [a community 2 hr. walk west from Sta. Cecilia] and Playa de Oro, upper Río San Juan, 200-400 m. (AMNH 85171, 85186-85189). Along trail in second-growth forest.

B. Colombia: Chocó: 2 km. above Playa de Oro, south bank upper Río San Juan, 210 m. (AMNH 85172-85185, 86957-86978). Most specimens in relatively well-drained forest near river, but some were found in adjacent swamp forest; nos. 85178-85180 were taken about 1.5 km. inland (south) of the others, in swamp forest near low hills. All specimens were on the ground or on low perches close to ground.

C. Colombia: Chocó: Quebrada Vicordó, about 5 km. upstream from Noanamá on middle Río San Juan, 100 m. (AMNH 86993, 86994). Forested hillside, the frogs seeming most common in areas where forest was interspersed with small banana plantations.

D. Colombia: Chocó: Quebrada Docordó, about 10 km. upstream from its junction with middle Río San Juan, 100 m. (AMNH 86979-86984, from ridge on south side Quebrada Docordó [sampled for toxins]; AMNH 86985-86989, ridge on north side [not sampled for toxins]). Forest on low ridges on opposite sides of Docordó River. Frogs on ground and on low vegetation up to 1 m. aboveground; several sleeping at night, on leaves about 0.3-0.6 m. aboveground.

– Colombia: Chocó: Pangala on lower Río San Juan (AMNH 88242-88282, west bank Río San Juan; AMNH 88283-88470, east bank). Collection obtained by Borys Malkin, mostly from Chocó Indians.

E. Colombia: Cauca: Quebrada Guanguí, ½ km. above junction with Río Patia, a tributary of upper Río Saija (AMNH 85938-85971, 86634, 88841-88864). Frogs obtained in well-drained areas, in hillside and ridgetop forest, on ground and low perches.

F. Colombia: Cauca: about 8 km. airline SE Guapi, Quebrada Salero, a branch of Quebrada Temuey on south side lower Río Guapi, 20 m. (AMNH 88488-88498). Densely forested low ridge, adjacent to swamp forest in tidal region; frogs mostly calling from perches about 5 cm. to 1 m. aboveground.

G. Colombia: Nariño: Guayacana, 500 m. (AMNH 85048-85158, 86635-86640). Frogs mostly purchased from local residents, but a few caught on ground in second-growth forest and around bases of large trees near pasture edge.

H. Ecuador: Pichincha: Río Baba, 5-10 km. SSW Santo Domingo de los Colorados, 500 m. (AMNH 89579-89601). Abundant in forest being cleared for farmland; frogs quite terrestrial, many on ground and others calling from leaves and logs, usually close to ground (well below 1 m.).

– Ecuador: Pichincha: "about 10 miles [15 km.] S Santo Domingo de los Colorados" (AMNH 88207-88224). Frogs purportedly caught in "banana plantation"; purchased from dealer.

– Ecuador: Pichincha: Estación Biologica Río Palenque, about 40 km. SSW Santo Domingo de los Colorados, 300 m. (AMNH 89574-89578). Frogs common in dense forest and forest edge, including banana plantations adjacent to forest; on ground and calling from low vegetation to about 0.6 m. aboveground.

APPENDIX 4: ENVIRONMENTAL IMPACT STATEMENT

In recent years, man has become cognizant of his fearful reproductive potential and the consequences to the natural environment. A worldwide increase in conservation awareness has caused the word "ecology" to pass from the scientific community into the public domain, and governments have promulgated laws intended to protect what remains of native biotas. These laws are encouraging, although some have the unintended effect of stifling bona fide scientific research that might lead to a better understanding of some of the problems. Our own investigations of poison-dart frogs have not been hampered however, and we express appreciation and gratitude to our foreign colleagues and to the authorities in the eight tropical countries to which these studies have led us. We feel an indebtedness to provide a statement of the consequences of these studies, and to comment on measures that might be considered in the contemplation of conservation legislation.

Investigations of the skin toxins of poison-dart frogs have led to the discovery of compounds having novel structural and pharmacological properties, and several of these compounds already have proved to be invaluable tools in biomedical research (e.g., see essays by Witkop, 1970, 1971). It is expected that some compounds will go into clinical testing as potential anesthetic or therapeutic agents—possibilities that are stimulating attempts at syntheses of dendrobatid toxins in laboratories around the world. Knowledge of these toxins also is providing basic information pertinent to the systematics and biology of the animals themselves, as we have attempted to show in the present paper. However, none of this could have come about without the preliminary isolation and study of toxins from large numbers of several species of frogs in Panama (*Dendrobates auratus*, *D. pumilio*) and in Colombia (*Phyllobates aurotaenia*, *Dendrobates histrionicus*).

Some species of dendrobatid frogs occur in exceedingly dense local populations, but we have been concerned whether collection of large numbers might suppress local population sizes to

a harmful extent—and we have been prepared to cease collection if the frogs seemed less abundant than in some previous year. Fortunately, in no single instance did our activities appear to adversely influence the frog population; in fact, on several occasions the frogs seemed *more* abundant, causing us to suspect that populations had been harvested only to the point of stimulating a maximum rate of increase in the population growth curve (but other factors may also have been involved). Consider, for instance, *Dendrobates histrionicus* at the Colombian town of Guayacana. Frogs were purchased from local residents for a specified period, after which we left town hurriedly in order to stop the influx of specimens:

DATE	NO. FROGS PURCHASED	APPROXIMATE NO. HOURS	ESTIMATED NO. FROGS PER HOUR
Jan. 29, 1970	900	5.0	180
Nov. 30, 1971	1200	7.5	160
Oct. 17, 1972	2000	10.0	200
Feb. 25, 1974	3500	4.5	778

In most other cases, we collected personally and therefore obtained smaller numbers of specimens, but never have we noticed an apparent decrease in population sizes on return trips to the same locality. Such large numbers of frogs were necessary for initial structural determination of the toxins and to provide sufficient material for preliminary pharmacological studies. Laboratory syntheses of the toxins are expected to obviate such pressures on the frog populations, which, however, seem remarkably resilient.

In our judgment, no laws are necessary at this time to protect dendrobatid frogs specifically, although areas of their natural habitats are urgently in need of protection from encroaching human populations. In fact, the geographically restricted *Dendrobates lehmanni* may be in danger of extinction because of habitat destruction; commercial exploitation of this species seems to have had no serious effect to date, although this situation might change, as the habitat is being increasingly destroyed (e.g., see fig 27 and p. 251). Colombian authorities might consider introducing this beautiful frog into the forest preserve in the Río Verde drainage, which is a short distance from the type locality, on the opposite side of the Anchicayá Valley (map 2). *Dendrobates lehmanni* seemingly does not occur in the Río Verde area (see p. 251), but, before making an introduction, a search should be made of the forest preserve to see if there is any resident *Dendrobates* with which *D. lehmanni* might be thrown into competition.

We do not consider importation of dendrobatid frogs into temperate-zone countries to pose an "ecological threat," because escapees certainly would be unable to survive the winter months. One species (*Dendrobates auratus*) has been purposely introduced into Hawaii. A few species conceivably might be able to survive in subtropical regions, as in southern Florida, but, even so, it seems unlikely that any native species would be threatened by these small, diurnal frogs. Dendrobatids generally do not pose a public health problem, inasmuch as only a few species of *Phyllobates* seem unsafe to handle.

DECLARACIÓN DEL IMPACTO SOBRE EL MEDIO AMBIENTE

En años recientes, la humanidad se ha vuelto más y más consciente de su imponente potencial reproductivo y las consecuencias sobre el medio ambiente natural. Un aumento mundial en conciencia conservacionista ha hecho pasar la palabra "ecología" de la comunidad científica al dominio público, y los gobiernos han promulgado leyes intentando proteger lo que queda de los sistemas bióticos nativos. Estas leyes son prometedoras, solo que algunas aunque no intencionalmente inhiben investigación científica auténtica que podría conducir a un mejor entendimiento de algunos problemas. Sin embargo nuestras investigaciones en ranas venenosas no han sido impedidas, y expresamos nuestro reconocimiento y gratitud a nuestros colegas del extranjero y a las autoridades de los ocho países tropicales a los cuales nos condujo nuestra investigación. Sentimos una obligación de declarar las consecuencias de estos estudios, y de comentar en las medidas que podrían tomarse en cuenta en la consideración de legislación conservacionista.

Investigación de las toxinas de la piel de ranas venenosas ha conducido al descubrimiento de compuestos con nuevas propiedades estructurales y farmacológicas, y varios de estos compuestos ya han probado ser instrumentos valiosisimos en investigaciones biomédicas (por ejemplo, ensayos

por Witkop, 1970, 1971). Se espera que algunos compuestos sean probados clinicamente como potenciales agentes anestésicos o terapéuticos—estas posibilidades estan estimulando esfuerzos dirigidos hacia la síntesis de toxinas dendrobátidas en laboratorios alrededor del mundo. El conocimiento de estas toxinas también está suministrando información básica pertinente a la sistemática y biología de los propios animales, algo que hemos tratado de demostrar en el presente artículo. Sin embargo, nada de esto hubiera sido posible sin el aislamiento y estudio preliminar de toxinas de un gran número de ranas de varias especies en Panamá (*Dendrobates auratus*, *D. pumilio*), y en Colombia (*Phyllobates aurotaenia*, *Dendrobates histrionicus*).

Algunas de las especies de ranas dendrobátidas se presentan en grupos de población extremadamente densos, sin embargo nos ha preocupado si el recolectar un gran número de ranas podría reducir hasta a un nivel peligroso el tamaño de los grupos de población locales—y hemos estado prontos a parar la recolección si la ranas parecían ser menos abundantes que el año anterior. Felizmente, en ningún caso nuestras actividades parecieron tener una influencia adversa en la población de ranas; en efecto, en varias ocasiones las ranas parecieron ser *más* abundantes, haciendonos sospechar que las poblaciones habían sido cosechadas únicamente hasta el punto de estimular una velocidad máxima de crecimiento en la curva de crecimiento de población (pero otros factores también pueden haber influido). Consideremos, por ejemplo, *Dendrobates histrionicus* en el pueblo colombiano de Guayacana. Las ranas se compraron de residentes locales por un período de tiempo especificado, después del cual partimos apresuradamente para parar el influjo de especímenes:

FECHA	NO. RANAS COMPRADAS	NO. APROXIMADA DE HORAS	NO. APROXIMADO DE RANAS POR HORA
Enero 29, 1970	900	5.0	180
Noviembre 30, 1971	1200	7.5	160
Octubre 17, 1972	2000	10.0	200
Febrero 25, 1974	3500	4.5	778

En la mayoría de los casos, nosotros recolectamos las ranas personalmente y consecuentemente obtuvimos un número menor de especímenes, pero al regresar a la misma localidad nunca notamos una disminución aparente en el tamaño de las poblaciones. Un número tan grande de ranas fue necesario inicialmente para la determinación estructural de las toxinas y para proveer material suficiente para estudios farmacológicos preliminares. Se espera que síntesis de las toxinas en laboratorios disminuyan la presión sobre las poblaciones de ranas, las cuales sin embargo parecen recuperarse rápidamente.

En nuestra opinión, en este momento no es necesario imponer leyes para proteger las ranas dendrobátidas específicamente, aunque las áreas de su habitat natural están en urgente necesidad de protección en contra de las proximidad de poblaciones humanas. De hecho, *Dendrobates lehmanni*, la cual esta geográficamente restringrida, corre peligro de extinción a causa de la destrucción de su medio ambiente; explotación comercial de esta especie parece no haber tenido ningún efecto serio hasta la fecha, aunque la creciente destrucción del medio ambiente puede cambiar esta situación (fig. 27 y p. 251). Las autoridades colombianas podrían considerar la introducción de esta hermosa rana en la reserva forestal del Río Verde, la cual está a corta distancia de la localidad tipo al otro lado del Valle Anchicayá (mapa 2). *Dendrobates lehmanni* aparentemente no habita en la zona del Río Verde (ver p. 251) pero antes de introducirle, debe de llevarse a cabo uno investigación en la reserva forestal para ver si existe alguna *Dendrobates* nativa con la cual *D. lehmanni* pueda ser forzada a competir.

No consideramos que la importación de ranas dendrobátidas hacia países de la zona templada imponga una "amenaza ecológica" porque es evidente que ranas escapadas no podrían sobrevivir durante los meses invernales. Una especie (*Dendrobates auratus*) ha sido intencionalmente introducida en Hawaii. Es concebible que unas pocas especies puedan sobrevivir en regiones subtropicales, tales como en el sur de Florida, pero, aun así parece poco probable que ninguna especie nativa sería amenazada por estas pequeñas ranas diurnas. Los dendrobátidos generalmente no presentan un problema de salud pública en cuanto a que sólo unas pocas especies de *Phyllobates* parecen ser peligrosas de manejar.

LITERATURE CITED

Albuquerque, Edson X., Eric A. Barnard, Tieh H. Chiu, Antonio J. Lapa, J. Oliver Dolly, Sten-Erik Jansson, John W. Daly, and Bernhard Witkop
1973. Acetylcholine receptor and ion conductance modulator sites at the murine neuromuscular junction: Evidence from specific toxin reactions. Proc. Natl. Acad. Sci. USA, vol. 70, pp. 949-953.

Albuquerque, Edson X., John W. Daly, and Bernhard Witkop
1971. Batrachotoxin: Chemistry and pharmacology. Science, vol. 172, pp. 995-1002.

Amadon, Dean, and Lester L. Short
[In press.] Treatment of subspecies approaching species status. Syst. Zool.

Anon.
1971. Frogs with poisonous skin secretions. Zoonooz, Zool. Soc. San Diego, vol. 44, no. 3, pp. 18-19.

Aratani, M., L. V. Dunkerton, T. Fukuyama, Y. Kishi, H. Kakoi, S. Sugiura, and S. Inoue
1975. Synthetic studies on histrionicotoxins. I. A stereocontrolled synthesis of (±)-perhydrohistrionicotoxin. Jour. Organic Chem., vol. 40, pp. 2009-2011.

Atz, James W.
1970. The application of the idea of homology to behavior. *In* Aronson, Lester R., et al. (eds.), Development and evolution of behavior: Essays in memory of T. C. Schneirla. San Francisco, W. H. Freeman, pp. 53-74.

Ayala, Francisco J.
1974. Biological evolution: Natural selection or random walk? Amer. Sci., vol. 62, pp. 692-701.

Baldwin, Thomas O., and Austin Riggs
1974. The hemoglobins of the bullfrog, *Rana catesbeiana*. Partial amino acid sequence of the β chain of the major adult component. Jour. Biol. Chem., vol. 249, pp. 6110-6118.

Berthold, Arnold Adolph
1845. Ueber verschiedene neue oder seltene Reptilien aus Neu-Granada und Crustaceen aus China. Nachr. Georg-Augusts Univ. und K. Ges. Wiss. Göttingen, 1845, no. 3, pp. 37-48.
1846. [Same title, except first word rendered "Über."] Göttingen, pp. 1-32 + pls. 1-3 [hand-colored in AMNH copy]. Preprinted (reprinted?), with added title page, from Berthold, "1847" [1846?], *vide infra.*
"1847" [1846?]. [Same title.] Abhandl. K. Ges. Wiss. Göttingen, vol. 3 for 1845-1847, Abhandl. Phys. Cl., pp. 3-32 [matching pp. 3-32 in separate, *vide supra*] + pls. 1-3 [uncolored in copy at Acad. Nat. Sci. Philadelphia]. Title page of journal = 1847, possibly issued in parts.

Bogert, Charles M.
1960. The influence of sound on the behavior of amphibians and reptiles. *In* Lanyon, W. E., and W. N. Tavolga (eds.), Animal sounds and communication. Amer. Inst. Biol. Sci., publ. no. 7, pp. 137-320.

Boulenger, George Albert
1913. A collection of batrachians and reptiles made by Dr. H. G. F. Spurrell, F.Z.S., in the Choco, Colombia. Proc. Zool. Soc. London, 1913, pp. 1019-1038, pls. 102-108.

Breder, C. M., Jr.
1946. Amphibians and reptiles of the Rio Chucunaque drainage, Darien, Panama, with notes on their life histories and habits. Bull. Amer. Mus. Nat. Hist., vol. 86, art. 8, pp. 375-436, pls. 42-60.

Brown, Keith S., Jr., and Woodruff W. Benson
1974. Adaptive polymorphism associated with multiple Müllerian mimicry in *Heliconius numata* (Lepid. Nymph.). Biotropica, vol. 6, pp. 205-228.

Bunnell, Pille
1973. Vocalizations in the territorial behavior of the frog *Dendrobates pumilio.* Copeia, 1973, no. 2, pp. 277-284.

Cei, J. M., V. Erspamer, and M. Roseghini
1967. Taxonomic and evolutionary significance of biogenic amines and polypeptides occurring in amphibian skin. 1. Neotropical leptodactylid frogs. Syst. Zool., vol. 16, pp. 328-342.

Cochran, Doris M., and Coleman J. Goin
1970. Frogs of Colombia. U. S. Natl. Mus. Bull. no. 288, pp. i-xii, 1-655.

Cole, Charles J.
1970. Karyotypes and evolution of the *spinosus* group of lizards in the genus *Scelo-*

porus. Amer. Mus. Novitates, no. 2431, pp. 1-47.

Cole, Charles J., Charles H. Lowe, and John W. Wright
1968. Karyotypes of eight species of toads (genus *Bufo*) in North America. Copeia, 1968, no. 1, pp. 96-100.

Corey, E. J., John F. Arnett, and Gary N. Widiger
1975. A simple total synthesis of (±)-perhydrohistrionicotoxin. Jour. Amer. Chem. Soc., vol. 97, pp. 430-431.

Cronquist, A.
1974. Chemical plant taxonomy: A generalist's view of a promising specialty. *In* Bendz, G., J. Santesson, and V. Runnström-Reio (eds.), Chemistry in botanical classification. Proc. 25th Nobel Symposium. Stockholm, Nobel Foundation; New York, Academic Press, pp. 29-39.

Crump, Martha L.
1972. Territoriality and mating behavior in *Dendrobates granuliferus* (Anura: Dendrobatidae). Herpetologica, vol. 28, pp. 195-198.

Daly, John W., George B. Brown, Monica Mensah-Dwumah, and Charles W. Myers
[MS.] Classification of skin alkaloids from Neotropical poison-dart frogs (Dendrobatidae).

Daly, John W., Isabella Karle, Charles W. Myers, Takashi Tokuyama, James A. Waters, and Bernhard Witkop
1971. Histrionicotoxins: Roentgen-ray analysis of the novel allenic and acetylenic spiroalkaloids isolated from a Colombian frog, *Dendrobates histrionicus*. Proc. Natl. Acad. Sci. USA, vol. 68, pp. 1870-1875.

Daly, John W., and Charles W. Myers
1967. Toxicity of Panamanian poison frogs (*Dendrobates*): Some biological and chemical aspects. Science, vol. 156, pp. 970-973 + cover photo.

Daly, John W., Takashi Tokuyama, and Gerhard Habermehl
1969. Froschgifte. Isolierung und Struktur von Pumiliotoxin C. Justus Liebigs Ann. Chem., vol. 729, pp. 198-204.

Dole, Jim W., and Pedro Durant
1974. Courtship behavior in *Colostethus collaris* (Dendrobatidae). Copeia, 1974, no. 4, pp. 988-990.

Duellman, William E.
1966. Aggressive behavior in dendrobatid frogs. Herpetologica, vol. 22, pp. 217-221.
1967. Additional studies of chromosomes of anuran amphibians. Systematic Zool., vol. 16, pp. 38-43.
1970. The hylid frogs of Middle America. Mus. Nat. Hist. Univ. Kansas, monogr. no. 1, pp. i-xii, 1-753, pls. 1-72 (bound in 2 vols.).

Dunn, Emmett Reid
1941. Notes on *Dendrobates auratus*. Copeia, 1941, no. 2, pp. 88-92.

Dunn, Emmett Reid, and L. C. Stuart
1951. Comments on some recent restrictions of type localities of certain South and Central American amphibians and reptiles. Copeia, 1951, no. 1, pp. 55-61.

Estes, Richard
1970. Origin of the Recent North American lower vertebrate fauna: An inquiry into the fossil record. Forma et Functio, vol. 3, pp. 139-163.

Estes, Richard, and Osvaldo A. Reig
1973. The early fossil record of frogs. A review of the evidence. *In* Vial, James L. (ed.), Evolutionary biology of the anurans. Columbia, Missouri, Univ. Missouri Press, pp. 11-63.

Fukuyama, T., L. V. Dunkerton, M. Aratani, and Y. Kishi
1975. Synthetic studies on histrionicotoxins. II. A practical synthetic route to (±)-perhydro- and (±)-octahydrohistrionicotoxin. Jour. Organic Chem., vol. 40, pp. 2011-2012.

Funkhouser, John W.
1956. New frogs from Ecuador and southwestern Colombia. Zoologica, New York Zool. Soc., vol. 41, pt. 2, pp. 73-80, pl. 1.

Goodman, Donald E.
1971. Territorial behavior in a Neotropical frog, *Dendrobates granuliferus*. Copeia, 1971, no. 2, pp. 365-370.

Grzimek, Bernhard
1970. Grzimeks Tierleben. Enzyklopädie des Tierreiches. Zürich, Kindler Verlag, pp. 1-568.

Habermehl, Gerhard, H. Anders, and Bernhard Witkop
1975. Synthese von rac.-Pumiliotoxin C. Naturwissenschaften, vol. 62, p. 345.

Haffer, Jürgen
1974. Avian speciation in tropical South America. Publ. Nuttall Ornith. Club, no. 14, pp. i-viii, 1-390.

Holmquist, R., and Thomas H. Jukes
1975. Species-specific effects and the evolutionary clock: A reply to Penny. Jour. Molecular Evol., vol. 4, pp. 377-381.

Hoogmoed, M. S.
1971. *Dendrobates*, een kleurrijk genus. Het Aquarium, vol. 41, no. 8, pp. 182-189 (also issued in German *in* Aquar. Terrar. Zeitschr., 1971, vol. 24, no. 1, pp. 1-7).

Hull, David L.
1967. Certainty and circularity in evolutionary taxonomy. Evolution, vol. 21, pp. 174-189.

Ibuka, Toshiro, Yasuo Inubushi, Ikutaro Saji, Kiyoshi Tanaka, and Norio Masaki
1975. Total synthesis of *dl*-pumiliotoxin C hydrochloride and its crystal structure. Tetrahedron Letters, no. 5, pp. 323-326.

Johnson, David F., and John W. Daly
1971. Biosynthesis of cholesterol and cholesterol acetate in dendrobatid arrow poison frogs. Biochem. Pharmacol., vol. 20, pp. 2555-2559.

Jukes, Thomas H., and Richard Holmquist
1972. Evolutionary clock: Nonconstancy of rate in different species. Science, vol. 177, pp. 530-532.

Kim, Yong H., George B. Brown, Harry S. Mosher, and Frederick A. Fuhrman
1975. Tetrodotoxin: Occurrence in atelopid frogs of Costa Rica. Science, vol. 189, pp. 151-189.

Kimura, Motoo
1968. Evolutionary rate at the molecular level. Nature, vol. 217, pp. 624-626.

Kimura, Motoo, and Tomoko Ohta
1971a. Protein polymorphism as a phase of molecular evolution. Nature, vol. 229, pp. 467-469.
1971b. Theoretical aspects of population genetics. Princeton, New Jersey, Princeton Univ. Press, pp. i-ix, 1-219.

King, Jack Lester, and Thomas H. Jukes
1969. Non-Darwinian evolution. Science, vol. 164, pp. 788-798.

King, Mary-Claire, and Allan C. Wilson
1975. Evolution at two levels in humans and chimpanzees. Science, vol. 188, pp. 107-116.

Lapa, A. J., Edson X. Albuquerque, J. M. Sarvey, John W. Daly, and Bernhard Witkop
1975. Effects of histrionicotoxin on the chemosensitive and electrical properties of skeletal muscle. Exp. Neurol., vol. 47, pp. 558-580.

Märki, Fritz, and Bernhard Witkop
1963. The venom of the Colombian arrow poison frog *Phyllobates bicolor*. Experientia, vol. 19, pp. 329-338 [1-10 in reprint].

Margoliash, E.
1969. Homology: A definition. Science, vol. 163, p. 127.

Martin, William F.
1972. Evolution of vocalization in the genus *Bufo*. *In* Blair, W. Frank (ed.), Evolution in the genus *Bufo*. Austin and London, Univ. Texas Press, pp. 279-309.

Mosher, H. S., F. A. Fuhrman, H. D. Buchwald, and H. G. Fischer
1964. Tarichatoxin-tetrodotoxin: A potent neurotoxin. Science, vol. 144, pp. 1100-1110.

Myers, Charles W.
1974. The systematics of *Rhadinaea* (Colubridae), a genus of New World snakes. Bull. Amer. Mus. Nat. Hist., vol. 153, art. 1, pp. 1-262.

Myers, Charles W., and John W. Daly
1971. Comment on the proposed designation of a new type-species of *Dendrobates* Wagler, 1830. Bull. Zool. Nomenclature, vol. 28, p. 141.

Nieuwenhuizen, [Arend] van den
1972. [Black and white photograph of "*Dendrobates tinctorius histrionicus*" (= *Dendrobates lehmanni*)]. Lacerta, 1972, no. 2, cover.

Oppolzer, Wolfgang, Wolfgang Fröstl, and Hans Peter Weber
1975. The total synthesis of (±)-pumiliotoxin-C. Helvetica Chim. Acta, vol. 58, pp. 593-595.

Polder, W. N.
1973. Over verzorging en voortplanting in gevangenschap van *Dendrobates azureus* en enkele andere Dendrobatidae; Het Aquarium, vol. 44, no. 1, pp. 16-22.
1974a. [Same title followed by "(2)".] *Ibid.*, vol. 44, no. 7, pp. 186-191. (German version in Aquar. Terrar. Zeitschr., vol. 27, no. 1, pp. 28-32, 1974, with some changes in illustrations.)
1974b. [Same title followed by "(III)".] *Ibid.*, vol. 44, no. 12, pp. 324-330. (German version in Aquar. Terrar. Zeitschr., vol. 27, no. 7, pp. 244-249, 1974, with reduced number of illustrations.)

Prager, Ellen M., and Allan C. Wilson
1975. Slow evolutionary loss of the potential for interspecific hybridization in birds: A manifestation of slow regulatory evolution. Proc. Natl. Acad. Sci. USA, vol. 72, pp. 200-204.

Sarich, Vincent M., and Allan C. Wilson
1967. Immunological time scale for hominid evolution. Science, vol. 158, pp. 1200-1203.

Savage, Jay M.
1968. The dendrobatid frogs of Central America. Copeia, 1968, no. 4, pp. 745-776.

Savage, Jay M., and W. Ronald Heyer
1967. Variation and distribution in the tree-frog genus *Phyllomedusa* in Costa Rica, Central America. Beitr. Neotrop. Fauna, vol. 5, no. 2, pp. 111-131.

Senfft, Walter
1936. Das Brutgeschäft des Baumsteigerfrosches (*Dendrobates auratus* Girard) in Gefangenschaft. Zool. Garten, Leipzig, vol. 8, pp. 122-131.

Silverstone, Philip A.
1971. *Dendrobates* Wagler, 1830 (Amphibia: Anura): Proposed designation of type-species under the plenary powers. Bull. Zool. Nomenclature, vol. 27, pp. 262-264.
1973. Observations on the behavior and ecology of a Colombian poison-arrow frog, the kõkoé-pá (*Dendrobates histrionicus* Berthold). Herpetologica, vol. 29, pp. 295-301.
1975. A revision of the poison-arrow frogs of the genus *Dendrobates* Wagler. Nat. Hist. Mus. Los Angeles County, Sci. Bull. no. 21, pp. [i-vi], 1-55.

Simpson, Beryl B.
1973. Contrasting modes of evolution in two groups of *Perezia* (Mutisieae; Compositae) of southern South America. Taxon, vol. 22, pp. 525-536.

Simpson, George Gaylord
1960. Notes on the measure of faunal resemblance. Amer. Jour. Sci., vol. 258-A, pp. 300-311.

Tokuyama, Takashi, John W. Daly, and Bernhard Witkop
1969. The structure of batrachotoxin, a steroidal alkaloid from the Colombian arrow poison frog, *Phyllobates aurotaenia*, and partial synthesis of batrachotoxin and its analogs and homologs. Jour. Amer. Chem. Soc., vol. 91, pp. 3931-3938.

Tokuyama, Takashi, K. Uenoyama, G. Brown, John W. Daly, and Bernhard Witkop
1974. Allenic and acetylenic spiropiperidine alkaloids from the Neotropical frog, *Dendrobates histrionicus*. Helvetica Chim. Acta, vol. 57, pp. 2597-2604.

Toner, Mike
1972. Florida fast becoming a zoo of imports scientists warn. Trop. Fish Hobbyist, vol. 20, no. 8, pp. 45-48 (reprinted from Miami Herald).

Trueb, Linda
1968. Variation in the tree frog *Hyla lancasteri*. Copeia, 1968, no. 2, pp. 285-299.

Vanzolini, P. E., and Ernest E. Williams
1970. South American anoles: The geographic differentiation and evolution of the *Anolis chrysolepis* species group (Sauria, Iguanidae). Arq. Zool., São Paulo, vol. 19, pt. 1, pp. 1-124 + 5pls.; pt. 2, pp. 125-298.

Villa, Jaime
1972. Anfibios de Nicaragua. Managua, Inst. Geogr. Nac. and Banco Central de Nicaragua, frontispiece + pp. [i-x], 1-218.

West, Robert C.
1957. The Pacific lowlands of Colombia. Louisiana State Univ. Studies, soc. sci. ser., no. 8, pp. i-xiv, 1-278 + intercalated maps.

Wilson, Allan C., Linda R. Maxson, and Vincent M. Sarich
1974. Two types of molecular evolution. Evidence from studies of interspecific hybridization. Proc. Natl. Acad. Sci. USA, vol. 71, pp. 2843-2847.

Wilson, Allan C., Vincent M. Sarich, and Linda R. Maxson
1974. The importance of gene rearrangement in evolution: Evidence from studies on rates of chromosomal, protein, and anatomical evolution. Proc. Natl. Acad. Sci. USA, vol. 71, pp. 3028-3030.

Winter, William P., Kenneth A. Walsh, and Hans Neurath
1968. Homology as applied to proteins. Science, vol. 162, p. 1433.

Witkop, Bernhard
1970. Quimica y farmacologia de la batraciotoxina. Rev. Fac. Farmacia Univ. de los Andes, vol. 7, nos. 10-11, pp. 51-100.
1971. New directions in the chemistry of natural products: The organic chemist as a pathfinder for biochemistry and medicine. Experientia, vol. 27, pp. 1121-1138 + color pl.