

# Olfaction and Predator Detection in *Coleonyx brevis* (Squamata: Eublepharidae), With Comments on the Functional Significance of Buccal Pulsing in Geckos

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**ABSTRACT** Squamate reptiles rely heavily on two nasal chemical senses in directing most of their behavior: nasal olfaction and vomeronasal function. For most behaviors in most species, the vomeronasal system is the predominant sense. It has been suggested, however, that geckos are unusual in the extent to which they rely on nasal olfaction rather than vomeronasal function. In this study, we use defensive tail display as a behavioral bioassay to examine the context and relative use of olfaction vs. vomeronasal function in a eublepharid gecko, *Coleonyx brevis*. When presented with appropriate snake-predator skin chemicals in the absence of relevant visual stimuli, *C. brevis* exhibits a defensive tail display that has been shown to be adaptive in defending against snake predators. We demonstrate that olfactory cues alone are sufficient to provoke the behavior and that geckos precede the display in many cases with "buccal pulsing," a behavior that we suggest is an olfactory sampling mechanism analogous to mammalian sniffing. Our results support the gecko-olfaction hypothesis and demonstrate that geckos use nasal olfaction to discriminate among potential predator species. We discuss alternative hypotheses for the origin of species-specific, chemosensory predator identification in *Coleonyx*. © 1996 Wiley-Liss, Inc.

The importance of the chemical senses in squamate biology is now universally acknowledged (see reviews by Burghardt, '70, '80; Halpern, '80, '83, '87, '92; Mason, '92; Schwenk, '95). Indeed, nearly all aspects of squamate behavior and ecology are mediated to a greater or lesser extent by chemical information, including prey choice, prey location, foraging behavior, self and individual recognition, kin recognition, mate choice, mate location, shelter location, den location, trail following, aggregation, predator identification, exploratory behavior, defensive behavior, courtship behavior, and copulatory behavior (references above).

Despite the plethora of cases in which we know chemoreception to be important, we remain surprisingly ignorant about the functional delineation of the three chemosensory systems available to squamates (Graves, '93; Schwenk, '95). Of these, gustation (taste) is arguably the least important, despite the ubiquity of oral and lingual taste buds in most species (Schwenk '85). Of the two nasal-chemical senses (nasal olfaction and vomeronasal function), the vomeronasal system (VNS) is known or assumed to be the predominant sensory mode in most scincomorphan and anguimorphan

squamates, especially caenophidian snakes (summarized in Halpern, '92; Schwenk, '93b). However, very few studies have examined explicitly the function of the squamate nasal-olfactory system (NOS) (reviewed by Halpern, '92; Schwenk, '93a); therefore, its significance in mediating much of squamate behavior remains unexplored.

Although developmentally coupled, the NOS and VNS become anatomically separate in squamates: the vomeronasal epithelia are sequestered within two bulb-like organs in the snout above the palate (the vomeronasal, or Jacobson's, organs [VNO]), and in adults VNO-nasal confluence is lost and a VNO-oral connection is established (Parsons, '59; Halpern, '92; Schwenk, '95). Nasal and vomeronasal sensory epithelia are innervated separately by main and accessory olfactory nerves, respectively, which project to the main and accessory olfactory bulbs (Halpern, '92). Indeed, the systems remain distinct through the telencephalon at least

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as far as the amygdaloid nuclei, where "cross-talk" between the systems and with other sensory and motor systems might occur (Lohman and Smeets, '93; Martínez-García, et al., '93).

Given the anatomical segregation of the two nasal-chemosensory systems, it is not surprising that each requires different physical mechanisms for stimulation. Sensory transduction at the nasal epithelium occurs when volatile chemicals present in the air are inhaled through the external nares into the nasal cavities. Air exits the nasal cavities into the mouth via the internal nares (choanae). Vomeronasal stimulation, on the other hand, is believed to occur only when chemicals are delivered into the mouth by the tongue (Kahmann, '32; Halpern and Kubie, '80; Graves and Halpern, '89). Such orally introduced chemicals do not make their way into the nasal cavities via the internal nares (Halpern and Kubie, '80; Graves and Halpern, '89). Environmental chemicals are sampled during tongue-flicking (TF), a behavior in which the tongue is protruded and oscillated to varying degrees. During TF, the tongue tip might touch the substrate or an object and thereby sample adherent, relatively nonvolatile chemicals, or it might oscillate in the air and collect volatiles for transfer to the VNO. The relative importance of volatile vs. nonvolatile chemical sensing by the tongue-vomeronasal system remains an open question (for discussion, see Halpern, '92; Schwenk, '95), but overall the vomeronasal system seems specialized for the sensation of nonvolatile chemicals.

Halpern ('83:169) noted that "the intimate relationship between the olfactory and vomeronasal senses in activating and directing the behavior of vertebrates should provide fertile ground for future research." The prevailing view of the functional relationship between the NOS and VNS in squamates is articulated by the Cowles and Phelan ('58) hypothesis, which states that initial detection of volatile chemical cues by the olfactory system triggers TF and the vomeronasal system, which then acts as a proximate chemoreceptor. Schwenk ('95) integrated this hypothesis with gustation in the context of recent findings and proposed a nested hierarchy of system function and information retrieval for the three chemosensory systems: 1) NOS stimulation by airborne volatiles; 2) stimulation of TF and VNS function; 3) VNS analysis of volatiles with TF to air; 4) VNS localization of chemical source through klinotaxis or tropotaxis; 5) VNS analysis of nonvolatile components of chemical source with TF to substrate; and 6) gustatory analysis of prey with tongue/oral con-

tact (if a predatory sequence). Each of these stages is potentially integrated with information from other sensory systems.

Unfortunately, as Halpern ('92) has pointed out, even those studies that purport to do so have not actually tested the Cowles and Phelan ('58) hypothesis, and a crisp, functional distinction between the nasal chemosensory systems remains elusive, particularly with regard to the sensation of volatiles (Schwenk, '95). The difference in physical mechanisms of stimulation noted above, however, opens the door to controlled behavioral observations that could help to tease apart the functional province of each system. Caution is called for, however; although TF is widely accepted as *prima facie* evidence of vomeronasal function, Halpern ('92) and others (e.g., Schwenk, '85; Schwenk and Mayer, '91; Graves and Halpern, '89; Greenburg, '93; Delheusy and Bels, '94) have pointed out that TF might occur in nonchemosensory contexts and/or for reasons other than vomeronasal stimulation. Current data, therefore, support the notion that TF (or at least snout/mouth contact) is a necessary but not sufficient criterion for vomeronasal functioning in squamates and that behaviors elicited in the absence of TF or other oral contact must be mediated by other senses.

Gekkotan squamates provide ideal candidates for study of the nasal chemical senses for several reasons: 1) both nasal chemical senses are well developed in geckos (Eublepharidae and Gekkonidae) and pygopodids (Pygopodidae) (Schwenk, '93a,b, and references therein); however, 2) strong circumstantial evidence suggests that geckos are unusual among squamates in the degree to which they use nasal olfaction (Schwenk, '93a), (i.e., the NOS seems to be the predominant sensory mode in some behaviors rather than the VNS; this contention is supported by recent, independent observations [Delheusy and Bels, '94]); 3) gekkotans are mostly nocturnal and/or fossorial (those that are diurnal surface dwellers, such as the day geckos, *Phelsuma*, are almost certainly derived from nocturnal ancestors), and nocturnality has been associated with the evolution of enhanced olfactory abilities in other tetrapod lineages (mammals: Crompton and Jenkins, '78, '79; birds: Healy and Guilford, '90); 4) some eublepharid geckos exhibit a defensive tail display in response to chemical cues from predatory snakes (see below) that can be used as a behavioral bioassay for NOS/VNS function; 5) All gekkotans exhibit typical squamate TF and most also perform an unusual "buccal

pulsing" behavior in which the floor of the mouth is rapidly depressed and elevated in the absence of TF. The behavior resembles buccal pumping characteristic of many amphibians (Brainerd et al., '93) and may serve to draw air into the nasal cavities for NOS stimulation.

In this paper, we exploit these characteristics of gekkotan lizards to test further the hypothesis that geckos utilize nasal-olfactory cues, in the absence of VNS stimulation, to initiate important adaptive behaviors. By using TF as the minimum requirement for VNS function and defensive tail display as a bioassay for chemosensory predator detection, we show that nasal olfaction alone is sufficient for elicitation of the behavior and does not act merely as a trigger for VNS function, as suggested by the Cowles and Phelan ('58) hypothesis. We further suggest that buccal pulsing represents olfactory, not respiratory, behavior and is analogous to mammalian sniffing. Such sniffing behavior may be unique among squamates and further strengthens the position of gekkotans as mammalian analogues and ideal models for tests of dual olfactory function in tetrapod vertebrates (Schwenk, '93a).

### ***Behavioral bioassays and discriminating power***

When examining chemosensory-mediated responses, it is important to distinguish between an organism's simple examination of a stimulus and its capacity to identify the source of the stimulus. In this context, behavioral bioassays are particularly useful. However, results that conclusively demonstrate identification can be difficult to obtain in tests involving some behavioral bioassays. For example, TF is used frequently to demonstrate chemosensory identification of food, and the main datum collected is TF number. However, unless TF is followed by a specific behavior that conclusively demonstrates identification (for example, prey attack), one cannot distinguish between the simple examination of a source and its identification.

In contrast, predator-specific defensive behaviors provide conclusive bioassays of predator identification. Several species of eublepharid geckos exhibit a predator-specific tail display behavior during encounters with predators. Specifically, when a predator is identified by a gecko, the gecko positions its body with its tail toward the approaching predator, elevates the tail from the horizontal, locomotory position into a vertical arch, and then slowly undulates it from left to right (Congdon et al., '74; Dial and Fitzpatrick, '81). Ex-

perimental studies have shown that this functions to attract the predator's attack to the tail, which is then autotomized, resulting in the lizard's escape (Dial and Fitzpatrick, '83). In some lizard species, tail display functions also to signal to a predator that it has been sighted by the prey organism (Dial, '86; Pough, '88); however, there are no data indicating this to be the function in eublepharid geckos. Instead, in these lizards, tail display is used almost exclusively in a predator-encounter context (Dial et al., '89).

Two criteria are required of a behavioral pattern used as a bioassay (O'Connell, '77). First, the functional basis of the behavior during naturally occurring events must be understood; second, the behavior should occur specifically in response to a particular stimulus. Tail display in eublepharid geckos satisfies both criteria and is therefore a useful bioassay in the analysis of chemosensory systems.

## **MATERIALS AND METHODS**

### ***Species***

*Coleonyx brevis* is a small (mean adult snout-vent length [SVL] = 54 mm), nocturnal, terrestrial, eublepharid gecko that is found primarily in the Chihuahuan Desert of southwestern North America; it is distributed in western Texas, southeastern New Mexico, and northeastern Mexico (Dixon, '70). When approached by snake predators, this species exhibits the defensive tail display described above, which frequently results in tail autotomy. In one study (Dial, '78a), 68% of the males and 80% of the females collected at one locality had regenerated tails.

*Hypsiglena torquata* (mean SVL = 280 mm) is a nocturnal, terrestrial, colubrid snake that is distributed widely throughout most of the southwestern US and northern Mexico. It occurs sympatrically with *C. brevis* over approximately 100% of the gecko's distribution (calculated from distribution maps in Conant and Collins, ['91]). *H. torquata* is a saurophagous (lizard-eating) predator, and stomach-content analysis (Dial, unpublished data) indicates that *C. brevis* constitutes a considerable portion of its diet. Autotomized, banded-gecko tails are frequently found in *H. torquata* stomachs as well (Dial, unpublished data).

*Sonora semiannulata* (mean SVL = 214 mm) is a nocturnal, terrestrial, colubrid snake that is distributed in the southwestern US and northeastern Mexico. It occurs sympatrically with *C. brevis* over approximately 100% of the gecko's distribution (calculated from distribution maps in Conant and Collins ['91]). Its diet comprises a wide vari-

ety of small arthropods, including insects, centipedes, scorpions, and spiders (Conant and Collins, '91). It is not known to eat lizards.

*Gyalopion canum* (mean SVL = 164 mm) is a nocturnal, terrestrial, colubrid snake that is distributed in western Texas, southern New Mexico, southeastern Arizona, and north-central Mexico. It occurs sympatrically with *C. brevis* over approximately 85% of the gecko's range (calculated from distribution maps in Conant and Collins, '91). It feeds almost exclusively on spiders (Conant and Collins, '91) and is not known to eat lizards.

*Phyllorhynchus decurtatus* (mean SVL = 396 mm) is a nocturnal, terrestrial, colubrid snake. It is a Mojave and Sonoran Desert species that is distributed in southwestern Arizona, southern Nevada, southern California, Baja California Norte, Baja California Sur, and Sonora, Mexico (Stebbins, '85). Thus, it does not occur sympatrically with *C. brevis*. However, it does occur sympatrically with *Coleonyx variegatus*, a close relative of *C. brevis* (Grismer, '88), upon which it preys (Dial et al., '89).

### **Specimen collection and maintenance**

All geckos ( $n = 22$ , 14 males, 8 females) and three of the snake species (*H. torquata*,  $N = 4$ , all males; *S. semiannulata*,  $N = 2$ , both males; *G. canum*,  $N = 2$ , both males) were collected in June, 1992, near Study Butte, Brewster County, Texas. All individuals of *P. decurtatus* ( $N = 3$ , all males) were collected in May, 1992, at Borrego Springs, San Diego County, California.

Lizards were maintained in the lab individually in plastic boxes (32 × 18 × 10 cm) with a paper towel substrate. Each container was exposed to a natural (June) photoperiod and kept in a thermal environment with a diel range of 24–32°C. This temperature regime falls within the range of field temperatures of this lizard species during June (Dial, '78b). Geckos were fed small domestic crickets twice weekly. The snakes were housed individually in similar boxes and were not fed prior to experiments. All lizards and snakes were supplied with water ad libitum.

### **Experimental procedures**

We tested for chemosensory mechanisms by presenting geckos with a variety of chemical stimuli. Geckos were tested in their home cages. All stimuli were presented on cotton-tipped, wooden applicators (Burghardt, '67). In each trial, a stimulus was applied to the swab (see below), and then the swab was moved slowly to a position 1–2 cm in front of a gecko's snout; no swab-gecko contact

occurred. The swab tip was held in that position until the gecko responded. If no response occurred within 60 sec, the swab was slowly removed. By presenting chemical stimuli on an applicator, all biologically relevant visual stimuli were removed and whatever visual stimulus the swab provided the gecko was held constant and controlled for each trial/individual.

Six experimental conditions were presented to geckos in random order, as follows: 1) Distilled water was used as a neutral/humidity control (cotton tips were dipped into water and the excess was blotted with tissue paper); 2) a commercial cologne (Paco Rabanne®) was used as a "pungency control" (tips were dipped into a 1:1 solution of cologne and distilled water, and the excess was blotted dry); 3–6) integumentary chemicals from the four experimental species of snakes were obtained by rolling dry swab tips across the dorsal, lateral, and ventral skin surfaces of snakes, between the neck and the abdominal regions (skin in this region of the snake body has been shown to contain semiochemicals [Mason, '92]). No skin chemicals were collected from the head or cloacal/tail regions. To maximize the amount of skin chemicals absorbed by the swab tips, individuals within a particular snake species were chosen in sequence. Individual snake specimens were "swabbed" only once per day.

Each trial was scored for six categories of gecko response, as follows: 1) no response; 2) tongue-flick; 3) labial lick; 4) buccal pulsing; 5) tail display; and 6) flight. Only adult geckos with complete (original or regenerated) tails were used. Dependence of behavioral responses to experimental stimuli was tested using the G-Test of Independence (Sokal and Rohlf, '81). Significance was evaluated at  $P = 0.05$ .

## **RESULTS**

Responses to all chemical stimuli are presented in Table 1. No intersexual differences in gecko responses were noted; thus, data for both sexes were pooled for comparisons among stimuli. Geckos responded with a putative sampling technique (TF or buccal pulsing) and/or defensive tail display more frequently to snake skin chemicals than to either of the controls ( $G = 15.44$ ,  $df = 2$ ,  $P < 0.001$ ). The cologne-pungency control was the only stimulus that elicited labial licks, and they occurred in 100% of the trials with cologne ( $G = 129.18$ ,  $df = 5$ ,  $P < 0.001$ ).

Eighty-six percent of the geckos tested with skin secretions from *H. torquata* (the *C. brevis* preda-

TABLE 1. Behavioral responses of *Coleonyx brevis* to six chemical stimuli: Distilled water, cologne, and integumentary chemicals from four species of snakes (*Gyalopion canum*, *Sonora semiannulata*, *Phyllorhynchus decurtatus*, and *Hypsiglena torquata*)<sup>1</sup>

Response	Chemical stimulus											
	Water (N = 22)		Cologne (N = 22)		<i>G. canum</i> (N = 15)		<i>S. semiannulata</i> (N = 7)		<i>P. decurtatus</i> (N = 22)		<i>H. torquata</i> (N = 22)	
	Number	%	Number	%	Number	%	Number	%	Number	%	Number	%
NR	14	64	0	0	7	47	5	71	21	95	3	13
LL	0	0	22	100	0	0	0	0	0	0	0	0
TF only	8	36	0	0	8	53	2	29	1	5	0	0
TF, TD	0	0	0	0	0	0	0	0	0	0	3	14
TF, TD, FL	0	0	0	0	0	0	0	0	0	0	1	5
BP only	0	0	0	0	0	0	0	0	0	0	0	0
BP + TD	0	0	0	0	0	0	0	0	0	0	4	18
TD only	0	0	0	0	0	0	0	0	0	0	10	45
TD + FL	0	0	0	0	0	0	0	0	0	0	1	5

<sup>1</sup>Stimuli were presented on cotton-tipped swabs, and gecko responses were recorded for 60 sec/individual. BP = buccal pulsing; FL = flight; LL = labial lick; NR = no response; TD = tail display; TF = tongue flick.

tor) exhibited tail display, and tail display occurred exclusively in response to *H. torquata* chemicals ( $G = 60.10$ ,  $df = 5$ ,  $P < 0.001$ ). There was no change in orientation during the display. Seventy-nine percent of trials that resulted in tail display involved no TF (i.e., only 21% of the trials in which a display occurred were preceded by TF). However, in 21% of the tail-display trials, buccal pulsing preceded the display. Nine percent of the trials with skin chemicals from *H. torquata* resulted in rapid flight away from the swab tip. The only response elicited by skin chemicals from *G. canum*, *S. semiannulata*, and *P. decurtatus* (nonsaurophagous or nonsympatric predators) was TF.

## DISCUSSION

### Predator detection and discrimination

The results of these experiments demonstrate clearly that *C. brevis* is capable of using integumentary chemicals alone, in the absence of visual cues, to detect the presence of a potential predator. Tail display occurred in 86% of the trials with skin chemicals from a primary gecko predator (*H. torquata*) but never occurred in trials using sympatric, nonsaurophagous species (*G. canum* and *S. semiannulata*) or a nonsympatric gecko predator (*P. decurtatus*). These data indicate that snake-integumentary chemical cues are sufficient for geckos both to identify a sympatric predator species and to discriminate among snake species. In both posture (movement from a horizontal to a vertical position; angle of curvature) and kinematics (timing and form of caudal undulations), tail displays were identical to those exhibited during staged, laboratory encounters between geckos and

live *H. torquata* (see Dial, '78a). However, in none of the trials did a gecko position its body with its displayed tail toward the swab tip, as is frequently done in response to live snakes (see Dial and Fitzpatrick, '81). Apparently, appropriate visual cues are necessary to orient the response.

Chemosensory predator detection in the absence of TF has heretofore been reported only for *Coleonyx* (*C. brevis*: this study; *C. variegatus*: Dial et al., '89). All other cases of chemosensory-based predator detection and identification in squamates have involved tongue-flicking and, presumably, VNS mediation (reviewed by Schwenk, '93a).

### Olfaction as an important sensory mode

Results of this study provide strong evidence for the use of olfaction, in the absence of vomeronasal function, in identification of a predator species and initiation of an adaptive behavioral response. We base this conclusion on the following observations. First, of the trials that resulted in tail display, 79% were not preceded by a tongue flick. Given that experimental data thus far indicate that TF is a minimum requisite for VNS function, it is reasonable to conclude that vomeronasal function was not involved in these trials. Thus, the NOS is implicated in sensation of chemically laden air received during inhalation. Second, in those tail display trials that occurred in the absence of TF, 21% were preceded by buccal pulsing, a possible accessory sampling mechanism for nasal olfaction. TF and buccal pulsing never occurred in the same trial. In all of the buccal pulse trials, buccal movements were initiated only after swab presentation to the gecko. This suggests

that snake-skin chemicals were delivered initially to the nasal epithelium by normal inhalation, which, in turn, activated buccal pulsing. In the trials that included buccal pulsing, 100% resulted in tail display, which occurred from 3–8 sec after the initiation of pulsing.

The frequency of TF observed in control and nonpredator snake trials suggests two possible interpretations: 1) the absence of relevant olfactory cues in these trials initiated TF and supplementary VNS exploration, (i.e., when the gecko failed to identify a chemical cue with its NOS, it initiated VNS function for finer-grained analysis of the stimulus); 2) TF to nonpredator cues reflects baseline, exploratory behavior that is suppressed in the presence of predator odor so as not to attract the attention of the snake. In any case, since no behavior was initiated after TF, we cannot determine if the cue was identified as not dangerous or merely remained unidentified (see discussion of bioassays above). However, both hypotheses suggest the use of nasal olfaction to detect and identify a predator species.

Our observations are not consistent with a strict interpretation of the Cowles and Phelan ('58) hypothesis, which implies that only biologically relevant odors trigger TF and VNS function. In this case, NOS detection of a highly relevant stimulus in fact obviated TF and further VNS investigation, and it was the absence of interpretable cues that triggered the VNS. Therefore, the functional relationship between the NOS and VNS will depend on the relative sensitivity and discriminatory ability of each system. Finally, these observations also highlight Halpern's ('92) concern regarding the use of TF number as a direct index of increasing biological relevance; in fact, TF rate might be unrelated to biological importance of the test odor or, as in this case, inversely related.

#### **Cautionary notes**

Our conclusions regarding the use of olfaction are based on several reasonable but untested assumptions. First, we assume that the absence of TF indicates an absence of vomeronasal stimulation. This has been tested experimentally for two species using a nonvolatile chemical stimulant (Halpern and Kubie, '80; Graves and Halpern, '89). There remains the possibility that volatile chemicals (of the type that must be involved in our experiments), unlike nonvolatiles, could make their way through the oral cavity into the VNO, presumably by inhalation through the mouth. If true, then VNS function could occur in the absence of

TF. All available evidence suggests that this is not the case, but it remains untested. Second, we assume that buccal pulsing results in air drawn into the nasal cavities through the external nares. It is conceivable that buccal pulsing either does not move air or that it draws air in through the mouth and not through the nose. Finally, we assume that if buccal pulsing does draw air in through the nose, its biological role (*sensu* Bock and von Wahlert, '65) is for nasal olfaction. This is strongly supported by the context of buccal pulsing reported here and our ongoing behavioral observations. However, geckos exhibit buccal pulsing during normal, exploratory behavior, and it could serve other roles, including lung ventilation, respiratory cooling, and/or oral gas exchange. We are presently testing geckos using flow transduction and cineradiography to assess patterns of air movement during buccal pulsing (Schwenk, Brainerd, and Dial, in preparation). Our preliminary results are consistent with an olfactory role for buccal pulsing in geckos.

#### **Functional significance of buccal pulsing**

If, as we argue, buccal pulsing represents olfactory sampling, then it can be reasonably considered a type of sniffing behavior. Our preliminary observations indicate that buccal pulsing occurs in many species of gecko during exploratory behavior and investigation of novelties and that it is sometimes accompanied by pressing the snout into the substrate (Schwenk, Brainerd, and Dial, in preparation). Such snout contact could conceivably facilitate VNS stimulation as well (Halpern and Kubie, '80; Graves and Halpern, '89), though once again context and the absence of TF suggest that it is an olfactory and not a vomeronasal behavior. A putative buccal pulsing–olfaction mechanism also occurs in alligators (Weldon and Ferguson, '93) and possibly some turtles (personal observation).

Molecular transduction of chemicals at the olfactory epithelium must occur via the interface between chemically laden air and the fluid covering the sensory epithelial cells. Actual transduction of the cell membrane must be preceded by diffusion of volatile chemicals into the overlying fluid; the rate of such diffusion is limited by the presence of a boundary layer in the air overlying the fluid coat (Vogel, '94). Buccal pulsing could serve not only to draw a larger volume of air into the nasal cavities but also to increase the velocity gradient at the boundary layer. This would maximize the rate of diffusion of chemicals into the fluid available for molecular transduction, po-

tentially increasing the speed of discriminatory ability of olfactory processing (Vogel, '94). Such olfactory enhancement has obvious survival value in the case of predator detection and initiation of a defensive display.

Buccal pulsing might be used by some squamates in other contexts. For example, Deban et al. ('94) showed that buccal pulsing is used as a pumping mechanism for thoracic inflation in crevice-wedging chuckwalla (*Sauromalus obesus*, Iguanidae). They suggested that other species (including *Coleonyx variegatus*) might use buccal pumping for lung ventilation, but there is little evidence to support this. Indeed, in geckos and possibly other lizards, buccal pulsing often occurs in the absence of the rib movements associated with lung aspiration (Schwenk, Brainerd, and Dial, in preparation). Therefore, there is no necessary relationship between buccal pulsing and lung ventilation as suggested by Deban et al. ('94). Buccal pulsing is relatively common in varanid lizards (e.g., Bels et al., '95), and in this group it is involved in threat display (Bels et al., '95) and lung ventilation (E. Brainerd, personal communication). In any case, although buccal pulse pumping for respiration is quite common in amphibians (Brainerd et al., '93), it is certainly atypical in squamates (Gans, '70; Perry, '83). Buccal pulsing for the purpose of olfaction might be limited to gekkotan squamates; however, some other lineages, such as xantusiids and amphisbaenians (see Schwenk, '93a), are good candidates for study in this context.

#### **Labial licks and the use of cologne as a pungency control**

Labial licks were observed in 100% of trials using a commercial cologne and in no other context. Cologne is used routinely in cotton-applicator tests of chemoreception in squamates (e.g., Cooper, '90a,b, '91, '92, '95; Cooper and Alberts, '90, '91; Cooper and Vitt, '86; Dessault and Krekorian, '91; López and Salvador, '92; López and Martín, '94; many others). Labial licking occurs in many squamates after a feeding bout, and in geckos it is associated with facial (and eye) cleaning. Its occurrence only in the presence of cologne suggests that the cologne is unpleasant to the geckos. They do not attempt additional discrimination by means of TF or buccal pulsing. These observations suggest that alcohol-containing cologne is not an appropriate pungency control because it may act as an irritant, suppressing TF and other chemosensory behavior. Irritation introduces a factor other than

pungency and therefore confounds interpretation of results, especially when traditional scoring methods (e.g., TF number or Tongue Flick Attack Score, [Cooper and Burghardt, '90]) are used. Appropriate controls might include biological odors outside individual and genetic/historical experience of the species and individual tested (e.g., sardine extract in the case of non-fish-eating species).

#### **Observations on the origin of chemosensory predator identification in *Coleonyx***

The genus *Coleonyx* comprises seven species whose phylogenetic relationships are shown in Figure 1 (based on Grismer, '88). *Coleonyx mitratus* and *C. elegans* are tropical-forest geckos distributed in southern Mexico and Central America. With the exception of *C. fasciatus* (an arid, tropical-thorn scrub gecko from Sonora, Mexico), the remaining species occur in southwestern deserts: *C. switaki* and *C. variegatus* in the Sonoran and the Mojave-Sonoran Deserts, respectively, and *C. reticulatus* and *C. brevis* in the Chihuahuan Desert. The first outgroup to *Coleonyx* comprises four genera, all with Old World distributions (see Dial and Grismer, '92). The availability of a cladogram for *Coleonyx* (Fig. 1) permits preliminary observations on the origin of species-specific, chemosensory predator identification (CPI) within the genus.

This study and Dial et al. ('89) demonstrate the defensive tail display and CPI are present in at least two species of *Coleonyx* (Fig. 1). However, it is important to note that each species responds with the display to a different species of snake: in the case of *C. brevis*, *H. torquata* (this study), and in the case of *C. variegatus*, *P. decurtatus* (Dial et al., '89). In addition, *C. brevis* responds to its sympatric snake predator (*H. torquata*) but does not respond to odors of the nonsympatric gecko predator, *P. decurtatus*. *Coleonyx variegatus* is sympatric with both *H. torquata* and *P. decurtatus* and is a natural prey species of both snakes. However, it has been tested only with *Phyllorhynchus* chemical cues.

Given the observations above, we can recognize two components of CPI: first, the capacity to use olfaction to recognize predator odors, and, second, the ability to discriminate among potential predators and to identify the appropriate sympatric species. Given the few data available, a conservative estimate for the origin of CPI in *Coleonyx* is in the ancestor of *variegatus* + *brevis* + *fasciatus*, indicated by the stippled bar in Figure 1. Based on these data, this represents the most parsimonious hypothesis for the evolution of CPI in this

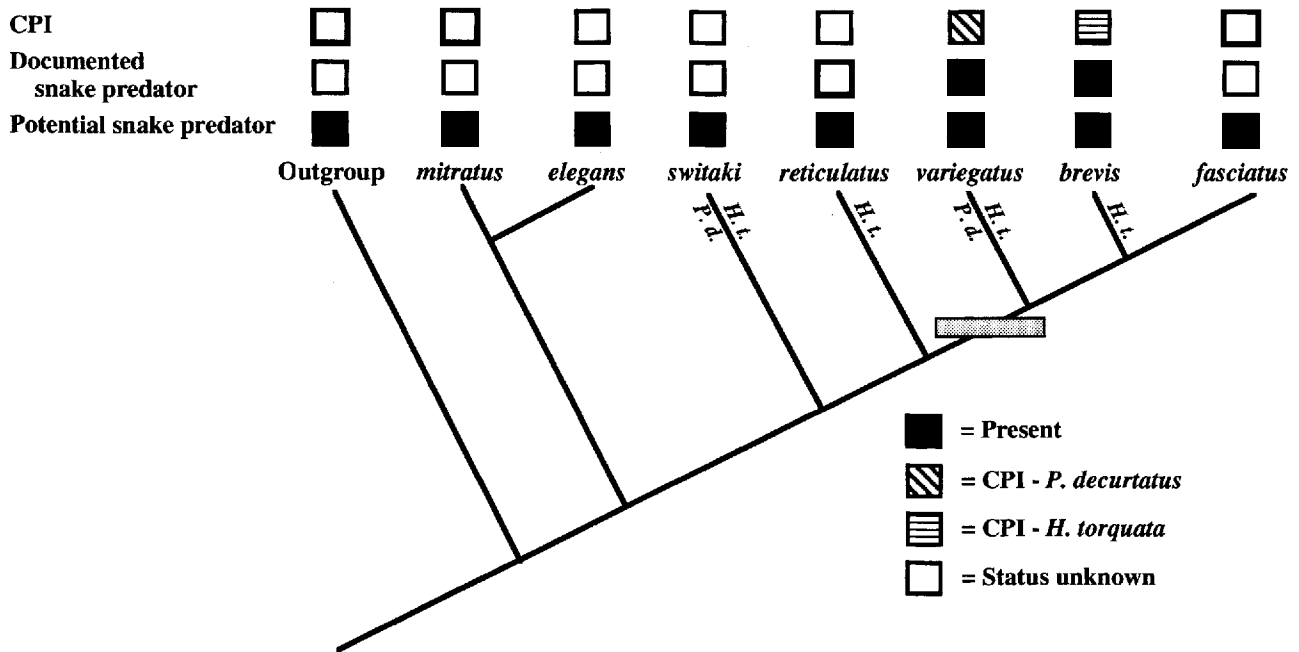


Fig. 1. A cladogram showing phylogenetic relationships and the distributions of predator-related characteristics of species within *Coleonyx* and in the first outgroup. Sympatric snake predators known to eat *Coleonyx* are indicated along branches of four gecko species (*H.t.* = *H. torquata*; *P.d.* = *P. decurtatus*); however, actual predation (based on stomach-content analyses or field observations) has been demonstrated

only for *C. variegatus* and *C. brevis*. The stippled bar indicates a conservative interpretation for the point of origin of chemosensory predator identification (CPI), but species-specificity of the behavior probably arises along the terminal branches of the clade. Future work on the origin of CPI can be organized to test hypotheses suggested by the current pattern of associations.

clade of lizards. As more data are gathered, it is likely that the conclusion regarding its origin will be pushed farther toward the root of the tree.

In addition to the evolution of the appropriate anatomy and physiology enabling CPI, we must account for the species-specificity of the behavior. In this case, three alternative, possibly overlapping, explanations for the origin of species-specificity are possible: 1) the historical association between gecko species and sympatric-predator species suggests that species-specificity is the result of selection, which fine-tunes the system so that geckos respond only to sympatric predators; 2) possibly, species-specificity arose as a learned behavior based on experience and results from individual gecko survival of encounters with different snake species (however, see Van Damme et al., '95); and 3) it may represent a diet-induced characteristic, in which geckos recognize an odor acquired by snakes from conspecific individuals that were eaten.

Present data are inadequate to test these hypotheses. However, the information summarized in Figure 1 suggests that the genus *Coleonyx* provides an excellent system for pursuing questions regarding the origin of CPI and the interplay of evolutionary history, adaptation, and individual

experience in shaping complex behaviors. For example, given the origin of CPI at the point shown in Figure 1, we predict its presence in *C. fasciatus*, assuming a snake predator is demonstrated to prey on this species. Evolutionary response of this species in the absence of a snake predator might be even more instructive. Similarly, adaptive trade-offs might be examined in *C. reticulatus*, in which the slender, partially prehensile tail is used while climbing (Dial, '78a). Such a role for the tail might preclude its use in display owing to the increased likelihood of its loss. If there is a causal relationship between the evolution of defensive tail display and the evolution of CPI (presently unknown), the adaptive trade-off in tail evolution could affect the evolution of CPI in this species.

In conclusion, we note that the sensory basis of gecko behavioral ecology must be interpreted with caution. Our further support of Schwenk's ('93a) gecko-olfaction hypothesis suggests that the typical assumption of VNS mediation of gecko behavior (e.g., Cooper, '95) is not robust and that the use of tongue-flick rate as an assay of biological relevance of chemical cues is particularly problematic in this specious clade, as it may be in others (Halpern, '92).

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