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Prey Chemical Discrimination and Strike-Induced Chemosensory Searching in Lizards: Their Absence in a Crotaphytid Lizard (*Crotaphytus collaris*) and a Proposal for Research in Zoos

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Lizards in scleroglossan families of actively foraging carnivores and the herbivorous iguanids use the tongue to gather chemical samples to detect, identify, and locate food prior to attack, and to relocate lost food. In contrast, previously studied iguanian families other than Iguanidae lack lingually mediated prey chemical discrimination (PCD) and do not exhibit lingually mediated chemosensory searching behavior for bitten prey that has escaped or been lost (SICS = strike-induced chemosensory searching). In the present study, experimental tests showed that PCD and SICS are absent in *Crotaphytus collaris,* a member of the previously unstudied family of iguanian ambush foragers, Crotaphytidae. Available data suggest that in active foragers natural selection favors use of chemical cues to locate hidden prey, whereas in ambush foragers natural selection favors immobility to avoid detection by predators and SICS precludes simultaneous ambush. In most families the states of PCD and SICS are retained from the ancestors, but when foraging mode shifts, a change in chemosensory behavior appears to be induced. A proposal is made for a research program involving herpetologists at zoos. © 1996 Wiley-Liss, Inc.

Key words: chemosensory behavior, Squamata, Iguania, Crotaphytidae

INTRODUCTION

In squamate reptiles, tongue flicking serves to sample chemicals from the external environment for analysis by vomerolfaction, the sense associated with the vomeronasal system [Graves and Halpern, 1989; Cooper and Burghardt, 1990a;

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Halpern, 1992]. Volatile molecules in the air and nonvolatile molecules on substrates are transferred to the oral cavity and thence through the paired vomeronasal ducts that open at the roof of the mouth and lead to the chemosensory epithelia of the vomeronasal organs [reviewed by Burghardt, 1970; Halpern, 1992; Mason, 1992]. Tongue flicking and vomerolfaction allow diverse squamates to make important chemosensory discriminations about prey, predators, and conspecifics [reviewed by Halpern, 1992; Mason, 1992; Cooper, 1994a].

Pheromonal communication appears to be very widespread in both Iguania and Scleroglossa [Mason, 1992; Cooper, 1994a], but relatively little is known about the distribution of chemosensory detection of predators. The distribution of lingually mediated discriminations of prey has by no means been completely characterized, but is more thoroughly known than those for social and predator-related chemosensory behaviors. The major findings have been that the distribution is more restricted than for pheromonal communication, and is strongly affected by phylogeny and foraging behavior.

Since 1987 the first author and colleagues have been conducting comparative studies of prey chemical discrimination and strike-induced chemosensory searching, two major chemosensory behaviors used in food acquisition. Prey chemical discrimination (PCD) refers to the ability to detect lingually sampled prey chemical stimuli and distinguish them from other substances. Although several methods have been used to study PCD, most methods rely on counts of tongue flicks upon exposure to prey and control chemicals. Biting inanimate objects labeled with prey chemicals is another response that may be used independently or in combination with tongue-flick rates to detect possible differential responses among stimuli.

Strike-induced chemosensory searching (SICS) is seeking via chemosensory examination of an area to relocate prey that has been bitten and then released voluntarily or escaped. The search of SICS is accomplished by tongue-flicking substrates during locomotion through the area [Chiszar and Scudder, 1980]. Locomotion increases the likelihood of encountering the prey trail, which may then be scent trailed in some taxa [e.g., viperid snakes, Chiszar and Scudder, 1980; varanid lizards, Auffenberg, 1981]. To demonstrate SICS, it is necessary to establish that release or escape of prey is followed by a poststrike elevation in tongue-flick rate (PETF) and increased locomotion.

In most lizard families the presence or absence of PCD and SICS has been determined historically by retention from ancestors. The two major divisions of squamate reptiles are Iguania and Scleroglossa [Estes et al., 1988; Frost and Etheridge, 1989]. Iguanians tend to be territorial and not use the chemical senses to detect prey, whereas scleroglossans tend to be nonterritorial and use chemical senses to detect and identify prey (Stamps, 1977; Cooper, 1994b]. In Iguania, PCD and either PETF or SICS are absent in four of five families studied, being absent in Chamaeleonidae [Cooper, 1989a, 1994c], Polychrotidae [Cooper, 1989a; DePerno and Cooper, 1994], Phrynosomatidae [Cooper, 1989, 1994c], and Tropiduridae [DePerno and Cooper, 1993], but present in Iguanidae [Krekorian, 1989; Cooper and Alberts, 1990, 1993). In contrast, they are present in all but two of the 10 scleroglossan lizard families studied. PCD and PETF occur in Eublepharidae [Dial, 1978; Dial et al., 1989; Cooper, 1995a; DePerno, 1994], Lacertidae [Cooper, 1990a, 1991a]; Teiidae [Cooper, 1990a, 1993a], Scincidae [Loop and Scoville, 1972; Burghardt, 1973; Nicoletto, 1985; Cooper and Vitt, 1989; Cooper, 1992a], Gerrhosauridae [Cooper, 1992b]; Anguidae [Cooper, 1995b]; Helodermatidae [Cooper, 1989b; Cooper and Arnett, 1995], and Varanidae [Cooper, 1989b,c, 1993b]. The only scleroglossan families known to lack PCD and SICS in at least one species are Gekkonidae [DePerno, 1994] and Cordylidae [Cooper and Van Wyk, 1994; Cooper et al., 1995]. In all 15 lizard families, both PCD and PETF or SICS are present, or both are absent, suggesting that the conditions in which natural selection favors PCD prior to attack also favor use of tongue flicking to relocate prey.

Foraging mode is an even better predictor of PCD and SICS than phylogeny. Although variation exists within each mode in amounts of foraging time spent moving, speed, distance covered, and percent of attacks on prey from ambush, two distinct foraging modes occur in lizards [e.g., Huey and Pianka, 1981; McLaughlin, 1989]. Ambush foraging consists of remaining immobile at an ambush site, rushing to attack when prey passes nearby. Active foraging entails search for prey while moving through the habitat. As tongue flicking is linked to movement in both types of foragers [Evans, 1961; Cooper et al., 1993], it can be predicted that ambush foragers do not exhibit PCD or SICS and that active foragers show both behaviors.

These expectations have been strongly verified. All lizard ambush foragers studied lack PCD and SICS, including representatives of Chamaeleonidae, Polychrotidae, Phrynosomatidae, Tropiduridae, Gekkonidae, and Cordylidae, whereas all actively foraging lizards studied use PCD and PETF/SICS, including representatives of Eublepharidae, Lacertidae, Teiidae, Scincidae, Anguidae, Helodermatidae, and Varanidae [references cited above; Cooper, 1994a,b]. Although the foraging mode of gerrhosaurid lizards has not been studied, casual observations suggest that they may forage actively (Bill Branch, personal communication). If so, the presence of PCD and PETF agrees with the prediction for active foragers. In the only other family for which data are available, Iguanidae, the substitution of an herbivorous diet for ancestral carnivory and ambush presumably freed the lizards from selective constraints on tongue flicking while attempting to ambush. By tongue flicking, ambushers would reveal their location to prey and predators without being able to detect prey chemicals except at the ambush site. Grazing herbivores can evaluate visually located potential food items chemically by tongue flicking without disrupting crypticity because their exposed locations and movements while grazing make them detectable to visually oriented predators.

As part of the ongoing comparative project we present data here on lingually mediated PCD, PETF, and SICS in the collared lizard, *Crotaphytus collaris*. This species belongs to Crotaphytidae, a family of iguanian ambush foragers (Cooper and Fox, personal observations). It was predicted and experimentally confirmed that PCD, PETF, and SICS are all absent. Implications of these findings and need for studies of additional taxa are discussed. Fairly detailed descriptions of the experimental techniques are included in hopes of stimulating studies by zoo personnel.

MATERIALS AND METHODS

Subjects and Maintenance

Adult collared lizards were collected in Oklahoma and transported to Indiana University–Purdue University Fort Wayne, where they were housed in an AAALAC-accredited animal care facility. During acclimation to laboratory conditions and during the studies, room temperature was 24–27°C, and a combination of fluorescent, ultraviolet, and incandescent light was provided on a 14 hr L:10 hr D cycle. Lizards were housed individually in 50 × 26 × 31 cm glass terraria, each of which contained a pea gravel substrate and water bowl, and was covered by a removable screen top. Lizards were provided with water ad libitum and fed domestic crickets to satiation three times per week.

PREY CHEMICAL DISCRIMINATION

Chemical stimuli derived from the integuments of prey (crickets) and two control stimuli on cotton-tipped applicators were presented to lizards in a standardized manner used for studies of lingually mediated PCD for nearly 30 years [see Burghardt 1967, 1970; Cooper and Burghardt, 1990]. To ascertain the presence or absence of PCD, we tested the responses of lizards to three stimuli; distilled water as a control for response to an odorless substance, diluted cologne (Mennen Skin Bracer, Spice Scent) as a pungency control for response to an odorous nonfood substance, and cricket integumentary chemicals.

Stimuli were prepared by first dipping the cotton tip of a wooden applicator into distilled water, then shaking the swab to remove excess water that might induce licking. The pungency control was prepared by dipping the moistened swab into cologne and then shaking out any excess cologne. Because the concentrated cologne initially might be noxious, but volatilizes rapidly, the swab is not presented to a lizard until it does not irritate human nose or eyes when sniffed. A standard dilution of cologne might be used, but this crude procedure has been satisfactory. Prey stimuli were prepared by pressing a wetted swab against a cricket and rolling the swab over the cricket's integument. Similar prey stimuli have reliably induced lizards in numerous families to tongue flick at elevated rates and sometimes to bite the swab [e.g., Cooper 1990b, 1994a,b].

Each lizard (n = 18) was presented all three stimuli, one at a time, in a randomized block design. The sequence of presentation was counterbalanced to preclude confounding of any stimulus effect with sequential bias. To begin a trial, the experimenter slowly approached a lizard's cage, removed the screen, and held a swab 1.5 cm anterior to the lizard's snout. Numbers of tongue flicks were recorded for 60 sec. If a lizard did not tongue flick in the first 50 sec, the swab was gently brought into contact with its snout, a procedure that often elicits tongue flicking, and any responses were recorded for the next 10 sec. It was planned to terminate the trial upon biting and record the latency to bite the swab in seconds. This would have permitted analysis of bite frequency, latency to bite, and the tongue-flick attack score (TFAS). TFAS is a widely used measure of response to prey chemical stimuli that incorporates both tongue flicking and biting [for details see Burghardt, 1970; Cooper and Burghardt, 1990b]. Each lizard was presented one stimulus at a time with intertrial intervals of at least 30 min. Observations were conducted at 1050–1600 hr.

Two further important experimental considerations are hunger level and temperature. The lizards were not fed for 6 days prior to testing to ensure responsiveness to prey. At the conclusion of the experiment, the lizards fed readily. Control of temperature is necessary in studies of tongue-flicking responses to prey chemicals because tongue-flicking rates vary markedly with temperature [Stevenson et al., 1985; Cooper and Vitt, 1986; Van Damme et al., 1990]. As long as the animals feed normally, the exact temperature is less important than maintaining a limited temperature range because a wide range of experimental temperatures could substantially increase error variance. Room temperature was 27°C during the experiment, but heat lamps adjacent to the cages provided a thermal gradient allowing the lizards to thermoregulate.

For lizards that tongue flick fairly frequently in all stimulus conditions, statistical analysis of number of tongue flicks and TFAS is typically done using parametric analysis of variance for a single-factor experiment with repeated measures [Cooper and Burghardt, 1990b]. However, because the collared lizards tongue flicked so infrequently, the tongue-flick distributions seriously violated the assumption of normality. Therefore, the data were analyzed using nonparametric Friedman two-way analysis of variance, which can be followed by multiple comparisons if the main stimulus effect is significant [Zar, 1984].

Negative results must be carefully considered because a null hypothesis cannot be proven. However, if results from similar studies with positive results are available, statistical power analysis can give an estimate of the probability that a real effect will be detected using a given sample size. If there is no significant effect despite use of a sample size corresponding to a high probability that a real effect would be detected, one may be confident that no effect exists. A power analysis was performed [as in Cooper, 1989a, based on Winer, 1962]. Another approach might be to compare results of similar experiments for species that obviously detect prey chemicals and those that do not appear to do so. Highly significant results would be obtained in many cases, but we have not taken this approach because the experiments have been done at different times and under slightly different conditions.

TONGUE FLICK RATES and CHEMOSENSORY SEARCHING

PETF and SICS were assessed by standard methods for nonvenomous snakes and lizards [e.g., Cooper, 1989c, 1992a,b, 1994c; Cooper et al., 1989]. Because lizards rarely release prey voluntarily, experimental studies of PETF and SICS must include forcible removal of the prey from the lizard's mouth. Removal in many species requires the experimenter to handle the lizard, which could strongly affect subsequent behavior. A mechanical disturbance control is included in the experiment to simulate the effects of handling the lizard during removal of prey. The handling matches that during removal except that there are no chemical and mechanical stimuli in the oral cavity associated with biting and removal of prey. Effects of these stimuli have been studied in a snake and lizard [Cooper et al., 1989; Cooper, 1992a]: An animal was grasped, a swab bearing either prey chemicals or water was inserted into its mouth and rubbed on its oral epithelium, and its subsequent tongue flicking and searching behavior were recorded. In the complete absence of any difference in intraoral or other mechanical disturbance, the prey chemicals elicited much more tongue flicking and searching movement than did the control.

The mechanical disturbance control and the experimental (strike) condition together comprise the minimum set of conditions needed to establish the presence of PETF and SICS in species that do not voluntarily release prey. Two additional control conditions are usually included to assess the effects of seeing and perhaps smelling the prey, and of the general setting on tongue flicking and movement. In the sight condition a lizard is allowed to see the prey and perhaps detect it via airborne chemical cues, but is not allowed to approach the prey. Comparison with the mechanical disturbance control permits assessment of handling effects in the absence of attack on prey. In the context condition no visual or chemical prey stimuli are presented, but the experimental milieu is otherwise identical to that in the sight condition. Comparison of responses in the sight condition with those in the context condition allows detection of any effect of seeing the prey.

Each lizard was tested in these four conditions in an incompletely counterbalanced sequence. In the context condition a thread was held 20 cm anterior to a lizard's snout for 10 sec and then removed. In the sight condition a large adult cricket tethered by a thread tied between abdomen and thorax was held 20 cm anterior to a lizard's snout. It was removed after 10 sec if the lizard did not approach, or immediately if the lizard approached before 10 sec. Removal was necessary to prevent tongue flicking or attack. In the mechanical disturbance condition prey were presented as in the sight condition, but the lizard was allowed to approach and tongue flick the prey. If the lizard lunged toward or prepared to bites the cricket, the cricket was removed with one hand and simultaneously the lizard was grasped and handled to simulate the mechanical disturbance caused by removing the prey from its mouth in the strike condition. In such experiments if a lizard bites the cricket before it can be withdrawn, the trial is terminated and is conducted after the usual intertrial interval. In the strike condition, the primary experimental condition, the prey was introduced as above, but the lizard was permitted to bite the prey. As soon as the lizard bit, it was grasped and the prey was removed from its mouth.

Numbers of tongue flicks and bouts of locomotion were recorded in two successive minutes, starting with removal of experimental stimuli in the sight and context conditions or when initial flight movements after being handled stopped (after at most a few seconds) in the mechanical disturbance and strike conditions. Each of 17 individuals was tested once per day. Room temperatures during testing were 24–26°C, but heat lamps warmed the cages, permitting the lizards to maintain body temperatures in the 30s°C. They readily ate under these conditions. Observations were conducted at 1100–1600 hr.

Two distinct types of lingual protrusions are (1) tongue flicks, in which the tongue is protruded anteriad and well beyond the mouth, frequently touching a substrate, and (2) labial licks, in which the tongue is wiped on the labial scales without being projected far. Because only tongue flicks contacting substrates can be used to sample nonvolatile prey scent, data were analyzed separately for tongue flicks and labial licks. PETF is inferred if the main condition effect for tongue flicks is significant and the tongue-flick rate is higher in the strike than in the control conditions. SICS is detected only if PETF is accompanied by increased locomotion. Increase in locomotion is demonstrated statistically by significantly more movements in the strike than in the mechanical disturbance condition.

Significance tests for main effects were conducted using Friedman two-way analysis of variance [Siegel, 1956] with $\alpha = 0.05$, two tailed. Multiple comparisons were conducted following detection of significant main effects [Zar, 1984]. Differences in numbers of individuals performing behaviors between conditions were assessed by sign tests. Although multiple tests were performed without adjustment of alpha, the probabilities obtained are sufficiently low that significance is obvious except where noted.

TABLE 1. Tongue flicks by *Crotaphytus collaris* (n = 18) in 1 min in three experimental conditions*

	Condition		
Statistic	Cricket	Cologne	Water
Initial 50 sec			
$\bar{X}$	0.39	0.56	0.33
SE	0.33	0.23	0.18
Range	0–6	0–3	0–3
Final 10 sec			
$\bar{X}$	0.22	0.22	0.06
SE	0.12	0.13	0.06
Range	0–2	0–2	0–1
Entire 60 sec			
$\bar{X}$	0.61	0.77	0.39
SE	0.34	0.26	0.18
Range	0–6	0–3	0–3

*Data are presented separately for the initial 50 sec, the final 10 sec after the swab was touched to the anterior labials of individuals that had not previously tongue flicked, and the entire 60 sec. SE = 1.0 standard error.

RESULTS

Prey Chemical Discrimination

Few individuals tongue flicked in any of the conditions (five in responses to cricket chemicals, seven to cologne, and five to distilled water), and those individuals that did performed very few tongue flicks. No lizard included in the study bit the swab in any condition. Data for one lizard were discarded because it bit before tongue flicking so that the attack could not represent a response to lingually sampled chemical cues. Another individual was added to complete the design.

The low mean numbers of tongue flicks, low proportions of individuals tongue flicking, and their similarities across conditions (Table 1) indicate a low propensity to investigate the experimental stimuli by tongue flicking and absence of any differential response to prey chemical stimuli. Numbers of tongue flicks did not differ significantly among conditions for the entire minute ($X_r^2 = 0.78$, df = 2, $P > 0.10$), during the initial 50 sec ($X_r^2 = 1.00$, df = 2, $P > 0.10$), or during the final 10 sec after nonresponding individuals were touched by the swab ($X_r^2 = 0.33$, df = 2, $P > 0.10$).

Based on a phi value of 0.59, a sample of 18 lizards, and a two-tailed $\alpha = 0.05$, the power indicated is greater than 0.9. Table B.11 in Winer [1962] does not show curves for $P > 0.9$, but by visual extrapolation the power level for the current experiment is close to 0.95. That value would indicate that the chance that a true significant difference among conditions was present, but went undetected, is only approximately 0.05. Beyond the statistical lack of PCD, there was a very clear difference in the reaction to the swab by *C. collaris* and species that show PCD. Collared lizards appeared to pay less attention to the swabs and directed far fewer tongue flicks toward them.

TABLE 2. Numbers of tongue flicks (TF), labial licks (LL), and movements in 2 min by 17 *Crotaphytus collaris* in studies of poststrike elevation in tongue-flicking rate

			Condition			
Data	Minute	Statistic	Strike	Pull	Sight	Context
TF	One	$\bar{X}$	0.3	0.2	0.1	0.0
		SE	0.2	0.1	0.1	0.0
		Range	0–2	0–2	0–1	—
	Two	$\bar{X}$	0.1	0.0	0.0	0.0
		SE	0.1	0.0	0.0	0.0
		Range	0–1	—	—	—
LL	One	$\bar{X}$	14.7	1.1	0.2	0.0
		SE	1.7	0.6	0.2	0.0
		Range	3–28	0–9	0–3	—
	Two	$\bar{X}$	1.8	0.0	0.0	0.0
		SE	0.7	0.0	0.0	0.0
		Range	0–10	—	—	—
Movements	One	$\bar{X}$	0.6	0.6	0.4	0.1
		SE	0.2	0.2	0.2	0.1
		Range	0–2	0–2	0–2	0–1
	Two	$\bar{X}$	0.4	0.2	0.1	0.0
		SE	0.1	0.1	0.1	0.0
		Range	0–2	0–1	0–1	—

Poststrike Elevation in Tongue-Flicking Rate and Strike-Induced Chemosensory Searching

The lizards performed very few tongue flicks in any condition, the maximum number for any individual being two for a single minute or even the entire 2 min (Table 2). Numbers of tongue flicks did not differ significantly among conditions either in minute one ($X_r^2 = 0.67$, df = 3, $P > 0.10$) or minute two $X_r^2 = 0.11$, df = 3, $P > 0.10$). Only seven of the 17 lizards tongue flicked at all, four in the strike condition, two each in the mechanical disturbance and sight conditions, and none in the context condition.

Much higher frequencies of labial licks than tongue flicks occurred in the strike condition, and numbers of labial licks in the control conditions were much lower than in the strike condition; they were as low as numbers of tongue flicks in the context and sight conditions, and slightly higher in the mechanical disturbance condition (Table 2). The main effect for labial licks was highly significant in minute one ($X_r^2 = 30.23$, df = 3, $P > 0.001$), but not significant in minute two ($X_r^2 = 6.78$, df = 3, $P > 0.05$). In the first minute, numbers of labial licks were significantly greater in the strike condition than in the control conditions ($P < 0.001$ each). There were no significant differences in numbers of labial licks among the control conditions.

In the first minute, numbers of individuals that labial licked at least once were 17 in the strike condition, four in the mechanical disturbance condition, one in the sight condition, and none in the context condition. Significantly more individuals labial licked in the first minute in the strike condition than in the control conditions (sign tests, $P < 0.002$ each). In minute two, eight individuals labial licked at least once in the strike condition, but none labial licked in any other condition. The number of individuals that labial licked in minute two was significantly greater in the strike condition than in the control conditions (sign tests, $P = 0.008$ each). Numbers of individuals labial licking in the control conditions did not differ significantly in either minute ($P > 0.10$). There was no detectable relationship between numbers of tongue flicks and numbers of labial licks in the strike condition in minute one (Spearman rho $= 0.28$, $n = 17$, $P > 0.05$).

Although over half of the individuals moved at least once in the 2 min in the strike and mechanical disturbance conditions (Table 2), movements were all very brief and were not associated with lingual protrusion or apparent search for food. As movement almost invariably increased the distance between the lizard and the experimenter, the movements probably reveal defensive withdrawal rather than search for prey. Number of movements did not differ significantly among conditions in minute one ($X_r^2 = 4.94$, df $= 3$, $P > 0.10$) or minute two ($X_r^2 = 1.84$, df $= 3$, $P > 0.10$). Numbers of individuals that moved (minute 1, minute 2) were (7,5) in the strike condition, (9,3) in the mechanical disturbance condition, (5,2) in the sight condition, and (1,0) in the context condition. The only significant difference according to unprotected sign tests was that a greater number of individuals moved in minute one in the mechanical disturbance condition than in the context condition ($P = 0.022$). Given that 12 comparisons were made, six for each minute, this result should not be considered significant.

DISCUSSION

Prey Chemical Discrimination

Although negative results should be interpreted cautiously, the power analysis strongly suggests that PCD would have been found if present in *C. collaris*. The very low numbers of tongue flicks combined with absence of attacks on swabs and the relative lack of concentrated investigation of the swabs reinforce the statistical absence of PCD. Collared lizards typically detect prey visually and charge to the attack without prior tongue flicking (Cooper and Fox, personal observations). All of these considerations point to an absence of PCD in *C. collaris*. The present results, therefore, are consistent with previous findings (cited above) that ambush foragers and members of carnivorous iguanian families lack lingually mediated pCD. Furthermore, the behavior of *C. collaris* in the PCD experiment was very similar to that of other ambush foragers and carnivorous iguanians that do not use PCD [Cooper, 1989a, 1994c; DePerno and Cooper, 1993, 1994; Cooper and Van Wyk, 1994]. Although a latent capacity to discriminate among prey chemicals and other chemical stimuli is possible, it is clear that tongue flicking is not used to detect or identify prey before attack in *C. collaris* or the other taxa that experimentally lack PCD.

Tongue Flick Rates and Chemosensory Searching

Given the lack of any significant differences among conditions in either tongue-flick rates or number of movements, SICS was not demonstrated in *C. collaris*. In earlier studies of SICS using the same experimental design, PETF and/or SICS have been detected readily in other squamates despite much smaller sample sizes: $n = 7$ [Cooper, 1989c]; $n = 8$ [Cooper, 1991a; Cooper et al., 1995], $n = 9$ [Cooper and Arnett, 1995], and $n = 10$ [Cooper, 1991b, 1993a]. A null hypothesis cannot be proven, but it was obvious from very low tongue-flick rates, absence of tongue flicking by most lizards, and the very low movement frequency that the lizards were not searching for prey by tongue flicking. There was some indication that disturbance due to handling the lizards may have caused a slight (nonsignificant) increase in the frequency of movement in the mechanical disturbance condition. We conclude that SICS is absent in *C. collaris*. This result extends the findings of previous studies cited showing SICS to be absent in carnivorous igaunians and ambushers.

A possible alternative interpretation of the results is that collared lizards might exhibit SICS in the absence of experimenters, but suppressed it defensively during the experiment. This is a reasonable possibility because ambush foragers use crypsis dependent upon immobility as a major defense against detection by predators [Vitt and Price, 1982]. However, lizards in two other iguanian families observed from a blind also failed to reveal any indication of SICS, suggesting that defensive behavior does not account for the lack of SICS [Cooper, 1994c, unpublished observations on *Agama agama* and *Sceloporus clarkii*]. Visual detection and attack without tongue flicking in the field (Cooper and Fox, personal observations) suggest that absence of PETF as well as PCD is real, not an observer effect.

The increase in labial licking in minute one and the greater numbers of individuals performing labial licks in minutes one and two after biting prey do not indicate PETF or SICS. Labial licking cannot contribute to search for chemical prey cues on substrates beyond the lizard's body. Dissociation between labial licking and SICS is emphasized by the high frequency of labial licking while the lizards were otherwise motionless.

Possible functions of labial licking include repositioning after a biting attack, lingual cleaning, or procurement of additional prey chemicals from the labials [Bels et al., 1994; Cooper, 1949c]. Labial licking is distinct from lingual protrusions during pharyngeal transport and packing while feeding [Delheusy and Bels, 1992; Bels et al., 1994]. It resembles lingual cleaning cycles at the end of feeding [Bels et al., 1994], and might represent low-amplitude cleaning cycles. However, the large numbers of labial licks seem inconsistent with lingual repositioning or cleaning, leaving a chemosensory function most likely. Labial licking in the mechanical disturbance condition might also be an attempt to sample airborne prey chemicals. If labial licking serves to sample for volatile airborne prey chemicals, for prey chemicals previously deposited on the labials, or possibly to free chemicals adhering to the tongue for delivery to the vomeronasal system, it is possible that collared lizards and other iguanians showing elevated labial licking after biting prey [DePerno and Cooper, 1993, 1994; DePerno, 1994] use chemical cues to focus attention on particular prey types. Such search for prey would continue to be visual, but the search parameters might be specified by chemical cues [Cooper, 1994c]. This possibility remains to be studied. Whatever the functions of labial licking may be, the statistical independence of tongue flicks and labial licks suggests that these behaviors have different functions.

Relationships Among PCD, SICS, Foraging Mode, and Phylogeny

The predictions that PCD, PETF, and SICS are absent in crotaphytids because they are carnivorous iguanians and ambush foragers are verified for collared lizards. This adds another family of lizards that conforms to the relationships discussed above. Although a few families of iguanians have not been studied, representatives of all of the most speciose iguanian families except Iguanidae are now known to lack PCD and SICS [Cooper, 1994a,b,c; Deperno and Cooper, 1993, 1994. In Scleroglossa, representatives of all large families except Gymnophthalmidae have been studied and found to exhibit both PCD and PETF or SICS if active foragers, and to lack these behaviors if ambush foragers [Cooper, 1993a, 1994a,b].

Although data are lacking for a number of families and only a few species have been studied in any family, an interim assessment suggests that phylogeny determines the states of PCD, PETF, and SICS in a large majority of lizard families, but the influence of foraging mode overrides that of phylogeny when evolutionary changes in foraging mode create a conflict in predictions based on phylogeny and foraging ecology. Cladistic relationships among iguanian families are unclear, but it is highly unlikely that the predatory crotaphytids evolved from the herbivorous iguanids. In all other scenarios crotaphytids have retained from their ancestors both their foraging mode and lack of lingually mediated chemosensory behaviors related to prey [Cooper, 1994b].

The pattern of phylogenetic inertia in chemosensory behaviors in the absence of changes in foraging mode is extended by the present findings. Changes in state of chemosensory behavior in families for which foraging mode has changed from the ancestral condition support action of natural selection as hypothesized in the Introduction: Absence of PCD and PETF or SICS in Gekkonidae [Cooper, in press; DePerno, 1994] and Cordylidae [Cooper and Van Wyk, 1994; Cooper et al., 1995] and their presence in Iguanidae suggest that natural selection related to foraging success in active foragers and to antipredatory behavior in ambush foragers drives the changes in chemosensory behavior.

Zoos: Future Research and Possible Use in Husbandry

Further elucidation of these relationships depends on study of chemosensory behavior in (1) representatives of several families for which no data exist and (2) additional species chosen from divergent clades and ecological types within some of the large lizard families. In most lizard families all species studied have the same foraging mode, but some variation has been reported in Lacertidae, Scincidae, and Gekkonidae [reviewed by Cooper, 1994a,b], and wide variation occurs in Pygopodidae [Webb and Shine, 1994]. Intrafamilial variation in foraging mode offers outstanding opportunities for testing the hypotheses regarding the relative influence of foraging mode and phylogeny.

Herpetologists in zoos are especially well placed to contribute to studies of taxa that cannot normally be obtained in sufficient sample sizes for experimentation. Table 3 shows selected unstudied taxa for which important information might be obtained by collaboration among several biologists collecting data for one to a few specimens each. The experimental techniques are very easy to learn and use. By practicing on a few individuals in taxa having known chemosensory behaviors, zoo biologists can quickly develop the skills needed to test the important taxa. The authors would welcome the opportunity to accumulate data collected by multiple biologists for the taxa listed in Table 3 and to publish the results in multiauthored papers. Because chemical stimuli presented must be standardized and size of the swab appropriate for a given species, please contact W.E.C. before collecting data.

As shown in Table 3, the iguanian families for which zoos may provide the best opportunity for study are Hoplocercidae and Opluridae, especially the former. Corytophanids have not been studied, but the first author will shortly obtain specimens from a commercial dealer. The scleroglossan lizard families for which no data are available are Xantusiidae, Gymnopthalmidae, and Xenosauridae. In addition, no data

TABLE 3. Lizard taxa especially suitable for collaborative study of PCD and SICS in zoos

	Taxon	
Data lacking	Iguania	Scleroglossa
PCD and SICS in any species	Hoplocercidase Opluridae	Xantusiidae Gymnophthamidae Xenosauridae
SICS in any species		Amphisbaenia
PCD and SICS in active foragers		Gekkonidae
PCD and SICS in ambush foragers		Scincidae Lacertidae

are available for PETF and SICS in amphisbaenians. Contrary to the prevailing mode for their families, a few lacertids and skinks have been characterized as ambush foragers, and a few gekkonids as active foragers [reviewed by Cooper, 1994a,b; for Scincidae see Costanzo and Bauer, 1993]. Any opportunity to study such species should be taken. In addition, study of species representing branches of the very large families Scincidae and Gekkonidae that are distantly related to taxa already studied might uncover unsuspected variation. Finally, neither PCD nor SICS has been studied in the primitive scolecophidian snakes of the families Leptotyphlopidae and Typhlopidae, but the presence and duration of SICS in these snakes have important evolutionary implications.

Work with xantusiids and some gekkonids may prove difficult because these lizards have very highly developed olfactory systems as well as well developed vomerolfactory systems [Gabe and Saint Girons, 1976]. In pilot tests of a few specimens, some individual *Xantusia vigilis* and *Phelsuma madagascariensis* bit cotton swabs bearing either prey chemicals or cologne before tongue flicking, suggesting that they detected volatile chemicals by olfaction. Others performed many tongue flicks in response to cologne. It may prove necessary to block the external nares with vaseline or tissue adhesive to test for lingually mediated PCD and SICS in those species that may rely also on olfaction. Cologne may also prove to be an inappropriate control. Chemical stimuli from plants such as lettuce or carrot that are detected by herbivorous lizards might be a good substitute.

Direct applications of squamate chemosensory behavior in husbandry have been few, but labeling unacceptable prey such as rodents with chemicals of natural prey has been widely used by snake enthusiasts. For lizards, this method is likely to work only for species that use the tongue to gather chemical samples while searching for food. Using a different, but closely related technique, WEC has induced a number of reluctant gila monsters to eat mice by swabbing the mice with raw egg. Some snakes capable of SICS, and presumably some lizards, appear to form chemical search images [Melcer and Chiszar, 1989]. The probability of feeding by reluctant individuals might be enhanced by labeling the cages with prey chemicals. Because novel surroundings stimulate searching behavior, including elevated tongue-flicking rates [De Fazio et al., 1977; Burghardt et al., 1986], labeling cages with prey chemicals before transferring the squamate to the new cage might be especially effective.

CONCLUSIONS

1. PCD and SICS are absent in the crotaphytid lizard *Crotaphytus collaris*.
2. The absence of these behaviors is a retention of the ancestral condition, but in other families in which foraging mode changes, so does the presence or absence of PCD and SICS.
3. An increase in labial licking after biting prey suggests a chemosensory function for this behavior, perhaps related to formation of a visual search image based on chemical cues.
4. Zoo collections are well suited for study of some taxa that are rare or difficult to obtain.

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