Food-chemical discrimination and correlated evolution between plant diet and plant-chemical discrimination in lacertiform lizards

**William E. Cooper, Jr., Janalee P. Caldwell, Laurie J. Vitt,
Valentín Pérez-Mellado, and Troy A. Baird**

Abstract: Lizards use chemical cues to locate and identify prey and plant food, assess the nutritional quality of food, and detect plant toxins. Among insectivorous lizards, all actively foraging species studied respond strongly to prey chemicals sampled lingually, but ambush foragers do not. Much recent research has been devoted to assessing differential responses to food and nonfood chemicals (i.e., food-chemical discrimination) by omnivorous and herbivorous species and determining whether correlated evolution has occurred between plant diet and plant-chemical discrimination. We conducted experimental studies of food-chemical discrimination by two species of teiid lizards, the omnivorous *Cnemidophorus murinus* and the actively foraging insectivorous *Ameiva ameiva*. The omnivore distinguished both prey and plant chemicals from control substances. The insectivore exhibited prey-chemical, but not plant-chemical, discrimination, as indicated by tongue-flicking and biting. A comparative analysis using concentrated-changes tests showed that correlated evolution has occurred between plant consumption and plant-chemical discrimination in a major lizard taxon, Lacertiformes. These results extend and strengthen previous findings of similar correlated evolution to a new group and add to a growing database indicating that omnivorous lizards use chemical cues to assess both prey and plant foods.

Résumé : Les lézards utilisent des indices chimiques pour localiser et identifier leur proies et les plantes qui leur servent de nourriture, pour évaluer la qualité de leur nourriture et pour détecter les toxines végétales. Chez les espèces de lézards insectivores, toutes les espèces étudiées qui recherchent activement leur nourriture réagissent fortement à des substances chimiques provenant de proies et déposées sur leur langue, alors que les espèces qui chassent à l'affût ne réagissent pas. Beaucoup de travaux récents cherchent à évaluer les réactions différentes aux substances chimiques provenant d'aliments ou d'autres sources (i.e., discrimination alimentaire chimique) chez les espèces omnivores et herbivores et à déterminer s'il y a une évolution corrélative du régime alimentaire végétarien et de la discrimination des substances chimiques végétales. Nous avons étudié en laboratoire la discrimination des substances chimiques alimentaires chez deux lézards téiidés, l'omnivore *Cnemidophorus murinus* et l'insectivore à quête active de proies *Ameiva ameiva*. L'omnivore est capable de distinguer les substances chimiques provenant de plantes ou de proies des substances témoins. L'insectivore sait discriminer les substances chimiques provenant de proies, mais pas celles venant de plantes, si on se base sur ses comportements de claquement de langue et de morsure. Une analyse comparative à l'aide de tests de changements concentrés montre qu'il y a une évolution corrélative de la consommation des plantes et de la discrimination des substances chimiques végétales chez un taxon majeur de lézards, les lacertiformes. Nos résultats appuient les données antérieures sur l'évolution corrélative et apportent des exemples tirés d'un nouveau groupe; ils s'ajoutent à une banque croissante de données qui appuient l'hypothèse selon laquelle les lézards omnivores utilisent les indices chimiques pour évaluer leur nourriture d'origine tant animale que végétale.

[Traduit par la Rédaction]

Introduction

Because animal senses serve to detect adaptively important stimuli, similar sensory capacities have often evolved in response to similar detection problems. For example, when vision is restricted or inapplicable, as in many nocturnal taxa, hearing is important for detection of prey and for social communication, and chemical senses are important for detecting stimuli in the absence of the emitter (Dusenbury 1992). The ability to locate, identify, and respond to food using chemical cues is are crucially important for many animals. Among these, species having similar diets may be predicted to convergently evolve responsiveness to chemical components that are widespread or universal in their foods.

Received 31 July 2001. Accepted 25 February 2002. Published on the NRC Research Press Web site at http://cjz.nrc.ca on 26 April 2002.

W.E. Cooper, Jr.[1] Department of Biology, Indiana University – Purdue University at Fort Wayne, Fort Wayne, IN 46805, U.S.A.
J.P. Caldwell and L.J. Vitt. Sam Noble Oklahoma Museum of Natural History and Department of Zoology, 2401 Chautauqua Avenue, University of Oklahoma, Norman, OK 73072, U.S.A.
V.P. Pérez-Mellado. Departamento de Biología Animal, Universidad de Salamanca, 37071 Salamanca, Spain.
T.A. Baird. Department of Biology, University of Central Oklahoma, 100 North University Drive, Edmond, OK 73034, U.S.A.

[1] Corresponding author (e-mail: cooperw@ipfw.edu).

Can. J. Zool. **80**: 655–663 (2002) DOI: 10.1139/Z02-040

Many lizards use lingually sampled chemical cues to locate and identify food (Cooper 1994*a*, 1994*b*). Among insectivores, correlated evolution has occurred between foraging mode and chemosensory response to prey; active foragers discriminate between prey and control chemicals but ambush foragers do not (Cooper 1995, 1997, 2000*a*). Recent studies of responses to prey chemicals have shown that omnivorous and herbivorous lizards in both of the major lizard taxa, Iguania and Scleroglossa, respond strongly to both prey chemicals and plant chemicals regardless of foraging mode (e.g., Cooper and Alberts 1990; Cooper 2000*c*, 2000*d*, 2000*e*, 2000*f*; Cooper and Flowers 2001; Cooper and Pérez-Mellado 2001*a*). Within Scleroglossa, correlated evolution has occurred between plant diet and plant-chemical discrimination (Cooper 2002), presumably to maintain a correspondence between diet and the ability to detect chemical cues related to the identity and nutritional status of potential plant foods. Herbivorous and omnivorous lizards exhibit plant-chemical and prey-chemical discrimination (e.g., Cooper and Alberts 1990; Cooper 2000*c*, 2000*d*, 2000*e*, 2000*f*; Cooper and Flowers 2001; Cooper and Pérez-Mellado 2001*a*, 2001*b*), but insectivores exhibit prey-chemical discrimination only if they are active foragers, which applies only to Scleroglossa (Cooper 1994*a*, 1995, 1997; Perry 1995).

Lacertiformes is a species-rich scleroglossan taxon consisting of three families: Lacertidae, Teiidae (macroteiids), and Gymnophthalmidae (microteiids, the sister-group of Teiidae). With the exception of a few species of lacertids (Huey and Pianka 1981; Arnold 1990, 1993; Perry et al. 1990; Cooper 1994*a*, 1999), all lacertiforms are believed to be active foragers (Cooper 1994*a*; Perry 1995), although quantitative data are lacking for gymnophthalmids.

Prey-chemical discrimination has been detected in all lacertiform species studied, with a single possible exception. These species include several lacertids: two species of *Acanthodactylus* (Cooper 1999), two of *Gallotia* (Cooper and Pérez-Mellado 2001*b*), three of *Lacerta* (Nelling 1996; Marcos-Leon 1999; Cooper and Pérez-Mellado 2002), five of *Podarcis* (Cooper 1990, 1991; Marcos-Leon 1999; Cooper and Pérez-Mellado 2001*a*; Cooper et al. 2002), one of *Psammodromus* (Marcos-Leon 1999), and two of *Takydromus* (Nelling 1996; Cooper et al. 2000). Goose and Bels (1990) found no evidence for prey-chemical discrimination by *Lacerta viridis*, but their study was inconclusive because they used a very small sample and did not use standard methods for such studies. The only known exception to the rule that insectivorous ambush foragers do not exhibit prey-chemical discrimination is a lacertid, *Acanthodactylus boskianus*, that spends a greater proportion of time moving than most ambush foragers but less than typical active foragers, and is capable of prey-chemical discrimination (Perry et al. 1990; Cooper 1999). Prey-chemical discrimination has been studied in fewer species of teiids and not at all in gymnopthalmids. The teiids that have been shown to be capable of prey-chemical discrimination are two species of *Tupinambis* (Cooper 1990; Yanosky et al. 1993) and the insectivorous *Cnemidophorus gularis* (Cooper et al. 2000).

Plant-chemical discrimination has been studied in fewer lacertiform lizards. It is present in several omnivorous lacertids (*Gallotia caesaris* and *Gallotia simonyi*, Cooper and Pérez-Mellado 2001*b*; *Podarcis lilfordi*, Cooper and Pérez-Mellado 2001*a*; *Podarcis sicula*, Cooper and Pérez-Mellado 2002) and the omnivorous teiid *Tupinambis teguixin* (Yanosky et al. 1993). In contrast, plant-chemical discrimination is absent in the fewer insectivorous lacertiform species studied, the lacertids *Lacerta perspicillata* (Cooper and Pérez-Mellado 2002), *Podarcis hispanica* (Cooper and Pérez-Mellado 2001*a*), *Podarcis muralis* (Cooper et al. 2001), and *Takydromus sexlineatus* (Cooper et al. 2000), plus the teiid *C. gularis* (Cooper et al. 2000). Too few species have been studied to determine whether correlated evolution between plant diet and plant-chemical discrimination has occurred within Lacertiformes.

Ecologists have long used comparative studies of numerous species to investigate relationships between ecological and behavioral factors, but until the mid-1980s they routinely failed to consider the phylogenetic relationships among the species studied (Brooks and McLennan 1991; Harvey and Pagel 1991). Because closely related species tend to have similar traits, correlations between ecological factors are often inflated by considering each species to provide an independent data point. Numerous statistical techniques have been developed that make it possible to determine whether changes in two ecological traits have occurred in a correlated fashion independently of the relationships among the species (Harvey and Pagel 1991). Demonstrations of correlated evolution between traits can support hypotheses of adaptive relationships between traits, but additional evidence is required to establish causal relationships.

The primary goal of this study was to assess whether plant diet and plant-chemical discrimination are evolutionarily correlated in Lacertiformes, using modern methods that take into account the phylogenetic relationships among taxa. Because fewer than 3% of lizard species are herbivores or omnivores (Iverson 1982; Cooper and Vitt 2002), the greatest difficulty in studies of correlated evolution is to obtain data concerning sufficient independent origins of plant consumption. We selected *Cnemidophorus murinus* for study because this teiid species evolved omnivory (Dearing and Schall 1992) independently of *T. teguixin* (Presch 1974), the only other omnivorous teiid for which data are available. Prey- and plant-chemical discrimination had not been studied in *C. murinus*, but this species was deterred from consuming artificial foods by the addition of quinine in very low concentrations (Schall 1990). A secondary goal was to increase the size and diversity of the database on prey- and plant-chemical discrimination for Teiidae by adding two species, including a member of a previously unstudied genus, *Ameiva*.

Material and methods

Animals and maintenance

Adult *C. murinus* were collected on Bonaire Island, Netherlands Antilles, in March 2001. Wild-captured adult *Ameiva ameiva* were purchased from a commercial dealer (Strictly Reptiles). The lizards were transported to Indiana University – Purdue University at Fort Wayne (IPFW), where they were housed in an accredited animal-care facility. They were kept singly in glass terraria (50 × 31 × 27 cm), each of which contained indoor–outdoor carpet, a water bowl, and an opaque plastic shelter and was covered by a screen lid. All the side walls of each terrarium were covered with white paper to reduce disturbance to the lizards caused by movements of experimenters and other lizards. We have found that covering the walls greatly improves responsiveness of some lizard species to experimental stimuli. A 12 h light : 12 h dark light cycle was maintained using fluorescent lighting. Testing was delayed for several days after arrival at IPFW to allow the lizards to acclimate to laboratory conditions. *Cnemidophorus murinus* were supplied with and ate crickets, mealworms, lettuce, and fruits prior to testing. *Ameiva ameiva* ate crickets but refused to eat plants palatable to many omnivorous and herbivorous lizard species. The ambient temperature was 29°C during testing and maintenance, but the lizards were able to thermoregulate in a thermal gradient produced by heat lamps at one end of each cage. The lizards were active and fed readily under laboratory conditions.

Experimental procedures

Experiments were conducted to determine whether the lizards discriminate between chemical cues from animal prey and plants that are palatable to herbivorous lizards. For *C. murinus*, 20 individuals were selected randomly for testing from a larger laboratory population. For *A. ameiva*, all 20 individuals available were tested. Deionized water served as an odorless control to assess the strength of responses attributable to the experimental milieu rather than to specific chemical stimuli. For *C. murinus*, cologne (Mennen Skin Bracer, spice scent) was used as a pungency control, i.e., a control for responses to compounds having easily detected chemical cues unrelated to food. The cologne lacked any floral or fruity aroma to human observers. For *A. ameiva*, plants not eaten can be considered additional pungency controls. Responses differing significantly from those to an odorless control permit the inference that the stimulus has been detected, but not that it can be discriminated from other detectable chemicals unrelated to food. Pungency controls are useful because differential responses between odorous nonfood stimuli and food stimuli permit the conclusion that the lizards have discriminated between detectable food and nonfood stimuli. The absence of prey-chemical discrimination does not necessarily imply that lizards do not detect prey chemicals or even recognize them, only that they do not respond strongly to them. The experimental stimuli were the exoskeletal surface of crickets (*Acheta domesticus*) and Bartlett pears. These stimuli plus banana and romaine lettuce were used for tests of *A. ameiva*.

For *C. murinus*, chemical stimuli were presented on ceramic tiles (15 × 15 cm) because most lizards would not respond to hand-held cotton swabs, but fled instead. Water stimuli were prepared by immersing a cotton swab in deionized water and rubbing the swab on the entire upper surface of a tile. Cologne stimuli were prepared similarly using a 3:1 solution (deionized water to cologne) of Mennen Skin Bracer, spice scent. The cologne was diluted to eliminate the aversive responses (Cooper 1998*a*, 1998*b*) sometimes observed with undiluted cologne (Dial and Schwenk 1996; Cooper 1998*a*, 1998*b*). Prey stimuli were prepared by rubbing a cricket firmly on the tile surface to ensure transfer of surface chemicals to the swab. Plant-food stimuli were prepared by rubbing the freshly cut surface of a pear on the tile. Pears were used as plant stimuli because the lizards readily ate them in laboratory and field. For *A. ameiva*, which responded to hand-held cotton swabs, stimuli were prepared as above except that swabs were not rubbed on tiles but were rolled on the surfaces of crickets and plants (including the flesh of a banana and leaves of romaine lettuce). Pears, bananas, and romaine lettuce are rare or absent from the natural diets of the lizards but are appropriate stimulus sources because herbivorous and omnivorous lizards eat a wide variety of plants species and plant parts, including leaves and fruits (Cooper and Vitt 2002). In Scleroglossa, plant-eating species respond to chemical cues from such plants, but insectivorous–carnivorous species do not (Cooper 2002).

We used randomized-blocks designs in which each individual was tested once in all conditions. These designs have the advantage of minimizing the sample size needed by controlling for variability of responsiveness among individuals. To preclude bias due to the order of testing, the sequence of stimuli tested was varied among individuals in an incompletely counterbalanced design. For the experiment with *C. murinus*, there were 24 possible sequences. One sequence beginning with each of the four stimulus types was randomly excluded from the experiment. For the experiments with *A. ameiva*, there were 12 possible sequences. The planned designs included one complete replication and a partial second replication with four sequences randomly omitted. The lizards were not fed for 3 days prior to the experiments or during the experiments to ensure motivation to feed.

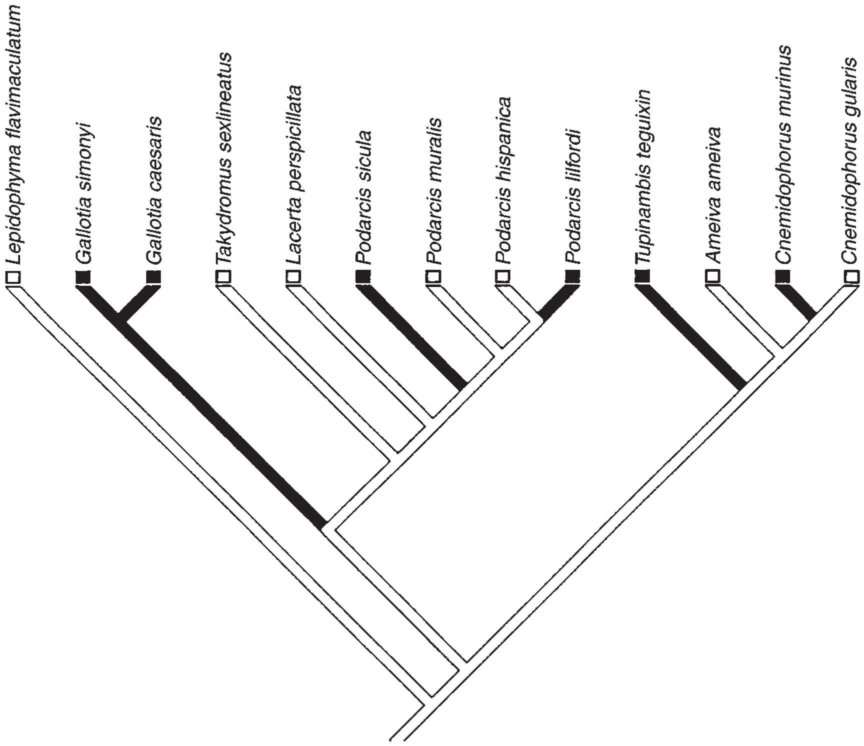
Trials with *C. murinus* were conducted from 09:30 to 17:00 CST. Each lizard was tested once per day on 15–18 March 2001. To begin a trial, an experimenter transported the lizard from its home cage to a transparent plastic test chamber having a 15 × 15 × 1 cm ceramic tile as its floor in an adjacent room. He then withdrew to observe the lizard through a one-way mirror. Beginning with the first tongue-flick directed to the tile, the number of tongue-flicks in 120 s was recorded, as were any responses possibly related to feeding. If a lizard failed to tongue-flick within 10 min, it was discarded from the experiment.

Two experiments with *A. ameiva* were conducted. In one of these, the stimuli were cricket surface, romaine lettuce leaf, and deionized water; responses to banana, pear, and cologne were tested in the other experiment. To begin a trial, the experimenter, moving slowly and being careful to minimize disturbance, approached a lizard's home cage and removed its lid. He next moved a swab to a position 1–1.5 cm anterior to the lizard's snout. Beginning with the first tongue-flick, the number of tongue-flicks directed to the swab in 60 s was recorded if the lizard did not bite the swab. If the lizard bit the swab within 60 s of the first tongue-flick, the number of tongue-flicks prior to the bite and the latency to bite were recorded.

Statistical analyses of experimental data

The data analyzed for *C. murinus* were the numbers of tongue-flicks, which provide a convenient bioassay of the intensity of chemosensory investigation (Cooper and Burghardt 1990). Variances of the raw data ($F_{\max} = 36.11$, df = 4,54, $P < 0.01$) were significantly heterogeneous using approximate Hartley's $F_{\max}$ tests (Winer 1962). Because violation of the assumption of homogeneity of variance could not be removed using logarithmically transformed data ($F_{\max} = 3.37$, df = 4,54, $P < 0.01$), parametric analysis of variance could not be used. The data were analyzed using nonparametric

Fig. 1. One of four alternative phylogenies of Lacertoidea used for tests of correlated evolution between plant consumption and plant-chemical discrimination. Solid branches indicate omnivory and plant-chemical discrimination. Open branches indicate insectivory and absence of plant-chemical discrimination.



Friedman's two-way analysis of variance (Zar 1996). Following detection of a significant main effect, differences among pairs of stimulus conditions were tested for significance using nonparametric procedures described by Zar (1996).

For *A. ameiva*, the variables examined were number of tongue-flicks, number of individuals that bit swabs, and the tongue-flick attack score for repeated-measures experiments (TFAS(R); Cooper and Burghardt 1990). TFAS(R) is the best overall indicator of response strength when some lizards bite and tongue-flick. It combines the effects of tongue-flicking and biting, giving heavier weight to a bite than to any number of tongue-flicks because a bite reflects a predatory attack. If a lizard does not bite in a trial, its TFAS(R) is the number of tongue-flicks in that trial. If it bites, TFAS(R) is the sum of two terms, one being 60 minus the latency to bite in seconds, the other being the maximum number of tongue-flicks performed by that lizard in any one of its trials.

Data on tongue-flicks and TFAS(R) for *A. ameiva* were analyzed by analysis of variance for a single-factor experiment having a repeated-measures (randomized blocks) design (Winer 1962). Hartley's $F_{\max}$ tests were used to verify homogeneity of variance of raw or, if necessary, logarithmically transformed data. When main effects were found to be significant, differences between pairs of stimulus means were tested for significance using Newman–Keuls tests (Winer 1962). Differences in the number of individuals that bit were analyzed by a sign test for cricket versus the control.

Because prey- or plant-chemical discrimination might exist but fail to be detected because of inadequate statistical power, we calculated the power when results were not significant (Zar 1996) using a ϕ coefficient of 3.92, based on an experiment with *Corucia zebrata* (Cooper 2000*g*). Significance tests were two-tailed, except where indicated elsewhere and justified by directional prediction, with $\alpha = 0.05$. Data are presented as the mean ± 1.0 standard error (SE).

Correlated evolution

Lacertoidea consists of Lacertiformes plus Xantusiidae (Estes et al. 1988). We included the insectivorous xantusiid *Lepidophyma maculatum* as an outgroup of Lacertiformes to permit assessment of correlated evolution in the broader taxon, Lacertoidea. The phylogenetic relationships of the lacertiform lizards in this study were taken from Presch (1974) for Lacertiformes, Presch (1974) and Wright (1993) for Teiidae, and Fu (1998), Harris et al. (1998), and Van Damme (1999) for Lacertidae. Because of disagreements among authors and uncertainty regarding some relationships, we reconstructed the evolution of plant diet and plant-chemical discrimination using the TRACE routine of MacClade (Maddison and Maddison 1992), using four phylogenies that differ in the placement of the genus *Takydromus* and *Podarcis muralis*. One of these phylogenies is shown in Fig. 1. Data for analysis were obtained from the present study and previous experimental works on lacertids and teiids cited above and on *Lepidophyma flavimaculatum* (Cooper 2000*b*).

We examined the hypothesis that correlated evolution has occurred between plant consumption and plant-chemical discrimination for all four phylogenies, using concentrated-changes tests (Maddison 1990). The concentrated-changes test takes into account the phylogenetic relationships among species and thereby avoids the overestimation of correlations due to shared phylogeny that plagues traditional correlations which treat each species as an independent data point. Maddison's (1990) test, which is appropriate for detecting correlated evolution between two binary variables, assumes that an independent variable causes changes in the dependent variable (Maddison 1990). In this case the hypothesis is that evolutionary acquisition of plant consumption (the independent variable) causes plant-chemical discrimination (the dependent variable) to evolve.

The concentrated-changes test was criticized by Sillén-Tullberg (1993) as being subject to bias if taxa not representative of the group are selected, but was subsequently found to perform well in simulation studies by Lorch and Eadie (1999), who found it to err by being conservative when only gains, but not losses, occur for a trait, which is the case for our data. The concentrated-changes test has the important advantage of requiring fewer changes in traits than other tests to attain sufficient statistical power to detect correlated evolution (Ridley 1983; Sillén-Tullberg 1993). Calculations were performed using MacClade 3.01 (Maddison and Maddison 1992).

Results

Cnemidophorus murinus

The sample size was reduced to 19 because one lizard stopped tongue-flicking after the second trial. The number of tongue-flicks varied significantly among conditions ($\chi^2 = 33.29$, df = 3, $P < 1 \times 10^{-5}$), with many more tongue-flicks in the animal- and plant-food conditions than in either of the control conditions (Fig. 2). Ranges of numbers of tongue-flicks were 5–105 for crickets, 6–62 for pear, 1–23 for cologne, and 4–21 for deionized water. The number of tongue-flicks in response to cricket stimuli was significantly greater than in response to cologne ($P < 0.001$) and deionized water ($P < 0.01$). The number of tongue-flicks elicited by pear stimuli was significantly greater than that elicited by cologne ($P < 0.001$) and deionized water ($P < 0.025$). The differences between the two food stimuli and the two control stimuli were not significant ($P > 0.10$).

The only other behaviors that might have been food-related were an attempt to bite a tile bearing cricket stimuli by the lizard that performed the most tongue-flicks and an attempt to dig at a tile bearing cricket stimuli by another individual. *Cnemidophorus* spp. frequently locate food by digging, presumably in areas where their chemical cues are detected (W.E. Cooper, Jr., unpublished field observations). Lizards spent substantial portions of trials climbing the walls of the test chamber, apparently attempting to escape.

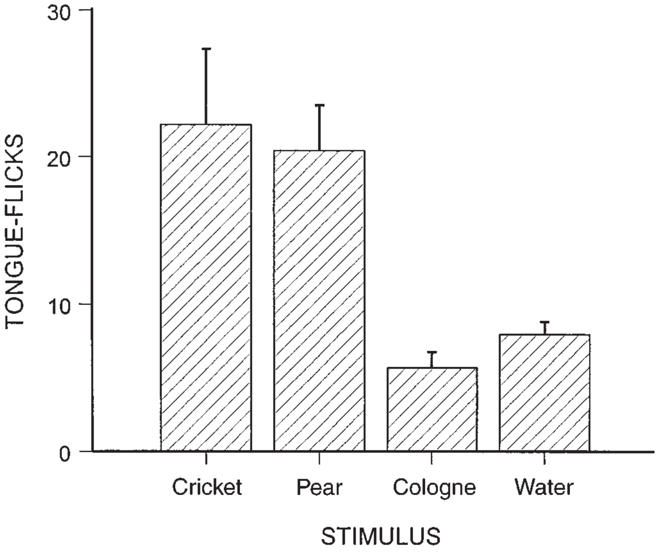
Ameiva ameiva

Fifteen of the 20 individuals completed both experiments; the others failed to respond at all or responded in only one trial.

Cricket–lettuce–water

Variances of tongue-flicks were significantly heterogeneous for the raw data ($F_{\max} = 3.39$, df = 3,28, $P < 0.01$) but were rendered homogeneous by logarithmic transformation ($F_{\max} = 2.38$, df = 3,28, $P > 0.05$). Numbers of tongue-flicks for the transformed data differed significantly among conditions ($F = 10.58$, df = 2,28, $P < 0.00038$; Table 1). Cricket stimuli elicited significantly more tongue-flicks than romaine lettuce ($P < 0.0014$) or deionized water ($P < 0.00057$). Numbers of tongue-flicks did not differ significantly between romaine lettuce and deionized water ($P > 0.10$).

Fig. 2. Mean numbers of tongue-flicks in 60 s by *Cnemidophorus murinus* responding to chemical stimuli from cricket, pear, cologne, and deionized water. Error bars represent 1.0 SE.



Few individuals bit swabs (five in the cricket condition and none in the other conditions). For lizards that bit in response to cricket stimuli, latency to bite was 12.17 ± 2.97 s, with the range 5–26 s. Significantly more individuals bit in response to crickets than in response to deionized water ($P < 0.032$, one-tailed) or romaine lettuce and deionized water combined (sign test with $P = 0.33$ for cricket and 0.67 for the other stimuli combined, $P < 0.0079$).

Variances of TFAS(R) differed significantly for the raw data ($F_{\max} = 16.87$, df = 3,28, $P < 0.01$) but were homogeneous for the logarithmically transformed data ($F_{\max} = 1.29$, df = 3,28, $P > 0.10$). TFAS(R) for the transformed data varied significantly among stimulus conditions ($F = 27.55$, df = 2,28, $P < 1.0 \times 10^{-6}$; Table 1). TFAS(R) was significantly greater in response to cricket stimuli than to romaine lettuce ($P < 0.00013$) or deionized water ($P < 0.00015$), but did not differ between romaine lettuce and deionized water ($P > 0.10$). The statistical power was >0.99.

Banana–pear–cologne

Variances of tongue-flicks were homogeneous ($F_{\max} = 1.83$, df = 3,28, $P > 0.05$). Numbers of tongue-flicks did not differ significantly among conditions ($F = 0.65$, df = 2,28, $P > 0.10$; Table 1). Only one individual bit weakly in the pear condition and none in the other conditions. Variances of TFAS(R) differed significantly for the raw data ($F_{\max} = 4.01$, df = 3,28, $P < 0.01$), but were homogeneous for the logarithmically transformed data ($F_{\max} = 1.28$, df = 3,28, $P > 0.10$). TFAS(R) for the transformed data did not differ significantly among stimulus conditions ($F = 0.88$, df = 2,28, $P > 0.10$; Table 1). Power was >0.99.

Table 1. Numbers of tongue-flicks and tongue-flick attack scores (TFAS(R)) for *Ameiva ameiva* in 60-s swab trials using various stimuli.

	Number of tongue-flicks			TFAS(R)		
	Mean	SE	Range	Mean	SE	Range
Cricket–lettuce–water						
Cricket	15.2	2.9	4–47	32.3	6.3	4–71
Romaine lettuce	6.6	1.5	1–20	6.6	1.5	1–20
Deionized water	7.1	1.6	1–17	7.1	1.6	1–17
Banana–pear–cologne						
Banana	7.3	2.7	1–44	7.3	2.7	1–44
Pear	7.1	2.0	1–33	10.5	5.3	1–84
Cologne	9.8	2.6	1–36	9.8	2.6	1–36

Correlated evolution

For all four phylogenies there were five independent origins of omnivory and plant-chemical discrimination. In all phylogenies, wherever one of the two variables changed, the other variable on the same branch changed, indicating that little or no delay occurred between the acquisition of plant diet and plant-chemical discrimination. No evolutionary losses of omnivory or plant-chemical discrimination occurred. Thus, omnivory and plant-chemical discrimination were perfectly correlated in Lacertiformes.

The correlations were significant for all phylogenies. For the phylogeny shown in Fig. 1, the probability of no relationship between plant diet and plant-chemical discrimination was <0.0024 for Lacertiformes and <0.0015 for Lacertoidea. For a phylogeny differing from that shown in Fig. 1 only in that *P. muralis* was the sister-species of the *P. lilfordi* – *P. hispanica* clade, the associations were significant at $P < 0.0027$ for Lacertiformes and $P < 0.0016$ for Lacertoidea. For the third phylogeny, which differed from that shown in Fig. 1 only in that the genus *Takydromus* was the sister-group of all lacertids other than the genus *Gallotia*, the correlations were significant at $P < 0.0027$ for Lacertiformes and $P < 0.0017$ for Lacertoidea. The final phylogeny differed from that shown in Fig. 1 in the positions of both *Takydromus* and *P. muralis*. For this phylogeny, plant diet and plant-chemical discrimination were significantly associated at $P < 0.0030$ for Lacertiformes and $P < 0.0018$ for Lacertoidea.

Discussion

Food-chemical discrimination

Cnemidophorus murinus discriminated both prey chemicals and plant chemicals from the control stimuli, as indicated by the significantly greater numbers of tongue-flicks in response to cricket and pear stimuli than to either of the controls. In contrast, *A. ameiva* exhibited prey-chemical discrimination but not plant chemical discrimination. Three results show that *A. ameiva* discriminated prey chemicals from other substances: the significantly greater number of tongue-flicks and greater TFAS(R) in response to cricket chemicals than to romaine lettuce or deionized water, and the significantly greater number of individuals that bit swabs bearing cricket stimuli than the other stimuli. The absence of any significant differences in responses of *A. ameiva* to plant and control stimuli combined with the high statistical power of the tests suggests that these animals lack plant-chemical discrimination. *Ameiva ameiva* showed no strong responses to chemical cues from three plant species, including two known to elicit elevated rates of tongue-flicking and (or) biting by omnivorous lizards (e.g., romaine lettuce – *C. zebrata* (Cooper 2000*b*) and pear – *C. murinus*).

The results were as predicted: both prey- and plant-chemical discrimination for an omnivore, and prey-chemical, but not plant-chemical, discrimination for an actively foraging insectivore. Prey-chemical discrimination has been demonstrated in all teiids and actively foraging lizard species tested. Because numerous species representing all major families of active foragers except Gymnophthalmidae have been studied (e.g., Cooper 1994*a*, 1995, 1997, 2000*a*, 2000*d*, 2000*e*, 2000*f*), it seems likely that prey-chemical discrimination is universal, or nearly so, in actively foraging lizards.

Both prey- and plant-chemical discrimination have been demonstrated in all omnivorous and herbivorous species of scleroglossans studied (Cooper 2002). It is not surprising that omnivorous species which evolved from actively foraging ancestors capable of prey-chemical discrimination retained this ability when plant-chemical discrimination evolved. This appears to be the case for *C. murinus*, which continues to consume prey frequently (Dearing and Schall 1992). However, even herbivorous species such as the skink *C. zebrata* (Cooper 2000*b*) and some iguanids (Cooper and Alberts 1990; Cooper and Flowers 2001; Cooper and Lemos-Espinal 2001) are capable of prey-chemical discrimination. It is quite unlikely that the herbivorous skink might have retained prey-chemical discrimination as an ancestral trait that is no longer useful. Neither is it necessary to conclude that herbivores' responses to prey chemicals are solely due to chemical attributes shared with plants. Such phylogenetic inertia and correlated sensitivity need not be invoked because *C. zebrata* continues to eat some animal prey (McCoy 1980), as do the herbivorous iguanids (Minnich and Shoemaker 1970; Nagy 1973; Nagy and Shoemaker 1975; Pianka 1986; Durtsche 1999, 2000; Cooper and Vitt 2002).

Correlated evolution

Sensory systems evolve to detect biologically important, predictable stimuli, including those associated with food (Dusenbury 1992). Thus, convergent evolution of responsiveness to widely occurring food stimuli is predicted, particularly to chemical stimuli common to many palatable plants (Cooper 2002). Correlated evolution has occurred between plant diet and plant-chemical discrimination within Lacertoidea and Lacertiformes. For the dataset examined, this finding is robust under various phylogenetic hypotheses. Because there are several untested omnivorous lacertids and teiids (Van Damme 1999; Cooper and Vitt 2002), ample opportunities exist for further testing of the evolutionary correlation, but the perfect correspondence between shifts to plant diet and responsiveness to plant chemicals in Lacertoidea and in Scleroglossa as a whole suggests that the present finding of correlated evolution is correct.

In most of the lacertiform and other omnivorous and herbivorous species tested, plant consumption is substantial throughout the activity season or is important during some prolonged portion of the year. This is the case for *C. murinus*, for which plants constitute most of the diet throughout the year (Dearing and Schall 1992). Thus, plant-chemical discrimination is likely to be important for identifying diverse plant foods and their ripeness and for detecting toxins, as in *C. murinus* (Schall 1990). Because the ancestors of omnivorous lacertids and teiids were active foragers that presumably were capable of prey-chemical discrimination, all that was needed for plant-chemical discrimination to evolve was the development of responsiveness to chemical cues associated with plants. Because plant and animal foods contain similar major categories of organic compounds, although in different concentrations, the transition to prey-chemical discrimination might have been relatively rapid, consistent with the joint transition to plant diet and to plant-chemical discrimination in the same species.

Among omnivorous and herbivorous species tested, both prey- and plant-chemical discrimination occur in all species of scleroglossans (Cooper 2002) and all but one species of iguanians (e.g., Cooper and Alberts 1990; Cooper 2000*c*; Cooper and Flowers 2001; Cooper et al. 2001). The exceptional species, *Sceloporus poinsettii*, failed to discriminate either prey or plant chemicals from control stimuli (Cooper et al. 2001). This species appears to be an insectivorous ambush forager at some places and times, but consumes flowers in substantial quantities during their brief periods of availability in its arid habitats (Barbault et al. 1985).

Possible reasons for the absence of plant-chemical discrimination in *S. poinsettii* include phylogenetic constraints on its acquisition in the family Phrynosomatidae, failure of plants, owing to their infrequent consumption, to pass a threshold of minimum selective importance required to bring about its evolution, and a lack of adequacy of visual cues for evaluating the plants consumed. No evidence exists for sensory constraints within Phrynosomatidae. Indeed, *Sceloporus occidentalis* utilizes conspecific chemical cues to distinguish sex (Duvall 1979). On the other hand, during the short time when blooming flowers are present, they are readily detectable visually because their position on plants, shape, and coloration contrast with those of other plant parts, and their nutritional quality is unlikely to vary enough to require assessment by means of chemical cues. The tight correspondence between plant-chemical discrimination and omnivory suggests that chemical cues are reliably helpful for locating and (or) assessing the quality of plant foods by a large majority of omnivorous lizard species.

Acknowledgments

This work was partially supported by a grant to W.E.C. from the Cleveland Metroparks Zoo for travel to Bonaire Island to collect *C. murinus* and by a minigrant for research from Indiana University – Purdue University at Fort Wayne. The Sam Noble Oklahoma Museum of Natural History provided funds for research in Bonaire to J.P.C. and L.J.V.

References

Arnold, E.N. 1990. Why do morphological phylogenies vary in quality? An investigation based on the comparative history of lizard clades. Proc. Roy. Soc. Lond. B Biol. Sci. **240**: 135–172.

Arnold, E.N. 1993. Phylogeny and the Lacertidae. *In* Lacertids of the Mediterranean region. *Edited by* E.D. Valakos, W. Bohme, V. Perez-Mellado, and P. Maragou. Hellenic Zoological Society, Athens. pp. 1–16.

Barbault, R., Ortega, A., and Maury, M.E. 1985. Food partitioning and community organization in a mountain lizard guild of northern Mexico. Oecologia, **65**: 550–554.

Brooks, D.R., and McLennan, D.A. 1991. Phylogeny, ecology, and behavior: a research program in comparative biology. University of Chicago Press, Chicago.

Cooper, W.E., Jr. 1990. Prey odor detection by teiid and lacertid lizards and the relationship of prey odor detection to foraging mode in lizard families. Copeia, 1990: 237–242.

Cooper, W.E., Jr. 1991. Responses to prey chemicals by a lacertid lizard, *Podarcis muralis*: prey chemical discrimination and poststrike elevation in tongue-flick rate. J. Chem. Ecol. **17**: 849–863.

Cooper, W.E., Jr. 1994*a*. Chemical discrimination by tongue-flicking in lizards: a review with hypotheses on its origin and its ecological and phylogenetic relationships. J. Chem. Ecol. **20**: 439–487.

Cooper, W.E., Jr. 1994*b*. Prey chemical discrimination, foraging mode, and phylogeny. *In* Lizard ecology: historical and experimental perspectives. *Edited by* L.J. Vitt and E.R. Pianka. Princeton University Press, Princeton, N.J. pp. 95–116.

Cooper, W.E., Jr. 1995. Foraging mode, prey chemical discrimination, and phylogeny in lizards. Anim. Behav. **50**: 973–985.

Cooper, W.E., Jr. 1997. Correlated evolution of prey chemical discrimination with foraging, lingual morphology, and vomeronasal chemoreceptor abundance in lizards. Behav. Ecol. Sociobiol. **41**: 257–265.

Cooper, W.E., Jr. 1998*a*. Evaluation of swab and related tests as a bioassay for assessing responses by squamate reptiles to chemical stimuli. J. Chem. Ecol. **24**: 841–866.

Cooper, W.E., Jr. 1998*b*. Prey chemical discrimination indicated by tongue-flicking in the eublepharid gecko *Coleonyx variegatus*. J. Exp. Zool. **281**: 21–25.

Cooper, W.E., Jr. 1999. Supplementation of phylogenetically correct data by two species comparison: support for correlated evolution of foraging mode and prey chemical discrimination in lizards extended by first intrageneric evidence. Oikos, **86**: 97–104.

Cooper, W.E., Jr. 2000*a*. An adaptive difference in the relationship between foraging mode and responses to prey chemicals in two congeneric scincid lizards. Ethology, **106**: 193–206.

Cooper, W.E., Jr. 2000*b*. Chemical discrimination of potential food items by a xantusiid lizard, *Lepidophyma flavimaculatum*. J. Herpetol. **34**: 323–325.

Cooper, W.E., Jr. 2000*c*. Chemosensory discrimination of plant and animal foods by the omnivorous iguanian lizard *Pogona vitticeps*. Can. J. Zool. **78**: 1–5.

Cooper, W.E., Jr. 2000*d*. Correspondence between diet and food chemical discriminations by omnivorous geckos (*Rhacodactylus*). J. Chem. Ecol. **26**: 755–763.

Cooper, W.E., Jr. 2000*e*. Food chemical discriminations by the omnivorous scincid lizards *Tiliqua scincoides* and *Tiliqua rugosa*. Herpetologica, **56**: 208–216.

Cooper, W.E., Jr. 2000*f*. Responses to chemical cues from plant and animal food by an omnivorous lizard, *Gerrhosaurus validus*. J. Herpetol. **34**: 616–619.

Cooper, W.E., Jr. 2000*g*. Food chemical discriminations by an herbivorous lizard, *Corucia zebrata*. J. Exp. Zool. **286**: 372–378.

Cooper, W.E., Jr. 2002. Convergent evolution of plant chemical discrimination by omnivorous and herbivorous scleroglossan lizards. J. Zool. (Lond.). In press.

Cooper, W.E., Jr., and Alberts, A.C. 1990. Responses to chemical food stimuli by an herbivorous actively foraging lizard, *Dipsosaurus dorsalis*. Herpetologica, **46**: 259–266.

Cooper, W.E., Jr., and Burghardt, G.M. 1990. A comparative analysis of scoring methods for chemical discrimination of prey by squamate reptiles. J. Chem. Ecol. **16**: 45–65.

Cooper, W.E., Jr., and Flowers, M. 2001. Plant chemical discriminations by an herbivorous iguanid lizard, *Sauromalus ater*. Amphib.-Reptilia, **22**: 69–80.

Cooper, W.E., Jr., and Lemos-Espinal, J.A. 2001. Coordinated ontogeny of food preferences and responses to chemical food stimuli by a lizard, *Ctenosaura pectinata* (Reptilia: Iguanidae). Ethology, **107**: 639–653.

Cooper, W.E., Jr., and Pérez-Mellado, V. 2001*a*. Food chemical cues elicit general and population-specific effects on lingual and biting behaviors in the lacertid lizard *Podarcis lilfordi*. J. Exp. Zool. **290**: 207–217.

Cooper, W.E., Jr., and Pérez-Mellado, V. 2001*b*. Omnivorous lacertid lizards (*Gallotia*) from El Hierro, Canary Islands, can identify prey and plant food using only chemical cues. Can. J. Zool. **79**: 881–887.

Cooper, W.E., Jr., and Pérez-Mellado, V. 2002. Responses to food chemicals by two insectivorous and an omnivorous species of lacertid lizards. Neth. J. Zool. In press.

Cooper, W.E., Jr., and Vitt, L.J. 2002. Distribution, extent, and evolution of plant consumption by lizards. J. Zool. (Lond). In press.

Cooper, W.E., Jr., Paulissen, M.A., and Habegger, J.J. 2000. Discrimination of prey, but not plant, chemicals by actively foraging, insectivorous lizards, the lacertid *Takydromus sexlineatus* and the teiid *Cnemidophorus gularis*. J. Chem. Ecol. **26**: 1623–1634.

Cooper, W.E., Jr., Habegger, J.J., and Espinoza, R.E. 2001. Responses to prey and plant chemicals by three iguanian lizards: relationship to plants in the diet. Amphib.-Reptilia, **22**: 349–361.

Cooper, W.E., Jr., Pérez-Mellado, V., and Sillero, N. 2002. Responses to food chemicals by the insectivorous lacertid lizard *Pordarcis muralis*. Amphib.-Reptilia. In press.

Dearing, M.D., and Schall, J.J. 1992. Testing models of optimal diet assembly by the generalist herbivorous lizard *Cnemidophorus murinus*. Ecology, **73**: 845–858.

Dial, B.E., and Schwenk, K. 1996. Olfaction and predator detection in *Coleonyx brevis* (Squamata: Eublepharidae), with comments on the functional significance of buccal pulsing in geckos. J. Exp. Zool. **276**: 415–424.

Durtsche, R.D. 1999. The ontogeny of diet in the Mexican spiny-tailed iguana, *Ctenosaura pectinata*: physiological mechanisms and ecological consequences. Doctoral dissertation, University of Oklahoma, Norman.

Durtsche, R.D. 2000. Ontogenetic plasticity of food habits in the Mexican spiny-tailed iguana, *Ctenosaura pectinata*. Oecologia, **124**: 185–195.

Dusenbury, D.B. 1992. Sensory ecology. W.H. Freeman, New York.

Duvall, D. 1979. Western fence lizard (*Sceloporus occidentalis*) chemical signals. I. Conspecific discriminations and release of a species-typical visual display. J. Exp. Zool. **210**: 321–326.

Estes, R., De Queiroz, K., and Gauthier, J. 1988. Phylogenetic relationships within Squamata. *In* Phylogenetic relationships of the lizard families. *Edited by* R. Estes and G. Pregill. Stanford University Press, Stanford, Calif. pp. 119–281.

Fu, J. 1998. Toward the phylogeny of the family Lacertidae: implications from mitochondrial DNA 12S and 16S gene sequences (Reptilia: Squamata). Mol. Phylogenet. Evol. **9**: 118–130.

Goose, V., and Bels, V.L. 1990. Analyse comportementale et fonctionnelle des touchers linguaux lors de l'exploration et de la prise de nourriture chez le lezard vert (*Lacerta viridis*, Laurenti 1768). Bull. Soc. Herpetol. Fr. **53**: 31–39.

Harris, D.J., Arnold, E.N., and Thomas, R.H. 1998. Relationships of lacertid lizards (Reptilia: Lacertidae) estimated from mitochondrial DNA sequences and morphology. Proc. R. Soc. Lond. B Biol. Sci. **265**: 1939–1948.

Harvey, P.H., and Pagel, M.D. 1991. The comparative method in evolutionary biology. Oxford University Press, Oxford.

Huey, R.B., and Pianka, E.R. 1981. Ecological consequences of foraging mode. Ecology, **62**: 991–999.

Iverson, J.B. 1982. Adaptations to herbivory in iguanine lizards. *In* Iguanas of the world. *Edited by* G.M. Burghardt and A.S. Rand. Noyes Publications, Park Ridge, N.J. pp. 60–76.

Lorch, P.D., and Eadie, J.M. 1999. Power of the concentrated changes test for correlated evolution. Syst. Biol. **48**: 170–191.

Maddison, W.P. 1990. A method for testing the correlated evolution of two binary characters: Are gains or losses concentrated on certain branches of a phylogenetic tree? Evolution, **44**: 539–557.

Maddison, W.P., and Maddison, D.R. 1992. MacClade: analysis of phylogeny and character evolution. Version 3.0. Sinauer Associates, Sunderland, Mass.

Marcos-Leon, M.B. 1999. Ecolofisiologia y estrategias de obtencion de alimento en cuatro especies de Ladertidae. Doctoral thesis, University of Salamanca, Salamanca, Spain.

McCoy, M. 1980. Reptiles of the Solomon Islands. Wau Ecology Institute, Wau, Papua New Guinea.

Minnich, J.E., and Shoemaker, V.H. 1970. Diet, behavior and water turnover in the desert iguana, *Dipsosaurus dorsalis*. Am. Midl. Nat. **84**: 469–509.

Nagy, K.A. 1973. Behavior, diet and reproduction in a desert lizard, *Sauromalus obesus*. Copeia, 1973: 93–102.

Nagy, K.A., and Shoemaker, V.H. 1975. Energy and nitrogen budgets of the free-living desert lizard *Sauromalus obesus*. Physiol. Zool. **48**: 252–262.

Nelling, C. 1996. Responses to prey odors by three species of lacertid lizards: prey odor discrimination, aged odor detection, strike-induced chemosensory searching, and chemosensory search images. M.S. thesis, Shippensburg University, Shippensburg, Pa.

Perry, G. 1995. The evolutionary ecology of lizard foraging: a comparative study. Doctoral dissertation, University of Texas, Austin.

Perry, G., Lampl, I., Lerner, A., Rothenstein, D., Shani, E., Sivan, N., and Werner, Y.L. 1990. Foraging mode in lacertid lizards: variation and correlates. Amphib.-Reptilia, **11**: 373–384.

Pianka, E.R. 1986. Ecology and natural history of desert lizards. Princeton University Press, Princeton, N.J.

Presch, W. 1974. Evolutionary relationships and biogeography of the macroteiid lizards (family Teiidae, subfamily Teiinae). Bull. South. Calif. Acad. Sci. **73**: 23–32.

Ridley, M. 1983. The explanation of organic diversity: the comparative method and adaptations for mating. Oxford University Press, Oxford.

Schall, J.J. 1990. Aversion of whiptail lizards (*Cnemidophorus*) to a model alkaloid. Herpetologica, **46**: 34–39.

Sillén-Tullberg, B. 1993. The effect of biased inclusion of taxa on the correlation between discrete characters in phylogenetic trees. Evolution, **47**: 1182–1191.

Van Damme, R. 1999. Evolution of herbivory in lacertid lizards: effects of insularity and body size. J. Herpetol. **33**: 663–674.

Winer, B.J. 1962. Statistical principles in experimental design. McGraw–Hill, New York.

Wright, J.W. 1993. Evolution of the lizards of the genus *Cnemidophorus*. *In* Biology of whiptail lizards (genus *Cnemidophorus*). *Edited by* J.W. Wright and L.J. Vitt. Oklahoma Museum of Natural History, Norman. pp. 27–81.

Yanosky, A.A., Iriart, D.E., and Mercolli, C. 1993. Predatory behavior in *Tupinambis teguixin* (Sauria: Teiidae). I. Tongue-flicking responses to chemical food stimuli. J. Chem. Ecol. **19**: 291–299.

Zar, J.H. 1996. Biostatistical analysis. Prentice Hall Inc., Upper Saddle River, N.J.