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Food Chemical Cues Elicit General and Population-Specific Effects on Lingual and Biting Behaviors in the Lacertid Lizard *Podarcis lilfordi*

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ABSTRACT Actively foraging lizards are capable of identifying prey using only chemical cues sampled by tongue-flicking, and the relatively few omnivorous and herbivorous lizards tested similarly can identify both animal and plant foods from chemical cues. Whether lizards that eat plants respond to cues specific to preferred plant types and whether there is geographic variability in responses to cues from various plants correlated with the importance of those plants in local diets is unknown. In three populations of an omnivorous lacertid, the Balearic lizard *Podarcis lilfordi*, we studied chemosensory sampling and feeding responses to chemical cues from plant and animal foods presented on cotton swabs. Each lizard population is endemic to one islet off the coast of Menorca, Balearic Islands, Spain. Lizards in all three populations discriminated chemical cues from plant and animal foods from control substances. Our results extend findings of prey chemical discrimination and plant chemical discrimination in omnivores, increasing confidence that correlated evolution has occurred between plant diet and chemosensory response to palatable plants. There were no consistent differences among populations in tongue-flicking and biting responses to stimuli from flowers of syntopic and allopatric plant species. The lizards may respond to cues indicative of palatability in a wide range of plant species rather than exhibiting strong responses only to locally available plant species. Nevertheless, tongue-flicking and biting frequencies varied among plant species, perhaps indicating food preferences. In addition, there were differences among populations in tongue-flick rates, latency to bite, and licking behavior. Licking was observed in only one lizard population as a response to floral chemicals from only one of the plants species tested, raising the possibility of a population-specific linkage between identification of a particular plant species and performance of an appropriate feeding response. *J. Exp. Zool. 290:207–217, 2001.* © 2001 Wiley-Liss, Inc.

Many lizards use chemical cues sampled by tongue-flicking to locate and identify food (Cooper, '94a,b), but much less is known regarding the relationship between diet and chemosensory response strength or about responses to chemical cues from palatable plants by omnivorous and herbivorous species. Neither is there any information about variation in chemosensory responses among populations within species, especially in relation to diet.

Snakes respond much more strongly to chemical cues from preferred prey types than from prey eaten rarely or not at all. This has been demonstrated within species of garter snakes of the natricine genus *Thamnophis* at single sites (Burghardt, '67, '70b). Even more convincing are studies that have shown that strength of chemosensory response varies geographically within species with changes in diet. Such geographic variation has been found in garter snakes (Burghardt, '69, '70a; Arnold, '92; Burghardt and Schwartz, '99) and in the colubrines *Masticophis flagellum* (Cooper et al., '90) and *Coluber constrictor* (Cooper et al., 2000).

Because most lizards are generalist predators of small animals, it has been assumed, but not demonstrated, that differential responses to chemical cues from diverse prey are unlikely.

Omnivory and herbivory, present in a small per-

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centage (ca. 3%) of lizard species (Pough, '73; Iverson, '82; King, '96), present an alternative opportunity to examine correspondence between diet and chemosensory response in lizards. Omnivorous species that use chemical cues to detect food may be predicted to respond strongly to chemical cues from palatable plants as well as animal cues. Because they occasionally consume animal prey, the same applies to herbivores.

Study of several lizard species that consume plants has confirmed these predictions (Cooper and Alberts, '90; Cooper 2000a,b, in press a,b; Cooper and Flowers, in press; Cooper et al., in press a). In contrast, species that do not eat plants do not exhibit elevated tongue-flick rates or increased probability of biting in response to plant chemical stimuli (e.g., Cooper and Hartdegen, '99, Cooper, 2000c; Cooper and Habegger, 2000). These interspecific findings are suggestive, but further information is needed to ascertain the generality of this correspondence between diet and chemosensory response. Correlated evolution between plant diet and chemosensory responsiveness must be demonstrated to establish the interspecific relationship.

Prey chemical discrimination and foraging mode have undergone correlated evolution (Cooper, '95, '97, '99). Species of actively foraging, insectivorous/carnivorous lizards respond strongly to prey chemicals, exhibiting increased tongue-flick rates and biting stimulus sources (Cooper, '95, '97, '99, 2000a). Such species do not respond strongly to chemical cues from plants palatable to herbivorous and omnivorous species (e.g., Cooper and Hartdegen, '99, Cooper, 2000c; Cooper and Habegger, 2000). Ambush-foraging insectivores do not use chemical cues to locate and identify prey chemicals (Cooper, '95, '97, '99), but the few species of omnivores and herbivores derived from ambushing insectivores that have been tested respond strongly to chemical cues from both plants and animals (e.g., Cooper and Alberts, '90, '91; Cooper, 2000b, in press a; Cooper and Flowers, in press). Available evidence is consistent with the hypothesis that correlated evolution has occurred between plant consumption and responsiveness to plant chemicals, but the evidence is based on data from relatively few plant-eating species and requires confirmation and extension to additional taxa.

The lacertid lizard *Podarcis lilfordi*, a medium-sized species endemic to the Balearic islands, provides an opportunity to assess the degree of correspondence between geographic variation in chemosensory behavior and diet. This species is omnivorous, consuming diverse prey and flowers and fruits of diverse plant species (Pérez-Mellado and Corti, '93; Pérez-Mellado and Traveset, in press). It also consumes nectar (Pérez-Mellado and Casas, '97). It occurs off the coast of Menorca and Mallorca on numerous islets having different plant communities; common food plants on some islands are absent on others (Pérez-Mellado and Traveset, 1999 for the Cabrera Archipelago; Pérez-Mellado, unpublished data for Islets off Menorca).

We predicted that *P. lilfordi* would exhibit both prey chemical discrimination and plant chemical discrimination because it is an omnivore derived from actively foraging ancestors (Cooper, '95, '97). We further predicted that if the lizards responded differentially among plant species, they would respond more strongly to syntopic plants than to plants not present their islets. For two islets, Sargantana and Colom, we selected plant species present on both islands and other plant species present on one, but not both of the islets, as stimulus sources. We additionally selected plant species to permit assessment of effects of syntopy on chemosensory responses in the Aire lizard population. All plants tested are included in the diet where present (Pérez-Mellado, unpublished data). A significant interaction between chemosensory response and plant type, with stronger responses to plant species present than to absent plant species, would indicate correspondence between diet and chemosensory response among populations. We also predicted that any qualitative differences among lizard populations from different islets in responses to chemical cues from a particular plant species would reflect inter-islet differences in the abundance of the plant species.

MATERIALS AND METHODS

Animals and maintenance

Adult lizards were collected in late May and early June, 2000 by noosing on the islets of Sargantana (n = 17) and Colom (n = 20), using pieces of pear as bait only on the former, and on Aire (n = 20) by trapping in a plastic cooler baited with pear. They were transported to the laboratory on Menorca, where they were housed individually in plastic terraria (40 × 26 × 26 cm or 46 × 26 × 26 cm) containing an indoor-outdoor carpet substrate and a water bowl. The sides of each cage were covered by white paper to reduce distraction by the surroundings and disturbance by movements of the investigators. The light cycle was natural for the region, provided by a window. The thermal cycle was that of the region with additional heat supplied by incandescent bulbs suspended above the terraria. The room temperature during experiments was 28–29°C. The lizards had the opportunity to thermoregulate by basking and were alert and active during trials and readily ate in the same conditions at other times.

Experimental procedures and analyses

Larval *Tenebrio molitor* (mealworm) was selected as a stimulus source representing animal prey. Flowers of *Capparis spinosa* (Capparidaceae), *Helichrysum stoechas* (Compositae), and *Galactites tomentosa* (Compositae) were chosen as plant stimuli to permit assessment of the responses of lizards from the Colom and Sargantana populations to chemical stimuli from syntopic and allopatric plants. *Capparis spinosa* was present on both islets at the time of the experiment, *H. stoechas* being absent on Sargantana, and *G. tomentosa* being absent on Colom. We also studied responses by lizards from Aire to chemical cues from flowers of *H. stoechas*, which has never been recorded on Aire, and *G. tomentosa*, which has been recorded previously on Aire, but was absent in 2000. Cologne (Mennen Skin Bracer, Spice Scent) served as a pungency control, i.e, a control for responses to an odorous, nonfood substance. The cologne was diluted to three parts water to one part cologne by volume to eliminate aversive properties of undiluted cologne (Dial and Schwenk, '96; Cooper, '98a,b). Deionized water was an odorless control. We prepared stimuli by dipping the cotton tip of a 15-cm wooden applicator into deionized water and then adding other stimuli if necessary. After excess water was removed by flicking the wrist, the moistened swab was then rolled firmly over the surface of a mealworm or a flower, or was dipped into diluted cologne.

To conduct a trial, an experimenter approached a lizard's cage and positioned the cotton swab 1–2 cm anterior to the lizard's snout, moving slowly to avoid eliciting escape behaviors. Starting with the first tongue-flick, an experimenter recorded the number of tongue-flicks directed to the swab in 60 sec, the occurrence of biting and its latency in seconds, and the occurrence of licking. Licking was distinct from tongue-flicking in that the anterior dorsal surface of the tongue was pressed against the swab, whereas only the tines of the tongue and usually their ventral surfaces contacted the swab during tongue-flicks.

Behaviors of each lizard from Colom and Sargantana were recorded in response to all six stimulus types. The sequence of stimuli was partially counterbalanced among individuals to avoid possible sequential bias, and at least 30 min elapsed between trials. Variables analyzed statistically were number of tongue-flicks, number of individuals that bit, latency to bite, number of individuals that licked, and the tongue-flick attack score. The tongue-flick attack score for repeated measures experiments, TFAS(R), is the number of tongue-flicks if the lizard does not bite in that trial. If the lizard bites the swab, TFAS(R) is the sum of the maximum number of tongue-flicks by that individual in any one of its six trials and (60 minus latency to bite in seconds). This measure combines tongue-flicks with predatory attack to give the best overall measure of response strength (Burghardt, '70b; Cooper and Burghardt, '90; Cooper, '98a).

For each species tongue-flicks, latency to bite, and TFAS(R) were analyzed using analysis of variance for a single-factor experiment having a repeated measures (randomized blocks) design (Winer, '62). Data were examined for heterogeneity of variance and extreme departure from normality. When variances were significantly heterogeneous (as approximated by Hartley's Fmax tests), significance tests were conducted using logarithmically transformed data [log (x + 1)]. When main stimulus effects were significant, comparisons between pairs of condition means were made using Newman-Keuls tests.

Differences among conditions in numbers of individuals that bit and numbers of individuals that licked swabs were examined using Cochran Q tests. When main effects were significant, paired comparisons were made using binomial tests. Due to the large numbers of possible paired comparisons, significance levels were adjusted lower by a sequential Bonferroni procedure (Wright, '92). Probabilities reported for the binomial test are not adjusted, but values stated to be significant reflect the Bonferroni adjustment.

To examine possible differences in responses to chemical cues from plants by the populations from the two islets Colom and Sargantana, we conducted 2 × 3 factorial analyses of variance for numbers of tongue-flicks, latency to bite, and TFAS(R). The factors were population and plant species (all three plant species tested). The difference in frequency of licking between populations was assessed for significance using a Fisher exact probability test. For all statistical tests, $\alpha = 0.05$. Tests were two-tailed unless otherwise stated as justified by directional prediction.

Similar tests were conducted for *P lilfordi* from Aire, but in two separate experiments. In experiment one each lizard responded to chemical cues from *H. stoechas* (present), *G. tomentosa* (absent during the tests, but present in prior years), and deionized water. In experiment two they responded to cues from the yellow composite flower *Asteriscus aquaticus* (Compositae, abundant in the year of testing), fruits of *Dracunculus muscivorus* (Araceae, a major dietary item in some seasons and years, but scarce in late May to early June of 2000), and deionized water. In both experiments, stimulus sequences were partially counterbalanced and the minimum intertrial interval was 30 min. The variables and statistical analyses were as described for the Sargantana and Colom populations. However, for one case in which variances remained significantly heterogeneous after logarithmic transformation, differences in latency to bite were examined for significance using the nonparametric Friedman two-way analysis of variance (Zar, '96). We also conducted analyses of variance to compare responses of all three lizard populations for the two plant species used as stimulus sources for all three, using the same statistical procedures as in comparisons between the Colom and Sargantana populations.

RESULTS

Sargantana

Lizards from Sargantana and Colom responded strongly to chemical cues from mealworm, and all populations responded strongly to chemical cues from syntopic plants. For the Sargantana population, number of tongue-flicks (logarithmically transformed) differed significantly among conditions (Table 1; F = 7.70; df = 5, 80; $P < 0.001$). The lizards performed significantly more tongue-flicks in response to stimuli from *Capparis spinosa* and *Galactites tomentosa* than to mealworm, cologne and deionized water (Table 2). The only other significant differences were that in the *H. stoechas* condition the lizards tongue-flicked less frequently than to *G. tomentosa* and more frequently than to deionized water (Table 2).

TABLE 1. Responses of Podarcis lilfordi *from Sargantana to chemical cues presented on cotton swabs for 60 sec*

	MW	CS	HS	GT	CL	WA
Tongue-flicks						
$\bar{X}$	9.8	17.1	11.1	27.6	8.4	6.2
SE	2.9	3.3	1.7	6.8	1.8	1.8
Range	1-18	3-46	2-23	3-113	1-23	1-30
Number that bit	14	3	5	5	2	3
Latency to bite						
$\bar{X}$	14.8	52.8	45.5	49.1	53.4	51.0
SE	5.3	4.3	6.0	4.9	4.6	4.9
Range	1-60	5-60	2-60	2-60	1-60	2-60
Number that licked	1	0	0	7	0	0

MW, mealworm; CS, *Capparis spinosa*; HS, *Helichrysum stoechas*; GT, *Galactites tomentosa*; CL, cologne; WA, deionized water.

Some individuals bit in all conditions, but far more bit in response to mealworm cues than in any of the other conditions (Table 1). These differences were significant, with more individuals biting in response to mealworm cues than to *C. spinosa* ($P < 0.00025$), *H. stoechas* ($P < 0.002$), *G. tomentosa* ($P < 0.002$), cologne ($P < 0.001$), and deionized water ($P < 0.001$). The results for latency to bite (Table 1) were similar to those for numbers of individuals that bit. Latency to bite differed significantly among conditions (F = 13.14; df = 5, 80; $P < 0.0016$), and the only significant differences were in comparisons between mealworm and other stimuli. The lizards bit at shorter latency in the mealworm condition than in all other conditions ($P < 0.001$ each).

TFAS(R) varied significantly among conditions (Fig. 1; F =11.62; df = 5, 80; $P < 0.001$). TFAS was significantly greater in the mealworm condition than in all other conditions ($P < 0.001$ each). TFAS(R) was significantly greater in response to *G. tomentosa* than to cologne ($P = 0.045$, one-tailed) or deionized water ($P = 0.03$, one-tailed). Although response to *H. stoechas* were considerably stronger than to cologne and deionized water, the differences were not significant. No other differences closely approached significance.

Licking occurred almost exclusively in trials with *G. tomentosa* (Table 1). The number of individuals that licked swabs differed significantly among conditions (Q = 31.05, df = 5, $P < 0.001$). Substantially more individuals licked in the *G. tomentosa* condition than in the mealworm condition ($P < 0.016$) and all other conditions ($P < 0.008$

TABLE 2. Significant levels of differences in numbers of tongue-flicks between pairs of stimulus conditions for the Sargantana population of Podarcis lilfordi

	CS	HS	GT	CL	WA
MW	< 0.045	NS	< 0.003	NS	NS
CS		NS	NS	< 0.016	< 0.002
HS			< 0.043	NS	< 0.034
GT				< 0.001	< 0.001
CL					NS

MW, mealworm; CS, *Capparis spinosa*; HS, *Helichrysum stoechas*; GT, *Galactites tomentosa*; CL, cologne; WA, deionized water.

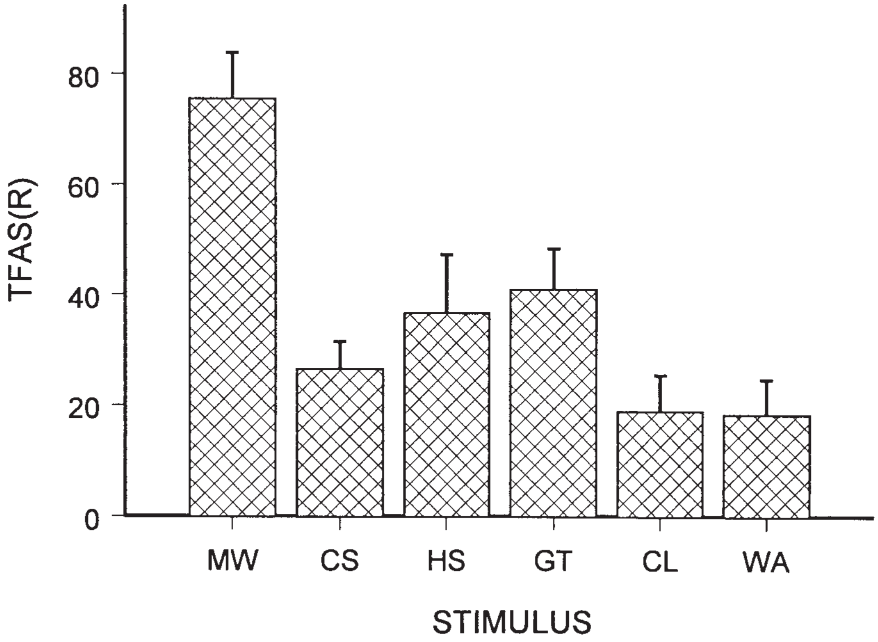

Fig. 1. Mean + SE of tongue-flick attack scores [TFAS(R)] for *Podarcis lilfordi* from Sargantana in response to chemical stimuli from mealworm (MW), *Capparis spinosa* (CS), *Helichrysum stoechas* (HS), *Galactites tomentosa* (GT), cologne (CL), and deionized water (WA).

each), but these differences were not significant after Bonferroni adjustment. However, the binomial probability that seven individuals would bite in response to *G. tomentosa* and only one would bite in all other conditions combined is < 0.001.

Colom

Lizards from Colom also responded strongly to chemical cues from mealworms and plants. Numbers of tongue-flicks did not vary greatly among conditions for the Colom population (Table 3). The variances were intractably heterogeneous, but results of the analysis of variance (F = 0.73; df = 5, 95; $P > 0.60$) agreed with the nonparametric Friedman test in indicating no significant differences among conditions ($\chi^2 = 4.27$, df = 5, $P > 0.50$).

Numerous individuals bit in all conditions, including the control conditions (Table 3). The number of individuals that bit differed significantly among conditions ($\chi^2 = 19.80$, df = 5, $P < 0.005$). Significantly more lizards bit in response to *G. tomentosa* stimuli than to stimuli from cologne (binomial $P < 0.001$). The only other difference that approached significance after Bonferroni adjustment was the greater number that bit in the mealworm condition than in the cologne condition (binomial $P < 0.008$, one-tailed). Latency to bite differed significantly among conditions (Table 3; F = 7.12; df = 5, 95; $P < 0.001$), with the shortest latencies in response to stimuli from *G. tomentosa* and mealworms. Latency to bite was significantly longer in response to cologne and deionized water, respectively, than in response to mealworm ($P < 0.002$ and $P < 0.017$) and *G. tomentosa* ($P < 0.001$ and $P < 0.015$). Mean latencies to bite for *C. spinosa* and *H. stoechas* were significantly shorter than for deionized water ($P < 0.002$ and $P < 0.013$, respectively), but not for cologne ($P > 0.10$ each). No other differences approached significance.

For the Colom population TFAS(R) provided a

TABLE 3. Responses of Podarcis lilfordi *from Colom to chemical cues presented on cotton swabs for 60 sec*

	MW	CS	HS	GT	CL	WA
Tongue-flicks						
$\bar{X}$	3.8	5.7	6.0	5.4	4.4	4.8
SE	1.1	1.3	1.4	1.1	0.9	0.6
Range	1-22	1-23	1-23	1-20	1-18	1-9
Number that bit	18	16	15	19	9	13
Latency to bite						
$\bar{X}$	9.6	15.7	21.1	8.4	39.8	29.1
SE	4.0	5.3	5.6	3.1	5.8	5.6
Range	1-60	1-60	1-60	1-60	1-60	2-60
Number that licked	0	0	0	0	0	0

MW, mealworm; CS, *Capparis spinosa*; HS, *Helichrysum stoechas*; GT, *Galactites tomentosa*; CL, cologne; WA, deionized water.

clearer picture of differences in response strength among conditions than either tongue-flicks or numbers of individuals that bit (Fig. 2). TFAS(R) differed significantly among conditions (F = 7.87; df = 5, 95; $P = 0.001$). Responses to the animal and plant stimuli were all substantially greater than to the two control stimuli. Probabilities associated with differences from cologne and deionized water, respectively, were < 0.011 and < 0.001 for *G. tomentosa*, < 0.016 and < 0.001 for mealworm. In contrast to latency to bite, TFAS(R) for *C. spinosa* and *H. stoechas* was significantly greater than for deionized water ($P < 0.008$ and $P < 0.004$, respectively). No other differences were significant.

No individuals from the Colom population licked swabs.

Aire

In experiment one for the Aire population, the lizards exhibited strong responses to chemicals from the two plant species (Table 4). Numbers of tongue-flicks did not differ significantly among stimuli (Table 4; F = 1.31; df = 2, 38; $P > 0.10$ for logarithmically transformed data). Numbers of individuals that bit differed significantly among conditions (Table 4; $\chi^2 = 8.00$, df = 2, $P < 0.02$). The only significant difference between pairs of conditions was the greater number of individuals that bit in response to cues from *G. tomentosa* than deionized water (binomial $P < 0.016$, one-tailed). No individuals licked swabs.

TFAS(R) was much stronger in response to the two syntopic plants than to deionized water (Fig. 3). The main effect of TFAS(R) was significant (F = 8.77; df = 2, 38; $P < 0.001$). Response strength to the plant species did not differ significantly, but TFAS(R) was significantly greater to chemical stimuli from *G. tomentosa* ($P = 0.0013$) and *H. stoechas* ($P = 0.002$) than to deionized water.

In experiment two for the Aire population, the lizards exhibited a different pattern, responding strongly to chemical cues from flowers of one syntopic plant food species, but not to edible fruits from another species commonly eaten by the lizards (Table 4). Numbers of tongue-flicks were somewhat greater in response to fruits of *Dracunculus muscivorus* than the other stimuli, but the main effect was not significant (Table 4; F = 2.86; df = 2.38; $P = 0.069$). Many individuals bit swabs bearing cues from *A. aquaticus* and deionized water, but only one bit a *D. muscivorus* swab (Table 4). A significantly greater number of individuals bit swabs in the *A. aquaticus* condition than in the *D. muscivorus* condition ($P < 0.001$). Significantly fewer individuals bit in the *D. muscivorus* condition than in the deionized water condition ($P = 0.006$). No individuals licked swabs. Latency to bite differed significantly among conditions ($\chi^2 = 17.39$, df = 2, $P = 0.001$).

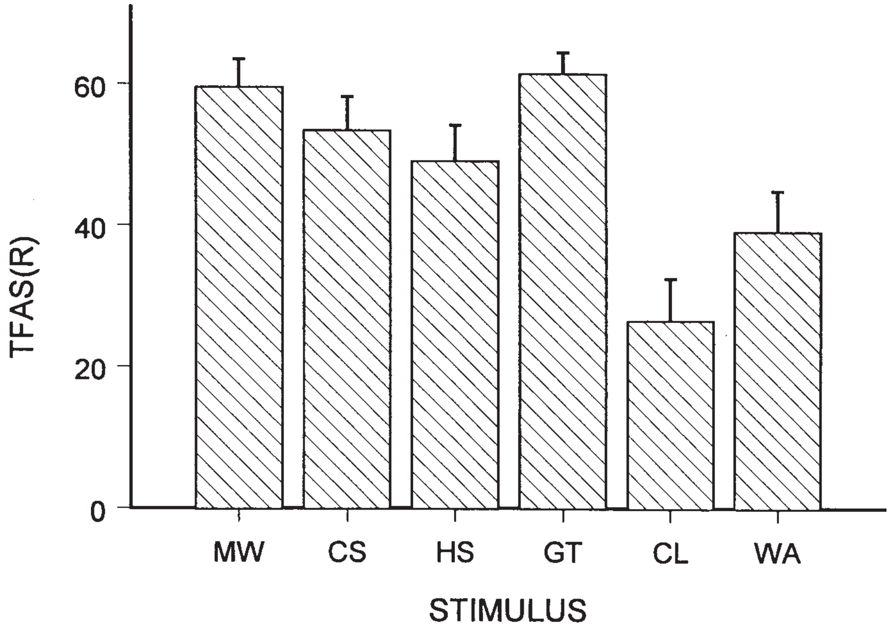

Fig. 2. Mean + SE of tongue-flick attack scores [TFAS(R)] for *Podarcis lilfordi* from Colom in esponse to chemical stimuli from mealworm (MW), *Capparis spinosa* (CS), *Helichrysum stoechas* (HS), *Galactites tomentosa* (GT), cologne (CL), and deionized water (WA).

TABLE 4. Responses by Podarcis lilfordi *from Aire to chemical stimuli in 60 sec swab test*

Experiment one	HS	GT	WA
Tongue-flicks			
$\bar{X}$	12.2	9.6	5.7
SE	2.6	2.1	1.1
Range	1-35	1-34	1.18
Number that bit	11	13	7
Latency to bite			
$\bar{X}$	28.8	25.4	40.2
SE	6.5	6.2	6.2
Range	1-60	1-60	1-60
Number that licked	0	0	0
Experiment two	AA	DM	WA
Tongue-flicks			
$\bar{X}$	6.4	10.2	5.8
SE	1.4	1.5	0.9
Range	1-20	2-26	1-15
Number that bit	14	1	10
Latency to bite			
$\bar{X}$	22.9	57.2	37.2
SE	6.0	2.8	5.8
Range	1-60	4-60	2-60
Number that licked	0	0	0

AA, *Asteriscus aquaticus*; DM, *Dracunculus muscivorus*; GT, *Galactites tomentosa*; HS, *Helichrysum stoechas*; WA, deionized water.

TFAS(R) was unexpectedly low for *D. muscivorus* (Fig. 4). The main stimulus effect was significant (F = 8.19; df = 2, 38; $P < 0.002$ for logarithmically transformed data). TFAS(R) was significantly greater in response to chemical cues from *A. aquaticus* than from *D. muscivorus* ($P < 0.001$) and deionized water ($P = 0.04$). TFAS(R) was marginally lower to *D. muscivorus* than to deionized water ($P = 0.063$).

Comparisons of responses elicited by chemical cues from flowers in the two experiments revealed no difference in between *A. aquaticus* and *G. tomentosa* in tongue-flicks (F = 1.59; df = 1, 38, $P > 0.10$), number of individuals that bit ($P > 0.10$), of TFAS(R) (F = 0.37; df = 1, 38; $P > 0.10$). There were no significant differences between *A. aquaticus* and *H. stoechas* in number of individuals that bit ($P > 0.10$) or TFAS(R) (F = 0.30; df =1, 38; $P > 0.10$); but the number of tongue-flicks was marginally greater in response to *H. stoechas* than to *A. aquaticus* (F = 3.81; df = 1, 38; $P < 0.058$).

Interpopulational comparisons

Lizards from Sargantana and Colom responded strongly to chemical cues from flowers of syntopic food plants and from flowers of palatable plant present in the area, but not currently found on their respective islands. After logarithmic transformation, numbers of tongue-flicks differed significantly between islands (F = 30.80; df = 1, 35; $P = 0.001$), but not among plant species (F = 2.34; df = 2, 70; $P > 0.10$). The island by plant species interaction was not significant (F = 1.78; df = 2, 70; $P > 0.10$). Latency to bite was shorter for Colom than for Sargantana (F = 43.44; df = 1, 35;

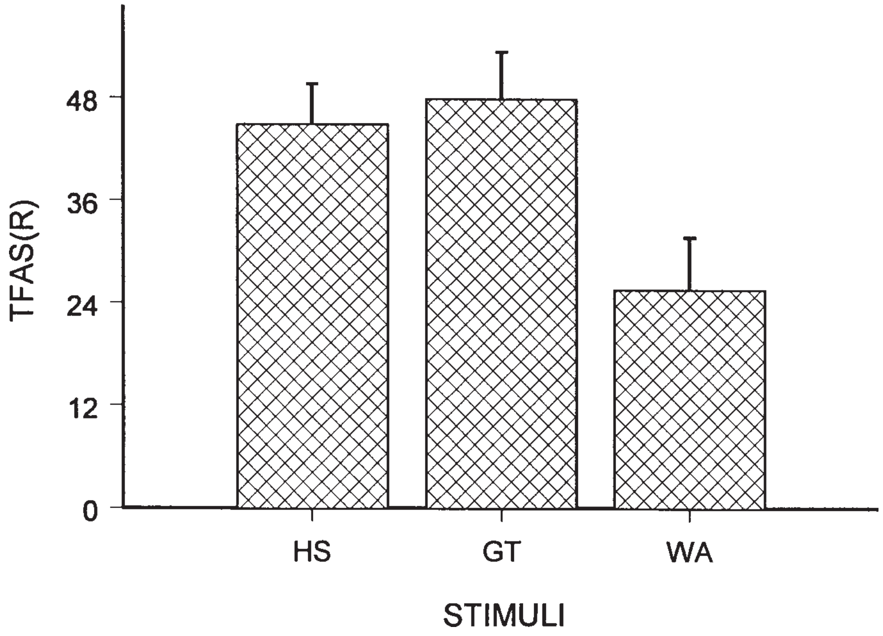

Fig. 3. Mean + SE of tongue-flick attack scores [TFAS(R)] for *Podarcis lilfordi* from Aire (experiment 1) in response to chemical stimuli from *Helichrysum stoechas* (HS), *Galactites tomentosa* (GT), and deionized water (WA).

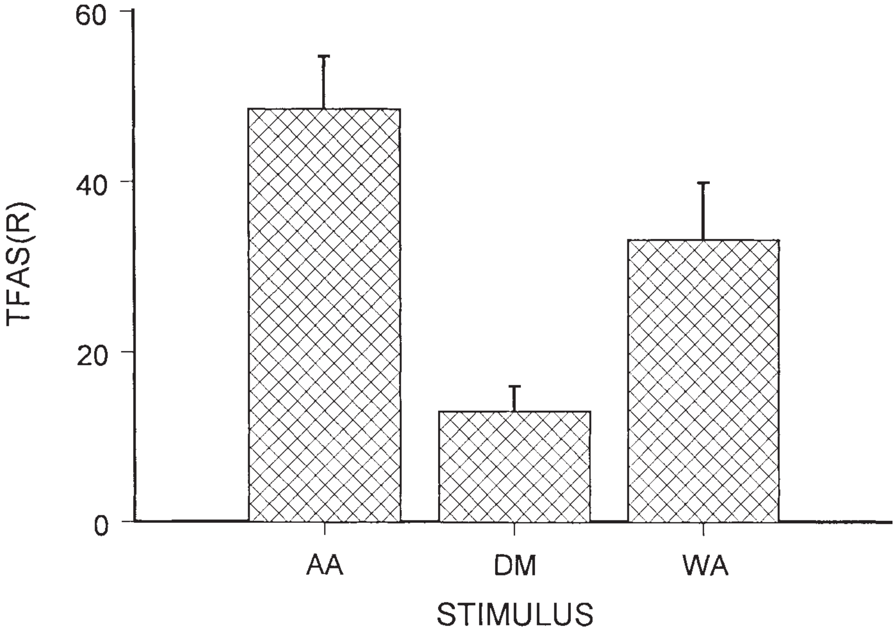

Fig. 4. Mean + SE of tongue-flick attack scores [TFAS(R)] for *Podarcis lilfordi* from Aire (experiment 2) in response to chemical stimuli from *Asteriscus aquaticus* (AA), *Dracunculus muscivorus* (DM), and deionized water (WA).

$P < 0.001$), but did not differ among the three plant species (F = 1.01; df = 2, 70; $P > 0.10$). The island by plant species interaction was not significant (F = 2.11; df = 2, 70; $P > 0.10$).

A similar pattern was observed for TFAS(R), for which the island effect was significant (F = 9.416; df = 1, 35; $P < 0.005$), but the stimulus effect (F = 2.56; df = 2, 70; $P = 0.085$) and the interaction were not (F = 0.99; df = 2, 70; $P > 0.10$). Comparisons of the two islands showed no difference in rank TFAS(R) for *H. stoechas* (U = 143.5; n = 17, 20; $P > 0.10$) or *G. tomentosa* (U = 162; n = 17, 20; $P > 0.10$). However, lizards from Colom responded more strongly to *C. spinosa* than did lizards from Sargantana (U = 75.5; n = 17, 20; $P < 0.01$).

In the comparisons between lizard populations from all three islets for *H. stoechas* and *G. tomentosa*, there were differences in numbers of tongue-flicks and latency to bite among populations, but not between plant species for logarithmically transformed data. Neither was the plant × islet interaction significant. Number of tongue flicks differed significantly among lizard populations (F = 7.64; df = 2, 54; $P < 0.002$), but not among plant species (F = 1.40; df = 1, 54; $P > 0.10$), and the population × plant species interaction was not significant (F = 2.49; df = 2, 54; $P < 0.093$). Lizards from Sargantana tongue-flicked at higher rates than those from Aire ($P < 0.03$) and Colom ($P < 0.001$). Tongue-flick rates did not differ significantly between the Aire and Colom populations ($P < 0.092$). Latency to bite differed significantly among the three islets (F = 11.34; df = 2, 54), being significantly longer for the Sargantana lizards than for those from Aire ($P < 0.006$) and Colom ($P < 0.001$). The plant species (F = 1.04; df = 1, 54; $P > 0.10$) and interaction effects (F = 1.13; df = 2, 54; $P > 0.10$) were not significant.

For TFAS(R) the variances were heterogeneous even for the logarithmically transformed data, but less so than for the raw data. For the transformed data, TFAS(R) differed significantly among lizard populations (F = 4.79; df = 2, 54; $P < 0.013$). TFAS(R) was significantly lower in the Sargantana population than in the Aire ($P < 0.009$) and Colom ($P < 0.04$) populations. TFAS(R) also differed significantly among plant species (F = 4.31; df = 1, 54; $P < 0.04$), with slightly stronger responses to *G. tomentosa* than to *H. stoechas*. The interaction effect was not significant (F = 1.01; df = 2, 54; $P > 0.10$).

Licking of swabs bearing chemical cues from *G. tomentosa* differed dramatically between the two populations. Slightly over one third of the lizards from Sargantana, where *G. tomentosa* is abundant, rapidly and repeatedly licked swabs bearing chemical cues from that plant. In contrast no lizards from Colom or Aire licked swabs. This difference was significant (Fisher $P < 0.004$ each).

DISCUSSION

Like other previously tested species of omnivorous lizards, *P. lilfordi* from populations on all three islets responded strongly to chemical cues from palatable plants, and those from the two islets examined responded strongly to animal prey cues, discriminating both types of cues from control substances. The presence of prey chemical discrimination is indicated by the significantly shorter latency to bite, by greater TFAS(R) in the mealworm condition than in the control conditions for both Sargantana and Colom, and by the significantly greater proportion of individuals that bit swabs in the mealworm condition than in the control conditions in the Sargantana population.

Prey chemical discrimination occurs in all species of active foragers tested (e.g, Cooper, '94a, '95, '97), including other lacertid species representing several genera (*Acanthodactylus boskianus*, Cooper, '99; *Lacerta agilis*, Nelling, '96; *L. monticola*, Marcos León, '99; *Takydromus sexlineatus,* Nelling, '96; Cooper et al., in press b; *T. septentrionalis,* Nelling, '96; *Podarcis bocagei,* Marcos León , '99; *P. hispanica*, Cooper, '90; Marcos León, '99; *P. muralis,* Cooper, '91; and *Psammodromus algirus,* Marcos León, '99). The present data thus extend findings suggesting that lingually mediated prey chemical discrimination is ubiquitous in actively foraging lizards. It also occurs in *Aacanthodactylus scutellatus*, in which ambush foraging was secondarily derived (Cooper, '99), suggesting that it may be universal or nearly so in Lacertidae.

Plant chemical discrimination by *P lilfordi* is demonstrated in several ways. For the Sargantana population it is indicated by the significantly greater numbers of tongue-flicks in response to cues from flowers of *C. spinosa* and *G. tomentosa* than to the control conditions, and by the significantly higher TFAS(R) to *G. tomentosa* cues than to the controls. For the Colom lizards, the best evidence is the significantly greater latency to bite and TFAS(R) in response to *G. tomentosa* than to the control stimuli. Aire lizards exhibited plant chemical discrimination in both experiments. In experiment one this is indicated by the greater TFAS(R) in response to *G. tomentosa* and *H. stoechas* than to deionized water. In experiment two, it is shown by the significantly greater TFAS(R) to stimuli from *A. aquaticus* than from deionized water.

Studies of responses to chemical cues from plant and animal food by a limited number of omnivorous, herbivorous, and carnivorous lizard species have shown that omnivorous and herbivorous species respond strongly to both animal and plant chemicals. This is true for plant eaters derived from ambush foragers (e.g., Cooper and Alberts, '90; Cooper and Flowers, in press), as well as those derived from active foragers. The present findings of chemical discrimination of plant and animal chemicals from control substances by *P. lilfordi* add to a growing number of species of active foragers in which plant chemical discrimination appears to have been added to the chemosensory repertoire in association with adoption of an omnivorous diet. By providing another independent comparison within Lacertidae between the insectivorous *T. sexlineatus* (Cooper et al., in press b) and the omnivorous *P. lilfordi*, the presence of plant chemical discrimination in *P. lilfordi* contributes to the data base needed to test the hypothesis that there has been correlated evolution between plant diet and plant chemical discrimination in lizards.

Our results support the existence of a broad correspondence between plant diet and chemosensory responsiveness to plant chemicals, but suggest a complex relationship between plant species and chemosensory evaluation. It is clear that strong responses are not restricted to plants normally encountered. Comparisons of tongue-flicking and biting responses to chemical cues from three plant species gave no evidence for stronger response to syntopic than allopatric plants. Responses were stronger [TFAS(R)] to cues from *C. spinosa* by lizards from Colom than Sargantana, but *C. spinosa* is present on both islets.

Lizards in the Sargantana population exhibited more tongue-flicks to one of two syntopic plant species, *G. tomentosa*, than to the allopatric *H. stoechas*, and responses to the allopatric *H. stoechas* did not differ significantly from controls for numbers of tongue-flicks or TFAS(R). However, TFAS(R), numbers of individuals that bit, and latency to bite did not differ significantly among plant species. For the Colom population, there were no significant differences among plant species for tongue-flicks, bites, latency to bite, or TFAS(R), giving no support for differential response to chemical cues from flowers among the three plant species. For the Aire population the only evidence suggesting possible differential response among flowers is the greater tongue-flicking rate in response to *H. stoechas* than to the other two species tested, but the differences were not significant.

Three bits of evidence show that responses by

P. lilfordi vary among plant species. First, chemosensory behaviors differed greatly between stimuli from *A. aquaticus* flowers and *D. muscivorus* fruits in experiment two, with greater response strength to the former indicated by significant differences in number of lizards that bit and TFAS(R). Because the lizards readily consume fruits of *D. muscivorus* (Pérez-Mellado et al., 2000), these differences may indicate that the lizards rely more on visual than chemical cues for feeding on *D. muscivorus* or that the fruits of this species provide weaker chemical cues than the flowers. Second, in the comparison between all three lizard populations, TFAS(R) was greater in response to *G. tomentosa* than to *H. stoechas*. One possible factor affecting this difference is differences between plant species in concentrations of nutrients such as sugars and proteins.

Third, licking differed greatly among plant stimuli and islands, being observed only in response to stimuli from *G. tomentosa* by lizards from Sargantana, which had been feeding on *G. tomentosa* at the time of capture. Because *G. tomentosa* is absent on Colom, the greater incidence of licking in response to cues from this plant by lizards from Sargantana might be an adaptive response to feeding on nectar evolved by the Sargantana population. Because *G. tomentosa* was present on Aire in recent years, but not in 2000, the absence of licking in response to cues from *G. tomentosa* by lizards in the Aire population suggests that either this population did not evolve the licking response or that the response, if present, may be strongly affected by recent feeding experience.

Our results show that *P. lilfordi* responds strongly to chemical cues from a variety of palatable plants, including allopatric species. There is some evidence for differential tongue-flicking and biting responses between cues from flowers of different plant species. Chemical cues from flowers of all species elicited stronger responses than did those from the fruit of another species, even though the fruits are an important food source (Pérez-Mellado et al., 2000). Because we tested responses only to parts of plants eaten by lizards, the responses to inedible parts of edible species are uncertain, but would presumably be weaker.

The difference in licking responses between populations and plant species show a differential feeding response between plants by the lizards based on chemical cues. The differences in licking in response to stimuli from flowers of *G. tomentosa* among lizard populations might be a consequence of recent availability (especially on Aire), but the comparison between Sargantana and Colom populations might reflect an adaptation acquired in syntopy by lizards from Sargantana, but not by those from Colom where *G. tomentosa* is allopatric.

The feeding behavior of *P. lilfordi* on flowers varies among plant species, even within islets. They eat whole flowers of some plant species, but lick flowers of species such as *Chrithmum maritimum* (Pérez-Mellado and Casas, '97; Pérez-Mellado, unpublished observations). Because licking is a feeding response, the differences in licking among plant species may reflect interspecific differences among flowers in availability and accessibility of nectar. This hypothesis is supported by our unpublished data showing that the lizards lick swabs bearing sugar solutions.

Comparisons of tongue-flicking and biting responses among island populations did not reveal any major differences in responses to plants related to syntopy, but the populations differed in the strength of responses to all plants. This was shown by (1) the differences in response to three plant species between Colom and Sargantana for tongue-flicks, latency to bite, and tongue-flick attack score, and (2) the higher tongue-flick rate, greater latency to bite, and lower TFAS(R) in response to cues from two plant species for the population from Sargantana than the other lizard populations. We cannot explain the differences among populations using the present data. They might be transient, due to factors such as differences in relative abundance of plant and animal foods among islets, or genetically fixed.

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