

## Behavioural variation in natural populations. VI. Prey responses by two species of garter snakes in three regions of sympatry

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**Abstract.** Diets and congenital reactions to prey were studied in the garter snakes *Thamnophis elegans* and *T. sirtalis* at three sites where they occur in sympatry in northern California and Washington. Recently ingested prey retrieved from the stomachs of wild-caught snakes indicated that *T. sirtalis* was primarily an anuran predator at all three sites. In contrast, *T. elegans* was primarily a slug predator at two sites, but at a third site leeches, anurans and fish dominated the diet. Congenital reactions to prey were studied by measuring chemoreceptive responses to prey odours by naive, newborn snakes. Within each snake species, chemoreception scores showed litter differences within localities as well as geographical variation. Geographical differences in chemoreceptive responses generally coincided with geographical differences in diet, suggesting evolutionary responses to selection.

Studies of intraspecific geographical differences may offer our best hope for dissecting the causes of behavioural evolution. Although differences between species are usually more pronounced than geographical variation within species, species often differ in so many aspects of behaviour, morphology and ecology that diagnosis of selective pressures is difficult. Diagnosis within species may be more trustworthy because confounding variables are not so prevalent. The other virtue offered by studies of geographical differences is that small evolutionary steps can be revealed. Geographical variation is the most elementary data ordinarily available in microevolutionary studies, unless populations are studied over a series of generations.

Despite the pivotal empirical and theoretical roles of geographical variation (Mayr 1963; Gould & Johnston 1972), this phenomenon has been little studied by behaviourists (Boch 1957; Sawada 1963; Littlejohn 1965; Ferguson 1971; Krebs & Kroodsma 1980; Arnold 1981a; Drummond & Burghardt 1983; Luyten & Liley 1985). What are the small steps by which patterns of behaviour evolve, populations differentiate and species differences arise? Presumably the microevolution of behaviour proceeds by gradual shifts in the means of statistical distributions, but data bearing on this point are rare. Aside from the paucity of data, the effect of experience is a special technical problem

plaguating studies of intraspecific variation in behaviour. Population differences may reflect differences in experience. In the present study of chemoreception, this problem was circumvented by testing newborn, naive snakes. Furthermore, Burghardt's (1971) perturbations of maternal diet and Arnold's (1981c) hybridization studies failed to detect prenatal maternal effects.

The adaptive significance of geographical differences in behaviour can be assessed by checking for correlation with environmental factors that are presumed to exert selection and by testing for parallel geographical variation in two or more species. In the present study, the correlational approach was pursued by looking for associations between prey availability and diet, on the one hand, and the feeding responses of naive snakes, on the other hand. The argument is that geographical differences in feeding responses that correspond with geographical differences in prey availability or diet are likely to present responses to selection. However, geographical differences in behaviour may coincide by chance with geographical differences in putative selective pressures. Parallel geographical variation in a second species provides evidence for independent response to selection and so can increase our confidence in identification of selection pressures. The utility of testing for parallel geographical variation in multiple species has long been appreciated by morphologists (Klauber 1941; James 1970;

Hagen & Gilbertson 1972) but has seldom been used by behaviourists.

Geographical variation in the feeding responses of two species of garter snakes (*Thamnophis elegans* and *T. sirtalis*) were examined and compared. It may be helpful to the reader to anticipate certain key results. Both species showed geographical variation, and in most cases the geographical differences coincided with differences in prey availability and/or diet. The two species showed parallel geographical variation in behavioural responses to fish odour, but responses to the odours of other prey did not show parallel geographical variation. In the statistical analyses of geographical variation, I will first concentrate on an overall test for parallelism (across all odour types) and then consider each odour type separately.

*Thamnophis sirtalis* is found from coast to coast in North America, typically in riparian habitats. Throughout its range it is largely a predator on anurans and/or earthworms (Fitch 1965; White & Kolb 1974; Kephart 1982; Gregory 1984). *Thamnophis elegans* is restricted to western North America and is often found far from water, as well as in riparian habitats. Its diet is more varied than that of *T. sirtalis* and often includes slugs, annelids, fish, mammals, birds, reptiles and amphibians. Geographical variation in diet is more pronounced than in *T. sirtalis* (White & Kolb 1974; Gregory 1978, 1984; Arnold 1981a; Kephart 1982).

Chemoreception is a major sensory channel for prey identification in garter snakes and other natricine snakes. Prey odours are sufficient to elicit prey attack in naive, newborn natricines (Burghardt 1967, 1970a, 1980; Burghardt & Pruitt 1975). When cotton-tipped swabs saturated with aqueous or chemical washes from the surface of the prey are presented to naive snakes, they commonly give a sustained tongue-flicking response and sometimes attack the swabs. This response is readily quantified by counting tongue flicks or scoring the incidence of attacks. Nerve lesion studies indicate that the tongue-flicking response to prey odours is mediated by the vomeronasal (Jacobson's) organs (Halpern & Frumin 1979). Tongue-flicking is a mechanism for delivery of odours to the vomeronasal organs in the roof of the mouth (Halpern & Kubie 1980, 1983). Congenital differences between snake species in chemoreceptive response to prey odours can mirror major dietary differences. For example, naive newborn individuals of the crayfish-eating genus *Regina* show a pronounced tongue-flick

response to crayfish odour, but fish-eating relatives do not (Burghardt 1967, 1968). Congenital, chemoreceptive responses to some prey odours are readily modified by experience, but responses to other prey odours are ontogenetically and experientially stable (Fuchs & Burghardt 1971; Arnold 1978; Mushinsky & Lotz 1980).

One of my study species (*T. elegans*) shows geographical variation in chemoreceptive responses to prey. In a comparison of coastal and inland populations in California, Arnold (1981a) found that naive, newborn coastal snakes showed a stronger response to slug, leech and plethodontid salamander odours, but inland snakes showed a stronger response to fish odour. Similarly, Drummond & Burghardt (1983) found that newborn inland *T. elegans* showed a greater aquatic foraging response to live fish than coastal snakes. All of these differences, except leech response, are consistent with geographical differences in diet and prey distribution. Coastal snakes are terrestrial foragers that specialize on slugs, whereas inland snakes are aquatic foragers that prey on fish. Coastal snakes encounter plethodontid salamanders, but inland snakes are allopatric with these prey. The lower response of inland snakes to leeches was enigmatic, because inland snakes routinely prey on leeches, whereas coastal snakes do not. Furthermore, Arnold (1981a) was unable to detect geographical differences in chemoreceptive response to a variety of anuran and salamander prey, even though differences could have been expected on the basis of diet and prey distribution. Thus, dietary differences were only partially successful in predicting geographical differences in the chemoreceptive responses of newborn *T. elegans*.

The failure of some chemoreceptive responses to reflect geographical differences in *T. elegans* diet could not be attributed to absence of genetic variation. Arnold (1981a) found significant among-litter variation in one or both populations in chemoreceptive responses to all prey except plethodontid salamanders. Ayres & Arnold (1983) found significant among-litter differences in both populations in their tendencies to feed on slugs. However, an investigation of among-litter covariation in responses to different prey, revealed genetic coupling between chemoreception scores (Arnold 1981b). In particular, a strong positive genetic coupling between responses to slugs and leeches helped explain the enigmatic responsiveness of coastal snakes to leech odour.

## MATERIALS AND METHODS

### Diet Data

I determined the diets of the snakes by forcing recently captured animals to regurgitate (Carpenter 1952). The procedure does not harm the snake. In addition, information on the diets of *T. elegans* and *T. sirtalis* at the collecting sites was taken from the following sources: coastal California (Humboldt County) *T. elegans* from Arnold (1981a, unpublished data); coastal California (Humboldt County) *T. sirtalis* from Arnold (1978, unpublished data); inland California (Lassen County) *T. elegans* and *T. sirtalis* from Kephart (1982); coastal Washington (Pierce County) *T. sirtalis* from Arnold & Wassersug (1978, unpublished data). The diet results represent data pooled over a number of years and over various sites in each region. Thus in inland California, data for 22 localities covering 338 km<sup>2</sup>, including the two localities where snakes were obtained for the behavioural work, were collected over a 7-year period (1974–1980; see Kephart 1981, 1982; Kephart & Arnold 1982). Kephart's field notes were consulted in order to allocate items to some categories (e.g. anurans versus salamanders). In coastal California, data from six localities spanning 46 km of Humboldt County coastline (including the two localities where snakes were obtained for behavioural work) were collected over a 9-year period (1977–1985). In coastal Washington, data from the three collecting sites were accumulated over a 6-year period (1973–1978).

### Subjects for Behavioural Tests

Newborn snakes used in the chemoreception and feeding trials were the laboratory-born progeny of gravid females collected in three regions of sympatry: coastal California (Humboldt County), coastal Washington (Pierce County), and inland California (Lassen County). It was not always possible to collect gravid females of both species at exactly the same site, because one species was usually less common. Nevertheless, it was possible to find collecting sites in each region that were ecologically similar, relatively close together and where both species occurred in sympatry. In coastal California, five female *T. elegans* were obtained at one locality and six female *T. sirtalis* were obtained from a locality 36 km away. In coastal Washington, a total of five female *T. elegans* were collected at two localities 11.6 km apart. A total of five female

*T. sirtalis* were collected at these two coastal Washington localities and at a third site, midway between the first two sites. In inland California, five female *T. elegans* were collected at one locality and five female *T. sirtalis* were collected at a site 16 km away. The numbers of neonates per litter that were used in chemoreception tests were: 6, 8, 9, 11 and 14 for *T. elegans* and 3, 4, 6, 7, 13 and 13 for *T. sirtalis* from coastal California; 6, 7, 8, 9 and 14 for *T. elegans* and 8, 8, 8, 10 and 13 for *T. sirtalis* from Washington; 6, 7, 9, 11 and 16 for *T. elegans* and 7, 9, 10, 11 and 12 for *T. sirtalis* from inland California.

All gravid females were collected in June and July of the same year and shipped by air back to the laboratory within 14 days of capture. I housed the females individually in the laboratory at 27°C and withheld food until after their litters were born in August and early September. I separated newborn snakes from their mothers within 18 h of birth and then housed the neonates individually at 27°C in plastic boxes measuring 9 × 17 × 31 cm with a paper towel substrate and a water dish. Newborn snakes were maintained without food until the postnatal age of 14 days, so that naive reactions to prey odours could be recorded. *Thamnophis elegans* and *T. sirtalis* are born with yolk reserves, and the 14-day waiting period permitted the depletion of those reserves.

### Chemoreception Tests

At age 14 days and again at age 15 days each snake was presented with the following sequence of odours: control (distilled water); slug, *Ariolimax californicus*; slender salamander, *Batrachoseps attenuatus*; boreal toad tadpoles, *Bufo boreas*; minnows, *Hesperoleucus symmetrica*; and rough-skinned newt, *Taricha granulosa*. These prey, or close relatives, are sympatric with one or more snake populations and represent the major prey categories in the diets of one or both snake species.

Prey odours were presented on cotton-tipped swabs with wooden handles, 15 cm long (Chesebrough-Ponds, 5-5405). Odiferous swabs were prepared by swirling batches of 10–30 swabs at a time among the bodies of 40 g of prey mixed with 200 ml distilled water. Fish and toad tadpole swabs were prepared by freezing those prey and later swirling swabs among the recently thawed bodies, equilibrated at 20°C. Slug, *A. californicus*, salamander, *B. attenuatus* and newt, *T. granulosa*

swabs were prepared by swirling the swabs among live prey at 20°C. Control swabs were simply swirled in distilled water. Several hundred swabs of each type were prepared at one time and kept frozen in polyethylene bags until needed. Before chemoreception tests the swabs were thawed for 0.5 h and then kept in separate test tubes immersed in crushed ice during the presentation trials.

During a chemoreception trial, a swab was held stationary 1 cm in front of the snake's head. The number of tongue-flicks that contacted the cotton-tip during a 1-min interval (beginning when the first tongue-flick touched the swab) was visually counted and constituted the snake's chemoreception score. On each of the two test days, the different swabs were presented to each snake at intervals of 40 min. Swabs were used once and then thrown away. Arnold (1981a) gives further details of the presentation protocol.

#### Statistical Analysis of Chemoreception

All statistical analyses were performed on the natural logarithm of the number of tongue flicks/min (see Arnold 1981a for examples of frequency distributions). In those cases in which a snake attacked the swab before 1 min had elapsed, the snake's score was pro-rated to an estimate of the number of tongue flicks for a full minute, using the number of tongue flicks that occurred up until the time of attack and the elapsed time from the first tongue flick (recorded to the nearest 0.1 s with a stop watch). To check the validity of this simple pro-rating scheme, I plotted the number of tongue flicks against elapsed time until attack for each prey odour type. These plots revealed linear relationships with zero intercepts. Cooper & Burghardt (1990) refer to this scoring scheme as the projected tongue-flick rate. A separate statistical analysis of the number of swabs attacked was not attempted because attack rates were generally low (mean = 3%, range = 0–27%).

The data for each individual consist of response scores to six different prey odours on trial day 1 and to the same six prey odours on trial day 2. Individuals are members of litters, litters are nested within localities ( $N=3$ ) and localities are crossed with species ( $N=2$ ). One can imagine a factorial analysis that tests for the main effects of individual and prey odour type and their interaction. Such an analysis is inappropriate because the responses of an individual to different odour types (or to

the same odour type) are not independent. Two alternative modes of analysis do not make such assumptions of independence: repeated measures and profile analysis.

One can imagine a univariate repeated measures analysis in which the within-subject factor of prey odour type is nested within trials. There are two problems with this approach. First, although such a repeated measures approach allows correlation between prey odour types, it assumes that all pairs of odour types have the same correlation (Winer 1971; Bock 1975). Previous work, however, indicated that some pairs of odour-type responses are highly correlated but others are not (Arnold 1981b). Second, such an analysis puts emphasis on within-subject differences among odour type but, as argued later, these differences may not be biologically informative. For these reasons I used a multivariate analysis of trial profiles. This approach allows heterogeneity in correlation among prey odour types, and it allows scrutiny of litter, population and species differences in the responses to each prey odour type. See Harris (1975, pp. 125–127) for a detailed discussion of univariate repeated measures models versus multivariate models of the type used here.

Habituation scores were analysed by computing a multivariate analysis of variance (MANOVA) in score profiles using the general linear model (GLM) of the Statistical Analysis System (SAS 1985). A habituation score (profile) was computed for each individual's response to each odour type by subtracting the ln score at the second trial from the ln score at the first trial. These habituation scores for each odour type were then used as the dependent variables in the analysis. Litter identity, species and locality were treated as classification variables. The statistical model tested for species and locality main effects, for species  $\times$  locality interaction and for random litter effects within species and locality combinations. Habituation to the control swab was analysed as part of the vector of habituation scores, rather than as a covariate, because control habituation showed no significant species, locality, litter or species  $\times$  locality effects. In this and all subsequent analyses, significance tests were based on type III sums of squares, which control for the presence of other effects in the model (SAS 1985). Significance tests for this and other MANOVAs were based on Wilks' criterion.

The multivariate analysis of habituation scores was also used to test the null hypotheses that the

responses to each odour type showed no change from the first to the second trial (i.e. that grand means equalled zero). These tests were conducted by creating a dummy variable,  $J=1$ , for all individuals and then including that dummy variable as an independent variable in the model. The NOINT option was used in the model statement to suppress calculation of a second intercept term by GLM. Simms & Burdick (1988) describe the procedure and give an example of the relevant programming statements in SAS.

Average chemoreception scores over the two trials were analysed with a similar MANOVA. An average score for each individual was computed for each odour type by taking the sum of the ln scores at the two trials and dividing it by two. As before, litter, species and locality were treated as classification variables and the GLM model tested for species and locality main effect, species  $\times$  locality interaction and for random litter effects within species  $\times$  locality combinations. Average response to the control (distilled water) swab was treated as a covariate, because preliminary analyses revealed statistically significant litter and locality effects for that variable. In addition to analysing score averages, the above GLM model was separately fitted to scores at each of the two trials, using control score at the relevant trial as a covariate.

A separate MANOVA of average chemoreception scores was also computed within each snake species to examine patterns of geographical variation. Litter and locality were treated as classification variables, and average control score was used as a covariate. The GLM model tested for locality effects using the type I among litter mean square in the denominator to compute  $F$ -ratios. To determine which locality means differed, Tukey's studentized range test (honestly significant different test) was used with  $\alpha=0.05$ . This test controls the maximum experimentwise error rate and corrects for unequal sample sizes (SAS 1985).

#### Feeding Tests

Feeding tests in which actual prey were offered to the snakes were conducted after the completion of the chemoreception tests. Beginning at the post-natal age of 16 days each snake was offered a small, whole, thawed prey (guppy, *P. reticulata*) or standard sized ( $2 \times 4$  mm) piece of thawed prey (*A. californicus* body wall, *B. boreas* tadpole tail). Each prey was presented on 10 consecutive days. At each

presentation ingestion or refusal was recorded and any uneaten prey from the preceding day was removed. The sum over 10 days of the number of trials resulting in ingestion constituted the snake's feeding score. Each *T. elegans* was tested for 10 days with slug and then for 10 days with toad tadpoles. The number of *T. elegans* with feeding scores is less than the number given chemoreception tests because one coastal California litter ( $N=14$ ) was inadvertently omitted from feeding tests, and because seven coastal California and two Washington snakes died during the feeding tests. *Thamnophis sirtalis* were not tested with slug, since pilot work indicated that slugs are virtually never eaten in the field or laboratory. Instead *T. sirtalis* were tested on toad tadpoles, *B. boreas* and fish, *P. reticulata*. Instead of testing each snake on both prey, half of each litter was arbitrarily tested on each prey.

## RESULTS

#### Prey Availability

The occurrence of major prey categories at the three study sites is summarized in Table I. The coastal sites in California and Washington are qualitatively identical in prey occurrence. Inland California differs from the two coastal sites in lying beyond the geographic limits of plethodontid salamanders and newts, *Taricha*. Slugs are virtually absent at the inland site. They are found at only a few isolated springheads.

#### Diets in the Field

*Thamnophis sirtalis* is primarily an anuran predator at all three sites (Fig. 1). Fish are also eaten at all three sites but constitute an important element in the diet only at the inland California site. *Thamnophis elegans* is primarily a slug predator in coastal California and perhaps in Washington as well, but in inland California, outside the range of most slugs, the diet is made up of leeches, anurans and fish (Fig. 1).

#### Chemoreceptive Responses to Prey Odours

Average chemoreception scores at each of the two trials are given in Table II. The lowest average score was 1.1 tongue flicks/min ( $=0.11$  on the ln scale) for Washington *T. sirtalis* responding to the

Table I. Prey availability at each of the three study sites

Prey	Study sites		
	Coastal CA	Coastal WA	Inland CA
Earthworms	+	+	+
Leeches	?	?	+
Slugs	+	+	-
Ambystomatid salamanders	+	+	+
Plethodontid salamanders	+	+	-
Newts	+	+	-
Toads	+	+	+
Fish	+	+	+

+: presence; -: absence; ?: questionable occurrence.

distilled water control and the highest was 19.1 tongue flicks/min ( $=2.95$  on the ln scale) for Washington *T. elegans* responding to the odour of toad tadpoles.

While comparison of chemoreceptive scores to the same prey are meaningful among populations or between species, comparisons between prey types may be misleading. Test swabs for a particular prey (e.g. slugs) were prepared in a batch and had a standardized odour content. Test swabs for different prey may have had different concentrations of odours because prey types may differ in how readily odours were transferred to swabs (e.g. due to differences in prey surface structure, mucus release, etc.). Thus, population and species differences in chemoreceptive response to, say, slug versus toad swabs may or may not be biologically informative. Such comparisons are avoided in the following discussions.

The average attack rates to swabs were low (4% for *T. elegans*, 2% for *T. sirtalis*). Control and newt swabs were never attacked by either snake species. The highest attack rates were to slug, fish and toad swabs by *T. elegans* and to toad and fish swabs by *T. sirtalis*. Population differences in attack rate within each snake species to these swab types followed the pattern for tongue-flick scores.

#### Habituation Effects

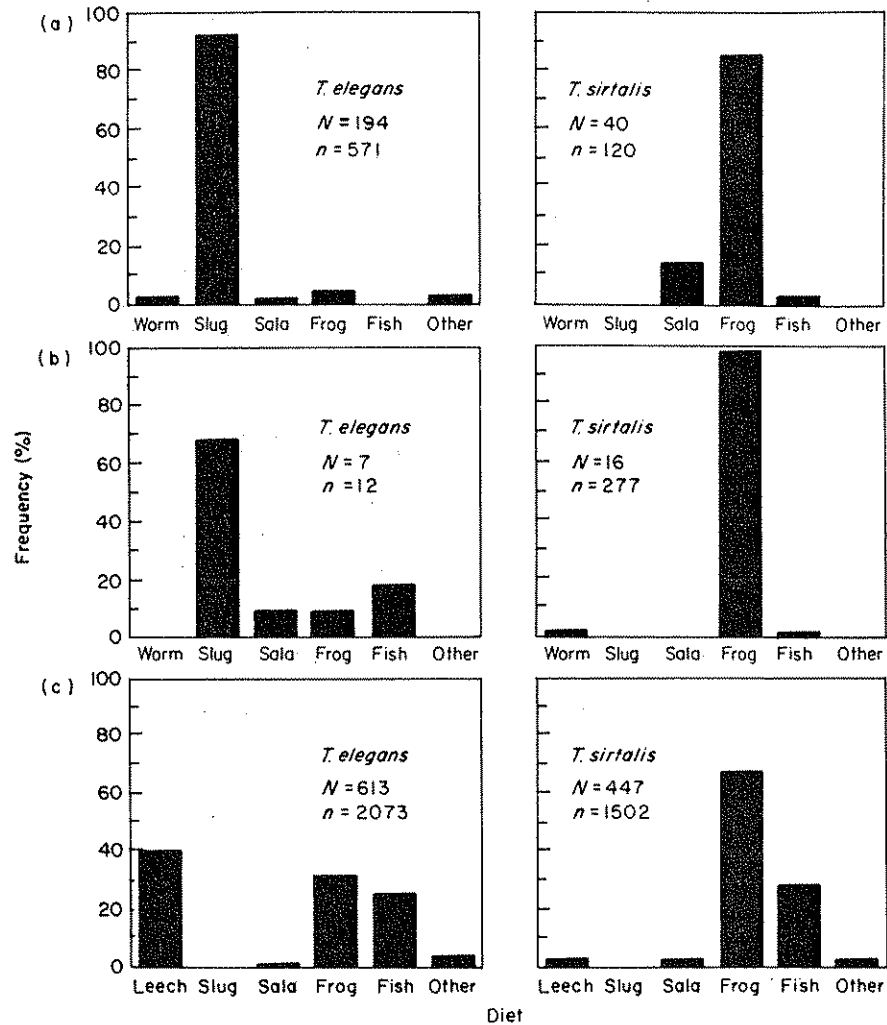
All test odours elicited lower responses at trial 2 than trial 1 ( $F$ -values for the six test swabs ranged from 15.4–172.9,  $df=1, 252$ ,  $P<0.01$ ). The average per cent reduction from the first to the second trial ranged from 8 to 60% in *T. elegans* and from 31 to 67% in *T. sirtalis*, depending on test odour (computed from data in Table II).

The MANOVA for differences between scores at trials 1 and 2 revealed significant litter effect and species  $\times$  locality interaction but no significant species or locality effects (Table III). The individual ANOVAs revealed that the overall litter effect arose from significant habituation effects to salamander and newt odours. Species  $\times$  locality interaction in score differences for toad and newt odour were the main contributors to the significant, overall interaction term.

#### Average Chemoreception Response

The results of a MANOVA and individual ANOVAs for average chemoreceptive response at the two trials are reported in Table IV. The MANOVA revealed significant overall regression of score averages on control averages, species and locality effects, species  $\times$  locality interaction and litter effects. The ANOVAs showed that responses to two or more prey odours contributed to each of these significant effects. In particular, all of the prey odours except fish showed significant species  $\times$  locality interactions. Thus, *T. elegans* and *T. sirtalis* did not show parallel geographical variation in behavioural responses to most prey.

Significant species  $\times$  locality interaction in habituation scores for toad and newt (Table III) complicate the interpretation of interaction terms for score averages for those prey. The interaction in score averages (Table IV) might be due to habituation effects exclusively. Accordingly, separate ANOVAs were computed for both toad and newt odour scores at trials 1 and 2. For both prey species, the ANOVAs revealed significant ( $P<0.01$ ) species  $\times$  locality interaction at both trials. Thus,



**Figure 1.** Diets of *T. elegans* and *T. sirtalis* in the three regions of sympatry: (a) coastal California, (b) coastal Washington, and (c) inland California. Bars indicate the proportion of prey items from each category.  $N$  = the number of snakes with prey items in their stomachs;  $n$  = the total number of prey items. The Worm category denotes earthworms. The Sala (salamander) category represents *Ambystoma gracile* larvae in Washington and coastal California and *A. macrodactylum* larvae in inland California. The Frog category represents *Rana aurora* and *H. regilla* in coastal California; *Rana catesbeiana* and *B. boreas* in Washington; *B. boreas*, *H. regilla* and *Scaphiopus intermontanus* in inland California. The Other category denotes mammals and lizards for coastal California *T. elegans*; mammals, lizards and snakes for inland California *T. elegans*; earthworms, crustaceans and insects for inland California *T. sirtalis*.

species  $\times$  locality interaction in score averages for toad and newt odour could not be attributed to habituation effects.

To determine the nature of geographical variation within *T. elegans* and *T. sirtalis*, a separate MANOVA was conducted on each snake species, testing for locality effects and litter differences within localities. Average response to control was used as a covariate. The results are summarized in

Fig. 2. The MANOVA revealed significant ( $P < 0.001$ ) overall locality and litter effects in both snake species. *Thamnophis elegans* showed significant geographical variation in average responses to slug, toad and fish odours ( $P < 0.001$ ), while *T. sirtalis* showed significant geographical variation in responses to slug ( $P < 0.001$ ), salamander and fish odours ( $P < 0.01$ ). *Thamnophis elegans* showed significant ( $P < 0.001$ ) differences among

Table II. Average ( $\pm$ SE) chemoreception scores to prey odours by naive, newborn snakes at trial (1) and trial (2)

Subject	Trial	N	Prey odours					Newt
			Control	Slug	Sala	Toad	Fish	
<i>T. elegans</i>								
Coastal California	(1)	48	0.27 $\pm$ 0.08	2.55 $\pm$ 0.23	1.70 $\pm$ 0.20	1.93 $\pm$ 0.24	1.39 $\pm$ 0.20	0.24 $\pm$ 0.08
	(2)	48	0.17 $\pm$ 0.06	1.70 $\pm$ 0.28	0.74 $\pm$ 0.17	1.56 $\pm$ 0.22	1.13 $\pm$ 0.18	0.35 $\pm$ 0.09
Coastal Washington	(1)	44	0.71 $\pm$ 0.11	2.25 $\pm$ 0.20	2.31 $\pm$ 0.20	2.95 $\pm$ 0.14	2.03 $\pm$ 0.15	1.06 $\pm$ 0.14
	(2)	44	0.54 $\pm$ 0.09	1.67 $\pm$ 0.21	0.79 $\pm$ 0.12	1.66 $\pm$ 0.18	1.17 $\pm$ 0.15	0.64 $\pm$ 0.12
Inland California	(1)	49	0.51 $\pm$ 0.10	0.85 $\pm$ 0.15	1.97 $\pm$ 0.21	2.84 $\pm$ 0.18	2.37 $\pm$ 0.17	0.42 $\pm$ 0.09
	(2)	49	0.38 $\pm$ 0.09	0.78 $\pm$ 0.12	0.80 $\pm$ 0.14	2.21 $\pm$ 0.18	1.55 $\pm$ 0.22	0.30 $\pm$ 0.09
<i>T. sirtalis</i>								
Coastal California	(1)	46	0.37 $\pm$ 0.08	0.50 $\pm$ 0.12	1.93 $\pm$ 0.21	2.18 $\pm$ 0.22	1.21 $\pm$ 0.20	0.90 $\pm$ 0.18
	(2)	46	0.30 $\pm$ 0.08	0.16 $\pm$ 0.07	0.72 $\pm$ 0.17	1.37 $\pm$ 0.21	0.98 $\pm$ 0.19	0.18 $\pm$ 0.08
Coastal Washington	(1)	47	0.39 $\pm$ 0.10	0.51 $\pm$ 0.13	1.30 $\pm$ 0.18	1.92 $\pm$ 0.20	1.14 $\pm$ 0.18	0.23 $\pm$ 0.08
	(2)	47	0.11 $\pm$ 0.05	0.27 $\pm$ 0.09	0.29 $\pm$ 0.09	1.35 $\pm$ 0.21	0.71 $\pm$ 0.15	0.13 $\pm$ 0.05
Inland California	(1)	49	0.78 $\pm$ 0.13	1.19 $\pm$ 0.17	1.85 $\pm$ 0.18	2.35 $\pm$ 0.19	1.83 $\pm$ 0.19	0.59 $\pm$ 0.12
	(2)	49	0.47 $\pm$ 0.09	0.87 $\pm$ 0.15	0.74 $\pm$ 0.13	1.25 $\pm$ 0.17	1.17 $\pm$ 0.17	0.39 $\pm$ 0.10

Slug: *A. californicus*; Sala: the plethodontid salamander *B. attenuatus*; Toad: *B. boreas*; Fish: *H. symmetrica*; and Newt: the salamandrid salamander *T. granulosa*.

Table III. Results of analyses of variance of differences in chemoreception scores at trials 1 and 2 for the five prey types

Source of variation	df	Control	Slug	Sala	Toad	Fish	Newt	MANOVA
Species	1	NS	NS	NS	NS	NS	NS	NS
Locality	2	NS	NS	NS	NS	NS	NS	NS
Species $\times$ locality	2	NS	NS	NS	*	NS	*	**
Litter	25	NS	NS	*	NS	NS	**	**
Model	30	NS	NS	NS	NS	NS	NS	**
Error	252							
Total	282							

See Table II and text for identity of prey odours.

\* $P < 0.05$ ; \*\* $P < 0.01$ .

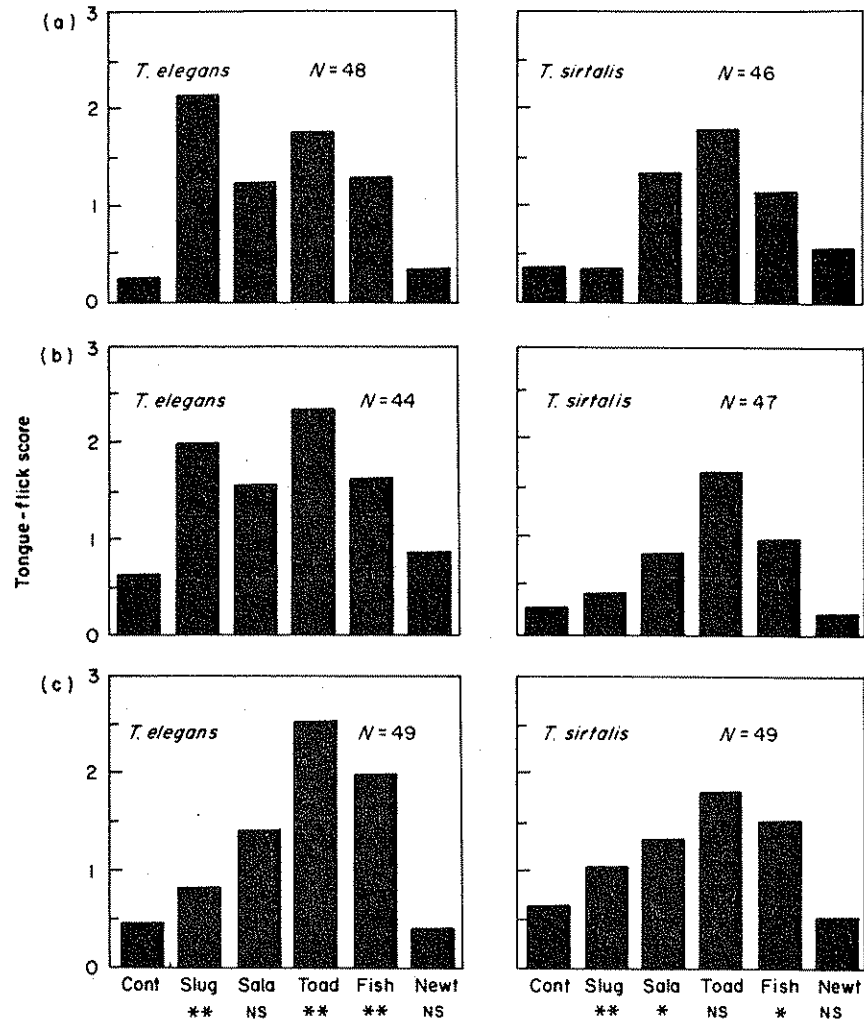
Table IV. Results of analyses of variance of average chemoreception scores to five prey types

Source of variation	df	Slug	Sala	Toad	Fish	Newt	MANOVA
Control	1	*	**	*	NS	**	**
Species	1	**	NS	*	**	*	**
Locality	2	*	NS	NS	**	NS	**
Species $\times$ locality	2	**	*	**	NS	**	**
Litter	25	**	**	**	**	**	**
Model	31	**	**	**	**	**	**
Error	251						
Total	282						

See Table II and text for identity of prey odours.

\* $P < 0.05$ ; \*\* $P < 0.01$ .





**Figure 2.** Average chemoreceptive responses to five prey types by naive, newborn *T. elegans* and *T. sirtalis* from three regions of sympatry: (a) coastal California, (b) coastal Washington, and (c) inland California. The heights of the vertical bars indicate average chemoreception score (in number of tongue flicks/min) over two trials. The sources of test odours are distilled water (Control), slug, plethodontid salamander, toad tadpoles, fish and newt. Species identities of odour sources are given in Table II and in the text.  $N$  = the number of snakes in each sample. The significance levels for  $F$ -tests of population differences within each species are shown at the bottom of the figure: \* $P < 0.01$ ; \*\* $P < 0.001$ .

litters in average response to all prey odours except newt, while *T. sirtalis* showed significant ( $P < 0.01$ ) litter effects only for toad and fish odours.

Tukey's studentized range tests were computed in the ANOVAs just described to determine which localities differed in average chemoreception scores in each snake species. In *T. elegans* the coastal Washington and coastal California sites showed higher responses to slug odour than the inland California site. *Thamnophis elegans* from the inland

California and coastal Washington sites showed higher responses to toad odour than *T. elegans* from the coastal California site. Finally, with respect to fish odour, *T. elegans* from inland California showed a higher response than those from coastal California. *Thamnophis sirtalis* showed a geographical trend in responses to slug odour that was the opposite of the *T. elegans* pattern, with *T. sirtalis* from the inland California site showing a higher response than those from

the Washington and coastal California sites. Responses to salamander odour by snakes from the coastal and inland California sites were higher than those of snakes from the Washington site. In addition, the sample of *T. sirtalis* from the inland California site showed a higher response to fish odour than the Washington sample.

#### Feeding Tests

The feeding scores of both species showed bimodal distributions within populations (Figs 3 and 4). Each distribution consisted primarily of one group of individuals with high scores (8–10) and another group with low scores (0–1). For simplicity I will refer to snakes with scores equal to or greater than 5 as prey-eaters and snakes with scores less than 5 as prey-refusers.

Results of feeding tests of *T. elegans* on slugs showed pronounced geographical variation (Fig. 3) which paralleled the chemoreception results, with the coastal California sample showing a predominance of slug-eaters, the inland California sample showing a predominance of slug-refusers and the Washington sample showing an intermediate distribution ( $\chi^2 = 34.0$ ,  $df = 2$ ,  $P < 0.001$  for contrast between numbers of slug-eaters and slug-refusers among all three populations;  $\chi^2 = 11.1$ ,  $df = 1$ ,  $P < 0.001$  for coastal California versus Washington). Toad-eating in *T. elegans* predominated in all three regions without striking geographical differences, but the inland California sample showed a somewhat higher incidence of consistent toad-eaters.

Fish- and toad-eating snakes predominated in all the *T. sirtalis* samples (Fig. 4), and there was no clear evidence of geographical variation in feeding responses to either of these prey.

## DISCUSSION

#### Litter and Geographical Differences in Responses to Prey Odours

Trial differences and trial averages in chemoreceptive response showed striking differences in litter and geographical variation. Generally speaking, trial differences did not show litter, geographical or species differences (Table III). In contrast, average chemoreceptive responses generally did show litter, geographical and species differences (Table IV). Apparently, there is more genetic variation within populations underlying average response than there is underlying habituation.

Perhaps for this reason, habituation is less prone to geographical and species differentiation. The adaptive significance of habituation is also harder to understand.

The prevalence of litter differences within localities in the present study calls into question Burghardt's (1970b) conclusion that newborn *T. sirtalis* from the midwestern U.S.A. show geographical variation in prey responses. In that study three localities were represented by a total of four litters. Consequently geographical differences could not be separated from litter differences within localities. Similarly, Dix (1968) did not take litter differences into account in his comparisons of newborn *T. sirtalis* from Florida and Massachusetts. Thus, these studies of eastern *T. sirtalis* may have simply detected litter differences rather than true geographical differences.

#### Bimodality in Distributions of Feeding Scores

Ayres & Arnold (1983) observed striking bimodalities in the distributions of slug feeding scores in large samples of naive *T. elegans*. Arnold (1981a) presented evidence that such bimodality reflects a threshold response to slug odours. The present study revealed bimodality in feeding responses of *T. elegans* and *T. sirtalis* to anuran tadpoles, and of *T. sirtalis* to fish.

#### Potential Selection Pressures

The availability of prey at each site is an indication of the potential selection pressures acting on the two species. The most conclusive statement that can be made from a consideration of prey availability is that there is no selection favouring predatory response to plethodontid salamanders and newts at the inland site. Both of these prey types do not occur at that locality. Selection for response to slugs is weak or non-existent since those prey are rare and localized at the inland site. Selection may favour predatory response to other prey that are present at all the sites, but from prey availability alone it is difficult to predict whether selection is stronger at one locality than the others. Snake diets give a better indication of the actual pressures acting on the behavioural responses to prey types.

#### Selection Pressures Inferred From Snake Diets

The contrasting geographical differences in the diets of *T. elegans* and *T. sirtalis* suggest that selection is an important cause of species differences in chemoreception. In *T. sirtalis*, anurans dominate

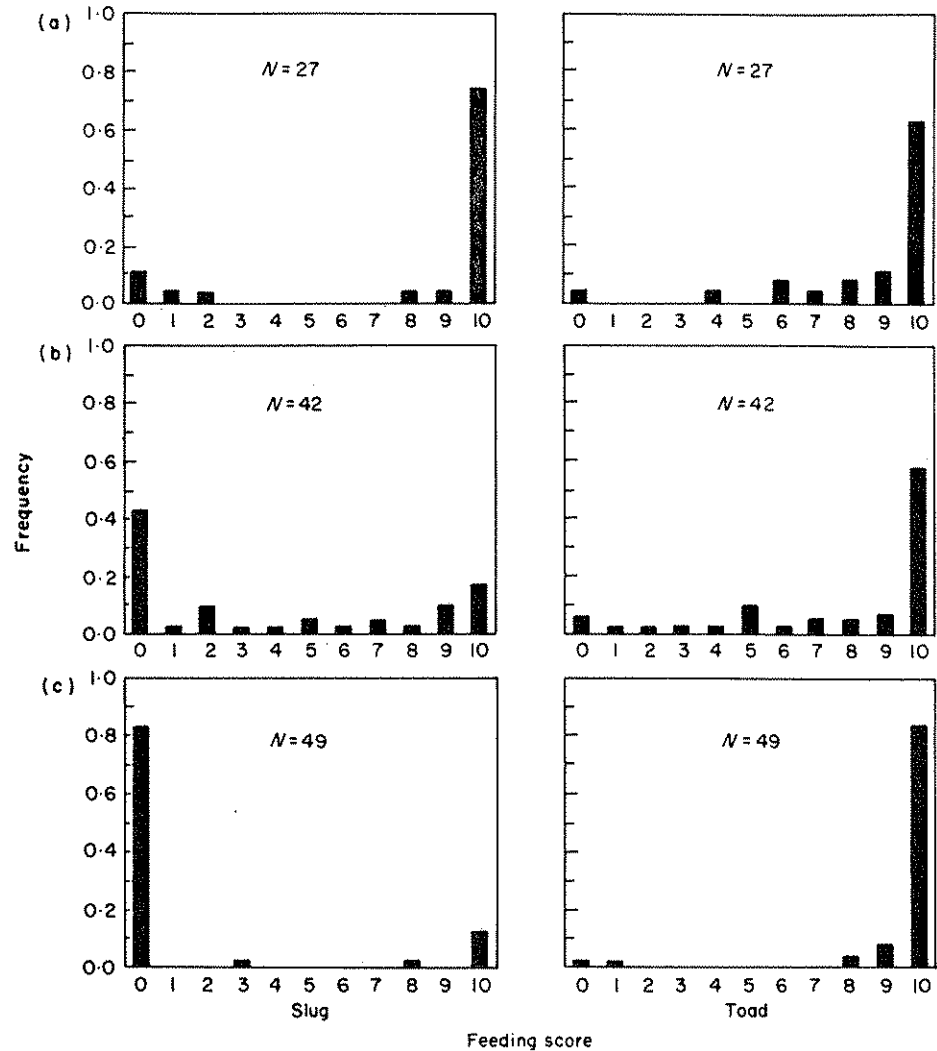


Figure 3. Feeding responses to slugs, *A. californicus*, and toad tadpoles, *B. boreas*, by newborn *T. elegans* from three regions: (a) coastal California, (b) coastal Washington, and (c) inland California. Small pieces of freshly thawed prey were presented. A score of 10 indicates consistent ingestion over 10 trials, while a score of 0 indicates persistent refusal.  $N$  = the number of snakes in each sample.

the diet at all three sites. Inland *T. sirtalis* may experience stronger selection for fish recognition, since fish are a relatively more important element in the inland diet. The incidence of minor elements of the diet (annelids and salamanders) is roughly similar at all the sites although the coastal California population might experience stronger selection for salamander recognition (Fig. 1). Thus geographical uniformity in diet suggests that *T. sirtalis* experiences a similar pattern of selection on most chemoreceptive responses at all three sites, but perhaps

experiences stronger selection for fish recognition at the inland site. In contrast, *T. elegans* shows striking geographical variation in diet that may reflect geographical differences in selection. Slugs dominate the diets of coastal California and Washington populations, suggesting strong selection for chemoreceptive recognition of slugs at those sites. In contrast, inland *T. elegans* experience virtually no selection for slug recognition. Although recent field work in the Eagle Lake region has turned up sparse populations of slugs (*Agriolimax* sp.) at

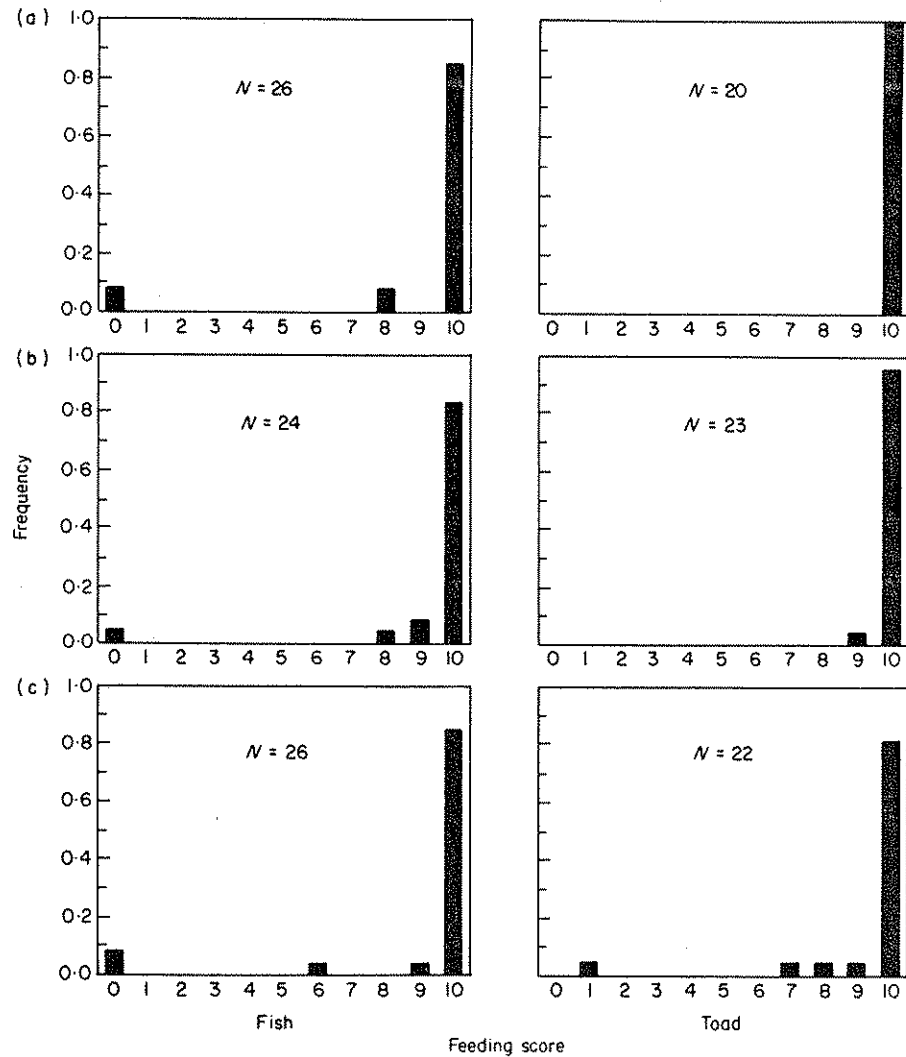


Figure 4. Feeding responses to fish, *Poecilia reticulata*, and toad tadpoles, *B. boreas*, by newborn *T. sirtalis* from three regions: (a) coastal California, (b) coastal Washington, and (c) inland California. Conventions as in Fig. 3.

two springheads, as well as a few stomach records for *T. elegans*, this arid inland site is outside the range of most slug species (Arnold 1981a). The other likely geographical difference in selection on prey recognition concerns fish. Fish are an important element in the diet of the inland population and perhaps in the diet of the Washington population as well (Fig. 1). Aquatic foraging by *T. elegans* was routinely observed at the inland California site, but never observed during several years of field work at the coastal California site. Similarly, drying pools with stranded fish were never observed at the

coastal California site. Thus, selection for chemo-receptive recognition of fish is probably strong at the inland California and Washington sites but non-existent at the coastal California site. The diet data also suggest that *T. elegans* might experience stronger selection for anuran recognition at the inland site.

Although the present results are an improvement on some past studies of geographical variation in garter snake predatory behaviour in which no studies of local prey availability and diet were conducted (Dix 1968; Burghardt 1970b), better data on

selection pressures are needed. It should be possible to score the predatory behaviour of large cohorts of neonates, mark and release these individuals and then recapture them so that their behaviour can be related to growth and survivorship in the field. Such a longitudinal study was attempted at the inland site with *T. elegans* but too few recaptures were made to permit estimates of selection.

#### Selection Inferred from Geographical Variation in Behaviour

The pattern of geographical variation in behaviour constitutes a third line of evidence for selection. We can ask whether geographical variation within species is in the same direction as geographical differences in selection that are inferred from prey availability and diet. The chemoreceptive responses of *T. elegans* to slugs, toad and fish are three cases in point. Geographical differences in these three patterns of behaviour (Fig. 2) parallel differences in selection inferred from diet (Fig. 1), suggesting evolutionary responses to selection. Similarly, in *T. sirtalis* geographical differences in chemoreceptive responses to salamanders and fish parallel differences in selection inferred from diet.

The only example of geographical variation in behaviour that is not consistent with differences in diet is the response of *T. sirtalis* to slugs. These prey did not occur in *T. sirtalis* diets at any of the sites, yet inland snakes showed a much stronger reaction to slug odour than snakes from the other two sites. In a study of *T. elegans*, Arnold (1981b) detected a genetic correlation between chemoreceptive responses to slugs and leeches. Inland *T. sirtalis* do prey on leeches (Fig. 1), so perhaps their higher reaction to slug odour represents a correlated response to selection for leech recognition. To put this explanation on a sound footing, however, we would first need to determine whether a genetic correlation between reactions to slugs and leeches prevails in *T. sirtalis* as it does in *T. elegans*.

Second, we can ask whether the two species differ in their patterns of geographical variation. The prediction that the two species should show similar responses to selection and hence, similar geographical variation, is based on the assumptions that: (1) the two species experience the same selection pressures at each site and (2) the mirroring of selection by evolved behaviour is not clouded by genetic constraints and drift. The fact that *T. elegans* and *T. sirtalis* do not show parallel geographical variation can probably be attributed to

species differences in the geographical pattern of selection. The most striking instance of non-parallel geographical variation is in chemoreceptive response to slug odour. In *T. elegans*, the coastal populations showed the strongest response to slug odour, whereas in *T. sirtalis*, the inland population showed the strongest response. In *T. elegans*, the pattern probably reflects a direct response to selection for slug recognition in coastal populations (where slugs are important prey), while the *T. sirtalis* pattern may reflect a correlated response to selection for leech recognition in the inland population. Two other sources of non-parallel geographical variation in these two species are their responses to salamanders (*T. sirtalis* showed geographical differences, *T. elegans* did not) and toads (geographical variation in *T. elegans* but none in *T. sirtalis*). These patterns are reflected in dietary differences and therefore probably represent species differences in selection. Although, the overall trend is for non-parallel geographical variation in the two snake species, it should be noted that in both species, the inland populations showed stronger responses to fish odour. That common pattern is reflected in the diets of both species, suggesting parallel geographical trends in selection for fish recognition.

The lack of geographical variation in behavioural responses to some prey probably reflects uniformity in selection rather than an absence of genetic variation in behaviour. Litter differences within populations for most prey responses were detected for both *T. elegans* and *T. sirtalis* in this study and in past studies of *T. elegans* (Arnold 1981b; Ayres & Arnold 1983). In this study the failure to detect litter differences in behavioural responses of *T. sirtalis* to slugs and salamanders, and in behavioural responses of both snake species to newt odour can probably be attributed to small sample sizes.

#### Chemoreceptive Response to Toxic Newts

Brodie & Brodie (1990, 1991) have recently studied geographical variation in the resistance of *T. sirtalis* to the skin toxin (tetrodotoxin) of the newt *T. granulosa*. Oregon populations of *T. sirtalis* are much more resistant to tetrodotoxin than Vancouver Island populations. Brodie & Brodie (1991) argue that this geographical difference reflects an 'arms-race' between predator and prey because Vancouver Island newts are also much less toxic than Oregon newts. While my

inland California site is outside the distribution of newts, *T. granulosa* does occur at both the coastal California and Washington sites. Nevertheless, I did not find newts in the diets of either *T. sirtalis* or *T. elegans* at either of these sites. However, the absence of newts from the diet cannot be taken as evidence of newt avoidance because diet sample sizes are relatively small. Despite these ecological unknowns, the present results complement Brodie & Brodie's findings by demonstrating litter, geographical and species differences in chemoreceptive responses to newt odour (Table IV).

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