Geographic Differences in Behavioral Responses of Hatchling Lizards (Eumeces okadae) to Snake-predator Chemicals

AKIRA MORI AND MASAMI HASEGAWA

Abstract: Antipredator and tongue flicking responses of hatchlings of Eumeces okadae were compared between Kozu-shima Island, where they co-occur with snake predators, and Hachijo-kojima, a snake-free island. In Experiment 1 lizards from both islands showed higher tongue flick rates to cotton swabs bearing snake and prey chemicals than to controls. Lizards from Hachijo-kojima emitted more tongue flicks to snake chemicals than those from Kozu-shima. In Experiment 2 lizards from Hachijo-kojima showed higher tongue flick rates to cotton swabs bearing non-saurophagous, allopatric snake chemicals than to control stimuli. Lizards from Kozu-shima exhibited tail wave display, which may deflect attacks to the autotemous tail, more frequently to saurophagous snake chemicals than to non-saurophagous snake and control stimuli, but there were no significant differences in tongue flick rates among the three chemicals for these lizards. In Experiment 3 lizards were introduced into an unfamiliar terrarium treated with snake chemicals. Lizards from Hachijo-kojima emitted more tongue flicks in cages chemically labelled by snakes than in control cages. No significant differences were observed in tongue flick rates between snake labelled and control cages in Kozu-shima lizards, and their tongue flick rates in snake labelled cages were significantly lower than those of Hachijo-kojima lizards. There were no differences in the frequency of tail waves, wall-climbing, movements, or immobility between snake labelled and control cages in lizards from both islands. Based on the higher tail wave frequency and lower tongue flick rates in Kozu-shima lizards than in Hachijo-kojima lizards, we hypothesize that lizards from Kozu-shima have evolved the ability to recognize chemical cues from snake predators after a few tongue flicks and that the higher tongue flick rates by lizards from Hachijo-kojima indicate less efficient recognition or lack of recognition of predator chemicals.

Key words: Eumeces okadae; Elaphe quadrivirgata; Geographic variation; Tongue flick frequency; Antipredator response

Recently, increasing attention has been paid to the importance of geographic variation in behavior to understand the evolutionary process of behavior (Foster and Endler, 1999). Although behavioral geographic variations are usually considered as consequences of adaptive diversifications, observed variations do not necessarily reflect the adaptive consequences in a given population because several factors limit the evolution of adaptive phenotypes (Riechert, 1999). The hierarchies of spatial scales of environmental variation, natural selection, and gene flow are primal factors that produce a variety of evolutionary outcomes (Thompson, 1999). In this regard, island populations are ideal to evaluate adaptive geographic variation because the hierarchies of the spatial scales are often explicit.

Antipredator behavior is a phenotype suitable to examine possible geographic variation. Survival of prey largely depends upon its ability to escape from predation by employing appropriate antipredator mechanisms (Edmunds, 1974; Endler, 1986). Among a variety of potential antipredator mechanisms, prey has to employ the most effective one against a given predator. To do this, an ability of accurate predator recognition by a prey is essential. Thus, natural selection should favor the development and maintenance of the ability to recognize potential predators and to exhibit appropriate antipredator behaviors under high predation pressure. If the kinds and intensity of predation pressure have been altered or relaxed under particular ecological conditions, prey species may modify or lose antipredator mechanisms (Blázquez et al., 1997; Downes and Shine, 1998; Griffiths et al., 1998; Coss, 1999; Margurran, 1999). Therefore, we can expect that the ability to recognize specific predators as well as to exhibit effective antipredator behavior will vary among different popu-

Accepted 30 Nov. 1999
lations of the same species under different predation regimes (Downes and Shine, 1998).

*Eumeces okadae* is an ideal subject for comparative study of geographic variation in antipredator behavior. The lizard is a diurnal, ground-dwelling scincid endemic to the Izu Islands, Japan. The island populations of *E. okadae* can be classified into three types based on their major syntopic predators, namely, birds, snakes, and carnivorous mammals (Hasegawa, 1994a, b). These populations exhibit divergence in life history traits, degree of sexual dimorphism, and mating system possibly as adaptive ecological consequences to different predation regimes (Hasegawa and Moriguchi, 1989; Hasegawa, 1994a, b). For example, populations with avian predators are late-maturing with biennial reproduction of large eggs, whereas those with ophidian predators are early maturing with annual reproductions of small eggs (Hasegawa, 1994a). With these backgrounds, it is likely that the ability of predator recognition and the antipredator behaviors of *E. okadae* have diverged among the populations with different predator fauna.

Chemical senses are critically important to many squamate reptiles, including *Eumeces*, in several ecological contexts such as foraging, social relationships, and predator avoidance (Halpern, 1992). For chemical discrimination and recognition, vomerolfaction, the sense supported by the vomeronasal system (Cooper and Burghardt, 1990), plays the most important role (Halpern, 1992). The ability of chemical detection and discrimination through the vomeronasal system has been evaluated by counting the number of tongue flicks emitted toward chemical stimuli (Cooper, 1998a) because the tongue acts as a chemical sampling device that retrieves chemicals from the external environment and transports them to the vomeronasal system (Halpern, 1992). In several species of *Eumeces*, especially those of New World, the ability of chemosensory recognition of prey, predator, and conspecifics has been experimentally demonstrated or suggested by showing differences in tongue flick rates (Cooper, 1990a; Cooper and Vitt, 1984a, b, 1986a, b, 1989).

In the present study, we examine the differences of predator recognition ability of *E. okadae* between island populations with and without a predator. Specifically, we compare the innate responses of tongue flick rates by the lizards to snake chemicals between the snake-present and snake-free islands. We also compare antipredator behaviors of the lizards between the islands.

**GENERAL METHODS**

**Animals and Their Maintenance**

The Izu Islands, consisting of nine inhabited islands, are located off the south coast of central Japan, linearly from north to south over a distance of approximately 230 km between 32°29' and 34°43' N (Fig. 1). Lizards were collected from two islands, Kozu-shima (18.6 km²) and Hachijo-kojima (3.1 km²), located at 34°12' N, 139°9'E and 33°6' N, 139°45'E, respectively. The major predator of *E. okadae* on Kozu-shima is a diurnal colubrid snake, *Elaphe quadrivirgata*, whereas a possible predator on Hachijo-kojima is a thrush, *Turdus celaenops* (Hasegawa, 1994a, b). No snakes exist on Hachijo-kojima (Hasegawa, unpublished data).

Gravid females of *Eumeces okadae* collected from the islands in May 1994 were reared in captivity until they laid eggs. For each island, twelve hatchlings of *E. okadae* randomly selected from two clutches were used for experiments.
The lizards were marked by toe-clipping and kept in two groups in plastic cages (23 x 15 x 13 cm). Lizards from the two islands were randomly assigned to each cage, and the cagemates were changed sporadically and haphazardly. This treatment was done to acclimate the lizards to both cages (see method section in Experiment 1). Each cage was provided with moistened sandy soil and dead leaves for substrate, a small stone for shelter, and a water dish. The cages were usually placed outdoors near the laboratory building of the Natural History Museum and Institute, Chiba. The lizards were fed mealworms (larvae of *Tenebrio molitor*), woodlice (*Porcellio scaber*; Isopoda), and land hoppers (*Paciforchestia* sp.; Amphipoda) every day.

**Data Analyses**

In the following three experiments, we examined the effects of chemical stimuli and islands on behavioral responses of lizards. For variables which had a high proportion of zero value, we used non-parametric tests because of extreme non-normality. In this case, Friedman two-way ANOVA or Wilcoxon signed ranks test was used for comparisons among chemical stimuli, and a two-tailed Mann-Whitney U-test was used for comparisons between islands (Siegel and Castellan, 1988).

For variables in which the proportion of zero values was not too high, we made repeated measures analysis following a Multivariate approach, MANOVA, using software by JMP (version 3), SAS Institute Inc. We examined the main effects of chemical stimuli (repeated measures), islands, and clutches (nested within islands), and effects of interaction between chemical stimuli and islands. For these analyses, data were first examined for heterogeneity of variance by Bartlet's tests. If the assumption of homogeneity of variance was not met, the data were transformed into common logarithm or square root, and heterogeneity of variance was re-examined. If this transformation did not satisfy the assumption, appropriate non-parametric tests were done to examine the effects of chemical stimuli and islands separately, and interaction was not examined.

The Tukey-Kramer test was used for multiple comparisons for parametric data. Multiple comparison tests preceded by Friedman two-way ANOVA were made following Siegel and Castellan (1988). When appropriate multiple comparison tests were unavailable, the level of statistical significance for individual comparison was calculated by the Bonferroni method (Sokal and Rohlf, 1995). We set an experimentwise error rate at 0.05 for each variable of each experiment.

**Experiment 1**

The aim of this experiment was to examine the behavioral responses of predator-naive hatchling lizards to snake chemicals and to compare the responses between Kozu-shima and Hachijo-kojima.

**Methods**

Preparation and presentation of chemical stimuli followed a well-established procedure employed for the examination of the ability of chemical discrimination by the vomeronasal organ in Squamata (Burghardt, 1970; Cooper, 1990b, 1998a). The following three chemical stimuli on cotton swabs were presented to the lizards: 1) Control stimulus: a cotton tip was dipped into distilled water and excess drops were flipped away. 2) Predator stimulus: the swab was placed in contact with the integument of *Elaphe quadrivirgata* collected from Kozu-shima, and rolled over the snake's external surface. 3) Prey stimulus: the swab was rolled over the external surface of a mealworm. A new stimulus was used for each lizard.

Before the experiment, lizards kept in one home cage were moved to the other cage and left undisturbed for 5 min. A lizard was then gently removed from the latter cage and placed into the former cage. Because all lizards were acclimatized to both cages, they readily responded to the stimuli without showing any exploring or freezing responses in the test cage. After terminating the trial, the tested lizard was replaced with another one. Two to three min after the introduction, the tip of a swab, to which a stimulus was applied, was presented 1–2 cm in front of the snout of the lizard, and the number of tongue flicks directed to the swab was counted for 60 sec or until the lizard bit the swab, whichever came first. The frequency of tail wave behavior was also recorded. Tail wave was defined as slow undulating movements of the entire tail or its posterior portion from side to side, continued for more than one sec. Tail wave (and tail wag or tail vibration) is considered an antipredator display of several species of lizards, although its exact functional mechanisms are controversial (Cooper and Vitt, 1985; Hasson et al., 1989; Mori, 1990; Magnusson, 1996; Cooper, 1998b). Each lizard was tested with the three stimuli in a counterbalanced sequence with an intertrial in-
terval of 1 min at the age of 12 or 13 days. Ambient temperature during the experiment was approximately 28°C.

Results

There were significant effects of stimuli and interaction on the number of tongue flicks (MANOVA with log transformed data, the former, Wilk’s $\Lambda=0.26$, $dF=2, 19$, $F=26.87$, $p<0.001$; the latter, Wilk’s $\Lambda=0.59$, $dF=2, 19$, $F=6.48$, $p<0.01$; Fig. 2). There were no significant effects of islands and clutches on the number of tongue flicks (the former, Wilk’s $\Lambda=0.95$, $dF=1, 20$, $F=1.00$, $p>0.05$, the latter, Wilk’s $\Lambda=0.86$, $dF=2, 20$, $F=1.66$, $p>0.05$). Lizards from Kozu-shima flicked the tongue less frequently in response to controls than to predator and prey chemicals (Tukey-Kramer tests, the former, $q=5.75$, the latter, $q=8.47$ [critical $q$ value=4.15], $p<0.05$). In lizards from Hachijo-kojima, the number of tongue flicks emitted in response to predator chemicals was significantly larger than those in response to controls and prey chemicals (the former, $q=9.14$, the latter $q=4.51$), and the number emitted in response to prey chemicals was significantly larger than in response to controls ($q=4.63$). Significant differences in tongue flick rates between the islands were detected only for predator chemicals: lizards from Hachijo-kojima flicked the tongue more frequently than those from Kozu-shima ($q=4.65$). Four and one lizards from Kozu-shima and Hachijo-kojima, respectively, bit the swab tip treated with a mealworm.

In the predator chemical condition, three and one lizards from Kozu-shima and Hachijo-kojima, respectively, exhibited tail wave. One lizard from each island showed tail wave in the prey chemical condition, and one from Kozu-shima waved its tail in the control condition. No lizards from Hachijo-kojima moved their tails in the control condition. There were no significant differences in the frequencies of tail wave between islands for any stimuli (U-tests, control, $z=1.00$, predator, $z=1.07$, prey, $z=0.00$, all $p>0.05$). There were also no significant differences in the frequencies of tail wave among stimulus conditions for either island (Friedman tests, Kozu-shima, $x^2=1.00$, $p>0.05$, Hachijo-kojima, $x^2=2.00$, $p>0.05$).

Discussion

Higher tongue flick responses to prey chemicals than to controls and the subsequent bites on the swab with prey chemicals indicate that the lizards are able to detect and discriminate the prey chemicals, presumably using the vomeronasal system. In addition, the results indicate that the experimental procedures widely used for squamate reptiles to examine chemosensory ability (Cooper, 1998a) are also valid for E. okadae.

When responding to snake chemicals, lizards from both islands showed higher tongue flick rates than to control stimuli, but lizards from Hachijo-kojima emitted more tongue flicks than those from Kozu-shima. Does this imply that lizards from Hachijo-kojima are able to recognize snake chemicals more efficiently? Before answering this question, we need to clarify whether the high tongue flick response of Hachijo-kojima lizards is a response specific to predator chemicals. The different responses to snake chemicals between the islands might reflect instead differences in responsiveness to unfamiliar, novel stimuli. The next experiment was conducted to examine this possibility.

**EXPERIMENT 2**

This experiment was conducted to examine the responses of lizards to chemicals from unfamiliar, novel stimuli. Because artificial chemicals such as cologne, which has been widely used as a control for odorant stimuli (see Cooper, 1998a for review), may not be an appropriate control (Dial and Schwenk, 1996; Cooper,
we did not use them. Alternatively, we used chemicals from a non-saurophagous snake allopatric with *E. okadae*, as an odorant, novel control stimulus.

Methods
Three chemical stimuli were presented. Control and snake predator stimuli were prepared in the same way as in Experiment 1. Snake predator chemicals were obtained from *Elaphe quadrivirgata* from Tadanae-jima Island (Fig. 1). Chemical stimuli from a non-saurophagous snake was obtained by rolling a swab over the external surface of a colubrid snake, *Rhabdophis subminiatus*. This species, predominantly feeding on amphibians and fish (e.g., Keng and Tat-Mong, 1989; Cox, 1991; David and Vogel, 1996), occurs widely in subtropical and tropical Asia, but does not occur in Japan. The room temperature ranged from 27 to 29°C during the experiment. Other experimental procedures and recorded variables were the same as in Experiment 1, except that the lizards were tested at the age of 30 or 31 days.

Results
There were significant effects of stimuli on the number of tongue flicks (MANOVA with log transformed data, Wilk's $\Lambda=0.51$, $dF=2$, 19, $F=9.19$, $p<0.005$; Fig. 3A). There were no significant effects of islands, clutches, or interaction on the number of tongue flicks (islands, Wilk's $\Lambda=0.97$, $dF=1$, 20, $F=0.70$, clutches, Wilk's $\Lambda=0.81$, $dF=2$, 20, $F=2.30$, interaction, Wilk's $\Lambda=0.91$, $dF=2$, 19, $F=0.89$, all $p>0.05$). Multiple comparison tests showed that lizards from Hachijo-kojima emitted tongue flicks significantly more frequently in response to non-saurophagous snake chemicals than to control stimuli (Tukey-Kramer test, $q=5.22$ [critical $q$ value=4.15], $p<0.05$).

The frequency of tail wave was significantly affected by chemical stimuli (MANOVA with square root transformation, Wilk's $\Lambda=0.68$, $dF=2$, 19, $F=4.43$, $p<0.05$; Fig. 3B). There were no significant effects of islands, clutches, or interaction on the frequency of tail wave (islands, Wilk's $\Lambda=0.97$, $dF=1$, 20, $F=0.63$, clutches, Wilk's $\Lambda=0.79$, $dF=2$, 20, $F=2.58$, interaction, Wilk's $\Lambda=0.81$, $dF=2$, 19, $F=2.27$, all $p>0.05$). Multiple comparisons revealed that lizards from Kozu-shima exhibited tail wave more frequently in response to saurophagous snake chemicals than in response to controls and non-saurophagous snake chemicals (the former, $q=5.33$, the latter, $q=4.17$ [critical $q$ value=4.15], $p<0.05$).

Discussion
Although statistical tests did not show significant interaction effects, response patterns were apparently different between the islands. Higher tongue flick response of Hachijo-kojima lizards to non-saurophagous snake chemicals, as well as to snake chemicals in Experiment 1, may suggest higher sensitivity to novel stimuli of the

![Chemical stimuli presented](image-url)
lizards than those of Kozu-shima. On the other hand, lizards from Kozu-shima frequently exhibited tail wave when exposed to sauropagous snake chemicals, implying the recognition of predator chemicals by the lizards. These different response patterns between the islands can be explained when we consider the differences of the functions of the behaviors: tongue flick is a behavioral attempt to obtain information about the external environment, whereas tail wave can be considered as a behavioral output directed toward predators (see General Discussion). On the other hand, compared to Experiment 1, tongue flick rates toward *Elaphe quadrivirgata* decreased in lizards from both islands. This may be due to habituation effects accompanying repeated testing (Burghardt and Schwartz, 1999).

**EXPERIMENT 3**

In this experiment we examined differences in other behavioral reactions between islands. Lizards were introduced into an unfamiliar terrarium treated with snake chemicals or into a control terrarium. In addition to tongue flicks and tail waves, three behavioral responses were examined and compared between the islands: escape response, number of movements, and immobility. Temporal changes of tongue flick rates was also examined over three consecutive minutes.

**Methods**

Snake terraria (200 × 150 × 80 mm) were unfamiliar cages in which a hatchling of *Elaphe quadrivirgata* had been kept for 11 to 13 days and removed immediately before the experiment. The snakes were hatched in captivity from the eggs laid by females collected from Tadanae-jima Island. The terrarium was made of polypropylene corrugated board and provided with paper floor covering and a small water dish, which were removed before the experiment. Blank, clean terraria, used as controls for responses to the experimental setting, were the same as the snake terrarium in material and size. No snake had been kept in the control terrarium for at least 10 months before the experiment.

A total of 48 cages (24 snake and control cages each) were prepared, and each cage was used for only one trial. Half of the lizards were tested in a snake cage first and the remaining were tested with a blank cage first for each island. Approximately 60 min separated snake and control tests for each lizard. The room temperature ranged from 27 to 29°C during the experiment. The lizards were tested approximately two hours after terminating Experiment 2.

In each trial, a lizard was gently removed from the home cage by hand and introduced into the experimental terrarium. The lizard was then left undisturbed, and the number of tongue flicks emitted were counted for three min by an experimenter positioned besides the terrarium. Behaviors were recorded by 8mm video camera-recorder, and the following four behavioral variables were recorded for each trial by video tape analysis:

1. **Tail wave**: the frequency of tail waves was counted.
2. **Escape response**: a lizard either approaches the wall of the cage by walk or jumps onto the wall, and stands in an upright position against the wall performing scratching movements with the forelegs. This behavior was considered as an attempt to escape from the cage. The frequency of such escape responses was counted.
3. **Number of movements**: the bottom of the cage was divided into eight equal sections, and the frequency of times a lizard changed sections was counted.
4. **Immobility**: when a lizard did not exhibit any movement of the body for more than 10 sec except for tongue flicks and breathing movements of the abdomen, we recorded that the lizard was immobile. The frequency of bouts of immobility was counted.

![Fig. 4. Mean number of tongue flicks performed in 180 s by hatchling *Eumeces okadae* from snake-present (Kozu-shima) and snake-free (Hachijo-kojima) islands in two experimental situations (Experiment 3). Bars indicate 1 SE. Identical alphabetical letters above the histograms denote values that do not differ significantly between the cages within each island. An asterisk above the histograms indicates significant differences between the islands.](image-url)
Movements and immobility were recorded to evaluate the propensity for spontaneous immobility, which is considered one of the antipredator responses of reptiles (Greene, 1988; Van Damme et al., 1990; Mori, 1991).

**Results**

Both islands and stimuli significantly affected the number of tongue flicks (MANOVA with square root data, the former, Wilk's $\Delta=0.49$, $dF=1$, 20, $F=20.85$, $p<0.001$, the latter, Wilk's $\Delta=0.65$, $dF=1$, 20, $F=10.72$, $p<0.005$; Fig. 4). Lizards from Hachijo-kojima flicked their tongue more frequently in snake cages than in control cages ($q=4.83$ [critical $q$ value $=3.77$], $p<0.05$), whereas those from Kozu-shima did not show significant differences between the cages ($q=1.46$). In snake cages, lizards from Hachijo-kojima emitted more tongue flicks than those from Kozu-shima ($q=7.04$). There were no significant differences in the number of tongue flicks performed in control cages between the islands ($q=3.67$). There were no significant effects of clutches and interaction on the number of tongue flicks, although the latter was marginally non-significant (the former, Wilk’s $\Delta=0.90$, $dF=2$, 20, $F=1.16$, $p>0.05$, the latter, Wilk’s $\Delta=0.85$, $dF=1$, 20, $F=3.48$, $p=0.077$).

To analyze the changing pattern of tongue flick rates, observation duration (180 sec) was equally divided into three time blocks (early, middle, and late 60 sec), and three-way repeated measures ANOVA was performed, with islands and cages as main factors and time blocks as repeated measures. Both main factors had significant effects on the tongue flick rates in snake cages (island, $dF=1$, 44, $F=24.83$, $p<0.0001$, cage, $dF=1$, 44, $F=8.21$, $p<0.01$; Fig. 5).

**Table 1.** Comparisons of behavioral responses of hatchling *Eumeces okadae* between snake-present (Kozu-shima) and snake-free (Hachijo-kojima) islands in two experimental situations (control and snake cages: Experiment 3). Figures in the Table are means ($\bar{x}$), standard errors (SE), and ranges of occurrence frequencies of each variable.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Cage</th>
<th>Kozu-shima</th>
<th>Hachijo-kojima</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$\bar{x}$</td>
<td>SE</td>
</tr>
<tr>
<td>Tail wave</td>
<td>snake</td>
<td>0.33</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Escape response</td>
<td>snake</td>
<td>1.08</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>1.58</td>
<td>1.00</td>
</tr>
<tr>
<td>No. of Movements</td>
<td>snake</td>
<td>23.8</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>28.1</td>
<td>7.2</td>
</tr>
<tr>
<td>Immobility</td>
<td>snake</td>
<td>2.75</td>
<td>0.48</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>2.00</td>
<td>0.41</td>
</tr>
</tbody>
</table>
declined over minutes. No significant effects were obtained in interactions (island vs time, \( dF=2, 88, F=1.67 \), cage vs time, \( dF=2, 88, F=1.40 \), island vs cage vs time, \( dF=2, 88, F=0.93 \), all \( p>0.05 \)).

Tail wave was exhibited only by a single lizard from Kozu-shima in a snake cage. There were no significant differences in the frequency of escape response between the islands in both snake and control cages, although the former was marginally non-significant (U-tests, the former, \( z=2.14, p=0.0162 \), the latter \( z=0.14, p>0.05 \), critical \( p \) value=0.0125 for multiple comparison; Table 1). There were no significant differences in the frequency of escape response between snake and control cages for both islands (Wilcoxon tests, Kozu-shima, \( z=0.11, p>0.05 \); Hachijo-kojima, \( z=1.89, p>0.05 \)).

There were no significant differences in the frequency of movements either between islands or between snake and control cages (the former, U-tests, snake cage, \( z=0.12, control, z=0.06 \), the latter, Wilcoxon tests, Kozu-shima, \( z=1.21 \), Hachijo-kojima, \( z=1.51 \), all \( p>0.05 \); Table 1). Neither main factors nor interaction significantly affected the frequency of immobility, although interaction was marginally non-significant (MANOVA, islands, Wilk's \( \Lambda=0.94 \), \( dF=1, 20, F=1.17, p>0.05 \), stimuli, Wilk's \( \Lambda=0.99 \), \( dF=1, 20, F=0.24, p>0.05 \), clutches, Wilk's \( \Lambda=0.78 \), \( dF=2, 20, F=2.83, p>0.05 \), interaction, Wilk's \( \Lambda=0.83 \), \( dF=1, 20, F=4.26, p=0.052 \); Table 1).

Discussion

Again, lizards from Hachijo-kojima showed higher tongue flick responses than those from Kozu-shima in tests with snake chemicals. On the other hand, there were no detectable differences between the islands in the frequencies of putative antipredator responses. Even tail wave frequency showed no island differences, but this is obviously due to low frequency of occurrence of this behavior under the present experimental conditions.

In the following section, we discuss the possible causes of the discrepancies in the response patterns between the islands, among the experiments, and between tongue flicks and the other behavioral responses.

**GENERAL DISCUSSION**

Consistently higher tongue flick responses of lizards from Hachijo-kojima to snake chemicals than those of Kozu-shima throughout the experiments indicate the repeatability of this trait, at least during the first month after hatching. One possible implication of this result is that lizards from Hachijo-kojima can discriminate and recognize snake chemicals more effectively than those of Kozu-shima. However, the results of antipredatory tail-waving suggest the opposite.

It has been demonstrated, both in snakes and lizards, that prey animals respond with higher tongue flick rates to chemicals of sympatric predators than to those of allopatric predators, non-predators, and/or control stimuli (Weldon, 1982; Thoen et al., 1986; Cooper, 1990a; Van Damme et al., 1990, 1995). A recent study on intraspecific variations of antipredator behavior also showed that lizards sympatric with a snake predator exhibit more tongue flicks toward the snake chemicals than those allopatric with the snake (Downes and Shine, 1998). Our findings differ from these studies in that lizards sympatric with a snake predator exhibit fewer tongue flicks to the snake chemicals than those allopatric with the snake. On the other hand, our results are similar to those of several studies that showed either the absence of differences in tongue flick responses between predator and control stimuli (Simon et al., 1981; Weldon et al., 1990; Phillips and Alberts, 1992) or lower responses to predator chemicals than to other stimuli (Chiszar et al., 1978; Dial et al., 1989).

Several factors may account for the different results among these studies. Interpretation of the functional significance of tongue flicking is complicated by the different experimental procedures. For instance, tongue flicks directed toward cotton swabs and those exhibited in empty cages may have different biological and contextual functions. Attempts to obtain information by tongue flicking may change throughout testing if the testing duration is long. Initial tongue flicks may be emitted to detect and identify the chemical stimuli, whereas subsequent flicks may be employed to obtain further detailed information such as the intensity or distribution of chemicals (Thoen et al., 1986). Comparisons among a variety of stimuli within a single population and comparisons among populations using the same stimuli may require different interpretations of the relative frequency of tongue flicks. Foraging tactics of predators (sit-and-wait or active foraging) may affect the reaction of prey animals to predator chemicals (Downes and Shine, 1998). Another complication is that some species may rely on nasal olfaction rather than vomeronasal for chemical detection (Dial et al., 1989; Dial and Schwenk, 1996). As critically reviewed by Cooper...
indicate the ability to rapidly recognize the relations. In other words, the lower tongue flicking with snake predators should recognize snake chemicals sooner than those in snake-free populations, necessary for accurate predator identification.

Reducing the amount of chemical information should improve predator recognition ability by animals suffering intensive predation pressure. A delayed reaction may lead to death. Thus, prey should adopt appropriate antipredator behavior as soon as possible because delayed reaction may lead to death. Therefore, we hypothesize that geographic differences in tongue flick rates of E. okadae reflect the adaptive response of Kozushima lizards to the snake predator and a reduced ability of snake recognition of Hachijo-kojima lizards. This hypothesis is supported by the evidence that Kozushima lizards exhibited antipredatory tail waving more frequently than Hachijo-kojima lizards.

However, higher tongue flick rates of Hachijo-kojima lizards over those of Kozu-shima lizards do not necessarily indicate an inability of predator recognition of the former. Indeed, higher tongue flick responses to snake chemicals than to control stimuli in Experiments 1 and 3 show that lizards of Hachijo-kojima detected the chemicals, and the higher number of tongue flicks in Experiment 1 to predatory snake stimuli than to either water or mealworm stimuli indicates that they discriminated among the stimuli. However, it is not certain whether the lizards recognized the snake stimuli or were merely investigating an unknown stimulus.

In Experiments 1 and 3 no geographic differences were detected in antipredator reactions. There are, at least, four possible, mutually not exclusive, explanations for the absence of differences. First, the concentration of chemicals contained in the swab or on the bedding materials may have been insufficient to induce the lizards to employ antipredator behaviors. Second, the test duration might not have been long enough to elicit antipredator reactions, although this is unlikely. Third, some of the behavioral variables in Experiment 3 may not have been adequate to evaluate the differences in antipredatory responses between the islands. In the brief trials, movements, wall-climbing, and immobility may be determined primarily by the novel setting and not reflect responses to chemical stimuli (Thoen et al., 1986). Detailed analysis with subdivision of moving patterns (such as crawl, slow walk, and walk [e.g., Thoen et al., 1986; Van Damme et al., 1995]) might reveal responses to predator chemicals, but preliminary observations do not seem to show any such responses.

Finally, occurrence of tail wave suggests that lizards of both islands retain essentially similar antipredator reactions. Both Eumeces okadae on the Izu Islands and its closely related species in the Japanese main islands, Eumeces latiscutatus (Kato et al., 1994), are sympatric with Elaphe quadrivirgata over most of their geographic ranges (Sengoku et al., 1996). As for Eumeces okadae, three island populations are free from...
any snake predator, and eight other populations are sympatric with *Elaphe quadri virgata* (Hasegawa, 1994a, b, Hasegawa and Moriguchi, 1989). This distribution pattern suggests that allopatry of *Eumeces okadae* with *Elaphe quadri virgata* is derived and that the lizards on the snake-free islands have experienced relaxation of selection by snake predation. It is generally supposed that under the relaxed selection, disintegration of phenotypes related to predator recognition and predation avoidance may occur (Coss, 1999). However, disintegration is not always observed, and behavioral characters are retained for long periods of time after selection has been relaxed. Although the lizards of Hachijo-kojima may have reduced ability to rapidly recognize snake chemicals, they may retain the antipredator reactions.

Two causal factors for the retention of ancient behavioral characters could be considered here. Gene flow is an agent that may reduce or eliminate fitness consequences of local natural selection on behavioral traits (Storfer and Sih, 1998; Thompson, 1999). Hachijo-kojima is located far south of other areas where *Eumeces okadae* occurs in sympatry with *Elaphe quadri virgata*. The closest sympatric area is Mikurajima island, located approximately 80 km north of Hachijo-kojima (Fig. 1). Overseas dispersal of *E. okadae* by rafting from such snake-present islands to Hachijo-kojima is unlikely because the current of this region (Kuroshio Current) flows from southwest to northeast, impeding the possibility of gene flow. The second possible explanation is that if the isolation of the populations has occurred recently in an evolutionary time scale, ancient behavioral patterns may still be retained even under relaxed selection. Given that *E. okadae* shows a variety of ecological and morphological differentiations among the Izu Islands (Hasegawa, 1994a, b), the time span seems to be long enough to allow the differentiation of behavioral characters.

Our hypothesis on the evolution of intraspecific variation of tongue flick frequency should be tested with multiple populations with and without predators. Geographic differences of antipredator reactions should be also examined to clarify whether geographic variation of tongue flick frequency is associated with that of antipredator responses.

**Acknowledgments.**—We are grateful to W. E. Cooper, Jr. and R. Shine for kindly reviewing the manuscript. This study was partially supported by a Grant-in-Aid from the Japan Ministry of Education, Science, Sports, and Culture to M. Hasegawa (A06740587).

**Literature Cited**


FOSTER, S. A. AND J. A. ENDLER. 1999. Geographic
DIAL, B. E., P. J. WELDON AND B. CURTIS. 1989. Olfaction and
DE FAZIO, A., C. A. SIMON, G. A. MIDDENDORF AND


Department of Zoology, Graduate School of Science, Kyoto University, Sakyo, Kyoto 606–8502, JAPAN (AM) and Department of Ecological Science, Natural History Museum and Institute, Chiba, Aoba-cho, Chiba 260–8682, JAPAN (MH)