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Responses of the lizard *Lacerta vivipara* to predator chemical cues: the effects of temperature

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Abstract. The thermal dependence of chemoreceptive and behavioural responses of the lizard *Lacerta vivipara* to chemical cues of the predatory snake *Vipera berus* were studied. The lizards were observed in cages that had previously been inhabited by a viper and their behaviour was compared with that of lizards in unfamiliar, but clean cages. The lizards' body temperatures were controlled by placing the cages in environmental chambers. Although tongue-flick rates were clearly temperature-dependent, snake chemicals elicited increased tongue-flick rates at all temperatures (20–35°C). Body temperature therefore seems to influence the intensity of chemosensory examination, but does not seem to affect its function within the temperature range considered. Snake chemical cues also induced a shift in general behaviour of the lizards. When confronted with the stimuli, they changed their normal behaviour (an alternation of activity bouts and basking) towards long periods of immobility, interrupted by jerky, hesitant walks. The effects of temperature on this reaction primarily concerned thermoregulatory behaviour: in the control experiments, basking was prolonged at low temperatures whereas in the viper experiments, it lasted longer at high temperatures.

Behavioural and physiological adjustments to changes in environmental temperature have been described for many diurnal reptiles (for a review see Avery 1982). However, the scarcity of studies examining the effects of temperature on important whole-animal functions thwarts the integration of thermal physiology and ecology (Huey & Stevenson 1979). Most existing studies have focused on sprint speed in lizards (e.g. Bennett 1980; Hertz et al. 1983; Crowley 1985; van Berkum 1986; Huey & Bennett 1987). Locomotion in lizards can be measured with relative ease and seems ecologically relevant, as many species rely on fast movements to catch prey or to avoid predatory attacks. However, only Christian & Tracy (1981) have actually demonstrated that temperature-induced variations in sprint speed influence the abilities of lizards to escape predation. We feel that several factors may obscure the assumed relation between sprint velocity and fitness, at least in some species. First, fast dashes are but a component of lizard foraging or escape behaviour. Second, lizards may use behavioural adjustments to compensate for the reduction in running abilities at suboptimal body temperatures (Rand 1964; Hertz et al. 1982; Crowley & Pietruszka 1983).

The reliability of predictions of ecological performance may thus benefit from studies of the thermal sensitivity of other components of anti-predator and foraging behaviour, and from the examination of possible overall shifts in such behaviour. Studies of the temperature dependence of perceptive functions related to ecologically relevant behaviours seem especially rewarding, as perception is of key importance in eliciting subsequent actions.

Lizards use their flexible, forked tongues to sample molecules in the surroundings and to deliver them to a second chemoreceptive centre (Jacobson's organ) in the roof of the mouth. Because of the ease of observation, tongue flicking allows one to estimate an animal's investigation of a stimulus source. Accordingly, the rate of tongue flicking has been used as a quantitative index of 'interest' in a variety of stimuli (for a review see Simon 1983). Tongue-flick rates are temperature-dependent in the skink *Eumeces laticeps* (Cooper & Vitt 1986) and the garter snake *Thamnophis sirtalis* (Stevenson et al. 1985).

In this paper, we report on the thermal

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dependence of chemoreceptive and behavioural responses towards snake chemical cues in the cool-temperate lizard *Lacerta vivipara* Thoen et al. (1986) showed that this lizard is able to detect chemicals from its natural predator, the common viper, *Vipera berus*. This can be inferred from a dramatic increase in the number of tongue extrusions, and from a general shift in behaviour in the presence of snake chemicals (Thoen et al. 1986). Such a response system, which consists of a readily quantifiable perception mechanism and easily recognizable elicited behavioural actions, provides a unique opportunity to study the effect of body temperature on the lizards' chemoreceptive abilities and on associated behavioural responses.

We address the following questions. (1) Is the lizard's chemoreceptive response to viper chemicals temperature-dependent? (2) Does the behavioural reaction to the chemicals change with body temperature?

METHODS

Animals and their Maintenance

Lacerta vivipara is a small (adult body length: 50–60 mm; mass: 3–4 g), ground-dwelling lizard that behaves like a typical heliotherm (Avery 1976). It most frequently inhabits well-vegetated and rather humid places. We caught lizards for our experiment in a patch of moist heathland in the Belgian national nature reserve 'de Kalmthoutse Heide' (Kalmthout (51°25'N, 4°25'E), province of Antwerp, Belgium). Field body temperatures of active lizards at this site averaged ($\pm$SD) 29·9$\pm$3·51°C (N=1063, range: 16·2–37·8), but varied considerably seasonally (Van Damme et al. 1987). In a thermogradient study, *L. vivipara* selected body temperatures between 24 and 38°C ($\bar{X}\pm$SD=32·1$\pm$2·34, N=1645; Van Damme et al. 1986).

We conducted experiments on 10 adult male lizards. They were housed in soil-filled plastic terraria containing heather and mosses. A 75-W bulb suspended 20 cm above the containers provided heat and light for 8 h a day. Food (crickets and mealworms) and water were provided ad libitum. All lizards were tested within 2 weeks of their capture.

A male common viper was caught at 'het Groot Schietveld' (51°20'N, 4°30'E), ca. 15 km from the site where the lizards were captured. This snake preys on *L. vivipara* (Pielowski 1962; Presst 1971) and is sympatric with this lizard over large parts of Western and Northern Europe, but is absent at our lizard-collecting site.

Experiments

Experiments were performed between 4 and 24 September 1986. Individual lizards were introduced into two differently treated terraria (50 × 50 cm; substrate consisting of soil and mosses): (1) Control: an unfamiliar, but otherwise untreated terrarium; (2) Viper: an unfamiliar terrarium in which the viper was housed during the night, but removed immediately before an experiment.

For both treatments, we tested each individual lizard once at each of five different temperatures (20, 27·5, 30, 32·5, 35°C), which span the range of body temperatures when the lizard is active. Tests were run in a randomly selected order of temperature and experimental treatment; only one series of tests (treatment/temperature) was performed daily. To control the lizard's body temperature, we placed the terraria in small environmental chambers. A 100-W heat-bulb, mounted in the roof of these chambers, provided heat. Thermostats (precision 0·3°C), connected to thermistors situated in the centre of the terraria, controlled temperature within the chambers. We calibrated the termostats prior to an experiment by monitoring the body temperatures of dead lizards placed in the terraria with an electronic thermometer (precision 0·1°C). A 20-W cold fluorescent lamp provided illumination in the chamber. We observed the lizard's behaviour through a one-way mirror on the front of the chamber.

In each test, we observed continuously the lizard's behaviour for 10 min, starting immediately after its introduction into the terrarium. A detailed and continuous recording of the behaviour was obtained through the use of a GWBASIC-program, run on a personal computer. We distinguished between the following behavioural acts (see also Thoen et al. 1986).

(1) Tongue flicks: the lizard extrudes and rapidly retracts its tongue, regardless of whether the tongue touches the substrate or is 'waved' in the air.

(2) Walk: continuous, relatively fast, forward movement.

(3) Slow motion: slow stalking movement.

(4) Run: very fast movement, often over only a short distance.

(5) Bask: the lizard rests under heat bulb with the ribs spread laterally.

(6) Stand up: the lizard stands upright against the wall of the terrarium and performs scratching movements with the forelegs.

(7) Dig: the lizard burrows a (shallow) hole in the substratum of the terrarium, using its forelegs (this behavioural act was not reported by Thoen et al 1986).

(8) No move: lizard does not move and does not spread its ribs laterally as when it is basking

We recorded the number of tongue flicks and the duration of the other behavioural acts for the total course and for the different 1-min intervals of each test.

Statistical Analysis

The duration of the distinct behavioural acts and the number of tongue flicks in different temperature and experimental treatments (Control/Viper) were compared using two-way analyses of variance To quantify the overall behavioural responses to the experimental situations, we performed a principal component analysis on the individual scores for each behaviour We tested for differences between experimental and temperature treatments and individual lizards, using three-way analyses of variance on the projections of the individual scores on the first three principal axes. Temporal changes in tongue-flick rates and the duration of the behavioural acts within experimental situations were examined by regressing mean number of tongue flicks per min against time The resulting slopes were compared between temperature treatments by analysis of covariance (ANCOVA).

RESULTS

Tongue Flicking

Tongue-flicking rates are clearly temperature-dependent (Table I). In both the Control and Viper situations, the number of tongue flicks per 10-min observation period increased with temperature At all temperatures, tongue-flick rates were higher in the Viper situation than in the Control experiment (Table I).

In some of the experimental situations, tongue-flick rates obviously changed during the course of the observation period (Fig. 1). In the Control situation, there was a significant negative linear relation between the number of tongue-flicks per 1-min interval and time at 27 5, 30, 32 5 and 35°C. At these temperatures, the rate of decline was similar (ANCOVA, $F=15\,9$, $df=3$, $P>0\,10$). In the 20°C Control experiment, tongue-flick rates were invariant throughout the observation period. In the Viper situation, the number of tongue flicks declined significantly with time at 32 5 and 35°C, but not at the lower temperatures.

Not all behaviour was attended by the same tongue-flick rate (Table II). The lizards extruded their tongue more often when moving (Walk, Slow motion) than when sitting still (No-move, Bask). Hence, one could argue that the observed thermal dependence of tongue-flick rate merely results from differences between temperatures in the duration of the distinct behavioural acts (see further) However, tongue flicking during Slow motion, Walk and No move was temperature-dependent (ANOVA, all $P<0\,01$).

Behaviour

The lizard's behaviour consisted basically of an alternation of resting (No move), basking and moving (Walk, Slow motion). The No move act lasted consistently longest in the Viper terrarium ($P<0\,001$), but did not change with temperature (Table I) The total duration of the time the lizards spent moving (Slow motion and Walk) differed between experimental treatments (two-way ANOVA, $P<0\,001$), but not between temperatures ($P>0\,2$). At most test temperatures, the lizards were more mobile in the Control than in the Viper situation; the only exception was the 20°C-test, in which the lizards moved more in the Viper situation. Although temperature did not affect the summed duration of Walk and Slow motion, there were obvious effects of temperature and cage treatment on the way in which the lizards moved Slow motion was rarely seen in the Control terrarium, but occurred far more often in the Viper experiments, especially at high temperatures. The Run behaviour was seldom observed. Stand up and Dig are two behaviour patterns typical for lizards that are placed in unfamiliar terraria (R. Van Damme, D. Bauwens, D. Vanderstighelen & R. F. Verheyen, unpublished data); their duration decreased in the presence of snake chemicals (Table I).

The most striking result was the combined effect of temperature and experimental treatment on basking duration. In the Control tests, basking was

Table I. Duration (s/10 min) of distinct behavioural acts and frequency of tongue flicks (number/10 min) of *Lacerta vivipara* in different experimental (Viper/Control) and temperature treatments ($\bar{X} \pm$ SE, $N = 10$)

	Control situation					Viper situation					Effect of		
Behaviour	20°	27·5°	30°	32·5°	35°	20°	27·5°	30°	32·5°	35°	Exp. × Temp.	Exp.	Temp.
No move	138±14	121±19	138±17	124±26	115±18	253±28	306±34	261±34	292±44	254±23	NS	**	NS
Walk	281±18	280±66	322±42	295±60	331±60	187±36	131±37	133±39	54±21	56±15	**	**	**
Slow motion	0± 0	0± 0	0± 0	5±10	0± 0	117±39	104±40	137±36	154±30	189±36	**	**	**
Run	0± 0	0± 0	1± 0	0± 0	6± 2	3± 2	0± 0	1± 0	0± 0	0± 0	**	NS	**
Bask	125±20	90± 1	38±11	93± 7	39±14	0± 0	34±19	51±27	84±31	73±30	**	**	**
Stand up	40± 6	28± 6	69± 9	45±10	67±11	31±10	24± 9	14± 6	14± 8	14± 8	**	**	*
Dig	14± 7	80± 3	28± 1	36± 5	36± 1	9± 5	0± 0	3± 3	1± 1	12± 4	**	**	**
Tongue flicks	76± 9	120± 9	117± 7	181±12	160±12	184±19	185±24	287±21	292±30	309±25	**	**	**

Three-way ANOVA: $*P < 0{\cdot}01$; $**P < 0{\cdot}001$.

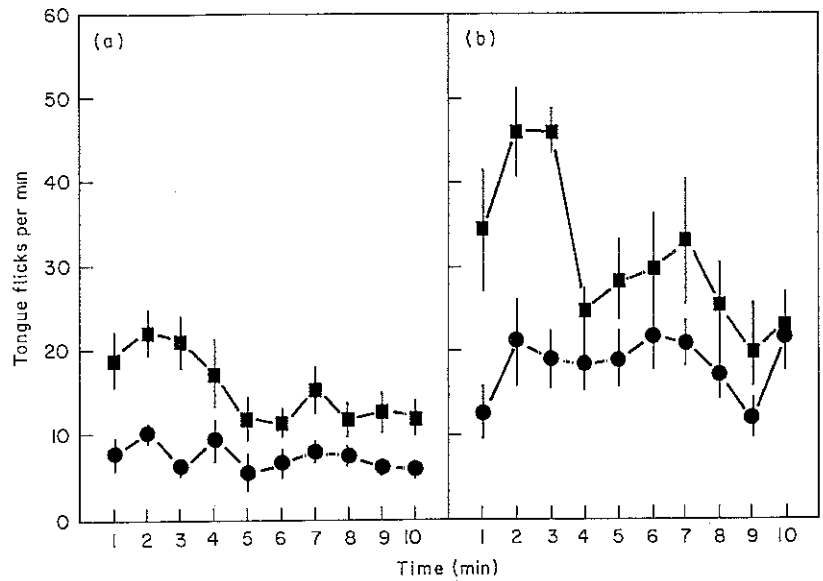

Figure 1. Tongue-flick frequency (number per min ± SE) during the course of the observation period at two distinct body temperatures (●: 20 and ■: 35°C). (a) Control situation; (b) viper cues present

Table II. Tongue-flick frequencies (number/s; $\bar{X} \pm$ SE) associated with distinct behavioural acts

	Control situation		Viper situation	
Behaviour	20°C	35°C	20°C	35°C
No move	0·10±0·02 (9)	0·15±0·02 (10)	0·13±0·02 (10)	0·37±0·07 (10)
Walk	0·20±0·02 (10)	0·35±0·02 (10)	0·44±0·04 (9)	0·83±0·11 (9)
Slow motion	*	*	0·54±0·07 (8)	0·97±0·06 (10)
Run	0·00±0·00 (3)	0·06±0·05 (8)	0·03±0·03 (3)	*
Bask	0·04±0·01 (10)	0·20±0·07 (8)	*	0·15±0·11 (9)
Stand up	0·11±0·03 (10)	0·21±0·04 (10)	0·25±0·08 (8)	0·43±0·15 (8)
Dig	0·02±0·01 (8)	0·09±0·03 (9)	0·04±0·04 (3)	0·09±0·06 (5)

The number of individuals that exhibited the specific behavioural act is shown in parentheses.
*Behaviour not observed.

prolonged at low temperatures and declined at higher temperatures. In the Viper experiments, in contrast, basking was completely abandoned at 20°C but became gradually more important at more elevated temperatures.

The duration of Slow motion per 1-min interval declined throughout the course of the Viper tests at all but the lowest temperature. The mean duration of Bask in the Viper tests increased over the observation period at 30, 32·5 and 35°C, but not at the lower temperatures.

We used principal component analysis to quantify overall behaviour in distinct experimental treatments. In this analysis, the raw data were the total duration of each behavioural act during the 10-min course of the individual tests. The five extracted principal axes accounted for 41·6, 20·2, 13·9, 10·9 and 8·23% of the total variation. The alignment of the tests along the component axes was studied by considering their projections on these axes (Fig. 2).

The first principal component was positively correlated with the total duration of No move ($r=0·72$, $P<0·001$) and Slow motion ($r=0·82$, $P<0·001$) and negatively with Walk ($r=-0·92$, $P<0·001$) and Stand up ($r=-0·68$, $P<0·001$). The scores on the first principal component differed according to experimental treatment (three-way

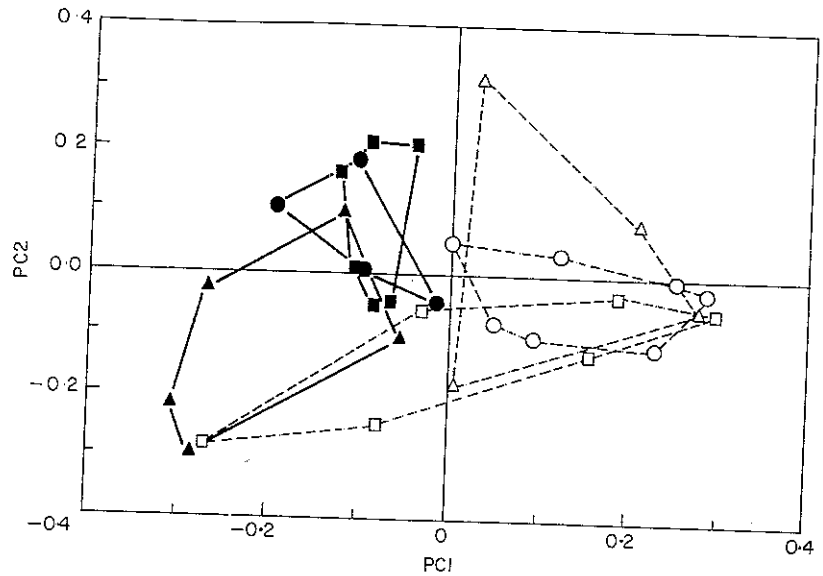

Figure 2. Projections of the behavioural scores in the individual tests on the PC1, PC2 space obtained by a principal component analysis. For each experimental/temperature treatment shown, minimum convex polygons were constructed by connecting extreme projections; projections situated within the polygons are not shown. □, ○, △: Viper situation; ■, ●, ▲: Control. □, ■: 20°C; ○, ●: 27·5°C; △, ▲: 35°C.

ANOVA, $P<0{\cdot}001$) and to the test temperature ($P<0{\cdot}05$). No consistent behavioural differences between individuals were detected ($P>0{\cdot}5$). The Viper tests scored high on the first component axis, which means that the lizards showed more of the No move and Slow motion behaviour, and less of the Walk and Stand up behaviour. The Control tests had low values. The 20°C-tests scored low relative to other temperatures in both Viper and Control situations (Fig. 2).

Bask was the only behaviour that correlated with the second principal axis ($r=0{\cdot}89$, $P<0{\cdot}001$). In the Control situation, high temperature tests had low scores (i.e. the lizards basked little), whereas low temperature tests had high scores (the lizards basked more). In the Viper situation, the opposite was found: high temperature tests produced high scores, low temperature test projections were low (Fig. 2). The position of the projections on the remaining axes did not differ between stimuli or temperature situations.

DISCUSSION

Our results confirm the findings of Thoen et al. (1986) that *L. vivipara* detects chemicals from its predator, the viper. This was evidenced by (1) an important increase in tongue-flick rates and (2) a general shift in behaviour in the Viper trials.

Temperature altered the lizards' responses to snake chemicals in a quantitative rather than in a qualitative way. Tongue-flick rates were clearly temperature-dependent in both Control and Viper situations. However, viper chemicals elicited an obvious increase of tongue-flick rates at all temperatures, indicating that even at lower body temperatures, lizards detected the presence of snake chemicals. Body temperature hence influences the intensity of chemosensory examination, but it does not seem to affect its function, at least not within the range of body temperatures used in our study. A study of the thermal dependence of hearing in lizards (Werner 1972) provided analogous conclusions: although cochlear potentials and evoked responses in the brain are clearly affected by temperature, no conclusive evidence on the thermal dependence of actual 'hearing' has been published.

The lizards' behavioural responses to viper chemicals were also temperature-dependent. When confronted with snake chemicals, they changed their 'normal' behavioural pattern, an alternation of activity bouts (Walk), resting (No move) and basking, towards a pattern that was characterized by long periods of immobility (No move), interrupted by jerky, hesitant walks (Slow motion).

Temperature effects on this reaction primarily concerned thermoregulatory behaviour In the Control situation, the lizards basked more frequently and for longer periods at low temperatures This is in general agreement with observations on other heliothermic lizards and ectotherms (Dreisig 1984). In the Viper experiments, we found the reversed situation: lizards abandoned basking at low temperatures and resumed thermoregulatory behaviour at high temperatures. We can think of two, not mutually exclusive, hypotheses that may explain the observed behavioural shifts One is purely mechanistic, the other has a more functional basis. Both hypotheses assume that the lizards associate the detection of viper chemicals with the nearby presence of this predator.

(1) Body temperature may affect the lizards' perception of the environment, through its influence on different components of the neural system and on central nervous coordination (Prosser & Nelson 1981). At higher body temperatures, the lizards should be able to explore the experimental cage more adequately, and to perceive more quickly that no predator is near by. They therefore resume more rapidly their 'normal' behaviour. Three observations seem to support this hypothesis. (1) Viewed over the 10-min observation course, the mean number of tongue flicks per 1-min interval declined more rapidly at higher temperatures. We noted a significant temporal decline in the number of tongue flicks at most temperatures in the Control situation, but only at 32 5 and 35°C in the Viper situation. (2) The duration of the Slow motion behaviour decreased in time at all but the lowest temperature. (3) In the Viper terraria, the lizards resume basking sooner at high temperatures

(2) The duration of Basking may be mediated by a compromise between the costs and benefits of thermoregulatory behaviour (Huey & Slatkin 1976) In the assumed presence of a predator, basking at low temperatures may not be rewarding, as the costs (being conspicuous) do not meet the benefits (increased fleeing abilities) Lizards that maintain relatively high body temperatures need less time to raise their temperatures to levels that are optimal for sprinting and/or they may feel more 'confident' to escape possible attacks.

Our results suggest that the relation between temperature and responses to predator stimuli is not a straightforward one We feel that fitness predictions based on one component of a lizard's escape behaviour (e g sprint velocity) may be premature, at least in some species and/or situations. If feasible, a field study of the anti-predator behaviour and escape performance at different body temperatures should be conducted to establish such a relation.

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