

Heat in evolution's kitchen: evolutionary perspectives on the functions and origin of the facial pit of pitvipers (Viperidae: Crotalinae)

Aaron R. Krochmal^{1,*}, George S. Bakken^{1,†} and Travis J. LaDuc^{2,‡}

¹Department of Life Sciences, Indiana State University, Terre Haute, IN 47809, USA and ²Section of Integrative Biology, University of Texas, Austin, TX 78712, USA

*Author for correspondence at present address: Department of Natural Sciences, University of Houston – Downtown, One Main Street, Houston, TX 77002, USA (e-mail: KrochmalA@uhd.edu)

†Present address: Department of Ecology and Organismal Biology, Indiana State University, Terre Haute, IN 47809, USA

‡Present address: Texas Natural History Collections, Texas Memorial Museum, 10100 North Burnet Road, PRC 176/R4000, Austin, TX 78758, USA

Accepted 3 September 2004

Summary

Pitvipers (Viperidae: Crotalinae) possess thermal radiation receptors, the facial pits, which allow them to detect modest temperature fluctuations within their environments. It was previously thought that these organs were used solely to aid in prey acquisition, but recent findings demonstrated that western diamondback rattlesnakes (*Crotalus atrox*) use them to direct behavioral thermoregulation, suggesting that facial pits might be general purpose organs used to drive a suite of behaviors. To investigate this further, we conducted a phylogenetic survey of viperine thermoregulatory behavior cued by thermal radiation. We assessed this behavior in 12 pitviper species, representing key nodes in the evolution of pitvipers and a broad range of thermal environments, and

a single species of true viper (Viperidae: Viperinae), a closely related subfamily of snakes that lack facial pits but possess a putative thermal radiation receptor. All pitviper species were able to rely on their facial pits to direct thermoregulatory movements, while the true viper was unable to do so. Our results suggest that thermoregulatory behavior cued by thermal radiation is a universal role of facial pits and probably represents an ancestral trait among pitvipers. Further, they establish behavioral thermoregulation as a plausible hypothesis explaining the evolutionary origin of the facial pit.

Key words: pitviper, facial pit, behavioral thermoregulation, thermal detection, supranasal sac, snake.

Introduction

Senses are typically viewed as generalized and serving multiple functions (e.g. Schnitzler et al., 2003). For example, vision plays a key role in general navigation as well as in the acquisition of both prey items and potential mates. However, studies of the eponymous facial pit of pitvipers (Viperidae: Crotalinae) have focused on predation to the near exclusion of more generalized functions. One of the key features of the formalized hypothetico-deductive method known as 'strong inference' is the generation and testing of all possible hypotheses to avoid the fallacy of affirming the consequent (Platt, 1964). Important functions may be ignored by investigators focused on a single hypothesis, particularly when dealing with complex problems in behavior and evolution, and this appears to be the case for the facial pits.

The facial pits are located midway between the eye and nostril on either side of the head. These organs respond to emitted thermal radiation and thus allow the snake to detect environmental temperatures from a distance (Bullock and Diecke, 1956; de Cock Buning, 1983). Facial pits have been well documented as aids in both prey detection and strike

direction (Noble and Schmidt, 1937; Bullock and Diecke, 1956; de Cock Buning, 1983; Kardong and Mackessy, 1991; Kardong, 1992; Kardong and Berkhoudt, 1999). Consequently, most authors consider them to be specialized prey acquisition aids that lack additional utility.

However, this ability to detect modest fluctuations in emitted thermal radiation could be used as part of a generalized sense used to examine variation in surrounding surface temperature (Bullock and Diecke, 1956; Goris and Nomoto, 1967; Greene, 1997). In addition to predation, proposed uses of a generalized thermal radiation sense include behavioral thermoregulation (Bullock and Barrett, 1968), predator detection (Greene, 1992) and den site selection (Sexton et al., 1992). Of these, only thermoregulation has been tested experimentally. Using a variety of arenas in which thermal radiation was the only available cue, Krochmal and Bakken (2003) showed that western diamondback rattlesnakes (*Crotalus atrox*) could make correct thermoregulatory decisions when their facial pits were functional but lost this ability when their facial pits were blocked. These results demonstrate that information from the

facial pits can direct thermoregulation and support the hypothesis that facial pits serve a generalized sensory function.

Krochmal and Bakken (2003) further noted that *C. atrox* is a derived North American pitviper that inhabits the American Southwest. There, surface temperature shows large daily variations and can be lethally high (50–70°C) for several hours each day (G.S.B., unpublished data). Clearly, such microclimates can create selective pressures favoring quick and efficient detection of thermal refugia (Huey, 1982, 1991). However, other species of pitviper typically inhabit more benign thermal environments where selective pressures for efficient thermoregulation are less evident. For example, Wills and Beaupre (2000) showed that timber rattlesnakes (*Crotalus horridus*) in the Ozark Mountains never experience operative temperatures during the active season that are so extreme as to drive them underground. Accordingly, the ability to make thermoregulatory decisions in response to thermal radiation detected by the facial pits might be present only in species that are frequently exposed to high temperatures, such as *C. atrox*.

These studies raise several questions. (1) Although all pitvipers can detect thermal radiation, is the use of this information to direct thermoregulatory behavior an adaptation to hot environments or is it common to all pitvipers? (2) If the use of thermal radiation to guide thermoregulation is not universal among pitvipers, is it restricted to particular clades or (3) has it evolved repeatedly in distant lineages in response to recent selective pressure? (4) Is the facial pit a generalized sense organ or does it typically serve a single function? (5) If the facial pit routinely serves multiple functions, which best represents the selective force that initially drove its evolution? (6) What might intermediate forms between cutaneous thermoreceptors and the facial pit have looked like?

To begin to answer these questions, we looked for major phylogenetic and ecological trends in the presence or absence of a thermoregulatory response to thermal radiation within the family Viperidae. We focused on the pitvipers, as they all possess a highly refined thermal radiation sensor and form a taxonomically and ecologically diversified group. Additionally, we included a single species of true viper (Viperidae: Viperinae), a closely related subfamily that lacks facial pits. Breidenbach (1990) reported that two true vipers, the puff adder (*Bitis arietans*) and the Russell's viper (*Daboia russelii*), preferentially strike at warm targets, suggesting that these (and perhaps other) true vipers possess a thermal radiation sensor. The identity of this sensor is not obvious, but neurological and histological studies suggest that the supranasal sac, an organ common to all true vipers, might be a thermal radiation receptor (York et al., 1998).

In the present study, we examined 12 species of pitviper and a single species of true viper for the presence or absence of behavioral thermoregulation mediated by thermal radiation. We chose species that represented both key nodes in the evolution of pitvipers and a broad range of thermal environments. We used a simple Y-maze test (Krochmal and Bakken, 2003) to determine whether animals possessed the ability to direct thermoregulatory movements using only

thermal radiation cues. Practical limits on the number of individuals of each species that could be obtained and tested prevented us from using quantitative differences in performance to detect subtle ecological and taxonomic patterns. However, by sampling widely both taxonomically and ecologically, the presence or absence of this behavioral ability can disclose any major ecological or phylogenetic trends and provide direction for more detailed studies.

Materials and methods

Study animals

We selected 12 species of pitvipers on the basis of current phylogenetic hypotheses for extant pitviper taxa (LaDuc, 2003; Parkinson et al., 2002). We included eight North American pitvipers: the copperhead [*Agkistrodon contortrix* (Linnaeus 1766); $N=3$], the cottonmouth [*Agkistrodon piscivorus* (Lacépède 1789); $N=5$], the western massasauga [*Sistrurus catenatus* (Rafinesque 1818), $N=3$], the rock rattlesnake [*Crotalus lepidus* (Kennicott 1861); $N=5$], the ridgenosed rattlesnake [*Crotalus willardi* Meek 1905; $N=4$], the timber rattlesnake (*Crotalus horridus* Linnaeus 1758; $N=3$), the western rattlesnake (*Crotalus viridis* (Rafinesque 1818); $N=5$) and the blacktailed rattlesnake (*Crotalus molossus* Baird and Girard 1853; $N=4$). In addition, we sampled two South American lineages by including the urutú (*Bothrops alternatus* Duméril, Bibron and Duméril 1854; $N=5$) and the eyelash palm pitviper [*Bothriechis schlegelii* (Berthold 1846); $N=3$]. Finally, we included a member of the most basal pitviper clade, the Malayan pitviper [*Calloselasma rhodostoma* (Kuhl 1824); $N=4$], and a member of the sister taxon to New World pitvipers, the mamushi [*Gloydius blomhoffii* (Boie 1826); $N=4$]. Together with published data on the western diamondback rattlesnake (*Crotalus atrox* Baird and Girard 1853) (Krochmal and Bakken, 2003), these species represent all three North American genera and sample the deep nodes in the pitviper tree as well as more recent diversification events (Fig. 1). The focal pitviper species typically inhabit a wide variety of habitats, including deserts, grasslands and brush, mixed deciduous woodlands, mountains, tropical forests and swamps.

As our representative true viper, we selected the puff adder [*Bitis arietans* (Merrem 1820); $N=5$]. Puff adders are one of the true vipers reported to preferentially strike at warm targets (Breidenbach, 1990). They also frequent hot environments, and thus one might expect puff adders to direct thermoregulatory behavior using thermal radiation cues as do *C. atrox*.

Captive housing and maintenance routines conformed to established safety protocols for venomous snakes (Gans and Taub, 1964). All animals were housed individually in glass aquaria lined with newspaper and were provided with water and a shelter box. Snakes were maintained on a diet of laboratory mice on an irregular feeding schedule and were housed on a 12 h:12 h light:dark cycle for between 6 months and 10 years before experimentation began. Apart from *A. piscivorus*, all North American pitvipers were maintained at a

constant temperature (27°C); *A. piscivorus* and all additional species tested were housed under a 12 h:12 h light:dark and 30°C:23.5°C temperature cycle. All North American pitvipers, excluding *C. willardi* and *A. piscivorus*, were wild-caught by T.J.L.; *C. willardi* were captive-born progeny of wild-caught adults, and *A. piscivorus* and all additional species were obtained from commercial suppliers (*A. piscivorus* and

B. schlegelii – Exotic Gems Reptile, Fort Myers, FL, USA; remainder – Glades Herp, Inc., Fort Myers, FL, USA).

Disabling thermal radiation sensors

To test whether facial pits or supranasal sacs aid in thermoregulation, we compared the behavioral performance of snakes with functional organs to their performance with disabled organs. We temporarily disabled the facial pits using an established procedure (Krochmal and Bakken, 2003). Briefly, we lightly anesthetized snakes with isoflurane (Abbott Laboratories, North Chicago, IL, USA), plugged each facial pit cavity with a small polystyrene ball and glued a piece of aluminum foil over the facial pit using a latex adhesive (Skin Bond®, Smith-Nephew Inc., Fort Largo, FL, USA). To temporarily disable the supranasal sacs, we glued a thin piece of aluminum foil over each opening, being careful to avoid blocking the nares.

Our experimental design required that snakes tested with functional radiation sensors first received a sham blocking procedure. This consisted of anesthetizing snakes, inserting and removing a polystyrene ball from the facial pit cavity (if present) and holding a glue-laden foil patch 1 mm from the facial pit or supranasal sac for approximately 20 s.

Experimental conditions and apparatus

To encourage active behavioral thermoregulation in our study animals, we subjected them to a substantial but sub-lethal heat stress and provided a thermal refuge that could be identified from a distance only by using thermal radiation as a cue. We conducted experiments in a Y-maze (Krochmal and Bakken, 2003), as the failure of animals to perform under highly simplified conditions would be a strong indication of the absence of behavioral ability. The Y-mazes were fashioned from polyvinylchloride (PVC) plumbing components and transparent, rigid polycarbonate tubing (8.9 cm o.d./8.2 cm i.d.). The center of each maze was a four-branch PVC Y, and the two side branches were composed of 35 cm sections of open-ended polycarbonate tube. The center branch of the Y was fitted with an acrylic window rather than a tube. The snake’s behavior at the decision point was videotaped through this window using a video camera with a 90° field of view. The base of the Y consisted of a removable snake carrier used to introduce snakes into the maze at the start of each trial. The carrier was built from a 45 cm long polycarbonate tube with a PVC cap on the distal end. The proximal end was joined to a PVC coupling fitted with a sliding door. The end of each maze branch (the ‘goal’) was made of a similar carrier, which was wrapped with copper heat exchange coils and inserted into a sleeve of insulation. We attached the carrier and goals to the maze by inserting the open-ended tube into the coupling and securing it with a cap screw.

We maintained the main part of the maze at a uniform temperature of 40°C by placing it in one of two walk-in environmental chambers (model 08805 or 08083; Hotpack Corporation, Philadelphia, PA, USA) set at 40°C. We regulated one goal at the stressful ambient temperature (40°C) and the

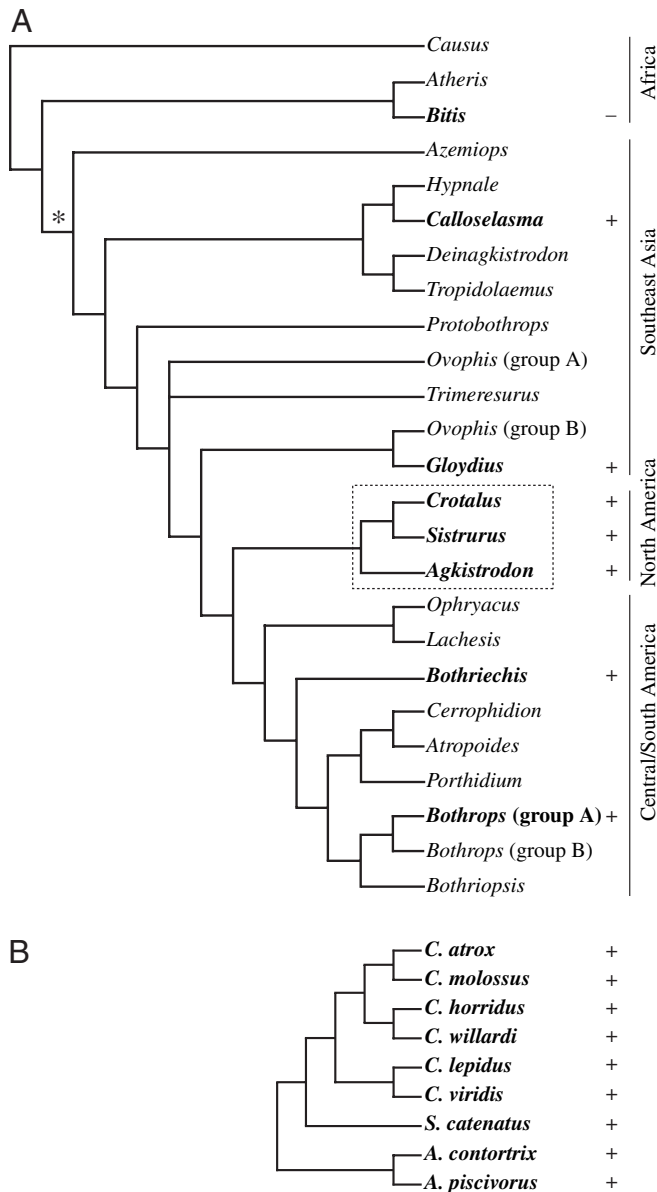


Fig. 1. (A) Phylogenetic relationships and geographic distribution of viper taxa used in this study (modified from Parkinson et al., 2002) and presence (+) or absence (-) of behavioral thermoregulation mediated by thermal radiation cues. Genera in bold indicate use in the present study, with *Bothrops* group A containing *B. alternatus*. The asterisk denotes the origin of the facial pit. Phylogenetic relationships of the genera enclosed by the broken box are shown in greater detail in B. (B) Phylogenetic relationships of North American pitvipers used in the present study (genera *Agkistrodon*, *Crotalus* and *Sistrurus*; modified from LaDuc, 2003) and presence of behavioral thermoregulation mediated by thermal radiation cues (+).

other (the 'refuge') at 30°C by circulating water from two temperature-controlled water baths through the copper coils.

In our experiment, we used temperature as both the stimulus and the reward for behavioral thermoregulation. The 30°C temperature of the thermal refuge approximates the preferred body temperatures of a variety of pitvipers (Cowles and Bogert, 1944; Fitch, 1960; Hirth and King, 1969; Moore, 1978; Beck, 1995, 1996; Wills and Beaupre, 2000). We selected 40°C as the stressful ambient temperature on the basis of available data on upper temperature limits (Cowles and Bogert, 1944; Fitch, 1960; Krochmal and Bakken, 2003). Of the 720 trials conducted under this temperature regime, we observed heat stress responses such as gaping or loss of righting response in only nine trials (all involving *C. lepidus*; 1.25% of total trials). These animals were immediately removed from the experiment and recovered fully.

If individuals of one of the species consistently failed to distinguish between goals under these conditions, we modified experimental conditions and tested them again. We increased the ambient temperature to 50°C to increase both motivation and signal strength relative to the 30°C refuge. We also reduced maze branch lengths to 0.75 m to provide a stronger thermal signal at both the 40°C and 50°C ambient temperatures. Manipulating these two variables generated a total of four Y-maze experiments corresponding to each combination of branch length (1.0 m or 0.75 m) and ambient temperature (40°C or 50°C). These procedures were only used on a single species and, while doing so, we observed no signs of heat stress in the animals tested under these conditions.

To ensure that experimental snakes were responding solely to thermal radiation, we eliminated or randomized unintentional cues. First, we randomized the location of the refuge (right or left branch) and the overall orientation of the maze within the environmental chamber (three-way rotational symmetry of Y-maze) to prevent learning or confounding refuge temperature with some unknown directional cue. This yielded six unique maze configurations under which each snake was tested. Thus, the number of trials per species was $6N$, where N is the number of individual snakes; trials were conducted twice, once under each sensory condition (see below). Second, we used multiple mazes and cleansed each maze thoroughly between trials to remove directional odor trails. Third, we randomized the association between a particular goal assembly and its temperature and circulated water around both goals at the same rate. Finally, we verified the absence of thermal gradients using thermographic imaging (ThermaCAM® PM575; FLIR, North Billerica, MA, USA) and an array of fine (40 AWG) thermocouples suspended in the air space and embedded in the maze substrate.

Experimental design and statistical analyses

Snakes of each species were divided into two subgroups. At the start of the experiment, one subgroup had its thermal radiation receptors blocked, while the other received the sham procedure and retained functional receptors. After being

tested, snakes with functional receptors had them blocked, while snakes with blocked receptors had the blockages removed. Previous studies tested for lasting effects of blocking and unblocking the facial pits and found none (Haverly and Kardong, 1996; Krochmal and Bakken, 2003). As our questions related to the behavior of species, the experimental unit was the individual snake. Therefore, for each snake and treatment (blocked or unblocked) we characterized overall performance in the maze by calculating a single proportion of success, equal to the number of initial movements toward the thermal refuge divided by the total number of trials. For each individual trial, we scored each snake as having moved toward the thermal refuge when its head was 5 cm past the central branch point. When all six trials were complete for each snake-treatment (functional or disabled pits) combination, we computed the proportion of success and applied an arcsin transformation. We then compared, within a treatment group, the mean for all individuals of a species to the *a priori* null hypothesis using one-sample *t*-tests. The null hypothesis was that snakes would attain a mean proportion of success of 0.5, representing the 1 in 2 possibility of finding the refuge by chance. Our alternative hypothesis was that snakes with functional pits would locate a refuge more frequently than predicted by chance. Thus, we report one-tailed *P* values. We scored the ability of a species to use thermal radiation to direct behavioral thermoregulation as present (+) if $P < 0.025$ when pits were functional and $P > 0.20$ when pits were blocked. It was scored absent (–) if $P > 0.2$ both when pits were open and when pits were blocked. Results were considered indeterminate if $0.20 > P > 0.025$ for one or both treatments.

Results

Pitvipers

When tested with functional pits, all 12 pitviper species were able to locate the thermal refuge far more often than predicted by chance alone (Table 1). These same individuals subsequently lost this ability when their facial pits were temporarily disabled (Table 1). All pitvipers tested with the choice of 30°C vs 40°C refuges at a distance of 1 m were therefore scored positive for the ability to direct thermoregulatory movements *via* radiative cues (all $P < 0.025$). Accordingly, this behavior was present in species with diverse thermal ecologies and phylogenetic positions.

True viper

Under the same conditions where the pitvipers displayed the ability to respond to thermal radiation cues, the one true viper tested, *B. arietans*, was unable to locate the thermal refuge with either functional or disabled supranasal sacs (Table 1). Thus, we repeated the testing using the modified protocols (see Materials and methods). As some *B. arietans* populations inhabit particularly hot environments, we raised the stressful temperature to 50°C to increase motivation. Also, because the supranasal sac lacks sensitivity-enhancing features of the facial

Table 1. The presence of a functional facial pit enables thermoregulation mediated by thermal radiation cues, but the presence of a functional supranasal sac in true vipers does not

Species	Sensor	Mean ± S.E.M.	<i>t</i>	d.f.	<i>P</i>
<i>C. molossus</i>	Open	0.78±0.04	7.40	3	0.0025
	Blocked	0.52±0.06	0.31	3	0.3900
<i>C. horridus</i>	Open	0.82±0.01	32.00	2	0.0005
	Blocked	0.44±0.11	0.50	2	0.3330
<i>C. willardi</i>	Open	0.78±0.04	7.40	3	0.0025
	Blocked	0.53±0.02	1.00	3	0.1955
<i>C. lepidus</i>	Open	0.82±0.08	4.20	4	0.0070
	Blocked	0.57±0.04	1.63	4	0.0890
<i>C. atrox</i> *	Open	0.78±0.04	6.53	11	0.0001
	Blocked	0.52±0.04	0.52	11	0.3080
<i>C. viridis</i>	Open	0.73±0.05	4.28	4	0.0065
	Blocked	0.47±0.06	-0.46	4	0.3345
<i>S. catenatus</i>	Open	0.78±0.05	5.19	2	0.0175
	Blocked	0.48±0.08	-0.30	2	0.3975
<i>A. piscivorous</i>	Open	0.79±0.03	9.41	4	0.0005
	Blocked	0.49±0.04	-0.32	4	0.3820
<i>A. contortrix</i>	Open	0.77±0.05	5.43	2	0.0160
	Blocked	0.56±0.06	1.00	2	0.2115
<i>B. alternatus</i>	Open	0.79±0.03	9.61	4	0.0005
	Blocked	0.47±0.06	-0.59	4	0.2935
<i>B. schlegelii</i>	Open	0.81±0.01	31.00	2	0.0005
	Blocked	0.53±0.03	2.00	2	0.0920
<i>G. blomhoffii</i>	Open	0.78±0.05	7.70	3	0.0025
	Blocked	0.51±0.06	0.12	3	0.4570
<i>C. rhodostoma</i>	Open	0.78±0.04	7.40	3	0.0025
	Blocked	0.51±0.06	0.12	3	0.4570
<i>B. arietans</i>	Open	0.47±0.06	-0.46	4	0.3345
	Blocked	0.49±0.07	-0.20	4	0.4250
<i>B. arietans</i> [†]	Open	0.47±0.05	-0.73	4	0.2525
	Blocked	0.48±0.06	-0.35	4	0.3725
<i>B. arietans</i> [‡]	Open	0.52±0.04	0.54	4	0.3105
	Blocked	0.48±0.06	-0.35	4	0.3725
<i>B. arietans</i> [§]	Open	0.49±0.07	-0.20	4	0.4250
	Blocked	0.51±0.04	0.32	4	0.3820

Means and standard errors for the proportions of success of animals with both functional ('open') and disabled ('blocked') thermal radiation sensors. All *P* values are one-tailed. *Data from Krochmal and Bakken (2003); †data collected at an ambient temperature of 50°C; ‡data collected at 50°C and with maze branches of 0.75 m; §data collected at 40°C and with maze branches of 0.75 m.

Data reported for *B. arietans* were collected from the same individuals.

pit (see Discussion), we retested animals with the distance to the refuge shortened (0.75 m vs 1.0 m) to increase signal strength. In all four combinations of experimental temperatures and distance to goal, puff adders still failed to locate the thermal refuge more often than dictated by chance, regardless of whether supranasal sacs were functional or disabled (Table 1). Thus, we conclude that *B. arietans* was unable to direct thermoregulatory movements when the only cue was thermal radiation.

Discussion

Phylogeny, ecology, and function of the facial pit

In our broad phylogenetic survey, we found the ability to use thermal radiation to direct thermoregulatory behavior to be present in all pitviper taxa studied. It appears probable that most or all pitvipers can utilize thermal radiation cues to guide behavioral thermoregulation, as we examined representative species from all North American genera, two Central and South American clades and two Asian lineages. We sampled

key nodes in the phylogeny, including the most basal North American pitviper (*A. contortrix*; Parkinson et al., 2000), a member of the clade from which all New World pitvipers arose (*G. blomhoffii*; Parkinson et al., 2002) and a member of the most basal pitviper clade (*C. rhodostoma*; Parkinson et al., 2002).

The ability to make thermoregulatory decisions based on thermal radiation was present in pitvipers typically found in habitats with diverse thermal conditions, including desert scrub/grassland (*C. atrox*, *C. viridis*) and open scrub habitats (*S. catenatus*), temperate forests (*C. horridus*), swamps (*A. piscivorus*) and tropical forests (*B. schlegelii*). Accordingly, the manifestation of thermoregulation mediated by thermal radiation does not seem to be driven by ecological factors.

Our first three questions – whether or not the use of thermal radiation cues in thermoregulation is restricted to particular environments, whether or not it is restricted to particular pitviper clades and, if it is restricted to particular habitats or clades, whether it is ancestral or derived – are answered by the apparent universality of facial pit-mediated thermoregulatory behavior. This capability appears to be neither an adaptation to hot environments nor an evolutionary legacy restricted to particular lineages of pitvipers. Instead, the use of thermal radiation cues to aid behavioral thermoregulation appears to be an ancestral trait among pitvipers, a point emphasized by the presence of this behavior in the most basal pitviper clade.

As for the fourth question – whether the facial pit is a special or general sense organ – our data demonstrate that, like prey acquisition, the use of thermal radiation cues to direct behavioral thermoregulation is a widespread, if not universal, function of facial pits. Thus, the case is strengthened for the hypothesis that the facial pits are all-purpose sensors that can potentially inform a range of behaviors. Other potential functions, including den site selection and predator detection, should also be tested experimentally to further validate the hypothesis.

True vipers and the supranasal sac

In contrast to the pattern we observed in pitvipers, we found no evidence that *B. arietans*, the only true viper investigated, used thermal radiation cues to inform thermoregulatory behavior. These snakes were unable to locate the thermal refuge more often than by chance when tested under the same experimental conditions as the pitvipers. When tested with increased thermal signal strengths and elevated levels of motivation, *B. arietans* was still unable to rely on thermal radiation to direct thermoregulatory movements. Thus, we conclude that *B. arietans* lacks either the ability to sense thermal radiation or the ability to use such cues to mediate thermoregulatory behavior.

Although published reports suggest that *B. arietans* and at least one other true viper can sense thermal radiation (Breidenbach, 1990), their sensitivity to thermal radiation signals and the physiological mechanism of their thermal radiation detectors present intriguing questions. The putative

radiation sensors, the supranasal sacs, are small organs with slit-like openings located adjacent and dorsal to each nostril (York et al., 1998). The gross structures of these organs are not obviously suited to detecting relevant targets by thermal radiation. In the facial pits of pitvipers, thermal radiation is sensed when it is absorbed by the pit membrane, causing differential heating of the nerve endings. Wet tissue is highly opaque to thermal radiation and has a high heat capacity (specific heat capacity ~0.8), in both cases because of high water content. Thus, the thermal receptor must be located as near the air–body interface as possible. However, the receptor can then be heated and cooled by convection and evaporation. Consequently, stray air currents may produce false signals. The thermal receptors of pitvipers are shielded from convection by being located in deep pits. Another limit to sensitivity of thermal radiation receptors is that the heat generated by absorbed thermal radiation is efficiently conducted away by wet, vascularized tissue. Conductive heat loss is minimal in facial pits because the thermoreceptors in them are located on a suspended membrane. Consequently, the thermoreceptors are insulated from conductive heat loss by the pocket of gas between the membrane and the back of the pit. By contrast, the supranasal sac of true vipers is a simple structure with no suspended membrane (York et al., 1998). Further, the minute opening can admit but little thermal radiation. Consequently, a given source of thermal radiation would produce less change in temperature in a supranasal sac than it would in a facial pit, making a supranasal sac a less sensitive thermal radiation receptor than a facial pit.

Both anatomical and behavioral evidence thus indicate that, if the supranasal sac is a thermal radiation receptor, it is probably a poor one. Thus, the supranasal sac of true vipers is one possible model for the form and function of an early, comparatively insensitive, thermal radiation receptor within the family Viperidae. Better knowledge of sensitivity of the supranasal sac to thermal radiation and of its behavioral functions might improve our understanding of the origin of thermal radiation sensors.

The origin of the thermal radiation sense

Based on our results, we hypothesize that within the family Viperidae, the ability to use thermal radiation to mediate thermoregulatory decisions is unique to pitvipers and might have arisen concurrently with the facial pits. However, this apparent phylogenetic pattern must be interpreted conservatively, as we only surveyed a single species of true viper and we did not examine *Azemiops feae*, the purported sister taxon to the pitvipers (Parkinson et al., 2002). The range of species studied needs to be expanded significantly to test this hypothesis fully.

The ability to mediate behavioral thermoregulation using only thermal radiation as a cue appears to be an ancestral trait among pitvipers. The simple phylogenetic and ecological presence or absence pattern that we found indicates that the use of thermal radiation cues to direct thermoregulatory movements arose very early in the history of pitvipers. As this

behavior was absent in the only true viper studied, it is possible that this behavior co-originated with the pit organ.

More detailed conclusions will require, at a minimum, a broader survey of the family Viperidae, particularly a more thorough study of the true vipers. Within the pitvipers, quantitative comparison of the relative performances among species and among populations within a species believed to have been confined to a defined thermal environment for evolutionarily significant periods, or both, might yield useful phylogenetic patterns. However, because our ecological and phylogenetic enquiries produced no clear indication of which adaptive forces may have driven the evolution of the facial pits, we suspect that the questions of origins may need to be addressed by other means.

First, a thorough study of the behaviors utilizing facial pit input may illuminate its evolutionary origins. Roles proposed for the facial pit include prey acquisition (e.g. Noble and Schimdt, 1937), behavioral thermoregulation (Bullock and Barrett, 1968), predator detection (Greene, 1992) and den site selection (Sexton et al., 1992). Until recently, prey acquisition was the only confirmed role of facial pits and was therefore commonly considered the adaptive force that drove their initial evolution. Behavioral thermoregulation and prey acquisition currently stand as the only documented roles of facial pits. Both now constitute viable alternative hypotheses for the selective force that drove evolution of the crotaline facial pit.

Further progress requires that hypotheses regarding the biological functions and evolutionary origins of the thermal radiation sense (e.g. Greene, 1992; Sexton et al., 1992) be subjected to empirical scrutiny and accepted or rejected on the basis of appropriate quantitative models and behavioral experiments. These investigations should address the characteristics of the physical (i.e. infrared) signals available for the conjectured function and whether these could be detected given the physiological capabilities of hypothetical intermediate states in the evolution of the facial pits.

For example, Greene (1992) showed that the facial pits arose simultaneously with several unique defensive displays and indirectly demonstrated that pitvipers rely more on confrontation with enemies than on locomotor escape compared with true vipers. Though there is no direct evidence supporting an antipredator role of facial pits, this compelling indirect evidence linking the origin of the facial pits with the origin of defensive behaviors, combined with the likely thermal signal emitted by a moderate-sized predator, dictates that such a utility be the focus of future experimentation.

Second, the precursor to the facial pit was probably comparatively insensitive to emitted thermal radiation. Accordingly, it follows that the behavior that could be accomplished most easily with minimal thermal sensitivity probably drove its origin. There are no systematic experimental studies assessing the strength of the thermal radiation signal from various sources relevant to ophidian biology. However, sufficient information is available to allow a coarse assessment of the relative merits of prey acquisition and thermoregulation

as the hypothetical selective forces that drove the evolution of the thermal radiation sense.

For example, the physical nature of emitted thermal radiation supports a thermoregulatory origin of facial pits (Krochmal and Bakken, 2003). Using physical principles, the signal strength presented to the pit organ by a target can be roughly defined as temperature contrast \times solid angle, where solid angle is approximately target cross-sectional area/distance. Environmental features probably emit far stronger thermal radiation signals than do prey items. Over ecologically relevant distances, typical prey items present small targets with surface temperatures that, because of fur or feather insulation, frequently differ little from ambient surface temperatures (e.g. Hill et al., 1980; Shine and Sun, 2003). By contrast, environmental features relevant to behavioral thermoregulation exhibit larger temperature differences, often 40°C or more (fig. 2 in Stoujesdisk and Barkman, 1992; G.S.B., unpublished data), and subtend larger solid angles than do prey items. They therefore present a larger thermal signal than do most prey items. Consequently, it is likely that an early, comparatively limited, thermal radiation detector could sense environmental features more easily than it could prey items. These physical considerations suggest behavioral thermoregulation to be the more plausible of these two hypotheses for the evolutionary origins of the facial pits (Krochmal and Bakken, 2003).

To draw firmer conclusions, systematic quantitative experimental studies of typical thermal signals are needed. Such studies should be further augmented by more extensive examinations of the neurophysiological mechanisms underlying thermal radiation detection. Knowledge of the threshold sensitivity of the supranasal sacs as well as the neuronal pathways through which thermal information is sensed and processed in true vipers would be particularly helpful, as it would allow for meaningful comparisons with crotaline facial pits. Additional well-designed studies of the behavioral responses of various true viper species to thermal radiation signals would be highly valuable as well.

When combined, phylogenetic, ecological, physical and physiological studies of the thermal radiation sense may confirm the phylogenetic root of facial pit-mediated thermoregulatory behavior and could shed light on the other functions and evolutionary origins of the facial pit.

We thank M. Hao, A. Lynott, C. Oufiero, T. Roth and M. Van Sant for assisting with blocking facial pits. Funding for this work was supported by grants from Sigma Xi, The American Society of Ichthyologists and Herpetologists and the Indiana Academy of Science to A.R.K., and by NSF grant BSR 99-70209 to G.S.B. Scientific collecting permits from Texas Parks and Wildlife (D. Riskind, R. Roegner), Arizona Game and Fish Department, and New Mexico Department of Game and Fish (C. Painter) facilitated the collection of live rattlesnakes from Texas (#SPR-1017-912) and New Mexico (authorization no. 3064). All experimental procedures were conducted under the jurisdiction of the Indiana State

University Institutional Animal Care and Use Committee (protocol #02-15: A.R.K./G.S.B.) and University of Texas Animal Care and Welfare Committee (protocol #99031201).

References

- Beck, D. D.** (1995). Ecology and energetics of three sympatric rattlesnake species in the Sonoran Desert. *J. Herpetol.* **29**, 211-223.
- Beck, D. D.** (1996). Effects of feeding on body temperatures of rattlesnakes: a field experiment. *Physiol. Zool.* **69**, 1442-1455.
- Breidenbach, C. H.** (1990). Thermal cues influence strikes in pitless vipers. *J. Herpetol.* **24**, 448-450.
- Bullock, T. H. and Barrett, R.** (1968). Radiant heat receptors in snakes. *Commun. Behav. Biol. A* **1**, 19-29.
- Bullock, T. H. and Diecke, F. P. J.** (1956). Properties of an infra-red receptor. *J. Physiol.* **134**, 47-87.
- Cowles, R. B. and Bogert, C. M.** (1944). A preliminary study of the thermal requirements of desert reptiles. *Bull. Am. Mus. Nat. Hist.* **83**, 265-296.
- de Cock Buning, T.** (1983). Thermal sensitivity as a specialization for prey capture and feeding in snakes. *Am. Zool.* **23**, 363-375.
- Fitch, H. S.** (1960). Autecology of the copperhead. *Univ. Kans. Publ. Mus. Nat. Hist.* **8**, 417-476.
- Gans, C. and Taub, A. M.** (1964). Precautions for keeping poisonous snakes in captivity. *Curator* **7**, 196-205.
- Goris, R. C. and Nomoto, M.** (1967). Infrared reception in oriental crotaline snakes. *Comp. Biochem. Physiol.* **23**, 879-892.
- Greene, H. W.** (1992). The ecological and behavioral context for pitviper evolution. In *Biology of the Pitvipers* (ed. J. A. Campbell and E. D. Brodie, Jr), pp. 107-117. Tyler, TX: Selva.
- Greene, H. W.** (1997). *Snakes: The Evolution of Mystery in Nature*. Princeton, NJ: Princeton University Press.
- Haverly, J. E. and Kardong, K. V.** (1996). Sensory deprivation effects on the predatory behavior of the rattlesnake, *Crotalus viridis oregonus*. *Copeia* **1996**, 419-428.
- Hill, R. W., Beaver, D. L. and Veghte, J. H.** (1980). Body surface temperatures and thermoregulation in the black-capped chickadee (*Parus atricapillus*). *Physiol. Zool.* **53**, 305-321.
- Hirth, H. F. and King, A. C.** (1969). Body temperatures of snakes in different seasons. *J. Herpetol.* **16**, 101-102.
- Huey, R. B.** (1982). Temperature, physiology, and the ecology of reptiles. In *Biology of the Reptilia*, vol. 12 (ed. C. Gans and F. H. Pough), pp. 25-91. London: Academic Press.
- Huey, R. B.** (1991). Physiological consequences of habitat selection. *Am. Nat.* **137**, S91-S115.
- Kardong, K. V.** (1992). Proximate factors affecting guidance of the rattlesnake strike. *Zool. Jb. Anat.* **122**, 233-244.
- Kardong, K. V. and Berkhoudt, H.** (1999). Rattlesnake hunting behavior: correlations between plasticity of predatory performance and neuroanatomy. *Brain Behav. Evol.* **53**, 20-28.
- Kardong, K. V. and Mackessy, S. P.** (1991). The strike behavior of a congenitally blind rattlesnake. *J. Herpetol.* **25**, 208-211.
- Krochmal, A. R. and Bakken, G. S.** (2003). Thermoregulation is the pits: use of thermal radiation for retreat site selection by rattlesnakes. *J. Exp. Biol.* **206**, 2539-2545.
- LaDuc, T. J.** (2003). Allometry and size evolution in the rattlesnake, with emphasis on predatory strike performance. *Ph.D. Thesis*. University of Texas.
- Moore, R. G.** (1978). Seasonal and daily activity patterns and thermoregulation in the southwestern speckled rattlesnake (*Crotalus mitchelli pyrrhus*) and the Colorado desert sidewinder (*Crotalus cerastes laterorepens*). *Copeia* **1978**, 432-442.
- Noble, G. K. and Schmidt, A.** (1937). The structure and function of the facial and labial pits of snakes. *Proc. Am. Philos. Soc.* **77**, 263-288.
- Parkinson, C. L., Zamudio, K. M. and Greene, H. W.** (2000). Phylogeography of the pitviper clade *Agkistrodon*: historical ecology, species status, and conservation of cantils. *Mol. Ecol.* **9**, 411-420.
- Parkinson, C. L., Campbell, J. A. and Chippindale, P. T.** (2002). Multigene phylogenetic analysis of pitvipers, with comments on their biogeography. In *Biology of the Vipers* (ed. G. W. Schuett, M. Höggren, M. E. Douglas, and H. W. Greene), pp. 93-110. Eagle Mountain, UT: Eagle Mountain Publishing.
- Platt, J. R.** (1964). Strong inference. *Science* **146**, 347-353.
- Schnitzler, H. U., Moss, C. F. and Denzinger, A.** (2003). From spatial orientation to food acquisition in echolocating bats. *Trends Ecol. Evol.* **18**, 386-394.
- Sexton, O. J., Jacobson, P. and Bramble, J. E.** (1992). Geographic variation in some activities associated with hibernation in Nearctic pitvipers. In *Biology of the Pitvipers* (ed. J. A. Campbell and E. D. Brodie, Jr), pp. 337-345. Tyler, TX: Selva.
- Shine, R. and Sun, L. X.** (2003). Attack strategies of an ambush predator: which attributes of the prey trigger a pit-viper's strike? *Funct. Ecol.* **17**, 340-348.
- Stoujesdisk, P. H. and Barkman, J. J.** (1992). *Microclimate: Vegetation and Fauna*. Knivsta, Sweden: Opulus Press.
- Wills, C. A. and Beaupre, S. J.** (2000). An application of randomization for detecting thermoregulation in timber rattlesnakes (*Crotalus horridus*) from northwest Arkansas. *Physiol. Biochem. Zool.* **73**, 325-334.
- York, D. S., Silver, T. M. and Smith, A. A.** (1998). Innervation of the supranasal sac of the puff adder. *Anat. Rec.* **251**, 221-225.