Eye and Pit Size are Inversely Correlated in Crotalinae:
Implications for Selection Pressure Relaxation

Yang Liu,1 Qin Chen,1 Theodore J. Papenfuss,2 Fang Lu,3* and Yezhong Tang1

1Department of Herpetology, Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu, China
2Department of Amphibians and Reptiles, Museum of Vertebrate Zoology, University of California, Berkeley, California
3Department of Ophthalmology, West China Hospital, Sichuan University, Chengdu, China

ABSTRACT Mate, prey, and predator recognition often depend on the integration of information from multiple sensory modalities including visual, auditory, and/or olfactory inputs. In Crotalinae, the eyes sense visible light while the pit organs detect infrared (IR) radiation. Previous studies indicate that there is significant overlap between the eye and pit sensory fields and that both senses are involved in recognition processes. This study investigated the relationships between eye and pit sizes in this taxonomic group as a function of phylogeny and habitat. In view of the fact that pit orientation depends largely on snout shape, pit vipers were grouped as follows: 1) arboreal, 2) terrestrial with rounded snout, and 3) terrestrial with pointed snout. The pit orientations and habitat patterns were fully independent of the Crotalinae phylogenetic tree. The phylogenetic generalized least squares model showed that both eye and pit areas were not of significantly phylogenetic relatedness, implying alternatively a strong effect of adaptation on eye and pit sizes. Negative correlations between relative eye and pit areas in terrestrial (both pointed and rounded snouts) and arboreal species were statistically significant. Our results suggest that the eyes and pits function in a complementary fashion such that selection for IR-perception relaxes selection pressures on the visual system and selection for visual discrimination relaxes selection pressures acting on the IR-system. J. Morphol. 277:107–117, 2016.

KEY WORDS: infrared perception; vision; pit character; phylogeny; adaptation

INTRODUCTION

Crotalinae snake species (pit vipers) are unique among animals capable of detecting middle and long wavelength infrared (IR) radiation insofar as pit vipers are capable of IR-imaging (Campbell et al., 2002). The pit organ located between the eye and nostril on each side of the viper's face serves as an IR-detector. Behaviorally, the facial pit appears to function as an "eye" because it can compensate for visual deprivation (Kardong and Mackessay, 1991; Kardong and Berkhoudt, 1999). Pit vipers perceive a visual-thermal multispectral image (Newman and Hartline, 1982; Moiseenkova et al., 2003). This conclusion is supported by the fact that the resolution of the thermal image is improved by neural image sharpening mechanisms in the medulla (Stanford and Schroeder, 1980) and by the fact that ascending pathways conveying IR and visual information converge in the forebrain (Berson and Hartline, 1988). In addition, the IR-senses normally work in concert with the visual system in facilitation of prey detection, identification, and localization (Ford and Burghardt, 1993). Nevertheless it is still unclear if the IR-system and visual system normally work in parallel since the physical characteristics of the visible (0.38–0.75 μm) and IR (0.8–30 μm) radiation emitted by endotherms are different.

It is thought that the evolution of the IR-sensory system has made Crotalinae the most species-rich taxon in the Viperidae, containing over 200 species (Malhotra and Thorpe, 2004; Castoe and Parkinson, 2006; Orlov et al., 2009; David et al., 2011; Pyron et al., 2013). Pit vipers inhabit various habitats, with major radiations in both the Old and New Worlds (Zhao et al., 1998; McDiarmid et al., 1999). An abundant diversity of pit viper species and ecological patterns provides an excellent opportunity to investigate morphological adaptation associated with the evolution of multimodal sensory processing.

In pit vipers, eye and pit sensory fields largely overlap. Structurally, both visual and IR-inputs are projected to the pit viper optic tectum where a layer of IR-sensitive neurons is located subjacent to the visual layer (Berson and Hartline, 1988).
Functionally, the infrared and visual spatiotopic tectal maps have similar but not identical axes; the IR-magnification is greater than that for classical vision (Hartline et al., 1978). Nevertheless visual and IR-information are projected in register spatially and temporally to the midbrain and the available evidence indicates that the snakes can use either modality for hunting prey. 

Chen et al. (2012) found that snakes with either the pit organs or eyes occluded on both sides of the face exhibit about 75% success in prey capture. Similarly, occlusion of both eyes or both pits constrict strike angles during prey capture to the same extent, within 30° to the left and right sides of the midline (Chen et al., 2012). In addition, congenitally blind pit vipers using IR-imaging have been shown to aim and strike at prey as accurately as normal siblings under a variety of conditions (Kardong, 1986; Kardong and Mackessy, 1991). Finally, neurophysiological studies support the idea that binocular thermal stereopsis occurs in the viper midbrain (Goris and Terashima, 1973; Berson and Hartline, 1988).

In view of these studies, we hypothesized that selection favoring IR-perception could relax the selection pressures on development of the visual system because these two modalities can function in a complementary and perhaps independent way. Thus, we predicted that eye and pit sizes would be inversely correlated across the whole Crotalinae, yet vary independently with phylogeny.

MATERIALS AND METHODS

Morphological Measurements

A total of 167 specimens were examined, belonging to all 24 genera, including 59 species of Crotalinae (Table 1). The data were obtained from specimens stored in the Museum of Vertebrate Zoology, UC Berkeley and the National Museum of Natural History of the Smithsonian Institution, USA. All specimens used for size measurement had been fixed in ethanol for prolonged periods. In order to eliminate the effect of asymmetry on the relationship between eyes and pits, biometric measures were performed exclusively on the left side.

For each specimen, left ED (eye diameter, the longest diameter of the visible spectacle, Fig. 1A) and PL (the pit length of one side, Fig. 1B) were measured to the nearest 0.2 mm, with a vernier caliper. Five to six photos were taken for each specimen with the focus centered on the eye or pit, so that the eye and pit areas could be measured using Image J (Collins, 2007) with ED and PL used as scales for normalizing. In order to minimize allometric effects on the analysis, the residuals of the eye and pit area variables were computed and used in statistical tests. When more than one set of measurements on the same species was available, the data were averaged so extra weight was not given to any species. Equal numbers of adult male and female specimens were included if possible, to minimize bias in these estimates which might be due to sexual dimorphism and allometry (Thomas et al., 2006; Werner and Seifan, 2006).

Ecological Classification

Information concerning the habitats and daily activity patterns of species was based on 1) our own long-term field experiences with behavioral observations and specimen collection; 2) influential monographic books (Zhao et al., 1998; Zhao, 2006) and 3) the published literature.

In this study, the genera Bothriopsis (Kwiatkowski and Burt, 2011), Bothriechis (Campbell and Smith, 2000), Trimeraurus (Malhotra and Thorpe, 1997), and Tropidolaemus (Tsai et al., 2012) were considered as arboreal. All other genera were categorized as terrestrial, and then grouped into two patterns based on their snout shapes and pit orientations, which are shown in Table 1.

Although most pit vipers show no preference for being active diurnally or nocturnally, primarily nocturnal foragers were selected by searching the snake ecology literature. These included Bothropoides jararaca (Sazima, 1992), the genus Bothrops (Wasko and Sasa, 2009), Calloselasma rhodostoma (York, 1984), Oophis monticola (Malhotra et al., 2011), Oophis okinavensis (Mori et al., 2002), Protobothrops mucrosquamatus (Huang et al., 2007), and Trimeraurus stejnegeri (David et al., 2001).

Construction of Phylogenetic Trees

The time-calibrated tree of Crotalinae was derived from a recent comprehensive phylogenetic tree of 4,161 species of Squamata (Pyron and Burbrink, 2014). The species not included in this study were pruned from the analyses with package “caper” (Orme et al., 2012; Han and Fu, 2013).

Data Analysis

Phylogenetic generalized least squares regression analysis (PGLS) was performed in R 3.136 3.1.2 to test the degree of dependence of the biometric variables on the phylogenetic covariance (R Development Core Team, 2012). Residuals of the eye and pit areas were calculated in order to minimize allometric scaling (Revell et al., 2007). A phylogenetic parameter i (Pagel, 1999) was used as the measure of phylogenetic signals for continuous traits, ranging from 0 (no phylogenetic signal, equivalent to a “star” phylogeny) to 1 (consistent with Brownian motion, BM), while intermediate values imply that the data support a model with status between “star” phylogeny and BM. Its optimum value and confidence limits were estimated using the “pgls” function in packages “caper” with a maximum likelihood method when performing a correlation analysis (Orme et al., 2012). The relationship between the eye and pit areas in Crotalinae was evaluated using PGLS, applying a covariance matrix based on Brownian and Ornstein–Uhlenbeck (OU) motion models of evolution with functions “pml” “corgBrownian” (Freckleton et al., 2011), and “corMartin” (Martins and Hansen, 1997) in packages “nlme” (Abell and Ribera, 2011), and “ape” (Paradis et al., 2004). We used the estimated value of alpha to create the correlation structure in the OU motion model.

Next statistical analyses were performed using SPSS 11.5 for windows. All data were examined for assumptions of normality and homogeneity of variance, using Kolmogorov–Smirnov and Levene’s test, respectively. Pearson correlation analysis was applied to examine the possible compensation of biometric data after transforming to residuals. Statistical significances were tested with the two-tailed test.

RESULTS

Snout Shape and Pit Orientation

There were three snout shape patterns and corresponding pit orientations in pit vipers. All pit vipers were grouped into these patterns based on snout shapes and habitats: 1) arboreal, 2) terrestrial with rounded snout, and 3) terrestrial with pointed snout. All arboreal species have a marked constriction or grooving of the face in front of the
## TABLE 1. List of species studied

| Scientific name          | N | Catalogue numbers          | Habitat   | Activity rhythm | Snout shape | Eye area (mm³) | Pit area (mm³) |
|--------------------------|---|----------------------------|-----------|----------------|-------------|---------------|---------------|
| *Agkistrodon bilineatus* | 2 | MVZ Herp 200829 MVZ Herp 229768 | Terrestrial | No preference | Pointed     | 22.38         | 2.09          |
| *Agkistrodon contortrix* | 4 | MVZ Herp 38467 MVZ Herp 38468 MVZ Herp 38469 MVZ Herp 46773 | Terrestrial | No preference | Pointed     | 15.48         | 1.42          |
| *Agkistrodon piscivorus* | 2 | MVZ Herp 204941 MVZ Herp 229768 | Terrestrial | No preference | Pointed     | 16.61         | 1.57          |
| *Atropoides indomitus*   | 2 | MVZ Herp 267166 MVZ Herp 267167 | Terrestrial | No preference | Rounded    | 8.7           | 1.65          |
| *Atropoides mexicanus*   | 2 | MVZ Herp 267168 USNM 110426 | Terrestrial | No preference | Rounded    | 13.32         | 2.59          |
| *Atropoides nummifer*    | 2 | MVZ Herp 219559 MVZ Herp 215983 USNM 32580 USNM 32581 | Terrestrial | No preference | Rounded    | 7.99          | 1.08          |
| *Bothriechis aurifer*    | 2 | MVZ Herp 201398 MVZ Herp 265926 | Arboreal   | No preference | Pointed     | 18.92         | 1.74          |
| *Bothriechis bicolor*    | 2 | MVZ Herp 159460 USNM 46511 | Arboreal   | No preference | Rounded    | 13.26         | 3             |
| *Bothriechis lateralis*  | 2 | MVZ Herp 207374 MVZ Herp 207375 | Arboreal   | No preference | Rounded    | 10.46         | 0.62          |
| *Bothriechis nigroviridis* | 4 | MVZ Herp 24228 MVZ Herp 215983 USNM 32580 | Arboreal   | No preference | Pointed     | 5.81          | 0.69          |
| *Bothriechis schlegelii* | 2 | MVZ Herp 68689 MVZ Herp 204271 | Arboreal   | No preference | Pointed     | 9.07          | 1.93          |
| *Bothriechis thalassinus* | 2 | MVZ Herp 267175 USNM 561799 | Arboreal   | No preference | Pointed     | 6.88          | 0.37          |
| *Bothriopsis lineatata*  | 2 | USNM 36995 USNM 36996 | Arboreal   | No preference | Rounded    | 10.63         | 1.24          |
| *Bothrocophias hyoprora* | 2 | MVZ Herp 175374 USNM 165302 | Terrestrial | No preference | Pointed     | 10.46         | 1.94          |
| *Bothrhopoides diporus*  | 4 | MVZ Herp 127510 MVZ Herp 134155 MVZ Herp 134156 USNM 165503 | Terrestrial | No preference | Rounded    | 16.32         | 0.67          |
| *Bothrhopoides jararaca* | 4 | MVZ Herp 93070 MVZ Herp 134151 MVZ Herp 134152 MVZ Herp 134164 | Terrestrial | Nocturnal     | Rounded    | 18.92         | 1.22          |
| *Bothrhopoides neuwiedi* | 1 | MVZ Herp 134157 | Terrestrial | No preference | Rounded    | 12.56         | 1.28          |
| *Bothrops asper*         | 2 | MVZ Herp 78100 MVZ Herp 160199 | Terrestrial | Nocturnal     | Pointed     | 16.47         | 1.03          |
| *Bothrops atrox*         | 4 | MVZ Herp 42044 MVZ Herp 197141 MVZ Herp 197142 MVZ Herp 197143 | Terrestrial | Nocturnal     | Pointed     | 11.34         | 0.95          |
| *Bothrops brazili*       | 2 | MVZ Herp 16342 MVZ Herp 163343 | Terrestrial | Nocturnal     | Pointed     | 9             | 1.35          |
| *Bothrops jararacussu*   | 2 | MVZ Herp 134153 MVZ Herp 134154 | Terrestrial | Nocturnal     | Pointed     | 26.41         | 2.66          |
| *Calloselasma rhodostoma* | 4 | MVZ Herp 222320 MVZ Herp 222321 MVZ Herp 222322 MVZ Herp 222323 | Terrestrial | Nocturnal     | Pointed     | 10.29         | 0.74          |
| *Cerrophidion godmani*   | 8 | MVZ Herp 40460 MVZ Herp 40463 MVZ Herp 40468 MVZ Herp 104700 MVZ Herp 109421 MVZ Herp 109422 MVZ Herp 264336 MVZ Herp 264338 | Terrestrial | No preference | Rounded    | 16.97         | 1.77          |
| *Crotalus cerastes*      | 2 | MVZ Herp 27022 MVZ Herp 173702 | Terrestrial | No preference | Rounded    | 14.58         | 1.37          |
| *Crotalus lepidus*       | 6 | MVZ Herp 22404 | Terrestrial | No preference | Rounded    | 9.62          | 1.17          |
| Scientific name                  | N  | Catalogue numbers                  | Habitat | Activity rhythm | Snout shape | Eye area (mm$^2$) | Pit area (mm$^2$) |
|----------------------------------|----|-----------------------------------|---------|-----------------|-------------|------------------|------------------|
| MVZ Herp 44755                   | MVZ Herp 67194                    | MVZ Herp 67200                  | MVZ Herp 68225                  | MVZ Herp 229857 |
| Crotalus pricei                  | 2  | MVZ Herp 59314                    | Terrestrial | No preference | Rounded | 5.94 | 0.89 |
| Crotalus scutulatus              | 4  | MVZ Herp 11429                    | Terrestrial | No preference | Rounded | 13.85 | 2.13 |
| Crotalus viridis                 | 4  | MVZ Herp 10227                    | Terrestrial | No preference | Rounded | 8.81 | 1.43 |
| Deinagkistrodon acutus           | 4  | MVZ Herp 23891                    | Terrestrial | No preference | Pointed | 16.54 | 1.83 |
| Garthius chaseni                 | 1  | USNM 134136                       | Terrestrial | No preference | Pointed | 10.63 | 2.65 |
| Gloydius blomhoffi               | 4  | MVZ Herp 22285                    | Terrestrial | No preference | Rounded | 9.95  | 1.42 |
| Gloydius breviceaudus            | 2  | MVZ Herp 12593                    | Terrestrial | No preference | Rounded | 7.16  | 1.57 |
| Gloydius halys caraganus         | 2  | USNM 14319                        | Terrestrial | No preference | Rounded | 7.07  | 0.69 |
| Gloydius intermedius             | 4  | MVZ Herp 211753                   | Terrestrial | No preference | Rounded | 5.23  | 0.95 |
| Gloydius strauchi                | 8  | MVZ Herp 216825                   | Terrestrial | No preference | Rounded | 6.56  | 1 |
| Gloydius ussuriensis             | 4  | MVZ Herp 61831                    | Terrestrial | No preference | Rounded | 7.74  | 2.25 |
| Hypnale hypnale                 | 2  | USNM 203372                       | Terrestrial | No preference | Pointed | 5.94  | 0.66 |
| Lachesis muta                   | 2  | MVZ Herp 163370                   | Terrestrial | No preference | Rounded | 15.83 | 2.22 |
| Lachesis stenophys              | 2  | USNM 165966                       | Terrestrial | No preference | Rounded | 10.69 | 1.46 |
| Mixcoatlus barbouri             | 2  | MVZ Herp 45253                    | Terrestrial | No preference | Rounded | 7.64  | 1.17 |
| Mixcoatlus melanurus            | 1  | USNM 108602                       | Terrestrial | No preference | Pointed | 7.64  | 1.52 |
| Ophryacus undulatus             | 2  | USNM 46345                        | Terrestrial | No preference | Pointed | 6.69  | 1.27 |
| Ovophis monticola               | 2  | MVZ Herp 226627                   | Terrestrial | No preference | Rounded | 6.49  | 2.64 |
| Ovophis okinavensis             | 4  | MVZ Herp 200851                   | Terrestrial | No preference | Pointed | 10.86 | 1.05 |
| Porthidium dumni                 | 2  | MVZ Herp 200832                   | Terrestrial | No preference | Pointed | 5.55  | 0.74 |
| Porthidium nasutum              | 2  | MVZ Herp 217608                   | Terrestrial | No preference | Pointed | 7.35  | 0.9 |

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downward pits, forming a wide receptive field of pits with downward orientation (Fig. 2). For the pointed snout pattern, the front of the snake's head constricts in the forward direction to form a cuneal shape (viewed from top, Fig. 3A) with an obvious tip point (viewed from side, Fig. 3B) so that the pit orientations are directed mainly forward. For the rounded snout pattern, the snout end does not appear pointed from any angle, so that the pit orientations are directed mainly laterally (Fig. 4).

Phylogeny Mapping

Although a few genera appeared to be nonmonophyletic in Crotalinae (Pyron et al., 2013), most species measured had stereotypically consistent patterns of pit orientation within their genera. Consequently, in these genera, we selected one species from each genus for reconstructing the phylogenetic tree, but used all species in the genera Ovophis, Mixcoatlus, and Rhinocerophis, because each of these contained species of varied pit orientations (Fig. 5). No consistent traits such as habitat, pattern of daily activity, or snout shape could be mapped on branches within any clade in the phylogenetic tree.

Relationships Between Eye Sizes and Pit Areas

In this study, lambda equaled 0.44, between 0 and 1, and significantly different from the upper (P < 0.001) but not the lower bounds (P = 0.06), indicating that the trait evolved approximately randomly. Eye and pit areas were not of significantly related to each other based on a BM model (PGLS: AIC = 286.73, r = -0.32, df = 56, t = -0.87, P = 0.10) or an OU motion model of evolution (PGLS: AIC = 276.82, r = -0.22, df = 56, t = -0.95, P = 0.09). Similarly, no significant correlation between eye and pit was found based on a nonphylogenetic model (Pearson correlation: r = -0.156, df = 56, P = 0.08).

For the PGLS model, eye and pit areas were significantly related to each other based on a BM model or

Table 1. (continued).

| Scientific name                  | N   | Catalogue numbers | Habitat    | Activity rhythm | Snout shape | Eye area (mm²) | Pit area (mm²) |
|---------------------------------|-----|-------------------|------------|-----------------|-------------|---------------|---------------|
| Protobothrops flavoviridis      | 2   | MVZ Herp 23904    | Terrestrial| No preference   | Rounded     | 19.79         | 2.02          |
|                                 |     | MVZ Herp 57922    |            |                 |             |               |               |
| Protobothrops macrosquamatus    | 6   | MVZ Herp 22325    | Terrestrial| No preference   | Rounded     | 7.5           | 2.35          |
|                                 |     | MVZ Herp 23907    |            |                 |             |               |               |
|                                 |     | MVZ Herp 218024   |            |                 |             |               |               |
|                                 |     | MVZ Herp 226628   |            |                 |             |               |               |
|                                 |     | MVZ Herp 230461   |            |                 |             |               |               |
|                                 |     | MVZ Herp 241450   |            |                 |             |               |               |
| Rhinocerophis alternatus        | 1   | MVZ Herp 200831   | Terrestrial| No preference   | Rounded     | 15.97         | 1.94          |
| Rhinocerophis ammodytoides      | 2   | MVZ Herp 127512   | Terrestrial| No preference   | Pointed     | 9.62          | 1.34          |
|                                 |     | MVZ Herp 134149   |            |                 |             |               |               |
| Rhinocerophis coticara          | 1   | MVZ Herp 200831   | Terrestrial| No preference   | Rounded     | 19.16         | 2.18          |
| Sistrurus catenatus             | 4   | MVZ Herp 79231    | Terrestrial| No preference   | Rounded     | 11.4          | 1.14          |
|                                 |     | MVZ Herp 226244   |            |                 |             |               |               |
|                                 |     | MVZ Herp 228856   |            |                 |             |               |               |
|                                 |     | MVZ Herp 228857   |            |                 |             |               |               |
|                                 |     | MVZ Herp 64700    |            |                 |             |               |               |
|                                 |     | MVZ Herp 110880   |            |                 |             |               |               |
| Sistrurus miliarius             | 2   | MVZ Herp 23898    | Terrestrial| No preference   | Rounded     | 11.16         | 0.79          |
|                                 |     | MVZ Herp 23903    |            |                 |             |               |               |
|                                 |     | MVZ Herp 224298   |            |                 |             |               |               |
|                                 |     | MVZ Herp 226618   |            |                 |             |               |               |
|                                 |     | MVZ Herp 226620   |            |                 |             |               |               |
|                                 |     | MVZ Herp 226622   |            |                 |             |               |               |
|                                 |     | MVZ Herp 226624   |            |                 |             |               |               |
|                                 |     | MVZ Herp 258152   |            |                 |             |               |               |
| Trimeresurus albolabris         | 8   | MVZ Herp 23898    | Arboreal   | No preference   | Pointed     | 8.5           | 0.76          |
|                                 |     | MVZ Herp 23903    |            |                 |             |               |               |
|                                 |     | MVZ Herp 224298   |            |                 |             |               |               |
|                                 |     | MVZ Herp 228856   |            |                 |             |               |               |
|                                 |     | MVZ Herp 226618   |            |                 |             |               |               |
|                                 |     | MVZ Herp 226620   |            |                 |             |               |               |
|                                 |     | MVZ Herp 226622   |            |                 |             |               |               |
|                                 |     | MVZ Herp 226624   |            |                 |             |               |               |
|                                 |     | MVZ Herp 258152   |            |                 |             |               |               |
| Trimeresurus gumprechti         | 1   | MVZ Herp 226641   | Arboreal   | No preference   | Pointed     | 17.05         | 1.85          |
| Trimeresurus macrops            | 1   | MVZ Herp 258153   | Arboreal   | No preference   | Pointed     | 8.7           | 1.02          |
| Trimeresurus stejnegeri         | 2   | MVZ Herp 224286   | Arboreal   | No preference   | Pointed     | 14.65         | 2.2           |
|                                 |     | MVZ Herp 226639   |            |                 |             |               |               |
| Trimeresurus vogeli             | 2   | MVZ Herp 222324   | Arboreal   | No preference   | Pointed     | 12.25         | 1.31          |
|                                 |     | USNM 95094        |            |                 |             |               |               |
| Tropidolaemus wagleri           | 4   | MVZ Herp 43702    | Arboreal   | No preference   | Pointed     | 17.41         | 3.69          |
|                                 |     | MVZ Herp 111878   |            |                 |             |               |               |
|                                 |     | MVZ Herp 200852   |            |                 |             |               |               |
|                                 |     | USNM 34506        |            |                 |             |               |               |

MVZ: the Museum of Vertebrate Zoology, UC Berkeley, USA.
USNM: the National Museum of Natural History of the Smithsonian Institution, USA.

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REVERSAL CORRELATION BETWEEN EYE AND PIT SIZES IN CROTALINAE

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an OU motion model of evolution for both terrestrial (Rounded snout: BM, $AIC = 147.04, r = 0.64, df = 25, t = -2.63, P = 0.015$; OU, $AIC = 147.12, r = -0.91, df = 25, t = -2.51, P = 0.019$; Pointed snout: BM, $AIC = 108.61, r = -0.59, df = 18, t = -1.55, P = 0.02$; OU, $AIC = 110.44, r = -0.43, df = 18, t = -1.2, P = 0.04$) and arboreal species (BM, $AIC = 64.53, r = -0.95, df = 13, t = -4.08, P = 0.001$; OU, $AIC = 59.94, r = -0.76, df = 13, t = -5.69, P < 0.001$). There were more species in the Pearson correlation analyses, because three species could not be assigned to a position in the phylogenetic tree. However, the results showed similar relatedness between the relative eye and pit areas in terrestrial species (Rounded snout: $r = -0.398, n = 28, P = 0.036$; Pointed snout: $r = -0.472, n = 18, P = 0.048$, Fig. 6) and arboreal species ($r = -0.667, n = 13, P = 0.013$, Fig. 7).

**DISCUSSION**

Radiations with wavelengths longer than about 1.2 μm are not sufficiently energy-rich to induce conformational changes in retinal (e.g., cis-retinal to all-trans retinal); thus, visual photoreceptors are not able to detect IR-wavelengths. Pit organs in snakes have evolved to respond to middle and long wavelength IR-radiation which emitted by endothermic animals, thus enabling these species to use both the visual and IR-systems to image prey (Hartline et al., 1978; Bakken and Krochmal, 2007).

Absolute and relative eye sizes typically vary independently with phylogeny among vertebrate taxa, reflecting the way species have become adapted to selection pressures related to habitat differences, daily activity patterns, availability of prey, foraging patterns, and predator avoidance strategies (Werner, 1969; Howland et al., 2004; Thomas et al., 2006; Liu et al., 2012). Clutch parameters may be associated with neonatal eye size in geckos (Werner and Seifan, 2006). The size of an animal’s eyes reflects the costs and benefits associated with adaptation (Land and Nilsson, 2002), evolving under strong selection pressures which might nevertheless have been relaxed by the innovation of pit organs in snakes.

In our analysis, the possible effects of each of the following needed to be considered: 1) asymmetry of the eye size between the left and right sides as well as possible lateralization of visual behavior (Deckel, 1995; Werner and Seifan, 2006; Razzetti et al., 2007), 2) sexual dimorphism of the eyes and/or the pits, and 3) allometric scaling of biometric measures resulting from the restriction of ratios between head sizes and those of eye and/or pit sizes. In order to insure that the analysis identified relationships between eye and pit size evolution, we excluded the influences of asymmetry and sexual dimorphism by measuring the eyes and pits on the left side only given that both sexes have the same relative eye and pit sizes. The allometric effect was minimized through computing residuals instead of ratios of eye/pit areas to head sizes.

**Large Sensor for Weak Signals**

Animals with IR-sensitivity possess many advantages in hunting, feeding, thermoregulation, predator avoidance, and locating sites for egg-laying, each of which increases survival and reproductive success (de Cock Buning, 1983; Kardong, 1986; Greene, 1992; Sexton et al., 1992; Krochmal and Bakken, 2003; Krochmal et al., 2004). Some snakes (Crotalinae, boas, and pythons) with high numbers of IR-sensitive receptors are able to detect temperature changes of 0.003°C or less, far smaller than insects and bats can detect. Moreover, these snakes can form IR-images (Bullock and Diecke, 1956). IR-radiation (≥1.2 μm) does not contain sufficient energy (≤1.0 eV) to trigger the kinds of transduction cascades underlying visual receptor activity. Thus, larger pits are necessary for sensing weaker IR-signals emitted by a warm body. Larger size can bring about better sensitivity but lower resolution which may be compensated for by image reconstruction in the brain, as is believed to occur in pit vipers (Sichert et al., 2006).

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Two critical functions of the eyes involve detecting light at low intensity (visual sensitivity) and distinguishing detail at a given light intensity (visual resolution). Both functions depend on the size of the eye (Walls, 1942; Land and Nilsson, 2002). More photons enter the eye per solid angle of image as the eye size increases. Thus, the absolute rather than relative (e.g., measurement divided by body index) size determines the sensitivity and resolution of the eyes (Thomas et al., 2006). In general, although phylogeny can influence eye morphological characters (Howland et al., 2004; Motani and Schmitz, 2011), ambient light levels appear to be more important factors determining eye size in vertebrates (Thomas et al., 2006; Hall, 2008; Schmitz and Wainwright, 2011; Liu et al., 2012).

Functional Complementation Between Infrared and Visual Systems

The facial pit consists of a flask-shaped cavity divided into inner and outer chambers by a suspended sensory membrane which is obviously larger than the cavity mouth (de Cock Buning, 1985; Gracheva et al., 2010). The cavities expand into a depression of the maxillary bone. Because, it

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functions as a camera-like device with pinhole optics, the anatomical characteristics of the pit including orientation, shape, and size are associated with the sensory field (Bakken et al., 2012). The sensory field of the IR-device substantially overlaps that of the eye. The sensory field overlap is also reflected by the neural spatiotopic maps for both systems (Hartline et al., 1978) as well as in the targeting areas (Chen et al., 2012).

IR-responsive neurons are located ventral to layer 7b of the optic tectum. The receptive fields of the tectal IR-neurons exhibit a similar topological arrangement as the receptive fields of visual neurons located dorsal to layer 7a (Kass et al., 1978). Bimodal neurons also exist and are located between the IR and visual layers and have been found to respond to both IR and visual stimuli, providing a possible basis for integration of the two systems (Newman and Hartline, 1981). Since both IR and visual information may be used for decision making, it is possible that the eye and pit on the same side of the head work in concert for targeting prey. These results are also consistent with a study showing that the predation performance of a congenitally blind rattlesnake was the same as that of normal siblings (Kardong and Mackessy, 1991). Taken together, these studies show that there is some degree of interchangeability in the way the IR and visual systems function for prey targeting in Crotalinae species.

**Adaptation Rather Than Phylogeny Matters**

Eye and pit sizes and ecological patterns were not consistently associated with specific clades, suggesting that these variations in eye and pit size in Crotalinae species result from selection pressures acting on each species and do not reflect characters associated with phylogeny. Interspecific variations in the exact position and orientation of the external opening of the facial pit might reflect the same side of the face, or when either both eyes or both pit organs were occluded (Chen et al., 2012). These results suggest that the eye and pit on the same side of the head work in concert for targeting prey. These results are also consistent with a study showing that the predation performance of a congenitally blind rattlesnake was the same as that of normal siblings (Kardong and Mackessy, 1991). Taken together, these studies show that there is some degree of interchangeability in the way the IR and visual systems function for prey targeting in Crotalinae species.

![Fig. 5. Phylogenetic tree derived from Pyron and Burbrink (2014). The other species were pruned using package “caper” in R i386 3.1.2 (Orme et al., 2012). Sun, no preference; Moon, primarily nocturnal; T, terrestrial; A, arboreal; R, rounded snout; P, pointed snout.](image1)

![Fig. 6. The correlations between the residuals of pit and eye areas in terrestrial species of Crotalinae.](image2)

![Fig. 7. The correlations between the residuals of pit and eye areas in arboreal species of Crotalinae.](image3)
ecological demands (Kohl et al., 2012). For instance, the three-dimensional geometry of the facial pit determines its orientation sensitivity and imaging properties (Bakken and Krochmal, 2007) and different snout shapes result in different pit orientations (Bakken et al., 2012). Differences in habitat affect eye size in Squamata (Werner, 1969; Liu et al., 2012). The light levels (i.e., scotopic vs. photopic) available to lizards influence eye shape to a greater degree than phylogeny (Hall, 2008). For colubrid snakes, EDs are larger in diurnal than in nocturnal species, and are not related to phylogeny (Liu et al., 2012). In Liolaemus lizards the parietal-eye size shows a weak relation to thermal tolerance rather than phylogeny (Labra et al., 2010).

Large pit organs could theoretically relax the selection pressure for large eyes (or vice versa) in Crotalinae snakes. Compared with the regular scales on the skin, the eyes and pits are more delicate and more easily injured. Natural selection would hence favor the development of the smallest possible fragile areas on the surface in order to minimize the chances of injury. It is notable that the evolution of trichromatic vision in primates relaxed selection pressure on the vomeronasal system, resulting in the loss of some olfactory receptor genes (Liman and Innan, 2003; Gilad et al., 2004). These trade-offs are consistent with the idea that as sensory systems become larger or more complex the associated neuronal networks must also enlarge despite the fact that overall brain size is usually constrained. Thus, specialized enlargement of one sensory system may be associated with reduction in the size of another. Such trade-offs may be especially prominent in animals with highly specialized sensory systems such as the IR, electric, and magnetic sensory systems as well as echolocation (Harvey and Krebs, 1990; Shen et al., 2013).

In conclusion, the orientation of the pit opening is influenced by snout shape, which brings about different degrees of sensory field overlap between the eyes and the pits. The two systems exhibit neurophysiological similarities and are used similarly behaviorally. As a result, eye size in Crotalinae varies inversely with pit area size. The relationship between eye and pit areas is virtually independent of phylogeny in Crotalinae.

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