

## How the Horned Lizard got its Horns - supporting online material

Kevin V. Young, Edmund D. Brodie, Jr. & Edmund D. Brodie III\*

Department of Biology, Utah State University, Logan, Utah, 84322-5305, USA

\*Department of Biology, Indiana University, Bloomington, Indiana, 47405-3700, USA

Correspondence: E. D. Brodie III: edb3@bio.indiana.edu, (812) 856-4572

### Material and Methods

The general rugose appearance and horny margin of the body and head of horned lizards are thought to increase crypsis by disrupting the outline of the body form against the background (S1, S2). Published accounts also assert that horns are used to repel or prevent ingestion by predators including other lizards, snakes, mammals and birds (S3-S5). Anecdotal observations suggest that predators occasionally die after eating horned lizards when the horns lodge in the throat (S6, S7), but such occurrences do not offer a fitness advantage to the prey, since they too die. A quantitative relationship (measurable as a covariance (S8, S9) between variation in horn length and the probability of survival in interaction with predators is necessary to establish selection on horn length. Moreover, an understanding of how the horns function to generate such a relationship is fundamental to determining the cause of selection and therefore adaptation (S9-S11).

We quantified selection on relative horn lengths of flat-tailed horn lizards, by comparing measurements of live lizards with those from skulls of lizards killed by shrikes and collected from their repository on bushes (primarily creosote). Collections were made on the Barry M. Goldwater Air Force Gunnery Range outside Yuma, Arizona, in 1996 and 1997. Skulls ( $n = 29$ ) and heads of live lizards ( $n = 155$ ) were scored for the width at the tips of the squamosal horns (“squamosal horn length”), the length of the parietal horns (“parietal horn length”, measured as the length of the longest horn), and the width at the tips of the parietal horns (“tip width”) (Fig. S1). We selected these variables because the flat-tailed horned lizard, *Phrynosoma mcalli*, has the longest relative horn sizes in the genus (S12-S14). Head length (not including horns) is closely correlated with snout-vent length ( $r=0.90$ ,  $n=122$ ,  $P<0.0001$ ) in live lizards and was used as an estimate of body size differences. Residual measures of parietal and squamosal horn

lengths, and tip width were used from linear regressions on head length. The residual measures provide estimates of relative horn dimensions controlling for differences in overall head (and body) size and allometry (S15), as well as reduce the multicollinearity in the data set.

We used univariate cubic splines (S16) to produce a visualization of the selection surface for individual characters. Surfaces were estimated using the program *glms v 4.0* (S16). Values of 1 were chosen to minimize generalized cross-validation scores ( $\lambda=2$  for relative squamosal horn length and  $\lambda = -2$  for relative parietal horn length). Multiple regression was used to provide quantitative estimates of the strength of selection on each character accounting for the correlations among traits. Nonlinear selection was investigated using quadratic regression (S8, S9). Analyses using principal component measures of morphological variation (not shown) provided qualitatively similar results to those reported here.

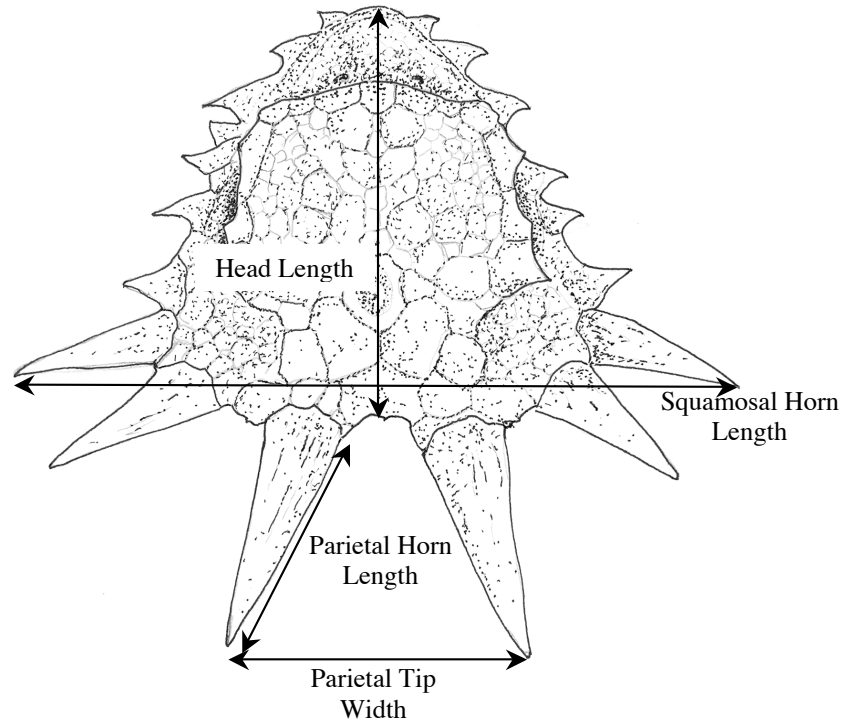
### **SOM text**

Selection analyses revealed significant directional selection on squamosal horn length and parietal horn length, but not on parietal horn tip width. No significant nonlinear selection was detected on any single trait or on the combination of horn characters. Nonlinear selection is notoriously difficult to detect and may require sample sizes of upwards of 500 individuals to provide sufficient power to detect in a multiple regression analysis (S17).

Two factors that might be suspected to produce false indications of selection in this study are skull shrinkage and sexual dimorphism. Neither appears to be a confounding factor after inspection of the data. Shrinkage of skulls after death is minimal. Intact skulls of freshly road-killed horned lizards were collected and measured immediately in 1998 and then dried for approximately 5 yrs and re-measured ( $n=2$ ). Comparisons revealed average shrinkage of 0.22 mm (2%) of parietal horn length and 0.95 mm (3.6%) of squamosal horn length. Not all horn lengths shrunk. This change could only account for up to a third of the horn length differences observed between live and shrike-killed lizards.

Sexual dimorphism is also minimal, with females having slightly shorter horn lengths relative to head size. Sex is not discernable in shrike-killed lizards. Measures of live lizards indicate that the average female squamosal horn length is 0.52 mm (2%) less than the population mean, and average female parietal horn length is 0.23 mm (2.3%) less than the population mean.

The shrike-killed sample of heads differed from the population mean by 10% or greater for both measures. Body size-adjusted measures (residuals) for the shrike-killed sample have non-overlapping 95% confidence intervals with either male or female lizards. Sex-biased predation, independent of horn length, therefore cannot explain the differences between the live and shrike-killed samples.



**Figure S1.** Dorsal view of horned lizard skull showing position and measurements of horn length characters examined for potential selection by loggerhead shrikes.

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