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Multiple Sensory Modalities in Diurnal Geckos Is Associated with the Signaling Environment and Evolutionary Constraints

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Synopsis To be effective, animal signals need to be detectable in the environment, but their development and expression require resources. For multimodal communication, investment in elaborating traits in one modality could reduce the elaboration of traits in other modalities. In *Cnemaspis* geckos, chemical signals for conspecific communication predated the evolution of visual signals, allowing us to examine the potential trade-off in signal elaboration and the current habitat associations with signal use. We studied five species of *Cnemaspis* and quantified visual (patch size, color characteristics) and chemical (secretory composition) traits in males, as well as key environmental parameters (temperature, humidity, light) in each of their habitats. Within species, we found some trade-off in the elaboration of signals, as the strength of several components in the visual and chemical modalities were negatively associated. Strength of some signal components in each modality was also independently associated with specific environmental parameters that affect their detection (visual traits) and persistence (chemical traits). Specifically, species with larger, brighter, and more saturated color patches were found in habitats where the brightness and chroma of light were lower. Furthermore, environments with higher substrate temperature and higher relative humidity harbored species that produced secretions with a higher percentage of saturated and aromatic compounds. Thus, the elaboration of multimodal signals in this group of *Cnemaspis* geckos seems to increase the efficiency of communication in the signaling-environment, but the strength of signals in different modalities is constrained by trade-offs in signal expression.

Introduction

The efficacy of animal communication is determined by the structure and content of signals (Endler 1993a), as well as the ambient environment in which communication occurs (Alberts 1992). Signals, which can be morphological, behavioral, or physiological products used for intraspecific interactions (Otte 1974; Candolin 2003; Bro-Jørgensen 2010), are under selection for high detectability and persistence in the environment (Endler 1993b). Higher response to a signal can be achieved by increasing the intensity or stability of the signal or by selecting microhabitats optimal for emission, transmission, and reception of the signal by receivers (Endler 1992). For example, unlike animals living in temperate grasslands, those that live in tropical forests, where temperatures are higher, have pheromones with higher average

molecular weight which enhances signal stability (Alberts 1992). Habitat selection for different lighting environments in *Anolis* lizards directly enhances detectability of their ultraviolet -reflective dewlap (Leal and Fleishman 2002). Studying signal structure and composition in the context of the local environment is, therefore, key to understanding their effectiveness in communication.

Multimodal signaling adds to the complexity of signal evolution and maintenance, especially when traits in different modalities compete for resources and are under directional selection to increase in intensity (Badyaev et al. 2002; Agrawal et al. 2010; Bourg et al. 2017). The development and expression of multiple traits require resources (Agrawal et al. 2010), and thus trade-offs may occur between trait components if resources are constrained (reviewed in

Zera and Harshman 2001). For example, in the cricket, *Gryllus rubens*, dietary nutrients are limited and results in a trade-off between stronger flight muscles and smaller ovaries in long-winged morphs, and the opposite pattern in short-winged morphs (Mole and Zera 1993). Trade-offs in the strength or intensity of signal components within or across sensory channels for social communication are also apparent. In many bird species, song complexity is negatively correlated with visual ornamentation (Ossip-Klein et al. 2013; Cooney et al. 2018). In *Sceloporus* lizards, species with more elaborate visual displays have fewer chemical secreting femoral pores (Ossip-Klein et al. 2013). Because of such trade-offs, lower expression of some signaling components may result in less than optimal transmission efficiency of that modality in the local habitat. However, if reduction in signal expression in one modality is associated with an increase in the strength of expression of traits in other modalities, the overall multimodal signal could still be effective in the environment, especially if signal components are redundant (Bro-Jørgensen 2010; see Partan and Marler 1999 for types of multimodal signals). Thus, the current signaling-environment is an important factor for the maintenance and optimization of multimodal signals.

Among lizards, geckos are versatile and use multiple signaling modalities to communicate with conspecifics. Most geckos are nocturnal (Pianka and Vitt 2003) and utilize chemical, acoustic, or visual signals for intraspecific communication (Schwenk 1993). Though chemicals are used for sex-recognition, as in *Eublepharis macularius* (Mason and Gutzke 1990) and *Hemidactylus flaviviridis* (Mahendra 1953), multimodal communication is common in species such as the tropical house gecko, *H. mabouia*, where chemical secretions, in association with chirp calls and postures, are used during courtship (Regalado 2003). Among diurnal geckos, visual communication, associated with the evolution of color in males, has been shown to be involved in sex-recognition in *Sphaerodactylus* (Regalado 2012). Visual signals in the diurnal gecko, *Gonatodes albogularis*, contain even more information, as the intensity of the yellow heads (present only in males) is also correlated with the quality of male territory (Ellingson 1994).

Like several other genera of diurnal geckos, *Cnemaspis* evolved from nocturnal ancestors (Röll 2001) that communicated primarily via chemical and/or acoustic signals (Cooper 1994; García-Roa et al. 2017; Mayerl et al. 2015). Males of many *Cnemaspis* species not only have femoral glands but only some species show sexual dimorphism in visual

traits, with male expression of colored gular patches. These visual traits are a relatively recent addition to signaling in this genus (Ellingson et al. 1995). In at least three species, *C. littoralis*, *C. indica*, (M.S. Kabir and M. Thaker, submitted for publication), and *C. mysoriensis* (Kabir et al. 2019), chemical secretions elicit behavioral responses from conspecifics of both sexes. Thus, from measurements in the lab and observations from the wild, chemical secretions were found to play a role in territory marking and in individual assessment during social interactions (Joshi 2020). In experimental manipulations, visual stimuli, in association with chemical stimuli, also elicit responses by at least one sex in *C. mysoriensis* (Kabir et al. 2019) and *C. littoralis* (Kabir and Thaker, submitted for publication). Field observations of *C. mysoriensis* and *C. littoralis* correspond with this, as males are seen to use postural changes and gular inflations during social interactions, a behavior that is common to many lizards with gular patches (Johnson et al. 2019).

Here, we study the expression of signals, and the local environmental conditions in *Cnemaspis*, to understand patterns of signal elaboration and potential trade-offs between the gain of traits in one modality against the pre-existing traits in a different modality. To understand trait elaboration, we measured the following chemical traits from wild populations of these five species: total number of compounds, percentage of aromatic compounds, and percentage of compounds that were saturated. We also measured the following visual traits: relative gular patch size (percentage patch size relative to body area), brightness, and chroma of gular patches. From the field sites of each species, we measured substrate temperature and relative humidity of the local environment as factors that could influence the effectiveness of chemical signaling, and brightness and chroma of habitat light as environmental factors that could influence visual signaling. We specifically tested two main hypotheses. (1) We hypothesized a trade-off between intensities of signals in different modalities, such that species with more intense signal components in one sensory modality will have less intense signal components in the other modality. For each signal modality independently, (2) we hypothesized that the degree of trait expression will correspond to local environmental conditions, such that it increases the efficacy of signaling in the environment. For chemical signaling traits, we predict that species in areas with higher temperature and relative humidity will have a higher percentage of aromatic and saturated compounds in their secretions, which are known to be resistant to degradation (Alberts

1992). For visual traits, we predict that species in habitats with poor light-conditions will have gular patches that are larger, brighter, and with higher chroma, relative to those species found in well-lit habitats, as these visual properties will increase the contrast of the gular patch thereby enhancing the perceptiveness of the signal to conspecifics (similar to Leal and Fleishman 2002; Leal and Fleishman 2004 in *Anolis*; but see Ng et al. 2013). Different hues can play an important role in increasing conspicuousness of signalers under different light conditions (Endler 1993b). However, since all the sexually dichromatic species of *Cnemaspis* in our study had very similar hue at the gular (yellow-orange), we did not predict hue changes across environments.

Methods

Study species

We focused on five species of *Cnemaspis* that are found in forested habitats and dwell in crevices of tree-trunks close to the ground. *Cnemaspis mysoriensis* is naturally found in low and mid elevation dry deciduous forests, but can also be found in anthropogenic structures (Giri et al. 2009); *C. amboliensis* is found in mid elevation forests; *C. littoralis* is found in low elevation moist deciduous forests; *C. gracilis* is found in low- and mid-elevation forests; and *C. indica* is found in high elevation shola forests (S. Pal et al., submitted for publication). Correspondingly, we captured adult individuals of all species from 5 to 10 sites within 3 km of their type locality: *C. mysoriensis* from the forested campus of the Indian Institute of Science (IISc, 13.02°N, 77.57°E, Karnataka, India), *C. littoralis* from the forested campus of St. Xavier's Arts and Science College (11.27°N, 75.79°E, Kozhikode, India), and Sarovaram Biopark (11.27°N, 75.79°E, Kozhikode, India), *C. gracilis* from Krishna Colony (11.00°N, 77.01°E, Tamil Nadu, India), *C. indica* from shola forests of Upper Bhavani (11.23°N, 76.52°E, Tamil Nadu, India), and *C. amboliensis* from Forest Park (15.96°N, 73.99°E, Maharashtra, India).

Adult individuals of all gecko species were caught by hand during their breeding and activity period (February), between 0830 h and 1700 h, in February of each year (2016–18), photographed, measured, and scraped for chemical secretions (see details below). These five species are similar in size (snout-to-vent length: 30 [\pm 2] mm). All are chemically sexually dimorphic; males, and not females, have either femoral glands only (*C. littoralis* and *C. indica*), or both femoral and precloacal glands (*C. mysoriensis*,

C. gracilis, and *C. amboliensis*). Additionally, males of *C. mysoriensis*, *C. littoralis*, and *C. gracilis* are sexually dichromatic, and have yellow gulars (see Supplementary Fig. S1). The remaining dorsal and ventral regions of these five species are chromatically similar in males and females. Species of *Cnemaspis* from India and Sri Lanka have been reported to reproduce throughout the year (Somaweera 2009; Sayyed 2019). During the field collection, geckos were seen engaging in social interactions with both males and females, and gravid females and eggs were also apparent. Thus, we expected to capture high chemical and visual trait expression associated with breeding activity during the sampling period. All individuals were released at their site of capture within 90 min.

Characterizing chemical compounds

We collected chemical secretions from males ($N=5$ per species) within 90 min of capture. Secretions, which are visible as a thin film on the ventral surface above the femoral and precloacal regions of these geckos, were gently scraped-off from using sanitized metal spatulas and were immediately stored in 1 mL of dichloromethane (Merck, HPLC grade) in sterilized glass vials with Teflon coated screw caps that were sealed with parafilm. To maximize detection of all chemical compounds, including those that are in low concentrations, we pooled secretions from 10 individual males of the same species in each vial ($N=5$ vials per species). A separate glass vial containing only dichloromethane was stored with each set of sample vials to test for possible impurities. Sealed vials containing secretions of all species were stored at -4°C in respective field sites for approximately 10–15 days before being transported on ice to the laboratory at IISc. Sealed samples of *C. indica* were stored and transported in cool and dark conditions as refrigeration in the field was not available. Once transported to the laboratory, all vials were stored at -20°C until analysis.

Chemicals were analyzed using gas chromatography and mass spectrometry (Agilent 5890B). Samples were first transferred to 1 mL GC vials (Borosil) and lyophilized to remove moisture. The dried samples were then placed in a water bath at 60°C with 40 μL of dry pyridine (Sigma Aldrich, GC grade) and 100 μL of N-Methyl-N-(trimethylsilyl)-trifluoroacetamide (MSTFA; Sigma Aldrich, GC grade) for 30 min for derivatization, after which they were concentrated to 30–40 μL under nitrogen gas before analysis (as per Kabir et al. 2019). We identified hydrocarbons and fatty acids by matching the mass spectra

and retention indices of the compounds with the respective standards (Sigma Aldrich 40147-U and CRM18918). Detection of aromatic compounds was performed by observing the presence of fragment peaks at definite m/z ratios (Lee 1998). We identified aromatic compounds and non-hydrocarbon, non-fatty acid compounds from the database of the National Institute of Standards and Technology (NIST/EPA/NIH Mass Spectral Library, Ver. 2.2, 2014). For analysis, we compared chemical richness, and not chemical abundance, across species because intraspecific variation in abundance of many compounds was greater than interspecific abundance (see also Baeckens et al. 2018). The presence or absence of compounds, however, was consistently maintained within replicate vials of each species and allow us to compare across species.

Characterizing visual signals

We quantified color on the gular of males of all species ($N=15$ for *C. mysoriensis*, $N=20$ for *C. littoralis*, $N=11$ for *C. gracilis*, $N=20$ for *C. indica*, $N=19$ for *C. amboliensis*) using spectrometry. Percentage reflectance of color from an area of 2 mm² at the gulars of all males was measured using an optic fiber probe connected to a spectrometer (JAZA2474 Ocean Optics, Dunedin, FL, USA). The probe had a customized holder that was internally blackened on all sides to prevent reflection of light from the walls of the holder and had an obliquely cut aperture, such that light incident on the skin of the lizard was at an angle of 45°. The PX lamp triggering was maintained at 10 ms and boxcar at 5; three reflectance readings were averaged per animal. Spectral reflectance was standardized initially with a white and black standard and all reflectance readings were recorded relative to those. All negative reflectance values were converted to “0” and the overall data were smoothed by a factor of “0.2” prior to analysis (in R package “pavo”). Data from 300 to 700 nm were used to calculate the brightness and chroma of the gular based on the segment classification method (Endler 1990; Smith 2014). Total brightness was calculated as the total area under the curve between 300 and 700 nm. For chroma, we first divided the reflectance spectrum into four equally divided segments: Segment A (“A”: 300–400 nm), Segment B (“B”: 400–500 nm), Segment C (“C”: 500–600 nm), and Segment D (“D”: 600–700 nm). We then calculated the difference between Segments D and B (LM) and Segments C and A (MS), which reflect the relative brightness values as converted into the opposing inputs of the long-

medium wavelength receptors (LM) and medium-short wavelength receptors (MS) expected for a lizard. Chroma was calculated with the formula $\sqrt{(LM^2 + MS^2)}$.

To determine whether gular color patches on the colored species of *Cnemaspis* are conspicuous to conspecifics, we calculated the unweighted Euclidean distance between the spectral reflectance of the gular on males of all species against a common background of tree bark. Since the microspectrophotometric (MSP) data of the cone cells in *Cnemaspis* are unknown, we used the MSP data from *G. albogularis* (from E. Loew, personal communication), a diurnal gecko in which males have a yellow head, similar to many species of *Cnemaspis*. Based on the spectral sensitivity (λ_{max}) of the pigments, three cone cells have been identified: P362, P475, and P542 (Ellingson et al. 1995). Cone cell density and receptor noise remain unknown, and thus, we used Euclidean distance-based methods to determine whether the brightness and chroma of the gular patch of male lizards of each species were conspicuous against their signaling background. Euclidean distance measures have been shown to be linearly related to the perceptual color distance, and therefore provides a reliable measure of the probability of detection of color by receivers (Fleishman et al. 2016).

We first used the R package “pavo” to smoothen the spectral reflectance of the gular patches (by a factor of 0.2) of all individuals and the habitat irradiance of all species. We also smoothed the reflectance of the tree barks measured at the field sites ($N=12$ barks) and averaged them to obtain the mean reflectance of tree bark. This was used as the common background against which we calculated the achromatic and chromatic Euclidean distance (see Fleishman et al. 2016). Next, we used these processed data, along with the MSP data mentioned above, to calculate the quantum catches of each photoreceptor type using the “vismodel” function in “pavo.” Results of these quantum catches were then used to calculate the unweighted Euclidean distance (chromatic and achromatic) using the “coldist” function in “pavo.”

Finally, from digital photographs of the ventral side of the lizards (using Canon EOS 600D camera with 18–55 mm lens), we measured the size of colored patches on the gular of all lizards ($N=15$ for *C. mysoriensis*, $N=20$ for *C. littoralis*, $N=11$ for *C. gracilis*, $N=20$ for *C. indica*, $N=19$ for *C. amboliensis*) by manually outlining the region in ImageJ (<http://imagej.nih.gov/ij/>). The surface area of the body, measured by outlining the body from snout

to vent excluding the limbs, was used to calculate the percentage of the body showing colored patches (percentage gular patch size). All measurements were taken three times for each photograph and the average value was used for analysis.

Characterizing the environment

To determine differences in environmental parameters at the microhabitat scale of lizards at the site of capture, we measured substrate temperature with an infrared thermometer (Fluke 62 Mini) and relative humidity with a digital hygrometer (Mastech SK11700) positioned ~ 2 m and ~ 0.5 m, respectively, from the ground. Recordings of temperature and humidity were taken exactly at the spot where lizards were caught. All measurements were taken once per individual lizard (per site) between 1400 h and 1700 h.

We characterized the irradiance of the habitat for all species by taking measurements between 0900 h and 1500 h under a clear sky with the irradiance probe, attached to an Ocean Optics Jaz spectrometer, pointing parallel to the ground (Fleishman et al. 1997) to mimic a lizard viewing its surroundings while perched. Brightness and chroma of the habitat in the 300–700 nm wavelength range were measured from the irradiance spectra (as per Endler 1993b).

Statistical analysis

To determine differences in visual (percentage gular patch size, brightness, and chroma of gular patch) and chemical traits (number of compounds, percentage of aromatic compounds, and percentage of saturated compounds) we performed Kruskal–Wallis tests across species. Pairwise comparisons of traits between species were performed with post hoc Dunn's test with Bonferroni correction (at $\alpha = 0.013$). To determine whether chemical composition differed across species, we performed a permutational Multivariate analysis of variance (PERMANOVA) by Jaccard distance, using the “adonis” function of the package “vegan” in R (version 3.6.3).

Trade-offs between visual and chemical traits across species were calculated with Kruskal–Wallis test between each visual trait (percentage gular patch size, patch brightness, and patch chroma) and chemical trait (number of compounds, percentage saturated compounds and percentage aromatic compounds). Chemical traits were first converted into categorical variables as three ranks of equal bin width based on the range of trait values across all species. Specifically, number of compounds was

classified as low (12–22), medium (23–32), and high (33–42); percentage saturated compounds were classified as low (6–19%), medium (20–32%), and high (33–45%); and percentage aromatic compounds were classified as low (0–6%), medium (7–13%), and high (14–20%).

To determine associations between chemical traits and the environment, we performed Kruskal–Wallis test between each of the chemical traits (percentage saturated compounds and percentage aromatic compounds) and environmental parameters (substrate temperature and relative humidity). All tests were followed with post hoc Dunn's test with Bonferroni correction for pairwise comparisons when relevant.

To determine associations between visual traits (percentage gular patch size, brightness of gular patch, and chroma of gular patch) and the environment (brightness of habitat light and chroma of habitat light), we performed linear regression models. For patch brightness, we used generalized linear models with gamma distribution since the linear models did not meet the criteria for homoscedasticity. For all statistical analyses with percentage gular patch size, data of only sexually dichromatic species were used ($N = 3$ species). For the remaining analyses, we used data of all species ($N = 5$ species). All statistical analyses were performed in R (version 3.6.3).

Results

Differences in visual and chemical traits across species

The five species of *Cnemaspis* differed significantly across many visual and chemical traits (Table 1). Specifically, percentage gular patch size ($H = 36.91$, $df = 2$, $P < 0.001$, Fig. 1a), patch brightness ($H = 43.27$, $df = 4$, $P < 0.001$, Fig. 1b), and patch chroma ($H = 67.86$, $df = 4$, $P < 0.001$, Fig. 1c), differed across species. Against the typical bark substrates and under natural irradiance conditions, these species also differed in the achromatic ($H = 44.87$, $df = 4$, $P < 0.001$, Fig. 1d) and chromatic visual contrasts of their gular patch ($H = 54.56$, $df = 4$, $P < 0.001$, Fig. 1e).

Among the chemical traits, species also differed in the total number of compounds ($H = 18.55$, $df = 4$, $P < 0.001$; Fig. 2a), percentage of aromatic compounds ($H = 21.55$, $df = 4$, $P < 0.001$; Fig. 2b), and percentage of saturated compounds ($H = 22.16$, $df = 4$, $P < 0.001$; Fig. 2c). A non-metric multidimensional scaling analysis (NMDS with stress value of 0.045) showed that the chemical composition in secretions of male glands differed across species,

Table 1 Pairwise comparison of visual and chemical traits across species

Pairwise-species	Patch size (%)	Patch brightness	Patch chroma	Achromatic contrast	Chromatic contrast	No. of compounds	No. of aromatic compounds	Saturated compounds (%)
Myso-Litt	$D = -3.51$, $P < 0.001$	$D = -3.93$, $P < 0.001$	$D = 2.08$, $P = 0.188$	$D = -2.65$, $P = 0.041$	$D = -1.41$, $P = 0.789$	$D = 3.51$, $P = 0.002$	$D = 3.32$, $P = 0.005$	$D = 4.31$, $P < 0.001$
Myso-Grac	$D = -6.04$, $P < 0.001$	$D = -6.51$, $P < 0.001$	$D = -0.36$, $P = 1.00$	$D = -6.49$, $P = 1.00$	$D = -4.11$, $P < 0.001$	$D = 2.08$, $P = 0.027$	$D = 3.54$, $P = 0.002$	$D = 2.88$, $P = 0.019$
Myso-Indi	—	$D = -3.26$, $P = 0.006$	$D = -3.96$, $P < 0.001$	$D = -3.68$, $P = 0.001$	$D = -5.27$, $P < 0.001$	$D = 0.65$, $P = 1.00$	$D = 0.31$, $P = 1.00$	$D = 1.08$, $P = 1.000$
Myso-Ambo	—	$D = -3.19$, $P = 0.007$	$D = -4.34$, $P < 0.001$	$D = -4.08$, $P < 0.001$	$D = -5.91$, $P < 0.001$	$D = 2.76$, $P = 0.029$	$D = 1.79$, $P = 0.367$	$D = 2.49$, $P = 0.063$
Litt-Grac	$D = -3.19$, $P = 0.002$	$D = -3.31$, $P = 0.005$	$D = -2.27$, $P = 0.115$	$D = -4.45$, $P < 0.001$	$D = -3.06$, $P = 0.011$	$D = -0.73$, $P = 1.00$	$D = 0.22$, $P = 1.00$	$D = -1.42$, $P = 0.777$
Litt-Indi	—	$D = 0.73$, $P = 1.000$	$D = -6.52$, $P < 0.001$	$D = -1.11$, $P = 1.000$	$D = -4.16$, $P < 0.001$	$D = -2.86$, $P = 0.021$	$D = -3.01$, $P = 0.013$	$D = -3.23$, $P = 0.006$
Litt-Ambo	—	$D = 0.75$, $P = 1.000$	$D = -6.89$, $P < 0.001$	$D = -1.58$, $P = 0.574$	$D = -4.87$, $P < 0.001$	$D = -0.75$, $P = 1.000$	$D = -1.53$, $P = 0.632$	$D = -1.81$, $P = 0.353$
Grac-Indi	—	$D = -3.92$, $P < 0.001$	$D = 3.22$, $P = 0.006$	$D = -3.51$, $P = 0.002$	$D = 0.45$, $P = 1.000$	$D = 2.13$, $P = 0.165$	$D = 3.23$, $P = 0.006$	$D = -1.81$, $P = 0.353$
Grac-Ambo	—	$D = 3.91$, $P < 0.001$	$D = -3.58$, $P = 0.002$	$D = 3.08$, $P = 0.010$	$D = -1.09$, $P = 1.000$	$D = -0.02$, $P = 1.000$	$D = -1.75$, $P = 0.403$	$D = -0.39$, $P = 1.000$
Indi-Ambo	—	$D = 0.03$, $P = 1.000$	$D = -0.46$, $P = 1.000$	$D = -0.48$, $P = 1.000$	$D = -0.76$, $P = 1.000$	$D = 2.11$, $P = 0.174$	$D = 1.48$, $P = 0.688$	$D = 1.42$, $P = 0.777$

Shown are values of post-hoc Dunn's Test with associated P -values. $N = 3$ species for patch size (%); $N = 5$ species for the remaining traits. Significant test values (at $P < 0.013$ after Bonferroni correction) are in bold.

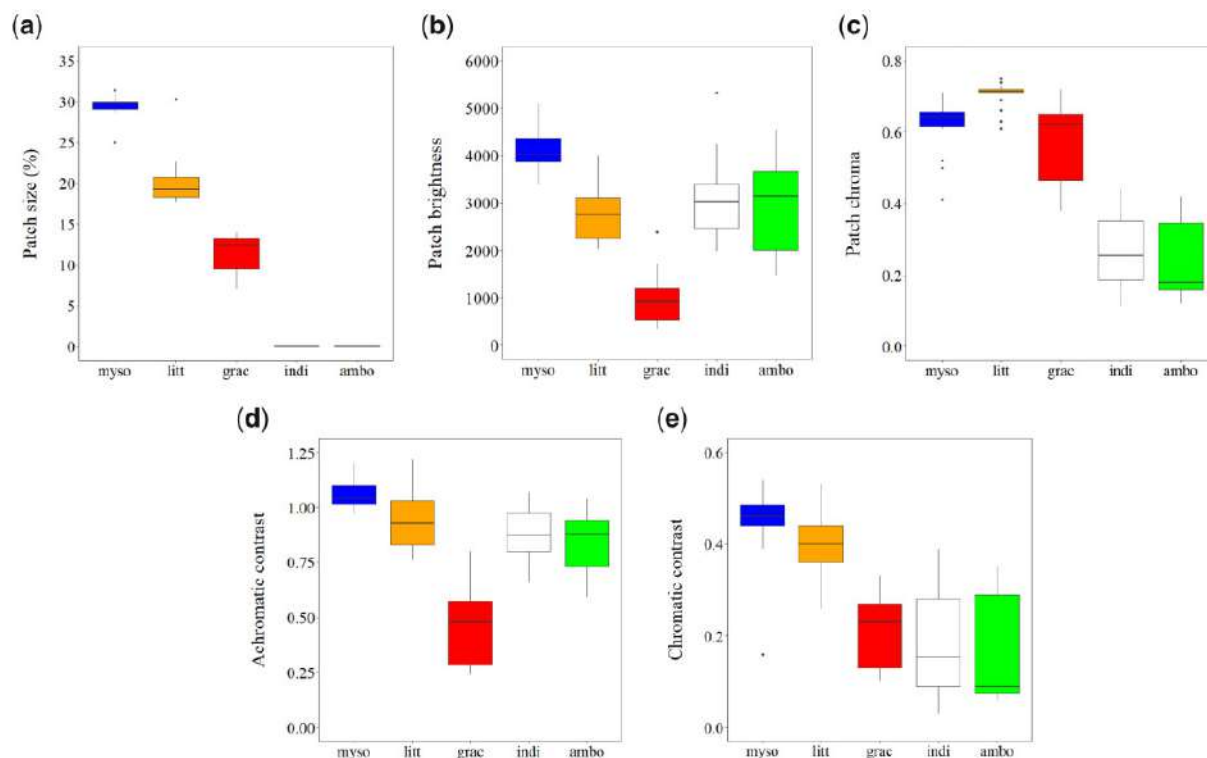


Fig. 1 Visual traits across species: (a) percentage patch size, (b) patch brightness, (c) patch chroma, (d) achromatic contrast, and (e) chromatic contrast. Boxplots show medians, quartiles, 5th and 95th percentiles, and extreme values. (myso = *C. mysoriensis*; litt = *C. littoralis*; grac = *C. gracilis*; indi = *C. indica*; ambo = *C. amboliensis*).

except between *C. gracilis* and *C. amboliensis* ($F_{4,20} = 62.17$, $P < 0.001$; Fig. 2d; Supplementary Table S2). Briefly, *C. gracilis* and *C. amboliensis* had qualitatively similar compounds, but the remaining species differed from each other in the number of chemical compounds, the identity of the chemical compounds, as well as the categories of chemical compounds (Supplementary Table S2).

Trade-offs between visual and chemical traits

Across species, we found that the number of compounds ($H = 26.70$, $P < 0.001$), percentage aromatic compounds ($H = 26.70$, $P < 0.001$), and percentage saturated compounds ($H = 26.70$, $P < 0.001$) were negatively associated with percentage of gular patch size. Similarly, number of compounds ($H = 16.29$, $P < 0.001$), percentage aromatic compounds ($H = 32.34$, $P < 0.001$; Fig. 3a), and percentage saturated compounds ($H = 26.89$, $P < 0.001$; Fig. 3b) were negatively associated with gular patch brightness. With gular patch chroma, we found some association with percentage aromatic compounds ($H = 62.49$, $P < 0.001$; Fig. 3c) and percentage saturated compounds ($H = 19.94$, $P < 0.001$; Fig. 3d), but no association with number of compounds ($H = 1.67$, $P = 0.141$). Specifically, species with medium ranked percentage saturated compounds and

percentage aromatic compounds had significantly lower gular patch chroma than those with low ranked or high ranked compounds (Table 2). Post hoc pairwise comparisons for trade-offs between ranks of chemical traits (low, medium, and high) are presented in Table 2.

Relationship between environmental conditions and chemical traits

Species with higher ranked percentage aromatic compounds ($H = 61.46$, $P < 0.001$; Fig. 4a) and percentage saturated compounds ($H = 54.28$, $P < 0.001$; Fig. 4b) were found in areas with higher substrate temperature. Similarly, species with higher ranked percentage aromatic compounds ($H = 36.90$, $P < 0.001$; Fig. 4c) and percentage saturated compounds ($H = 57.81$, $P < 0.001$; Fig. 4d) were found in areas of higher relative humidity. Post hoc pairwise comparisons between ranks of chemical traits are provided in Table 3.

Relationship between environmental conditions and visual traits

Percentage gular patch size of lizards was negatively associated with brightness of the environment ($t = -8.93$, $P < 0.001$; Fig. 5a), but did not vary with

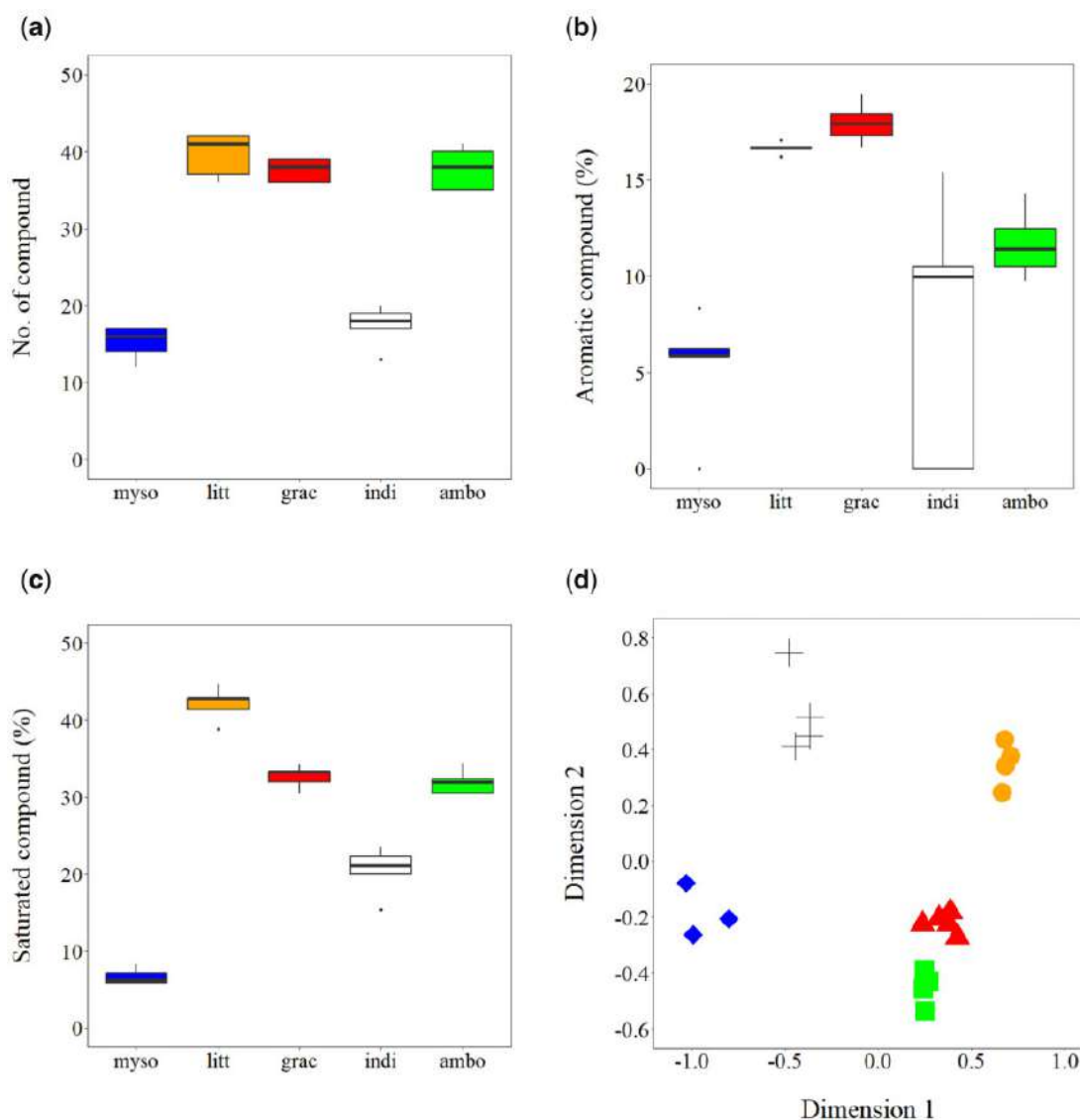


Fig. 2 Chemical traits across species: (a) number of compounds, (b) percentage of aromatic compounds, (c) percentage of saturated compounds, and (d) multi-dimensional scaling analysis of chemical compounds. Boxplots for (a), (b), (c) show medians, quartiles, 5th and 95th percentiles, and extreme values. For (d), blue diamond = *C. mysoriensis*, orange circle = *C. littoralis*, red triangle = *C. gracilis*, black cross = *C. indica*, green square = *C. amboiensis*.

chroma of the environment ($t = -1.01$, $P = 0.32$). Patch brightness was also negatively associated with brightness of the environment ($t = 5.94$, $P < 0.001$; Fig. 5b), but did not vary with chroma of the environment ($t = 0.79$, $P = 0.434$). Finally, patch chroma did not vary with brightness of the environment ($t = 0.79$, $P = 0.427$), but was negatively associated with chroma of the environment ($t = -2.27$, $P = 0.027$; Fig. 5c).

Discussion

We studied visual and chemical signaling traits in five species of diurnal *Cnemaspis* geckos to

determine patterns of signal elaboration across environmental conditions, and the potential trade-offs between signal components in the visual and chemical modalities. As expected from general patterns of diversification, species of *Cnemaspis* differed in several components of visual and chemical traits that are involved in intraspecific communication. Some of these traits, however, seem to vary in keyways that could enhance efficiency of communication in the local environmental conditions of their habitat. We also found some evidence for trade-offs between visual and chemical signaling traits, such that elaboration in one modality did not follow with elaboration in the other.

The amount and composition of chemical signals determine its intended function, as well as its temporal and spatial persistence in the environment (Alberts 1992; Baeckens et al. 2018). In *Cnemaspis*, we found considerable variation in chemical signaling traits, not only in the number of compounds but also in the percentage of aromatic and saturated compounds in their secretions. Several studies in lizards have found similar classes of compounds in glandular secretions (Escobar et al. 2001; Louw et al. 2007; Khannoon et al. 2011). For example, across *Liolaemus* species, 49 distinct compounds were found in the ventral secretions, among which, cholesterol and several carboxylic acids were common to all (Escobar et al. 2001). Signals with high compositional diversity, such as that found in *Cnemaspis*, are primarily used at close ranges to provide detailed information about the signaler (Alberts 1992).

Characteristics of chemical traits for these species were also positively associated with local environmental conditions (similar to Campos et al. 2020, but see Baeckens et al. 2015, for lacertids). We found that, in general, species in areas with high temperature and humidity produced secretions that had higher percentage of saturated and aromatic compounds. Similar patterns have been seen in lizards in the family Lacertidae, where species in xeric habitats have greater proportions of stable fatty acid esters and alcohols with higher molecular weight than species in mesic habitats (Baeckens et al. 2018). In *Sceloporus*, species living in hotter environments have lower proportions of unsaturated fatty acids, higher proportions of aldehydes, and no differences in proportions of saturated fatty acids (Campos et al. 2020). Higher number of aromatic and saturated compounds increases the overall stability of the secretions in those environments. Larger molecules, that are relatively less volatile, have lower diffusion rates and persist longer where deposited (Louw et al. 2007). Among the species we measured, however, *C. mysoriensis* produced lower than expected percentages of aromatic and saturated compounds, given the temperature of their environment. This may be explained by the fact that some of these compounds have multiple functions, and that abundance of a few compounds may override the lack of richness. For example, saturated compounds and alkyl ketones act as sex attractants in snakes (reviewed in Mason and Parker 2010), whereas some act as adhesives in lizards (Khannoon 2012). Aromatic compounds have been reported to have multiple functions including sex-attraction, territory marking, and recognition (Alberts 1992). Additionally, close

inspection of the constituent compounds in the secretions of *C. mysoriensis* showed that most of the compounds have high molecular weight (determined from retention time of elution), and may be a different way to decrease loss of chemicals by evaporation (Alberts 1992; Baeckens et al. 2018). This is in stark contrast to the secretions of *C. indica* that have fewer compounds of high molecular weight, yet are relatively rich in aromatic and saturated compounds. Thus, for signalers that use chemicals for short-distance communication, like *Cnemaspis*, it is beneficial to have at least some molecules with higher weight and stability for efficient communication, especially in environments with high temperature and humidity.

The evolution of visual signals in *Cnemaspis* seems to increase the conspicuousness of signalers to receivers, as some properties of visual signals are associated with properties of light in the habitat. Species with larger, brighter, and more saturated (higher chroma) color patches were found in duller habitats (i.e., brightness or chroma of light was lower). Color patches for sexual signaling that are conspicuous against natural backgrounds enhance detectability (Macedonia 2001; Endler and Day 2006). Contrast between the spectral reflectance of male gulars against tree bark reveals key differences in the conspicuousness of these patches to conspecifics. Specifically, chromatic contrasts were greater for the yellow gulars of *C. mysoriensis* and *C. littoralis* compared to the yellow gular of *C. gracilis*, and the white gulars of *C. indica*, and *C. amboiensis*. Further, achromatic contrast of these gulars was greatest for *C. mysoriensis* and lowest for *C. gracilis*, with intermediate values for the other species. Thus, irrespective of the absolute chroma of the yellow or white gulars of these geckos, males of *C. mysoriensis* and *C. littoralis* are highly conspicuous in their environment, while male gulars of *C. indica*, *C. amboiensis*, and *C. gracilis* are lower. The presence of the yellow gular, at least for *C. mysoriensis* and *C. littoralis*, seems to be enhancing efficiency of visual conspicuousness under the prevailing habitat conditions for communicating on tree-bark under greenish-yellow filtered light of forest canopy (Endler 1993a; Ellingson et al. 1995). Unlike the other dichromatic species, the yellow gular of *C. gracilis* does not seem to increase conspicuousness in their environment. The low intensity and conspicuousness of visual signals in *C. gracilis* may not hinder social communication, however, since this species produces a high diversity of chemical secretions.

Development, expression, and maintenance of traits is resource dependent, and increasing the

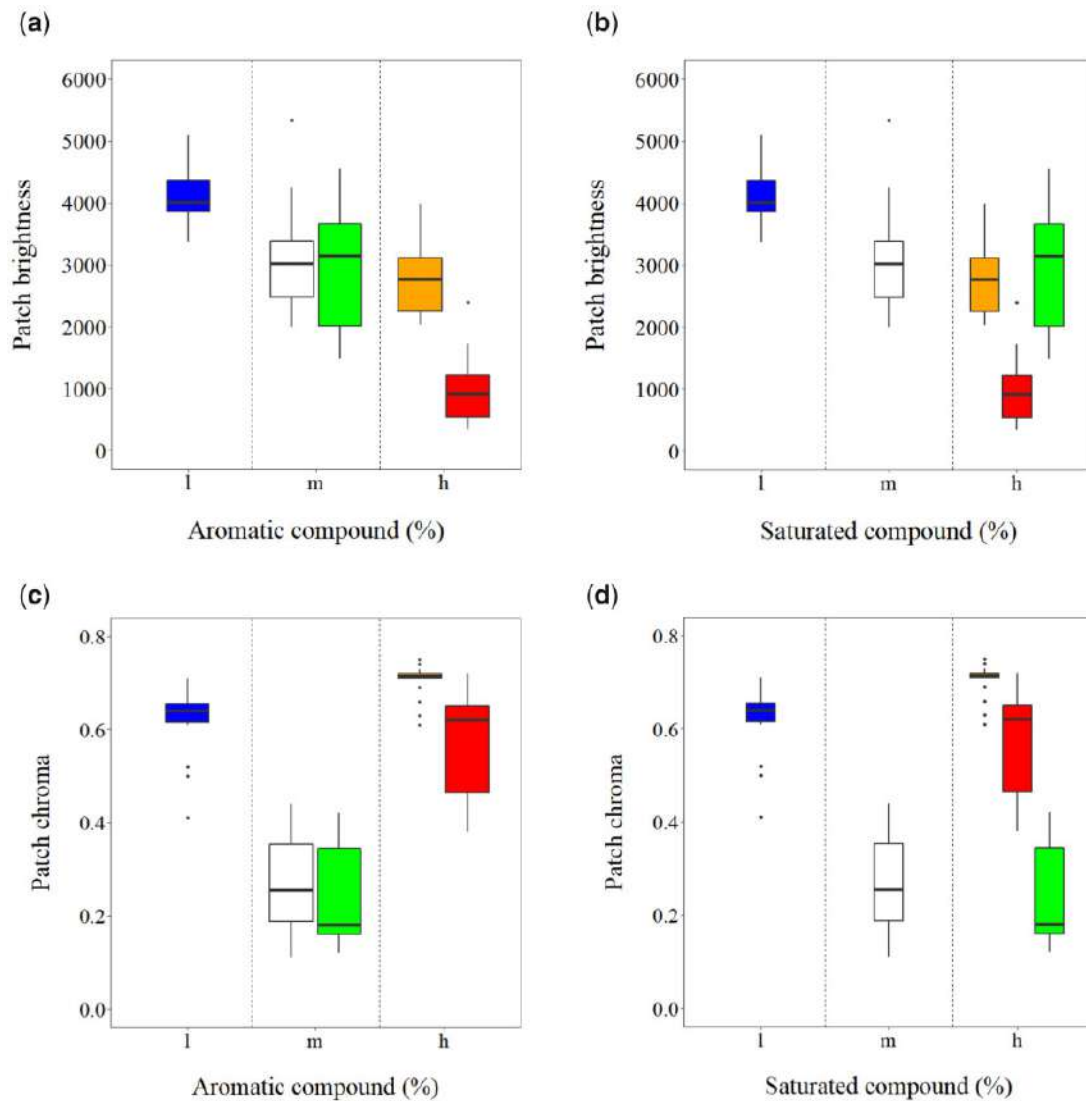


Fig. 3 Associations between visual and chemical traits: (a, b) Patch brightness and (c, d) Patch chroma with percent aromatic compound (left panel) and percentage saturated compound (right panel). Boxplots show medians, quartiles, 5th and 95th percentiles, and extreme values. Categorical ranks of chemical traits: “l” = Low, “m” = Medium, “h” = High. Blue = *C. mysoriensis*, orange = *C. littoralis*, red = *C. gracilis*, white = *C. indica*, green = *C. amboliensis*.

Table 2 Trade-offs between visual and chemical traits across five species of *Cnemaspis* geckos

Chemical traits	Rank comparisons	Visual traits		
		Patch size (%)	Patch brightness	Patch chroma
No. of compounds	Low vs. high	$D = 5.17, P < 0.001$	$D = 4.04, P < 0.001$	$D = -1.67, P = 0.141$
Saturated compounds (%)	Low vs. high	$D = 5.17, P < 0.001$	$D = 5.18, P < 0.001$	$D = 1.07, P = 0.424$
	Low vs. medium	—	$D = 3.26, P = 0.002$	$D = 3.95, P < 0.001$
	Medium vs. high	—	$D = 1.56, P = 0.178$	$D = 3.91, P < 0.001$
Aromatic compounds (%)	Low vs. high	$D = 5.17, P < 0.001$	$D = 5.67, P < 0.001$	$D = 1.29, P = 0.293$
	Low vs. medium	—	$D = 3.64, P < 0.001$	$D = 4.68, P < 0.001$
	Medium vs. high	—	$D = 2.81, P = 0.007$	$D = 7.61, P < 0.001$

Shown are pairwise post-hoc comparisons of chemical trait ranks (low, medium, and high) as Dunn's Test scores and associated P values. Significant test values (at $P < 0.05$) are in bold.

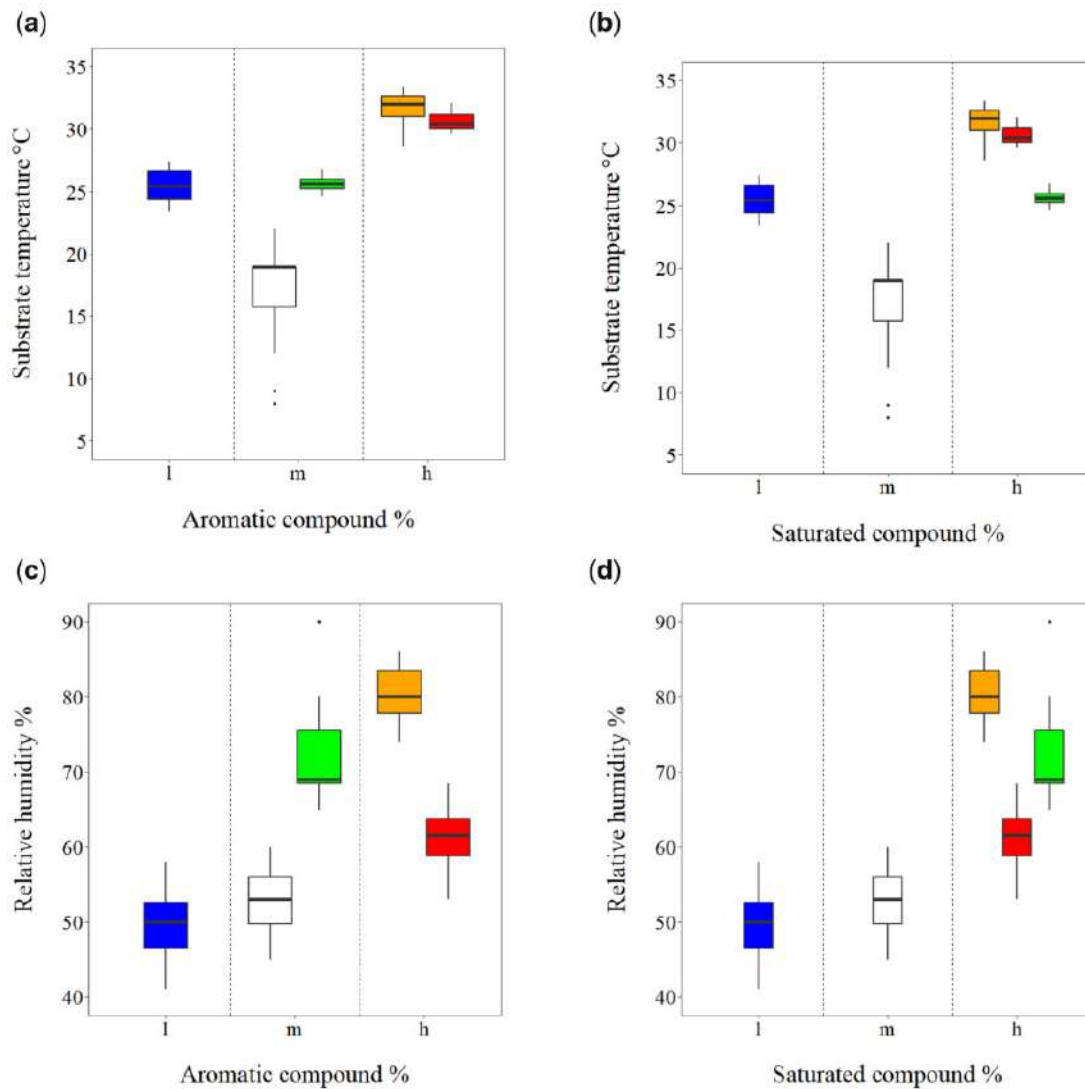


Fig. 4 Associations between chemical traits and environmental parameters: (a, b) substrate temperature, and (c, d) relative humidity with percentage of aromatic compound (left panel) and percentage of saturated compound (right panel). Boxplots show medians, quartiles, 5th and 95th percentiles, and extreme values. Categorical ranks of chemical traits: “l” = Low, “m” = Medium, “h” = High. Blue = *C. mysoriensis*, orange = *C. littoralis*, red = *C. gracilis*, white = *C. indica*, green = *C. amboliensis*.

Table 3 Relationship between chemical traits and environmental parameters across five species of *Cnemaspis* geckos

Chemical traits	Rank comparisons	Environmental parameters	
		Substrate temperature	Relative humidity
Saturated compounds (%)	Low vs. high	<i>D</i> = 2.95, <i>P</i> = 0.005	<i>D</i> = 6.27, <i>P</i> < 0.001
	Low vs. medium	<i>D</i> = 3.09, <i>P</i> = 0.003	<i>D</i> = 0.94, <i>P</i> = 0.524
	Medium vs. high	<i>D</i> = 7.28, <i>P</i> < 0.001	<i>D</i> = 5.77, <i>P</i> < 0.001
Aromatic compounds (%)	Low vs. high	<i>D</i> = 4.32, <i>P</i> < 0.001	<i>D</i> = 5.98, <i>P</i> < 0.001
	Low vs. medium	<i>D</i> = 1.67, <i>P</i> = 0.142	<i>D</i> = 3.41, <i>P</i> = 0.001
	Medium vs. high	<i>D</i> = 7.76, <i>P</i> < 0.001	<i>D</i> = 3.51, <i>P</i> < 0.001

Shown are pairwise post-hoc comparisons of chemical trait ranks (low, medium, and high) as Dunn's test scores and associated *P* values. Significant test values (at *P* < 0.05) are in bold.

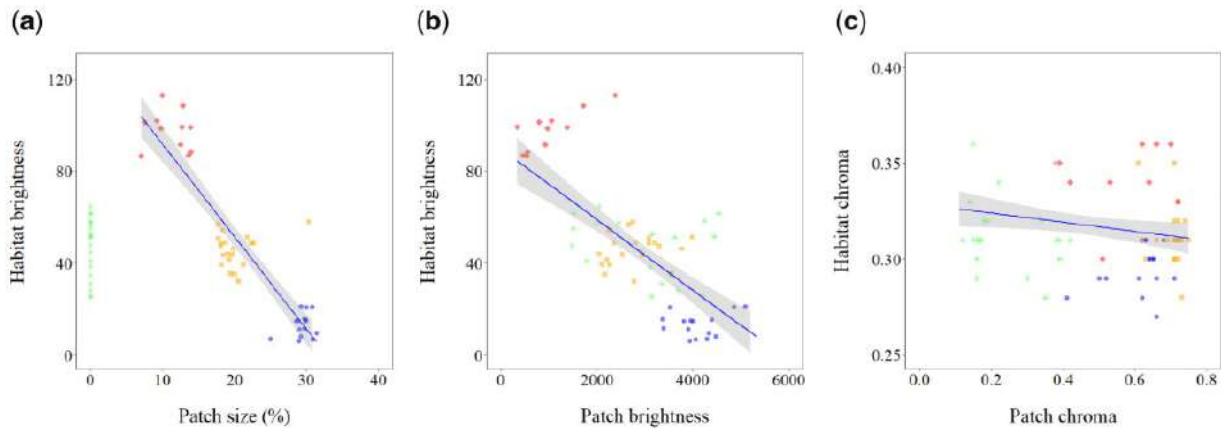


Fig. 5 Scatterplots showing associations between visual traits and the light environment: (a) percentage patch size ($N=3$ species) and (b) patch brightness ($N=5$ species) with habitat brightness, and (c) patch chroma ($N=5$ species) with habitat chroma. Blue = *C. mysoriensis*, orange = *C. littoralis*, red = *C. gracilis*, black = *C. indica*, green = *C. amboliensis*.

strength of features in one trait may draw from the allocation of resources from other traits. For species with multimodal signals, investing nutrients in the development and expression of one sensory channel may impose constraints on others (Partan 2013), leading to potential trade-offs between them. In *Cnemaspis*, we found that some chemical signal components were negatively associated with some visual traits. Specifically, we found that percentage gular patch size and gular brightness were higher for species with lower chemical traits, in particular the richness of chemical compounds, and the percentage of aromatic and percentage saturated compounds. A similar negative relationship between expression of visual and chemical signals has been seen in *Sceloporus* (Campos et al. 2020). Such macroevolutionary patterns between traits may arise from selection favoring one sensory channel over another, as a result of genetic correlations and functional redundancy (adaptive negative correlation; Agrawal et al. 2010). However, previous studies with *C. mysoriensis* have shown that chemical and visual signals are non-redundant for communication, at least for male–male social interactions (Kabir et al. 2019). Thus, the evolution of traits in the two sensory channels is probably independent of each other and under different environmental selection pressures for efficient transmission. Limitations of resources during the development of glands and chromatophores, or while maintaining chemical secretions and the brightness of the gular during adulthood may explain the resulting trade-off between the two sensory channels. Nevertheless, the expression of bright gular patches probably resulted in some resources being drawn away from the production of the pre-existing chemical signals.

Not all combinations of traits across modalities showed a negative relationship expected from a trade-off (see also Campos et al. 2020). Patch chroma, for example, was high for species with both low and high ranks of chemical traits, but not intermediate levels. One reason for this is that patch chroma and patch brightness show a weak negative correlation and thus strong chemical-visual relationships seen with patch brightness will not hold with patch chroma. Multiple components within the same modality often reveal inherent trade-offs. For example, number of head-bobs and the presence of a colored belly patch, which are both visual signal components, were found to be negatively correlated across *Sceloporus* spp. (Ossip-Klein et al. 2013). The conditions under which trade-offs within or across modalities occur, which include genetic and physiological, as well as nutritional and environmental, remain to be well understood.

Overall, we find no definitive pattern of selection favoring the pre-existing chemical channel over the visual signaling channel. But instead, the evolution and maintenance of multimodal traits appear to be associated with current habitat conditions. Further studies with larger number of species and a larger number of signaling traits in different modalities will provide stronger evidence for the relationships between signal trade-offs and the role of environment in shaping multimodal signals. Assessment of receiver responses to signals in each modality for all these species is now needed to fully elucidate the relative importance of these elaborated visual and chemical signals for intraspecific communication.

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Supplementary data

[Supplementary data](#) available at *IOB* online.

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