A macroevolutionary analysis of cryptic colouration in sexually dichromatic grasshoppers of the genus Sphenarium (Orthoptera: Pyrgomorphidae).

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Abstract

Background matching and disruptive colouration are defense mechanisms of animals against visual predators. Disruptive colouration tends to evolve in microhabitats that are visually heterogeneous, while background matching is favored in microhabitats that are chromatically homogeneous. This is one of the few comparative studies that have tested the relative impact of background matching and disruptive markings in the chromatic evolution. Controlling for the phylogeny, we explored the evolution of the colouration and the marking patterns in the sexual dichromatic and widely distributed Neotropical grasshoppers of the genus Sphenarium. These grasshoppers represent an excellent model to investigate the evolution of cryptic colouration on insects due to the heterogeneity of the environments where they have evolved. We found a correlation between the grasshoppers’ colouration and disruptive markings with the chromatic properties of their environments that was inferred by the levels of precipitation during the rainy season. The results suggest that colours and marks patterns could evolve due to predation pressures. Colour in both sexes could offer camouflage that is not perfectly background matched to a single habitat but instead offers a degree of resemblance to multiple backgrounds. Moreover, we found that males and females chromatic properties differ between them and precipitation levels where the species are found. This suggests that the sexes have diverged in their response to the environments, favoring the evolution of sexual dichromatism in these grasshoppers.

1 Introduction

In many animal taxa, colouration can be adaptative in several ways, including social signaling, thermoregulation, protection from ultraviolet light, and antipredator defences (Cott 1940, Cuthill et al. 2017). Cryptic colouration is adaptative because it reduces the signal produced by an organism; it thus becomes less visually conspicuous to potential predators (Bond 2007, Théry and Gomez 2010). Two of the most spread strategies to reach cryptic colouration are background matching (BM) and disruptive colouration (DC) (Stevens et al. 2006a, Stevens and Merilaita 2009, Quicke 2017). BM is the resemblance between the colours and patterns of an organism with its surroundings (Hughes et al. 2019). DC is the concealment between the colours and patterns of an organism’s outline due to contrasting markings that break or distract the attention of the predators from the organism’s outline (Stevens and Cuthill 2006, Stevens and Merilaita 2009). BM can be favoured in chromatically homogeneous habitats (Robledo-Ospina et al. 2017, Orton and McBrayer 2019); its success depends on the colouration of the environment and the probability that an individual stays on the backgrounds where it is cryptic (Merilaita et al. 1999, Kang et al. 2016). However, BM can be ineffective at reducing the risk of detection when animals are in motion in heterogeneous environments (Ioannou and Krause 2009). DC can co-occur with BM and enhances concealment from various predators (Cuthill et al. 2005, Schaefer and Stobbe 2006, Fraser et al. 2007, Adams et al. 2019). DC success could be maximized when some marks on the organism’s surface
match the background and other marks have high contrast with the rest of the body (Stevens and Merilaita 2009). DC also has evolved in visually heterogeneous microhabitats because it breaks the outlines of the organisms independently of the variable background patterns and can conceal an organism while it is in motion (Stevens et al. 2006a,b). The evolution of BM colours represents a compromise between matching closely a single background or adopting a generalist strategy where the individuals resemble multiple backgrounds to some extent but not perfectly (Hughes et al. 2019). On the other hand, DC could be independent of the background colouration and depends on the organisms’ markings and shape (Merilaita and Lind 2005). DC could be more adaptative than BM in individuals with high vagility, which have a low probability of staying in specific backgrounds or inhabiting heterogeneous colour habitats (Théry 2007, Théry and Gomez 2010, Robledo-Ospina et al. 2017).

Because BM and DC strategies decrease the probability of detection by predators, selection will act on organisms so that they match the geographical variation in substrate colour (Endler, 1990, Stuart-Fox and Ord 2004, Rosenblum 2006, Marshall et al. 2015, Hantak and Kuchta 2018, Adams 2019). However, if males and females occupy different microhabitats because of their different sexual roles, natural selection could favour a divergence between females and males (Slatkin, 1984), thereby favouring sex-specific cryptic colour patterns (Forsman and Appelqvist, 1999, Medina et al. 2016, Ramírez-Delgado and Cuevadel Castillo 2020, Cuevadel Castillo et al. 2021). Sexual dichromatism studies have focused mainly on vertebrates (Font et al. 2009), and its evolution has been explained by differences in the selective pressures imposed by visual predators in only a few cases (Orton and McBrayer 2019). In invertebrates, the study of the evolution of sexual dichromatism has focused on arthropods, mainly also in the context of sexual selection (see Quicke 2017 and references there), although it is still poorly documented in the context of cryptic colouration (Forsman and Appelqvist 1999, Ramírez-Delgado and Cuevadel Castillo 2020, Cuevadel Castillo et al. 2021). For instance, in some arthropod species, BM and DC tactics have evolved closely with the environment. Jumping spiders exhibit BM in relatively flat chromatic backgrounds, whereas in dynamically changing backgrounds, they exhibit DC (Robledo-Ospina et al. 2017). Also, crabs living in homogeneous backgrounds evolved BM, whereas those living in heterogeneous backgrounds have evolved DC (Price et al. 2019).

In many grasshopper species, cryptic colouration has evolved as an anti-predator strategy (Tsurui et al. 2010, 2013, Edelaar et al. 2019, Camacho et al. 2020); even different morphs from a grasshopper species have been described as disruptive or showing resemblance to their environment (Tsurui et al. 2013, Forsman 2018, Ramírez-Delgado and Cuevadel Castillo 2020). Grasshoppers of the genus Sphenarium are found in a wide variety of environments and show wide variation in their colour and marking patterns (Sanabria-Urbán et al. 2017). Moreover, some species of this genus exhibit a notorious sexual dichromatism; BM is associated with females and DC to males (Ramírez-Delgado and Cuevadel Castillo 2020, Cuevadel Castillo et al. 2021, Ramírez-Delgado and Cuevadel Castillo 2023). In S. zapotecum, in visually heterogeneous areas, predators spent more time searching for striped male morphs with lower BM and higher disruptive properties. On the other hand, females with high BM improve survival and significantly increase predator searching time (Ramírez-Delgado and Cuevadel Castillo 2023). Sphenarium grasshoppers are the prey of many visually oriented vertebrates, including birds, mammals, reptiles (Kevan 1977), and humans, which have extensively consumed species of the genus since pre-Columbian times (Ortiz de Montellano 1978, Ramos-Elorduy and Pino-Moreno 1989).

The Sphenarium genus consists of 17 flightless univoltine generalist herbivorous species distributed from central Mexico to northern Guatemala in a high altitudinal margin (0 to >2600 masl, Sanabria-Urbán et al. 2015). Because the differences in altitude and climate associated with their distribution are substantial, these species are found in rainy and dry deciduous tropical forests, temperate forests, shrublands, and grasslands. In low seasonal environments, the vegetation remains mainly greenish all year round, while in highly seasonal environments, there is a transition between brownish and greenish tonalities. They hatch in May and June during the rainy season and die during the coldest months (December to February) (Sanabria-Urbán et al. 2017). Females are larger than males (Sanabria-Urbán et al. 2017), and both sexes are polygamous (Cuevadel Castillo and Núñez-Farfán 1999, Cuevadel Castillo and Núñez-Farfán 2002). The males tend to search actively for females, whereas females are less mobile and can be found close to the ground when they are
about to lay their eggs (Ramírez-Delgado and Cueva del Castillo 2020).

The diversity of environments these grasshoppers inhabit allows us to analyse the potential adaptive chromatic cryptic divergences between them. Despite the implication of DC and BM on the evolution of the chromatic patterns of the species, very few comparative studies have tested their relative impact on the chromatic evolution of lineages (Caro and Koneru 2021). Moreover, given that many cryptic species are sexually dichromatic, focusing on these taxa may reveal profound implications regarding the evolution of intraspecific chromatic variation with respect to selective pressures imposed by visual predators on the members of each sex.

In this study, we explored the relationship between the colouration patterns of males and females of the grasshoppers of the genus Sphenarium and their environment marking patterns whilst controlling for phylogenetic effects. Because matching colouration between habitats and individuals can be impacted by seasonal and geographic climatic variation (Caro et al. 2016), we investigated the relationship between the environment and the grasshoppers’ chromatic patterns by using the precipitation patterns in the localities and months where the adult grasshoppers were located. Since environmental colouration is strongly related to water availability, i.e. different colour and patterns are expected from different levels of green vegetation and projected shadows of the vegetation (Yom-Tov and Geffen 2006), and due to local adaptation and BM, we further expected to find a positive relationship between environmental colouration and the marking patterns of males and females. Also, we hypothesized that this relationship would be stronger in females than males because of their lower mobility (Ramírez-Delgado and Cueva del Castillo 2020, Cueva del Castillo et al. 2021). We predicted a positive relationship between the colouration of the grasshoppers and their environment with precipitation, and a positive relationship between the marking patterns of the grasshoppers and their environment with precipitation, due to the high contrast between lights and shadows associated with dense vegetation. Furthermore, if the selective pressure on males’ and females’ chromatic patterns differ, we would expect a divergence in their chromatic coevolution.

2 Methods

2.1 Image acquisition

Between October and September 2017, and 2018. We obtained photographs of male and female adults of the 17 Sphenarium taxa, as well as their backgrounds in 17 localities across in central and southern Mexico (figure 1). The places varied in elevation from 77 to 2,374 masl (table S1). Details of number of photographs taken per species, sex, and data obtained for colour and pattern analyses are provided in supplementary material (table S2). During data collection, the geographic position and elevation of each locality was recorded using a GPS-map 60CSx (Garmin, Kansas City, USA). In each location, we walked at a slow and steady pace whilst looking for grasshoppers in areas of approximately 100 m²; we registered the exact point where grasshoppers were found, and we caught them by hand. Once collected, the grasshoppers were placed in a cooler for approximately five minutes; this allowed us to lower the temperature and activity of individuals. We then placed each grasshopper back in the site where it was collected, we took a photograph of it against the background where it was found. We took the photographs below a white diffuser umbrella on sunny days. In the photographs we included a colour checker card (X-Rite Color Checker Passport 2, Munsell Color Laboratories), which also included a size and a grey colour scale. The photographs were taken with a Canon EOS 70D digital camera, fitted with 18-55 mm, f/3.5-5.6 lens. The settings of the camera, lens, and illumination were constant in all photographs. The aperture of the sensor was set to f-stops f/5.6; the values of light sensitivity (ISO) were set to 400; the focal distance was set at 55 mm; the camera was held at approximately 40 to 50 cm apart from the objective. The only setting that was adjusted between photographs was shutter speed; in this way we prevented overexposure in photographs. After the photographs were taken, the grasshoppers were temporarily placed in a plastic bag in order to avoid their potential recapture, and they were released after all the caught individuals in a given locality were photographed. We stored the images as RAW format. The camera has no modifications to allow ultraviolet sensitivity, so the photographs only contain information of the visible light spectrum; note, however, that grasshoppers have very little ultraviolet light reflectance (Tsurui et al. 2010). To obtain the photographs and make them objectively measurable we
follow the suggestions outlined for Stevens et al. (2007), and Troscianko and Stevens (2015).

2.2 Image analysis

To analyze the images, we used the Multispectral Image Calibration and Analysis (MICA) plugin (Troscianko and Stevens 2015) available for ImageJ software (Schneider et al. 2012). MICA plugin uses linear data from the raw images, controls the light conditions variation with the gray scales from the colour checker and creates a multispectral image made of a stack of the images corresponding to long wave (R Channel), medium wave (G channel), and short wave (B channel). The multispectral image made it possible to take objective measurements from different channel reflectances, in order to later compare the colour and pattern between our photographs. From the multispectral images, we measured the reflectance of the RGB channels of the grasshoppers’ dorsal surface, and in the same photo, a similar area adjacent to the grasshoppers was considered the background surface of the grasshoppers. We also performed a granularity analysis on both surfaces.

2.2.1 Colour analysis

We estimated brightness, saturation and hue from the RGB reflectance data. The three parameters allowed us to separate the achromatic (brightness) and chromatic (saturation and hue) properties of the images. Brightness refers to intensity of light on the image. Hue is determined by the dominant wavelength of the visible spectrum. It is the attribute that permits colours to be classified as red, yellow, green, blue, or an intermediate colour, and saturation pertains the amount of white light mixed with a hue. High-saturation colours contain little or no white light.

Brightness was obtained using the means of the three channel values: \((R + G + B) / 3\). Saturation was calculated as the Euclidean distance between completely white, and the RGB values obtained in our photographs. By following this approach, large distance values represent high saturation. We obtained two hue values: \(\text{Hue}1 = R / G\), and \(\text{Hue}2 = (R + G) / B\). These were calculated this way following the principle of opponent channels, which is based in the way opponent colour channels work to detect colour (Osorio and Vorobyev 2005).

We must point out that the analyzes are interpreted from a human visible spectrum perspective. Spectral sensitivity can be different in other possible predators such as birds or mice, and their prey detectability could involve elements that we did not consider in this study (Théry and Gomez 2010). Nonetheless, humans’ processing capabilities are similar to those of natural predators, especially birds (Dukas and Kamil 2001), and some studies have noticed that human “predation” can predict predation by other visual predators under natural conditions (Karpestam et al. 2013). Moreover, in central and southern Mexico, \(S. \ zapotecum\) and other species of the genus have been traditional elements of the human diet since pre-Columbian times (Ortiz de Montellano 1978, Ramos-Elorduy and Pino-Moreno 1989).

2.2.2 Pattern analysis

We performed a granularity (energy) analysis to measure the patterns in our photographs. This method roughly resembles the way animals decompose the visual information in different spatial frequencies (Stevens 2011). The photographs had to meet the following minimum pixel scale for this analysis 1 mm: 15 pixels; if the photographs did not meet this requirement, they were not used for pattern analysis (Troscianko and Stevens 2015). We used the average pixel reflectance of red and green channels to calculate the energy spectrum of grasshoppers and their background across 15 filters ranging from 2 to 256 pixels, in increments of multiples of 2. We obtained three descriptive values from this process: (i) overall pattern contrast: the amount of energy across all scales; (ii) the dominant marking size: the scale of dominant marking contrast (the filter where the highest value of energy is reached); and (iii) pattern diversity: the proportion between dominant marking contrast and rest of the measured energy.

2.3 Climatic data acquisition

Because the \(Sphenarium\) species complete their reproductive cycle during the wettest and the driest periods
of the year (Sanabria-Urban et al. 2015), we obtained values from high-resolution climate surfaces (Fick and Hijmans 2017) of the precipitation of the wettest (PTW) and the driest (PDT) trimesters of the year for each sampling location. We expected green tonalities to be positively related with the amount of humidity and the amount of primary productivity of the plants, and brownish tonalities stronger associated with dryer environments (Yom-Tov and Geffen 2006).

2.4 Comparative analyses

To correct for the phylogenetic non-independence of taxa, we used the phylogenetic relationships of the Sphenarium genus (Sanabria-Urban et al. 2017), and the phylogenetic generalized least squares (PGLS) method to test the association between (a) the colour and patterns of the grasshoppers, with (b) the colours and patterns of their environment and the climatic factors of the localities where the grasshoppers were found. PGLS were performed using the caper package (Orme et al. 2018) as implemented in R (ver. 4.0.1) (R Core Team 2020). PGLS is a comparative method that incorporates the phylogenetic autocorrelation of the data in the structure of errors (variance-covariance matrix; (Martins and Hansen 1997, Freckleton et al. 2002). In this case, the PGLS method was used to test the maximum likelihood of the evolutionary regression coefficient between traits (Pagel 1999). We also estimated the weighting parameter $\lambda$ to improve the data’s fit to the model and correct for phylogenetic effects in all generated PGLS models (Pagel 1999). $\lambda$ measures phylogenetic dependence of observed trait data (Pagel 1999, Freckleton et al. 2002): the unit value approaches one when related species resemble each other more than they resemble species drawn at random from a phylogenetic tree (Blomberg and Garland Jr 2002).

2.5 Testing the association between the colouration of grasshoppers, their background colouration, and climatic factors.

We constructed four PGLS models for each sex in order to test for associations between the colouration parameters and climatic factors and background colouration. The natural log-transformed values of hue, saturation, and brightness of the grasshoppers were used as response variables, and the natural log-transformed values of hue, saturation, and brightness of the background, were used as respective explanatory variables. We further included the precipitation variables; ln(PWT and ln(PDT)) as explanatory variables in all models.

2.6 Sexual dimorphism in colour patterns

To test the divergence between the chromatic patterns of males and females, we performed major axis regression of the chromatic patterns of males on the chromatic patterns of the females. A slope steeper than 1 would mean that the divergence in the chromatic could be higher in males than females (>1), whereas a slope lower than 1(<1) would mean higher in females than males. We used the phylogenetic independent contrasts method (PIC method; Felsenstein 1985), as implemented by the R package caper (Orme et al. 2013) to control for the phylogenetic non-independence of species (Harvey et al. 1991). We examined the studentized residuals for outliers $>|\pm 3|$ and discarded from this analysis the outliers contrasts. Ultimately, we regressed the independent contrasts of the log-transformed values of hue 1 and 2, saturation, brightness, overall patterns’ contrast, dominant marking size, and pattern diversity of the male grasshoppers on their counterparts on females by fitting major axis regression using the R packages smatr (Warton et al. 2012).

3 Results

3.1 Association between grasshoppers’ colouration and background colouration + climatic factors

The PGLS models in which we tested the association between colouration parameters show a general strong, positive association between the colour parameters of male and female grasshoppers and their microhabitats (table 1A-F; figure 2). The only exception was hue2 (table 1G, H). Moreover, the analyses show a significant positive association between the brightness and saturation of the females with the driest trimester of the year (table 1A, B; figure 3a, b), and a significant positive association between male brightness with the wettest trimester of the year (Table 1E; figure 3b). The values of $\lambda$ are overall very low (zero or close to zero) (table 1A-H), which suggests a lack of phylogenetic signal and a rapid evolution of these colouration traits.
3.2 Association between climatic factors with grasshoppers and background patterns.

In females, there is a significant, positive relationship between pattern diversity and habitat (table 1K; figure 2e), whereas the PGLS models of males indicated that only the relationship between dominant marking contrast and the driest trimester of the year was significant and positive (table 1L; figure 2f). The $\lambda$ values were high for both, female pattern diversity and males dominant marking contrast (table 1K, L), which suggests high phylogenetic signal and slow evolution of traits.

3.3 Sexual dimorphism in colour patterns

The independent contrasts of the major axis regressions of males on females for hue 1 and 2, saturation, and brightness, overall patterns’ contrast and dominant marking size were positive and highly correlated, whereas the patterns diversity evolves independently in males and females (table 2). The rate of divergence of brightness, saturation and hue2 was similar in males and females, while the divergence rate of hue1, overall patterns’ contrast and dominant marking size was higher on males than females (slope stepper than 1; figure 4c, e, f).

4 Discussion

The findings from our study contribute significant insights into the intricate evolution of colouration among Sphenarium grasshopper species. The observed disparities in colour patterns among these species are attributed to localized adaptations to distinct environmental conditions. It is evident that natural selection has favoured the development of cryptic colouration in both male and female grasshoppers. However, a nuanced gender-specific divergence is discernible in their responses to environmental cues. Notably, the brightness of males exhibits a positive correlation with the wettest trimester of the year, while the brightness and saturation of females are positively associated with the driest trimester. Intriguingly, despite the absence of direct correlations with climatic variables, female mark patterns demonstrate a positive connection with their backgrounds. Conversely, male patterns show no such relationship with their surroundings, although a significant link between male marks (overall pattern contrast) and the driest trimester is evident. An interesting aspect that emerges is the relatively rapid evolution of chromatic patterns compared to the evolution of marking patterns.

The disparity in the pace of evolutionary divergence between background resemblance and disruptive markings can be rationalized by the varied payoffs associated with each strategy. In scenarios where a species colonizes a new, vibrant, and uniform environment, natural selection tends to hasten the convergence of colouration with the new environment (referred to as BM). Conversely, the adaptability inherent in disruptive markings allows for a reduced divergence among species that face novel environments or alterations in their distribution range. In this context, the chromatic patterns exhibited by Sphenarium grasshoppers may embody a generalized strategy that moderately matches various backgrounds without precisely mimicking any one. It’s worth noting that the benefits derived from cryptic strategies differ between males and females. The divergence rate of hue1, overall pattern contrast, and dominant marking size are notably higher in males than in females. Conversely, the evolution of pattern diversity has proceeded independently within both genders, indicating a distinct niche specialization concerning light and shadow contrast for males and females. Nonetheless, the parallel evolution rates of brightness, saturation, and hue2 in males and females can be attributed to similar selective pressures faced by different species within their respective habitats.

The divergence in chromatic patterns within Sphenarium grasshoppers finds its basis in the diverse environments where the genus has diversified (as evidenced by Sanabria-Urbán et al. 2017) and the behavioural disparities between males and females. The synchronization of Sphenarium life cycles with rainy seasons, as discussed by Sanabria-Urbán et al. (2015), likely underlies the strong associations between chromatic patterns and environmental conditions exhibited across all species. This linkage is attributable to the profound influence of precipitation on primary productivity (Yom-Tov and Geffen 2006), resulting in alterations in vegetation colour and shadow patterns. Furthermore, the variations in male and female populations due to precipitation fluctuations on different timescales are notable. For instance, the protandrous nature of males in certain species aligns their maturation with the onset of the rainy season, maximizing mating opportunities.
During this phase, male brightness synchronizes with light-green shades, and their marks may serve them well during the subsequent driest trimester, characterized by deeper contrasts between light and shadow. In contrast, females, strategically maturing later than males, exhibit colour and mark patterns that correlate with vegetation plots and backgrounds, which they frequent during their reproductive season.

Males' active pursuit of females, often atop plants, juxtaposes the stationary female behaviour, placing them closer to the ground where they lay eggs. The pattern of marks on the dorsal surface of these grasshoppers serves dual purposes, providing BM and potentially contributing to DC, as postulated by Robledo-Ospina et al. (2017). Such highly contrasting markings might disrupt predators’ visual perception or divert attention from the organism’s contours, an adaptive advantage, as Merilaita (1998) and Hughes et al. (2019) suggested. However, the potential implications of these marking patterns on processes such as sexual selection warrant consideration (with reference to Cueva del Castillo and Cano-Santana 2001). Notably, the divergence rate in male marking patterns surpasses that of females, indicating heightened natural selection pressure on these male-specific traits. Remarkably, in the case of S. zapotecum, predators allocate more time to locate striped male morphs with reduced BM and increased disruptive characteristics and females with heightened background resemblance. This behaviour augments prey survival (Ramírez-Delgado and Cueva del Castillo 2023).

It is conceivable that these grasshoppers adopt a form of camouflage that offers partial resemblance to multiple backgrounds rather than a perfect match to a singular habitat. Alternatively, their camouflage may operate somewhat autonomously from BM strategies (as posited by Hughes et al. 2019). Nevertheless, the apparent convergence between Sphenarium chromatic patterns and their environments stems from a blend of differential predation dynamics and proactive habitat selection. This phenomenon parallels other grasshopper species (as demonstrated by Edelaar et al. 2017 2019, Heinze et al. 2022).

The survival of these grasshoppers hinges on their ability to navigate diverse predator threats, including avian, mammalian, reptilian, and arthropod predators (Kevan 1977), as well as human predation (highlighted by Sanabria-Urbán and Cueva del Castillo 2020). Their chromatic and achromatic visual cues have evolved in response to the distinct searching strategies of these predators. Remarkably, the resemblance of marking patterns to the environment extends to chromatic and achromatic variables. However, while chromatic cues excel in close-range searches, achromatic information proves more effective in long-distance prey detection (Schaefer and Stobbe 2006, Cazetta et al. 2009). Notably, human behaviour as a predator mirrors that of natural predators, particularly birds, a similarity exploited in some studies to predict predation by other visual predators in natural settings (Karpestam et al. 2013). This intriguing correspondence has likely influenced the evolution of Sphenarium grasshoppers’ chromatic patterns.

Considering the relative scarcity of phylogenetic comparative studies concerning cryptic colouration evolution, our research undoubtedly contributes to a foundational comprehension of colour evolution within the context of selection pressures imposed by visual predators. Nonetheless, it is imperative to acknowledge that future investigations into habitat preference and the exploration of other potential functions of grasshopper colouration, such as thermoregulation or UV radiation protection, hold the potential to unveil additional factors shaping the evolution of colouration within this cohort of neotropical grasshoppers.

5 References


### 6 Tables

Table 1. PGLS models of *Sphenarium* grasshoppers on coloration and pattern parameters for both sexes.

<table>
<thead>
<tr>
<th>Response variable</th>
<th>Explanatory variable</th>
<th>D.f.</th>
<th>t</th>
<th>F</th>
<th>Multiple $R^2$</th>
<th>P</th>
<th>$\lambda$</th>
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<tbody>
<tr>
<td>A) Brightness</td>
<td>Bg-Brightness</td>
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<td>13.637</td>
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<td></td>
<td>PDT</td>
<td>3.13</td>
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<td>0.008</td>
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<tr>
<td>B) Saturation</td>
<td>Bg-Saturation</td>
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<td>15.200</td>
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<td>C) Hue 1</td>
<td>Bg-Hue1</td>
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<td>Bg-overall patterns' contrast</td>
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Table 2. Major axis regressions values of the chromatic patterns of males on the chromatic patterns of the females.

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<th>Coloration /patterns traits</th>
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<th>UCL</th>
<th>LCL</th>
<th>r_w</th>
<th>D.f.</th>
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β: Coefficients of regressions, UCL and LCL: upper and lower 95% confidence intervals, r_w: Wald statistic values.

7 Figures and figure legends

Figure 1. Map of the sites where the photographs of Sphenarium grasshoppers were taken: S. purpurascens (19.31N, 99.19W), S. borrei (19.92N, 101.74W), S. occidentalis (18.66N, 101.65W), S. cypticum (18.18N,

Figure 2. Association between chromatic variables of \textit{Sphenarium} grasshoppers with the chromatic variables of their background, as tested using PGLS; (A) Brightness, (B) Saturation, (C) Hue1. Ordinary least squares regressions fitted are shown for illustrative purposes.

Figure 3. Relationship between chromatic variables of \textit{Sphenarium} grasshoppers: (a) Female brightness and PDT, (b) male brightness and PWT, (c) female saturation and PDT, (d) male saturation and PWT (e) male overall patterns contrast and PDT, and (e) Female pattern diversity and background pattern diversity. Note these graphs are shown only for illustrative purposes and were created with ordinary least squares linear models.
Figure 4. Major axis regressions (Solid lines) of the chromatic components of males on the chromatic components of the females of *Sphenarium* grasshoppers: (a) PIC brightness, (b) PIC saturation, (c) PIC hue 1, (d) PIC hue 2, (e) PIC Overall contrast patterns, (f) PIC Dominant marking size and (g) PIC Pattern diversity. Dotted lines: Slope = 1.