



**UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO**

**POSGRADO EN CIENCIAS BIOLÓGICAS**

**FACULTAD DE ESTUDIOS SUPERIORES IZTACALA**

**TENDENCIAS EVOLUTIVAS EN PATRONES CROMÁTICOS DE**

**CHAPULINES DEL GÉNERO *Sphenarium***

**(Orthoptera: Pyrgomorphidae)**

**TESIS**

QUE PARA OPTAR POR EL GRADO DE:

**DOCTOR EN CIENCIAS**

PRESENTA:

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**Los Reyes Iztacala, Tlalnepantla, Estado de México, 2022**



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FACULTAD DE ESTUDIOS SUPERIORES IZTACALA

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Directora General de Administración Escolar, UNAM  
P r e s e n t e

Me permito informar a usted que en la reunión ordinaria del Comité Académico del Posgrado en Ciencias Biológicas, celebrada el día **25 de abril de 2022** se aprobó el siguiente jurado para el examen de grado de **DOCTOR EN CIENCIAS** del estudiante **RAMÍREZ DELGADO VICTOR HUGO** con número de cuenta **307154615** con la tesis titulada **“Tendencias evolutivas en patrones cromáticos de chapulines del Género *Sphenarium* (Orthoptera: Pyrgomorphidae).”**, realizada bajo la dirección del **DR. RAÚL CUEVA DEL CASTILLO MENDOZA**, quedando integrado de la siguiente manera:

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Sin otro particular, me es grato enviarle un cordial saludo.

**ATENTAMENTE**  
**“POR MI RAZA HABLARÁ EL ESPÍRITU”**  
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**COORDINADOR DEL PROGRAMA**



**DR. ADOLFO GERARDO NAVARRO SIGÜENZA**



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## Resumen

La coloración tiene múltiples funciones en los organismos, como defensa ante factores abióticos, comunicación intra e interespecífica y camuflaje. El camuflaje es un mecanismo de defensa contra depredadores que se orientan visualmente y puede dividirse en enmascaramiento y coloración críptica. La coloración críptica comprende varias estrategias que impiden que un organismo sea detectado, dos de las más estudiadas e intuitivas son la coincidencia de fondo, es decir, asemejar en características cromáticas y patrones de marcas al fondo; y la coloración disruptiva, es decir, poseer marcas que impidan detectar las líneas que forman el contorno del cuerpo de los organismos. La coincidencia de fondo depende estrechamente de la coloración y patrones del ambiente para ser efectiva, por ende, esperaríamos que la evolución de esta estrategia ocurra en estrecha relación con la coloración ambiental y los factores relacionados a los colores del ambiente, como factores climáticos: En contraste, la coloración disruptiva puede ser exitosa independiente del ambiente. En insectos es común que utilicen la coloración críptica como mecanismo defensivo. Dependiendo de la coloración del ambiente y el comportamiento de los individuos estos pueden tener coloraciones y marcas que les brinden protección por coincidencia de fondo y coloración disruptiva.

En este trabajo se analizó la coloración chapulines del género *Sphenarium*. Las 17 especies de este género habitan una gran variedad de ecosistemas en un amplio margen altitudinal (0 a < 2900 msnm) desde el centro de México al norte de Guatemala y poseen una amplia variación en patrones cromáticos. Los objetivos del trabajo fueron saber qué tipo de coloración críptica poseen estos chapulines, si existe variación en las estrategias usadas por los sexos y si la evolución de la coloración y los patrones esta asociada a la coloración del ambiente y de las características climáticas.

En general, hembras y machos poseen colores que pueden proveer cripsis por coloración disruptivas y la coincidencia de fondo, sin embargo los patrones de marcas en machos proveen una mayor disrupción de su contorno, estas diferencias en estrategias crípticas median el dicromatismo sexual en estos chapulines, probablemente asociado a diferencias en conducta en los sexos. Después de controlar por la filogenia, la evolución de los patrones cromáticos de estos chapulines ocurre en estrecha relación con los colores del ambiente, lo que puede significar una rápida adaptación de la coloración con tal de empatar la coloración del ambiente. Respecto a los patrones de marcas, éstos evolucionan asociados a la cantidad de precipitación, probablemente como respuesta a ambientes heterogéneos con una gran variedad de coloración en hojas y parches de luz y sombra.

## Abstract

Coloration in animals has multiple functions, such as defense against abiotic factors, intra- and interspecific communication, and camouflage. Camouflage is a defense mechanism against visually oriented predators and is divided into masquerade and cryptic coloration. Cryptic coloration comprises several strategies that prevent an organism from being detected.

Two of the most studied and intuitive cryptic strategies are background matching, i.e., resembling the environment in color and patterns of marks. Disruptive coloration, i.e., having marks that prevent detecting the lines that form the contour of the organism's body. The background matching depends closely on the coloration and patterns of the environment to be effective. Therefore, we would expect that the evolution of this kind of crypsis occurs in close relation to the environmental coloration and the factors related to the environment's colors, such as climatic factors. Conversely, the efficacy of disruptive coloration could be independent of the environment. In insects is common for them to use cryptic coloring as a defensive mechanism. Depending on the environment's coloration and the individual's behavior, they may have colorations and marks that protect due to background coincidence and disruptive coloration.

This work analyzed the coloration of the grasshoppers of the genus *Sphenarium*. The 17 species of this genus inhabit a great variety of ecosystems in a wide altitudinal range (0 to < 2900 masl) from central Mexico to northern Guatemala and have a notable variation in chromatic patterns. The work's objectives were to know what type of cryptic coloration is used by these grasshoppers, if there is variation in the strategies used by the sexes, and if the evolution of coloration and patterns is associated with the environment's coloration and climatic factors.

In general, females and males have colors that can provide crypsis by disruptive coloration and background coincidence. However, the marking patterns in males provide a more significant disruption of their body outline; these differences in cryptic strategies mediate sexual dichromatism in these grasshoppers, probably associated with differences in behavior between the sexes. After controlling for the phylogeny, the evolution of these grasshoppers' chromatic patterns occurs in close relationship with the colors of the environment, which may mean a rapid adaptation of the coloration to match the coloration of the environment. Regarding the marks, these evolve associated with the amount of precipitation, probably in response to heterogeneous environments with a great variety of coloration in leaves and patches of light and shade.

## Introducción general

El estudio de la coloración en los seres vivos ha sido una de las principales fuentes de evidencia para sustentar la teoría de la selección natural quizá debido a que éste es uno de los caracteres más llamativos de los seres vivos y uno de los más estudiados (Darwin, 1859; Poulton, 1890; Endler, 1990; Bond, 2007). Debido a que la coloración modula las relaciones de los organismos con su ambiente, puede tener profundos efectos en su adecuación, entre los que destacan mecanismos para afrontar cambios en la temperatura o la exposición a la radiación ultravioleta, la comunicación inter e intraespecífica y las defensas anti-depredadores (Cott, 1940; Théry y Gomez, 2010; Cuthill *et al.*, 2017).

El camuflaje es una de las principales funciones que se ha atribuido a la coloración en animales, ya que favorece que un organismo pase inadvertido ante posibles depredadores que se orientan visualmente, lo cual puede incrementar sus posibilidades de no ser detectado por depredadores potenciales (Théry y Gomez, 2010; Merilaita, Scott-Samuel y Cuthill, 2017). El camuflaje se puede categorizar en enmascaramiento y cripsis. El enmascaramiento ocurre cuando un organismo aparenta ser y es detectado como un objeto que puede ser encontrado en su entorno, como hojas, flores, ramas u otros objetos (Skelhorn *et al.*, 2010; Stoddard y Stevens, 2010). La cripsis incluye una gran variedad de tácticas que disminuyen la posibilidad de que los organismos sean detectados, las dos tácticas más documentadas son la coincidencia de fondo (Merilaita y Lind, 2005; Michalis *et al.*, 2017) y la coloración disruptiva (Merilaita y Lind, 2005; Stevens y Merilaita, 2009a).

La coincidencia de fondo provee cripsis por medio de marcas y colores que asemejan las marcas y colores del entorno (Stevens y Merilaita, 2009b; Kang *et al.*, 2015). Este tipo de cripsis favorece que la variación en los patrones cromáticos de los organismos generalmente coincide con la variación geográfica en el color del sustrato (Endler, 1990; Hantak y Kuchta, 2018). A diferencia de la

coincidencia de fondo, la coloración disruptiva provee crípsis al favorecerse marcas y parches de colores que dificulten la detección del contorno de un organismo (Stevens y Cuthill, 2006). El contorno de los organismos es una de las características más fácilmente identificables por depredadores visuales (Bond, 2007). Marcas y colores que irrumpen las orillas de los objetos creando orillas falsas o capturen la atención de los depredadores hacia otra característica son efectivas estrategias crípticas independientes de la coloración del entorno (Merilaita, 1998; Stevens and Cuthill, 2006).

En un ambiente cromáticamente homogéneo se podría favorecer la evolución de colores y marcas que provean coincidencia de fondo, independientemente de la movilidad y conducta de los organismos (Ahnesjö y Forsman, 2006; Bond, 2007). En contraste, en un ambiente heterogéneo la coincidencia de fondo solo se favorecería en dos escenarios: a) si los organismos que utilizan esta estrategia tienen una baja movilidad y al mismo tiempo una alta probabilidad de posarse en sitios donde asemeje al fondo (Ahnesjö y Forsman, 2006; Bond, 2007) o b) si los organismos activamente buscan posarse en ambientes con similitudes cromáticas a las de ellos. En estos sitios heterogéneos la selección natural podría favorecer la evolución de la coloración disruptiva en organismos con alta movilidad y baja selectividad de sustrato sobre la coincidencia de fondo debido a que la primera genera la capacidad de ser críptico independientemente del sustrato.

Debido a la heterogeneidad cromática de los ambientes y la conducta de los organismos la coloración críptica óptima puede depender de una disyuntiva que maximice las propiedades crípticas al asemejarse a un ambiente o poseer una coloración que provea crípsis en múltiples microhabitats cromáticamente distintos (Merilaita, 1998; Merilaita, Tuomi y Jormalainen, 1999). En diferentes especies se ha documentado una disyuntiva entre su coloración asociado tipo de ambiente en que se encuentran los organismos y a su movilidad. Así, en el cangrejo de mar común (*Carcinus maenas*), las poblaciones que habitan ambientes cromáticamente homogéneos ha evolucionado la coincidencia de fondo, mientras que en organismos que habitan ambientes cromáticamente heterogéneos ha

evolucionado la coloración disruptiva (Price *et al.*, 2019). En arañas saltarinas (Salticidae), en las especies con poca movilidad se ha documentado la estrategia de coincidencia de fondo, mientras que en una especie con mayor movilidad se muestra una coloración disruptiva (Robledo-Ospina *et al.*, 2017). Asimismo, si los sexos de una misma especie habitan diferente microhabitats o tienen diferencias en comportamiento o movilidad se podría esperar que surgiera dicromatismo sexual asociado a distintas tácticas crípticas (Medina, Losos y Mahler, 2016; Forsman, 2018). Sin embargo, esta hipótesis ha sido poco explorada.

La diferencia en la coloración de los ambientes puede ser influida por factores climáticos, por ejemplo, la cantidad de precipitación puede estar asociada a la cantidad de hojas en la vegetación y al color de las hojas (Yom-Tov y Geffen, 2006), además la cantidad de parches de luz y sombra cerca del suelo también pueden estar relacionadas con la cantidad de follaje en arboles y arbustos. La correlación entre factores climáticos y la coloración en insectos típicamente esta asociada con las propiedades de termorregulación de las especies sin embargo esta asociación pocas veces a sido explorada con relación a las estrategias crípticas de los organismos.

En chapulines es común encontrar especies con coincidencia de fondo, con coloraciones disruptivas o en las que han evolucionado ambas estrategias (Forsman y Appelqvist, 1999; Tsurui, Honma y Nishida, 2010, 2013; Karpestam, Merilaita y Forsman, 2012, 2013, 2014; Baños-Villalba, Quevedo y Edelaar, 2018; Edelaar *et al.*, 2019; Camacho *et al.*, 2020). Sin embargo, no existen estudios comparativos filogenéticos en el orden Orthoptera que hayan abordado la evolución de la coloración y del dicromatismo sexual y su asociación con la coloración ambiental.

En este estudio se analizó la evolución de los patrones cromáticos en los chapulines del género *Sphenarium*. Este género está conformado por 17 especies de chapulines con un ciclo de vida anual cuyas relaciones filogenéticas están bien definidas (Sanabria-Urbán *et al.*, 2015, 2017). Estos chapulines se distribuyen desde el noreste de Guatemala hasta el centro de México en un amplio

margen altitudinal (0 hasta > 2900 m.s.n.m.). Las especies del grupo habitan en una gran variedad de ecosistemas, desde bosques de coníferas y tropicales, hasta zonas desérticas. Generalmente estos chapulines ocupan los estratos herbáceos y arbustivos. Su ciclo de vida ocurre estrechamente ligado al ciclo de lluvias de las zonas donde habitan, las ninfas eclosionan a principios de la temporada de lluvia (mayo y principios de junio) alcanzan el estadio adulto entre los meses de agosto y septiembre cuando aún hay una gran cantidad de hierbas y hojas verdes. Mueren en los últimos meses del año o a principios del año siguiente de acuerdo a la cantidad de alimento disponible asociado a la estacionalidad de los sitios donde habitan (Kevan, 1977; Sanabria-Urbán y Cueva del Castillo, 2020). Algunas especies tienen una amplia variedad de coloración que incorpora desde tonos negros, diversas tonalidades de café y verde con diversos patrones de marcas como bandas longitudinales y transversales y puntos sobre el tórax y el abdomen o presentan coloraciones lisas (sin marcas) en todo el cuerpo.

Debido a su amplio intervalo de distribución y a la diversidad de ambientes que ocupan, este género de chapulines es un modelo ideal para estudiar la evolución de la coloración y su potencial valor adaptativo. Por ello, los objetivos de este trabajo fueron: a) evaluar si la coloración y los patrones de marcas de estos chapulines proveen cripsis, ya sea por coincidencia de fondo y/o por coloración disruptiva en los ambientes donde se encuentran (este objetivo se probara de manera experimental en una especie y se evaluó por análisis de imágenes en las 17 especies), b) evaluar si hembras y machos difieren en su coloración (dicromatismo sexual) y si esto está asociado a la coloración sustratos en los que se encuentran en dos especies de chapulines del género *Sphenarium* c) explorar el efecto de la selección natural en la coloración de los chapulines respecto a la coloración de sus hábitats y a las características climáticas de su ambiente.

En el primer capítulo de la tesis se aborda el primer objetivo al evaluar el potencial criptico de la coloración de los chapulines de la especie *Sphenarium* mediante un experimento de detección con

humanos a través de una pantalla de computadora y un análisis de las características de las imágenes presentadas a los participantes del experimento. El segundo capítulo analiza el segundo objetivo mediante la evaluación de imágenes de chapulines de ambos sexos en dos especies con coloraciones contrastantes. Una de ellas con una alta variación en coloración y patrones de marcas y otra con una coloración relativamente homogénea. En el tercer capítulo se aborda el tercer objetivo mediante un análisis comparativo entre la coloración de los chapulines y la coloración de los sustratos a los que se hayan asociados las 17 especies de estudio, considerándose además los factores climáticos determinantes para su ciclo de vida. En conjunto estos tres capítulos ofrecen una panorámica integral sobre la evolución de la coloración en el género *Sphenarium*.

# Capítulo 1

## **Testing background matching and disruptive coloration on a neotropical grasshopper: a computer detection experiment with humans**

Manuscrito enviado a revisión a la revista *Entomologia Experimentalis et Applicata*





**Testing background matching and disruptive colouration in a sexually dichromatic grasshopper: a computer detection experiment.**

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# Testing background matching and disruptive colouration in a sexually dichromatic grasshopper: a computer detection experiment.

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**Running Head:** Crypsis and predation in a neotropical grasshopper

## **Abstract**

Cryptic colouration is an adaptative mechanism against predators. Colour patterns can become cryptic through background matching and colouration that disrupts the outline of an organism. Background matching could be advantageous in chromatically homogeneous microhabitats. Conversely, colouration disrupting the edges of organisms can be favoured in visually heterogeneous microhabitats.

Grasshoppers of the genus *Sphenarium* inhabit very heterogeneous environments and exhibit both strategies. Within the populations, adults of both sexes exhibit different colour patterns. Some have longitudinal and transverse bands over the thorax and abdomen, and some are plain in colour without any marks, showing great continuous variation between organisms with complex patterns and simpler patterns. However, males usually exhibit more variation in patterns and number of bands than females. In this study, we analysed the cryptic properties of the colour patterns of males and females of *Sphenarium zapotecum*, and we tested the effectiveness of background matching and disruptive colouration using humans as 'predators' in a computer detection experiment. We found that the females and males are dichromatic and seem to follow different colour cryptic strategies; males are more disruptive than females, whereas females have a high background matching with less disruptive elements. In addition, in visually heterogeneous areas, predators spent more time searching for striped male individuals with lower background matching and higher disruptive properties, In contrast, the image of a female individual with high background matching improves survival and significantly increases predators' searching time. Since background matching is associated with females and disruptive colouration to males, our results could help explain the evolution of sexual dichromatisms in this and other species of grasshoppers of the genus *Sphenarium*.

**Key words:** Background matching, Disruptive colouration, Grasshoppers, Crypsis, Predation, Survival, Sexual dichromatisms.

## **Introduction**

Colour patterns can be adaptive if they reduce the risk of visual detection by predators. Colouration improves survival if it conceals an organism's body from possible visual-oriented predators (Cott, 1940; Théry & Gomez, 2010; Cuthill et al., 2017). Colour patterns can be cryptic by background matching (colours that resemble the general colour of the visual background) or disruptive colouration (patterns that conceal the body outline of an animal; Norris & Lowe, 1964). Background matching can be easier to achieve in visually homogeneous microhabitats (Robledo-Ospina et al., 2017; Orton & McBrayer, 2019). However, it can be inefficient at reducing potential detection when organisms are in motion or stay in heterogeneous environments (Ioannou & Krause, 2009). On the other hand, disruptive colouration could evolve in visually heterogeneous microhabitats. Strips and dots could disrupt the outlines of the organisms independently of the background and it can be adaptive in organisms with high mobility (Stevens et al., 2006). Moreover, if females and males use different microhabitats owing to their different sexual roles (males being more mobile than females for search for potential mates), natural selection towards crypsis can favour a divergence in cryptic colour patterns between females and males (Forsman & Appelqvist, 1999; Medina et al., 2016; Ramírez-Delgado & Cueva del Castillo, 2020; Cueva del Castillo et al., 2021).

Most of the studies of sexual dichromatism have been focused on vertebrates (Badyaev & Hill, 2003; Font et al., 2009). It has been explained only by differences in selective pressures imposed by visual predators only a few times (Orton & McBrayer, 2019). Usually, sexual dichromatism is associated with differences in sexual selection pressures. For example, differences in colouration between females and males are found in many bird species. However, the females tend to be cryptic to decrease the risk of being predated, whereas males are conspicuous due to sexual selection pressures (Badyaev & Hill, 2003; Medina et al., 2017). In invertebrates, the study of the evolution of sexual dimorphism in colour patterns has focused on insects, mainly orthopterans and butterflies (Forsman &

Appelqvist, 1999; Ramírez-Delgado & Cueva del Castillo, 2020; van der Bijl et al., 2020; Cueva del Castillo et al., 2021); and spiders (Li et al., 2008).

In the case of orthopterans evidence suggest that males and females could have different ecological niches and favour different microhabitats and therefore different body colouration. Given that many cryptic species are sexually dichromatic, their study has profound implications regarding the evolution of intraspecific chromatic variation concerning selective pressures imposed by visual predators.

Cryptic colouration is typical in grasshoppers (Gillis, 1982; Eterovick et al., 1997; Forsman & Appelqvist, 1999; Ahnesjö & Forsman, 2006; Karpestam et al., 2012; Baños-Villalba et al., 2018). In pygmy grasshoppers, background matching and disruptive colouration have been related to the habitation of different habitats and grasshopper size (Tsurui et al., 2010, 2013). In contrast, *Sphenarium purpurascens* and *S. planum* show different patterns that could be associated with both cryptic strategies (Ramírez-Delgado & Cueva del Castillo, 2020; Cueva del Castillo et al., 2021). In these two species, males and females are sexually dichromatic. In a highly contrasting environment, both males and females used disruptive colouration as a cryptic strategy. In contrast, males and females followed different cryptic strategies in a heterogeneous environment. Males were more disruptive than females, and females exhibited high background matching with fewer disruptive elements.

In this work, we evaluated the cryptic properties of males and females of *Sphenarium zapotecum* grasshoppers. *S. zapotecum* is grasshopper species restricted to the outer southern slope of the Sierra Madre del Sur in Oaxaca. These grasshoppers are univoltine: nymphs emerge mainly at the beginning of the rainy season (June – July) and mostly become adults and reproduce during fall (from mid-September to mid-December). Following reproduction, oviposition and the highest adult mortality occur during winter, from mid-December to mid-February (Sanabria-Urbán & Cueva del Castillo, 2020). Adults show a large variation in chromatic patterns, and like other species of the genus, they are sexually dichromatic (Ramírez-Delgado & Cueva del Castillo, 2020; Cueva del Castillo et al., 2021).

Males usually have more complex and contrasting patterns than females, with stripes that cross both longitudinally and transversely and some bright spots in the first two segments of the abdomen in their dorsal area. Meanwhile, females tend to have areas coloured in even tones (flatter patterns) and sometimes have a clear transverse stripe along the thorax or a plain green or brown colour over their entire body (Sanabria-Urbán et al., 2017). They are predated by many visually-oriented vertebrates, including birds, mammals, and reptiles (Kevan, 1977).

We analysed the cryptic properties of the colour patterns of males and females of *S. zapotecum* and tested the effectiveness of background matching and disruptive colouration cryptic strategies using humans as ‘predators’ in a computer detection experiment. In a colour monitor, images of the grasshoppers individuals were displayed at random in a heterogeneous chromatic background. Given the different visual properties of males and females and because in other *Sphenarium* species, males usually are found in heterogeneous and contrasting chromatic environments, and females exhibited high background matching (Ramírez-Delgado & Cueva del Castillo, 2020; Cueva del Castillo et al., 2021), we expected that in visually heterogeneous backgrounds predators spent more time searching for striped male individuals with lower background matching and higher disruptive properties, whereas female individuals with high background matching improves survival and significantly increases predators’ searching time.

## **MATERIALS AND METHODS**

### *Image acquisition*

We took 51 digital photographs of adult males (32) and females (19) of *S. zapotecum* under field conditions at the edge of a pine-oak forest in the community of San Isidro Paz y Progreso in the state of Oaxaca (17.07 N, 97.83 W). The spread of grasshoppers was well defined by the open area around a

dirt road and the forest boundaries. This open area had a very similar vegetal composition dominated by grasses and some small herbs (lower than 20 cm), and the ground was mainly composed of brown soil. Taking advantage of the delimited distribution area of this population and the similarity of the space where they distribute, we took a photograph from a representative area of the background where the grasshoppers were found. This background represented the substrates and colours where both males and females can be found in the field. In an area of approximately 100 m<sup>2</sup> three people walked slowly searching for grasshoppers, which were collected by hand, put into a plastic tube (5 cm<sup>3</sup>), and placed in a cooler until they were unable to move. Each grasshopper with its background was photographed from a zenithal view with a Canon EOS 70D camera fitted with an 18–55 mm, f/3.5 – 5.6 lens. The focal distance was constant at 55 mm, the aperture of the camera was set to f-stops: f/5.6, the light sensitivity value (ISO) was set to 400 in all photographs, and the shutter speed was adjusted in every shot to keep from overexposing the pictures. A white diffuser umbrella was placed over each grasshopper to remove potential shadows. All photographs were taken approximately 50 cm away from the objectives and included a colorchecker card (X-rite Colorchecker Passport Photo 2, Munsell Color Laboratories) in the same plane as the grasshoppers. Additionally, the photograph of the background including the main characteristics of the area (grasses and herbs leaves) was taken under the same camera settings and conditions. The grasshoppers were released at the same places where they were collected after all the photographs were taken. Sampling was conducted in compliance with the Mexican law (Ley General del Equilibrio Ecológico y Protección Ambiental).

### *Image analysis*

Images of the 51 grasshoppers were analysed as raw images to measure the background matching and disruptive properties of all grasshoppers against their background. The total dorsal surface area of the grasshoppers, excluding appendices, and a similar-sized surface area of the adjacent background were

used to obtain measurements of the grasshoppers and their microhabitats. Gray reflectance standards from the ColorChecker card were applied to standardize the pictures. Photographs were scaled down to 15 pixels per mm.

Using the Multispectral Image Calibration and Analysis (MICA) Toolbox version 2.0 (Troscianko & Stevens, 2015) for ImageJ 1.52v (Schneider et al., 2012) software and taking advantage of the ColorChecker used in the photographs, we converted the images into human cone-catch images. The camera's spectral sensitivity was estimated using the Xrite ColorChecker colour patches, which reflectance of every patch is included in the MICA 2.0 using a cone-catch mapping function (More details Troscianko & Stevens, 2015). This conversion produces images based on the spectral sensitivities of a human visual system. Additionally, we used the colour adjacency analysis tools included in the MICA toolbox (van den Berg et al., 2020) to measure the proportions of the colours in the background image, revealing that 67% of green pixels correspond to grass and herbs, 21% of brown pixels correspond to dry grass and herbs, and 12% of near-black pixels correspond to the dark shady areas between some herbs.

### *Background matching*

From the cone-catch images, we measured background matching of the colour and brightness of the individuals against their background using chromatic and achromatic just notable differences (JND). The lower values of JND denote high resemblance between surfaces, whereas high JND values denote low resemblance between two surfaces (Osorio & Vorobyev, 2008; Troscianko & Stevens, 2015). We considered the background as a square of about 25x 25 mm around the grasshopper.

### *Disruptive coloration*



We evaluated the edge disruption of the grasshoppers using the GabRat tool implemented in the MICA toolbox. We used the GabRat tool to measure the ratio between false and coherent edges of the surfaces of the grasshoppers. The GabRat tool estimates the coherent and false edges of an object in an image. The analysis produces values ranging from zero to one, where values close to zero are non-disruptive. The GabRat tool is based on a Gabor band-pass filter (see Troscianko et al., 2017; Price et al., 2019). This is one of the best predictors of human detection time of disruptive targets, and it is superior to other pattern metrics algorithms tested in humans (see Troscianko et al., 2017). Before using this tool, we converted the cone-catch images into LAB images. LAB images fit roughly with human luminance and colour perception and allow us to measure chromatic disruption (Troscianko et al., 2017). These images are composed of three channels: L corresponds to an achromatic channel (lightness), and A and B to chromatic channels (red to yellow and blue to green, respectively) (Kim et al., 2000).

## **Detection Experiment**

For the detection experiment, we chose a photograph of a single female and three photographs of the males that represent most of the variation in the population (Fig. 1). The photograph of the female was named “PG”: a green female with an almost plain dorsal area, i.e. without any stripes or dots in the dorsal area (Fig. 1A). Because all the females from this population were similar, we only used this female individual in the study. The photographs of the males were named “PB”: a brown male with a mostly plain dorsal area (Fig. 1B); “SG”: a green male with black and brownish stripes and two white dots on the thorax (Fig. 1C); and “SD”: a darker green male with brown and black stripes and a couple of yellow dots on the thorax (Fig. 1D). The photographs of the individuals and the photograph of the background were transformed into a PNG (portable network graphic) format without any colour correction. This format allows us to manipulate the images for the experiments and keep the colours objectively measurable.

We removed the backgrounds from the photographs of the selected individuals and replaced them with a transparent canvas.

From the background image (see *Image acquisition*), we cut a rectangular area with a proportion of 16:9 in a landscape orientation containing green grasses and herbs and some brown dry herbs that was used as a background for the experiment. In this heterochromatic background any grasshopper can be found in the collecting place. We use the same background to control the complexity of the scene, which can affect the survival times, and it is not related to the coloration features of the individuals (Dimitrova & Merilaita, 2010). We created 12 artificial images (3 from the female individual and 9 from the male individuals). Each image contained a individual in a random position and orientation over the background. We used each individual (PG, PB, SG, SD) in three images, each of which included each individual in a different position and orientation (flipped horizontally, vertically, and flipped both vertically and horizontally). We randomly selected the images of the individuals that were flipped. After the flip transformations, we generated four different sets of orientations on the background image. Finally, we scaled all images down to 1920 × 1080 pixels. In all the images, the background and the grasshopper maintain their original proportions.

### *Experimental procedure*

We showed all the images to 18 men and 28 women between 20 and 24 years of age. These volunteers have normal vision, or corrected to normal vision. Each participant was individually placed in a dark room 50 cm away from a colour monitor with a resolution of 1920 × 1080 pixels and a size of 59.8 × 33.6 cm (Dell P2714H). Before the experiment was conducted, monitor colour was calibrated and set to 60% brightness. We verbally instructed each participant to search and use a mouse to click on the grasshoppers they detected in the images. The images were shown at random to every volunteer. A new image presented whenever they clicked on the image. The computer registered the delay between when

an image appeared and was clicked on (survival time). If the participant clicked on the grasshopper, correctly, we considered the response a successful predation event. If the volunteer clicked outside of the grasshopper, or if the individual was undetected for more than 60 seconds, we assumed that the grasshopper was not detected or it was wrongly detected, and it had the opportunity to escape. In both cases, we registered the survival time of the individuals at 60.01 seconds, the maximum time that a individual can survive in our experiment

## **Statistical analyses**

### *Comparison between dorsal grasshopper surface and background for males and females of *Sphenarium zapotecum**

We compared the colour dorsal surfaces grasshoppers' resemblance with de background (JND) and disruptive properties (GabRat) of the males and females using two tailed *t* test. JND data were transformed by quadratic roots to meet parametric test assumptions.

### *Detection Experiment*

The survival time of the individual images was analysed using the Cox hazard analysis (Cox, 1992). This analysis allows us to compare the probability at which a individual will be predated over time (hazard ratio) of the four individuals and the hazard ratio of the 12 images depending on the position and orientation of the individuals. Given the lack of independence of data from the same participant (Aalen, 1994), we used the participants as a random variable (frailty variable). The analysis was performed in R 4.0 (R Core Team, 2020) using the “survival” package (Therneau & Grambsch, 2000; Therneau, 2020).

## RESULTS

### *Comparison between dorsal grasshopper surface and background for males and females of *Sphenarium zapotecum**

The *t* test indicates significant differences between males and females on the background matching (JND) and disruptive colouration (GabRat) of the organism. Females are more similar to their background than the males (Chromatic JND  $t_{49} = -6.66$ ,  $P < 0.0001$ ; Fig 2A), (Achromatic JND  $t_{49} = -5.61$ ,  $P < 0.0001$ ; Fig 2B); However, having less disruptive properties than them (GabRat  $t_{49} = -4.32$ ,  $P < 0.0001$ ; Fig 2C).

The descriptive values of the image analysis on the images created for the experiment showed that the plain individuals (PG (female) and PB individuals) have better background matching (lower JND values) than the striped individuals (SG and SD individuals), and that striped individuals have more disruptive patterns (higher GabRat values; see table 1).

### *Detection Experiment*

The hazard analysis of the individuals, in which participants were included as a random variable (Fig 3), showed that the PG individual with higher background matching has the lowest risk to be predated, and that its predation risk was much lower than that of the PB individual (5.32 higher hazard ratio of PB compared to PG), whereas the hazard ratio of SG individual (with the highest disruptive elements) is 2.3 times higher than that of the PG individual. Moreover, no significant differences were found between individuals PB and SD and individuals SD and SG.

The hazard analysis showed that image 2 had the lowest hazard ratio (0.35 of the predation hazard of image 1) and the highest background matching when constructed with the PG individual. Images 1 and 3 (constructed with the PG individual and high background matching) have similar hazard ratios as

compared to images 7, 9 (constructed with the SG individual), and 12 (constructed with the SD individual with high disruptive colouration). Images 4, 5, and 6 (constructed with the PB individual) and images 8, 10, and 11 (constructed with the striped individuals) were twice as likely to be predated than image 1. No differences between images were found among the group of images constructed with the PB individual. The same pattern occurs for the groups of images constructed with the SG and SD individuals. However, differences were found between images of the PG individual, which could suggest that the hazard of this individual may depend on its position over the background.

## **DISCUSSION**

Our results indicate that this *Sphenarium zapotecum* population, like in other *Sphenarium* species (Ramírez-Delgado & Cueva del Castillo, 2020), males are less similar to their backgrounds and females have good chromatic background matching, this could explain why males are more disruptive than females. Moreover, the experimental study indicated that the lowest predation hazard was associated to the plain green female, which was related to a background matching strategy. However, the disruptive elements of male striped individuals seem to improve the probability of survival regarding non-disruptive, less effective elements related to background matching.

The differences in detection between the plain female (PG) and male (PB) individuals can probably be explained by the experimental background, which has a large proportion of green in the image. However, the heterogeneity of the background image used for this study improve the other male individuals (SG, SD) chances to survive relative to the PB individual. The stripes and clear dots on the individuals could make it harder for observers to find the edges of the image. The striped and plain individuals may have different concealment effects under conditions that involve movement (Hall et al., 2013, 2017). Thus, striped patterns can lower the possibilities that a prey will be captured after

being recognized due to motion-dazzle (marks that hinder the ability of the predator to calculate the speed and trajectory of the prey) (Stevens & Merilaita, 2009).

The payoff of background matching and disruptive colouration can be strongly affected by the environmental chromatic heterogeneity (Bond, 2007). Under chromatic homogeneous conditions, individuals with a high background matching could have a high survival expectation. Nevertheless, disruptive colouration could be more successful in a heterogeneous environment, although there may be a trade-off with being highly cryptic in this type of environment, between grasshoppers matching their background well enough and having disruptive patterns, which would give them a better chance to conceal themselves in different places.

The environmental complexity associated with the visual predators of this species may explain the evolution of sexual dichromatism and the diversity of the coloration and marking patterns in the males of this and other species of the genus *Sphenarium*. In the field females, and males of *S. purpurascens* and *S. planum* (Ramírez-Delgado & Cueva del Castillo, 2020; Cueva del Castillo et al., 2021) are found in different chromatic niches. Their survival expectative can be affected by differential predation associated with a heterogeneous background and their capacity to choose their activity places. Interestingly, the female of *S. zapotecum*, like the females of other *Sphenarium* species, tend to have flatter colouration (Sanabria-Urbán et al., 2017; Ramírez-Delgado & Cueva del Castillo, 2020), which seems to be associated with the highest background matching and the lowest risk of predation. The females' high colour-matching with their background could lower their detectability, especially if they have reduced mobility or if they can place themselves where their colour-match is high (Endler, 1978; Merilaita et al., 2017; Michalis et al., 2017). The sexual size dimorphism bias to females (Sanabria-Urbán et al., 2015), plus an increase in weight due to egg maturation, could explain the lack of mobility of *Sphenarium* females. On the other hand, males' marking elements could reduce the risk of boundary detection by potential predators (Endler, 1978; Merilaita, 1998; Cuthill et al., 2005;

Schaefer & Stobbe, 2006) and can be adaptative in organisms with high mobility in heterogeneous environments (Stevens & Cuthill, 2006; Stevens et al., 2006). We could expect high mobility in males because they usually actively search for females (Thornhill & Alcock, 1983), and males are more mobile than females in at least in one species of the genus, *Sphenarium purpurascens* (Ramírez-Delgado & Cueva del Castillo, 2020).

We must point out that our results and their interpretation must be treated with caution because of the lack of mobility of the preys plus potential differences between the visual sensitivities of humans and other possible predators like lizard's infrared vision or dichromatic mammals could affect the predation result. Nonetheless, we can consider humans as a natural predator of this species because 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 (Sanabria-Urban et al., 2017). Moreover, experimental use of humans as predators has been useful to test the concealment of an organism under controlled conditions (e.g. Tsurui et al., 2010, 2013; Karpestam et al., 2012, 2013; Todd et al., 2015). Humans' processing capabilities are similar to those of natural predators, specially birds (Dukas & Kamil, 2001), and some studies have noticed that human "predation" can predict predation by visual predators under natural conditions (Karpestam et al., 2013).

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**Author Contributions:** Both authors contributed equally to the conception and study's design, fieldwork, data analyzes, and writing of the paper.



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## FIGURE LEGENDS

Figure 1. Individuals and the background used for the experiment. A) Individual PG: plain green female, B) Individual PB: plain brown male, C) Individual SG: striped green male, D) Individual SD: striped darker green male, and E) the background.

Figure 2. Boxplots of background matching: properties chromatic JND (A), and Acromatic JND (B); and disruptive properties: GabRat (C), in the 3 cases there are significant differences according to the  $t$  test. JND data were plotted using square root transformations.

Figure 3. Means (upper number and squares), and confidence interval 95% (numbers between parenthesis and error bars) of the hazard ratio of the individuals 2-4 respect the hazard of the individual 1 (dotted line), P values (small numbers) from the frailty models and number of observations (N) are shown.

Figure 4. Means (upper number and squares), and confidence interval 95% (numbers between parenthesis and error bars) of the hazard ratio of the images 2-12 respect the hazard of the image 1 (dotted line), P values (small numbers) from the frailty models and number of observations (N) are shown.

Table 1. Background matching (Chromatic and Achromatic JND) and disruptive values (GabRat) of the individuals by image and their means (by individual). JND: Just notable differences. PG: plain-green individual, PB: plain-brown, SG: striped-green male SD: striped-dark male.

Image	individual	Chromatic JND by image	Chromatic JND by individual	Achromatic JND by image	Achromatic JND by individual	GabRat by image	GabRat by individual
1	PG	0.93		2.61		0.13	
2	PG	1.39	1.45	1.47	2.28	0.21	0.13
3	PG	2.03		2.76		0.05	
4	PB	2.32		3.69		0.08	
5	PB	3.04	2.76	3.96	3.89	0.08	0.13
6	PB	2.91		4.04		0.23	
7	SG	3.25		5.21		0.31	
8	SG	3.54	3.30	4.35	4.75	0.36	0.31
9	SG	3.11		4.68		0.27	
10	SD	5.12		4.26		0.3	
11	SD	3.84	4.36	5.12	5.05	0.35	0.27
12	SD	4.12		5.77		0.21	

Fig. 1

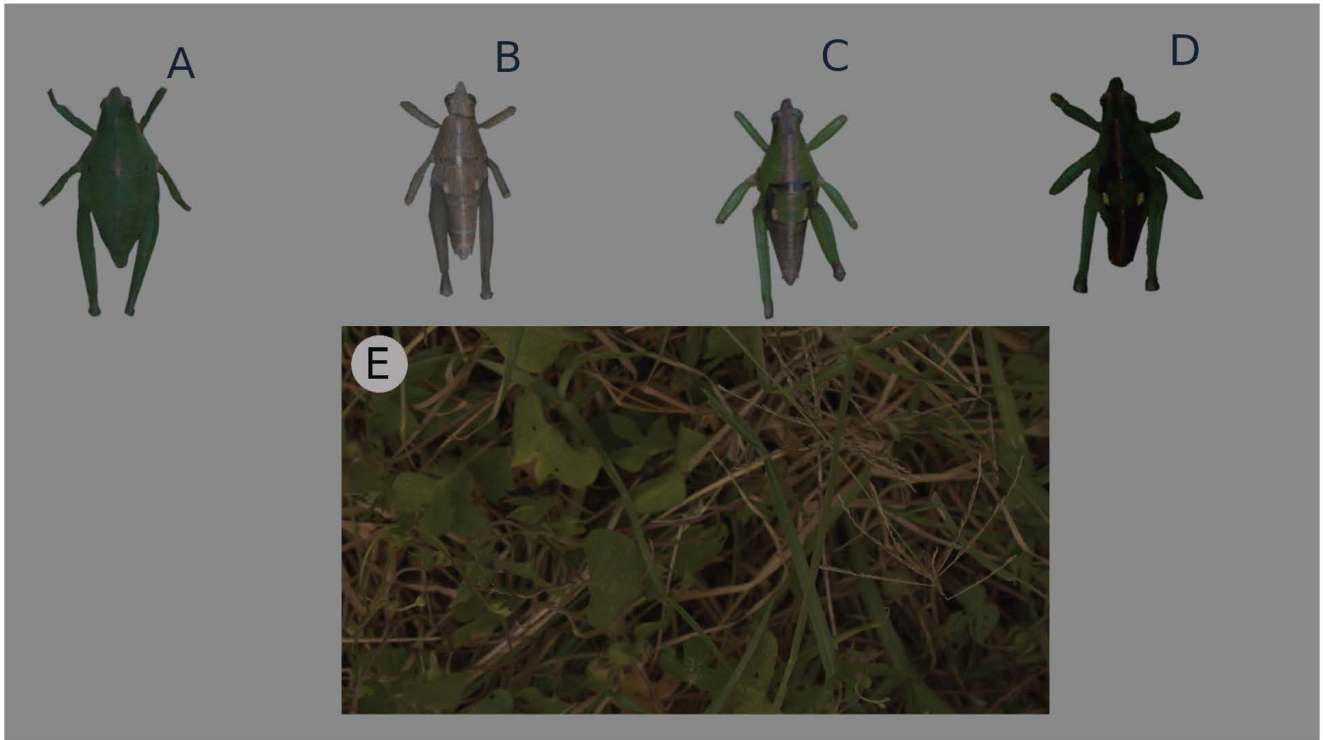


Fig. 2

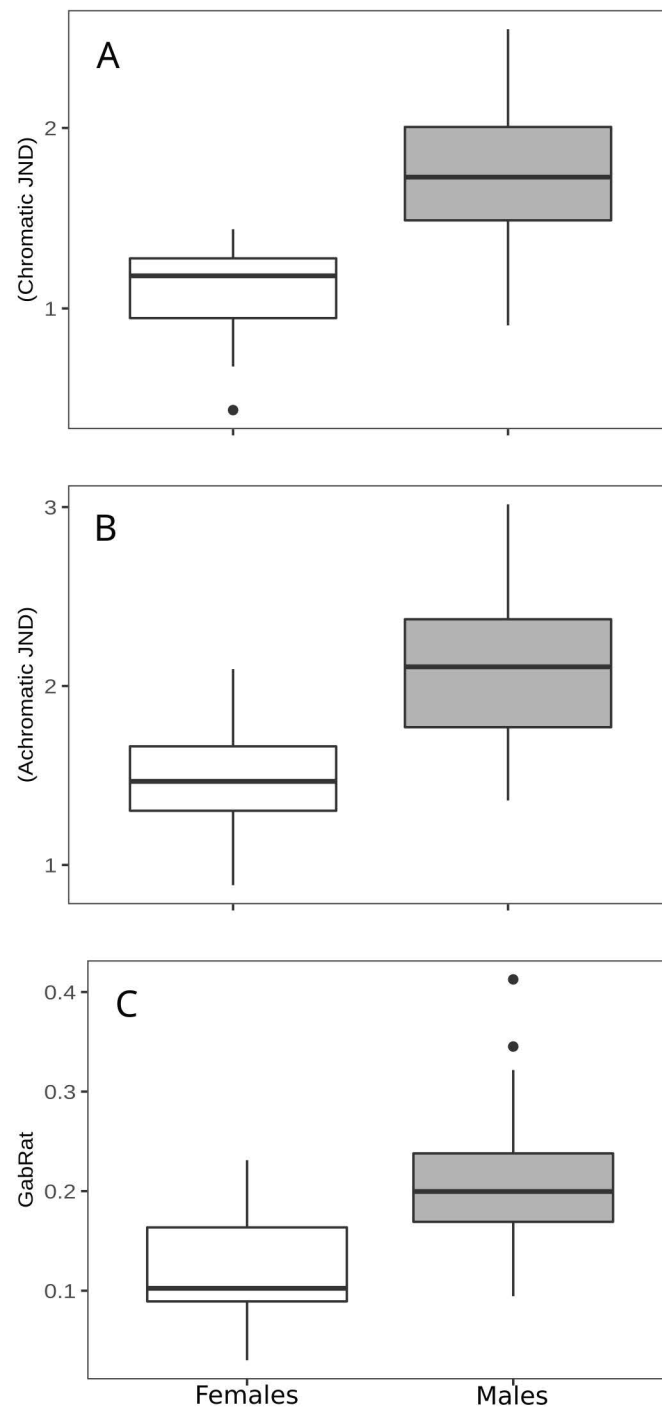




Fig. 3

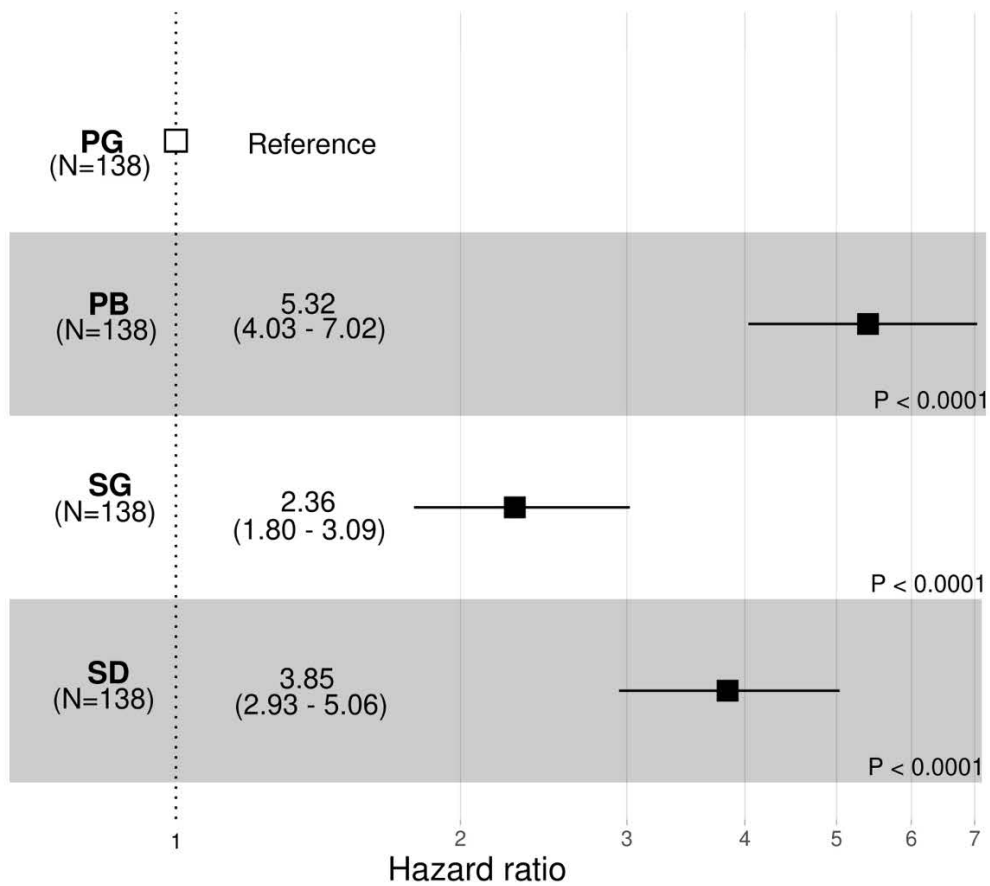
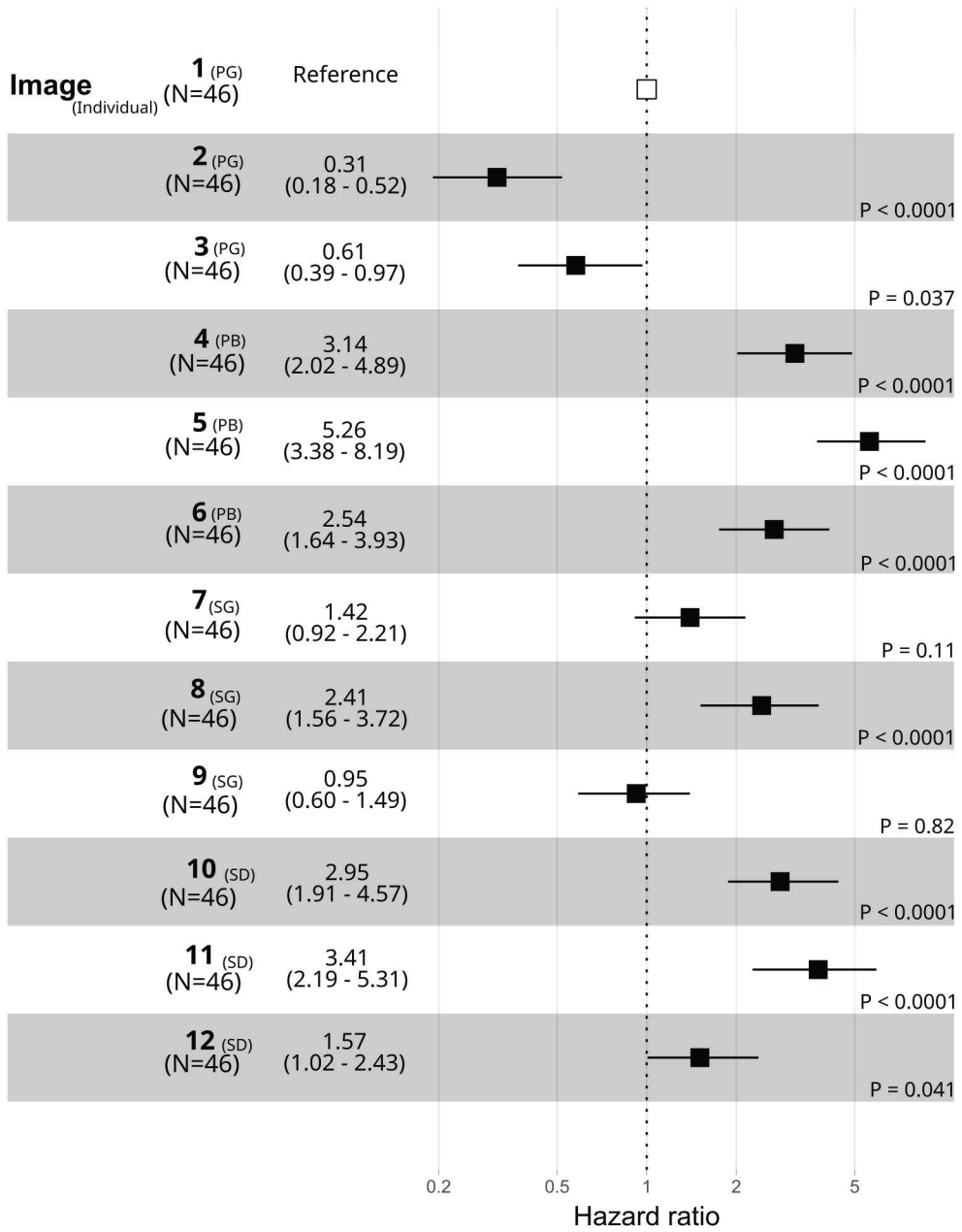


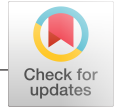
Fig. 4



## Capítulo 2

### **Background matching, disruptive coloration, and differential use of microhabitats in two neotropical grasshoppers with sexual dichromatism**

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# Background matching, disruptive coloration, and differential use of microhabitats in two neotropical grasshoppers with sexual dichromatism

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## Abstract

Cryptic coloration is an adaptative defensive mechanism against predators. Color patterns can become cryptic through background coloration-matching and disruptive coloration. Disruptive coloration may evolve in visually heterogeneous microhabitats, whereas background matching could be favored in chromatically homogeneous microhabitats. In this work, we used digital photography to explore the potential use of disruptive coloration and background matching in males and females of two grasshopper species of the *Sphenarium* genus in different habitats. We found chromatic differences in the two grasshopper species that may be explained by local adaptation. We also found that the females and males of both species are dichromatic and seem to follow different color cryptic strategies, males are more disruptive than females, whereas females have a high background matching with less disruptive elements. The selective pressures of the predators in different microhabitats and the differences in mobility between sexes may explain the color pattern divergence between females and males. Nevertheless, more field experiments are needed in order to understand the relative importance of disruptive and background matching coloration in the evolution of sexual dichromatism in these grasshoppers.

## KEYWORDS

background matching, crypsis, digital photography, disruptive coloration, grasshoppers, image analysis, sexual dichromatism

## 1 | INTRODUCTION

The relationship between organisms and their environment is mediated by coloration in many ways, including social signaling, thermoregulation, protection from ultraviolet light, and antipredator defenses (Cott, 1940; Cuthill et al., 2017 and references therein). In cryptic coloration, color patterns can be adaptative if they lower

the risk of being visually detected by predators. Crypsis is probably the most widespread form of concealment (Merilaita & Lind, 2005; Merilaita, Scott-Samuel, & Cuthill, 2017). Color patterns can become cryptic by multiple mechanisms, including background coloration-matching (colors that resemble the general color of the visual background) and disruptive coloration (patterns that conceal an animal's body outline; Merilaita, Tuomi, & Jormalainen, 1999; Norris

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& Lowe, 1964). Since crypsis reduces the probability of detection by predators, its variation usually matches geographic variation in substrate color (Endler, 1990; Hantak & Kuchta, 2018; Marshall, Philpot, Damas-Moreira, & Stevens, 2015; Rosenblum, 2006; Stuart-Fox & Ord, 2004). If females and males use different microhabitats, sexual dichromatism may evolve to better conceal them from visually oriented predators and could suggest differential crypsis values between sexes (Medina, Losos, & Mahler, 2016; Orton & McBrayer, 2019). Examples of crypsis mediating the coloration differences between females and males are found in many bird species. However, in these cases, often females are cryptic because of predation pressures, whereas males are conspicuous due to sexual selection (Badyaev & Hill, 2003; Medina et al., 2017). Nonetheless, if females and males utilize different microhabitats, natural selection for crypsis can favor the divergence between females and males in dorsal cryptic color patterns (Forsman, 1995; Forsman & Appelqvist, 1999; Medina et al., 2016).

Cryptic coloration is typical in grasshoppers (Ahnesjö & Forsman, 2006; Baños-Villalba, Quevedo, & Edelaar, 2018; Eterovick, Figueira, & Vasconcellos-Neto, 1997; Forsman & Appelqvist, 1999; Gillis, 1982; Karpestam, Merilaita, & Forsman, 2012), and yet no studies have addressed the evolution of sexual dimorphism in color patterns in this group of insects.

The *Sphenarium* genus is found in a wide variety of ecosystems, from northwest Guatemala to central Mexico (Sanabria-Urbán et al., 2015). *Sphenarium purpurascens* has a broad distribution range in central Mexico and lives in a wide variety of habitats, whereas *Sphenarium planum* has flatter color patterns and only lives in the Tehuacán Valley, a xeric area with less complex background chromatic patterns (Sanabria-Urbán, Song, Oyama, González-rodríguez, & Castillo, 2017). They are generalist herbivores; adults are found in herbs, grass, and bush leaves. In *S. purpurascens* females are less mobile than males and can be found close to the ground, where they lay their eggs (Camacho Castillo, 1999). On the other hand, males are easier to find in higher places, looking actively for females (R. Cueva del Castillo, personal observation). They are predated by many vertebrates, including birds, mammals, and reptiles (Kevan, 1977). Distinct species within the genus have different color patterns, but in general, these grasshoppers have longitudinal and transverse bands over the thorax and abdomen, showing great continuous variation; males usually exhibit more considerable variation in patterns variation and number of bands than females (Figure 1), who tend to have color areas in more uniform tone (flatter patterns, Sanabria-Urbán et al., 2017). Despite differences in color patterns between the sexes, there is no evidence of sexual selection acting on coloration. Males and females mate randomly with respect to male and female color patterns (Cueva del Castillo & Cano-Santana, 2001).

In this work, we explored the potential use of disruptive coloration and background matching in males and females of two grasshopper species of the *Sphenarium* genus in different microhabitats. Because both species are found in different environments and males and females may differ in their behavior due to their different reproductive roles (Camacho Castillo, 1999; Cueva del Castillo



**FIGURE 1** *S. purpurascens* grasshoppers. Male is mounting a female. Males have typically more bands and contrasting marking than females (photograph by Salomón Sanabria-Urbán)

& Cano-Santana, 2001; Cueva del Castillo, Núñez-Farfán, & Cano-Santana, 1999). Due to the more complex background chromatic patterns of Pedregal de San Ángel (see below), we expected that males and females of *S. purpurascens* showed more complex color patterns than *S. planum*.

## 2 | MATERIALS AND METHODS

### 2.1 | Study sites and image acquisition

Images of adult grasshoppers and their backgrounds were acquired in the middle of the rainy season, during the first and second weeks of October 2017, when most of the individuals in the populations were adults and the vegetation was still green. The photographs of *S. purpurascens* were taken at Pedregal de San Ángel, Mexico City (19°19'07.9"N, 99°11'33.7"W), whereas the photographs of *S. planum* were taken at the Tehuacán Valley, Puebla (18°33'27.9"N, 97°27'49.1"W). Even though *S. purpurascens* is widely distributed in central and south Mexico (Sanabria-Urbán et al., 2017), the Pedregal de San Ángel was chosen because its high environmental heterogeneity (see below), whereas *S. planum* was collected in the Tehuacán valley because this species has a narrow distribution (Sanabria-Urbán et al., 2017) and lives in a more homogeneous environment than *S. purpurascens*. Both localities gave us the opportunity to test potential different cryptic strategies associated with two contrasting environments.

The Pedregal de San Ángel is a place with a complex vegetal community and complex chromatic patterns in backgrounds, situated within the Trans-Mexican Volcanic Belt (Morrone, 2006) with a flora composition that has Neotropical and Nearctic affinities (Rzedowski, 1954; Rzedowsky, 1991). The photographs were taken in an area where the vegetation is dominated by oaks, grasses, herbs, and xerophytic scrubs. The ground is partially covered by leaf litter and black volcanic rocks. On the other hand, the Tehuacán Valley is situated in the Sierra Madre del Sur

(Dávalos-Álvarez, Nieto-Samaniego, Alaniz-Álvarez, Martínez-Hernández, & Ramírez-Arriaga, 2007), in xerophytic vegetation at the bottom of the valley (Pérez-Valladares et al., 2019). The area where the photographs were taken is dominated by xerophytic shrubs and some small herbs (lower than 30 cm), and the ground is mainly composed of brown soil and some sedimentary rocks. In both places, an area of approximately 100 m<sup>2</sup> was sampled. Three persons walked slowly over the area, searching for grasshoppers. Special care was taken to keep from disturbing any detected grasshoppers. When one was found, its location was first established, and then it was collected by hand, placed into a plastic bag (40 cm × 25 cm), and placed in a cooler until it was unable to move. Each grasshopper was returned to the same spot where it was first seen (usually on leaves or plant stems), and photographs were taken both of the dorsal view of the grasshopper and the background where it was returned. Grasshoppers that moved or escaped as a result of the approaching collectors were discarded from the study.

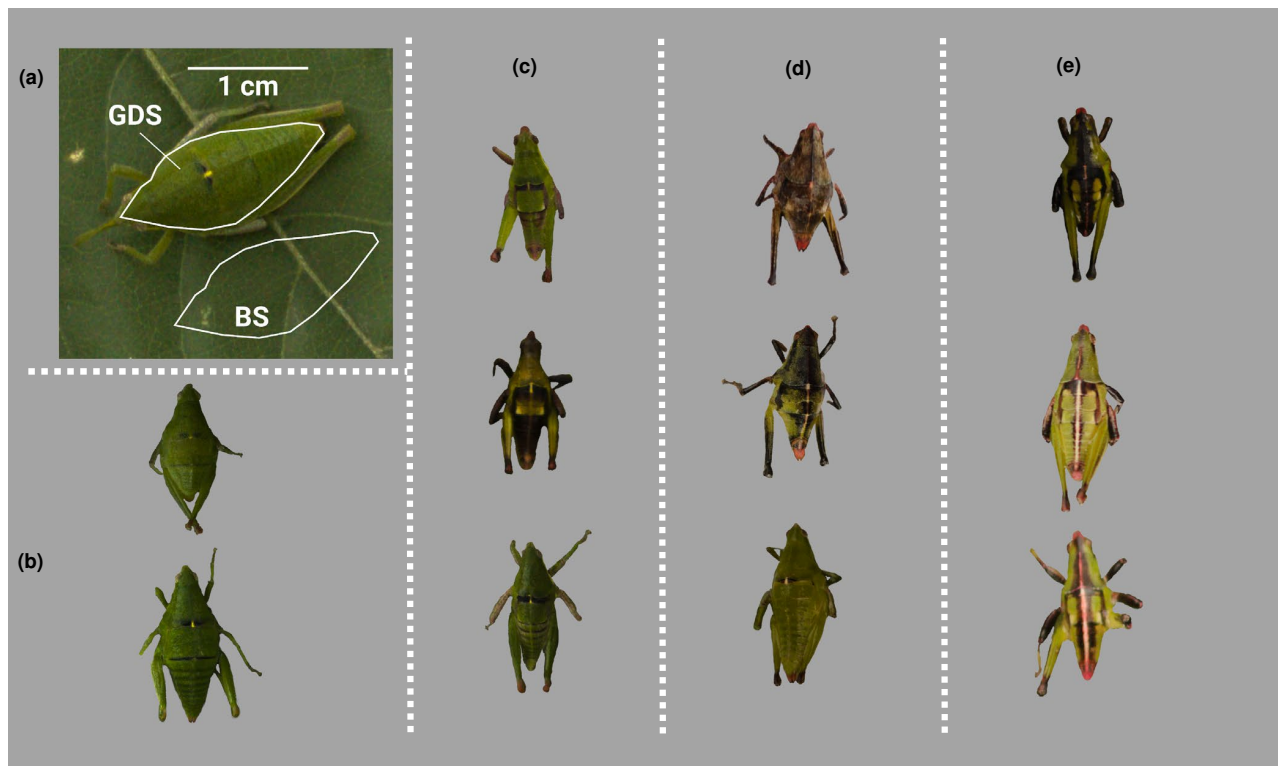
In all cases, photographs were taken with a Canon EOS 70D camera fitted with an 18–55 mm, f/3.5 – 5.6 lens. Camera modifications to allow sensitivity to the ultraviolet spectrum were not implemented, so our analysis is restricted to the visible spectrum. However, previous studies have shown marginal reflectance of ultraviolet light on grasshoppers (Tsurui, Honma, & Nishida, 2010). All photographs were taken under field conditions between 11:00

and 14:00 hr. in daylight. A white diffuser umbrella was placed over each grasshopper in order to remove potential shadows. All photographs were taken 40–50 cm away from the grasshopper and include a grayscale from a colorchecker card (X-rite Colorchecker Passport Photo 2, Munsell Color Laboratories) in the same plane as the grasshoppers and their background. The grasshoppers were released at the same places where they were collected after the photographs were taken.

Following the suggestions outlined by (Stevens, Párraga, Cuthill, Partridge, & Troscianko, 2007; Troscianko & Stevens, 2015) to take objective measurements from digital photographs, we took the photographs as follows: the focal distance was constant at 55 mm, the aperture of the camera was set to f-stops: f/5.6, the light sensitivity value (ISO) was set to 400 in all photographs, and the shutter speed was adjusted in every shot to keep from overexposing the pictures. Images were stored as .CR2 (Canon raw image format) to avoid information loss.

## 2.2 | Image analyses

We processed and analyzed the images with the Multispectral Image Calibration and Analysis (MICA) toolbox (Troscianko & Stevens, 2015) for ImageJ software (Schneider, Rasband, & Eliceiri, 2012). The MICA toolbox uses linear images from raw photographs and



**FIGURE 2** Dorsal view of *Sphenarium* grasshoppers used in this work: (a) *S. planum* female: In the image the dorsal (GDS) and background (BS) surfaces that were measured are shown. Additional images from *S. planum* (b) females, (c) males, and *S. purpurascens* (d) females, (e) males) are shown

gray standard patches of the color checker as controls for different light conditions, and creates stacks of three images, known as multispectral images, corresponding to the different channels of the visible spectrum: short wave: Blue (B), mediumwave: Green (G), and longwave: Red (R) (Troscianko & Stevens, 2015). We evaluated the coloration and color patterns of each grasshopper and its background. The total dorsal surface area of the grasshoppers, excluding appendices, and a similar-sized surface area of the adjacent background were used to obtain measurements of the grasshoppers and their microhabitats (Figure 2a). Gray reflectance standards from the ColorChecker card were applied to standardize the pictures. Photographs were scaled down to 17 pixels per mm.

### 2.2.1 | Pattern analysis

We performed a granularity analysis based on the Fast Fourier band-pass filtering to evaluate the color patterns. Band-pass filters allow information at different spatial scales to be separated (for details see Chiao, Chubb, Buresch, Siemann, & Hanlon, 2009; Stoddard & Stevens, 2010). Granularity analysis measures the standard deviation of pixel reflectance at different pixel scales, also known as filter sizes; this measurement is referred to as energy. The graphic representation of energy across the size of the different filters generates an energy spectrum, which is useful for comparing energy patterns between surfaces (Chiao et al., 2009). This analysis resembles how animals process the visual information, decomposing the spatial information into different spatial frequencies (Godfrey, Lythgoe, & Rumball, 1987; Stevens, 2011). Granularity analysis has been used to distinguish matches in background patterns (Chiao et al., 2009; Tyrie, Hanlon, Siemann, & Uyerra, 2015) and to mark contrasts, which are typically found in disruptive color patterns (Robledo-Ospina, Escobar-Sarria, Troscianko, & Rao, 2017). Granularity analysis has been used to measure the pattern markings of several species of animals, including zebras and lions (Godfrey et al., 1987), cuttlefish (Barbosa et al., 2008; Chiao et al., 2009), fish (Tyrie et al., 2015), and spiders (Robledo-Ospina et al., 2017), as well as eggs (Stoddard & Stevens, 2010; Yang, Hu, Ma, Liang, & Møller, 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 pixels to 256 pixels, in increments of multiples of  $\sqrt{2}$ . We obtained three descriptive variables from the energy spectrum: the maximum energy peak of the spectrum ( $e_{\max}$ ), the filter size where  $e_{\max}$  is reached ( $\text{Filter}_{\max}$ ), and the proportion of the  $e_{\max}$  compared to the rest of the spectrum ( $e_{\text{prop}}$ ), which respectively indicate contrast of the dominant marking, marking size, and pattern diversity.

### 2.2.2 | Color background matching analysis

Color background matching was evaluated by measuring individual pixel reflectance and calculating the mean reflectance values of the

multispectral image for the three channels (RGB) for the grasshoppers and their backgrounds. Spectral images are in a 16-bit scale, given this image format, the reflectance values range from zero to 65,535.

### 2.2.3 | Disruptive coloration

We evaluated the edge disruption of grasshoppers using GabRat tool implemented in MICA toolbox. GabRat tool measured the ratio between false and coherent edges of the grasshoppers' surfaces. This metric is one of the best predictors of human detection times on disruptive targets and superior to other pattern metrics algorithms tested in humans (see Troscianko, Skelhorn, & Stevens, 2018). GabRat tool is based on a Gabor band-pass filter (see Price, Green, Troscianko, Tregenza, & Stevens, 2019; Troscianko et al., 2018). This tool estimates coherent and false edges from an object in an image. The analysis produces values ranging from zero to one. Values  $>0.4$  are considered highly disruptive, and values  $<0.2$  are considered low disruptive (Price et al., 2019).

For the GabRat analysis, we use the multispectral image used in the granularity analysis. We obtained the GabRat values from the photographs of grasshoppers' dorsal surface. For this analysis, the size of the Gabor filter ( $\sigma$ ) ideally should match the acuity of the possible viewers in order to be effective. In this study, we use a  $\sigma$  value = 5 because it has been informative in analysis where the objects were scaled close to 17 pixels per mm (Price et al., 2019; Troscianko et al., 2018).

We obtained the GabRat value for R, G, B channels of the multispectral images, subsequently, we obtained the mean GabRat ( $\bar{X}$  GabRat) of the three channels for every grasshoppers' photograph.

## 2.3 | Statistical analyses

### 2.3.1 | Dorsal surface pattern comparisons by species and sexes

We performed a multivariate analysis of variance (MANOVA) considering species, sexes, and the interaction species  $\times$  sexes to explore the  $e_{\max}$ ,  $\text{Filter}_{\max}$ , and  $e_{\text{prop}}$  parameters for the dorsal surfaces of females and males of both species. Also, since the MANOVA was significant (see below), additional univariate analyses of variance (ANOVA) and honest significant differences Tukey's tests were performed to detect the significant parameters of the analysis.

### 2.3.2 | Background pattern comparisons by species and sexes

A MANOVA considering species, sexes, and the interaction species  $\times$  sexes was performed to explore potential differences between

the  $e_{\max}$ ,  $\text{Filter}_{\max}$ , and  $e_{\text{prop}}$  parameters of the backgrounds where the grasshoppers were placed. Additional univariate ANOVAs and honest significant differences Tukey's tests were performed to detect the significant parameters of the analysis.

### 2.3.3 | Comparison between dorsal grasshopper surface and background for females and males of *S. planum* and *S. purpurascens*

For the females and males of each grasshopper species, we performed paired *t*-tests comparing the dorsal surfaces of the grasshoppers to the background where they were placed.

### 2.3.4 | Grasshopper color background matching

In order to test the color background matching for females and males of the two grasshopper species, we performed major axis linear regressions between the color channels' reflectance of males and females of each species and their respective backgrounds. Because a high correlation and slopes near 1 between grasshoppers and their background RGB values, would denote background color matching (O'Hanlon, Feeney, Dockery, & Gormally, 2017), we compared if the slopes differed from 1 for those traits where the major axis regression was significant. We performed the slope comparisons using a likelihood ratio test (details in Warton, Wright, Falster, & Westoby, 2006) in smart 3 R package (Warton, Duursma, Falster, & Taskinen, 2012).

**TABLE 1** ANOVAs of (a) the  $e_{\max}$ , (b)  $\text{Filter}_{\max}$ , and (c)  $e_{\text{prop}}$  of the dorsal surface of the *Sphenarium* grasshoppers, and their backgrounds (d-f)

Source	df	Sum of squares	Mean square	F	p
a. ANOVA of $e_{\max}$ of the dorsal surface of <i>Sphenarium</i> grasshoppers					
Species	1	3.07	3.07	134.19	<0001
Sex	1	0.373	0.373	16.326	<0001
Specie $\times$ Sex	1	0.025	0.025	1.103	.295
Error	145	3.318	0.022		
b. ANOVA of $\text{filter}_{\max}$ of the dorsal surface of <i>Sphenarium</i> grasshoppers					
Species	1	0.462	0.462	5.858	.017
Sex	1	3.965	3.965	50.202	<0001
Species $\times$ Sex	1	0.475	0.475	6.01	.015
Error	145	11.454	0.079		
c. ANOVA of $e_{\text{prop}}$ of the dorsal surface of <i>Sphenarium</i> grasshoppers					
Species	1	0.012	0.012	6.74	.01
Sex	1	0.065	0.065	37.167	<0001
Species $\times$ Sex	1	0.009	0.009	5.004	.026
Error	145	0.252	0.025		
d. ANOVA of $e_{\max}$ of the background surface					
Species	1	1.716	1.716	30.32	<0001
Sex	1	0.334	0.334	5.908	.016
Specie $\times$ Sex	1	0.037	0.037	0.656	.419
Error	145	8.205	0.056		
e. ANOVA of $\text{filter}_{\max}$ of the background surface					
Species	1	0.229	0.229	1.017	.316
Sex	1	0.0001	0.0001	0.0006	.982
Species $\times$ Sex	1	0.3	0.3	1.323	.252
Error	145	32.934	0.227		
f. ANOVA of $e_{\text{prop}}$ of the background surface					
Species	1	0.003	0.003	0.931	.336
Sex	1	0.005	0.005	1.493	.224
Species $\times$ Sex	1	0.0002	0.0002	0.064	.801
Error	145	0.459	0.003		

Abbreviations: *df*, degrees of freedom; *F*, *F*-values; *p*, values.



### 2.3.5 | Disruptive coloration

An ANOVA and honest significant differences Tukey's test were performed to explore potential differences between  $\bar{X}$  GabRat considering species, sexes, and the interaction species  $\times$  sexes. Statistics were performed with R (R Core Team, 2018) and JMP 9.0 (2008; SAS Institute Inc.).

## 3 | RESULTS

We obtained photographs of the dorsal areas and backgrounds of 35 females and 44 males of *S. planum* and 42 females and 43 males of *S. purpurascens*. All photographs were used in the color analysis. For the pattern analysis of *S. purpurascens*, 15 images were excluded because they were below the pixel scale requirements (Troscianko & Stevens, 2015).

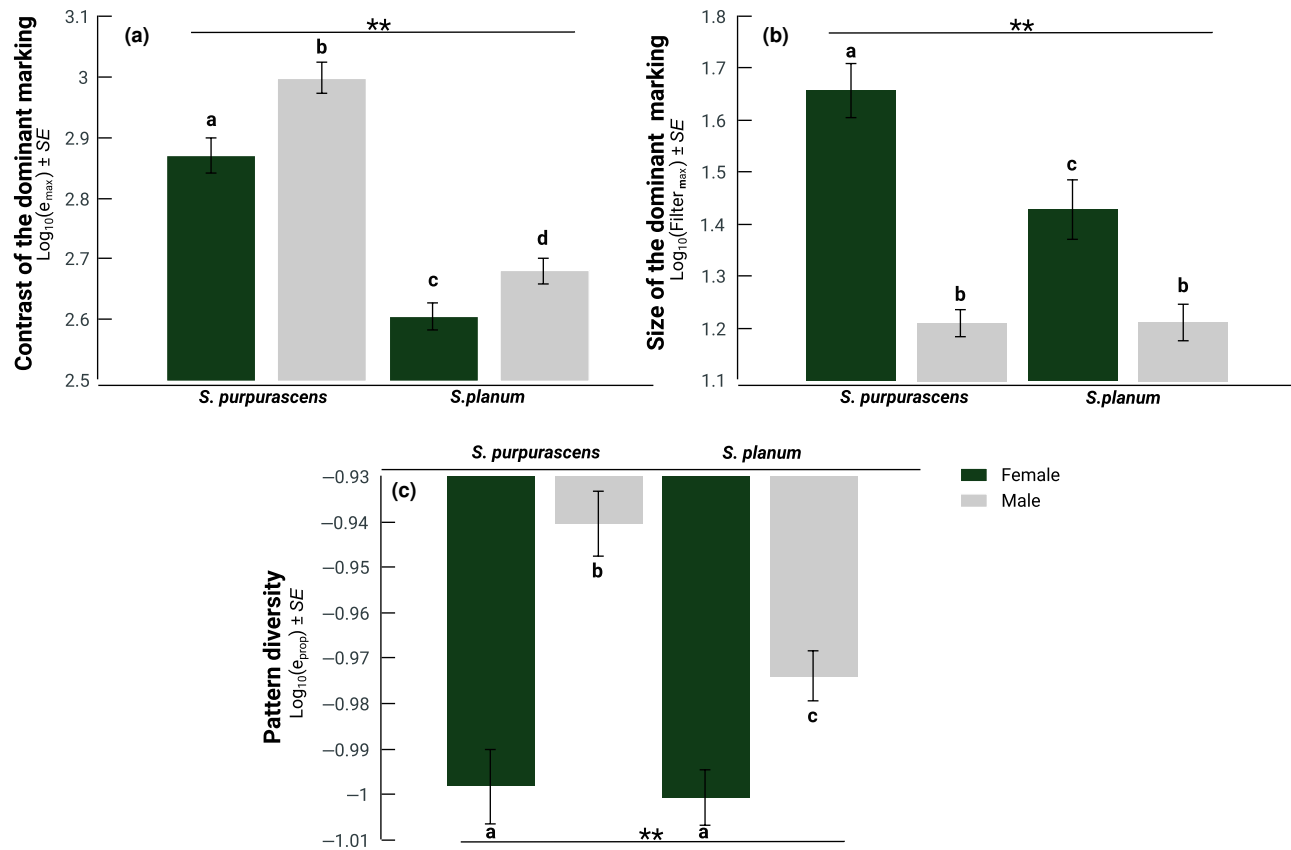
### 3.1 | Grasshopper dorsal surface pattern comparisons by species and sexes

The MANOVA indicates highly significant differences in the patterns' descriptive parameters (Wilks'  $\lambda = 0.29$   $F_{3,143} = 24.82$ ,

$p < .0001$ ), and the ANOVAs indicate that  $e_{\max}$  differs between species and sexes (Table 1a). Males of both species had a higher  $e_{\max}$  (Figure 3a), suggesting that their markings contrast more than those of females. As for the  $\text{Filter}_{\max}$ , we observed significant differences between species, sexes, and their interaction (Table 1b). *S. purpurascens*, and especially the females of the species, had the highest  $\text{Filter}_{\max}$  values (Figure 3b), which means that the markings of *S. purpurascens*, in particular those of its females, are larger than the markings in *S. planum*. Moreover,  $e_{\text{prop}}$  was also highly significant (Table 1c). There were differences between both species, between females and males, and the interaction between species and sex. The males of *S. purpurascens* had the highest  $e_{\text{prop}}$  values, which suggest that the dorsal marks of *S. purpurascens* are more heterogeneous than the marks of *S. planum* males and those of the females of both species (Figure 3c).

### 3.2 | Background pattern comparisons by species and sexes

The MANOVA indicates significant differences in the three analyzed parameters (Wilks'  $\lambda = 0.78$   $F_{3,143} = 4.03$ ,  $p < .0001$ ). The ANOVAs indicate that  $e_{\max}$  differs between the background associated with the species and sexes (Table 1d). The background associated with



**FIGURE 3** Means  $\pm$  standard error of the pattern parameters for the grasshoppers' dorsal surface: (a)  $e_{\max}$  (contrast of the dominant marks), (b)  $\text{Filter}_{\max}$  (size of the dominant marks), and (c)  $e_{\text{prop}}$  (pattern diversity). Bars with different letters denote differences between sexes, two stars denote differences between species according to HSD Tukey's test

*S. purpurascens* and the males of both species had a higher  $e_{\max}$  (Figure 4). We did not find significant differences in  $\text{Filter}_{\max}$  and  $e_{\text{prop}}$  (Table 1e,f). These results indicate that the background in Pedregal de San Ángel has higher contrast patterns than that of the Tehuacán Valley.

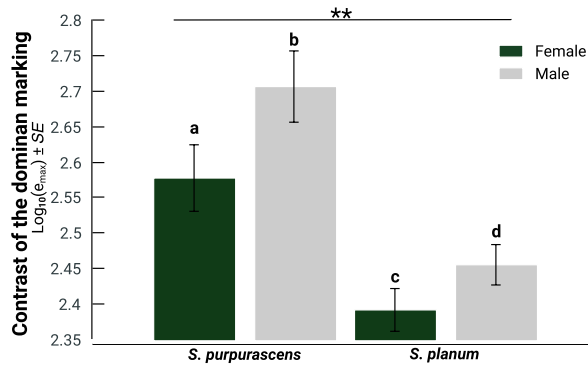
### 3.3 | Comparison between dorsal grasshopper surface and background for females and males of *S. planum* and *S. purpurascens*

For the females of *S. planum*, the differences between their dorsal area patterns and the background patterns where they were located were only significant for the  $e_{\max}$  values, whereas significant differences were found in  $e_{\max}$  and  $\text{Filter}_{\max}$  for the females of *S. purpurascens* (Table 2a). For the males of *S. planum*, the differences between their dorsal area and their background were significant for the  $e_{\max}$  and  $e_{\text{prop}}$

values, whereas significant differences were found in the three parameters  $e_{\max}$ ,  $\text{Filter}_{\max}$ , and  $e_{\text{prop}}$  for the males of *S. purpurascens* (Table 2b). Interestingly, both females and males in Pedregal de San Ángel showed patterns that contrasted the most with their environment, which is more visually heterogeneous than the Tehuacán Valley.

### 3.4 | Grasshopper color background matching

In females of *S. planum* and *S. purpurascens*, the type II regressions showed a strong association between the three reflectance channels (RGB) of the dorsal area and their background. Moreover, the slopes did not differ from 1 (Table 3), which means that the females' color background matching is high. On the other hand, in the males of both species, only the R channel showed a weak association and the slope was significantly different from 1 (Table 3, Figure 5), which suggests that color background matching is much lower in males than in females.



**FIGURE 4** Means  $\pm$  standard error of  $e_{\max}$  (contrast of the dominant marks) for the background surface. Bars with different letters denote differences between sexes according to HSD Tukey's test, two stars denote differences between species

#### 3.4.1 | Disruptive coloration

The  $\bar{X}$  GabRat values of the two grasshopper species are relatively low ( $<0.2$ ). Nevertheless, we found significant differences between species and sexes. *S. purpurascens* is more disruptive than *S. planum* (Table 4; Figure 6), and males are more disruptive than females. Nonetheless, the interaction between both variables (species  $\times$  sex) was not significant. Thus, the magnitude of the differences between males and males was similar between both species (Table 4; Figure 6).

## 4 | DISCUSSION

Our results show differences in color patterns, chromatic differences between females and males, and matching differences

**TABLE 2** Paired *t*-test comparisons of pattern parameters of the *Sphenarium* grasshoppers' dorsal surface and their background

Species—Sex	Pattern variables	Mean (SE) GDS	Mean (SE) BS	df	t	p
(a) <i>S. planum</i> —♀	$e_{\max}$	2.605 (0.022)	2.392 (0.029)	34	5.889	<0001
	$\text{Filter}_{\max}$	1.428 (0.057)	1.423 (0.106)	34	0.036	.971
	$e_{\text{prop}}$	-1.000 (0.006)	-1.01 (0.010)	34	1.176	.247
(b) <i>S. planum</i> —♂	$e_{\max}$	2.680 (0.021)	2.456 (0.028)	43	7.718	<0001
	$\text{Filter}_{\max}$	1.211 (0.036)	1.334 (0.071)	43	-1.680	.100
	$e_{\text{prop}}$	0.974 (0.005)	-1.005 (0.009)	43	3.061	.003
(c) <i>S. purpurascens</i> —♀	$e_{\max}$	2.867 (0.028)	2.553 (0.044)	41	6.948	<0001
	$\text{Filter}_{\max}$	1.655 (0.052)	1.4119 (0.063)	41	2.724	.009
	$e_{\text{prop}}$	-0.998 (0.008)	-1.008 (0.007)	41	0.955	.345
(d) <i>S. purpurascens</i> —♂	$e_{\max}$	2.998 (0.025)	2.7138 (0.049)	27	6.445	<0001
	$\text{Filter}_{\max}$	1.209 (0.025)	1.505 (0.065)	27	-4.155	.0002
	$e_{\text{prop}}$	-0.940 (0.007)	-0.994 (0.011)	27	3.939	.0005

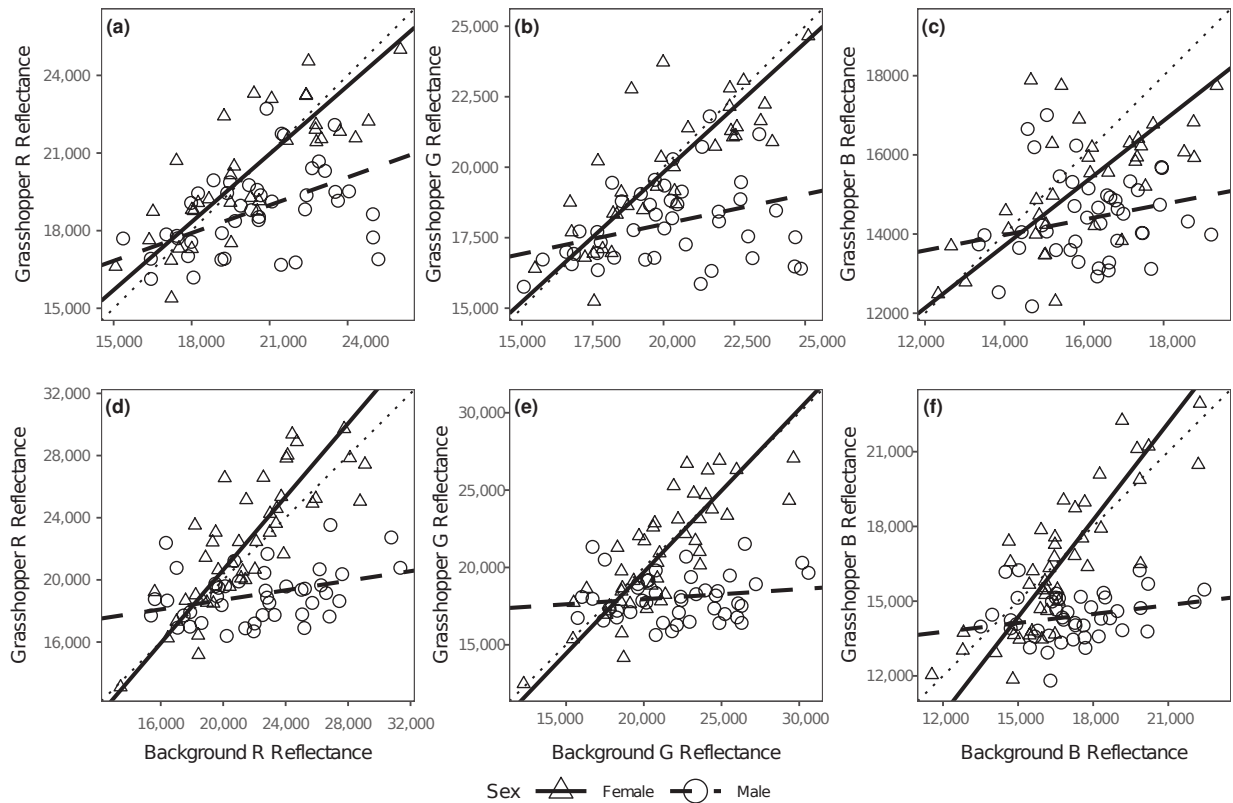
Note: Means and standard errors (SE) are shown.

Abbreviations: BS, background surface; df, degrees of freedom; GDS, Grasshoppers' dorsal surface; p = probability of error; t, *t*-test value.

**TABLE 3** Major axis regressions for the RGB values of the grasshoppers' dorsal area (Ra, Ga, Ba) as a function of their background (Rb, Gb, Bb), for females and males of *S. planum* and *S. purpurascens*

Reflectance regressed parameters	Sex	$\beta$	UCL	LCL	$r^2$	$r_s$	df	$p$
<i>S. planum</i>								
Ra to Rb	Male	0.35	0.66	0.10	0.15	-0.45	41	<01
	Female	0.87	1.14	0.66	0.65	-0.45	32	<b>.32</b>
Ga to Gb	Male	0.21	0.48	-0.03	0.06			
	Female	0.92	1.20	0.69	0.63	-0.11	32	<b>.53</b>
Ba to Bb	Male	0.19	1.31	-0.59	0.01			
	Female	0.78	1.23	0.47	0.36	-0.18	32	<b>.28</b>
<i>S. purpurascens</i>								
Ra to Rb	Male	0.15	0.30	0.01	0.09	-0.71	42	<0001
	Female	1.17	1.48	0.94	0.66	0.22	41	<b>.14</b>
Ga to Gb	Male	0.06	0.21	-0.08	0.01			
	Female	1.05	1.32	0.83	0.66	0.07	41	<b>.64</b>
Ba to Bb	Male	0.11	0.30	0.34	0.04			
	Female	1.29	1.62	1.04	0.68	0.01	41	<05

Note: Slopes of the regressions ( $\beta$ ), Upper (UCL; 97.5%) and Lower (LCL; 2.5%) Confidence Intervals, explained variance ( $r^2$ ) are shown. In addition,  $t$ -test values ( $r_s$ ), Degrees of freedom ( $df$ ), and  $p$  values ( $p$ ) of the analyses to test slopes different from 1 are shown for the regression that was significant. Slopes near 1 ( $\beta = 1$ ) between grasshoppers and their background RGB values denote background color matching, which is indicated by no significant differences are in bold.

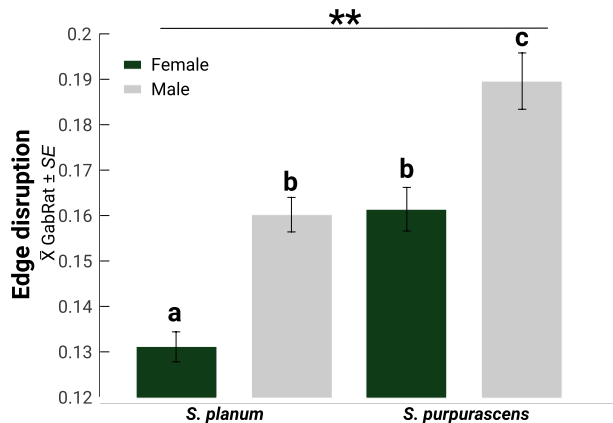


**FIGURE 5** Major axis linear regression for dorsal surface RGB and background RGB reflectances for *S. planum* (a, b, c) and *S. purpurascens* (d, e, f) grasshoppers. Triangles represent females and solid lines their slopes. Circles represent males and dashed lines and their slopes. Dotted lines:  $\beta = 1$ . For details see Table 3. Scale values are in units of 16-bit images from 0 to 65,535

**TABLE 4** ANOVA of GabRat of the dorsal surface of the *Sphenarium* grasshoppers species

Source	df	Sum of squares	Mean square	F	p
Species	1	0.02604	0.02604	32.376	<0001
Sex	1	0.03117	0.03117	38.753	<0001
Species × Sex	1	0.00001	0.00009	0.011	.916
Error	151	0.12146	0.0008		

Abbreviations: *df*, degrees of freedom; *F*, *F*-values; *p*, values.

**FIGURE 6** Means  $\pm$  standard error of  $\bar{X}$  GabRat (edge disruption) for the grasshoppers. Bars with different letters denote differences between sexes according to HSD Tukey's test. Two stars denote differences between species

between females and males and their microhabitat coloration in two *Sphenarium* grasshopper species. Moreover, we found differences in the disruptive properties between species and sexes, *S. purpurascens* is more disruptive than *S. planum*, and males across both species are more disruptive than females. The markings on males have a higher contrast, which also can indicate a disruptive function. On the other hand, females have a higher microhabitat color matching. As far as we know, this is the first study to show empirical data supporting the fact that sexual dimorphism in coloration could be associated with different cryptic strategies and microhabitat differentiation in arthropods.

The color pattern differences between *S. purpurascens* and *S. planum* can be attributed to local adaptation to different environmental conditions. *S. purpurascens* inhabits a complex environment with a wide diversity of plants, which probably leads to a wide variety of visual complexity patterns (more variety of shapes and details Dimitrova & Merilaita, 2010). This visual heterogeneity increases the possibility that both females and males were found in different background patterns. On the other hand, *S. planum* inhabits more homogeneous and less visually complex environments, which could explain why females and males were found in microhabitats with similar visual properties and had less marking patterns with less contrast.

The evolution of sexual dichromatism may be attributed to differences in the behavior of females and males associated with heterogeneous environments. *S. purpurascens* and *S. planum* seem

to follow two cryptic strategies: disruptive markings and matching coloration. Disruptive coloration could evolve in visually heterogeneous microhabitats because it breaks the outlines of the organisms independently of the variable background patterns, whereas background matching could be favored in chromatically homogeneous microhabitats (Orton & McBrayer, 2019; Robledo-Ospina et al., 2017). The marking elements associated with females and males could be cryptic if they reduce the risk of boundary detection by potential predators (Cuthill et al., 2005; Endler, 2006; Merilaita, 1998; Schaefer & Stobbe, 2006) and can be adaptive in organisms with a high mobility in heterogeneous environments (Stevens & Cuthill, 2006; Stevens, Cuthill, Windsor, & Walker, 2006). Nonetheless, this strategy is more evident in the males of both species. We could expect high mobility in males because they usually search for females actively, especially in protandrous species (Thornhill & Alcock, 1983). Interestingly, in Pedregal de San Angel, the males of *S. purpurascens* are protandrous (Cueva del Castillo & Núñez-Farfán, 1999), and they are also more mobile than females (Camacho Castillo, 1999).

Interestingly, males of both species have the highest contrast marking in the same spatial filters (Filter<sub>max</sub> 2.6) that predators may use to detect preys (Souza, Gomes and Silveira, 2011), which can reduce their risk to be detected by them. However, this hypothesis remains to be tested. Moreover, we cannot discard the idea that male coloration could be under female mate choice, even though males and females mate randomly with respect to their color patterns (Cueva del Castillo & Cano-Santana, 2001).

In both species, the females are less disruptive than males, but their background matching is higher than males. The color-matching with their background could lower their detectability, especially if they have reduced mobility or they are able to place themselves where the color match is high (Endler, 1978; Merilaita et al., 2017; Michalis, Scott-Samuel, Gibson, & Cuthill, 2017). In the Tehuacán Valley, the payoff for this strategy by females could be higher due to the environmental homogeneity. However, in a heterogeneous environment, it would depend on the individuals' ability to stay in a high matching microhabitat and/or reduce their mobility (Bond, 2007; Merilaita et al., 1999), as in fact occurs in the population of *S. purpurascens* in Pedregal de San Angel (Camacho Castillo, 1999). The sexual size dimorphism bias to females (Cueva del Castillo et al., 1999) and an increase in weight due to egg maturation can explain the lack of mobility of females. Moreover, environmental heterogeneity could explain the diversity of the females' colors in Pedregal de San Angel. However, it must be tested

whether females can evaluate and resettle themselves where their color match is high.

We must point out that our results are interpreted from a human visible spectrum perspective. Spectral sensitivity can be very 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 & Gomez, 2010). However, part of the human visible spectrum overlaps with the possible visible spectrum from other predators. Thus, mice use part of the visual human spectrum (green and red) to detect potential preys (Denman et al., 2018), and under certain conditions, birds and humans have shown similar performance in detection tasks (Dukas & Kamil, 2001; Michalis et al., 2017). Further studies on predation and escape behavior are needed to test the effectiveness of both coloration cryptic strategies that we suggest in this report.

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## CONFLICT OF INTEREST

None declared.

## AUTHOR CONTRIBUTIONS

Both authors contributed equally to the conception and study's design, fieldwork, data analyzes, and writing of the paper.

## DATA AVAILABILITY STATEMENT

Supplementary data associated with this study can be found at: <https://datadryad.org/stash/dataset/doi:10.5061/dryad.vhhmgqnpq>.

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## Capítulo 3

### **Macroevolutionary analysis of cryptic coloration in Neotropical grasshoppers of the Genus *Sphenarium* (Orthoptera: Pyrgomophidae)**

Manuscrito enviado a revisión a la revista *Evolution*





**A macroevolutionary analysis of cryptic coloration in sexually dichromatic grasshoppers of the genus *Sphenarium* (Orthoptera: Pyrgomophidae).**

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**Macroevolutionary analysis of cryptic colouration in sexually dichromatic grasshoppers of the Genus *Sphenarium* (Orthoptera: Pyrgomophidae). A comparative Analysis.**

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Running Title: *Sphenarium* cryptic colouration analysis

## **Abstract**

Background matching and disruptive colouration in animals are a primary defense against visual predators. Disruptive colouration might evolve in visually heterogeneous microhabitats, whereas background matching could be favoured in chromatically homogeneous microhabitats. This is one of the few phylogenetic comparative studies that have tested the relative impact of background matching and disruptive markings in the chromatic evolution of insect lineages. We explored the evolution of the colouration and the marking patterns in the sexual dichromatic and widely distributed Neotropical grasshoppers of the genus *Sphenarium*. They represent an excellent model to investigate the evolution of cryptic colouration on insects about their environment. We gathered grasshoppers' and microhabitat's colour, marking, and climatic information. The data was analyzed using phylogenetic general linear models. We found a correlation between the grasshoppers' colouration and their disruptive markings with the chromatic properties of their environment and precipitation levels suggest that colours and patterns could evolve due to predation pressures in these grasshoppers. 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 different grasshopper colouration associations between chromatic properties and precipitation levels, which suggests that sexes have diverged in their response to the environment, causing the sexual dichromatism in these grasshoppers.

Keywords: Crypsis, Background matching, Disruptive colouration, Sexual dichromatism, Grasshoppers, Comparative analysis, Image analysis

## **Introduction**

In many animal taxa colouration can be adaptative in several ways, including social signalling, 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 (Bond 2007; Théry and Gomez 2010). Two of the most spread strategies to reach cryptic colouration are (i) background matching (BM) and (ii) disruptive colouration (DC) (Merilaita et al. 1999; Stevens et al. 2006a; Stevens and Merilaita 2009). BM is the resemblance between the colours and patterns of an organism with its surroundings (Hughes et al. 2019). DC is the concealment 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). In contrast, DC could evolve 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 to stay in specific backgrounds, or inhabit heterogeneous colour habitats (Théry 2007; Théry and Gomez 2010;

Robledo-Ospina et al. 2017). Nonetheless, DC and BM are not mutually exclusive strategies, and could be equally successful (Schaefer and Stobbe 2006). DC's success could be maximised when some marks on the surface of the organism match the background, and other marks have high contrast with the rest of the body (Stevens and Merilaita 2009). In this scenario, DC organisms could maintain the association between their colour and that of the background. Because BM decreases 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). However, if males and females occupy different microhabitats because of their different sexual roles due to males being more than females vagile to search for potential mates, natural selection could favour a divergence between females and males, thereby favouring sex-specific cryptic colour patterns (Forsman and Appelqvist 1999; Medina et al. 2016; Ramírez-Delgado and Cueva del Castillo 2020; Cueva del 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, although it is still poorly documented (Forsman and Appelqvist 1999; Ramírez-Delgado and Cueva del Castillo 2020; Cueva del 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 relative 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 Cueva del 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. They are predated for a variety of visual-oriented predators like birds, mammals including humans, and reptiles. Moreover, some species of this genus exhibit a notorious sexual dichromatism that could be associated to different cryptic tactics in males and females (Ramírez-Delgado and Cueva del Castillo 2020; Cueva del Castillo et al. 2021). Despite the implication of DC and BM on the evolution of the chromatic patterns of the species, very few comparative phylogenetic studies have tested their relative impact on the chromatic evolution of lineages (Caro and Koneru 2021). Moreover, given that many cryptic species are sexual 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 and marking patterns of males and females of the grasshoppers of the genus *Sphenarium* and their environment colouration and marking patterns whilst controlling for phylogenetic effects. In addition, 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 where the grasshoppers were located. Since environmental colouration is strongly related to water availability, we expected green tonalities to be more abundant in environments closer to the tropics, due to the amount of humidity and the amount of primary productivity of the plants, whereas we expected brownish tonalities to exhibit stronger associations with dryer environments (Yom-Tov and Geffen 2006).

Due to local adaptation and BM, we further expected to find a positive relationship between environmental colouration and the coloration of males and females. Also, we hypothesised 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). Finally, we predicted a positive relationship between the green colouration of grasshoppers and their environment with precipitation, and a positive relationship between the diversity and contrast of the patterns of the grasshoppers with precipitation, due to the high contrast between lights and shadows associated to dense vegetation.

## **Methods**

### **Species of study**

The *Sphenarium* genus is constituted for 17 species that are distributed from central Mexico to northern Guatemala in a high altitudinal margin (0 to >2900 masl) and inhabit in a wide variety of environments along their distribution range (Sanabria-Urbán et al. 2015). These grasshoppers are flightless univoltine, generalist herbivorous. They hatch in May and June during the rainy season and die during the coldest months of the year (December to February)(Sanabria-Urbán et al. 2017). The species are sexually dichromatic (Ramírez-Delgado and Cueva del Castillo 2020; Cueva del Castillo et al. 2021) females are larger than males (Cueva del Castillo et al. 1999; Cueva del Castillo and Cano-Santana 2001), and both sexes are polygamous (Cueva del Castillo et al. 1999; Cueva del Castillo and Núñez-Farfán 2002). The males tend to search actively for females, whereas females are less mobile and can be founded close to the ground when they are about to lay their eggs (Ramírez-Delgado and Cueva del Castillo 2020). Since *Sphenarium* grasshoppers (Sanabria-Urbán et al. 2015)are sexually dichromatic and show a high interspecific colour variation, they represent an excellent model to investigate the evolution of cryptic colouration on insects.

### **Site of study**

We obtained photographs of male and female adults of the 17 *Sphenarium* species, as well as their backgrounds in several locations in central and southern Mexico (Figure 1), between October and September 2017, and 2018. The sampling criteria was similar in all locations (see below).

### **Image acquisition**

Between 2017 and 2018 we visited 17 localities across the distribution range of the species of the genus *Sphenarium*. The locations are scattered throughout central and southern Mexico. The places varied in elevation from 15 to 2,571 masl (See S1 Table). 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 m<sup>2</sup>; 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) (Stevens et al. 2007) and Troscianko and Stevens (2015) (Troscianko and Stevens 2015).

### **Image analysis**

To analyse 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 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 (Figure S1). We also performed a granularity analysis on both surfaces.

### **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 our images. 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:  $\text{Hue1} = R / G$ , and  $\text{Hue2} = (R + G) / B$ . 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).

### **Pattern analysis**

We perform 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 back-ground across 15 filters ranging from 2 to 256 pixels, in increments of multiples of  $\sqrt{2}$ . 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.

### **Climatic data acquisition**

Since we were interested in the influence of climatic factors on the environment colouration of adult *Sphenarium* grasshoppers, we obtained data on the precipitation of the wettest (PTW) and the driest (PDT) trimesters of the year for each sampling location, by recurring to the Worldclim climatic surface layers (Hijmans et al. 2005). These two climatic parameters correspond to the times of the year when adults of *Sphenarium* can be found naturally in their habitats (see (Sanabria-Urbán et al. 2015)).

### **Comparative analyses**

To correct for the phylogenetic non-independence of taxa, we used the phylogenetic relationships of the *Sphenarium* genus (Sanabria-Urbán et al. 2017), and the phylogenetic generalised 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  $\lambda$  in order to improve the fit of the data to the model and to 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): its 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).

### **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 *log*-transformed values of hue, saturation, and brightness of the grasshoppers were used as response variables, and the *log*-transformed values of hue, saturation, and brightness of the background, were used as respective explanatory variables. We further included the precipitation variables (*log*(PWT) and *log*(PDT)) as explanatory variables in all models.

### **Results**

We took 732 photographs of the 17 *Sphenarium* species and their backgrounds. Details of number of photographs taken per species and sex are provided in supplementary Table S2. The data obtained for colour and pattern analyses are also provided in supplementary material Table S2.

### **Association between grasshoppers' colouration and background colouration + climatic factors**

The PGLS models in which we tested the association between colouration parameters show a 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 3d, f), and a significant positive association between male brightness with the wettest trimester of the year (Table 1e; Figure 2e). 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.

## **Association between climatic factors with grasshoppers and background patterns**

In females, there is a significant, positive relationship between pattern diversity and microhabitat (Table 1k; Figure 2i), 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 2h). The  $\lambda$  values were high for both, female pattern diversity and males dominant marking contrast (Table 2k, l), which suggests high phylogenetic signal and slow evolution of traits. However, its important to notice that due to the low number of species used in this analysis the interpretation of lambda values should be cautious.

## **Discussion**

The differences in colour patterns between the species of *Sphenarium* can be attributed to adaptation to different environmental conditions. Our results suggest that the colouration of both males and females evolved to resemble the background colouration. However, they have diverged in their response to the environment. The brightness of males was positively associated with the wettest trimester of the year. In contrast, the brightness and saturation of females were positively associated with the driest trimester of the year.

Our results offer valuable insights into the evolution of *Sphenarium* grasshoppers' colouration. On the one hand, the marks (pattern diversity) of females showed a positive association with their backgrounds, even though their mark patterns were not associated with the climatic variables. On the other hand, the patterns of males were not associated with their background. Nonetheless, the relationship between male marks (overall pattern contrast) and the driest trimester of the year was significant. Interestingly, the evolution of the grasshoppers' chromatic patterns was much faster than the evolution of their marking patterns.

The differences in the rate of the evolutionary divergence of background resemblance and disruptive markings could be explained by the payoff differences between both strategies. If a species colonises a new, colourful and homogeneous environment, natural selection could favour fast adaptation (BM) to match the colours of the new environment. However, the versatility of the disruptive markings to face a diversity of background patterns may explain a lower divergence between species that face new environments, or environmental changes in their distribution range. Perhaps the chromatic patterns of the *Sphenarium* grasshoppers could represent a generalist (or compromised) strategy that may match several backgrounds to some extent, but none closely. These grasshoppers may be adopting a type of camouflage that is not perfectly matched to the background of a particular habitat, but instead offers a degree of resemblance to multiple backgrounds, or they may be using camouflage that works somewhat independently of BM (Hughes et al. 2019).

The divergence in the chromatic patterns in *Sphenarium* grasshoppers could be explained by the complex environments where the genus has diversified (Sanabria-Urbán et al. 2017) and the behavioural differences between females and males. The *Sphenarium* life cycles are synchronized to the rainy seasons (Sanabria-Urbán et al. 2015), which may explain why all the species showed strong associations between their chromatic patterns and their environments. One of the main reasons is that precipitation is strongly associated with the amount of primary productivity (Yom-Tov and Geffen 2006). In places with high precipitation, the production of green leaves and the posterior increase of litter can have a profound impact on the colours and shadows of the environment. In contrast, the brownish tonalities are common in places with low precipitation. However, the number of males and females varies with the amount of precipitation in different temporal scales. For instance, the males of some species are protandrous, and natural selection favours the synchronisation of male maturation with the beginning of the rainy season; this allows males to obtain a large number of matings (Nylin et al. 1993; Cueva del Castillo et al. 1999). At this moment of the grasshoppers' reproductive cycle, the

brightness of the males coincides with light-green colours. Interestingly, their marks can be adaptive in heterogeneous environments (Ramírez-Delgado and Cueva del Castillo 2020; Cueva del Castillo et al. 2021) and during the driest trimester of the year, when the contrast between lights and shadows can be deep than it is at the beginning of the reproductive season. Females, by contrast, could increase their fitness by maturing later than males (Cueva del Castillo and Núñez-Farfán 2002), their colours and the patterns of their marks could be associated with the vegetation plots and the background, where they can be found close to their oviposition sites at the end of the reproductive season (Cueva del Castillo et al. 1999). The males are often found on the top of plants, where they actively search for females (Ramírez-Delgado and Cueva del Castillo 2020; Cueva del Castillo et al. 2021). The females are less mobile than males and can be found near the ground, where they lay their eggs (Cueva del Castillo et al. 2021).

The pattern of the marks on the dorsal surface of *Sphenarium* grasshoppers provides BM, but it could also be related to DC (Robledo-Ospina et al. 2017). Highly contrasting markings could disrupt or distract the predator's perception from the outlines of the organism (Merilaita 1998; Hughes et al. 2019). However, the implications of marking patterns on processes such as sexual selection cannot be discarded (but see (Cueva del Castillo and Cano-Santana 2001)). Interestingly, the resemblance of marking patterns with the environment occurs on achromatic and chromatic grasshoppers' variables. Chromatic and achromatic visual cues can be related to the predator's different searching strategies; chromatic information is useful when searching in short distances, and achromatic information is useful when searching for preys on long distances (Schaefer and Stobbe 2006; Cazetta et al. 2009). In this work, we do not use potential predator visual systems, but these grasshoppers are usually predated by multiple animals, such as birds, mammals, reptiles, and other arthropods (Kevan 1977). All of them can use different searching strategies. However, humans can be considered predators of several species of the genus because they have collected and used these grasshoppers as a food resource since pre-

Columbian times (Sanabria-Urbán and Cueva del Castillo 2020), and in certain conditions, humans and birds perform similarly in detection tasks (Dukas and Kamil 2001; Michalis et al. 2017)

Other explanations for the colour variability of these grasshoppers could be associated with sexual selection. However, there is no evidence of sexual selection in other species of the genus (Cueva del Castillo and Cano-Santana 2001). Another possible explanation for the colour variation could be thermal regulations or ultraviolet radiation protection. The small males could lose heat faster than the bigger females (Punzalan et al. 2008). In general, a darker colouration in males could help them keep their corporal temperature high on cold days, stay mobile, and search for possible mates for more time (Bishop et al. 2016). These differences in thermoregulatory abilities could help to maintain sexual dichromatism. Also, more pigmented males could have better resilience to damage caused by ultraviolet radiation (Coelho et al. 2006; Cooper et al. 2016), which could be helpful if they spend more time under solar radiation. However, none of these hypotheses is mutually exclusive, with the evidence of the colouration on these grasshoppers causing differences in cryptic properties.

To summarise, there is a general lack of phylogenetic comparative studies on the evolution of cryptic colouration, and we believe the present study adds to the fundamental understanding of the evolution of colour with respect to selection pressures imposed by visual predators. We are currently conducting studies on predation and escape behaviour in *Sphenarium* grasshoppers to test the effectiveness of BM and disruptive colouration cryptic strategies in different chromatic environments. We acknowledge, nonetheless, that exploring other possible functions of grasshopper colouration, like thermoregulation or UV radiation protection, could provide us with more information on the possible factors involved in the evolution of the colouration of this group of neotropical grasshoppers.



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## Tables

Table 1. PGLS results of the *Sphenarium* grasshoppers for colouration and pattern parameters for both sexes. Bg: background; PDT: precipitation of the driest trimester; PWT precipitation of the wettest trimester, *D.f.*: degrees of freedom,  $\lambda$ : Pagel's Lambda. Data for the analysis was transformed by natural logarithms

Response variable	Explanatory variable	<i>D.f.</i>	t	F	Multiple R <sup>2</sup>	P	$\lambda$
a) ♀ Brightness	♀ Bg-Brightness		6.8707	13.6374		<0.0001	
	PDT	3,13	-3.1305	2.8311	0.8614	0.008	0
	PWT		0.9296	0.2496		0.369	
b) ♀ Saturation	♀ Bg-Saturation		7.2538	15.2006		<0.0001	
	PDT	3,13	3.1194	2.8111	0.8667	0.008	0
	PWT		-1.3203	0.5036		0.209	
c) ♀ Hue 1	♀ Bg-Hue1		6.6531	12.7873		<0.0001	
	PDT	3,13	-0.8487	0.2081	0.7697	0.411	0
	PWT		1.1106	0.3563		0.286	
d) ♀ Hue 2	♀ Bg-Hue2		1.8659	1.0058		0.084	
	PDT	3,13	1.4976	0.6479	0.3347	0.158	0
	PWT		-0.8525	0.2100		0.158	
e) ♂ Brightness	♂ Bg-Brightness		6.0130	10.4451		<0.0001	
	PDT	3,13	-1.4898	0.6412	0.8242	0.160	0.267
	PWT		2.4374	1.7163		0.030	
f) ♂ Saturation	♂ Bg-Saturation		6.5577	12.4232		<0.0001	
	PDT	3,13	1.1424	0.3770	0.8325	0.274	0.214
	PWT		-2.1176	1.2954		0.054	
g) ♂ Hue 1	♂ Bg-Hue1		3.6582	3.8660		0.006	
	PDT	3,13	-0.0818	0.0019	0.5137	0.936	0
	PWT		0.9360	0.2531		0.419	
h) ♂ Hue 2	♂ Bg-Hue2		1.8659	1.0058		0.084	
	PDT	3,13	1.4976	0.6479	0.3347	0.158	0
	PWT		-0.8525	0.2100		0.409	
i) ♀ Overall patterns' contrast	♀ Bg-overall patterns' contrast		1.4833	0.6356		0.161	
	PDT	3,13	-1.9302	1.0763	0.2702	0.076	0
	PWT		0.6102	0.1076		0.552	
j) ♀ dominant	♀ Bg-dominant		0.5522	0.0881		0.872	

marking size	marking size						
	PDT	3,13	0.1669	0.0080	0.1073	0.870	0
	PWT		-1.0360	0.3101		0.319	
k) ♀ Pattern diversity	♀ Bg-pattern diversity		2.9007	2.4307		0.012	
	PDT	3,13	-1.0221	0.3018	0.2630	0.325	0.603
	PWT		1.6296	0.7672		0.127	
l) ♂ Overall patterns' contrast	♂ Bg-overall patterns' contrast		0.8466	0.2071		0.412	
	PDT	3,13	-2.4439	1.7254	0.3156	0.029	0.588
	PWT		1.0517	0.3195		0.312	
m) ♂ dominant marking size	♂ Bg-dominant marking size		0.4398	0.0559		0.667	
	PDT	3,13	0.6179	0.1103	0.3347	0.547	0
	PWT		-1.3640	0.5375		0.196	
n) ♂ Pattern diversity	♂ Bg-dominant marking contrast		0.3289	0.0313		0.748	
	PDT	3,13	-1.1472	0.3802	0.3347	0.272	0
	PWT		0.5557	0.0892		0.588	

## Figures

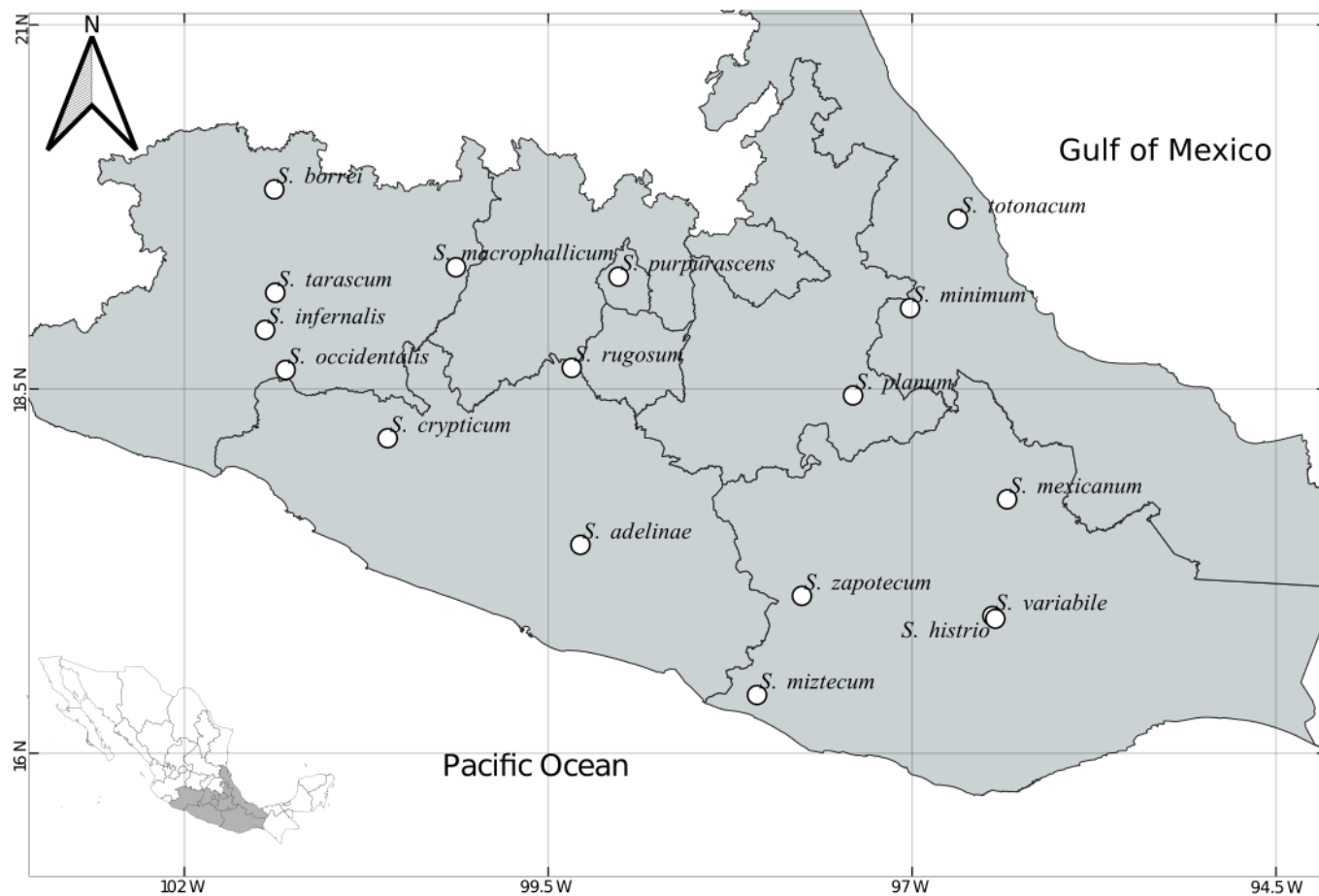


Figure 1. Map of the sites where the photographs of *Sphenarium* grasshoppers were taken: *S. purpurascens* (19.31N, 99.19W), *S. borrei* (19.92N, 101.74W), *S. occidentalis* (18.66N, 101.65W), *S. cypticum* (18.18N, 100.65W), *S. rugosum* (18.68N, 99.53W), *S. planum* (18.48N, 97.45W), *S. histrio* (16.93N, 96.42W), *S. variable* (16.93N, 96.42W), *S. miztecum* (16.37N, 98.47W), *S. adelinae* (17.43N, 99.47W), *S. macrophallicum* (19.38N, 100.39W), *S. tarascum* (19.20N, 101.73W), *S. infernalis* (18.94N, 101.81W), *S. zapotecum* (17.07N, 97.83W), *S. mexicanum* (17.75N, 96.31W), *S. minimum* (19.09N, 97.03W), and *S. totonacum* (19.72N, 96.68W).



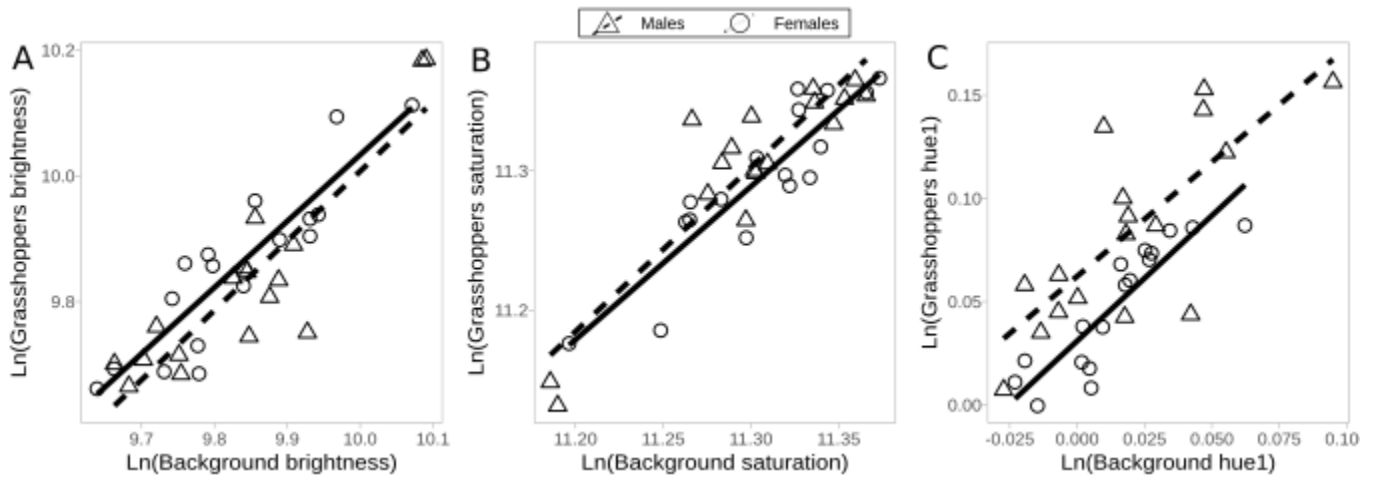


Figure 2. Association between chromatic variables of *Sphenarium* grasshoppers with the chromatic variables of their background, as tested using PGLS; (A) Brightness, (B) Saturation, (C) Hue1. Ordinary least squares regressions fitted are shown for illustrative purposes.

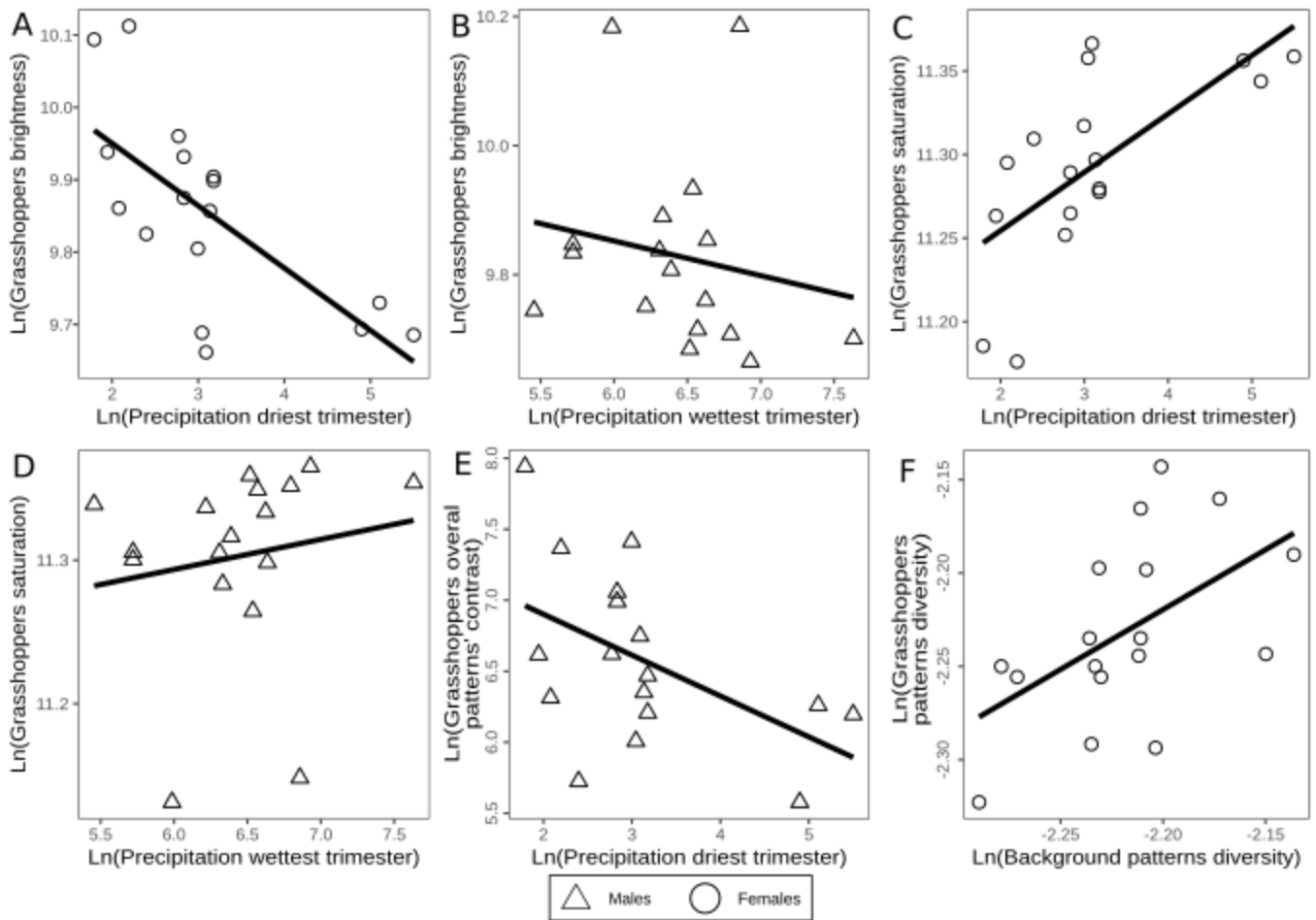


Figure 3. Relationship between chromatic variables of *Sphenarium* grasshoppers: (A) Female brightness and PDT, (B) male brightness and PWT, (C) female saturation and PDT, (D) male saturation and PWT, (E) male dominant marking size and PDT, and (F) Female pattern diversity and background pattern diversity. Note these graphs are shown only for illustrative purposes and were created with ordinary least squares linear models.

## Supplementary Tables

Table S1. Sites where *Sphenarium* species were collected. Locality names, geographic coordinates and their elevation.

Species	Locality name	Geographic Coordinates	Elevation (masl)
<i>S. purpurascens</i>	Pedregal de San Angel, Ciudad de México	19.31N, 99.19W	2324
<i>S. borrei</i>	Villa Jiménez, Michoacán	19.92N, 101.74W	1985
<i>S. occidentalis</i>	Churumuco, Michoacán	18.66N, 101.65W	190
<i>S. cypticum</i>	Near Ciudad Altamirano, Michoacan	18.18N, 100.65W	540
<i>S. rugosum</i>	Near Cacahuamilpa, Guerrero	18.68N, 99.53W	1523
<i>S. planum</i>	Tehuacán, Puebla	18.48N, 97.45W	1731
<i>S. histrio</i>	Near Mitla, Oaxaca	16.93N, 96.42W	1653
<i>S. variabile</i>	Near Mitla, Oaxaca	16.93N, 96.42W	1653
<i>S. miztecum</i>	Near Santiago Tepextla, Oaxaca	16.37N, 98.47W	77
<i>S. adelinae</i>	Near Chilpancingo, Guerrero	17.43N, 99.47W	1298
<i>S. macrophallicum</i>	Presa del Bosque, Michoacán	19.38N, 100.39W	1760
<i>S. tarascum</i>	Ario de Rosales, Michoacán	19.20N, 101.73W	1939
<i>S. infernalis</i>	Near La Huacana, Michoacán	18.94N, 101.81W	575
<i>S. zapotecum</i>	San Isidro Paz y Progreso, Oaxaca	17.07N, 97.83W	1563
<i>S. mexicanum</i>	Yetla, Oaxaca	17.75N, 96.31W	202
<i>S. minimum</i>	Near Cosomatepec de Bravo, Veracruz	19.09N, 97.03W	1479
<i>S. totonacum</i>	Tierra Blanca, Veracruz	19.72N, 96.68W	1090

Table S2. Chromatic and precipitation data for females and males of 17 grasshopper species of the genus *Sphenarium*. *n*: Sampled size, Grasshoppers Hue1 and Hue 2: G Hue1; G Hue 2, Background Brightness: B Brightness, Background Saturation: B Saturation, , Background Hue1 and Hue 2: B Hue1; B Hue2, Grasshoppers overall patterns contrast G OPS, Grasshoppers dominant marking size: G DMS, Grasshopper patterns diversity: G PD, Background overall patterns contrast B OPC, Background dominant marking size: B DMS, Background patterns diversity: B PD, PDT: Precipitation of the driest trimester (mm) PWT: Precipitation of the wettest trimester (mm).

Species	Sex	<i>n</i>	Grasshopper Brightness	Grasshopper Saturation	G Hue1	G Hue2	B Brightness	B Saturation	B Hue1	B Hue2	GabRat	G OPC	G DMS	G PD	B OPC	B DMS	B PD	PDT	PWT
<i>S. adelinae</i>	Female	18	18120.06	82222.30	1.04	0.78	17018.47	84098.96	1.01	0.81	0.17	419.41	36.92	0.10	234.67	22.20	0.11	20	690
<i>S. adelinae</i>	Male	22	20603.26	78002.31	1.14	0.75	19071.46	80588.84	1.01	0.79	0.21	1656.26	20.73	0.13	363.22	35.60	0.11	20	690
<i>S. borrei</i>	Female	20	19090.26	80570.76	1.07	0.76	17992.41	82418.64	1.02	0.81	0.15	502.54	40.94	0.11	403.98	45.15	0.11	23	595
<i>S. borrei</i>	Male	10	18167.83	82160.64	1.09	0.75	19453.90	79937.14	1.02	0.78	0.16	573.91	25.71	0.11	489.10	21.99	0.12	23	595
<i>S. crypticum</i>	Female	5	19162.99	80427.77	1.02	0.76	17317.53	83586.36	0.98	0.80	0.14	539.56	38.47	0.10	223.74	18.59	0.10	8	753
<i>S. crypticum</i>	Male	17	17330.72	83585.20	1.04	0.76	16656.08	84723.73	0.99	0.79	0.16	552.10	13.96	0.11	172.98	12.70	0.10	8	753
<i>S. histrio</i>	Female	27	19436.35	79959.28	1.06	0.77	17879.71	82626.50	1.02	0.80	0.17	542.42	43.66	0.10	317.32	32.10	0.11	17	305
<i>S. histrio</i>	Male	22	18907.22	80845.44	1.11	0.82	18861.69	80983.23	1.02	0.79	0.17	1161.15	13.39	0.13	365.14	32.58	0.11	17	305
<i>S. infernalis</i>	Female	8	20707.17	77904.95	1.01	0.77	20799.73	77866.38	1.01	0.75	0.14	582.94	56.14	0.12	524.26	55.19	0.11	7	562
<i>S. infernalis</i>	Male	12	19738.88	79488.58	1.04	0.75	20103.50	78860.48	1.04	0.77	0.20	747.60	31.60	0.11	432.85	49.56	0.12	7	562
<i>S. macrophallicum</i>	Female	17	20011.63	79028.64	1.09	0.76	20562.32	78076.89	1.04	0.77	0.12	495.93	44.77	0.11	437.96	31.94	0.12	24	550
<i>S. macrophallicum</i>	Male	15	18724.45	81234.70	1.09	0.72	18505.84	81591.29	1.03	0.77	0.19	644.76	24.54	0.10	405.44	21.14	0.11	24	550
<i>S. mexicanum</i>	Female	13	16082.06	85708.00	1.00	0.80	17657.35	83010.61	0.99	0.82	0.14	297.34	39.11	0.10	253.40	45.46	0.10	245	2065
<i>S. mexicanum</i>	Male	16	16335.67	85300.20	1.01	0.77	15722.06	86310.18	0.97	0.83	0.19	490.50	12.72	0.11	143.29	16.26	0.10	245	2065
<i>S. minimum</i>	Female	15	16209.63	85498.14	1.01	0.77	15715.47	86323.82	0.98	0.83	0.09	235.56	36.60	0.12	126.46	28.20	0.11	134	1022
<i>S. minimum</i>	Male	12	15762.27	86258.80	1.06	0.80	16036.23	85768.66	0.98	0.83	0.15	264.45	22.14	0.12	179.12	28.03	0.11	134	1022
<i>S. miztecum</i>	Female	24	24192.45	72072.83	1.08	0.68	21331.20	76769.07	1.03	0.78	0.17	728.95	29.77	0.11	487.96	25.71	0.11	6	951
<i>S. miztecum</i>	Male	28	26505.17	69450.37	1.15	0.70	24119.28	72086.76	1.05	0.75	0.20	2809.16	14.47	0.14	650.25	20.71	0.11	6	951
<i>S. occidentalis</i>	Female	17	24645.27	71399.37	1.07	0.67	23656.96	72862.32	1.03	0.73	0.16	709.06	34.26	0.11	327.79	74.06	0.11	9	398
<i>S. occidentalis</i>	Male	10	26454.81	68280.15	1.17	0.66	23984.26	72390.42	1.10	0.72	0.18	1581.81	13.66	0.14	670.16	17.09	0.13	9	398
<i>S. planum</i>	Female	43	18490.40	81589.16	1.02	0.76	18768.93	81085.47	1.00	0.80	0.13	263.54	43.45	0.10	431.07	41.30	0.11	11	234
<i>S. planum</i>	Male	34	17064.07	84025.50	1.04	0.78	18910.83	80844.94	1.02	0.80	0.16	306.49	61.31	0.11	449.75	18.80	0.11	11	234
<i>S. purpurascens</i>	Female	43	19896.90	79189.40	1.08	0.77	19726.22	79459.15	1.03	0.79	0.16	433.46	31.56	0.11	780.92	37.59	0.11	24	501
<i>S. purpurascens</i>	Male	44	17172.25	83843.16	1.05	0.78	20488.75	78152.29	1.00	0.79	0.19	496.64	28.00	0.11	966.32	14.77	0.12	24	501
<i>S. rugosum</i>	Female	26	16132.21	85629.48	1.04	0.79	16833.31	84425.40	1.00	0.79	0.14	376.69	43.16	0.11	186.74	20.26	0.10	21	676
<i>S. rugosum</i>	Male	35	16073.29	85730.63	1.06	0.81	17225.75	83739.06	0.99	0.82	0.18	376.69	43.16	0.11	213.88	23.16	0.10	21	676
<i>S. tarascum</i>	Female	18	21166.79	77024.98	1.06	0.76	19067.58	80604.10	1.02	0.79	0.13	593.72	50.83	0.11	301.94	36.77	0.11	16	762
<i>S. tarascum</i>	Male	33	19028.84	80687.47	1.10	0.75	18809.87	81035.64	1.02	0.78	0.20	751.02	24.46	0.12	354.27	36.12	0.11	16	762
<i>S. totonacum</i>	Female	8	16813.65	84444.38	1.02	0.79	17627.16	83056.94	1.00	0.78	0.16	519.50	37.66	0.11	323.19	61.56	0.12	165	713
<i>S. totonacum</i>	Male	9	16564.54	84877.03	1.05	0.80	17177.45	83808.83	0.99	0.82	0.19	523.30	30.65	0.11	227.19	52.01	0.11	165	713
<i>S. variabile</i>	Female	38	20573.09	78026.30	1.09	0.77	20561.92	78054.94	1.06	0.79	0.16	610.56	46.30	0.11	349.01	25.56	0.11	17	305
<i>S. variabile</i>	Male	22	18660.26	81268.61	1.13	0.82	19697.72	79499.34	1.06	0.80	0.16	1086.42	14.25	0.13	422.10	27.67	0.11	17	305
<i>S. zapotecum</i>	Female	18	15702.49	86373.98	1.09	0.79	15349.90	86971.74	1.03	0.80	0.12	317.52	28.17	0.11	177.96	32.36	0.11	22	893
<i>S. zapotecum</i>	Male	33	16443.98	85109.07	1.17	0.79	16359.43	85250.42	1.05	0.78	0.21	852.79	12.69	0.14	253.45	33.35	0.11	22	893

## Discusión y conclusiones generales

La coincidencia de fondo y la coloración disruptiva pueden ser adaptativas en los chapulines del género *Sphenarium*, ya que reducen las probabilidades de depredación. Sin embargo, la eficiencia de ambas estrategias antidepredación dependen del ambiente en el que se encuentren los chapulines. En ambientes con poca variación cromática la coincidencia de fondo puede reducir substancialmente la posibilidad de depredación (Bond, 2007; Akkaynak *et al.*, 2013). No obstante, aún en ambientes con mayor diversidad cromática la coloración disruptiva puede ser aún más eficiente al reducir el riesgo de detección. Curiosamente, en varias especies del género, las hembras tienden a mostrar una mayor coincidencia de fondo que los machos, mientras que éstos últimos muestran patrones disruptivos (Capítulo 2). Debido a que los machos pueden tener una mayor movilidad que las hembras, se puede esperar que estos se encuentren en entornos con una mayor variedad cromática y en los que la efectividad de la coincidencia de fondo puede ser menor. Por otra parte, las hembras al moverse menos que los machos, podrían tener una mayor probabilidad de ubicarse en entornos donde asemejaran el fondo. La divergencia de nichos de hembras y machos reforzada por las presiones de depredación ejercidas por depredadores orientados visualmente podría explicar, al menos parcialmente, el dimorfismo cromático en varias de las especies del grupo.

En varias especies ha evolucionado la cripsis por coincidencia de fondo o por coloraciones disruptivas de acuerdo al ambiente en el que se hallan (ejemplos en la introducción general). Sin embargo, se han documentado pocos casos en los que estas estrategias hayan favorecido la evolución del dicromatismo sexual. Así, en algunas lagartijas del género *Anolis* se ha registrado dicromatismo sexual probablemente asociado a presiones de selección impuestas por depredadores sobre machos y hembras, que generalmente se posan sobre diferentes sustratos (Medina, Losos and Mahler, 2016), mientras que en las mantis de las orquídeas, las hembras usan el enmascaramiento para ser detectadas

como flores y los machos se enmascaran como hojas (Svenson *et al.*, 2016). En este trabajo se propone al género *Sphenarium* como el primer caso documentado de artrópodos, hasta donde sé, con dicromatismo sexual mediado por distintas estrategias crípticas.

Después de controlar el efecto filogenético, en el género *Sphenarium* existe una asociación positiva entre características cromáticas y acromáticas de estos chapulines con su entorno. Probablemente estos patrones están relacionados con la respuesta a las presiones selectivas impuestas por diversos tipos de depredadores. Aparentemente la respuesta a las presiones locales de depredación pudo ser evolutivamente rápida, ya que la evolución de los patrones cromáticos y las relaciones filogenética de las especies es muy baja. Esta poca o nula inercia filogenética en rasgos de coloración también ha sido descrita en otros artrópodos, como en mantis (Svenson *et al.*, 2016) y odonatos (Cooper, Brown and Getty, 2016), y sugiere que al menos en artrópodos la evolución de la coloración podría estar sujeta a pocas restricciones filogenéticas, favoreciendo una alta diversificación y altos niveles de adaptación local.

No hay una relación entre la evolución de los colores ligada a factores ambientales, sin embargo, la diversificación de los patrones y marcas de las especies de *Sphenarium* estuvo ligada a la cantidad de precipitación asociadas a sus áreas de distribución. Así, en lugares con mayor precipitación quizá haya una mayor variedad y abundancia de vegetación, lo que generaría ambientes cromáticamente heterogéneos, con una mayor variedad de texturas, colores, sombras y formas que podrían favorecer a machos con una mayor contraste en su coloración corporal, ya que estas marcas favorecerían la coloración disruptiva. Sin embargo esta hipótesis debe ser probada por trabajos posteriores.

En trabajos como éste, la diferencia entre la sensibilidad del color de las cámaras o la visión de los humanos, puede diferir de la sensibilidad al color de los depredadores naturales (Stevens *et al.*, 2007); no obstante los chapulines *Sphenarium* son depredados por una gran variedad de organismos con una amplia variedad de sistemas visuales desconocidos, por lo que usar el sistema tricromático de

cámaras comerciales y humanos abarca la mayoría del espectro de luz que usan sus depredadores para identificarlos en su medio ambiente. Además, cabe aclarar que en otras especies de chapulines se hay muy poca reflectancia de luz ultravioleta (Tsurui, Honma and Nishida, 2010; Edelaar *et al.*, 2019).

Este trabajo es un acercamiento inicial para entender la evolución de los patrones de coloración de *Sphenarium*; un género de chapulines con una gran variación de coloración entre especies, poblaciones y sexos. Para entender a mayor profundidad la evolución de la coloración de estos organismos, trabajos futuros deberán adentrarse en el estudio de la variación de la coloración de estos organismos respecto a los cambios de coloración y patrones de marcas que ocurren en los entornos donde se encuentran a lo largo de su ciclo de vida, explorar los sistemas visuales de sus principales depredadores para entender como son percibidos, analizar a detalle las características conductuales de machos y hembras y como esto puede afectar la forma en que son percibidos por posibles depredadores, analizar la variación de la coloración durante la ontogenia de estos organismos y como estos cambios de coloración afectan la supervivencia de estos organismos, explorar otras posibles funciones de la coloración como la termorregulación y la protección UV, además de otros fenómenos que podrían moldear la composición de los colores encontrados en las poblaciones, como la selección dependiente de las frecuencias impuestas por la generación de imágenes de búsqueda en los depredadores.

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