Ethological study in two species of leaf-footed bugs (*Leptoglossus concolor and Leptoglossus macrophyllus)* (Hemiptera: Coreidae), and description of the behavioral repertoire in response to different looming stimuli.

Sofía Sánchez-Castro[1] (sofia_sanchez@javeriana.edu.co)[1]

[1]Undergraduate student in Biology, Faculty of Sciences, Department of Biology, Pontificia Universidad Javeriana, Bogotá D.C., Colombia.

Abstract

Antipredator strategies, such as aposematic and cryptic colorations, hidden colorations, and the production and sequestration of toxins are closely linked to behavioral repertoires in response to risk situations. These strategies include behaviors such as appearing larger, smaller, or moving slowly, among others. Species of the genus *Leptoglossus* exhibit cryptic colorations and a distinctive morphology in their hind legs, known as tibial expansions. It has been observed that these true bugs perform a lifting movement with one of these hind legs, whose function is yet to be determined. If this movement is a common response to stimuli indicating a possible predation risk, it could be considered an antipredator behavior. In this study, a total of 90 individuals of two species, *Leptoglossus concolor* (Walker, 1871) and *Leptoglossus macrophyllus* (Stål, 1870), were observed in three different locations in Colombia (Mariquita, Villeta, and Machetá). Various unpleasant stimuli were applied to these individuals to record their behavioral repertoires. Contrary to expectations, flying was the most frequent response to unpleasant stimuli of potential predation. Additionally, hidden coloration signals were proposed as a possible secondary antipredator strategy.

Key words: Behavior, Ethogram, leaf footed bug, Behavioral repertoire**.**

Resumen

Las estrategias antidepredadoras, como las coloraciones aposemáticas y crípticas, las coloraciones ocultas y la producción y secuestro de toxinas, están estrechamente ligadas a repertorios de comportamiento en respuesta a situaciones de riesgo. Estas estrategias incluyen comportamientos como parecer más grande, más pequeño o moverse lentamente, entre otros. Las especies del género *Leptoglossus* presentan coloraciones crípticas y una morfología distintiva en sus patas traseras, conocida como expansiones tibiales. Se ha observado que estos insectos realizan un movimiento de elevación con una de estas patas traseras, cuya función está aún por determinar. Si este movimiento es una respuesta común a estímulos que indican un posible riesgo de depredación, podría considerarse un comportamiento antidepredador. En este estudio se observaron un total de 90 individuos de dos especies, *Leptoglossus concolor* (Walker, 1871) y *Leptoglossus macrophyllus* (Stål, 1870), en tres localidades diferentes en Colombia (Mariquita, Villeta y Machetá). Se aplicaron diversos estímulos desagradables a estos individuos para registrar sus repertorios conductuales. Contrariamente a lo esperado, volar fue la respuesta más frecuente a estímulos desagradables de potencial depredación. Además, se propusieron señales de coloración oculta como posible estrategia secundaria antidepredadora.

Palabras clave: Comportamiento, Etograma, Chinche patas de hoja, Repertorio de comportamiento.

Introduction

Through evolution, insects have developed a wide variety of defense strategies. Common anti-predatory traits include cryptic coloration patterns to blend in and hide from predators, and sequestration and production of toxins for defense, often accompanied by aposematic colorations that serve as warning signals (Sugiura, 2020). These strategies may be accompanied by altered behaviors such as appearing larger, moving more slowly, or moving stealthily (Evans & Schmidt, 1990). An example of defensive behavior can be observed in the mantis species *Blepharopsis mendica* (Fabricius, 1775) (Insecta: Mantodea), which spreads its wings widely and raises its front legs when it feels threatened (Mirzaee et al., 2024).

Many species exhibit a diverse array of ornaments and colorations in their morphology, often intricately linked to their behavioral repertoire (Longbottom et al., 2022). Over evolutionary time, morphology and behavior have exhibited a correlated relationship, with various forms and colors sometimes serving distinct functions in interactions between individuals (Carranza, 2000). Sexual selection is proposed as a driving force behind the evolution of these conspicuous traits, though they may also serve as signals in social interactions among conspecifics, not necessarily limited to mating purposes. Indeed, another plausible explanation for these ornaments is their role as a predator deterrent (Longbottom et al., 2022). Behavior in insects has been studied in different contexts such as competition, commensalism, parasitism, parental care, altruism, and sexual selection (Choe & Crespi, 1997).

Behavioral studies within the family Coreidae (Insecta: Hemiptera) have predominantly centered on sexual selection (Eberhard, 1998; Miller, 2007; Emberts & Miller, 2016). Previous studies have focused on the dimorphism observed in the femurs of certain species like *Acanthocephala femorata* (Fabricius, 1775) and *Leptoglossus australis* (Fabricius, 1775) where it is explored the role of these structures in male-male combat and territorial defense (Mitchell, 1980; Miyatake, 1997). However, defensive strategies against predators within Heteroptera, the group to which Coreidea belongs, have primarily been investigated through the lens of chemical ecology because they possess a glandular system that secretes defensive substances against predators and serve as alarm cues for conspecifics (Aldrich, 1988; Gonzaga-Segura et al., 2013).

Species of the genus *Leptoglossus* (Coreidae), are known for being an agriculturally important group (Schaefer & Mitchell, 1983; Schaefer & Panizzi, 2000; Forthman et al., 2020), and are commonly referred to as guava bugs. Individuals are morphologically distinguished by flat expansions on their hind tibiae and cryptic coloration (Allen, 1969; Brailovsky, 2014). It has been observed that species of *Leptoglossus* sometimes exhibit a behavior of movement of the hind legs (pers. observations) like that observed in the species *Anisoscelis alipes* (Guérin-Méneville, 1831) (Coreidae). Longbottom et al. (2022) found no evidence of sexual dimorphism in hind leg morphology, and they propose that this movement might serve as an anti-predatory behavior. However, the behavioral repertoire of *Leptoglossus* species and the relationship between their behavioral responses and morphology remain insufficiently documented.

This study aims to delineate the behavioral repertoire of two *Leptoglossus* species: *L. concolor* and *L. macrophyllus* and assess the contexts in which the hind-leg raising response occurs, to elucidate the function of this movement. To address this goal, the following hypothesis was proposed: The hind leg raising movement is a distraction strategy for predators to attack a non-vital part of the body, thus facilitating escape via autotomy. If the hind leg raising movement serves as an anti-predatory response, then, it would be the most frequent response to the different looming stimuli.

Methods

Insect collection and mesh box observations

Initial observations were conducted in Mariquita, Tolima, from December 5-7, 2023, followed by observations in Villeta, Cundinamarca, from December 19-21, 2023. The localities were farms with scattered trees and grassland areas. However, the altitudes of both localities were different, with Mariquita at 495 masl and Villeta at 850 masl.

In Mariquita and Villeta, six and three adult specimens of *Leptoglossus concolor*, respectively, were collected and placed in mesh boxes measuring 50 x 50 x 50cm. These boxes were prepared with plant material sourced from the guava tree (*Psidium guajava*) where they were found, including leaves and branches. Each specimen was provided with half a guava and a water-filled lid. A 24-hour acclimatization period was observed post-collection to minimize disturbance during subsequent observations.

Four looming stimuli to be used were defined in this experiment. The term “looming stimuli” is used to denote stimuli perceived as unwanted or bothersome by individuals. It is not specifically designated as a predation risk stimulus due to the lack of clarity in determining how an animal perceives a situation as risky, since, for this purpose, additional variables such as metabolic rates would need to be measured, among others (Adamo & McKee 2017).

The four looming stimuli were applied successively to each individual: (1) approach with an outstretched hand, (2) touch with fingers, (3) touch with tweezers, and (4) hold the hind leg with tweezers. Responses to these stimuli were recorded: (1) stay still, (2) walk away, (3) fly. The stimuli were applied only once per individual in each location.

Following the experiment, the specimens were preserved in ETOH 75% for subsequent identification and accessioned into the museum of the Pontificia Universidad Javeriana (MPUJ) under current collection regulations.

Observations in natural conditions

Additional observations were conducted under natural conditions because in what unknown if confinement within mesh boxes could have altered the behavioral repertoire.

This second set of observations was made in Machetá, Cundinamarca, at “Rocas del Paraíso”. This environment was different from that of Mariquita and Villeta, with an altitude of 2094 masl, also it was not a farm but a section of not very dense forest that served as a passage for cattle. with an altitude of 2094 masl. The following ten looming stimuli were defined: (1) approach with an outstretched hand, (2) touch with a brush, (3) touch with a finger, (4) hold with fingers, (5) hold hind leg with tweezers covered with foam (so as not to harm the individual), (6) hold hind leg with fingers, (7) capture with tweezers gripping the pronotum, (8) capture with hands, (9) approach with tweezers, and (10) segregation of the aggregations.

Subsequently, potential responses were established based on observations conducted using the mesh box methodology. These responses included: (1) staying still, (2) extending both front and hind legs while flattening the body against the surface (Fig. 1), (3) walking away, (4) flying away, and (5) raising the hind leg (Fig. 2). Additional observations were documented, such as: Being either in aggregation or alone, feeding, or expelling odor.

During natural environment observations, a single stimulus was applied per observation per specimen, with each stimulus repeated six times across different individuals. For stimuli involving approaches with an outstretched hand, holding with fingers, capturing with tweezers gripping the pronotum, capturing with hands, and approaching with tweezers, each stimulus was applied for 10 seconds. The touching stimuli, using a brush or fingers, were administered only once per individual. For stimuli involving holding the hind leg with foam-covered tweezers and holding the hind leg with fingers, the duration of the holding was maintained for 8-10 seconds, or until the individual autotomized its leg. Lastly, to induce the disintegration of the aggregations, individuals were forcibly separated, after which six individuals that flew away from the aggregations were selected. These individuals were observed for 10 minutes each without applying other stimuli.

Observations took place on 90 individuals, and all of the data was recorded and tabulated, that was: first individual was subjected to the extended hand approach stimulus for 10 seconds, and the response was observed and recorded in the table of appendix A. Following this, a second individual underwent the same stimulus, and so forth until 6 applications of the same stimulus were completed on different individuals, with each response recorded (for supplementary information see appendix A).

Some individuals were collected and preserved in ETOH 75% for later identification. They were entered into the museum of the Pontificia Universidad Javeriana (MPUJ) with the codes: MPUJ_ENT0095301, MPUJ_ENT0095288, MPUJ_ENT0095300, MPUJ_ENT0095287, MPUJ_ENT0095299, MPUJ_ENT0095286, MPUJ_ENT0095298, MPUJ_ENT0095285, MPUJ_ENT0095284, MPUJ_ENT0095297, MPUJ_ENT0095296, MPUJ_ENT0095283, MPUJ_ENT0095295, MPUJ_ENT0095282, MPUJ_ENT0095294, MPUJ_ENT0095281, MPUJ_ENT0095280, MPUJ_ENT0095293, MPUJ_ENT0095291.

Figure 1. Dorsal and lateral view of the extension of front and hind legs with the body flattened on the surface (*Leptoglossus macrophyllus*).

Figure 2. Hind leg raising (different individuals of *Leptoglossus macrophyllus)*.

Statistical analyses

To support observations, statistical analyses were conducted using chi-square goodness of fit test with its respective null and alternative hypotheses: *H0*: Observed differences in responses may be due to chance, and *Ha*: There are significant differences between the responses. The test was conducted assuming a uniform (and equal) distribution in responses, with a significance level (α) of 0.05 and degrees of freedom (df) depending on the number of responses analyzed (Balakrishnan et al., 2013). When significant differences were found, a *post hoc* test of standardized residuals was conducted using the Past software 4.03 version (Hammer, 2001). This test identified the differences between the observed and expected values of the response frequencies.

Results:

Following initial observations conducted in the localities of Mariquita, Tolima, and Villeta, Cundinamarca, a total of 6 and 3 individuals were respectively observed and collected. The mesh box methodology was consistently employed in both locations, with recorded data documented in tables (for supplementary information see appendix A).

Observations recorded in Mariquita (fig. 3) reveal that responses differ across stimuli, varying according to the individual and often recurring regardless of the applied stimulus. The most prevalent response to the approach with an outstretched hand stimulus was to remain still. Conversely, the most common response to finger touch stimulus was walking away. Responses to the tweezers touch stimulus exhibited variability, with no single response prevailing over others. Lastly, the most frequent response to the stimulus of holding the hind leg with tweezers was an attempt to fly.

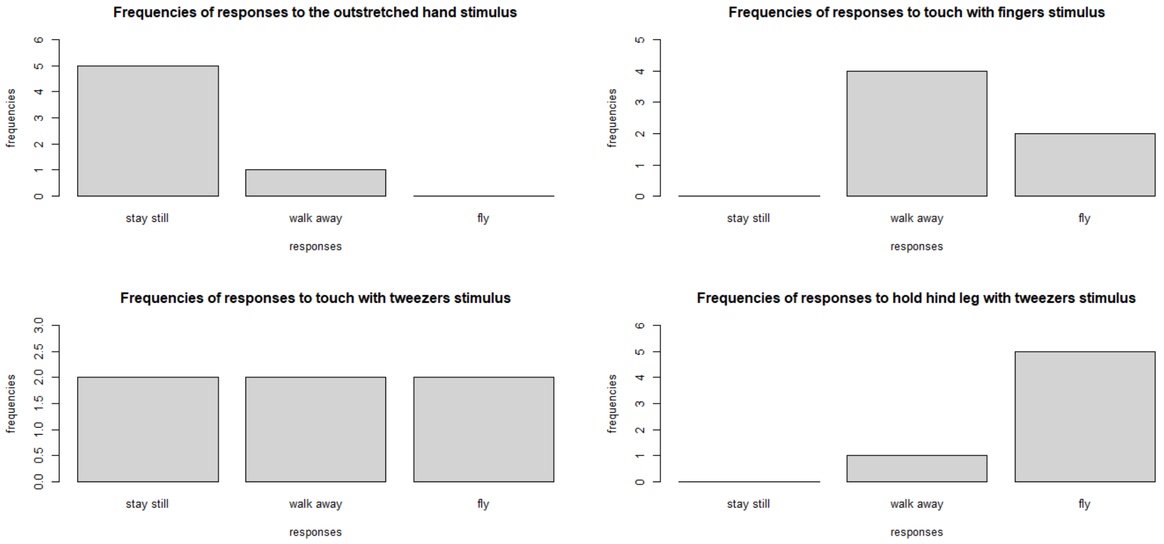


Figure 3. Frequencies of the responses to different stimuli (Mariquita, Tolima).

The stimuli “approach with outstretched hand” and “hold the hind leg with tweezers” presented significant differences in the responses (X^2, 2 df, α=0.05). In contrast, for the stimuli “touch with fingers” and “touch with tweezers”, presented non-significant differences in the responses (X^2, 2 df, α=0.05) (For supplementary information, see Appendix B).

The results of the *post hoc* test of standardized residuals were as follows:

Table 1: Standardized residuals for Mariquita.

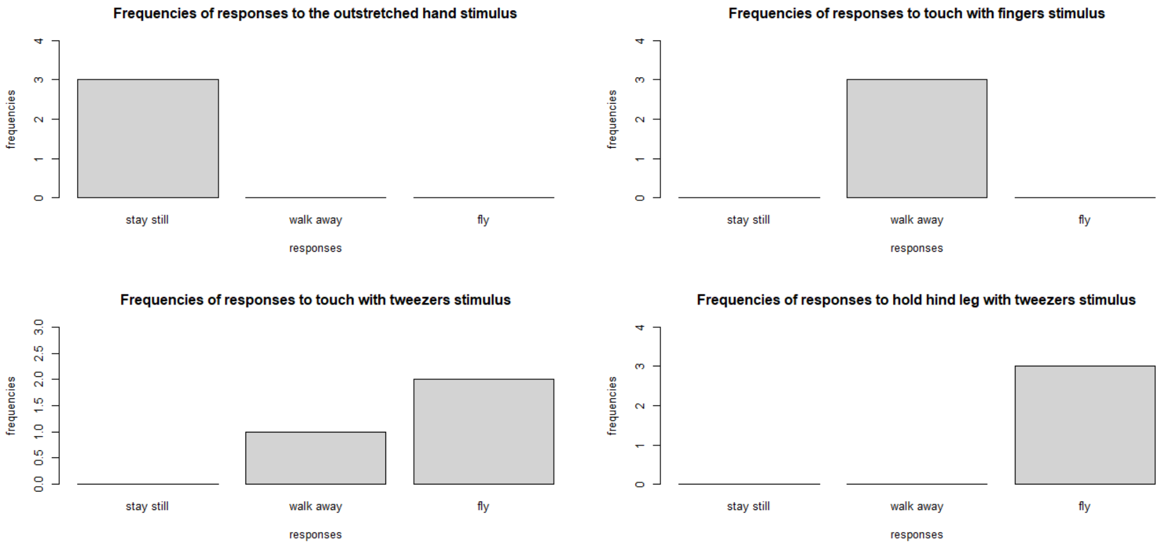
Responses	Observed	Expected
Stay still	1.5	0
Walk away	2	0
Fly	2.5	0

For the interpretation of standardized residuals (Table 1), the general rule of interpretation threshold was used, where values between -2 and 2 are considered non-significant, while values outside this range are considered significant. The obtained results indicate the following:

- For the response “Stay still”, with a value of 1.5, the observed value is close to the expected value, suggesting no significant difference.
- For “Walk away”, with a value of 2, it is at the threshold of being considered significant, indicating a slight over-representation compared to expected.
- As for “Fly”, with a value of 2.5 exceeding the threshold of 2, it suggests that the response “Fly” is significantly over-represented compared to expected.

In conclusion, for the Mariquita locality, the response “Fly” is more frequent than expected. The response “Walk away” is at the threshold of significance, while “Stay still” does not show a significant difference.

The observations conducted in Villeta (Fig. 4) demonstrate similarity in responses exhibited by individuals observed in Mariquita. Specifically, in response to the stimulus of approaching with an outstretched hand, individuals consistently remained still. Similarly, in reaction to the stimulus of touch with fingers, individuals consistently walked away. Finally, it was noted that in response to the touch with tweezers stimulus, flying was the predominant response, as observed similarly in response to the stimulus of holding the hind leg with tweezers.


Frequencies of responses to the outstretched hand stimulus

Frequencies of responses to touch with fingers stimulus

Frequencies of responses to touch with tweezers stimulus

Frequencies of responses to hold hind leg with tweezers stimulus

Figure 4. Frequencies of the responses to different stimuli (Villeta, Cundinamarca).

For Villeta data, the stimuli “approach with outstretched hand”, “touch with fingers” and “hold the hind leg with tweezers” were all 6, presenting significant differences in the responses (X^2, 2 df, α=0.05). Conversely, the stimulus “touch with tweezers”, presented non-significant differences in the responses.

The *post hoc* test of standardized residuals obtained from Villeta data were as follows:

Table 2: Standardized residuals for Villeta.

Responses	Observed	Expected
Stay still	-0.5	0
Walk away	0	0
Fly	0.5	0

As shown, there are no significant differences between the observed and expected values for any of the responses (“Stay quiet”, “Walk away”, “Fly”). This suggests that the observed distribution of responses does not significantly differ from what would be expected under the null hypothesis.

The results obtained in Mariquita and Villeta indicate that, despite being distant locations with different conditions in altitude, the behavioral preferences of the same species are similar. The individuals sampled from both locations predominantly exhibited the flight response over other possible responses.

Observations in natural environment Machetá, Cundinamarca

Description of the hind leg raise movement.

The behavior of raising the hind legs was observed in a total of four individuals when exposed to the stimulus of forced segregation in aggregations. Interestingly, this behavior was also noted in individuals not included in the experimental group (as part of incidental observations). The movement involves lifting one of the hind legs, starting with the femur and followed by the tibia slowly, until it reaches a fully extended position at 90 degrees or more from the body (Fig. 2). This position is maintained for a few seconds before the leg is gradually lowered, with the tibia descending first followed by the femur, until the leg rests back on the surface. After this movement, individuals mostly walk away until they find a new location, where they often remain still.

Based on the ethograms conducted with 90 individuals in Machetá, barplots were created for each stimulus, displaying the absolute frequencies of the responses (Fig. 5); it was observed that when presented with the stimulus of an outstretched hand approaching, the most common response was remaining still. However, flight was also observed as a response to this stimulus but not at the same frequency. Moreover, when the stimulus involved touching with a hand, individuals exhibited responses of staying still or walking away in equal proportions.

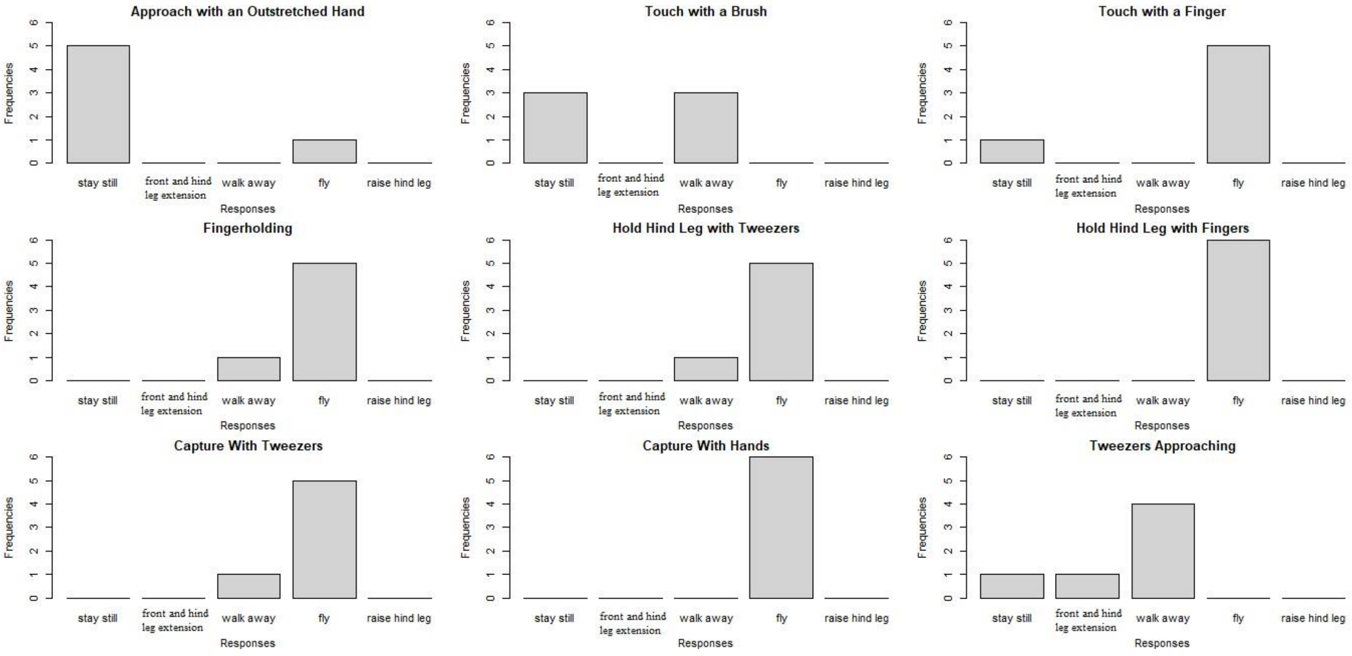


Figure 5. Absolute frequencies of each response to different stimuli in Machetá.

Individuals exposed to the stimulus of touching with fingers predominantly responded by flying, although some individuals also remained still. When presented with the stimuli of fingerholding (by the pronotum) and holding hind leg with tweezers, flying was the most frequent response, with instances of walking away observed but in lower proportions compared to those who flew (Fig. 5).

In the case of capturing with tweezers, flying was the predominant response, although some individuals chose to walk away. Finally, when exposed to the stimuli of holding hind leg with fingers and capture with hands, flying was consistently the response across all individuals studied (Fig. 5).

The X^2 test was conducted assuming a uniform distribution, with a significance level (α) of 0.05 and 4 degrees of freedom. The critical chi-square value for α = 0.05 and 4 degrees of freedom is 9,488 (Table B.1: Critical Values of the Chi-Square (X^2) Distribution.) (Zar, 2010)

Chi squared goodness for fit supported significant differences in the responses for “Extended hand approaching” (X^2, 4 df, α=0.05), “Touching with fingers” (X^2, 4 df, α=0.05), “fingerholding” (X^2, 4 df, α=0.05), “Holding hind leg with tweezers” (X^2, 4 df, α=0.05), “Holding hind leg with fingers” (X^2, 4 df, α=0.05), (X^2, 4 df, α=0.05) and “Capture with hands” (X^2, 4 df, α=0.05); while for the stimuli "Touching with a brush" and "Tweezers approaching" we accept the null hypothesis that there is no chance difference.

The *post hoc* test of standardized residuals obtained from Machetá data were as follows:

Table 3: Standardized residuals for Machetá

Responses	Observed	Expected
Stay still	-0,24343	0
Front and hind leg extension	-2,982	0
Walk away	-0,24343	0
Fly	6,7552	0
Raise hind leg	-3,2863	0

- For the responses “Stay still” and “Walk away”, with a value of -0,24343 both. They fall within the range of -2 to 2, so there are no significant differences.
- For “Front and hind leg extension”, with a value of -2,982 and “Raise hind leg” with a value of -3,2863. For both responses, there is a significantly difference between the observed and expected values, with the observation falling notably below the expected value.
- For “Fly”, with a value of 6,7552 exceeding the threshold of 2, it suggests that the response “Fly” is significantly over-represented compared to expected.

As shown in Table 3, responses “Stay still” and “Walk away” show no significant differences between the observed and expected values. In contrast, “Front and hind leg extension” and “Raise hind leg” occur less frequently than expected, whereas “Fly” occurs more frequently than expected. These results indicate that certain responses are significantly more or less common than anticipated under the model used.

The aggregation segregation stimulus was conducted by dispersing 6 aggregations over 6 different days and observing 6 individuals landing solo from those aggregations, totaling 36 observed individuals after the stimulus. It was noted that upon flying from the aggregations, they landed on plants distant from their initial location. The observed behavioral sequence generally consisted of flight, landing, remaining still, slowly walking towards a leaf, and pausing. Some settled on the leaf's underside, some on the upper surface, and a few on petioles and stems. After a while, some individuals flight again and landed elsewhere.

It is worth mentioning that the aggregation segregation stimulus was carried out by manually picking up an individual from the aggregation with fingers. Upon being handled, the individual's response was to attempt escape and immediately engage in chemical defense. After the expulsion of these substances, other individuals within the aggregation took flight, with some also displaying the same chemical defense before flying. The responses observed after the individual's flight (Fig. 6) illustrate that, when faced with forced segregation and landing elsewhere, 58% remained motionless, while 30% moved by walking to a different location upon landing, and finally, 11% were found with their hind leg raised.

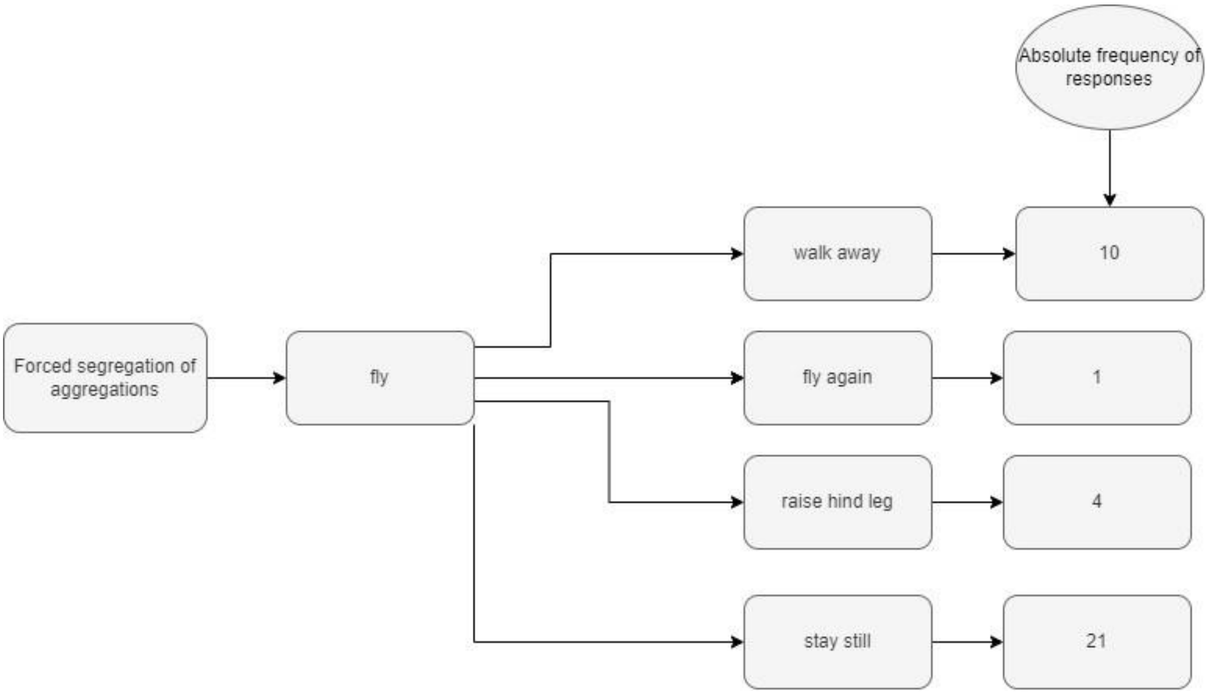


Figure 6. Diagram of forced segregation of aggregations stimulus with the frequency of the responses

Finally, a barplot (Fig. 7) was made to illustrate the distribution of relative frequencies, offering insight into the proportion of responses. The chart notably reveals that flying was the predominant response observed among individuals, accounting for 55%, followed by staying still and walking away, each at 18%. Hind leg raise followed at 7%. Conversely, the least prevalent response observed was the extension of both front and hind legs, representing only 2%.

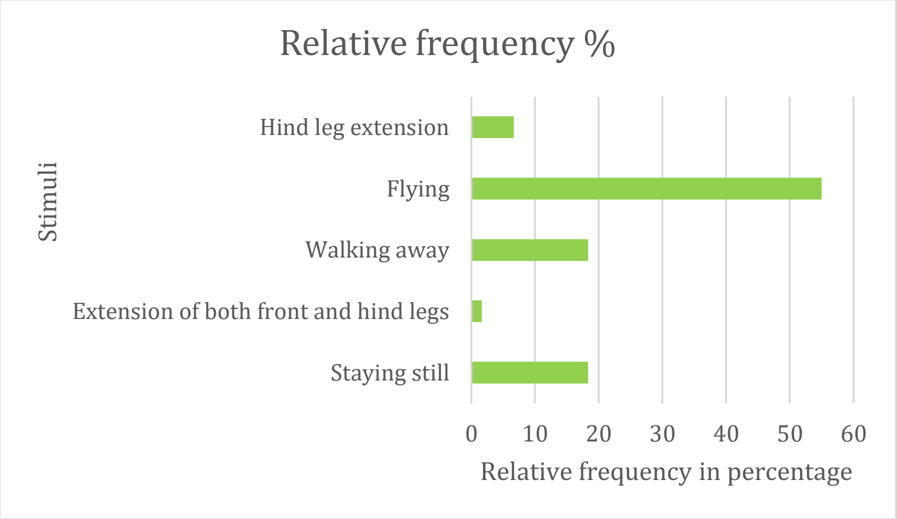


Figure 7. Relative frequency of responses (Machetá, Cundinamarca)

Aggregation behavior

Several additional observations were made, including whether the individual was in aggregations or alone. However, it was noted that the number of solitary individuals was minimal; out of the 90 individuals analyzed, only 9 were found to be alone, while the rest were in groups (Fig. 8). The aggregations were observed on both dry and withered leaves as well as on green leaves. The position of the individuals (on the underside or the upper side of the leaf) also varied depending on the color of the leaves.

Another noteworthy observation was the expulsion of odor, which typically occurred as individuals flew away, and even more frequently during instances of forced segregation in the aggregations. Odor expelling was perceived in 20 of the 54 individuals (without considering the 36 individuals of the stimulus segregation of the aggregations). Prior to taking flight 37% of the individuals often expelled the odor.

Figure 8. Aggregations of individuals *Leptoglossus macrophyllus*.

Bright coloration in abdomen

Seven of the sampled individuals were captured, and the area of the abdomen under the wings was observed. All seven exhibited iridescent coloration (Fig. 9). This coloration was also observed when they flew under the sun (see appendix for supplementary video).

Figure 9. Iridescent abdominal coloration (*Leptoglossus macrophyllus)*

As can be observed in Table 4, the most frequent response to any looming stimulus is typically flying, supported by the results of the *post hoc* test of standardized residuals.

Table 4. Absolute frequencies of responses to the nine stimuli.

	Stay quiet	Front and hind leg extension	Walk away	Fly	Raise hind leg
Extended hand approaching	5	0	0	1	0
Touching with a brush	3	0	3	0	0
Touching with fingers	1	0	0	5	0
fingerholding	0	0	1	5	0
Holding hind leg with twezzers	0	0	1	5	0
Holding hind leg with fingers	0	0	0	6	0
Capture with tweezers	0	0	1	5	0
Capture with hands	0	0	0	6	0
Tweezers approaching	1	1	4	0	0

Discussion:

This study is the first to investigate the behavior of *Leptoglossus* outside the context of sexual selection and chemical defenses. In this research, we found that *Leptoglossus* exhibits a rather diverse behavioral repertoire in response to different looming stimuli. In addition to its previously documented chemical defense behavior (Gonzaga-Segura et al., 2013), in this study it was observed that the behavioral repertoire of *Leptoglossus* in response to different looming stimuli is composed of different responses, which were: staying still, walking away, flying, raise one hind leg, and extending the front and hind legs flattening the body. Responses such as staying still, walking away, and flying were given at high frequencies. The responses obtained in relation to the applied stimuli demonstrate significant differences from chance, indicating that certain behaviors are preferred over others in the individual’s behavioral repertoire.

It remains uncertain how animals, especially insects, perceive and respond to predation risk situations. However, it is reasonable to assume that the repetitive “fight or flight” response can be an indicator of high predation risk (Adamo & McKee, 2017). These potential predation risk situations result in a repertoire of defensive behaviors against predators. Therefore, the looming stimuli applied to different individuals in Machetá, Villeta, and Mariquita provide a clear idea of that behavioral repertoire of *Leptoglossus* in defense against potential predators.

Some antipredator strategies are related to the morphology of individuals, which significantly influences their behaviors (Schmidt, 2009). Morphologies with bright colors warn predators of potential toxins or harmful substances, a phenomenon known as aposematism (Ruxton et al., 2019). Conversely, morphologies with cryptic colors enable individuals to blend into their surroundings, reducing detection by predators, a tactic called crypsis (Ruxton et al., 2019). In the case of *L. macrophylus* and *L. concolor*, their coloration is quite cryptic, featuring brown tones with some yellow details. Additionally, these species possess tibial expansions on their hind legs, like those of the species *Anisoscelis alipes*, although *Anisoscelis* displays bright and conspicuous colors (Brailovsky, 2016; Leavengood et al., 2024), unlike *Leptoglossus* which are mostly brown (Allen, 1969; Brailovsky, 2014). Both species exhibit specific movements with their hind legs; *Anisoscelis alipes* moves them in a waving pattern, alternating one leg and then the other (Longbottom et al., 2022), whereas *Leptoglossus macrophyllus* lifts one leg and holds it still for a few seconds. As the tibial structures in previous studies for *Leptoglossus* did not record dimorphism in the tibiae, they ruled out the function of these structures in male-male contest and it is suggested that possibly their function may be related to defense against predators or as camouflage (Miyatake, 1997).

The results obtained in this study suggest that the tibial structures in *L. macrophyllus* may not have sexual roles, as there appears to be no apparent dimorphism, similar to *Anisoscelis* (Longbottom et al., 2022). Typically, cryptic coloration is associated with behaviors such as slow movements or immobility, which help individuals blend into their environment and avoid detection (Matthews & Matthews, 2010), these slow movements, such as "hind leg raising" observed in the species *L. macrophyllus* appeared at a very low frequency in response to the looming stimuli applied. Therefore, based on the data obtained, this behavior could not be considered antipredatory behavior. However, it can be suggested as a possible crypsis behavior that is executed when alone, out of the safety of the aggregations, although this is not confirmed in this specific case.

Moreover, the behavior of "hind leg raising" observed in *Leptoglossus macrophyllus* was not witnessed in *Leptoglossus concolor*. However, the sample size for the latter species was smaller, and the occurrence of this behavior in *L. macrophyllus* was very rare. It is recommended to observe a larger sample size in *L. concolor* to determine whether the behavior of lifting the hind leg occurs or not. Nonetheless, the function of the "hind leg raising" behavior in *Leptoglossus macrophyllus* remains undetermined.

In addition to cryptic and aposematic colorations as antipredator mechanisms, there are hidden functional colorations that can become evident during an escape from a predator (flash display) or can serve as warning signals when the individual remains still (deimatic display) (Loeffler-Henry et al., 2019). These types of defenses are effective against visual predators such as birds and serve as secondary antipredator mechanisms that help evade or deter the predator (Emberts et al., 2020). For *Leptoglossus*, some bird species have been identified as predators (da Silva et al., 2021). In this study, it was observed that one of the most frequent responses of *Leptoglossus macrophyllus* to looming stimuli was to fly. During flight, in addition to releasing chemical defenses, a disruptive coloration is visible across the abdomen (Fig. 9) (see appendix for supplementary videos). This may indicate a possible secondary antipredator strategy of flash display in this species.

Another antipredator strategy observed in the species *Leptoglossus macrophyllus* was the formation of aggregations. These aggregations consisted of more than 20 individuals (Figure 13). They were found on both dry and withered leaves, as well as on green leaves. However, the location of the individuals varied depending on the state of the leaf. On withered and dry leaves, individuals were found on both the underside and the upper side of the leaf, and the aggregations consisted of a few individuals. In contrast, the aggregations on green leaves were denser, with a greater number of individuals, and they were located on the underside of the leaf, resembling clusters of dry leaves among the green ones (Fig. 8). These differences in the position of the aggregations of individuals on the leaves may support crypsis behavior, as they seek to blend with the background (Matthews & Matthews, 2010).

Moreover, groupings of distasteful individuals can benefit them by reducing the likelihood of predator attacks due to the “dilution” effect, which reduces the individual risk of attack, also, constant vigilance and defense are additional factors that benefit individuals in aggregations (Sillen-Tullberg & Leimar, 1988), the location of these aggregations among leaves formed clusters where distinguishing a single individual was difficult. When the aggregations dispersed and took flight, they always employed chemical defenses (odor), suggesting a strategy of predator repellence and a possible alarm signal to conspecifics (Gonzaga-Segura et al., 2013).

In conclusion, the behavioral repertoire of *Leptoglossus* is diverse, with flying being a common response. This behavior is always accompanied by chemical defenses, indicating that these individuals rely on their potential unpalatability. Additionally, the iridescent coloration displayed during flight is proposed as a flash display signal. Although the "raise one hind leg" response was not frequent, it is recommended to test another stimulus and use real predators, such as avian predators, and conduct experiments under more controlled conditions. Furthermore, differentiating between males and females could help determine if this behavior is sex-specific or if it occurs in both sexes.

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Appendix:

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