



Why does the Matador Bug, *Anisoscelis alipes* (Hemiptera: Coreidae), Wave its Brightly Colored Legs?

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Abstract Many animals exhibit elaborate traits and conspicuous behaviors. Sexual or social selection are often drivers of extreme trait elaboration and signalling, but conspicuous traits may also serve non-social functions. The matador bug, *Anisoscelis alipes* (Hemiptera: Coreidae), has large, brightly colored tibial expansions on its hind legs which are used to perform a stereotypic “waving” behavior, the function of which is unknown. If this phenotype functions primarily as a sexually selected aggressive signal, we predicted sexual dimorphism both in morphology and

behavior, as well as positive size allometry. Alternatively, if these traits function as non-sexual social signals, both male and females may perform waving behavior, and the frequency or rate of flag-waving should increase with proximity to conspecifics. Against some of our predictions, we found both males and females exhibited hind leg expansions which scaled isometrically with body size. We recorded 745 leg waves among 12 individuals and found no difference in the overall number of waves or the rate of waving between males and females. Further, the sex of individuals in the social environment did not predict the number of waves or rate of flag-waving. Sexual selection is often reported to be the main driver of

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many highly conspicuous traits, but our investigation of morphology, behavior, and natural history reveals little evidence for social or sexual selection as the primary driver of these conspicuous signals. We suggest future studies investigate a potential anti-predator function to explain the evolution of colorful and expanded tibial flags in other flag-legged bugs.

Keywords Insect behavior · Neotropics · Insect morphology · Elaborated traits · Anti-predator strategies · Conspicuous signals · Aposematism

Introduction

Many animals couple conspicuous and elaborate traits with behavioral displays. Yet, for most species we know little about the forces driving the evolution of these structures and their associated behaviors. The evolution of conspicuous traits may be driven by sexual selection, where signals are used in competition for mating opportunities (Darwin 1871; Lande 1980). Another, non-mutually exclusive possibility is that these traits are shaped by non-sexual social selection, where competition over resources rather than access to gametes may be the primary driver of conspicuous signals (West-Eberhard 1983; Lyon and Montgomerie 2012; Tobias et al. 2012; Okie et al. 2013; Webster et al. 2018; Falk et al. 2021). Finally, conspicuous traits and behavior may be shaped largely by natural selection, where traits act as decoys enabling prey to escape (Rubin et al. 2018), or as predator deterrent signals (Poulton 1890; Cott 1940; Edmunds 1974; Murphy 2006; Vidal-García et al. 2020; White and Umbers 2021). Some recent studies suggest that conspicuous traits likely serve multiple functions (Hebets and Papaj 2005; Pekár et al. 2017; Rojas et al. 2018; Perez and Backwell 2020; Shuker and Kvarnemo 2021). Signals used in mate-attraction, for example, may also act as predator deterrent signals (Rojas et al. 2018). In spite of this complexity, the prevailing evolutionary drivers of these signals can be deduced from the presence of sexual dimorphism in signal size, sex-specific signaling behavior or the social context in which signaling occurs (Falk et al. 2022; Bertram et al. 2004; Hebets and Papaj 2005; Vidal-García et al. 2020).

Exaggerated or elaborated sexually selected traits are often only present, or are at least more prominent,

in one sex (Clutton-Brock 2007; South and Arnqvist 2009). However, if traits are more strongly shaped by non-sexual social selection, both males and females may exhibit similar expression of trait elaboration and rates of display behavior, but individuals are more likely to perform signaling behavior when closer to conspecifics (West-Eberhard 1983; Dehn 1990; Webster et al. 2018). Predation is another pervasive selective force that shapes the evolution of many organismal traits, particularly coloration and patterns such as crypsis or warning colors (Edmunds 1974). Chemically defended organisms typically advertise their unpalatability via bright coloration, in the form of aposematism. Highly conspicuous traits may also act as decoys in the event of a predator attack, to deflect attack away from vital organs to more expendable body parts which can be regenerated or autotomized (Rubin et al. 2018). This deceptive “lure” tactic is found in lizards that use their brightly colored tails to distract predators (Watson et al. 2012; Bateman et al. 2014) and by lycaenid butterflies that twitch antenna-like wing extensions to create “false-heads” on their hindwing margins (Sourakov 2013; López-Palafox et al. 2015). However, predation and sexual selection are not necessarily mutually exclusive drivers of exaggeration. Conspicuous traits that serve an anti-predator function may also act as intersexual or social signals (Yang et al. 2016).

The family Coreidae (Hemiptera) is a charismatic, odorous group known for exaggerated leg traits, many of which are used in conspecific battles (Mitchell 1980; Miyatake 1997; Eberhard 1998; Okada et al. 2011). These weapons are often associated with increased muscle mass; some lineages, however, exhibit gracile femurs and elaborate tibial traits that likely serve another purpose. The coreid tribe Anisoscelidini (flag-footed bugs) often bear greatly expanded, colorful and flattened tibiae that are composed almost entirely of cuticle and have relatively little mass (Fig. 1). In this clade, the matador bug—*Anisoscelis alipes* (Hemiptera: Coreidae)—exhibits some of the most striking morphologies. Its tibial flags are brightly colored, and combined can account for more surface area than the rest of the dorsal body surface (Fig. 1 & S2). Thus tibial flags may allow individuals to exaggerate a visual signal with relatively low investment in metabolically active tissue (Eberhard et al. 2018; Somjee 2021; Somjee et al. 2021). Almost all evidence of femur exaggeration in coreids so far suggests this

Fig. 1 The matador bug, *Anisoscelis alipes*, has large conspicuous tibial plates on both hind legs with which they perform a stereotyped waving behavior, yet nothing is known about the selective pressures that shape these traits. (photo: Ana Endara)



evolutionary labile trait evolves through sexual selection (Mitchell 1980; Eberhard 1998; Miyatake 2002; Miller and Emlen 2010). Additionally, the matador bug has been frequently observed performing a stereotypic “waving” behavior with these hind legs (U. Somjee, *pers. obs.*) that we detail in this paper. This specific flag-waving behavior has not been formally described to the best of our knowledge.

In this study, we describe the morphology and behavior of the elaborate tibial flag in the matador bug for the first time. We record flag-waving behavior in experimentally manipulated social environments. To understand the evolutionary pressures driving the exaggerated hind tibia and conspicuous waving displays of the matador bug, we propose three hypotheses for its role and investigate the first two: 1) *sexual selection* – this trait is used in mate-attraction or signaling during intrasexual competition 2) *social selection*– this trait is used by both sexes in competitive signaling or 3) *natural/ecological selection*– this trait is used as an aposematic signal to advertise unpalatability or as a lure to misdirect predatory attacks to a less vital body part (see Table 2). If this trait primarily serves a sexually selected function, we predict that tibial flag morphology will be sexually dimorphic. We also expect to find steeper allometric scaling of the

trait in one sex if it is used as an intra-sexual signal of aggression for mates, as is the case in multiple other coreid species in which exaggerated leg traits function as sexually selected aggressive signals (Mitchell 1980; Eberhard 1998; Miyatake 2002; Miller and Emlen 2010; Somjee et al. 2015, 2018). Furthermore, if flag-waving functions as a sexually selected trait, we predict that one sex will wave more than the other and the bugs will alter their waving behavior depending on the sex of nearby individuals. Alternatively, if tibial flags act primarily as non-sexual social signals, we predict that flag-waving rates will not be influenced by the sex of other individuals in their social environment, and that flag-waving rates will increase when individuals are closer to each other. Lastly, it is possible that selection on the wave behavior of males and females may not be sexual or social, and is perhaps related to ecological factors such as predation.

Methods

Insect Collection and Rearing

Anisoscelis alipes were collected using a sweep net from a farm in Sona, Panamá (7.9318,-81.2997). We housed insects in large butterfly cages

(40×40×60 cm) and provided foliage from the area where they were collected, along with fresh maracuyá fruit (passion fruit; *Passiflora edulis f. flavicarna*) and a vial of water. The cage was regularly misted using a spray bottle to maintain humidity, especially while being transported. Within 24 h of collection, we transported insects to Gamboa, Panamá where they were housed in smaller butterfly cages (30×30×30 cm). Insects were provisioned with a moistened piece of paper towel, a maracuyá fruit and water so that insects could feed ad libitum. Cages were kept at ambient temperature and humidity and sheltered from the rain. These cages were misted twice daily; fruit and paper towels were replaced every two days.

Morphological Measurements

Specimens of *A. alipes* were collected between January 2020 and May 2021 from Central Panama (provinces: Panamá, Colón, Coclé & Veraguas). These insects were placed in vials and frozen for measurements. To measure the size and scaling relationships among body parts, we separated structures at natural break points of the exoskeleton. We placed these structures on a flat Styrofoam block covered with a glass plate to ensure they were flush against a flat surface for consistent measurements. Photos were taken with a scale for reference. We measured the surface area of hind leg tibial flags, as well as linear measures of left-front tibia, pronotum (a proxy for body size) and fore, and hind wing length. We used an average of both tibial flag surface areas for each individual insect. Many wild-caught insects were missing a hind leg; thus we measured either the left or right hind leg in cases where only a single hind leg was present. All measurements were carried out using Adobe Photoshop C4 (Adobe Systems Inc., San Jose, CA, U.S.A.).

Morphological Analysis

To compare the size and relative scaling relationship of flags within and among the sexes, we constructed separate general linear models (GLMs). We aimed to test if flags scale hypermetrically (disproportionately larger with increasing body size), hypometrically (disproportionately smaller with increasing body size)

or isometrically (in direct proportion to body size). Our initial model used tibial flag area as a response variable while body size and sex were included as explanatory variables. We used pronotum width² as our body size metric so that both continuous trait values were on the same scale for appropriate comparisons (Houle et al. 2011). We log₁₀ transformed all data to improve linearity, normality and homoscedasticity (Glazier 2013). To compare the scaling of flags to other traits we measured the front tibia length which does not appear to be elaborated, as well as the length of wings, which may be under selection to carry these elaborate structures. In separate GLMs we used front tibia length, forewing length and hindwing length each as explanatory variables while body size and sex were used as covariates. In the case of these linear measurements, we used a linear measure of pronotum width as our metric for body size (Houle et al. 2011). All statistical analyses were conducted in R statistical software v0.99.893 (R Core Team 2019). Under predictions from a function as aggressive signals under sexual selection (Eberhard et al. 2018), we expected that flags would scale hypermetrically, and that they would be disproportionately larger in males relative to females (supposing that females do not signal aggressively or signal less).

Social Environment Treatments

To examine the context-dependence of flag waving, we recorded behavior in two types of treatments: 1) same sex groups 2) mixed sex groups. We created two replicate groups of each same sex group (3 males and 3 females) and two replicates of each mixed sex groups:(i) 2 females and 1 male (ii) 1 female and 2 males. This group size is ecologically relevant – in the field, insects were most often found in groups of 2 or 3, but rarely in groups of more than 3 individuals within a square meter on a single host plant (U. Somjee, *pers. obs*). To minimize the chance that recent social interactions influenced our behavioral observations, we isolated individuals before treatments and randomized combinations of individuals for each treatment group such that individuals had been separated for 24 h prior to recording. In conditions of cultivation, *A. alipes* are most often active and found feeding and mating in the mid-morning and late afternoon (U. Somjee *pers. obs.*), thus we recorded social interactions for one hour at 1000 h and for one hour at 1700 h for each treatment.

Insects were introduced into a clean butterfly cage (30×30×30 cm) with the same set-up as the collection cages (described above). Two individuals were marked with a dot of non-toxic paint (Elmer's Painters marker) 1 white and 1 purple on the ventral side of their abdomen, which is unlikely to be in the visual range of other individuals. One individual remained unmarked, and this allowed us to track each of the three individuals throughout the hour-long behavioral trial. The standardized edges of the cubical observation cage allowed us to scale distances appropriately. We took note of the precise location of each individual relative to each other (± 1 cm) in the cage during waves (height, width, and depth of location in the chamber). In addition, we also recorded the location of each individual at 150 s intervals throughout each trial. The video camera (Sony–HDRCX405- 30 fps at 640×480p resolution) was set up approximately 50 cm away from the cage so that the entire cage fit neatly in the frame of the video. Video footage was later analyzed with a start time ~ 10 min after the experimenter left the room.

Behavioral Analysis

From the video footage, we classified waving behavior into discrete bouts. We defined the start of a discrete bout of waving behavior as the point when a wave was first initiated and the end of a bout when the leg was placed back on the ground and no waving subsequently took place for at least two seconds. Waving ranged from short bouts with the tibial flag lifted above the body and moved slightly, to more prolonged bouts where the flag appears to oscillate independent of the femur. For each bout of waving, we recorded the following variables: seconds into trial; unique bug identification (i.e., color mark or lack of mark; referred to as the focal individual); duration of the bout; number of waves per bout; and the distance between all three individuals.

Effect of Sex on Waving Behavior

To compare sex differences in waving behavior, we first compared the wave rate of males and females across all trials (6 males and 6 females) using an independent two sample t-test. We also used a t-test to compare whether individuals in mixed-sex groupings (2 females and 1 male; 1 female and 2 males) waved at different rates than same-sex groupings (3 females;

3 males). Next, we used a mixed-effects modelling approach to compare wave duration between males and females using the lme4 package (Bates et al. 2015). First, we compiled data for each wave bout ($n=318$), before fitting models with wave duration as the response variable. Sex of the individual and trial type (mixed-sex or same-sex) were included as fixed variables, while the trial ID and individual ID were included as random variables to account for the fact that the same individuals engaged in multiple bouts. In addition to wave duration, the same approach was used to model the number of waves per bout. For each model, we used a likelihood-ratio test to verify whether the inclusion of an individual's sex or trial type significantly affected response variables.

Effect of Proximity on Waving Behavior

We also tested whether waving behaviors were associated with proximity to other individuals. During every waving bout, we measured the distance from the waving individual to the closest other individual. To compare these measurements to instances when waves were not occurring, we also recorded the distances between individuals at standardized time points (every 150 s) in each trial. This provided a dataset representing distance between individuals when no waves occurred. We extracted the distance between the two closest individuals during these non-waving periods and compared this with the distance between a focal waving individual and the closest non-waving individual. This allowed us to get a measure of how close the nearest neighbor was when an individual was waving versus how close the nearest neighbour was when no waves were occurring in the group. The resulting data represented a minimum distance between individuals during waving timepoints ($n=318$) and non-waving timepoints ($n=187$). We then fit a mixed-effects model with minimum distance between individuals as the response variable. Presence of waving at each timepoint was included as a fixed predictor variable, and the trial ID was included as a random effect. This analysis was repeated with the mean distance between all three individuals in each trial as the response variable. For each model, we used a likelihood-ratio test to verify whether the presence of wave behavior was predictive of distance between individuals. All analyses were performed in R statistical software v3.5.3 (R Core Team 2019).

Results

Morphology:

We included 18 females and 19 males in our morphological analysis. Of these, six individuals (~16%) were already missing one hind leg when captured: in nature, individuals missing a hind leg are relatively common in this family (Embets et al. 2020). Female mean pronotum width was slightly larger than males (Table 1), but not significantly so ($t=1.81$, $df=1,35$, $p=0.08$). To assess whether juveniles exhibit tibial flags, pictures of 5 juveniles were taken at 4th and 5th instar stages (Fig. 4).

We found no significant effect of the interaction of sex and body size on flag-area (Walds $\chi^2=1.65$, $df=1,33$, $p=0.21$). We ran the model without the interaction term and found a difference in intercept: females were larger than males (Walds $\chi^2=139.9$, $df=1,34$, $p<0.001$) but did not have larger hind leg flags compared to males while accounting for body size (Walds $\chi^2=3.15$, $df=1,34$, $p=0.08$). In each of our other models that examined tibia length, forewing length, and hindwing length respectively we found no difference in slope (interaction term) between sex and body size (see Table 2).

Flag area scaled with similar slopes in relation to body size in both males and females (interaction; Walds $\chi^2=1.09$, $df=1,33$, $p=0.30$), and this scaling relationship did not differ from isometry ($F_{1,35}=157.2$, $p=0.64$). Front tibia length, forewing and hindwing length all scaled hypometrically with body size (Fig. 2).

Table 1 The absolute mean and standard deviation of morphological trait measures for female ($n=18$) and male ($n=19$) *Anisoscelis alipes*

Trait	Female		Male	
	mean	sd	mean	sd
Pronotum width (mm)	4.98	0.42	4.73	0.40
Fore wing length (mm)	13.13	0.87	12.35	0.93
Front tibia length (mm)	5.87	0.34	5.75	0.23
Hind wing length (mm)	10.40	0.59	9.87	0.66
Flag area (mm ²)	4.14	0.86	3.52	0.62

Behavior

Effect of Sex and Social Environment on Waving Behavior

In 8 hours of behavioral observation, we documented 745 individual leg waves clustered into 318 bouts from 6 females (161 bouts) and 6 males (157 bouts). All individuals in the experiment, irrespective of sex, exhibited waving behavior during experimental observations. Overall, the mean number of waving bouts were similar for both males and females across all trials ($t=-0.08$, $df=6.53$, $p=0.94$; males: 26.2 ± 8.0 SD, females: 26.8 ± 20.1). The mean wave rate of individuals in mixed-sex groups was not significantly different from the those in same-sex groups ($t=1.17$, $df=9.01$, $p=0.27$; mixed-sex: 21.67 ± 11.71 SD, same-sex: 31.3 ± 16.5). Neither the sex of a waving individual nor trial type were significant predictors of the number of waves per bout (sex: $\chi^2=1.92$, $p=0.17$; trial type: $\chi^2=0.47$, $p=0.49$), nor the duration of a bout (sex: $\chi^2=1.85$, $p=0.17$; trial type: $\chi^2<0.01$, $p=0.97$). Overall, we found that both sexes expressed similar waving frequencies across all social environments tested (Fig. 3).

Effect of Distance on Waving Intensity

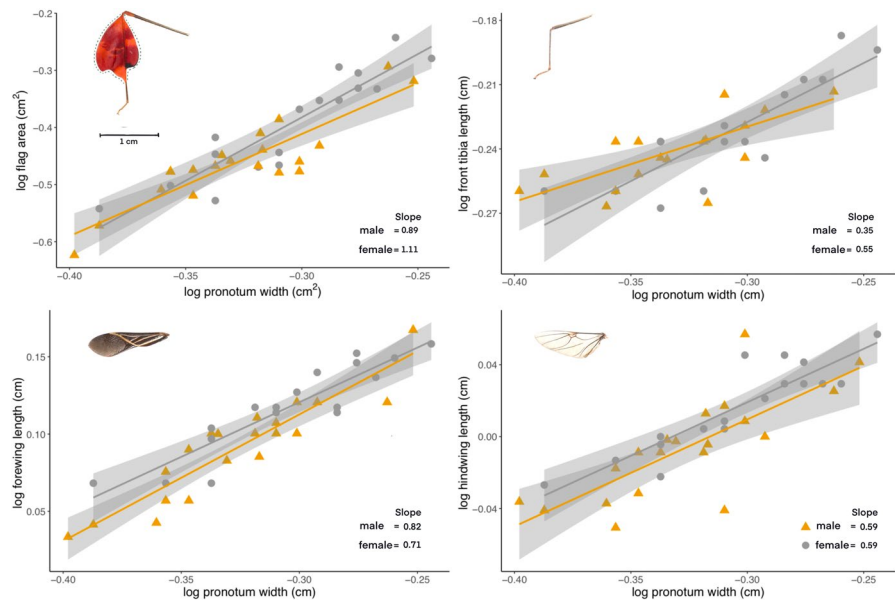
Did proximity to other individuals predict the frequency of waving? Likelihood ratio tests of our models found the opposite; insects were more likely to wave when the nearest neighbour was farther away rather than when closer together ($\chi^2=55.85$, $p<0.0001$). In addition, during waving bouts the mean distance among all three individuals was greater compared to when no waves were occurring ($\chi^2=10.14$, $p=0.001$).

Table 2 Results of 4 separate GLMs examining the relationship between relative trait size and sex. Each GLM examines the effect of sex on morphological trait size with body mass as a covariate. Linear measure of body size was squared when compared to flag surface area and all traits were log-transformed prior to analysis

Trait measured	Wald χ^2	d.f	p
Flag area	3.15	1.34	0.09
Front tibia length	2.23	1.29	0.14
Forewing length	0.50	1.33	0.49
Hindwing length	0.001	1.33	0.97

Error d.f. = 1 for all analyses

Fig. 2 The slope of the allometric relationship between trait size and body size scaled similarly in males and females for flag area, front tibia length, front wing length and hind wing length (total $n = 37$). The slope of the scaling relationship of flag area (a) scaled at or near isometry for both males (yellow triangles) and females (grey circles). Other putatively non-signalling traits, such as front tibia length (b), and fore (c) and hind (d) wing length scaled hypometrically (all inset photos are set to the same scale)



Discussion

The matador bug's striking morphology has contributed to its appearance on the covers of many popular books, and this species is a well-known pest in small-scale maracuyá (passion fruit, *Passiflora edulis*) farms in Panamá and Costa-Rica. Despite the apparent intrigue of this conspicuous trait, no published study to date has sought to explore hypotheses underlying the function of this exaggerated morphology. Previous work on this genus has focused mainly on its foraging behavior on its host plant, *Passiflora*, which may lend it similar chemical protection as *Heliconius* butterflies (Aldrich and Blum 1978; Aldrich 1988; Rodrigues et al. 2007).

We found no evidence for sexual dimorphism in tibial flags in the matador bug. Both males and females invest in these large, conspicuous signals (See Fig. 1). Tibial flags in both sexes scaled in direct proportion to body size, contrary to the positive allometric scaling of signals found in many sexually selected traits that function as threats (Eberhard et al. 2018). This absence of dimorphism in leg morphology stands in sharp contrast to many other coreid species, in which males often bear conspicuously larger exaggerated hind legs that are deployed in male-male competition and that often scale with positive allometry (Mitchell 1980; Miyatake 1997; Eberhard 1998; Somjee et al. 2018).

Furthermore, we found no sexual dimorphism in flag waving behavior. The sex of individuals in their social environment had no significant effect on the number of waves or rate of waving behavior. There was also no sex-difference in the distance of the closest individual in waving behavior of a focal individual. Whilst it is possible that these insects only deploy sexual signals under specific natural conditions (Bertram et al. 2004), sex-specific courtship and competitive behavior can typically be readily induced in semi-natural and artificial social environments in other insects in this family. Sex specific courtship has been observed in other insects where both males and females bear ornamentation (South and Arnqvist 2009). In some species of *Sabethes* mosquito, for example, both males and females have paddle-like structures on their mid-legs which only males use in courtship displays, but the presence of these ornaments in both sexes suggest they may have arisen from intralocus sexual conflict or mutual mate choice (South and Arnqvist 2008; Pennell and Morrow 2013). However, mutual mate choice is an unlikely explanation for the tibial ornament in *A. alipes*, because individuals waved at similar rates regardless of either sex or whether they were in mixed, or single-sex enclosures.

If not for attracting mates, why might these extravagant signals have evolved? Though sex did not predict morphology or behavior, we found that proximity to other individuals did impact waving behavior,

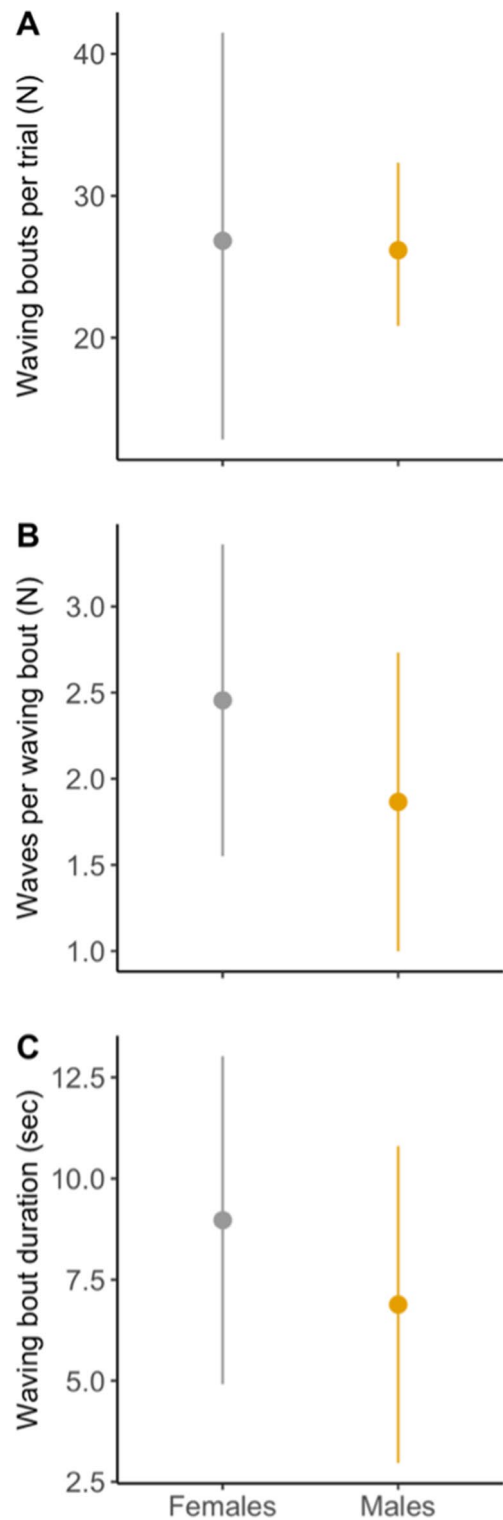
Fig. 3 In experimentally constructed social groups of the mator bug, *Anisoscelis alipes*, the number waving bouts (total bouts=318) and numbers of individual waves (total waves=745) did not differ between males (n=6) and females (n=6). In addition, we detected no difference in the rate of waving between males and females. These data were taken from video recordings of insects in a cage with no humans or other moving visual stimuli present. Data shows model outputs (Means \pm SE)

both in relation to waving frequency and bout duration. Individuals tended to be further apart when waves were occurring. Furthermore, waving behavior did not appear to prompt waves from other individuals. More than 30 s elapsed between one individual's wave bout and another's for over 90% of wave bouts in the experiment.

Our findings are consistent with an effect of conspecific density (dilution effect) underlying this behavior. That is, individuals might decrease anti-predation behaviors when closer to conspecifics purportedly because there is less individual risk of being preyed upon (Dehn 1990; Wrona and Dixon 1991). It is possible that this behavior may also act as a continuous, honest antipredation signal. Future studies that examine whether the rate of this signalling behavior in *A. alipes* changes or occurs more frequently when predators are present may provide further support for an antipredator function of these traits.

The predator lure hypothesis suggests that conspicuous traits may evolve in order to distract predators from essential parts of the body. A subsequent prediction is that lures are easily detached from the rest of the organism without severe harm. This finding is consistent with some findings from *A. alipes*, which have among the fastest autotomy rates in this family (Embets et al. 2016, 2020). Further, in our sample of wild caught *A. alipes* ~16% of individuals were already missing a single hind leg, suggesting that leg-loss may be a common phenomenon in this species. Relatively fast autotomy coupled with a highly conspicuous trait on the detachable body part suggests a deflective function for the tibial flag.

The bright, conspicuous coloration of the tibial flags could also indicate that this trait may act as a warning signal to visually-oriented predators (Rojas et al. 2018; Rößler et al. 2019). Many predators such as birds and spiders either show innate avoidance towards certain conspicuous colors such as red or yellow (Taylor et al. 2014), especially in contrast with black (Ruxton et al.



2018). They may quickly learn to avoid prey items that display such colors if the prey is in fact unpalatable

Fig. 4 Late instar (5th instar) matador bug nymphs, *Anisoscelis alipes*, bear colorful tibial flags and display “flag waving” behavior. This is consistent with the hypothesis that this morphology and behavior plays a role in predator defense (Photos: Ryan Dougherty)



(Poulton 1890; White and Umbers 2021). *A. alipes* appear to have considerable individual variation in color proportion and pattern, and likely sequester toxins from their host plants, usually cyanogenic passifloras (Aldrich and Blum 1978; Aldrich 1988). In addition, *A. alipes* produce an odorous volatile compound when disturbed (U. Somjee, pers. obs.). Collectively these findings suggest that these traits could function as aposematic signals, offering a non-mutually exclusive alternative to the predator lure hypothesis.

In favor of the defense hypothesis, we found that the exaggerated tibial flags (tibial expansion and conspicuous coloration) are already present and clearly distinguished in the 4th and 5th instar, when these insects are not yet sexually mature (Fig. 4) similar to

a closely related flag-footed bug, *Anisoscelis hymenipherus* (Alarcon and Cazorla 2020). Indeed, in another species of brightly colored coreid, nymphal defensive secretions provide effective antipredator defense (Prudic et al. 2008). In other insects, color change associated with mate attraction often develops at sexual maturity (Khan and Herberstein 2020) and in other coreid species, a vast majority of investment in sexually selected hind legs occurs only during the final molt to adulthood (Somjee et al. 2017). While a potential social function of these traits in other contexts cannot be discounted (Mitchell 1980; West-Eberhard 1983; Eberhard 1998), our findings provide no evidence for an intra-specific signal function (Table 3).

Table 3 Predicted functions of tibial flag morphology and waving behavior: related hypotheses and the extent of their empirical support. We found no empirical support for a sexually selected tibial flag function. Tibial flags were not sexual dimorphic and scaled isometrically with body size. In addition,

flag waving behavior was found in both males and females. We found some indication that flag waving could be socially selected but our findings were most consistent an anti-predator function

HYPOTHESES:	SEXUAL SELECTION	SOCIAL SELECTION	ANTIPREDATOR FUNCTION
FLAG SIZE	Larger in one sex	✗ Not necessarily larger in one sex	✓ Not necessarily larger in one sex
FLAG SLOPE	Scales with positive allometry when used as a threat	✗ Positive allometry or isometric	✓ Isometry or lower scaling
FLAGS PRESENT IN JUVENILES	Often absent in Juveniles	✗ Possibly present in Juveniles	✓ Possibly present in Juveniles
SOCIAL-DEPENDENT BEHAVIOR	Wave more when closer	✗ Wave more when closer	✓ Wave more when further apart
SEX-DEPENDENT BEHAVIOR	One sex wave more	✗ Both sexes wave equally	✓ Both sexes wave equally
SEX OF NEARBY INDIVIDUAL	More likely to wave when opposite sex near	✗ No effect of sex of nearest neighbor on waving	✓ No effect of sex of nearest neighbor on waving

It is also important to note that although we measured a large number of individual flag waves (~745), only a small number of individuals were used in behavioral trials ($n=12$), and more detailed relationships between flag-waving behavior and social context may have been masked by this relatively small sample size. Thus, other forms of social signals, perhaps in response to environmental cues cannot be ruled out. For example, non-dimorphic elaborate traits may still indicate fitness and increase reproductive success (Murphy 2007). It is also likely that these traits can serve multiple functions and are shaped by both predation and social selection (Rojas et al. 2018). The conspicuous nature of the tibial morphology and waving behavior may also allow conspecifics to eavesdrop on these signals to predators (Trillo et al. 2016), which could explain why they wave less when they are closer together.

Whilst our findings point towards a possible anti-predator function of tibial flags, it should be noted that our recordings were carried out in the absence of predators, or any moving visual stimulus apart from the conspecifics in the study. Jumping spiders for example have been observed consuming coreids (U. Somjee, *pers. obs.*), and our preliminary observations suggest that matador bugs increase waving rates in the presence of a real or simulated spider predator (see supp. mat. S2, S3). However, future experiments need to be conducted to test this hypothesis explicitly. We have anecdotally observed waving behavior when non-conspecifics (humans or other animals) approach these insects, suggesting that this may be a generalized response to approaching (looming) stimuli.

Our findings provide novel perspectives about how these elaborate hindleg traits evolved, what selection pressures act to shape them, and what their function is (Eberhard et al. 2018). Hindleg morphology represents a particularly evolutionary labile trait in this family of insects (Hemiptera: Coreidae), which comprises over ~3100 known species (Cumming and Le Tirant 2021). However, so far most of the evidence for this exceptional diversification of hindleg form in coreids suggests that these traits are shaped primarily by male-male competition for mating opportunities (Mitchell 1980; Miyatake 1997, 2002; Miller and Emlen 2010). Tibial expansions have been documented to function as social signals in at least one species of coreid (Eberhard 1998). In contrast to other

coreid species, we found no evidence here that sexual or social selection plays a role in shaping the evolution of these highly exaggerated traits. In *A. alipes*, both males and females possess this trait and both display flag-waving behavior at equal rates. Further, the ontogeny of trait development (these traits are present and colorful in juveniles) and the finding that insects in the wild are often missing a tibial flag all point to a potential anti-predator function of these exaggerated signals. Our findings provide morphological and behavioral data thus far lacking from the literature suggesting that non-social drivers of this specific signalling behavior are likely important yet entirely unexplored in the matador bug and other species of flag-footed bugs. While multiple functions of the elaborated traits are likely, our finding inspires future work to experimentally test the hypothesis of anti-predatory signalling in this group.

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Authors' Contributions CL, US, GG, and MJ designed the study. CL & CR collected the data. JF, US, and CL analysed the data. CL and US wrote the paper. US, CL, JF, DR, GG, JR provided critical feedback on writing and analysis.

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Data availability Data is in the DRYAD repository. <https://doi.org/10.5061/dryad.rbnzs7hc7>

Declarations

Ethics Approval and Consent to Participate The data was collected with approval from MiAmbiente research permit to Ummat Somjee (SC/A-33–19). The focal species in this study is not under protection. No approval from an ethics committee was required for this species.

Competing Interests We have no competing interests to declare.

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