

The evolution of multi-component weapons in the superfamily of leaf-footed bugs

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Abstract

Sexually selected weapons, such as the antlers of deer, claws of crabs, and tusks of beaked whales, are strikingly diverse across taxa and even within groups of closely related species. Phylogenetic comparative studies have typically taken a simplified approach to investigate the evolution of weapon diversity, examining the gains and losses of entire weapons, major shifts in size or type, or changes in location. Less understood is how individual weapon components evolve and assemble into a complete weapon. We addressed this question by examining weapon evolution in the diverse, multi-component hind-leg and body weapons of leaf-footed bugs, superfamily Coreoidea (Hemiptera). Male leaf-footed bugs use their morphological weapons to fight for access to mating territories. We used a large multilocus dataset comprised of ultraconserved element loci for 248 species and inferred evolutionary transitions among component states using ancestral state estimation. Our results suggest that weapons added components over time with some evidence of a cyclical evolutionary pattern—gains of components followed by losses and then gains again. Furthermore, our best estimate indicated that certain trait combinations evolved repeatedly across the phylogeny, suggesting that they function together in battle or that they are genetically correlated. This work reveals the remarkable and dynamic evolution of weapon form in the leaf-footed bugs and provides insights into weapon assembly and disassembly over evolutionary time.

Keywords: Alydidae, armaments, comparative analyses, Coreidae, correlated traits, weaponry

Introduction

From the fearsome tusks of prehistoric elephants to the branching “antlers” of antler flies, sexually selected weapons are as diverse as they are captivating. Along with stunning variability across taxa, weapons can be highly diverse within groups of closely related species (Emlen, 2008). For example, head weapons on extinct and extant members of the giraffe family include small ossicones, armored helmets, large flat expansions in the shape of butterfly wings, and paddle-like headgear that resembles the antlers of moose (Wang et al., 2022). The fascinating diversity of animal weapons can aid understanding of the evolutionary interplay of form and function because weapons have been selected to perform in physical combat (McCullough et al., 2016). For this reason, even small, intricate differences in morphology may reveal differences in fighting behavior across species and be tied to meaningful fitness consequences within species. A spotlight on weapon size alone can overlook the functional elements that contribute to fighting success (as described in Dennenmoser & Christy, 2013; Palaoro & Peixoto, 2022). Yet, the focus of most phylogenetic comparative studies of weapon morphology has been confined to major changes in weapon size or type, gains and losses of entire weapons, and shifts in location on the body (Cabrera & Stankowich, 2020; Dalebout et al., 2008; Emlen et al., 2005; Heinze et al., 2005; Hosoya

& Araya, 2005; Schutze et al., 2007). Work that considers the individual components of weapons can inform unresolved evolutionary questions about weapon assembly (Van Kleeck-Hann & Wiens, 2023). For example, such work can address whether extra components are added to weapons over time (Geist, 1966) as might be expected in an evolutionary arms race (Emlen, 2008) or run-away sexual selection (Moore et al., 2022). Furthermore, it can examine the extent to which certain sets of weapon components appear together repeatedly across the phylogeny, suggesting a coordinated function during battle.

Combat is often a full-body sport. Males engaged in male-male competition frequently launch their entire bodies toward rivals. Sexually selected weapons often serve as the contact points in battle, while supportive traits (*sensu* Okada et al., 2012; also “combat traits” *sensu* Rico-Guevara & Hurme, 2019) assist weapon function. For example, the ability to successfully use weapons can involve muscle contraction, posture adjustments made via the nervous system, and structures to hold the body to the substrate. Thus, in many ways, the entire body can be involved in combat. For the purposes of this study, the morphological weapons of sexual selection are defined as the elaborated structures that frequently contact other individuals during intrasexual contests. These weapons may be quite simple, such as the head horns of dik-diks, the

Received April 24, 2023; revisions received November 28, 2023; accepted January 19, 2024

Associate Editor: Tracey Chapman; Handling Editor: Alejandro Gonzalez-Voyer

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spurs of galliform birds, or the fangs of musk deer. In contrast, some animals can become highly weaponized, possessing weapon components stretching across vast areas of the body (e.g., Miyatake, 1997; Miller & Emlen, 2010; Dennenmoser & Christy, 2013; O'Brien et al., 2017; Figure 1). We can consider these to be weapon systems when one or more joints (fulcrums) are co-opted, and when multiple components operate in a coordinated fashion, heightening weapon functionality. Although weapon systems have been rarely studied in biomechanical detail outside the crustaceans (Bywater et al., 2015; Dennenmoser & Christy, 2013; Levinton & Allen, 2005; Sneddon et al., 2000), these systems may enable versatile combat maneuvers to exploit the prevailing context and may increase the capacity for rival manipulation, enabling a male to shift and hold a rival in position as he is pinched, punctured, or crushed. Since weapon components are often extremely tough, they may also serve as armor to protect the animal from bodily injury during combat. For all these reasons and more, weapon systems warrant further study. In addition, examining the evolution of multi-component weapons provides outstanding opportunities to trace the assembly and disassembly of weapons over time.

We examined the evolution of 15 components of a weapon system in a fascinating group of armed insects, the leaf-footed bugs and allies (Hemiptera: Coreoidea; Figures 1 and 2). This group includes ~3,300 species in five extant families, and it is one of only a few animal groups that produce weapons on the hind legs (Rico-Guevara & Hurme, 2019). In some species, the hind legs are slim and non-elaborated (Figure 2A); males in these species typically do not engage in male-male combat. In other species, the hind legs exhibit

extreme modifications including weapon components such as robust spines, club-like expansions, flags, and serrations (Figure 2B–G; CoreoideaSFTeam, 2022). In fact, it is the striking, elaborated hind legs that give the common name “leaf-footed bugs” to the Coreidae, the largest family within the Coreoidea. For simplicity, we will hereafter refer to this superfamily as the “leaf-footed bugs.”

The leaf-footed bugs: weapon morphology and behavior

The morphological elaborations on leaf-footed bug hind legs (Figure 2B–D) at times extend to parts of the thorax and abdomen (Figure 2E–G). Where such elaboration exists, it is typically greater in males and is used in fighting (e.g., Figure 1). Male fighting maneuvers are varied; males may lunge, kick, squeeze, slap, pierce, and tear at their rivals (Figure 1; Eberhard, 1998; Emberts & Wiens, 2021; Emberts et al., 2021; Fujisaki, 1981; Mitchell, 1980; Miyatake, 1993, 1995, 1997; Nolen et al., 2017; Okada et al., 2011; Tatarnic & Spence, 2013). Some species engage in escalated combat in a ventral-ventral position when hanging from a plant surface (e.g., the crusader bug, *Mictis profana* [Fabricius, 1803; Tatarnic & Spence 2013]), while others squeeze each other end-to-end (e.g., the heliconia bug, *Leptoscelis tricolor* Miller & Emlen, 2010; Westwood, 1842). Successful males establish territories on plant resources that females need for feeding and laying eggs. Species that fight differently likely experience differential selection on their form, leading to the evolution of varied weapon components. Indeed, many mictine species (such as *Mictis profana* and *Mictis longicornis* Westwood, 1842) possess a bizarre ventral horn—an

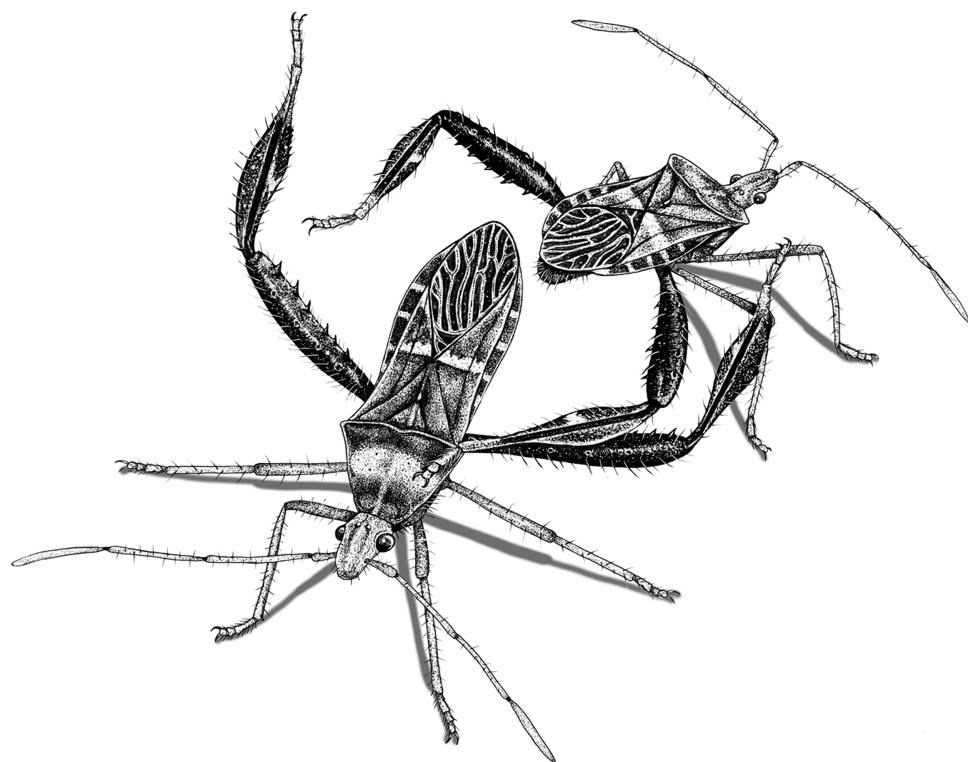


Figure 1. Male leaf-footed cactus bugs, *Narnia femorata* Stål, 1892, engaged in an end-to-end battle over a cactus territory where females mate, feed, and lay eggs. As seen here, many male leaf-footed bugs jockey into position, then press the spines on their femur into their opponent's body. Males of *N. femorata* show enlarged hind legs with spines and flags, including seven of the 15 weapon components studied (Table 1; Figure 4). Illustration by David J. Tuss.

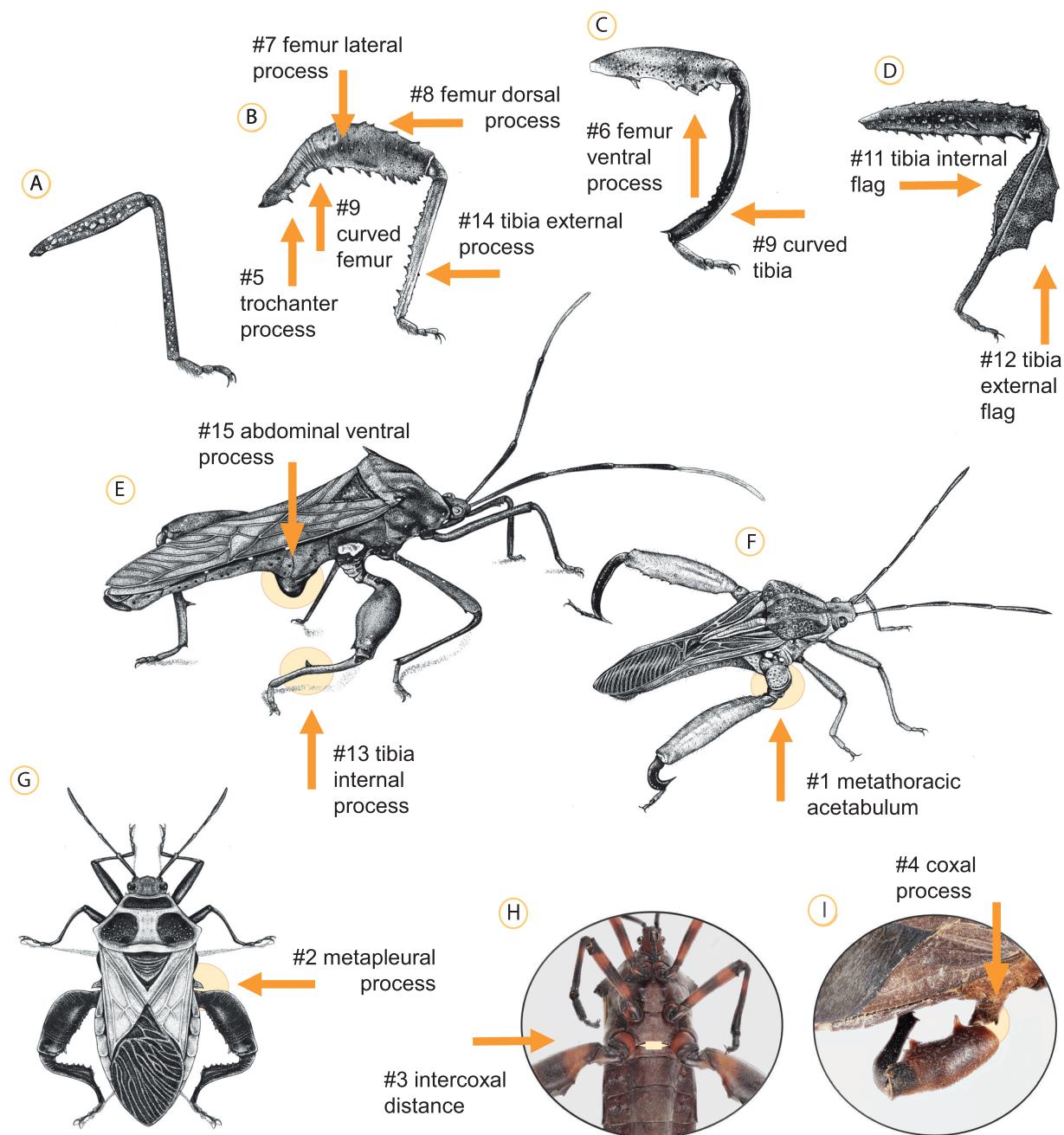


Figure 2. Illustrations of diverse hind leg and body shapes in the leaf-footed bug superfamily. Arrows and text show the weapon components examined. Featured are the hind legs of: (A) *Anasa tristis* (De Geer, 1773) with its simple hind leg (no weaponized components), (B) *Camptischium clavipes* (Fabricius, 1803), (C) *Hyalymenus subinermis* (Van Duzee, 1923), and (D) *Leptoglossus gonagra* (Fabricius, 1775). Full-body specimens include: (E) *Mictis longicornis* (Westwood, 1842), (F) *Alcocerniella limonensis* (Brailovsky, 1999), and (G) *Sagotylus confluens* (Say, 1832). (H) features a ventral view of *Thasus neocalifornicus* (Brailovsky & Barrera, 1994), and (I) shows the coxa of a hind leg of *Rhytidoris terminalis* (Burmeister, 1835). Illustrations by David J. Tuss.

abdominal sternal process—that is jabbed at the other male's horn during ventral-ventral contests (Figure 2F; Tatarnic & Spence, 2013). Fighting injuries are common in leaf-footed bugs and can include legs that are severed or missing, punctures to the legs and abdomen (G. Raina, *in prep*), and torn or punctured wings (G. Raina, *in prep*; Emberts & Wiens, 2021; Emberts et al., 2021).

As in elk (Metz et al., 2018) and many other species (Lane, 2018; Rojas & Burdfield-Steel, 2017), the sexually selected weapons of leaf-footed bugs have functions beyond male-male competition. For example, they serve an important role

in locomotion, and they are involved in predator defense. When attacked, some leaf-footed bugs squeeze attackers with their hind legs (Goodchild, 1977; C. W. Miller, personal observation), a defensive maneuver that can even draw blood (U. Somjee, personal communication). Interestingly, the hind legs of leaf-footed bugs do not appear to be used in mate choice; indeed, experimentally blinded female *Riptortus pedestris* (Fabricius, 1775) do not show differences in mating behavior (Numata et al., 1986), and in the absence of male-male dynamics, female *Narnia femorata* show no reluctance to mate with a male missing a hind limb (C. W. Miller, unpublished data).

Table 1. Morphological components and component state coding. Leg components correspond only to the hind legs (Components #3–14).

Component	Component states
1 Metathoracic acetabulum, visible in dorsal view	(0) Not or slightly expanded laterally in dorsal view; (1) Distinctly expanded laterally in dorsal view
2 Metapleural process	(0) Absent; (1) Present
3 Intercoxal distance	(0) Shorter than distance from coxa to lateral outer margin of metapleuron; (1) Equal to or longer than distance from coxa to lateral outer margin of metapleuron
4 Coxal process	(0) Absent or with very shallow tubercles; (1) Distinct tubercles and/or spines present
5 Trochanter process	(0) Absent or with very shallow tubercles; (1) Distinct tubercles and/or spines present
6 Femur ventral process	(0) Absent or with very shallow tubercles; (1) Distinct tubercles and/or spines present on the distal third or less; (2) Distinct tubercles and/or spines present on distal half or less; (3) Distinct tubercles and/or spines present on more than distal half
7 Femur lateral process	(0) Absent or with very shallow tubercles; (1) Distinct tubercles and/or spines present
8 Femur dorsal process	(0) Absent or with very shallow tubercles; (1) Distinct tubercles and/or spines present
9 Curved femur	(0) Straight or nearly so; (1) Distinctly curved basally
10 Curved tibia	(0) Straight or nearly so; (1) Curved toward the body; (2) Curved away from the body; (3) Sinuately curved
11 Tibia internal flag (=expansion)	(0) Absent; (1) Present
12 Tibia external flag	(0) Absent; (1) Present
13 Tibia internal process	(0) Absent or with very shallow tubercles; (1) Distinct tubercles and/or spines present
14 Tibia external process	(0) Absent or with very shallow tubercles; (1) Distinct tubercles and/or spines present
15 Abdominal ventral process	(0) Absent or with very shallow tubercles; (1) Distinct tubercles and/or spines present

Instead, chemical cues (Aldrich, 1988; Numata et al., 1986; Wang & Millar, 2000) and tactile/auditory cues (e.g., vibration, Numata et al., 1986; tapping, Miller, 2008; and stridulation, Shestakov, 2009) may be more influential in mate choice. Females rarely fight; when they do, it is typically with less intensity than males, and the conflict appears to center on feeding or oviposition sites (Eberhard, 1998; C. W. Miller, *personal observation*). Our focus in this study is the evolution of male weapon morphology, with work forthcoming on the evolution of sexual dimorphism in this superfamily.

Here, we provide the first phylogenetic analysis to investigate the evolution of male weapons across the leaf-footed bugs and one of the first studies across taxa that addresses the separate evolution of multiple weapon components (see also, Chow et al., 2021; Van Kleeck-Hann & Wiens, 2023). We capitalized upon the multi-component nature of the leaf-footed bug weapon by focusing on discrete components, rather than simplifying or generalizing body form. We used 243 ingroup taxa from the insect families Alydidae, Coreidae, and Rhopalidae, as well as five outgroup taxa in the Pentatomomorpha (Supplementary Table S1). Our sampling included a diverse representation of male hind-leg morphologies within leaf-footed bugs, as well as of the many subfamilies and tribes found across major biogeographic regions. We inferred a phylogeny of the superfamily using ultraconserved element (UCE) loci. We then investigated the evolutionary lability of each of these components with ancestral state estimation (ASE). We asked: (a) How evolutionarily labile are

weapons and their components? (b) Does the number of weapon components increase over time? (c) Do certain components co-occur repeatedly, suggesting genetic correlations or coordinated functions during battle?

Methods

Selection of morphological components for study

Numerous leaf-footed bugs, such as *Jadera haematoloma* (Herrich-Schäffer, 1847), *Savius diversicornis* (Westwood, 1842), and *Anasa tristis* (De Geer, 1773), have simple, streamlined bodies and legs (e.g., Figure 2A, Table 1). The females in such species are typically larger than males and exhibit a rounded abdomen, but sex differences in morphology are otherwise minor. Male-male competition has not been reported in these species. Males of other leaf-footed bug species show modifications to this simple body plan including sharp spines, curves, and flags (Figure 2B–G), and these characteristics are often associated with fighting (Figure 1). Our goal with this study was to understand the evolution of such character elaborations. We selected 15 characters that are commonly modified, vary widely in their expression, and are straightforward to score objectively and reliably. Hereafter, we refer to the characters as weapon components. The components, except for the presence/absence of a metathoracic acetabulum (Figure 2E; Component #1), are typically sexually dimorphic when they are elaborated. Indeed, most of these components directly contact, and even injure, rivals during competition.

Yet, much is unknown. Behavior has been documented in only a fraction of the thousands of leaf-footed bug species. Further, not all species with morphological elaboration engage in male-male competition (e.g., *Leptoglossus phyllopus* [Linnaeus, 1767], [Mitchell, 1980](#); *Anisoscelis alipes* [Guérin-Méneville, 1833], [Longbottom et al., 2022](#)). We embrace the rich spectrum in morphology and behavior across leaf-footed bugs, acknowledging that most traits in most species have multiple uses and that the uses vary across the phylogeny. Indeed, our ultimate hope is to encourage work that examines the evolutionary interplay of morphology and behavior in this intriguing group of insects.

Molecular data collection and phylogenetic inferences

For 216 taxa, we retrieved UCE sequence capture data from [Forthman et al. \(2019\)](#); [Kieran et al. \(2019\)](#); [Emberts et al. \(2020\)](#); [Forthman et al. \(2020\)](#); [Forthman et al. \(2022b\)](#); [Miller et al. \(2022\)](#) ([Supplementary Table S1](#)). We also downloaded genome sequences of *Halyomorpha halys* (Stål, 1855) (Pentatomidae) and *Oncopeltus fasciatus* (Dallas, 1852) (Lygaeidae) from NCBI to extract UCE sequences from scaffolds. We generated new sequence data for 30 taxa following DNA extraction, isolation, and library construction approaches described in [Forthman et al. \(2019, 2020, 2022b\)](#). In short, sequence capture was done using baits designed from two pentatomomorphan taxa ([Faircloth, 2017](#); see [Forthman et al., 2019](#)) and using the touchdown capture protocol from [Forthman et al. \(2022a\)](#). Enriched library pools were combined into a single pool in equimolar amounts prior to sequencing on a single Illumina HiSeq3000 lane (2×100) at the University of Florida's Interdisciplinary Center for Biotechnology Research. Sequence reads were demultiplexed, adapter-trimmed, deduplicated, error-corrected, and assembled into contigs following [Forthman et al. \(2022a\)](#). We used PHYLUCE v1.7.0 ([Faircloth, 2016](#)) to identify UCE loci from assembled contigs following ([Forthman et al., 2019, 2020, 2022a](#)). We also used the PHYLUCE to align UCE baits to two genome sequences (*Halyomorpha halys* and *Oncopeltus fasciatus*) and extract UCE loci with 500 bp of flanking nucleotides. A summary regarding newly generated read, contig, and UCE data is given in [Supplementary Table S2](#).

Loci were aligned individually with PHYLUCE using the --mafft setting ([Katoh & Standley, 2013](#); [Katoh et al., 2002](#)), and locus alignments were trimmed using trimAl v1.2 ([Capella-Gutiérrez et al., 2009](#)). Locus alignments with at least 50% and 70% of the total taxa were selected for analysis (referred to as “50p” and “70p” datasets, respectively). We also subsampled each of these datasets for the 25% most parsimony-informative loci (referred to as “25mi”), resulting in four datasets: 50p, 50p25mi, 70p, 70p25mi (see [Supplementary Table S3](#) for a summary of informative sites and number of UCE loci in each dataset).

For the 50p and 70p datasets, we concatenated locus alignments for maximum likelihood (ML) phylogenetic analysis, using the best model of sequence evolution and partitioning scheme identified by IQ-Tree v2.1.2 ([Minh et al., 2020](#)). For each dataset, ten separate partitioned ML analyses ([Chernomor et al., 2016](#)) were performed, with support measured by 1000 ultrafast bootstrap replicates (UFB; [Hoang et al., 2018](#)) and 1000 Shimodaira-Hasegawa-like approximate likelihood ratio test replicates (sh-alrt; [Guindon et al., 2010](#)). For all four datasets, we also inferred species trees from

optimal gene trees using ASTRAL-III v5.7.7 ([Mirarab et al., 2014b](#); [Sayyari & Mirarab, 2016](#); [Zhang et al., 2018](#)), which is a method statistically consistent with the multispecies coalescent (MSC) model ([Degnan & Rosenberg, 2006, 2009](#); [Kubatko & Degnan, 2007](#); [Roch & Steel, 2015](#)). In addition to the 50p and 70p datasets, we included the 50p25mi and 70p25mi datasets for species tree inference given that filtering for more informative loci has been shown to improve topological and branch lengths (in coalescent units) estimates in MSC analyses ([Forthman et al., 2022b](#); [Hosner et al., 2016](#); [Meiklejohn et al., 2016](#); [Mirarab et al., 2014a](#); [Sayyari & Mirarab, 2016](#); [Sayyari et al., 2017](#)). Prior to ASTRAL-III, we estimated individual gene trees using the best-fit model of sequence evolution for each locus alignment using IQ-Tree, with near-zero branch lengths collapsed. We assessed clade support using local posterior probabilities (LPP; [Sayyari & Mirarab, 2016](#)).

Prior to ASE, we transformed our 50p ML and 50p and 50p25mi MSC trees into ultrametric trees. First, we used IQ-Tree to estimate branch lengths as units of substitutions on the 50p and 50p25mi MSC topologies. We pruned out-group taxa and used the *chronos* function in the *ape* package v5.6.1 ([Paradis & Schliep, 2019](#)) with R v4.1.2 ([R Core Team, 2021](#)) to generate ultrametric trees under four models (correlated, discrete, relaxed, and clock) and four values of lambda (0, 0.1, 1, 10). For more specific details on our molecular data collection and phylogenetic inferences, see [Supplementary Materials](#).

Ancestral state estimation

For ASE, 13 components were coded as binary, while two (Components #6 and #10) were treated as multistate components. For Component #6, we assigned three categories for the distribution of the ventral femoral processes, when present. Coding ventral femoral processes as a multistate component rather than a binary present/absent component allowed us to explore whether species with more elaborated hind legs often have a more extensive distribution of spines and tubercles on the ventral surface of the femur compared to species with less elaborated legs. Similarly, for Component #10, the tibia can exhibit four distinctive categories of curvature, and we treated these as separate states to explore patterns of gains and losses relative to other components. We primarily coded trait data from available specimen material, but in some cases, data were retrieved from type images and/or taxonomic descriptions of sampled species.

We estimated ancestral states for each component on the 50p ML and 50p and 50p25mi MSC ultrametric trees using the *rayDISC* function in the R package *corHMM* v2.7 ([Beaulieu et al., 2013](#)), which uses an ML approach to estimate ancestral states and can accommodate multistate characters. We did not include the 70p and 70p25mi datasets as these resulted in the same topologies and similar branch lengths (see *Results* and [Supplementary Figures S1–S6](#); see [Supplementary Methods](#) for further details on generating ultrametric trees). We performed the analyses using marginal reconstruction to obtain the probability of each character state at internal nodes. We used marginal reconstruction since the joint reconstruction option only gives an optimal state for each node, and thus does not give a measure of uncertainty with respect to alternative states. We used the default “yang” root prior, which uses estimated transition rates to weight states at the root. Three models were tested: equal rates (ER),

symmetric rates (SYM) (for multistate components only), and all rates different (ARD). We performed a likelihood ratio test to determine the best-fit model for each component. The *rayDISC* function will output state probabilities at internal nodes, with probabilities summing to 1 for a given node. We considered a state with the majority probability to be the likeliest state for an internal node (e.g., exceeds 0.5 for binary traits), which was then used to count the number of transitions among states for a given component across a phylogeny. In instances where an internal node was ambiguous (i.e., all states are equally likely), the most parsimonious number of transitions involving that node was counted to be conservative in our estimates.

Ancestral states were also estimated using stochastic character mapping via the *make.simmap* function in the R package *phytools* v1.0 (Revell, 2012). This Bayesian method randomly samples discrete character histories that should approximate the posterior distribution of histories, given a tree and model of evolution. We performed 1000 simulations for each component, using the same best-fit models of evolution as in the *rayDISC* analysis. The prior distribution at the root node was also set to “estimated,” which estimates the stationary distribution of state frequencies. The function *densityMap* was used to summarize probabilities of each state along branches, while the posterior probabilities of each state at internal nodes were obtained using the *summary* function. Because we were interested in the number of transitions among component states—i.e., those that occur along branches as well as nodes—, we also estimated the posterior probability distribution of state changes for a given component across the phylogeny using the *density* function; we report the median number of component state transitions and the 95% high probability density (HPD) intervals.

Correlated weapon components

To evaluate whether two putative weapon components are correlated in a phylogenetic context, we used the *fitPagel* function in *phytools*. This function tests for significant correlations between two binary components by employing a commonly used ML method based on Pagel (1994). Pagel’s correlative test first estimates the rate of evolution of two traits under the null hypothesis that they evolve independently. Alternative models are then tested, in which the rate of evolutionary change in one trait depends on the state of the second trait—i.e., testing a dependency model. These alternative models can include a test of whether, for example, Component #1 is dependent on Component #2 or vice versa, as well as whether the two are interdependent. A likelihood ratio test is then used to evaluate whether the independent or any of the alternative models are favored.

Prior to analysis, we converted our two multistate components into binary ones (Component #6: ventral femoral processes [0] absent vs. [1] present; Component #10: tibia [0] straight vs. [1] curved). For components that were not coded as absent or present (i.e., Components #1, #3, #9, and #10), we treated the plesiomorphic state based on ASE results as “absent.” Lastly, we excluded sites (i.e., species) that had missing data for at least one component.

Maddison and FitzJohn (2015) demonstrated several evolutionary scenarios where trait correlation methods will produce misleading associations, which are discussed in further detail below. Maddison and FitzJohn (2015) made clear that there is no easy, objective solution to address these issues, and

to our knowledge, no analytical improvements to these methods have yet to address them. One way to ensure that statistically significant correlations are not incorrectly assigned is to plot ancestral state estimates for each trait onto a phylogeny, visually inspect the evolutionary patterns of trait-state gains between the two traits and evaluate if the evolutionary gains of one trait generally preceded or evolved simultaneously with the other trait or if there is evidence of the issues raised by Maddison and FitzJohn (2015). Below we describe how we assessed our results in light of Maddison & FitzJohn (2015).

The simplest scenario that can produce misleading correlative results is when at least one component has a single evolutionary origin (see the “Darwin’s Scenario” and Figure 1 in Maddison & FitzJohn, 2015). To address this situation a priori, we excluded component pairs from *fitPagel* testing if at least one component had only one evolutionary gain based on ASE results.

Correlated traits tests should produce significant results when there are replicated patterns of origins for both components throughout the phylogeny (see the “replicated co-distribution” and “replicated bursts” and Figure 1 in Maddison & FitzJohn, 2015). However, according to Maddison & FitzJohn (2015), it is possible to recover strong statistical associations between component pairs when replicated patterns of origins for these components occur in nearby clades, but this could be a *potentially* misleading result; the rate of origin for one component could potentially be explained by a third, unrelated component originating in a slightly larger clade that includes closely related “subclades” having independent gains of the second component (i.e., potential for unreplicated effects within lineages; Supplementary Figure S7A; Maddison & FitzJohn, 2015). The greater the number of evolutionary gains of a component and the more dispersed the replicated evolutionary patterns are in the phylogeny, the less concern there is for spurious associations (Maddison & FitzJohn, 2015). Thus, we plotted ancestral state estimates of both components onto a phylogeny and assessed if both components often occurred within the same clades that were relatively dispersed throughout the phylogeny. We excluded component pairs from *fitPagel* testing if one component originated within clades that were in relatively close proximity to the phylogenetic tree (e.g., Supplementary Figure S7A).

For the remaining component pairs, we conducted correlated traits tests using marginal reconstruction. The prior distribution at the root node was set to “estimated.” We used either the ER or ARD model of evolution (SYM = ER model with binary components) based on the best model found in our previous ASE analyses with *rayDISC*; if there were two different models for a pair of components (e.g., ER model for Component A and ARD model for Component B), we selected the most complex model for the *fitPagel* test given the use of a simpler model could potentially bias results due to model misspecification (Lemmon & Moriarty, 2004; Swofford et al., 2001). For each component pair, we performed three *fitPagel* analyses, with two dependency models (a different component treated as the dependent variable) and an interdependent model. A likelihood-ratio test was then used to determine whether one or more of the dependency and interdependency models were favored over the null hypothesis (i.e., two components are independent of one another). We adjusted *p*-values with the Benjamini–Hochberg correction for multiple

comparisons (Benjamini & Hochberg, 1995; Yang et al., 1994), with the statistical significance threshold set to $p < .05$.

To ensure that statistically significant correlations were supported by evolutionary patterns of trait-state gains among component pairs, we re-evaluated the ancestral state estimates for two components a posteriori to assess whether their evolutionary patterns matched predictions of significant dependency models. For example, when a *fitPagel* test supported a single dependency model (i.e., the second dependent and the interdependent models were not significant), we assessed whether the origin of one component generally preceded or evolved simultaneously with the other as would be predicted by the significant model. When patterns of component evolution did not appear to support the significant dependent model, we considered the correlated traits test to have produced a false positive result and did not report the two components as correlated (e.g., see *Supplementary Figure S7B*). If two dependency models were statistically supported but our evaluation suggested that only one of the two models was incorrectly supported, we still considered the correlated traits test to be accurate.

We report all *fitPagel* results in *Supplementary Material*, and for significant correlations recovered, side-by-side comparisons of the corresponding ASE results in *Supplementary Data File 1*. However, in *Results* section, we only report those statistically significant results we considered to be acceptable based on the criteria discussed above and our visual inspection of ASE results.

Results

Familial- to tribal-level phylogenetic relationships

Phylogenetic hypotheses among the families and subfamilies of leaf-footed bugs (*Figure 3*, *Supplementary Figures S1–S6*) were congruent with recent phylogenomic studies, which have supported the non-monophyly of Alydidae, Coreidae, Coreinae, and Meropachyinae (Emberts et al., 2020; Forthman et al., 2019, 2020, 2022b; Miller et al., 2022). Relationships among the tribes of Coreinae + Meropachyinae were also largely congruent with results of Forthman et al. (2020), however, we found several differences. For example, we did not recover a monophyletic Acanthocorini or Dasynini. While we found high support for Clade E (*Figure 3*) as the sister group of Clade D in our MSC analyses (LPP: 0.95–1.00; *Supplementary Figures S3–S6*; congruent with Forthman et al., 2020), our ML analyses found Clade E to be the sister group of Clade F + Clade G (*Figure 3*, *Supplementary Figures S1 and S2*), also with high support (UFB: 99–100; sh-alrt: 99.9–100). The phylogenetic position of Clade F was also unstable across our analyses (*Figure 3*, *Supplementary Figures S1, S2, and S6*). Lastly, we also continued to find support for the polyphyly of Anisoscelini and Hypselonotini following Forthman et al. (2020), but we recovered an additional lineage of Anisoscelini (all analyses) and one (ML analyses) to two lineages (MSC analyses) of Hypselonotini.

Diverse weapon trait combinations in the leaf-footed bugs

Our results are based on phylogenetic hypotheses and probabilistic models and should thus be regarded as best estimates given the analytical approaches employed. We found a rich diversity of weapon trait combinations. Several clades include multiple species with a high number of weapon components

(*Figure 4*). Processes off the ventral femora (Component #6) were the most common (170 coreoid taxa out of 243 sampled), followed by the internal tibial processes (Component #13; 111 taxa). Indeed, the presence of any weapon component was often accompanied by one or more spines coming off the ventral femora (Component #6; 146 taxa), though these spines also occurred by themselves (e.g., *Anasa scorbutica* [Fabricius, 1775]) (*Figure 4*). Species with knobs or spines distributed on more than half of the ventral femoral surface also had more elaborated hind legs compared to species with a more restricted distribution of processes. The laterally expanded metathoracic acetabulum (Component #1; five taxa), metapleural process (Component #2; 13 taxa), and coxal and trochanter processes (Components #4 and #5, respectively; 18 and nine taxa, respectively) were some of the least common components. All thoracic (Components #1 and #2) and abdominal (Component #15) components were found paired with elaborated hind-leg components, but not vice versa.

In reporting our ASE results based on our ML analysis, we estimated the number of transitions among component states by counting transitions among states with the highest probability at internal nodes (*Supplementary Figures S8–S53*). For stochastic character mapping, we report the estimated median number of transitions and HPD intervals (*Supplementary Figures S54–S104*). Our ML ASE results generally produced the most conservative estimates of the minimum number of trait gains and losses. Thus, we mainly focused on reporting results based on *rayDISC* analyses in the *Results* section, but results from all analyses are available in *Supplementary Materials*.

Most of our ASE analyses (except for Component #6, State 3 in the 50p25mi stochastic character mapping analysis) suggest the metathorax, hind legs, and abdomen of the last common ancestors of leaf-footed bugs (Node A) lacked weaponized components (*Figure 4*, *Supplementary Figures S8–S104*). Our analyses also suggest the last common ancestor of Alydidae + Coreidae (Node B) and Coreinae + Meropachyinae (Node C) also lacked weaponized components on the metathorax and abdomen (*Supplementary Figures S9, S10, S23–S25, S38–S40, S53–S55, S70–S72, and S87–S89*). Most of our ASE results also did not estimate any weaponized components for the hind legs in the last common ancestors of Node B (except analyses using the 50p25mi ultrametric tree; *Figure 4*, *Supplementary Figures S11–S22*). With respect to Node C, the majority of the ASE analyses also supported the presence of ventral femoral processes in the ancestor (*Figure 4*, *Supplementary Figures S29, S44, S59, S76, and S93*). Thus, our results from different analytical approaches and topologies suggest that “simple” legs (i.e., those lacking elaborations) were likely the ancestral condition in the superfamily or possessed a single weaponized component in the form of spines and tubercles on the femur.

Our best estimates from ASE analyses suggest that all components were convergently evolved within leaf-footed bugs, regardless of data filtering, topology, and analytical approaches (*Table 2*, *Supplementary Tables S4–S6*; *Figure 5*, *Supplementary Figures S8–S104*). All weapon component states were reconstructed with at least two or more gains (range of minimum number of trait-state gains = 2–19 [*rayDISC*] or 2–26 [stochastic character mapping]). The wider intercoxal distance (Component #3) and presence and distribution of femoral and tibial processes (Components #6, #13,

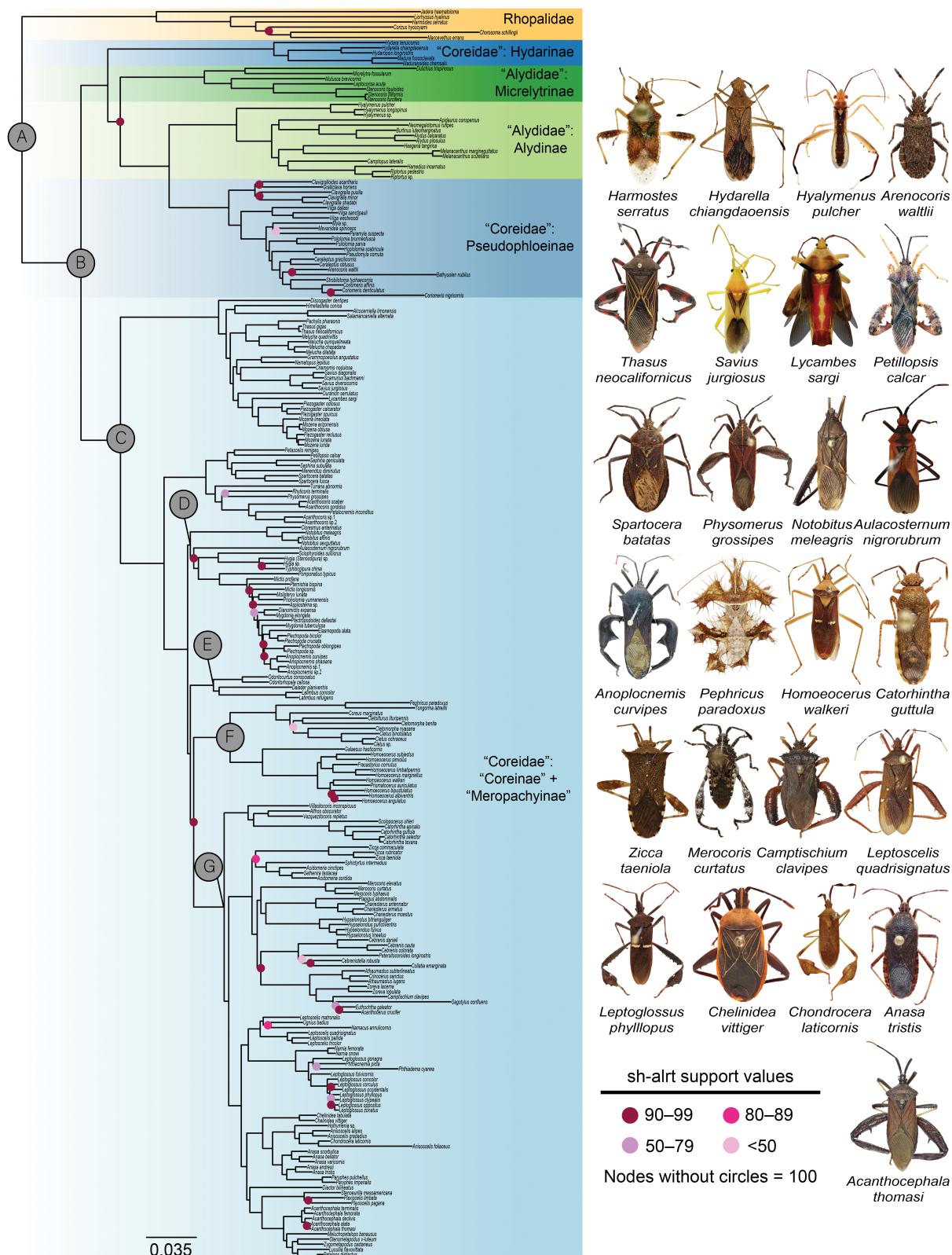


Figure 3. Maximum likelihood (ML) best tree based on the 50p concatenated alignment (outgroups not shown). Nodes labels A–G refer to clades discussed in the text. Colored circles at nodes represent instances when Shimodaira–Hasegawa-like approximate likelihood ratio test (sh-alrt) support is less than 100 (see *Data availability* for tree with all terminals and sh-alrt and ultrafast bootstrap values visible). Dorsal habitus images of select species are given to show a range of diversity within the Coreoidea (images not to scale). The families Alydidae and Coreidae, as well as the subfamilies Coreinae and Meropachyninae and several tribes within them, are not monophyletic.

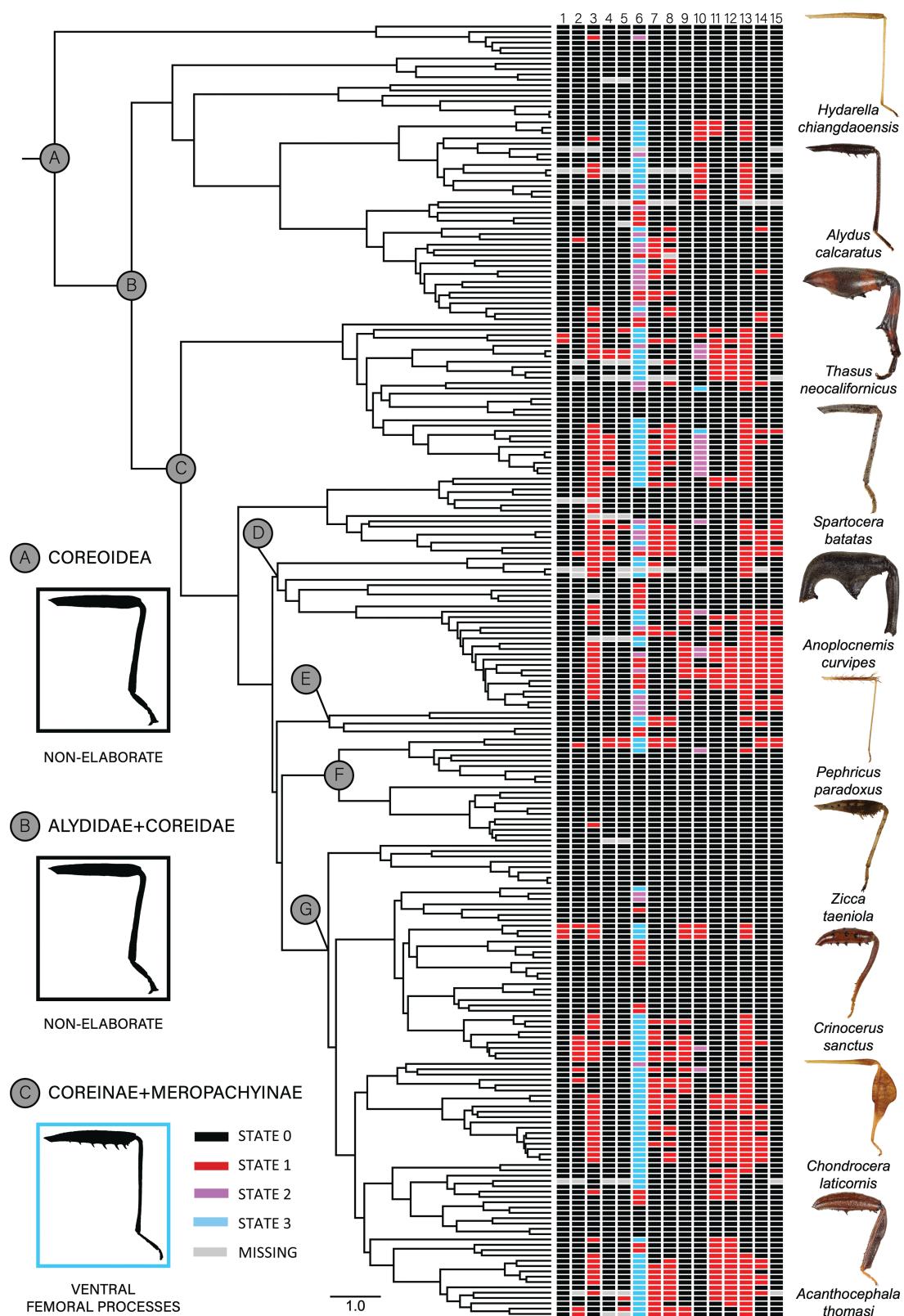


Figure 4. Ultrametric tree based on the 50p ML best tree, with components and component states displayed for terminal taxa on the right; images not to scale. Names of terminal taxa are removed for visualization purposes (refer to Figure 2 and Data availability for terminal names of this topology). Nodes labels A–G refer to clades discussed in the text. For select nodes, an illustration representing the general male hind leg morphology based on a majority consensus of ancestral state estimation (ASE) results across all analytical approaches and topologies is given; non-elaborate hind legs lack processes, flags, and curved femur and tibiae. In almost all ASE analyses, the last common ancestors of leaf-footed bugs (Nodes A [five out of six analyses] and B [four out of six]) lacked elaborated weapon components on the thorax, hind legs, and abdomen. The last common ancestor of “Coreinae” + “Meropachyinae” (Node C) was estimated to have ventral processes on the femur (five out of six analyses). Across the phylogeny, there is a rich diversity of weapon trait combinations, with species in several clades expressing a high number of weapon components.

Table 2. Summary of the minimum number of component state gains and losses across different ultrametric trees based on maximum likelihood (ML) ancestral state estimates and stochastic character mapping (SCM) (component state 0 not reported). For results specific to the 50p ML, 50p MSC, and 50p25mi MSC ultrametric trees, see [Supplementary Table S4](#). Abbreviations: ARD, all rates different model; ER, equal rates model; G-L, minimum gains minus minimum losses; Min, minimum; N, sample size; RD, *rayDISC* ML ASE analysis; SYM, symmetric model.

Component number	Model	Component states	N taxa	RD		SCM	
				Min gains	Min losses	Min gains	Min losses
1	ER	1	5	2	0	2	0
2	ARD	1	13	8	2	11	8
3	ER	1	89	19	3	22	5
4	ARD	1	18	6	4	8	8
5	ER	1	9	7	0	7	0
6	SYM (50p ML & MSC)/ ARD (50p25mi MSC)	1	36	18	2	25	1
		2	31	13	11	26	26
		3	103	13	26	15	37
7	ARD	1	59	15	12	21	22
8	ARD	1	55	14	18	21	31
9	ARD	1	27	5	7	7	11
10	SYM	1	18	4	2	4	2
		2	16	10	0	10	2
		3	2	2	1	2	0
11	ARD	1	53	5	11	7	15
12	ARD	1	47	5	9	7	12
13	ER	1	111	15	7	17	9
14	ARD	1	45	13	7	17	12
15	ARD	1	26	5	3	5	5

and #14) had the most independent gains estimated ([Table 2](#), [Supplementary Tables S4–S6](#)). Losses or reductions were estimated for 13 components (range of minimum number of trait-state losses = 1–18 [*rayDISC*] or 1–37 [stochastic character mapping]). The presence and distribution of femoral processes and presence of tibial flags exhibited a high number of losses (Components #6 [State 2], #7, #8, #11, and #12). Only losses of tibial flags (Components #11 and #12) outnumbered their evolutionary gains by about 2:1 across our ASE estimates, as well as the extensive distribution of spines and tubercles on the ventral surface of the femur (Component #6, State 3) based on stochastic character histories recovered. All other components and component states had more gains than losses or slightly more losses than gains reconstructed. We also evaluated whether branch lengths were associated with the number of evolutionary transitions. While some of the highest numbers of transitions occurred on relatively long branches ([Figure 5A](#)), in all other cases, long and short branches were associated with low to moderately high numbers of transitions, suggesting that these transitions did not occur in a “clock-like” fashion.

We also tested the hypothesis that weapons evolve with greater complexity over evolutionary time. Our results suggested a general accumulation of weapon components along internal branches for several clades in leaf-footed bugs ([Figure 5B](#)). However, we observed about 50 instances of reductions in weapon complexity, mostly near the tips of the tree, with two lineages having lost weapon components entirely. In about 20 cases, an initially more complex weapon began to exhibit reduced complexity in some clades, but then shifted back toward increasing complexity near the tips of the phylogeny ([Figure 5B](#), [Supplementary Figure S105](#)).

A complex network of correlations among weapon components

Our results from binary correlated traits tests suggested that many weapon components are significantly correlated with one another (26–35 component pairs), albeit with some differences when using different phylogenetic topologies ([Supplementary Table S7](#); [Figure 6](#), [Supplementary Figures S106 and S107](#)). The metathoracic acetabulum (Component #1) was the only component not correlated with any other components, regardless of tree topology. The intercoxal distance (Component #3), femoral processes (Components #6–#8), and tibial processes (Components #13 and #14) were estimated to have some of the highest numbers of statistically significant correlations with other components. In contrast, the metapleural process (Component #2), trochanteral processes (Component #5), and tibial flags (Components #11 and #12) had the lowest number of statistically significant correlations.

Discussion

Our ML and Bayesian ASE results suggest that the ancestor of leaf-footed bugs possessed simple hind legs and a streamlined body. Morphological elaborations then arose, including sharp spines, flags, curves, and serrations. The number of these elaborations increased over time in many clades. Yet we also found many examples of reductions, sometimes followed by rapid elaboration again, implying a cyclical nature of weapon complexity. We detected numerous instances of correlated evolution of weapon components, which allude to testable hypotheses of coordinated function during battle.

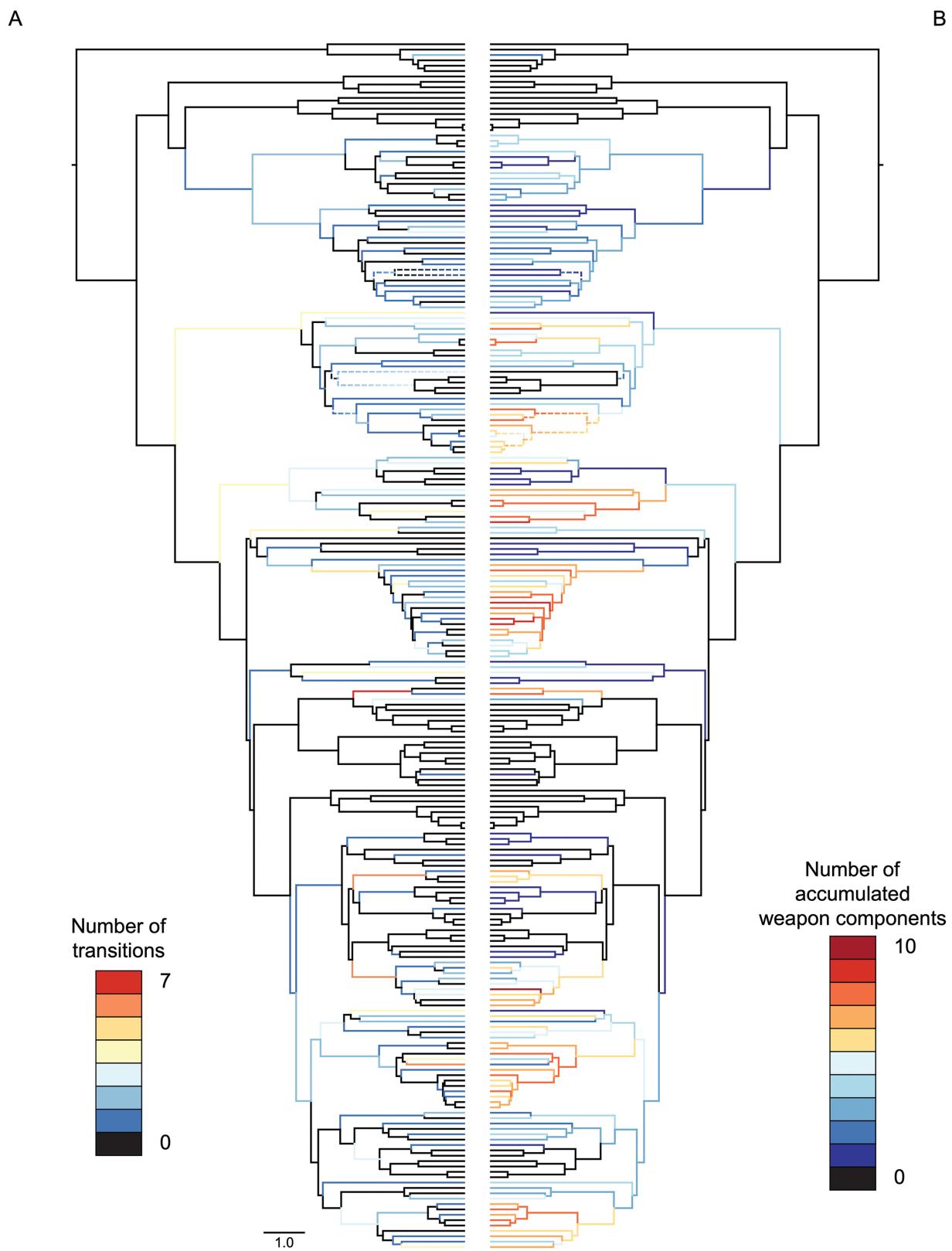


Figure 5. Summary of the total number of (A) inferred transitions (i.e., sum of total gains and losses) and (B) number of weapon states accumulated on branches based on *rayDISC* ASE analysis of the 50p ML ultrametric tree. Taxa with missing data and names of terminal taxa are removed for visualization purposes (refer to Figure 2 and *Data availability* for terminal names of this topology). Dashed lines indicate branches affected by at least one component having an ambiguous ancestral state; in this case, a color gradient is given to represent the range of the total number of transitions or weapon states along the branch components. Our results suggested that weapon components generally accumulated along internal branches of several clades, but there were many instances of subsequent reductions in weapon complexity over evolutionary time. In some cases, reductions in weapon components were followed by shifts back toward increasing the number of components.

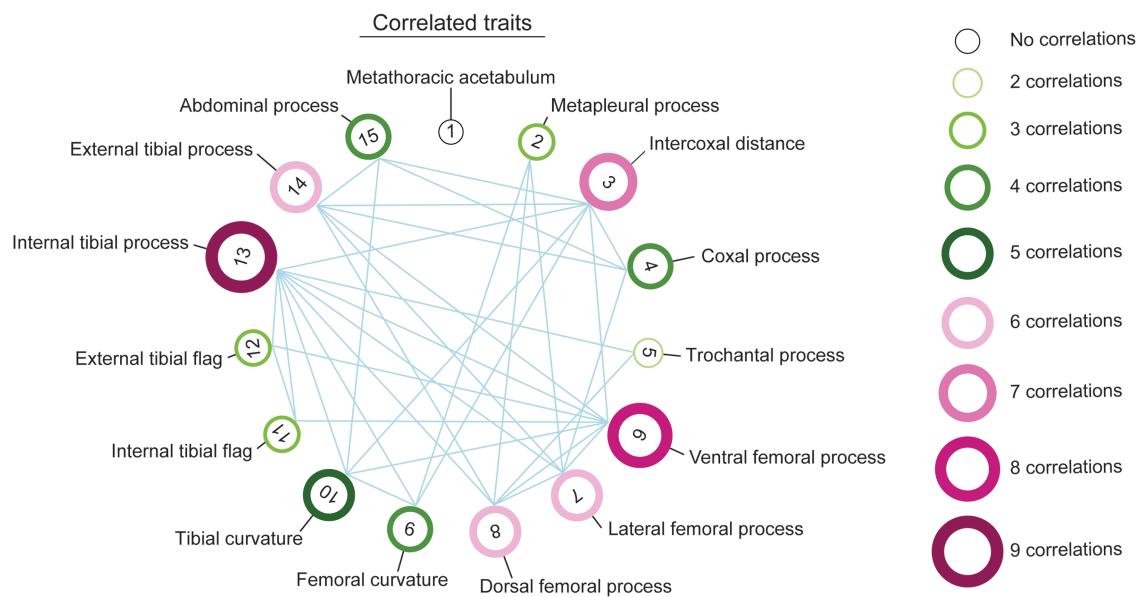


Figure 6. Correlated binary components based on *fitPage* analysis using the 50p ML ultrametric tree and evaluated using *rayDISC* ASE analysis. The size and color of circles around component numbers reflect the total number of statistically significant associations. Many of the weapon components examined are expressed alongside other components, with the intercoxal distance (#3), ventral femoral processes (#6), and internal tibial processes (#13) exhibiting a high number of correlations with other components.

A breathtaking level of diversity and complexity unfurled from the ancestor of leaf-footed bugs. Our best estimates suggest that multiple discrete weapon components evolved independently numerous times. For example, knobs and spines on the apex of the ventral side of the hind femur arose independently on at least 18 occasions, becoming one of the most common weapon elements (based on the *rayDISC* ASE analysis of the 50p ML topology, [Supplementary Table S4](#), [Supplementary Figure S14](#)). Curvature of the tibia away from the body evolved at least 10 times independently, while a tibia curved toward the body arose at least 4 separate times. The lability of weapon components is remarkable and reminiscent of the extreme evolutionary modifications in the jaws of marine wrasses and freshwater cichlids ([Wainwright et al., 2012](#)). Other studies of animal weapons have suggested high lability in location, size, and general type ([Dalebout et al., 2008](#); [Emlen et al., 2005](#); [Kim & Farrell, 2015](#)). Yet, few studies have examined the separate evolution of multiple weapon components. However, [Chow et al. \(2021\)](#) examined the evolution of five components of the decapod claw across 107 species, finding five independent origins of snapping behavior and showing that snapping appendages can evolve via multiple evolutionary pathways. [Van Kleeck-Hann and Wiens \(2023\)](#) investigated 11 weapon components in chameleons and found that each was gained at least 4 times and lost at least once. As in leaf-footed bugs, some chameleon species show almost all weapon components, while others possess none.

Our results support the hypothesis that greater weapon complexity evolved over time. Patterns of increasing weaponization have been previously hypothesized (e.g., [Emlen, 2008](#); [Moore et al., 2022](#)), but they have very rarely been tested using phylogenetic analyses. Across taxa, early animal weapons were likely small, sharp extensions such as spines, spurs, and fangs ([Emlen, 2008](#)). In the case of leaf-footed bugs, weapon elaboration likely started with structures bulging and projecting out of the ventral side of the hind femur

([Figure 2](#)). The addition of novel weapon components or the elaboration of existing components may provide an advantage in signaling a male's fighting prowess ([Clutton-Brock et al., 1979](#); [Searcy & Nowicki, 2010](#)), or it may directly yield a fighting advantage, for example, allowing a male to better grasp another male's body part more effectively during battle (e.g., tubercles used in beetles that clamp, [Eberhard, 1979](#); and fiddler crabs that grip, [Dennenmoser & Christy, 2013](#)).

The leaf-footed bugs likely experienced a proliferation of weapon components over time, but our results suggest that losses and reductions of these weapon components were also abundant. For example, *Petillopsis calcar* (Dallas, 1852) has seven weaponized components, while its close relative, *Sephina geniculata* Distant, 1881, possesses only one ([Figures 4 and 5, Supplementary Figures S8–S104](#)). *Camptischium clavipes* (Fabricius, 1803) exhibits 10 weaponized components, while its close relative, *Zoreva lacerna* Brailovsky & Barrera, 1982, has two ([Figures 4 and 5, Supplementary Figures S8–S104](#)). Looking across the phylogeny, we estimated that the dorsal side of the hind femur experienced at least 14 independent gains of spines and knobs followed by at least 18 losses or reductions in descendent taxa. Our analyses also suggested that curvature of the tibia toward the body was gained independently at least 4 times and then lost (i.e., reversal to a straight tibia) twice. The fact that some components were lost more often than others suggests that they may have been less functionally integrated with other components. Similarly, they may have provided fewer fitness benefits or greater costs, perhaps due to biomechanical compromises or energetic demands.

Weapons and other morphological elaborations cannot continue to become larger and more complex indefinitely. Large weapons may come with biomechanical costs. For example, when the horns of rhinoceros beetles experience an evolutionary increase in length, they generate weaker lifting forces ([Weber et al., 2023](#)). Importantly, even a modest weapon may be associated with costs that outweigh benefits

in some contexts (Miller, 2013; Miller & Svensson, 2014). For example, weapons may have high physiological demands during development, maintenance, or use (Basolo & Alcaraz, 2003; Somjee et al., 2018; O'Brien et al., 2019; but, see Kotiaho, 2001; McCullough & Emlen, 2013; McCullough et al., 2012). Thus, times of resource scarcity (Boggs, 2009) or heightened parasite loads (Hamilton & Zuk, 1982) may increase the costs and trade-offs associated with investing in a morphological structure with numerous components. In those cases, males with fewer or smaller components may achieve higher fitness than those investing in complex structures (Brockmann, 2001; Emlen, 2014). In addition, changes in predator abundance may raise the risks of predation for males that invest in certain bulky or conspicuous traits, thus selecting against some forms of elaboration (Møller, 1996; but, see Lane, 2018; Metz et al., 2018). Furthermore, given that these structures are likely often important in sexual selection, changes in resource distribution across the landscape and over time may make females more dispersed and less defensible (Emlen & Oring, 1977), reducing the benefits of weapon investment (Del Sol et al., 2021; Lüpold et al., 2014). Considering the many scenarios that should favor reduced weaponization, it is perhaps not surprising that weapon losses are so common. Indeed, the loss of sexually selected components has been documented in stalk-eyed flies, dung beetles, birds, artiodactyls, and more (Baker & Wilkinson, 2001; Caro et al., 2003; Chow et al., 2021; Kim & Farrell, 2015; Kimball et al., 2011; Menezes & Palaoro, 2022; Wiens, 2001). Interestingly, in leaf-footed bugs, our results suggest that a reduction in weapon complexity was, in some cases, followed by increasing complexity near the tips of the phylogeny, hinting at a cyclical nature to weapon elaboration. Weapon evolution may commonly involve such patterns, but tests of this hypothesis are scarce (Emlen, 2014 & D.J. Emlen, *personal communication*). The return of favorable conditions after a weapon loss or reduction may quickly select on a small knob, spine, and flag for expansion, leading to the regain of weapon components in a lineage.

Weapon forms can be associated with specific fighting styles (Caro et al., 2003; Eberhard, 1980; Geist, 1966; Lundrigan, 1996). The high plasticity and lability of behavior (West-Eberhard, 2003) suggest that changes in fighting style may take the lead in evolution, with morphology to follow (Emlen, 2008). The questions of why male fighting styles initially change and how such changes are retained are largely unaddressed. For many species, the structural context in which fights occur may be central. For example, competitions that occur in flat open spaces should take a different form than competitions that occur in tight burrows or dense vegetation (Cabrera & Stankowich, 2020; Eberhard, 1980; Emlen, 2008). Clades of animal species where males fight in a variety of structural contexts provide outstanding opportunities to investigate the role of the arena in the alteration of fighting behaviors. A wide range of host plant species is used by the ~3,300 species of leaf-footed bugs (Mitchell, 2000; Schaefer & Mitchell, 1983). Thus, males fight upon many different surfaces, such as the smooth shafts of bamboo (Miyatake, 1995), spiny cacti (Procter et al., 2012), or leafy, flexible legumes (Tatarnic & Spence, 2013). In some cases, a single leaf-footed bug species can use a wide range of host plants. For example, the well-armed Florida leaf-footed bug, *Acanthocephala femorata* (Fabricius, 1775), uses plants as strikingly different in structure as sunflower (*Helianthus annus*), white goosefoot (*Chenopodium album*), and yellow thistle (*Cirsium horridulum*) (Baranowski & Slater, 1986). Fighting surfaces should influence gripping, and the structure of the host plant will affect the space available for combat maneuvers. It would be fascinating to study the dynamics of selection on weapon components in *A. femorata* and other species that use multiple host plants.

All biological motion is subject to the laws of physics. As a result, mechanics and evolution are inescapably linked. Certain components of a weapon should thus be expected to correlate with other components, and together they should function in an integrated manner (Chow et al., 2021; Munoz et al., 2018; Nogueira et al., 2022). Palaoro & Peixoto (2022) recently called for studies to move away from simplified measures of weapons and focus on a better understanding of weapon functionality in less-studied taxa. Here, we detected 86 binary (presence/absence) trait combinations in 248 leaf-footed bugs. A network of evolutionary associations was estimated from our analyses (Figure 6). Correlations among components may indicate pleiotropy or linkage disequilibrium shaping the pathways of weapon evolution. Furthermore, the correlations suggest testable hypotheses of biomechanical function and integration. For example, when tibia curve (Component #10), it is typically away from the body, often with one or more prominent internal spines (Component #13; e.g., see tibia in Figure 2F). The curved, spined tibia is often associated with a curved femur (Component #9), which may act as a catching arch to help hold the opponent in place while the tibial spines pierce into its body. We also found that the metapleural process (Component #2), a spine that emerges laterally from the thorax, is found in species with curved femurs possessing dorsal or lateral projections (Figures 2 and 6), though how these structures would function together will remain unclear until behavioral analysis is pursued. Van Kleeck-Hann & Wiens (2023) examined eight pairs of adjacent characters on the head and body of chameleons and also found patterns of association: gular spines are positively associated with gular crests, tail spines with tail crests, and ventral crests with dorsal crests.

Evolutionary associations may highlight components at the heart of a functional weapon system. In leaf-footed bugs, the components with the greatest number of correlations with other components include bumps, knobs, or spines on the inside of the tibia (Component #13) and femur (Component #6) and an increased intercoxal distance (Component #3)—which is somewhat akin to broad shoulders in humans. The bumps, knobs, and spines may be useful for grip and/or as a concentrated force point during squeezing (Figures 2 and 6). Increased intercoxal distance was one of several traits that we included in our analyses without prior direct evidence that it was used in or contributed to success in aggressive interactions. However, the substantial number of correlations with other components suggest that it may be part of the morphological machinery of most leaf-footed bug weapons (see also Okada et al., 2012). Interestingly, tibia flags (Components #11 and #12) had among the lowest number of correlations, supporting behavioral evidence that the primary function of tibial flags is not combat (Longbottom et al., 2022; Mitchell, 1980).

Future work

Animal weapons provide a wealth of opportunities to understand the evolution of complexity, assembly, and integration.

Future work should continue to examine the evolutionary assembly of weapons by examining how individual components come together and are eventually lost. Furthermore, it is important to consider that diversity in animal weapons extends beyond what we can easily see and measure. For example, a functional weapon requires more than morphological expansions and other modifications (Lailvaux & Irschick, 2006; McCullough et al., 2014). Indeed, selection should also act on the internal structure and material properties of weapons to enhance functionality and reduce structural failure. Across (Swanson et al., 2013), and even within species (Woodman et al., 2021), weapons can vary in their ability to resist the rigors of combat. Our hope is that the patterns revealed in the current study will spark behavioral, biomechanical, and phylogenetic studies for many years to come.

Conclusions

For centuries, humans have been fascinated by the weapons animals use to engage in battle. Sexually selected weapons are surprisingly diverse in form, even across closely related species. Here, we use the leaf-footed bug superfamily as a model system to explore the evolution of a multi-component weapon. Our results suggest a general increase in weapon complexity over time with the addition of weapon components. However, many gains and losses occurred along the way. Our best estimates revealed that certain components were more likely to evolve with others and that some components, like hind femur bumps, knobs, and spines, were highly correlated with other components, suggesting they may be a crucial part of the weapon apparatus. These results illustrate the remarkable and dynamic evolution of weapon form in the leaf-footed bugs and suggest many future avenues for study across animal taxa.

Supplementary material

Supplementary material is available online at *Evolution*.

Data availability

Sequence read files of newly generated data are available on NCBI's Sequence Read Archive under BioProject PRJNA939638. Sequences of previously published data are available on NCBI under the accessions provided in online **Supplementary Material**. The character matrix, alignment, and phylogenetic tree data underlying this article are available in the FigShare project titled "The evolution of multi-component weapons in the superfamily of leaf-footed bugs" (https://figshare.com/projects/The_evolution_of_multi-component_weapons_in_the_superfamily_of_leaf-footed_bugs/161032).

Author contributions

Funding was acquired by C.W.M. All authors jointly conceived the study. M.F. assembled the dataset. R.T.K. and M.F. designed the analyses. M.F. carried out the analyses. M.F., R.T.K., and C.W.M. envisioned and produced the figures. C.W.M and M.F. jointly drafted the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Funding

This work was supported by the National Science Foundation Awards IOS-1553100 and IOS-2226881 to C.W.M. An

additional contribution was provided by the University of Florida Agricultural Experiment Station and the National Institute of Food and Agriculture, U.S. Department of Agriculture, HATCH under FLA-ENY-006407.

Conflict of interest: The authors declare no conflict of interest.

Acknowledgments

We thank E. V. Ginny Greenway, David Labonte, and Douglas Emlen for providing helpful feedback and for invigorating discussions. Christiane Weirauch, Dimitri Forero, Paul Masonick, Joe Eger, Petr Kment, Marcos Roca-Cusachs, Vasily Grebennikov, Takahisa Miyatake, Nik Tatarnic, Jason Cryan, Mark Deyrup, Wei Song Hwang, Li You, Oliver Keller, John Leavengood, Wanessa da Silva Costa, Sam Noble Oklahoma Museum of Natural History, Field Museum of Natural History, Florida State Collection of Arthropods, California Academy of Sciences, and National Museums of Kenya contributed specimens. We thank Bob McCleery, Cebisile N. Magagula, and the Savannah Research Center for facilitating specimen collection in Eswatini. South African specimens were collected under Ezemvelo KZN Wildlife permit #OP 172/2018 and the iSimangaliso Wetland Park Authority (World Heritage Site). Additional samples were collected with funding by University of Florida Research Abroad for Doctoral Students, Lee Kong Chian Natural History Museum fellowship, and National Science Foundation (NSF) OISE-1614015 to Zach Emberts (Australia permit #01-000204-1; Singapore permit #NP/RP17-012); the Society of the Study of Evolution Rosemary Grant; Smithsonian Tropical Research Institute Short Term Fellowship; and Systematics, Evolution, and Biodiversity Endowment Award (Entomological Society of America) to Ummat Somjee; and NSF DEB-0542864 to Michael Sharkey and Brian Brown. Nathan Friedman, Caroline Miller, and Min Zhao assisted with molecular benchwork.

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