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Functional Allometry of Sexually Selected Traits in *Tetraopes tetrophthalmus* Red Milkweed Beetles (Coleoptera: Cerambycidae)

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ABSTRACT

The allometry of sexually selected traits is highly variable. Some traits scale steeply in relation to body size while other traits scale more shallowly. Still others scale proportionately to body size. One explanation for this variability is trait function. Sexually selected traits should scale differently according to the function that they perform—the functional allometry hypothesis. Here, we investigate the functional allometry hypothesis in red milkweed beetles, *Tetraopes tetrophthalmus* (Coleoptera: Cerambycidae), a species that possesses multifunctional sexually selected traits. Males use their mandibles to eat and as weapons to fight rivals. Their antennae act as sensory organs and as tactile signals during copulatory courtship. We measured these sexually selected traits along with body size in both males and females. We also measured thorax and leg size, traits presumably not under sexual selection, as control traits. We found that tactile signal traits used in courtship were negatively allometric, while weapons, traits supporting the weapon, and control traits were isometric. Although male mandible weapons were isometric, we found that female mandibles were negatively allometric. Finally, weapon shape was also examined as this trait is important to fighting outcomes. We found that male mandible weapons have a rugose medial curve with a midpoint ledge that female mandibles lack. These results support the functional allometry hypothesis for variation in trait allometry. We discuss how interactions between the functions that these traits perform may influence the evolution of morphology.

1 | Introduction

Traits that evolve under sexual selection are highly variable in how their size scales relative to body size among adults (Bonduriansky 2007; Bonduriansky and Day 2003; Eberhard et al. 2009; Eberhard et al. 2018; Stern and Emlen 1999; Voje 2015). Such scaling is called static allometry (hereafter ‘allometry’) (Huxley 1924). Trait allometry can be described by first using $\log_{10}$-$\log_{10}$ transformations to place different traits of different size ranges in a comparable metric and examining the slopes of regressions of trait size on body size—that is, their allometric slopes (Huxley 1924). Traits that scale proportionately to body size have an allometric slope of one and are called isometric. Traits that are disproportionately large in large individuals (i.e., scale steeply on body size) have allometric slopes greater than one (positive allometry), whereas traits that are disproportionately small in large individuals (i.e., scale shallowly on body size) have allometric slopes less than one (negative allometry). Sexually selected traits span this range of variation in allometries (Bonduriansky 2007; Bonduriansky and Day 2003; Eberhard et al. 2018). Some traits, like the eye span of male stalk-eyed flies used as a visual aggressive signal, are disproportionately large in large individuals (Oliveira Vasconcelos et al. 2019) while others, like the ornamental feathers of some birds, are disproportionately smaller in large individuals (Cuervo and Møller 2001). Still other sexually selected traits, like the chelicerae of male ornate tiger spiders used in visual courtship, scale proportionately to body size (Costa-Schmidt and De Araújo 2008). This very broad range of variation in the allometry of sexually selected traits might arise from different statistical approaches. However, many studies that use the same approaches still observe allometric slope variation which is perplexing and invites investigation.

One explanation for the observed broad range of variation in the allometry of sexually selected traits is the functional allometry hypothesis (Eberhard et al. 2018; Rodríguez and Eberhard 2019). This hypothesis states that although all sexually selected traits play a role in sexual competition, different types of sexual traits are deployed in different ways and those different functions influence the allometric scalings that the traits evolve. For instance, body size is a strong determinant of success in one-on-one fights (Andersson 1994; Emlen 2008; Emlen 2014; Thornhill and Alcock 1983), and bluffing about one's size in an aggressive context is easily detected and punished (Berglund et al. 1996; Jenssen et al. 2005). Consequently, long-range aggressive signals should evolve to clearly indicate body size. The functional allometry hypothesis thus predicts steep allometric slopes for long-range aggressive signals (Eberhard and Rodríguez 2023; Eberhard et al. 2018; Rodríguez and Eberhard 2019). By contrast, long-range courtship signals are less likely to be tied to body size for functional success because mating partners may or may not attend to body size and bluffing in nonaggressive contexts is less risky. Long-range courtship signals are thus predicted to have shallower allometric slopes (Eberhard et al. 2018). Yet, another type of sexually selected trait is structures used in tactile courtship (e.g., genitalia that evolve to stimulate the mate under selection from cryptic mate choice) (Eberhard 1985; Eberhard 1996; Eberhard 2009a). Such tactile traits are selected to contact and adequately stimulate the body part that it contacts. Therefore, these traits are predicted to scale negatively with body size (Eberhard 2009a; Eberhard et al. 1999; Eberhard et al. 1998; Eberhard et al. 2018; Rodríguez and Eberhard 2019). Finally, structures that function in aggressive contact in fights (i.e., weapons) may vary in allometric scaling according to how the biomechanics of force application relate to weapon and body size (Dennenmoser and Christy 2013; Eberhard et al. 2018; McCullough 2014; McCullough et al. 2014; Rodríguez and Eberhard 2019). Predictions for weapons are thus harder to specify, although cases in which the aggressive signaling function can be disentangled from the force-application function suggest steeper and shallower scalings, respectively. These predictions are best stated for traits that have single and clear ('pure') functions (e.g., traits used exclusively as aggressive signals, as weapons, or in tactile courtship) (Eberhard et al. 2018; Rodríguez and Eberhard 2019). This is rare in nature. The next best thing for testing the functional allometry hypothesis is to assemble a broad sample of tests with different species.

Here, we examined several traits associated with male-male fights and tactile courtship to test the functional allometry hypothesis using red milkweed beetles, *Tetraopes tetrophthalmus* (Coleoptera: Cerambycidae). We used careful observation of behaviors and natural history (Rodríguez and Soley 2025) to identify traits used as weapons in male-male fights and in tactile signaling before and during copulation (DeLong et al. 2024). In fights over preferred mating sites on milkweed plants, male red milkweed beetles grapple with their mandibles (Figure 1, DeLong et al. 2024; Lawrence 1986; McCauley 1982; McLain and Boromisa 1987). In head-to-head encounters, males bite each other's mandibles, pushing and twisting against each other (DeLong et al. 2024; Lawrence 1986; McCauley 1982; McLain and Boromisa 1987). Males sometimes mount rivals, where they can also bite the opponent's body, legs, or antennae with their mandibles (DeLong et al. 2024). When males come across females, they quickly mount them, grasp their abdomen with their fore- and mid-legs, and gently tap their head and thorax with the tips of their antennae (i.e., antennation; Figure 1) (DeLong et al. 2024; Hanks and Wang 2017; McCauley 1982). Females may reject males by lowering the abdomen to prevent genital intromission or vigorously shaking them off their backs (McCauley 1982). If females allow copulation, males continue to occasionally antennate during intromission (Figure 1, DeLong et al. 2024). This antennation behavior fits the criteria to be considered copulatory courtship (i.e., tactile signaling): it is stereotyped and rhythmic, females are likely to sense it, and males never perform it outside the context of mounting or copulating with a female except when they mistake a male for a female (DeLong et al. 2024; Eberhard 1991; Eberhard 1994). In this case, mounted males will antennate and upon detecting the mistake, they will either dismount or begin fighting (DeLong et al. 2024; Lawrence 1986).

On the basis of the above description, we defined traits that function as weapons (male head and mandibles) and in tactile signaling (male antennae tips) in red milkweed beetles. We included the head of males as a weapon because it contains the muscles that control the application of force through the mandibles. We compared the allometric scaling of these traits with each other and with homologous female traits as a control. To ground these comparisons, we also asked whether males and females differ in body size. We also assessed the allometric scaling of other traits that the beetles do not use in fights or copulatory courtship and thus should not be under sexual selection as further controls: the length of the basal segment of the antennae, the length of the mid-leg femur, and the width of the thorax.

In the above comparisons, the functional allometry hypothesis makes the following predictions: the control traits should scale evenly with body size and similarly in males and females. The tactile signaling trait (male antennae tip) should scale more shallowly than the control traits and the female antennae tip. As discussed above, predictions for the weapon (male head and mandibles) are harder to specify. However, as these traits are

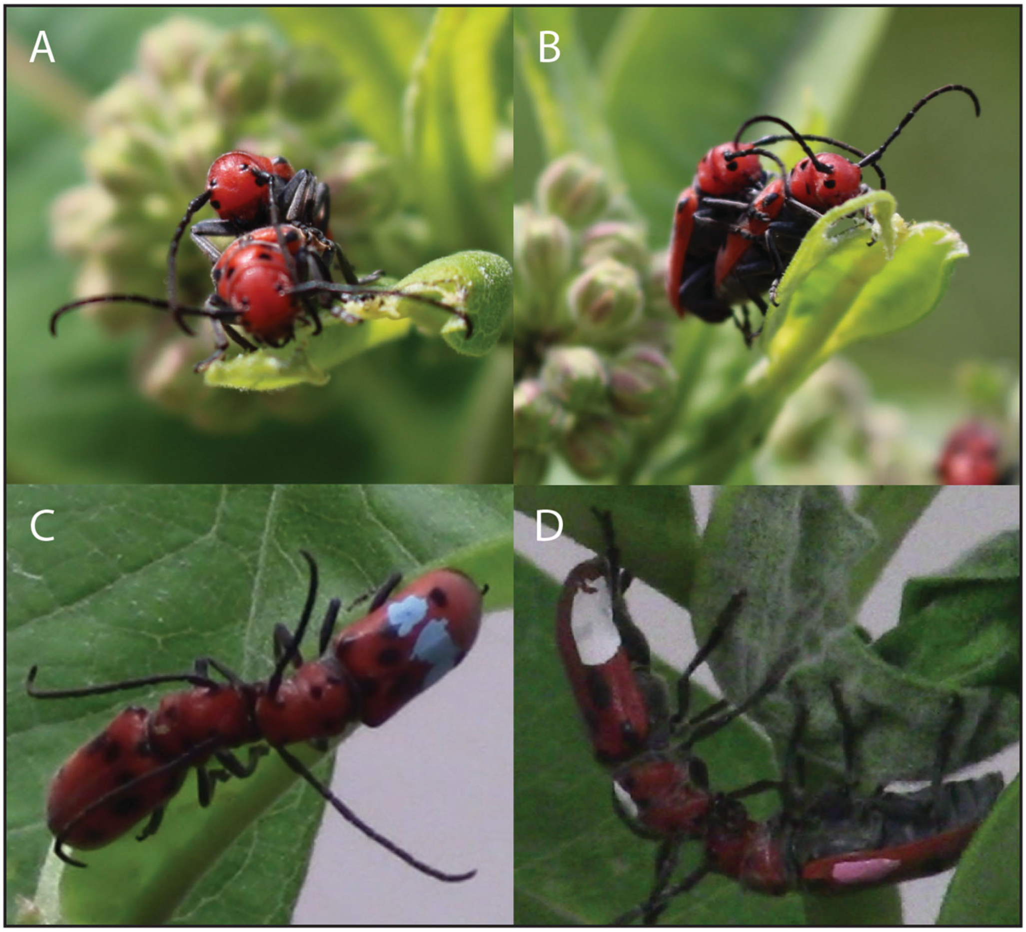

FIGURE 1 | Red milkweed beetle behaviors that inform the allometric scaling comparisons in this study. (A, B) Copulation depicting antennation on the head and thorax of females by males while grasping the female's abdomen with the fore- and mid-legs. (C, D) Male-male mandible grappling. (A, B) photos taken by Lauren A. Cirino. (C, D) stills come from videos of fighting behavior presented in DeLong et al. (2024).

not used as aggressive signals, they should not scale particularly steeply on body size, and their scaling should be sexually dimorphic as females do not use them to fight.

Note that red milkweed beetles of both sexes use their mandibles to feed (Chemsak 1963; Craig et al. 2024; DeLong et al. 2024) and their antennae to sense the environment (Haddad et al. 2023; Hanks 1999; Hanks and Wang 2017; Reagel et al. 2002). Thus, these male traits that we studied evolve under selective pressures to perform a combination of sexually and naturally selected functions compared to the female homologues and control traits that perform naturally selected functions.

The function of a trait may not only affect its allometric scaling but may also affect its shape. Like size, weapon shape is an important trait in determining fighting outcomes (Emlen 2008; Palaoro and Peixoto 2022). Male red milkweed beetles use their mandibles to fight and feed, whereas females only use them to feed. When fighting, males will bite and lock their mandibles together then push and twist one another to gain dominance (DeLong et al. 2024; Lawrence 1986; McCauley 1982; McLain and Boromisa 1987). Males, like females, also need to use their mandibles to feed, using them to puncture and slice through the leaves and flowers of milkweed (Chemsak 1963; Craig et al. 2024). Thus, mandible shape should evolve to allow males to perform both fighting and feeding functions while supporting only feeding behavior in females. We thus predicted the shape of the mandibles to be sexually dimorphic.

2 | Methods

2.1 | Mate Recognition and Pre-Copulatory Sensory Biology of *Tetraopes tetropthalamus*

Male *T. tetrophthalmus* locate mates by sensing plant volatiles with their antennae to identify a suitable milkweed host plant (Reagel et al. 2002). Once males have landed on a milkweed host, they search for females. *T. tetrophthalmus* males likely find females by using their antennae to identify short-range olfactory cues and/or contact pheromones like other cerambycids (e.g., Ginzel et al. 2003; Lu et al. 2007; Reagel et al. 2002; Wang et al. 1991). Cerambycid antennae tips (i.e., terminal segments) have chemoreceptors that help them distinguish conspecifics (Hanks 1999; Lopes et al. 2005; Lu et al. 2007; Wang et al. 2002). Males make contact with females by drumming or tapping the terminal segments of their antennae (i.e., antennation) onto the female's head or thorax during and after copulation (DeLong et al. 2024; Hanks and

Wang 2017). This behavior not only helps them detect pheromones, but it also provides tactile stimulation to the female (e.g., West-Eberhard 1984; Eberhard 1991), which likely increases their chances of achieving and maintaining a copulation (e.g., Mpho and Seabrook 2003). If males antennate conspecific males, they will recognize them as males and will periodically engage in fighting behaviors (DeLong et al. 2024; Wang et al. 2002). Vision may play a role in the decision to fight as body size plays a large role in the likelihood that males will engage in this behavior (Andersson 1994; Emlen 2008; Emlen 2014; Thornhill and Alcock 1983). Male *T. tetrophthalmus* are unlikely to use visual cues of the mandibles to determine whether they will engage in a fight since their mandibles do not protrude very far out of their heads (Figure 1).

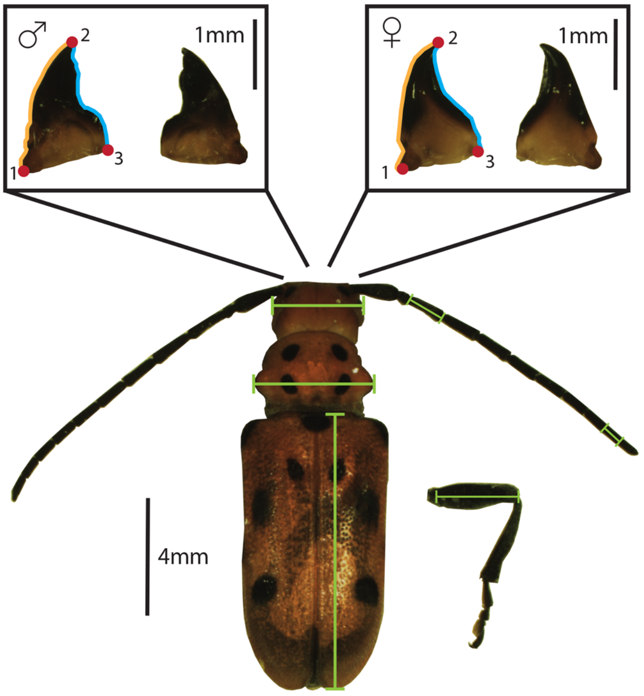

FIGURE 2 | Dorsal photograph of the red milkweed beetle traits we measured (green lines) including a dissected right mid-leg and mandibles: (traits from top to bottom of photograph) mandible size, antennae base, head width, thorax width, antennae tip, right elytra length, and right mid-leg length. We placed three landmarks (red points) and two semi-landmark curves (30 each) on the medial (point 2 to 3 highlighted in blue) and lateral (point 1 to 2 highlighted in orange) sides of the left mandible.

2.2 | Experimental Design

Red milkweed beetles live and feed on the common milkweed, *Asclepias syriaca* (Apocynaceae) (Chemsak 1963). We collected 123 adult red milkweed beetles ($n = 33$ females, $n = 90$ males) from the greater Milwaukee region in Wisconsin, USA in July 2022. We collected beetles from six field sites: Menomonee River Hills East (43°08’34”N 87°59’50”), Minooka park (42°59’13”N 88°12’07”W), Richfield (43°15’56”N 88°14’28”W), the Urban Ecology Center of Riverside Park (43°03’59”N 87°53’49”W), the University of Wisconsin–Milwaukee (UWM) campus (43°04’53”N 87°53’11”W), and the UWM Saukville Field Station (43°23’03”N 88°01’41”W). We determined the sex of these beetles by inspecting their genitalia. We used beetles for this study that were used in a previous behavior study where we kept females in groups of 5–10 on potted netted milkweed plants. We kept males separate on individual plants to prevent them from fighting. We kept the beetles under grow lights (14:10 light:dark) in our UWM laboratory for no more than 48 h.

To measure the beetles, we first cold euthanized and then photographed them by placing each beetle in an Olympus SZ61 microscope (Olympus, Tokyo, Japan) fitted with a Moticam 2500 camera (Motic, Richmond, BC, Canada) connected to a Macintosh computer. We used the program Motic Image Plus 2.0.10 (Motic, Richmond, BC, Canada) to take dorsal view photographs of the right antennae, head and thorax, and elytra (Figure 2). We dissected the mandibles and right midleg from the body and photographed them separately (Figure 2). We placed each beetles’ pair of mandibles on a granular substrate to allow them to lay flat and took dorsal view photographs. We included a scale bar in each photograph to calibrate our measurements.

We used ImageJ software (Abràmoff et al. 2004) to measure six traits from our photographs (Figure 2). We measured the length of the right elytron as a proxy for overall body size (Mason 1964; Scheiring 1977). We measured the length of the eighth segment of the right antennae distal to the head (antennae tip) as it is used in tactile signaling before and during copulation (DeLong et al. 2024). We measured the length of the first segment of the right antennae after the scape and pedicle (antennae base) as comparison for the antennae tip (Figures 1 and 2). We also measured the width of the head, thorax, and the length of the right mid-femur (Figure 2). We included the latter two as control traits for comparison.

We quantified mandible area and mandible shape using geometric morphometrics. We chose the left mandible to measure. We excluded three of our 123 photographs due to mandible damage during the dissection process, resulting in photographs of 120 beetles. Using TPSDig (Rohlf 2017), we placed three landmarks on the mandible: landmark 1 was placed on the proximal lateral corner of the mandible (Figure 2), landmark 2 was placed at the distal tip, and landmark 3 was placed on the proximal medial corner of the mandible (Figure 2). We then placed curves comprised of 30 evenly spaced semi-landmarks on the medial and lateral edges of the mandible. We used the scale bars in our photographs to calculate scale ratios for each photograph (mm/pixel). We loaded our landmark data into R (version 4.4.1) using the “geomorph” package (version 4.0.8). We removed the first and last semi-landmarks from each curve because they overlapped with the landmarks already on the mandibles leaving us with a total of 56 semi-landmarks per photograph. We then generated a slider file, designating each semi-landmark's connection to two other landmarks. We conducted a Procrustes alignment which optimized the location of our semi-landmarks by minimizing bending energy. We checked to see if our specimens were accurately landmarked by running several diagnostic analyses. These analyses included using (1) the function “plotOutliers” from the “geomorph” package to identify specimens with misplaced landmarks, (2) plotting the specimens before and after Procrustes alignment to identify any inaccurate landmarks or curves, and (3) examining the variation of each individual landmark across specimens. If we found any errors in landmark placement, we re-landmarked the specimen and re-ran the diagnostics described above until we no longer found any erroneous landmarks. We calculated each specimen's mandible area using the "geometry" package (version 0.5.0), based on the area enclosed by our landmarks and semi-landmarks.

2.3 | Statistical Analyses

We first tested for sexual dimorphism in body size in the beetles. We used a linear mixed model that had elytra length as the response variable and sex as the explanatory variable. We included collection site (six locations) as a random term in the model.

We estimated allometric slopes (*b*) by using $\log_{10}$-$\log_{10}$ ordinary least squares (OLS) regressions of each trait of interest on body size. This is the recommended method for estimating allometric slopes as it formally describes scaling of trait size on body size (Kilmer and Rodríguez 2017b). However, when measurement error is high for body size, this method may underestimate slopes (Kilmer and Rodríguez 2017b). We estimated error in the measure of body size in our study (elytra length) with a subsample ($n = 10$ beetles) of repeated positionings and photographs. The correlation between these repeated measurements was $r = 0.83$, suggesting there is a 17% error in our measurement of body size, and a corresponding risk of underestimating slopes by 17% (Kilmer and Rodríguez 2017b). Note, however, that even with higher measurement error, slopes are not necessarily underestimated (Al-Wathiqui and Rodríguez 2011). Further, as we use the same measure of body size for all slope estimates, the risk of underestimation is constant across all traits in our study, and our comparisons of relative slope steepness are unaffected.

We fit separate linear mixed models for each trait: antennae base, antennae tip, head width, thorax width, right midleg femur length, and mandible area (Figure 2). Before $\log_{10}$ transforming mandible area, we linearized it through a square root transformation. Henceforth, we refer to mandible size for this measure. We included each variable above as a separate response variable in each model. In all models, we included the following explanatory terms: body size (also $\log_{10}$ transformed), sex, and the interaction between body size and sex. We also included site as a random term. In these models, the main term for body size serves to estimate allometric slopes (*b*), the main term for sex tests for sexual dimorphism in trait sizes, and the interaction between body size and sex tests for sex differences in *b*. We used the function "glmmTMB" from the same named package in R to fit our linear mixed models.

We then conducted a secondary analysis using the slope estimates from the models described above as the response variable. This approach allowed us to view allometric slopes as traits which evolve. This analysis also allowed us to more directly compare slopes across trait function and sex. We ran an analysis of variance (ANOVA) test to examine slope differences between traits. We included trait function (tactile signaling, weapon, or control), sex, and the interaction between trait function and sex as the explanatory variables. We then used a Tukey's post hoc test ('TukeyHSD' in the *stats* package) to examine the differences between the means of each factor in the model.

We also examined whether mandible shape differed by sex, whether it correlated with body size, and whether this relationship differed by sex. To visualize the variation in shape in our data, we conducted a principal components analysis (PCA) using the "gm. prcomp" function from the *geomorph* package determining the amount of variation PC axes 1–4 captured. We then conducted a Procrustes ANOVA using the "procD. lm" function which allowed us to use all the dimensions of the shape data without the need for reducing its dimensionality via PCA. We constructed a model with our shape data as our response variable and included $\log_{10}$-transformed body size, sex, and the interaction between these two terms as explanatory variables. Since the interaction between body size and sex was not significant ($p > 0.3$) under a type III analysis, we ran the model again using a type II analysis. Type II tests evaluate the main effects while accounting for other main effects but not their interaction. This provides us with a better understanding of the main effects in the model, especially since the interaction is not significant. We also originally included site in the model, but since it was also not significant ($p > 0.2$), we removed it. We also tested for differences in shape disparity between males and females using the function "morphol. disparity" (*geomorph* package). Finally, we visualized the differences in mandible shape between males and females by conducting a Canonical Variate Analysis (CVA) using the *Morpho* package (version 2.12) that illustrates the variation captured by a single CVA axis. We also directly visualized the differences in mean mandible shape in males and females, before aligning our data and after Procrustes alignment. All linear models were run with a type III analysis unless otherwise indicated above. We completed all statistical analyses in R version 4.4.1 (R Core Team 2024).

3 | Results

3.1 | Sexual Dimorphism in Body and Trait Size

We found that female red milkweed beetles were larger than males (elytra length: $\chi^2 = 48.79$, df = 1, $p < 0.001$, Figure 3). Sex was not significant in any of the original trait models (Table 1), suggesting no sexual dimorphism in trait sizes. However, sex was marginally significant for mandible size (Table 1), suggesting that mandible size was larger in males compared to females. When we removed the body size × sex interaction term from the models where this term was not significant, sex became significant for antennae tip, antennae base, and midleg length, indicating that these traits were larger in males than females (Table 1, Figure 4).

3.2 | Allometric Scaling Analysis

In the initial linear mixed model analysis, all traits scaled with body size (i.e., body size was significant in every model; Table 1). The tactile signal trait (i.e., antennae tip) did not differ

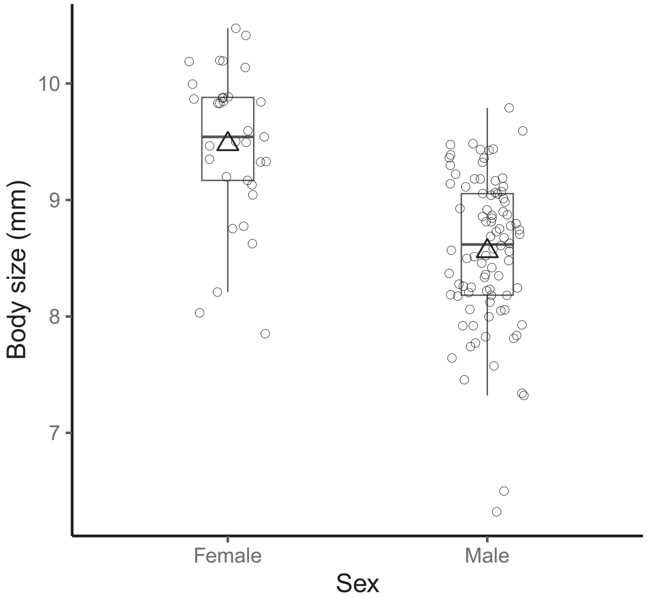

FIGURE 3 | Body size (i.e., elytra length) of red milkweed beetles. Female red milkweed beetles were larger than males. Box plots show the median (thick horizontal line) and mean (open triangle). Female body size ranged from 7.85 to 10.48 mm compared to males that ranged from 6.32 to 9.79 mm.

in scaling by sex (body size × sex interaction was not significant in Table 1), with allometric slopes (Table 1) corresponding to negative allometry (Table 1, Figure 4). By contrast, the weapon traits (i.e., mandible size and head) did differ in scaling by sex (significant and marginally significant body size × sex interactions for mandible and head size, respectively; Table 1). Scaling of weapon traits were isometric in males but negatively allometric in females (Table 1, Figure 4). The control traits (antennae base, mid-leg femur length, and thorax width) did not differ in scaling by sex and the interaction between body size and sex was also not significant for any of these traits (Table 1). Our allometric slope estimates (Table 1) are consistent with isometry or weak negative allometry for these control traits (Figure 4).

3.3 | Slope Comparison Analysis

This analysis showed that slopes varied according to trait function (tactile signal, weapon, control) and sex and that slope differences depended on sex (significant interaction term; Table 2). Male antennae tips (i.e., tactile signal) showed the shallowest slope, and male mandibles and head (i.e., weapons) along with the control traits for both sexes were closest to isometry (Figure 5). Interestingly, the sex difference was flipped between the antennae (shallower slope in males) and the mandibles, head, and control traits (steeper in males) (Figure 5).

Among the predicted slope differences, the post-hoc analysis identified the following as significant: male tactile signal versus male weapons ($p = 0.009$) and control traits (♂$p = 0.006$ and ♀$p = 0.01$); male weapons versus female tactile signal homologs ($p = 0.03$) and female weapon homologs ($p = 0.04$). Other non-predicted differences were also significant or marginally

TABLE 1 | Analysis of variation in trait size and allometric scaling according to trait function (tactile signal, weapon, or control) and sex.

	Tactile signal	Weapon		Control		
	Antennae tip	**Mandible size**	**Head**	**Antennae base**	**Midleg length**	**Thorax width**
Term	χ^2, p	χ^2, p	χ^2, p	χ^2, p	χ^2, p	χ^2, p
Body size	**30.08, < 0.001**	**73.30, < 0.001**	**76.35, < 0.001**	**46.83, < 0.001**	**52.66, < 0.001**	**51.98, < 0.001**
Sex	0.77, 0.38*	**3.42, 0.06**	2.43, 0.11	0.04, 0.84*	0.41, 0.52*	0.20, 0.65
Body size × sex	0.29, 0.59	**4.23, 0.04**	**3.01, 0.08**	0.00, 0.98	0.67, 0.41	0.27, 0.61
$b_♀$, $b_♂$	0.71 ± 0.1, 0.64 ± 0.07	0.77 ± 0.1, 0.97 ± 0.05	0.77 ± 0.08, 0.93 ± 0.05	0.95 ± 0.14, 0.95 ± 0.08	0.83 ± 0.13, 0.92 ± 0.06	0.93 ± 0.13, 0.99 ± 0.07

Note: We ran separate generalized linear mixed models for each trait and reported Wald χ^2 and p-values from those models (see text for explanation). We also report the slopes (b) with ± standard error for both females and males in the bottom row of the table. All degrees of freedom equal 1. We show all significant and marginally significant terms in bold.
*Removing the nonsignificant interaction changed the results for the main term for sex for the following traits.
Antennae tip – $\chi^2 = 82.63$, $p < 0.001$; Antennae base – $\chi^2 = 34.87$, $p < 0.001$; Midleg length – $\chi^2 = 21.42$, $p < 0.001$.

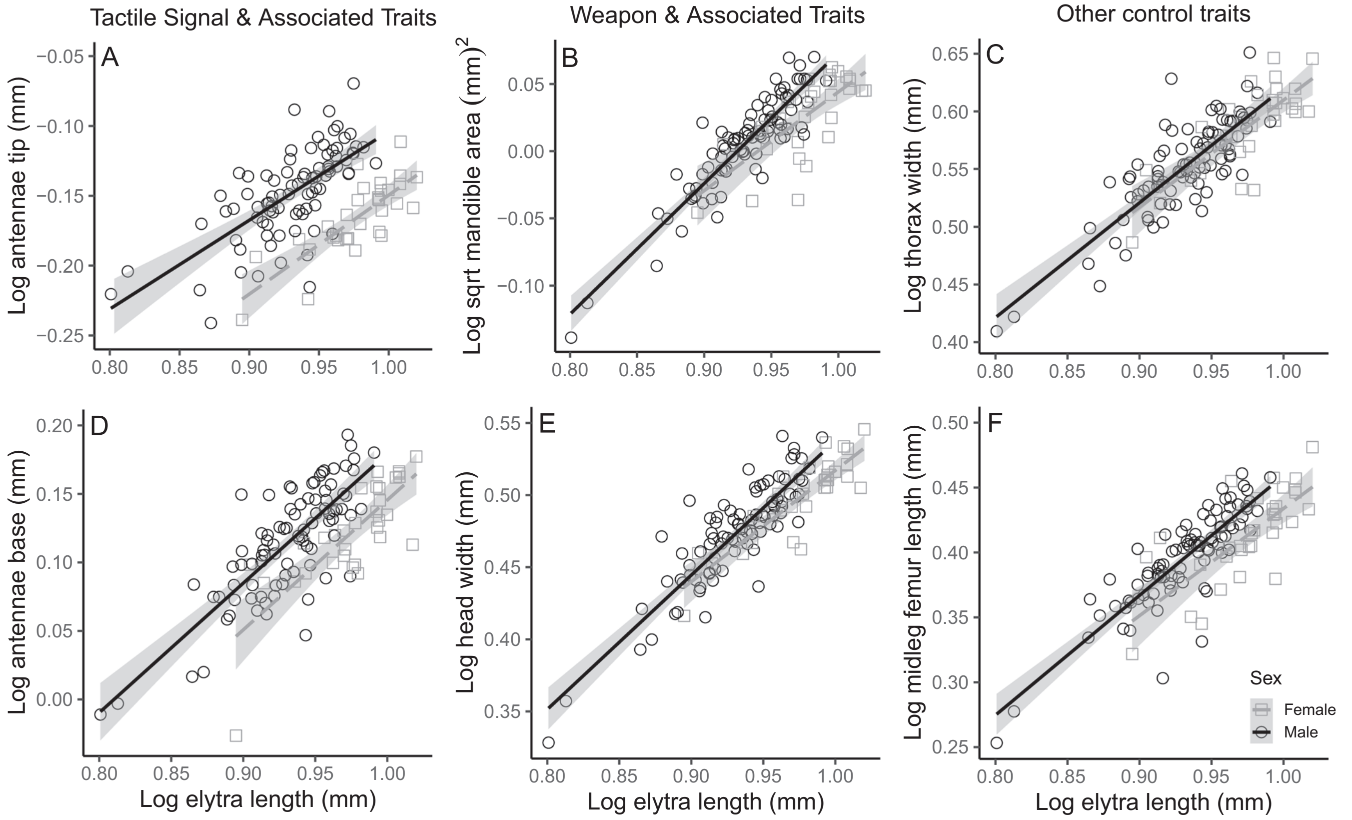

FIGURE 4 | Allometric scaling of traits used in copulatory courtship (left panel), male-male fights (center panel), and other control traits (right panel) in red milkweed beetles. (A) The antennae tip (tactile signal) is negatively allometric and (D) the antennae base (associated control trait for tactile signaling) is isometric in both males and females. (B) Mandible size (weapon) and (E) head (associated weapon trait) are isometric in males (weapon) and negatively allometric in females. (C) Thorax width and (F) mid-leg femur length, two other control traits, are isometric in both sexes. Individuals are represented by the points (gray squares = females; black circles = males) in all graphs. A regression line with 95% confidence intervals (shaded in gray) for each sex shows the slope of trait across body size (i.e., log elytra length).

TABLE 2 | Results of the analysis of variance test that examined the slopes between sex, trait function, and the interaction between the two terms.

Term	df (num, denom)	*F* value	*p* value
Function	**2, 6**	**25.06**	**0.001**
Sex	**1, 6**	**8.39**	**0.03**
Function × sex	**2, 6**	**5.84**	**0.04**

Note: All significant terms are bolded.

significant: male controls versus female weapon homologs ($p = 0.03$) and female tactile signal homologs (i.e., antennae tips) ($p = 0.02$); female tactile signal homologs versus female control traits ($p = 0.06$). All other trait slope comparisons were not significantly different from one another.

3.4 | Weapon Shape

We visualized the first four PCs that explained 83% of the total variation in our shape data (48%, 20%, 9%, and 6% for PCs 1-4, respectively). We plotted these PCs in two graphs to visualize and inspect variation in mandible shape (Figure 6). PCs 1 and 3 captured variation in the smoothness-rugosity of the medial curve of the mandible (Figure 6). PC 2 captured variation in the relative hooked-ness of the tip of the mandible (Figure 6). PC 4 captured variation in the presence of a ledge on the mandible medial edge (Figure 6).

We then analyzed weapon shape using all PCs generated by the PCA. We did not find a significant interaction between sex and body size (Table 3). However, we found that the shape changed with body size (Table 3). The medial curve of the mandible became smoother as body size increased (Table 3, Figure 7). We also found differences in shape by sex where female mandibles had a smoother medial curve that was more hooked than male mandibles, which had a rougher medial curve and was straighter (Table 3, Figure 7). We found no difference in shape disparity between males and females ($p = 0.83$) as females were only 4.2% more variable than males.

We further visualized significant differences in shape suggested by our above analysis between males and females using a CVA. This analysis produced one CV axis that captured 40.5% of the variation in shape (Figure 6).

4 | Discussion

Allometric scaling of sexually selected traits is highly variable. While 'pure' function traits are ideal to examine the reason for

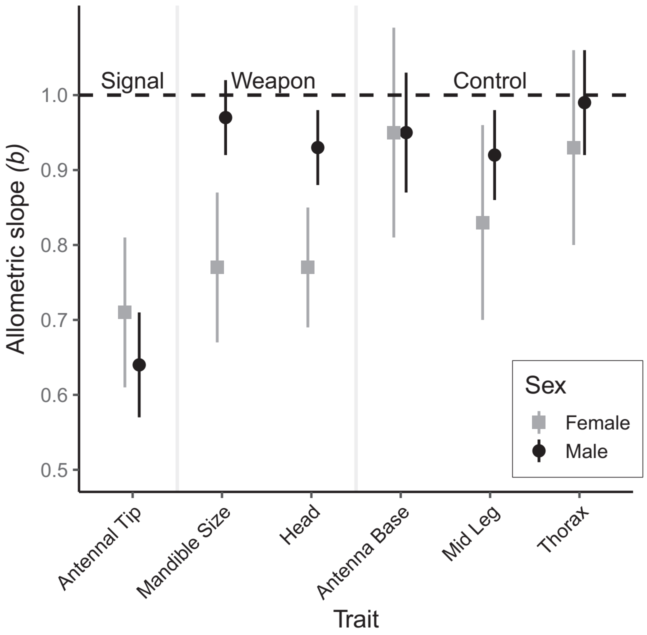

FIGURE 5 | Allometric slopes of all red milkweed beetle traits were compared by trait function and sex. The tactile signal trait has a shallower allometric slope than the weapon and control traits. The male mandible weapon and associated head trait had steeper slopes than female weapon homologs. The points represent the allometric slopes of each trait with ± SE bars. The black dashed line represents a perfectly isometric slope (1:1 body size-to-trait proportion).

this variability, such traits are rare in nature. Thus, we must examine a wide variety of multifunctional sexually selected traits from a diverse group of species to test hypotheses that explain this variability. We investigated the functional allometry hypothesis by examining red milkweed beetles. We measured their mandibles (used as a weapon by males and for eating by both sexes), antennae (used in tactile signaling during copulatory courtship by males and generally as a sensory organ by both sexes), and a variety of control traits. We found that the sexual function of the trait influences its allometric slope. The tactile signal trait scaled more shallow compared to other trait types (i.e., weapon and control traits) that scaled isometrically. The weapon and head (an associated trait that contains the muscles for the mandibles) had steeper slopes compared to their female homologues. We also found that the control traits scaled evenly with body size. Our results thus support the functional allometry hypothesis, even for traits that have a multifunctional purpose.

Male red milkweed beetles use the distal tips of their antennae in tactile signaling in addition to functioning as a part of the sensory system. Males antennate females' head and thorax during copulatory courtship (DeLong et al. 2024; Hanks and Wang 2017; McCauley 1982). Larger males should thus grow relatively shorter antennae and smaller males should grow relatively longer antennae to perform this function. In other words, body and antennae length together should be selected to contact and adequately stimulate the majority of females. If larger males with larger bodies grow antennae too long, then they will likely struggle to contact females' bodies. Similar shallow allometric slopes are seen in other tactile signaling traits, such as male genitalia, that stimulate and inseminate the female (Eberhard 2009b). Male antennae tip length also scales more shallowly than female antennae, although not significantly so in this study. Females only use their antennae to sense their environments (Haddad et al. 2023; Hanks 1999; Hanks and Wang 2017; Reagel et al. 2002), and it is possible that such a function may also select for shallow allometric scaling (Kilmer and Rodríguez 2017a), albeit perhaps not as strongly as the tactile signaling function.

Like the tactile signal trait, male mandibles are multifunctional. Male mandibles are used to eat and fight. Males use their mandibles to bite and lock their rivals' mandibles as they push and twist their opponent to jockey for territory and females (DeLong et al. 2024; Lawrence 1986; McCauley 1982; McLain and Boromisa 1987). Several beetle species that engage in male fights have weapons that are positively allometric (Kodric-Brown et al. 2006; McCullough et al. 2015; Painting et al. 2024), which tends to correspond to winning fights (Emlen 2008; Emlen et al. 2005; Palaoro and Peixoto 2022). However, red milkweed beetles have weapons that are isometric. Beetle species with positively allometric weapons usually use their weapons to hold their opponent some distance away from their body and leverage their weapon to force their opponent off a surface (Eberhard et al. 2018). Male red milkweed beetles fight closely head-to-head with their mandibles locked (DeLong et al. 2024; Lawrence 1986; McCauley 1982; McLain and Boromisa 1987). Positive allometry for this species may hinder their fighting ability due to the close proximity required to fight (Dennenmoser and Christy 2013; Eberhard et al. 2018; Geist 1966). However, male Helm's stag beetles also interlock their mandibles and push their opponent when fighting and their mandibles are positively allometric (Grey et al. 2025). Thus, fighting style alone may not fully explain the allometric slope of the mandible weapon in red milkweed beetles.

Biomechanics may also influence weapon allometry. Male red milkweed beetles also need their mandibles to function as cutting and chewing mouthparts, so they can eat as adults (Chemsak 1963; Craig et al. 2024). If the mandibles grow too large, bite force and cutting ability may decline (i.e., length of the mandible increasing relative to the width reduces its chewing functionality) (Clissold 2007). Female red milkweed beetle mandibles are negatively allometric, and they are only used to eat, which supports these functional constraints of these mandibles. Female red milkweed beetle mandibles are likely only acted on by natural selection causing the negative allometric slope we observe. Male stag beetles only use their mandibles to fight since they consume sap flow from trees which does not require chewing (Holloway 2007; Huang 2018). Since adult male stag beetle mandibles are not multifunctional, sexual selection likely only acts on this trait causing exaggeration (i.e., positive allometry). Male red milkweed beetle mandibles with their dual function, however, likely have two forces acting on them—natural selection, which likely keeps them smaller for feeding purposes and sexual selection, which likely pushes them larger for the fighting function. These two forces may stabilize the allometric pattern for this mandible trait in male red milkweed beetles (i.e., isometry) to optimize both functions.

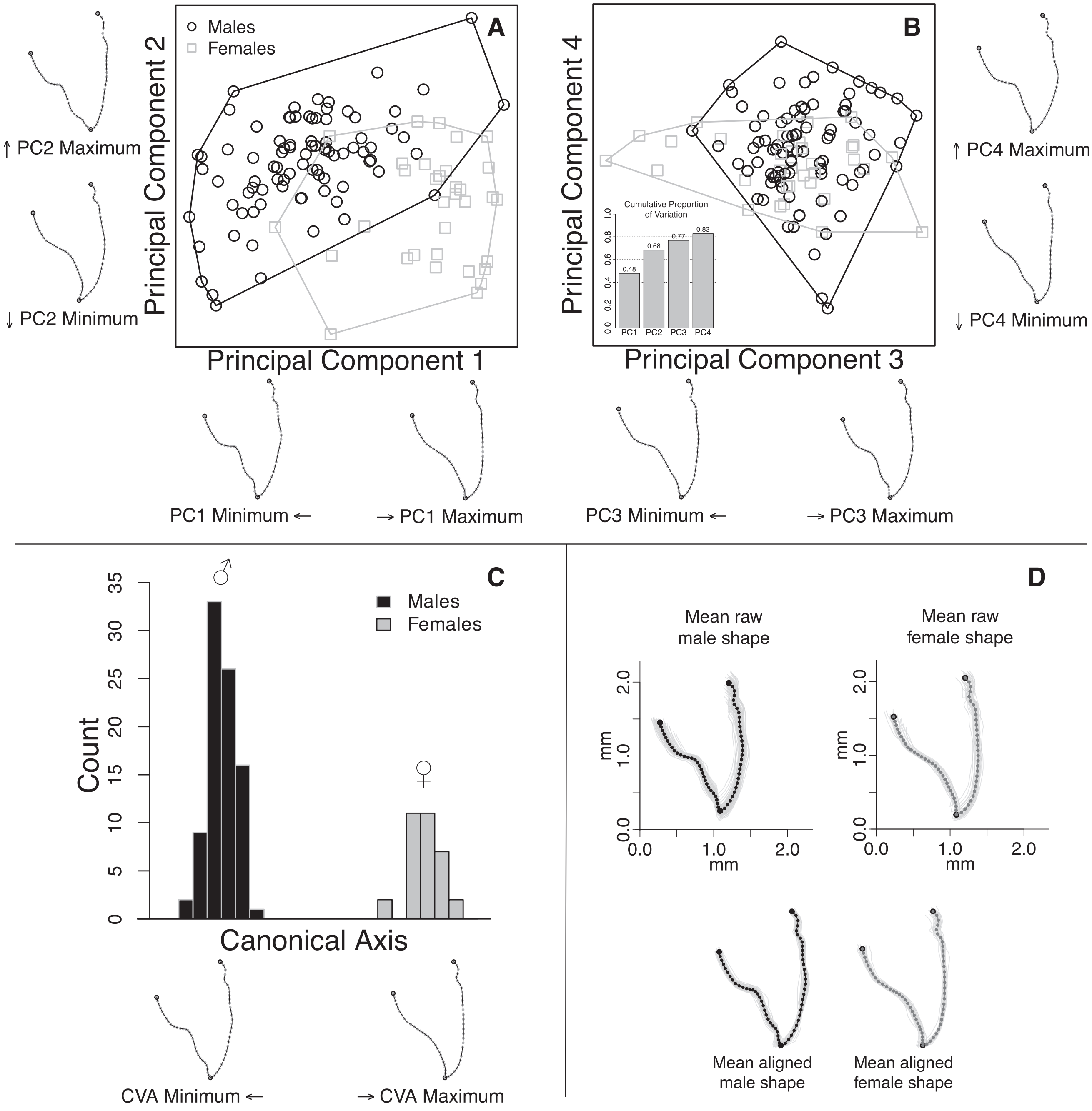

FIGURE 6 | Visualization of weapon shape (i.e., mandibles) of red milkweed beetles by sex. (A, B) Principal components 1 versus 2 and 3 versus 4 of red milkweed beetles mandible shape with projected shapes along each axis. The proportion of variance that each PC values contributed is plotted in the inset bar graph with graph B. (C) Canonical Variate Analysis (CVA) of mandible shape along with projected shapes. (D) Raw and aligned mandible shapes. Light gray lines represent individuals, and the black and dark gray dotted lines represent the mean of the individuals per sex.

The associated weapon trait (i.e., head size) that we measured in this study likely has supporting musculature to make the mandibles move (Clissold 2007; Goyens et al. 2014; Mills et al. 2016). We found that this trait follows a similar pattern of allometric scaling that we observed in mandible size for both sexes. Larger mandibles will likely need greater support and musculature to make them move to perform multiple functions in males while females only need them to support the chewing function.

In addition to the size of weapons and associated structures, weapon shape can also influence fighting outcomes within a species (Emlen 2008; Palaoro and Peixoto 2022). We found that weapon shape is sexually dimorphic. Weapon shape sex differences may have evolved due to the fighting function of male mandibles compared to females that only use their mandibles to eat (Chemsak 1963; Craig et al. 2024; DeLong et al. 2024; Lawrence 1986; McCauley 1982; McLain and Boromisa 1987). Males had rough medial mandibles and many had a ledge at the

TABLE 3 | Results of the Procrustes ANOVA test that examined mandible shape based on body size, sex, and the interaction between the two terms.

Term	df	*F* value	*p* value
Body size	**1**	**3.11**	**0.022**
Sex	**1**	**26.33**	**0.001**
Body size × sex	1	0.89	0.459

Note: All significant terms are bolded.

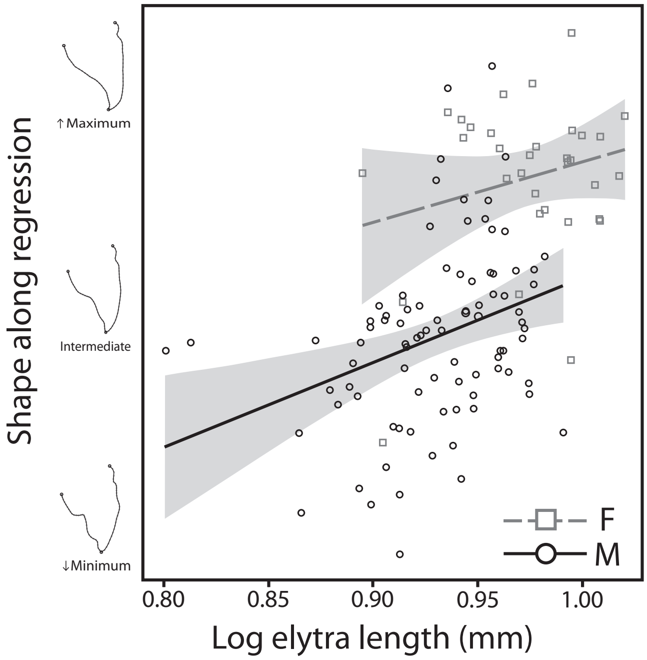

FIGURE 7 | Mandible shape varied with body size and sex in red milkweed beetles. Larger beetles had a smoother medial curve in their mandibles and females had overall smoother mandibles than males. Individuals are represented by the points (gray squares = females; black circles = males). A regression line with 95% confidence intervals (shaded in gray) is plotted for each sex.

medial midpoint compared to females that had a smoother medial mandible and lacked a ledge. These shape differences might be present because males engage a rival in a fight by biting their opponents' mandibles and then pushing and sometimes twisting to win a fight (DeLong et al. 2024; Lawrence 1986; McCauley 1982; McLain and Boromisa 1987). Because they push one another in fights, it seems reasonable that possessing a rough medial side of their mandibles, along with a ledge, would facilitate these fighting behaviors. Indeed, weapon shape adaptations reflect the fighting styles of males across species (McCullough et al. 2014). The structural and biomechanical properties of the weapon likely evolved to meet the fighting techniques of males (McCullough et al. 2014), and we expect the same to be true for red milkweed beetle males.

We also observed that in both sexes larger beetles had smoother medial mandibles to the point that larger males had female-like mandible shape. This pattern is not consistent with other studies on weapon shape (e.g., Goczał et al. 2019; Painting 2022). Perhaps large males are less likely to engage in male fights because they are large and the visual signal of body size is enough to make most would-be rivals retreat (Andersson 1994; Emlen 2008; Emlen 2014; Thornhill and Alcock 1983). Further, having a smoother medial mandible may be important for slicing through leaves and acquiring food. If large males are less likely to get into many fights based on their sheer size, a rough medial mandible may be less important for their reproductive success. It is possible, however, that the differences we observed between male and female mandibles are due to wear based on use and not a sexually selected shape adaptation. Male weapons can wear down after considerable use in some species (e.g., McEvoy et al. 2024). If this occurs in the mandible weapons of *T. tetrophthalmus*, then we would expect males that were collected early in the breeding season to have a different mandible shape compared to males collected later in the season. We did not find this result (Supplementary online material 1). Further, the exoskeleton of beetles is extremely tough and resistant to mandible bite damage and wear (Kundanati et al. 2019). Thus, it is unlikely that the mandible shape that we observed in this study is acquired over the lifetime of the beetle due to mechanical wear. The importance of weapon shape is understudied, however, and should be an avenue of future research.

5 | Conclusion

There is incredible variability in the allometry of sexually selected traits, and we have only begun to investigate the reason for this variation. Trait function rises to the top of possible explanations of these observations (Eberhard et al. 2018). Studies that focus on "pure" function traits have found that function does explain the variable allometry among sexually selected traits (Eberhard 2021; Eberhard et al. 2018). However, few traits are only used for only one purpose. Multifunctional traits are likely subject to conflicting sources of selection and may thus exhibit trade-offs in their morphology and performance (e.g., Bertram et al. 2021). Here, we explored two types of sexually selected traits—tactile signaling and weapons. We found evidence that the function of these traits explains their allometric scaling.

Author Contributions

Lauren A. Cirino: investigation, writing – original draft, methodology, validation, visualization, writing – review and editing, formal analysis, project administration, data curation, supervision. **Kirsten J. Lindemann:** investigation, validation, methodology, writing – review and editing, data curation. **Travis J. Hagey:** investigation, writing – review and editing, visualization, formal analysis, data curation, methodology. **Rafael L. Rodríguez:** conceptualization, investigation, funding acquisition, writing – original draft, writing – review and editing, methodology, formal analysis, supervision, resources, project administration.

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Ethics Statement

All our procedures adhered to the ASAB/ABS Guidelines for the use of animals in research as well as the legal requirements of the U.S.A. and all UWM guidelines.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that supports the findings of this study are available in the supporting material of this article.

Peer Review

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1002/jmor.70075.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.
AlloSlopeCompare. BodySizeRepeatability. Cirino beetle mandible analyses July2025. RMBAnalysis final. RMBLinearDat. RMBLinearDat. RMBmandibles. RMBslider. Supplementary material 1.