Research

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Muscle mass drives cost in sexually selected arthropod weapons

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Sexually selected weapons often function as honest signals of fighting ability. If poor-quality individuals produce high-quality weapons, then receivers should focus on other, more reliable signals. Cost is one way to maintain signal integrity. The costs of weapons tend to increase with relative weapon size, and thereby restrict large weapons to high-quality individuals who can produce and maintain them. Weapon cost, however, appears to be unpredictably variable both within and across taxa, and the mechanisms underlying this variation remain unclear. We suggest variation in weapon cost may result from variation in weapon composition—specifically, differences in the amount of muscle mass directly associated with the weapon. We test this idea by measuring the metabolic cost of sexually selected weapons in seven arthropod species and relating these measures to weapon muscle mass. We show that individuals with relatively large weapon muscles have disproportionately high resting metabolic rates and provide evidence that this trend is driven by weapon muscle mass. Overall, our results suggest that variation in weapon cost can be partially explained by variation in weapon morphology and that the integrity of weapon signals may be maintained by increased metabolic cost in species with relatively high weapon muscle mass.

1. Introduction

Sexually selected weapons are some of the most exaggerated and diverse structures in the animal world. They grow out of proportion with body size and other non-sexually selected structures [1–6], and when viewed across clades, they exhibit astounding diversity (e.g. [7–9]).

These weapons typically function as tools for intrasexual competition [9–15]. Animals use these structures to compete with same-sex rivals over direct access to mates [16–20], or over resources required by their mates [21–26]. Evidence also suggests that weapons function as intra- and intersexual signals. Weapon size often scales positively with overall body size [1–6,27], and overall body size typically reflects the genetic and environmental variation underlying individual fitness (hereafter referred to as 'quality' [28–32] (but see [33–35]). Through this connection, relative weapon size provides an effective signal of an opponent's resource-holding potential [25,33–36], and members of the opposite sex can use this metric to assess potential mates [25,37,38].

Honesty is essential to the function and persistence of sexual signals, and weapons are no exception [39–46]. If poor-quality animals can cheat and produce high-quality signals (i.e. large weapons), receivers should shift focus to other, more reliable indicators of quality. Cost is one way to maintain signal honesty
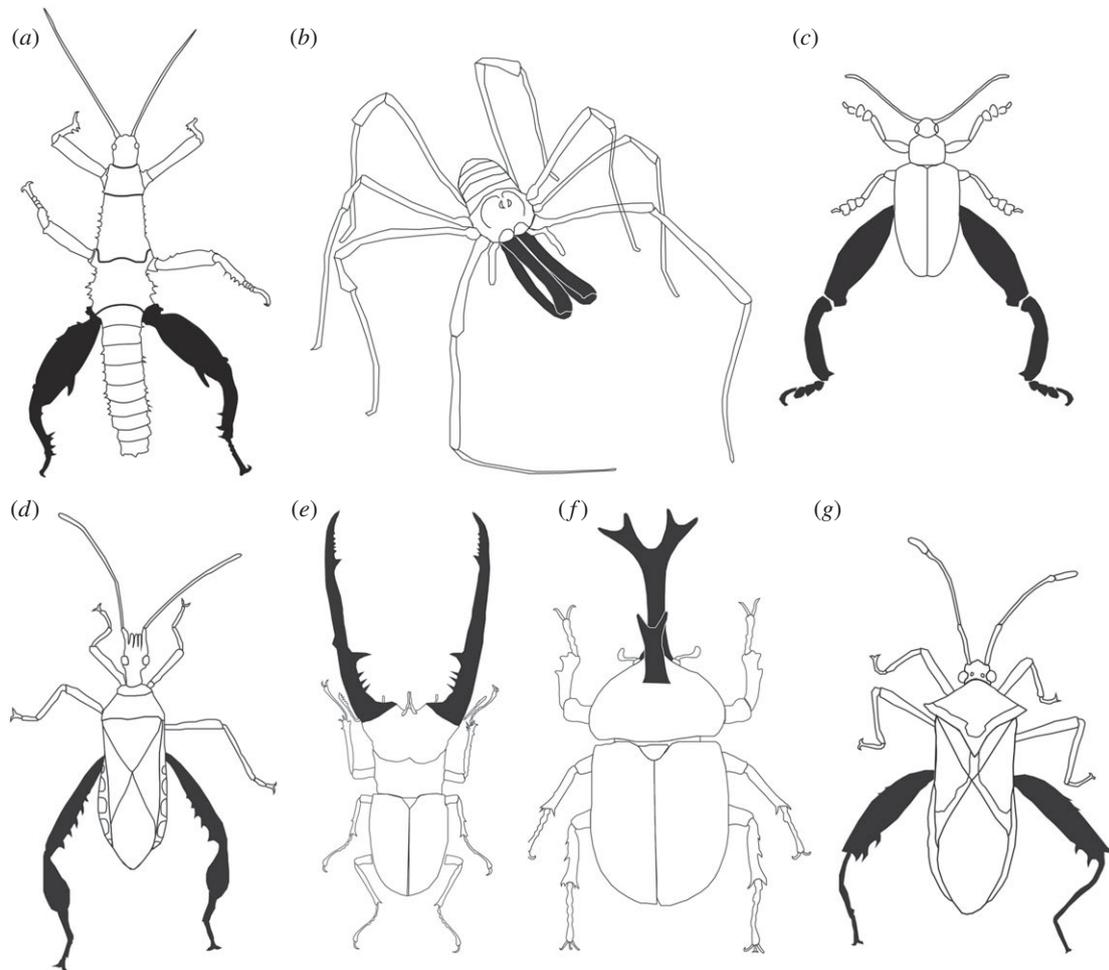
Figure 1. Study species with weapons defined by shaded area: (*a*) thorny devil stick insect, (*b*) New Zealand long-legged harvestman, (*c*) frog-legged beetle, (*d*) leaf-footed cactus bug, (*e*) Indonesian stag beetle, (*f*) Japanese rhinoceros beetle and (*g*) heliconia bug.

within a population, particularly when costs are steepest for poor-quality males [39,41–44,46,47]. Weapon cost tends to increase as structures increase in relative size [48–50]. Thus, large structures are both more conspicuous and more difficult to fake, which helps explain why sexual selection so often favours increases in weapon size [39–46].

The cost of sexually selected weapons has been examined from many perspectives and across a variety of taxa [48–57]. From this work, it is clear that the type (developmental, loco-motor, metabolic, etc.) and magnitude of these costs is highly variable [58–60]. Some species, for example, experience developmental costs as they invest resources in weapons at the expense of other traits [48,51,57]. Others endure heightened energy expenditure during locomotion, which results from the large weapons they carry (e.g. while running [49,52,54] and flying [50,52]). To date, the source of this variation remains unclear. However, we suggest that some types of weapon cost may be dependent on weapon composition—specifically, the amount of muscle associated with the weapon—and that identifying species with especially costly weapon morphology could help explain observed variation in weapon cost.

Here, we focus on the muscle content of sexually selected weapons and its relation to metabolic maintenance costs. Increased metabolism is an especially relevant measure of weapon cost, since variation in metabolic rate directly affects an animal's finite resource pool and impacts all other body functions. We predict that variation in weapon muscle mass, which is metabolically expensive to maintain [49,50,54,61,62], will help

explain species differences in the metabolic costs associated sexually selected weapons. Using measures of resting metabolic rate (RMR) and weapon muscle mass, we report the metabolic maintenance cost of sexually selected weapons in seven arthropod species (figure 1): thorny devil stick insects (Phasmatodea: Phasmatidae: *Eurycantha calcarata*), New Zealand long-legged harvestmen (Arachnida: Opiliones: Neopilionidae: *Forsteropsalis pureora*), frog-legged beetles (Coleoptera: Chrysomelidae: *Sagra femorata*), leaf-footed cactus bugs (Hemiptera: Coreidae: *Narnia femorata*), Indonesian stag beetles (Coleoptera: Lucanidae: *Cyclommatus* metallifer), Japanese rhinoceros beetles (Coleoptera: Scarabaeidae: *Trypoxylus dichotomus*) and heliconia bugs (Hemiptera: Coreidae: *Leptoscelis tricolor*). We show that, both within and across species, individuals with large weapon muscles relative to their body size have substantially higher RMRs than individuals with relatively small muscles, and we provide evidence that these trends are driven by muscle mass. We discuss our results in the context of honest signalling and costly weapons, and show that the observed variation in weapon cost is probably associated with variation in the morphology of the weapons studied.

2. Material and methods

(a) Study species
Species were chosen based on the presence of sexually selected weapons and ease of collection/sourcing through commercial

breeders (electronic supplementary material, table S1). Thorny devil stick insects [63,64], frog-legged beetles [20,65], leaf-footed cactus bugs [26,66] and heliconia bugs [67] all have enlarged hindleg weapons. These hindleg weapons are used to squeeze rivals and either (a) dislodge them from high value territories [66,67] or (b) remove them from receptive females mid-copula [20,65]. Indonesian stag beetles have enlarged mandibles used to dislodge rivals from potential nesting sites [68,69]. Japanese rhinoceros beetles have a twice-bifurcated head horn and a smaller, bifurcated thoracic horn, which are used to pry rivals from feeding sites frequented by potential mates [23,70]. New Zealand long-legged harvestmen have enlarged chelicera used to grapple opponents during contests over reproductive territories and/or females [71–74] (figure 1).

(b) Measurement of resting metabolic rate

Flow-through respirometry was used to measure carbon dioxide (CO_2) emission at rest (estimate of RMR) for stick insects ($n = 19$), frog-legged beetles ($n = 27$), leaf-footed cactus bugs ($n = 39$), stag beetles ($n = 13$), rhinoceros beetles ($n = 16$) and heliconia bugs ($n = 22$). The entire system was calibrated using pure N_2 and 2000 ppm CO_2.

CO_2 emission rate was measured for 1 h using a two-cell infrared analyser (Licor LI-7000, Licor, NE, USA) in differential mode. Dry, CO_2-free air was directed through the reference cell, which measured the fractional CO_2 concentration, then through a glass chamber containing the focal animal (2.2 l for stick insects; 14 ml for frog-legged beetles, leaf-looted cactus bugs, stag beetles and heliconia bugs; 500 ml for rhinoceros beetles) and into the measurement cell, which measured fractional CO_2 concentration of excurrent air. All gas circulated in 3 mm inner diameter plastic tubing. Air flow was controlled by a mass-flow controller (Unit instruments, CA, USA; 0–500 cm^3 min^{-1}), connected to controlling electronics (MFC-4, Sable Systems International, NV, USA). Flow rates were selected based on the body size and CO_2 production of each species to balance detectability and temporal resolution and were as follows: stick insects = 2 l min^{-1}, frog-legged beetles, stag beetles, rhinoceros beetles = 500 ml min^{-1}, leaf-footed cactus bugs, heliconia bugs = 250 ml min^{-1} [75]. Temperature was monitored using T-type thermocouples connected to a thermocouple meter (TC-1000, Sable Systems International). Activity was monitored visually or, when possible, using an activity detector (AD-1, Sable Systems International) and periods of activity were removed from the analysis. Before and after each trial, baseline CO_2 in the system was measured for 2 min with the experimental chamber empty. These measures were used to correct for baseline drift by constructing a linear model between CO_2 levels at the beginning and end of the trial and subtracting it from each CO_2 measurement.

Data were collected for frog-legged beetles using Laboratory Chart (v. 7.2, ADInstruments, AUS) receiving signals from an AD converter (PowerLab 8sp, ADInstruments). For all other species, data were collected using ExpeData (v. 1.1.9, Sable Systems International) receiving signals from an AD converter (UI2, Sable Systems International). AD converters received analogue signals from the two-cell infrared analyser, thermocouple meter and activity detector. The traces collected showed relative concentration of CO_2 (ppm) according to time (sampling frequency: 1 Hz). Raw measures were converted to molar rates of CO_2 production using known flow rate and the ideal gas law (equation (2.1)),

$$\dot{M}CO_2 = \frac{P * FR(F_e - F_i)}{R * T} \tag{2.1}$$

where $\dot{M}CO_2$ = rate of CO_2 production, P, pressure (1 ATM), FR, flow rate, F_e, excurrent CO_2 concentration, F_i, incurrent CO_2 concentration, R = gas constant (0.08206 1 atm K^{-1} mol^{-1}) and T = temperature. A continuous period of at least 20 min during which the animal was completely inactive was isolated, and

mean $\dot{M}CO_2$ production during this time was collected as an estimate of RMR. The first 10 min of each trial were excluded to avoid effects of handling.

Flow-through respirometry was not available for harvestmen. Instead, Warburg manometers were constructed [76]. Each animal ($n = 27$) was placed in a 60 ml syringe containing soda lime (Ca(OH)$_2$ and NaOH) soaked cotton wool. The syringe was then sealed to a graduated 1 ml syringe containing an ink filled water bubble. As the animal respired, CO_2 reacted with the soda lime and formed solid CaCO$_3$, which decreased gas volume and pressure within the syringe. This change in volume/pressure was measured via movement of the water bubble and collected as a measure of O_2 consumption and used as an estimate of RMR. Control manometers were run concurrently to correct for the baseline CO_2 and changes in atmospheric pressure. Measurements were collected over 20 min trials. Prior to each trial, animals were completely inactive for five minutes. Warburg manometers provide a high level of precision for measuring small organisms despite the lack the control provided by flow-through respirometry [75]. Still, to account for this lack of control, pressure and temperature fluctuation in the system was controlled for using a control manometer and CO_2 build up in the system was prevented by limiting trials to 20 min. We believe these actions allowed for reliable comparisons between data collected using Warburg manometers and flow-through respirometry. We recognize, however, the limitations associated with making direct comparisons using different methodologies and urge the reader to consider these limitations when interpreting our results.

(c) Morphological measures and muscle digestion/ dissection

All morphological measures were collected after measuring RMR (electronic supplementary material, table S2). For frog-legged beetles, stag beetles, rhinoceros beetles and harvestmen, weapon and body size were measured using digital callipers. For stick insects, leaf-footed cactus bugs and heliconia bugs, weapon and body size were measured from photographs using ImageJ v. 1.50i software (NIH, USA). Body mass of stick insects, harvestmen, leaf-footed bugs, rhinoceros beetles and heliconia bugs was measured directly using digital scales. Body mass of frog legged beetles was estimated using a linear model between body mass and body size PC1 (electronic supplementary material, table S2), which was constructed from a sample of frog-legged beetles collected from the same population at an earlier date. This appeared to be a reliable estimate of body mass for the frog-legged beetles measured here, but it should be noted that this method could have masked differences in individual quality encoded in deviations from the average relationship between body mass and overall size. Direct measures of body mass were unavailable for stag beetles. Instead, mean body mass for stag beetles was sourced from [52] and used as an estimate of mean stag beetle body mass.

Weapon muscle mass was measured using potassium hydroxide (KOH) digestion for frog-legged beetles, leaf-footed cactus bugs, stag beetles, rhinoceros beetles and heliconia bugs [77–79]. Weapon muscle mass was measured in harvestmen using papain digestion. Weapons and associated muscle (electronic supplementary material, table S2) were dissected, dried at 70°C (50°C for harvestmen) and weighed. After initial weighing, weapons were submerged in 10% KOH (18.5 U.ml^{-1} papain in 100 mM TRIS–HCl pH 7 buffer for harvestmen) and incubated at 70°C (room temperature for harvestmen) to digest soft tissue, primarily muscle (frog-legged beetles, stag beetles, rhinoceros beetles = 12 h; leaf-footed cactus bugs, heliconia bugs = 8 h; long-legged harvestmen = 72 h). After digestion, weapons were rinsed with water and dried at 70°C (50°C for harvestmen). Once dry, weapons were weighed a second time. The difference between first and second weighing was taken as an estimate of dry muscle mass.

For rhinoceros beetles, wet muscle mass was collected in place of dry muscle mass (using the protocol described above, except for drying steps), which likely overestimated weapon muscle mass compared to other species in this study. For stick insects, hind femurs were dissected, dried at 70°C for 24 h and weighed. Muscles were then manually dissected (due to their large size) and the hindleg was weighed a second time. The difference between first and second weighing was taken as an estimate of dry muscle mass. For species with paired weapons (stick insects, harvestmen, frog-legged beetles, leaf-footed bugs and heliconia bugs), weapon muscle mass was measured from a single weapon and multiplied by two to calculate total weapon muscle mass.

(d) Statistical analyses

All statistical analyses were performed in R v. 3.5.0 (R Core Development Team, 2018). For analyses of RMR, principal component analyses (PCA; R package FactoMineR [80]) were constructed separately for each species and used to estimate weapon and body size in an effort to capture the effects of overall body size on RMR (variables included in PCA summarized in electronic supplementary material, table S2). For all other analyses, a single, log-transformed linear measure of weapon and body size was used (electronic supplementary material, table S2, bold). This allowed for direct and transparent assessment of scaling relationships against isometry [81].

All data were log-transformed prior to analysis of scaling relationships. Two analyses were performed in each species to assess the relationship between RMR, weapon size and body size. First, RMR was regressed on body size and weapon size in the same model using ordinary least-squares (OLS) regression [82]. Second, residual RMR was regressed on residual weapon size using OLS regression to determine whether males with large relative weapon sizes (i.e. high residual weapon sizes) have high RMRs for their body size (i.e. high residual RMR). Residuals for the latter analyses were collected from separate OLS regressions of RMR on body size and weapon size on body size. Both analyses allowed us to assess the effect of weapon size on RMR while controlling for the effects of overall body size.

OLS regression was also used to assess the relationship between weapon size and body size and muscle mass and body size for all species. Relative weapon muscle mass was calculated first as muscle mass divided by linear measures of body size (relative muscle mass A) and second as muscle mass divided by body mass (relative muscle mass B). Measuring relative weapon muscle mass two ways provided two measures of relative weapon muscle mass for most animals, as well as a reliable measure of weapon muscle mass for stag beetles, where body mass was unavailable. Log mean relative weapon muscle mass was compared across species using means and 95% confidence intervals. For stag beetles, mean weapon muscle mass/mean body mass was used in place of mean relative weapon muscle mass (see above). RMR was then regressed on log relative muscle mass A and B in stick insects, frog-legged beetles, stag beetles (species with a significant relationship between residual RMR and residual weapon size) to determine the role weapon muscle mass plays in the observed trends (note: relative muscle mass B was not regressed on RMR in stag beetles).

3. Results

(a) Principal component analyses

In stick insects, harvestmen, frog-legged beetles, cactus bugs, stag beetles and rhinoceros beetles, principal component 1 (PC1) explained the majority of variation in every PCA and was used as the measure of weapon and/or body size (electronic supplementary material, table S2).

(b) Scaling relationships

Weapon size increased hyperallometrically ($\beta > 1$ for linear measurements) with body size in harvestmen ($\beta = 1.33 \pm 0.28$, $F_{1,25} = 22.28$, $p < 0.0001$), frog-legged beetles ($\beta = 1.43 \pm 0.14$, $F_{1,25} = 108.8$, $p < 0.0001$), stag beetles ($\beta = 1.975 \pm 0.69$, $F_{1,11} = 8.083$, $p = 0.016$) and heliconia bugs ($\beta = 2.064 \pm 0.26$, $F_{1,20} = 60.81$, $p < 0.0001$). Weapon size increased isometrically with body size in stick insects ($\beta = 2.206 \pm 0.38$, $F_{1,17} = 32.83$, $p < 0.0001$; isometry for area measurements, $\beta = 2$). Weapon size increased hypoallometrically with body size in cactus bugs ($\beta = 0.691 \pm 0.06$, $F_{1,37} = 113.2$, $p < 0.0001$) and rhinoceros beetles ($\beta = 0.662 \pm 0.07$, $F_{1,14} = 92$, $p < 0.0001$; figure 2).

RMR increased with weapon size in stick insects ($\beta = 1.263$, $F_{2,15} = 10.8$, $p < 0.001$), frog-legged beetles ($\beta = 0.259$, $F_{3,19} = 14.65$, $p < 0.001$) and stag beetles ($\beta = 0.133$, $F_{3,9} = 15.03$, $p < 0.001$), but there was no significant effect of body size on RMR and no significant interaction between weapon size and body size in these models (electronic supplementary material, table S3). There were no significant relationships between RMR and weapon size or body size in rhinoceros beetles, heliconia bugs, leaf-footed cactus bugs or harvestmen (electronic supplementary material, table S3). Residual RMR increased with residual weapon size in stick insects ($\beta = 1.05$, $F_{1,17} = 6.439$ $p = 0.021$), frog-legged beetles ($\beta = 0.254$, $F_{1,21} = 6.519$, $p = 0.019$) and stag beetles ($\beta = 0.131$, $F_{1,11} = 10.37$, $p = 0.008$), suggesting males with large relative weapon sizes experience higher RMRs than predicted by their body size (figure 2). Residual RMR decreased with residual weapon size in harvestmen ($\beta = -0.375$, $F_{1,24} = 6.561$, $p = 0.017$; figure 2). There was no significant relationship between residual RMR and residual weapon size in cactus bugs, rhinoceros beetles or heliconia bugs.

Muscle mass increased hyperallometrically ($\beta > 3$ for volumetric measurements [75]) with body size in stick insects ($\beta = 4.849$, $F_{1,17} = 27.29$, $p < 0.001$), frog-legged beetles ($\beta = 3.559$, $F_{1,22} = 207.5$, $p < 0.0001$) and stag beetles ($\beta = 3.507$, $F_{1,9} = 81.82$, $p < 0.0001$; figure 2). Muscle mass increased hypoallometrically with body size in cactus bugs ($\beta = 2.055$, $F_{1,37} = 11.86$, $p < 0.01$) and rhinoceros beetles ($\beta = 2.505$, $F_{1,55} = 204$, $p < 0.0001$; figure 2). In harvestmen and heliconia bugs, there was no significant relationship between muscle mass and body size.

Log relative weapon muscle mass for each species is summarized in figure 3 and electronic supplementary material, table S4. In stick insects, frog-legged beetles and stag beetles, log RMR increased with log relative weapon muscle mass A (stick insects: $\beta = 0.61$, $F_{1,17} = 28.55$, $p < 0.0001$; frog-legged beetles: $\beta = 1.367$, $F_{1,21} = 30.31$, $p < 0.0001$; stag beetles: $\beta = 1.99$, $F_{1,9} = 13.76$, $p < 0.01$) and log relative muscle mass B (stick insects: $\beta = 0.996$ $F_{1,17} = 29.65$, $p < 0.0001$; frog-legged beetles: $\beta = 1.988$, $F_{1,25} = 12.67$, $p < 0.01$; stag beetles: NA; electronic supplementary material, figure S1), suggesting the observed trends in RMR are indeed driven by high relative weapon muscle mass.

4. Discussion

We suggest that much of the variation observed in costly sexually selected weapons stems from variation in the types of weapons studied and their associated musculature. Here, we surveyed RMR as a metric of metabolic maintenance cost
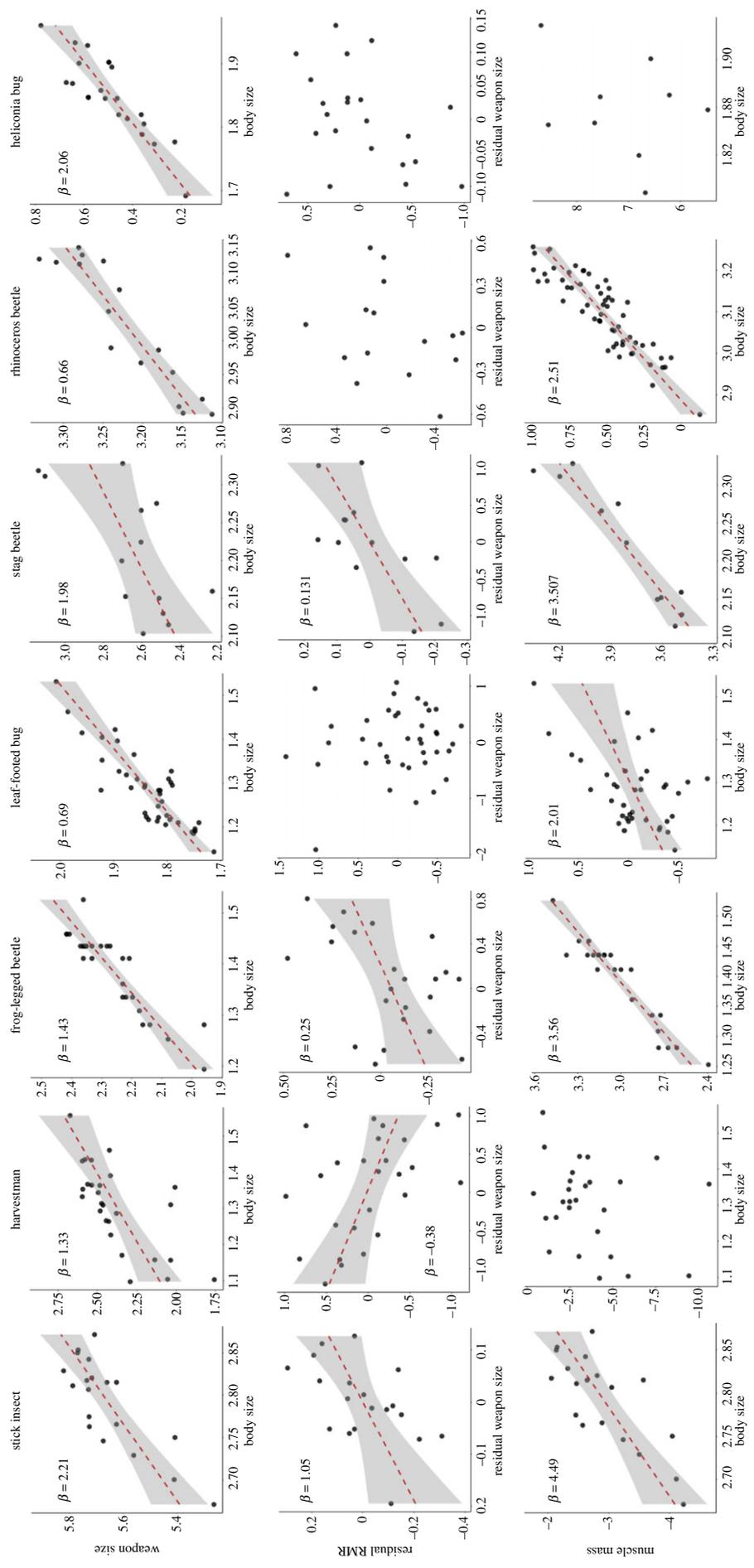
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Figure 2. Scaling relationship between log weapon and log body size (top), log residual resting metabolic rate and log residual weapon size (middle), and log muscle mass and log body size (bottom) for all species. Red dashed lines represent OLS regression. Shaded areas represent 95% confidence intervals around OLS regressions. Slopes (β) omitted for non-significant scaling relationships. (Online version in colour.)
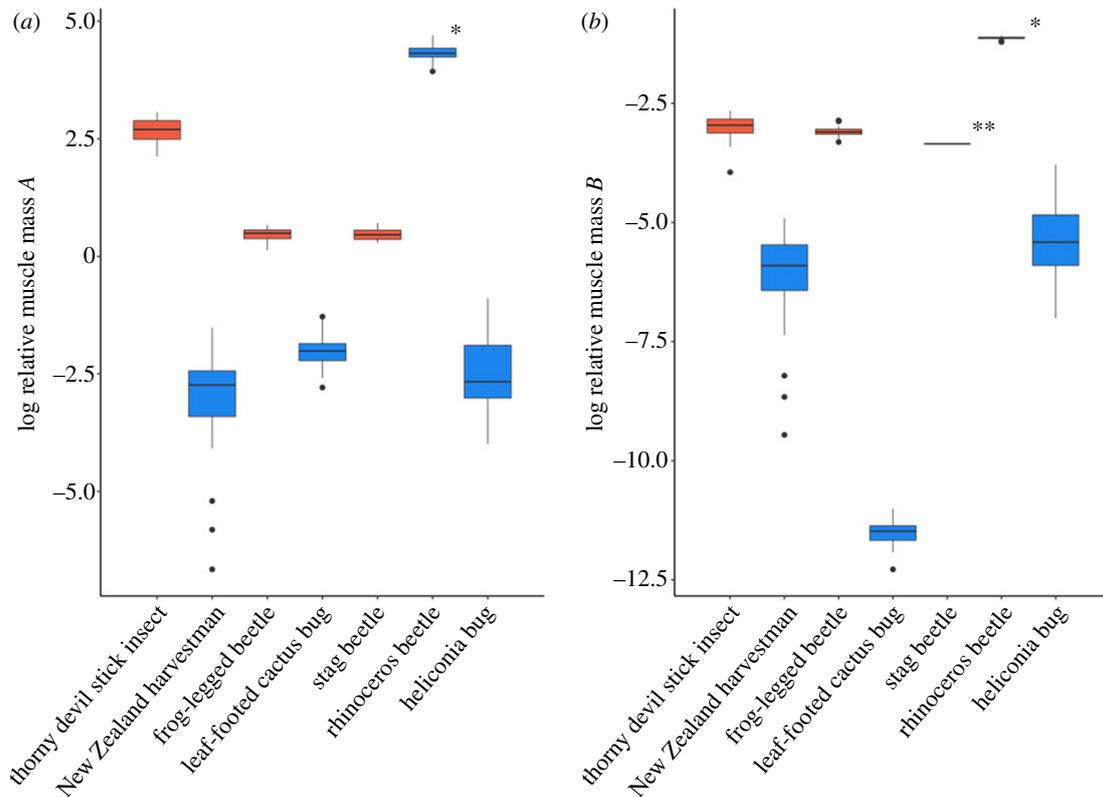
Figure 3. Boxplot of relative weapon muscle mass for all species. (a) relative muscle mass A = muscle mass/body size. (b) Relative muscle mass B = muscle mass/body mass. Shades of red indicate significant relationships between residual RMR and residual weapon size. Shades of blue indicate no significant relationship between residual RMR and weapon size. Error bars represent 95% confidence intervals around mean relative muscle mass. *Wet muscle mass was measured for rhinoceros beetles in place of dry muscle mass. **Relative muscle mass B calculated for stag beetles as mean muscle mass/mean body mass.

in seven arthropod species (figure 1) and related these measures to weapon muscle mass. In stick insects, frog-legged beetles and stag beetles, three species with high relative muscle mass (figure 3; electronic supplementary material, table S4) and steep scaling relationships between muscle mass and body size, there was a significant positive relationship between RMR and weapon size after controlling for body size (electronic supplementary material, table S3), and residual RMR increased with residual weapon size (figure 2). This suggests that males with large relative weapon sizes have unusually high RMRs, independent of their typically large body sizes. In addition, these species all had significant, positive scaling relationships between RMR and relative muscle mass (electronic supplementary material, figure S1), further suggesting these trends in RMR are driven by weapon muscle mass. By contrast, other species surveyed here showed shallow or nonsignificant scaling relationships between muscle mass and body size (figure 2), had low relative weapon muscle mass (figure 3), and either a negative or nonsignificant relationship between RMR and weapon size and/or body size (electronic supplementary material, figure S3; figure 2).

Our results suggest that when animals have high weapon-associated muscle mass that scales steeply with body size, they experience relatively high metabolic maintenance costs. These types of heavily muscled weapons should be especially prevalent in animals where performance depends on muscle content (rather than speed or strength-amplifying levers), and when hyperallometric scaling of weapon muscle mass is required to overcome mechanical disadvantage in large weapons [83–85]. Indeed, this is the case for both frog-legged beetles [83] and stag beetles [84], and in both systems residual RMR increased with residual

weapon size (figure 1). (Note: there is no published work to date on weapon force production in thorny devil stick insects.)

By extension, we suggest inconsistency in weapon literature regarding cost may, in part, result from interspecific variation in the way weapon strength is generated and the associated variation in weapon muscle mass. Leaf-footed cactus bugs, for example, show no perceptible strength [83] or metabolic cost associated with hindleg weapons and have relatively small muscles that scale hypoallometrically with body size (figures 2 and 3). The heliconia bugs measured here also showed no metabolic cost, and large-weaponed individuals did not have relatively large weapon muscles (figures 2 and 3). Recent work by Somjee et al. [86] does suggest weapon muscle contributes to RMR in this species, but RMR in that study scaled shallowly across weapon sizes, suggesting large-weaponed males experience low metabolic cost relative to their weapon size. In long-legged harvestmen, where relative weapon muscle mass is low and there is no significant relationship between weapon muscle mass and body size, residual RMR decreased as residual weapon size increased (figures 2 and 3). This pattern may result from biological variation in the way chelicera function across body/weapon sizes or may be an artefact of the way weapon/muscle size was measured. For example, weapon-associated muscle in the body, which was not measured here, may be important for weapon function in long-legged harvestmen. Additionally, physiologically relevant variation in harvestmen weapon morphology may not be fully captured by linear measurements of weapon size [71]. Finally, Japanese rhinoceros beetles show no locomotor, immune, developmental or metabolic cost associated with their large, hollow horns, and the prothoracic muscles

regulating weapon movement scaled hypoallometrically with body size (figure 2) [58–60]. Rhinoceros beetles did have relatively massive prothoracic muscles compared with weapon muscle mass in other species (figure 3), but this was probably an overestimation since wet muscle mass was measured in place of dry muscle mass. Overall, these trends could result from the difficulty associated with detecting patterns in data composed of small measurements with low variation. It is more likely, however, that weapon strength in each of these species is generated and maintained through modifications of the weapon lever system, rather than hyperallometric increases in weapon muscle (e.g. [69,85]), and that the resulting low muscle mass explains why costs have never been observed.

It should be noted, however, that weapon honesty is still expected in species where metabolic cost was not observed. These weapons still function as signals and should reliably display individual quality. Rather than metabolic costs driven by large muscles, species with small muscles may experience production costs resulting from differential resource allocation during weapon development [48,51,57] or locomotor costs, not from heavy, muscular weapons, but from bulky, otherwise lightweight structures [53,87]. Honesty may also be maintained through heightened condition-dependent development [28,32,47,88–96]. Sexually selected weapons are famously sensitive to developmental nutrition [95], the abiotic environment [97,98], parasite load [99] and stress [100]. When weapon growth is sensitive to these factors, only individuals of the highest quality can produce large weapons. Along with costs, condition dependence can effectively restrict the biggest weapons to the highest quality individuals, ensuring that weapon size persists as a reliable signal through time.

Overall, we suggest that much of the controversy surrounding the presence/absence of cost in weapon systems can be resolved, in part, by recognizing that both the type and magnitude of cost may be dependent on the composition of the weapon studied. Notably, we suspect some of the variation in weapon cost is driven by variation in the ways weapon force is generated and the associated variation in weapon muscle mass. The work presented here clearly suggests a relationship between weapon use, muscle content and cost across arthropod species. However, our interpretation of these results may have been limited by relatively small sample sizes, lack of phylogenetic control, and variation in the methodology used throughout. We therefore encourage those exploring the cost of sexually selected weapons to focus on large groups of species where robust phylogenetic methods are available [101], to use large samples sizes and consistent methodology when possible, and to direct their study using both a priori knowledge of the biomechanical mode of action of the structure and the behavioural ecology of the focal species.

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