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Males that drop a sexually selected weapon grow larger testes

Paul N. Joseph,[1,2] Zachary Emberts,[3] Daniel A. Sasson,[4] and Christine W. Miller[1,5]

[1] *Department of Entomology and Nematology, University of Florida, Gainesville, Florida 32608*

[2] *Current Address: Esophageal and Lung Institute, Allegheny Health Network, 4800 Friendship Avenue, Pittsburgh, Pennsylvania 15224*

[3] *Department of Biology, University of Florida, Gainesville, Florida 32611*

[4] *Whitney Laboratory for Marine Bioscience, University of Florida, St. Augustine, Florida 32080*

[5] *E-mail: cwmiller@ufl.edu*

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Costly sexually selected weapons are predicted to trade off with postcopulatory traits, such as testes. Although weapons can be important for achieving access to females, individuals of some species can permanently drop (i.e. autotomize) their weapons, without regeneration, to escape danger. We capitalized on this natural behavior to experimentally address whether the loss of a sexually selected weapon leads to increased testes investment in the leaf-footed cactus bug, *Narnia femorata* Stål (Hemiptera: Coreidae). In a second experiment, we measured offspring production for males that lost a weapon during development. As predicted, males that dropped a hind limb during development grew significantly larger testes than the control treatments. Hind-limb autotomy did not result in the enlargement of other nearby traits. Our results are the first to experimentally demonstrate that males compensate for natural weapon loss by investing more in testes. In a second experiment we found that females paired with males that lost a hind limb had 40% lower egg hatching success than females paired with intact males, perhaps because of lower mating receptivity to males with a lost limb. Importantly, in those cases where viable offspring were produced, males missing a hind limb produced 42% more offspring than males with intact limbs. These results suggest that the loss of a hind-limb weapon can, in some cases, lead to greater fertilization success.

KEY WORDS: Autotomy, compensation, fecundity, male–male competition, reproduction, sexual selection.

Sexual selection via male–male competition has resulted in the evolution of male sexual weapons (Andersson 1994). Larger, sturdier weapons are predicted to allow males to better compete for high-quality territories and mating opportunities, likely increasing reproductive success (Miyatake 1997; Eberhard 1998; Kelly 2008; Yamane et al. 2010; McCullough et al. 2016). However, many weapons are costly to produce and maintain (Emlen 2001; Allen and Levinton 2007; but see McCullough and Emlen 2013; McCullough and Tobalske 2013) and can compete for a limited supply of resources within an individual (Nijhout and Emlen 1998; Moczek and Nijhout 2004; Tomkins et al. 2005). Therefore, an individual that grows larger weapons is predicted to have fewer resources available to invest in other bodily structures (Kawano 1997; Tomkins et al. 2005; Yamane et al. 2010). Importantly, theory assumes a common developmental trade-off between costly traits, including many weapons and primary sexual traits (Parker et al. 2013). Allocation to weapons may be prioritized over allocation to primary sexual traits in those cases where females can be monopolized (Lupold et al 2014).

The theoretical framework for sexually selected trade-offs has generally been supported, with much of the existing work conducted on insects. Observational studies suggest a negative association between weapon size and the size of copulatory organs (Parzer and Moczek 2008), testes (Simmons et al. 1999; Fry 2006; Fitzpatrick et al. 2012; Lüpold et al. 2014; Dines et al. 2015), and ejaculates (Preston et al. 2001; Fry 2006; Liu et al. 2015). These studies have provided valuable and intriguing evidence that males lacking large weapons compensate for

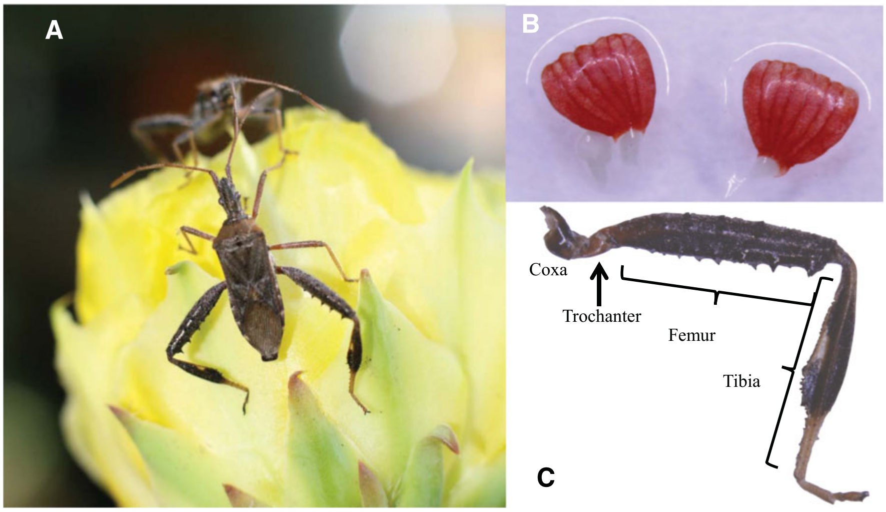

Figure 1. The leaf-footed cactus bug, *Narnia femorata*, including photographs of (A) the full body, (B) a pair of *N. femorata* testes, and (C) a hind limb with labeled segments. Photo credits: C. W. Miller, P. N. Joseph.

their reduced ability to acquire mates by investing more resources into each reproductive event. However, individual variation in the acquisition and allocation of available resources may confound correlations between traits (Reznick et al. 2000). Studies incorporating phenotypic engineering can provide powerful experimental complements to existing research, allowing a level of inference that cannot be achieved through observational studies alone (Zera and Harshman 2001; Simmons et al. 2017). Though few in number, experiments that have manipulated weapon growth (Fry 2006; Simmons and Emlen 2006) and testes growth (Moczek and Nijhout 2004) support a weapons-testes trade off (Simmons et al. 2017). However many phenotypic engineering studies use ablation methods, via micro-cauterization, which administer unnatural trauma to study subjects. Uneven survivorship across the experimental and control groups is a common result, which can affect the conclusions drawn from these studies. Furthermore, these studies do not address a fundamental question: when individuals are naturally unable to develop weaponry, do they grow larger testes?

Animals that routinely drop their sexually selected weapons provide a powerful and ecologically relevant opportunity to test questions about trait allocation and investment. Leaf-footed bugs in the family Coreidae are able to autotomize (drop) weaponized hind limbs to escape unfavorable situations (Emberts et al. 2016). Importantly, once the limbs are dropped they cannot be regenerated (Emberts et al. 2017). Thus, the process of regeneration does not compete for available resources and energy, as it does in species that regenerate their autotomized limbs, such as anoles and crabs (Dial and Fitzpatrick 1981; Backwell et al. 2000). Furthermore, after the permanent loss of a weapon, male leaf-footed bugs rarely secure access to females in a competitive environment (Emberts, *in review*). In hermit crabs (Yasuda and Koga 2016), jumping spiders (Taylor and Jackson 2003), and lobsters (O'Neill and Cobb 1979), males that lose a weapon are relatively unsuccessful in contest competition. Therefore, to maximize their reproductive success, theory suggests that these males should increase investment in postcopulatory traits, such as testes size (Parker et al. 2013).

The costliness of weapons may lead to a trade-off not only with testes but also with other nearby structures (Nijhout and Emlen 1998; Emlen 2001). The loss of a weapon, via autotomy, could allow for more resources to be allocated to nearby structures and increase their growth (henceforth refered to as the proximity hypothesis). Consquently, in cases where weapons and testes are in close proximity, an increase in testes size following weapon loss would not necessarily indicate a precopulatory/postcopulatory trade-off. Thus, to robustly test these alternatives, measuring the size of multiple nearby structures across experimental subjects is essential. Along these lines, if resources are simply being allocated to nearby structures, we might expect the testis closest to the site of weapon loss to grow larger than the other testis.

We conducted a phenotypic engineering experiment on the leaf-footed cactus bug, *Narnia femorata* Stål (Hemiptera: Coreidae; Fig. 1) to address allocation decisions when a weapon is autotomized. We followed up on this study with a second experiment to estimate the reproductive consequences of weapon autotomy for males. *N. femorata* possesses sexually dimorphic hind femurs (Miller et al. 2016) that are used as weapons in male contests (Procter et al. 2012). In escalated competitions, males kick and squeeze each other with their hind limbs until one male retreats (Nolen et al. 2017). Hind limbs in this species appear to be costly and condition-dependent traits (Miller et al. 2016; Allen and Miller 2017), and larger males are more successful in male–male contests (Procter et al. 2012; Nolen et al. 2017).

Previous observational findings suggested a trade-off might be present between weapons and testes in a wild population of another coreid species (Somjee et al. 2015), and we predicted that we would find such a tradeoff here. We measured the testes mass, body size, and size of four additional nearby morphological traits for *N. femorata* males that had a hind-limb weapon experimentally autotomized during development. We compared these males against males in three control groups: males that autotomized a nonweaponized mid limb; males that were handled in a similar manner, but without any autotomy; and males that did not receive any manipulation. An allocation trade-off between weapons and testes would be supported if individuals that lose a hind limb (relative to individuals that do not lose a hind limb) have larger testes for their body size. To test if the trade-off is due to the close proximity of these traits, we compared the size of nearby structures. We also looked for any difference in the relative size of the testis closest to the site of autotomy across our experimental and control groups.

Finally, we examined differences in the short-term reproductive success of males that automized a hind limb during development relative to intact males. Increased testis size may increase sperm production and lead to enhanced reproductive success, given that many males appear to be sperm limited (Schulte-Hostedde and Millar 2004; Simmons and García-Gonzalez 2008). Thus, based on the results of our first experiment, we predicted that pairings between females and males that autotomized a hind-limb weapon should lead to more offspring than pairings between females and intact males.

Methods

EXPERIMENT (1): EFFECTS OF HIND-LIMB LOSS ON TESTES SIZE

Animal husbandry and experimental design

Insects used in our first experiment were from the offspring of 29 mating pairs of wild caught *N. femorata* collected from Live Oak, FL between September and October 2015. Randomly paired males and females were placed in a plastic container consisting of a single potted prickly-pear cactus (*Opuntia mesacantha ssp. lata*, also called *O. humifusa*) with fruit. Mating pairs were able to mate and oviposit freely within their containers in a greenhouse maintained 21–32°C with a 14:10 h L:D photoperiod. Groups of newly hatched siblings (up to ten individuals) were housed in cups separate from their parents. Upon emergence as a fourth instar juvenile, each individual was housed in its own container with a cactus cladode and ripe fruit for feeding ad libitum, a standard practice in our laboratory (e.g. Miller et al. 2016). Insects raised under our laboratory conditions can grow as large as field-collected individuals, but they are on average smaller than insects captured from the wild, suggesting the nutrition available is suboptimal (e.g., Cirino and Miller 2017; Miller et al. 2016). We induced autotomy at the beginning of the fourth (penultimate) juvenile instar, since heteropteran testes undergo increased growth later in juvenile development (Economopolous and Gordon 1971; Dumser and Davey 1974). At this life stage, determining the sex of individuals is prohibitively difficult, thus we induced autotomy on individuals regardless of their sex. We did not use adult females in this first experiment.

We divided the 583 fourth-instar juveniles into one of four treatments: induced autotomy of the left hind limb (hind-limb autotomized), induced autotomy of the left mid limb (mid-limb control), handling stress (handling control), and unmanipulated (baseline control). Autotomy was induced by gripping a limb with a pair of forceps (Emberts et al. 2016) and gently using a paintbrush to incite an individual to self-autotomize. The process of autotomy in this species involves a lift-and-shift behavior by individuals that severs the limb at the joint between the trochanter and femur (Emberts et al. 2016). Autotomy was induced on the left mid limb (mid-limb control) to assess the effects of the removal of a typical locomotory limb on testes mass and the other traits. This limb is not enlarged and does not appear to be under direct sexual selection in this species. We tested for effects of handling stress on testes mass and other traits by establishing a third set of males that were brushed with the same paintbrush for a few seconds without having the forceps attached to a limb (handling control). Finally, we established a fourth set of males that were not contacted by the forceps or the paintbrush to serve as a baseline control. Individuals were left individually to feed ad libitum and develop to adulthood. Of the insects that survived to adulthood, 210 developed into females. Some insects escaped or autotomized an additional limb during their development. These insects were not used in further analyses. We froze 49 males from each treatment 14 days after adult emergence.

Testes mass

We removed pairs of testes from 196 thawed adult males, 49 for each of the four treatments. Each pair of testes was submerged in 70% EtOH and refrigerated until they were dried at 60°C for 24 hours in preweighed aluminum foil boats. We measured the mass of each pair of testes in the foil boats to the nearest microgram using a microbalance (Mettler Toledo XP6: Columbus, OH, USA).

Body size and structures close to the site of autotomy

To measure linear dimensions and area, we took a picture of 196 frozen males under a dissecting microscope (Leica M165C) with a digital camera (Canon EOS 50D). We used Image J (v1.46r; Schneider et al. 2012) to measure pronotum width as our metric of body size, since this simple measurement is highly correlated with overall body size in this species (Procter et al. 2012; Gillespie et al. 2014). For each male, we measured two components of the remaining (opposite side) hind limb, the right hind femur area and the right hind tibia area, to the nearest micrometer (Fig. 1C). We note that these two structures, though visually different, are probably not developmentally independent. Hind tibias are subtly enlarged and sexually dimorphic in this species, but they have no known use in sexual selection. Next, we measured two traits on the left side of the male, the same side as hind-limb autotomy: (1) the width of the left mid-limb femur, which is the closest nonweapon limb to the site of autotomy, and (2) the length of the hind left trochanter, the small second segment of the insect limb, between the coxa and the femur and immediately adjacent to the site of autotomy. These same-side measurements were performed for a random subset of hind-limb autotomized (mid limb and trochanter: $n = 20$) and baseline control males (mid limb: $n = 21$; trochanter: $n = 22$; Fig. 1B). To further test the proximity hypothesis, we tested whether the investment in left relative to right testis was affected by treatment. We measured the mass of individual left and right testes for a random subset of experimental ($n = 25$) and baseline control males ($n = 19$) to the nearest microgram using the microbalance described above.

EXPERIMENT (2): EFFECTS OF HIND-LIMB LOSS ON REPRODUCTIVE SUCCESS

The relationship between testes mass and fertilization success is a key assumption of many studies of sexual selection (Preston et al. 2003; Simmons and García-González 2008). Consequently, factors that increase the size of testes are generally assumed to increase the numbers of eggs fertilized per mating bout. We directly tested this assumption by investigating the effect of male hind-limb autotomy on the resulting number of hatched eggs when a male was paired with a female for just 24 hours. We predicted that the presence/absence of eggs should not be influenced by male autotomy because females will lay eggs even when they are not fertilized (C.W. Miller, *personal obs*).

Animal husbandry and experimental design

We reared individuals in Percival growth chambers with a 14:10 h L:D photoperiod, with 26°C during the day and 22°C at night. These individuals were the offspring from 13 pairs of first generation lab-reared *N. femorata*, that derived from wild individuals originally collected from Starke, FL (29.9804° N, 81.9848° W) in October 2016. Individuals were kept in groups of up to nine individuals until their fourth instar, at which point they were transferred to their own container. We then randomly assigned 134 juveniles of unknown sex to the autotomy treatment or control treatment. Autotomy was induced as described in Experiment 1. Fourteen to 20 days after adult eclosion, 54 males were transferred individually to a container with a virgin female, forming 54 mating pairs. Of the 54 males, 25 of these males had experienced autotomy and 29 were intact. The virgin females in Experiment 2 were raised under the same conditions as the males. We used 23 females that experienced autotomy, and 31 intact females. After 24 hours, the males were removed from the containers and were frozen for later morphological measurements. Each female remained in the container for an additional 14 days to oviposit freely, after which she was removed and frozen for later measurements. We counted eggs laid in the container and provided an additional 14 days for eggs to hatch, a sufficient amount of time for hatching (Vessels et al. 2013). We then noted hatching success (yes/no) and counted the number of hatched eggs for each female.

ANALYSIS

Experiment (1): Effects of hind-limb loss on testes size

We performed a simple chi-square analysis to examine the survivorship of the 583 individuals between the 4th instar (when the treatment was performed) and 14 days after becoming an adult. The linear measurements of pronotum width, mid-limb femur width, and left hind trochanter length were cube transformed, and the area measurements of hind femur area and hind tibia area were squared to convert their values to the same scale as mass for comparisons (see Tomkins and Simmons 2002). All morphological data were then $\log_{10}$ transformed to improve linearity, normality, and homoscedasticity. In all analyses of morphological traits, we assumed a normal trait distribution. We tested whether hind-limb removal during development affected adult male body size (measured as pronotum width) using a generalized linear model with the treatment (three controls and hind-limb removal) as a categorical explanatory variable. We found no effect of hind-limb removal on adult male body size (see below for statistical results), thus we proceeded to use body size as a covariate in five separate GLMs examining the effects of hind-limb removal on the size of five morphological traits relative to body size: testes mass, hind femur area of opposite limb, hind tibia area of opposite limb, mid-limb femur width on the same side, and hind-limb trochanter length on the same side. Our initial models were built with body size as a continuous covariate, treatment as a categorical factor, and the two-way interaction between body size and treatment. In our next analysis, we tested the effects of treatment (hind-limb removal and the three different controls) on the investment in left testes relative to right testes. Testes are naturally asymmetric, with the left testis approx. 15% larger than the right. Thus, we constructed a GLM model including left testis mass as the dependent variable, right testis mass as a continuous covariate, treatment as a categorical factor, and the two-way interaction between right testis mass and treatment.

Experiment (2): Effects of hind-limb loss on reproductive success

We performed a simple chi-square analysis to examine the survivorship of 134 males and females between the 4th instar (when the treatment was performed) and male–female pairing (14–20 days after becoming adult). We next constructed three GLMs, in all cases including female body size and male body size as covariates. In invertebrates, larger females typically produce more offspring, and including female body size in our model allowed us to control for this variation. Including male body size as a covariate allowed us to control for effects of overall size (larger males have larger testes). Sex cannot be determined at the fourth instar, therefore both males and females experienced autotomy in our study. We used both autotomized ($n = 23$) and intact ($n = 31$) females in Experiment 2 along with autotomized ($n = 25$) and intact ($n = 29$) males. Males and females were paired in all four combinations of autotomy and control. We included female treatment (autotomy or control) in our statistical models along with other main effects.

We restricted our analyses to specifically address our research questions: were eggs laid at all (yes/no), were these eggs viable (did they hatch yes/no), and how many hatchlings were produced? Our first GLM examined whether eggs were laid. We used the logit-link function and binomial distribution, with explanatory variables including the main effects of male treatment (autotomy or control), female treatment (autotomy or control), male body size, and female body size. Our second GLM examined whether or not any of the eggs laid hatched (yes/no), specifically for those pairs that did produce eggs. As above, we used the logit-link function and binomial distribution and the same explanatory variables. Our third model examined the number of hatchlings for the subset of pairs where at least one egg hatched, with the same explanatory variables as listed above. For these count data, we used a GLM assuming a Poisson distribution. All analyses were performed in IBM SPSS Statistics v.24.

Results

EXPERIMENT (1): EFFECTS OF HIND-LIMB LOSS ON TESTES SIZE

Individuals with hind limbs autotomized during the penultimate juvenile instar did not have higher mortality than those in the control treatments (approx. 19% mortality across 583 total individuals; $X^2 = 1.973$, d.f. = 3, $P > 0.20$). We found no effect of autotomy of the left hind-limb weapon on eventual adult body size (Wald $X^2 = 3.786$, d.f. = 1, $P = 0.286$). We next tested the effect of hind-limb autotomy on five morphological traits in adults, with body size as a continuous covariate in our models. As expected, the main effect of body size was statistically significant in all models, indicating that trait size increases with body size. A significant interaction between body size and treatment would indicate that the effect of treatment differed according to body size (a change in the *slope* of the scaling relationship due to treatment). We did not find the interaction between body size and treatment to be statistically significant for any of the measured traits (body size × treatment: testes mass, $P = 0.563$; right hind femur area, $P = 0.331$; right hind tibia area, $P = 0.539$; left mid-limb width, $P = 0.996$; left trochanter length, $P = 0.534$), thus this interaction was removed from all models. Removing the interaction allowed us to examine effects of treatment on scaling *intercept,* in other words, to examine if hind-limb autotomy results in larger trait size for any given body size.

Table 1. **Results of three separate GLM for effect of hind-limb loss with body size as covariate.**

Source	d.f.	Testes mass, wald X^2	Opposite-side hind femur area, wald X^2	Opposite-side hind tibia area, Wald X^2
Body size	1	198.467***	1791.895***	869.039***
Treatment	3	11.613**	0.116	1.014

Each response variable was $\log_{10}$ transformed.
$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

We found that hind-limb weapon autotomy during the penultimate juvenile instar led to the growth of larger testes (Table 1; Fig. 2). Males that experienced hind-limb autotomy had 15% greater testes mass relative to the testes of males from the control treatments. We did not find that autotomy was related to an increase in any other trait measured, including the nearby, same-side mid-limb femur (Table 1, 2; Fig. 2, 3B). Hind-limb autotomy (of the left hind limb in all cases) was related to a decrease in size for one trait—the left hind trochanter (Table 2; Fig. 3A). Trochanters are the small segment that directly connects to the femur and coxa, and is thus adjacent to the site of autotomy (Fig. 1C). This structure was notably reduced in males that autotomized the remaining portions of their limb during development.

If hind-limb autotomy leads to increased investment in nearby structures, then left testis mass might grow larger relative to right testes mass. We measured left and right testes mass for 44 individuals, 19 that received no manipulation and 25 with induced hind-limb autotomy. We constructed an initial model with left testes mass as the response variable, right testes mass as a continuous covariate, treatment as a categorical factor, and the interaction of right testes mass and treatment. The interaction was not found to be statistically significant ($P = 0.645$), and we removed it from our model. Right testis mass scaled with left testis mass as expected (Wald $X^2 = 268.517$, d.f. = 1, $P < 0.001$). Hind-limb removal did not increase left testis mass relative to right testis mass (Wald $X^2 = 0.615$, d.f. = 1, $P = 0.433$).

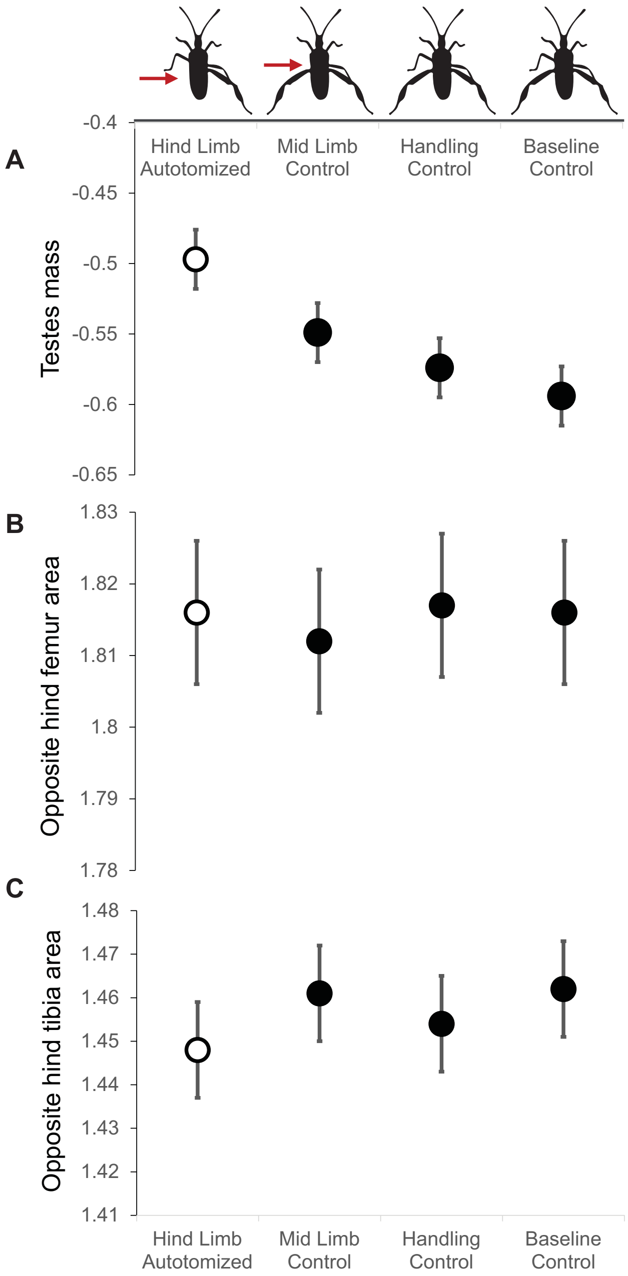

Figure 2. Males that dropped a left hind limb during development (hollow circles) had (A) significantly heavier testes than baseline control males (filled circles), yet did not differ in regards to (B) right (opposite-side) hind femur area or (C) right (opposite-side) hind tibia area. Estimated marginal means (±SE) were produced from the full model summarized in Table 1, where (A) testes mass (mg) was log transformed, and both (B) opposite right hind femur area (mm^2) and (C) opposite hind tibia area (mm^2) were squared, then log transformed before analysis. Red arrows point to the site of the autotomized limb for the hind-limb and mid-limb autotomy treatments.

Table 2. Results of two separate GLM for effect of hind-limb loss with body size as covariate.

Source	d.f.	Same-side mid-limb width, wald X^2	Same-leg hind trochanter length, wald X^2
Body Size	1	314.198**	5.297*
Treatment	1	1.165	279.049***

Each response variable was $\log_{10}$ transformed.
$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

EXPERIMENT (2): EFFECTS OF HIND-LIMB LOSS ON REPRODUCTIVE SUCCESS

In this experiment, and as above, individuals with hind limbs autotomized during the penultimate instar did not have higher mortality than those in the control treatment (approx. 13% mortality across 134 total individuals; $X^2 = 0.740$, d.f. $= 1$, $P > 0.20$). We tested the effects of weapon loss on reproductive success when males have a brief encounter with a female (and thus a limited time to mate and inseminate). We did not predict that male hind-limb removal would affect the probability that eggs were laid because females often produce eggs even when no mating has occurred. Thirty-five of the 54 females (65%) laid eggs during the two week egg-laying period (Fig. 4A). The females that laid eggs produced on average 23 eggs (range = 7–55 eggs). We found no effect of male treatment (autotomy or control), female treatment (autotomy or control), male body size, or female body size on the probability that eggs were laid (yes/no; Table 3). Next, we tested the effects of weapon loss on whether or not eggs hatched (in those cases where eggs were produced). Twenty-five of the 35 females that produced eggs (71%) had some hatching success (range = 5–46 live hatchlings). We found that females paired with males that autotomized a limb had substantially lower likelihood of hatching success than those paired with intact males (Table 3; Fig. 4B). We next examined the effect of male weapon autotomy on hatchling number, in those cases where females laid viable eggs. We found that females paired with males that had autotomized a limb during development produced 42% more live hatchlings. (Table 3; Fig. 4C).

Discussion

This study provides the first experimental evidence of a species naturally investing more resources in testes after the loss of a sexually selected weapon. The experimental removal of a hind limb resulted in increased overall growth of the testes without growth of other nearby structures. Further, the testis closer to the site of autotomy did not grow disproportionately larger than the testis on the other side. These results provide evidence of a

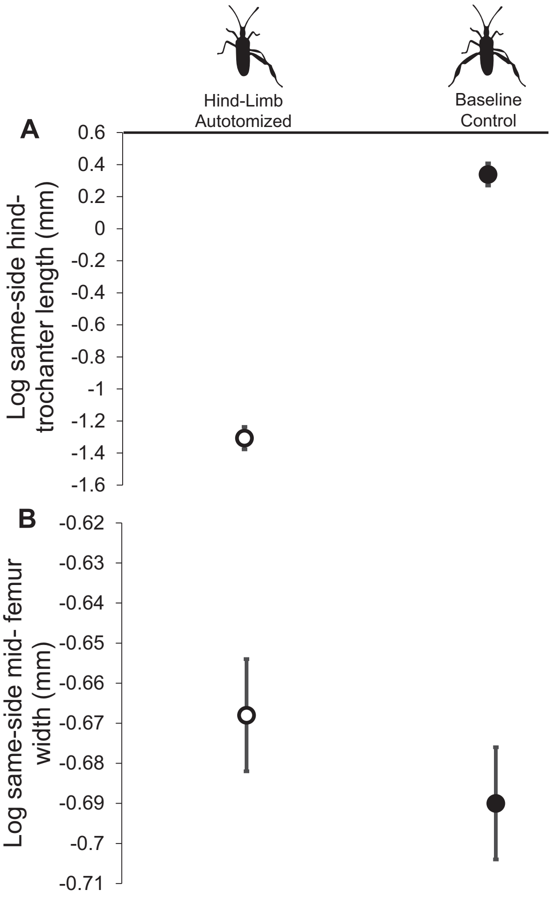

Figure 3. **(A) Hind-limb autotomized males (hollow circles) had significantly shorter trochanters as adults than baseline control males (filled circles). Autotomy for all males occured at the trochanter-femur joint, as is typical in nature. (B) We found no difference in the same-side middle-limb femur width of experimental males relative to baseline control males. Estimated marginal means (±SE) were produced from the full model shown in Table 2, where both (A) trochanter length (mm) and (B) mid-femur width were cube transformed, then log transformed before analysis.**

resource allocation trade-off between weapons and testes. In our second experiment, we found that males missing a hind limb had a fertilization advantage, but just in those cases where live offspring were produced.

Negative phenotypic correlations can exist between nearby structures. For example, across onthophagine beetles, those species with horns at the front of the head have proportionately smaller antennae, those with horns at the base of the head have proportionately smaller eyes, and those with horns on the thorax have proportionately smaller wings (Emlen 2001). Further, hormone manipulation in *O. taurus* that stunted horn development resulted in growth in the nearby compound eyes (Nijhout and Emlen 1998). We tested the proximity hypothesis to see if the removal of a hind limb during development led to the enlargement of traits in proximity. We included measurements of the mid-limb femur and the hind trochanter on the same size as the lost limb. Additionally, we measured the size of the opposite hind limb (femur area and tibia area) and the pronotum width, a proxy for body size. Finally, we measured the right and left testes individually to determine if the left testes grew larger than the right when the left hind limb was removed. We found no evidence to support the hypothesis that loss of a hind limb results in the growth of nearby traits. Instead, the loss of a hind limb resulted specifically in an increase in testes mass, despite the potential injury costs of weapon loss and autotomy in general. It is important to note that *N. femorata* is a hemimetabolous insect; as such, adult structures develop slowly as juveniles continue to acquire food from their environment. Thus, these results challenge the idea that morphological tradeoffs might be limited to closed systems, such as the pupae of holometabolous species (Moczek and Nijhout 2004; Tomkins et al. 2005). In a study conducted shortly after this one, Somjee et al. (2017) found evidence of a similar weapons-testes trade-off in another hemimetabolous insect in the same family (Coreidae). It is possible that resource allocation trade-offs are as prevalent in hemimetabolous species as the Holometabola.

Trade-offs between life-history traits have been demonstrated in a large number of laboratory and field studies (e.g., Reznick 1985; Partridge and Sibley 1991; Roff 1992, 2002; Stearns 1992; Gustafsson et al. 1994; Ots and Horak 1996; Sinervo and DeNardo 1996; Zuk 1996; Reznick et al. 2000; Preston et al. 2001; Parzer and Moczek 2008). In fact, evolutionary biological thought is rooted in the assumption that trait evolution is restricted or altered by fitness trade-offs (Stephens and Krebs 1986; Charnov 1989; Roff 1992, 2002; Stearns 1992; Futuyma 1998; Houston and McNamara 1999; Reznick et al. 2000; Zera and Harshman 2001). Yet, the question of whether males face a trade-off between weapons and testes, two traits important to fitness, has been rarely tested experimentally. Our study supports the findings of the few manipulative experiments where the halting of weapon development resulted in increased testes growth (Fry 2006; Simmons and Emlen 2006; Simmons et al. 2017). This study is the first to show that males that naturally lose their hind-limb weapon, as occurs frequently in nature (Emberts et al. 2016), grow larger testes. Hind-limb weapons in this family of insects are full of muscle, and thus likely to be metabolically expensive. Testes and ejaculates are also likely to be costly (Hosken 2001; Simmons and Roberts 2005; Simmons et al. 2017). Thus, it is not surprising that these structures should trade off in the context of this species. Figure 2A reveals a trend that the loss of a middle limb (small, but also full of muscle) could potentially be leading to a small increase in testes mass. Middle limbs are probably important to precopulatory sexual selection in this species, because they are involved in anchoring the male to the substrate during physical contests. Thus, a trade-off between middle limb investment and

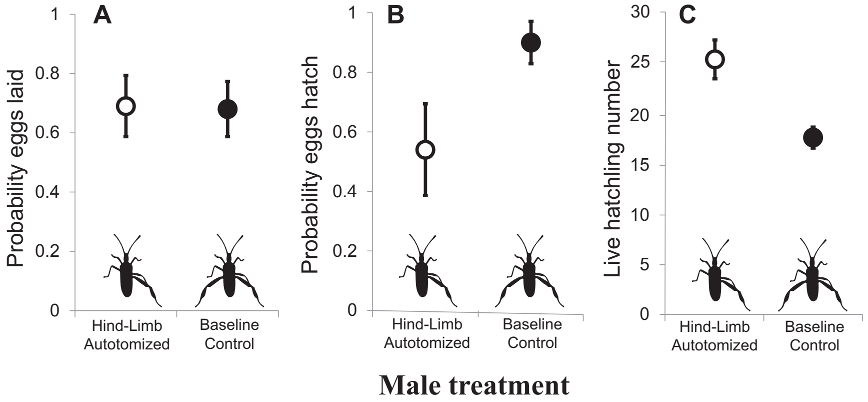

Figure 4. (A) Male hind-limb loss did not affect the probability that eggs were laid. (B) Eggs were less likely to hatch when females were paired with males with hind-limb autotomy; however, (C) if eggs did hatch, females paired with a male with a hind-limb removed produced a greater number of live hatchlings. Estimated marginal means (±SE) from the three GLMs summarized in Table 3.

Table 3. Results of three separate GLM using the binary distribution (eggs produced and egg hatch) and Poisson distribution (number of live hatchlings) on the effects of hind-limb autotomy on reproduction after two weeks of pairing.

Source	d.f.	Eggs laid, wald X^2	Egg hatch, wald X^2	Hatchling number, wald X^2
Male autotomy	1	0.001	3.983^{*}	11.926^{***}
Female autotomy	1	2.992	0.413	2.451
Male body size	1	0.584	1.926	2.451
Female body size	1	2.587	1.437	0.473

$^{*}P \leq 0.05$; $^{**}P \leq 0.01$; $^{***}P \leq 0.001$.

testes, if present, would not be contrary to the pre- and postcopulatory trade-off framework. However, the results observed here may also reflect a more general tradeoff between costly traits, not one limited to precopulatory and postcopulatory investments. For example, investment in building and maintaining muscle may more generally trade-off with investment in traits linked to reproduction, as has been documented in crickets (Zera and Denno 1997). We encourage future researchers to think broadly about trade-offs and, whenever possible, measure multiple costly life-history traits, not just those pertinent to sexual selection. Selection studies that measure the genetic covariances between many costly life-history traits would be particularly informative (Reznick 1985; Bell and Koufopanou 1986; Sterns 1989; von Schantz et al. 1995; Reznick et al. 2000; Yamane et al. 2010; Simmons et al. 2017). Trait investment should also be evaluated across multiple relevant ecological contexts (Reznick et al. 2000; Miller and Svensson 2014).

The relevance of allocation decisions to survival and reproduction is often inferred, but not tested. Here, and in many species, males missing weapons should be at a disadvantage. Males without weapons are typically subordinate in male contest competition (O'Neill and Cobb 1979; Berzins and Caldwell 1983; Smith 1992; Abello et al. 1994; Taylor and Jackson 2003; Yasuda and Koga 2016; Emberts et al. *in review*). Importantly, while male–male competition can limit mating for many males, it probably only rarely fully precludes males with small weapons and those without weapons from mating (e.g., Goldsmith and Alcock 1993; Emlen 1997; Simmons et al. 2004). In our first experiment, we found that males of *N. femorata* that lost a weapon during development grew larger testes. Our second experiment tested whether early weapon loss was linked to an increase in reproductive success in adulthood after a brief encounter with a female. Intriguingly, females paired with males missing a weapon were less likely to have their eggs hatch. These inviable eggs may be a result of females being less receptive to males missing a hind-limb weapon, perhaps via rejected mating attempts. Even virgin female *N. femorata* can be choosy, simply keeping their genital plates closed to avoid mating (Gillespie et al. 2014; Cirino and Miller 2017). It is also possible that males missing a hind-limb weapon may have greater rates of infertility than intact males. Autotomy is a form of injury (discussed in Emberts et al. 2017) and may result in infection, for example. Finally, males missing a hind-limb weapon may simply not court females as readily as intact males (Emberts et al. *in review*). Further work is needed.

Though many eggs did not hatch, when clutches did result in viable offspring, females mated to intact males produced an average of 17.79 live offspring, while females mated to males missing a hind-limb weapon produced on average 25.24 offspring, a notable 42% increase. This evidence suggests that males missing a hind-limb weapon can fertilize more eggs, as may be expected for males with larger testes. Thus, these results suggest both a reproductive cost and a fertilization benefit for males that lose a hind-limb weapon.

In conclusion, we have shown that males that lost a precopulatory sexually selected weapon developed larger testes for their body size and did not experience differential growth of other traits in close proximity, including the nearby testis. We examined fertilization consequences of losing a hind-limb weapon and found that, when offspring were produced, males missing a hind limb had a fertilization advantage. Our research provides a rare experimental example of a resource allocation trade-off shared between pre- and postcopulatory sexually selected traits (Moczek and Nijhout 2004; Fry 2006; Simmons and Emlen 2006; Somjee et al. 2017).

AUTHOR CONTRIBUTIONS

P.N.J., D.A.S., and C.W.M. designed the study. P.N.J. and Z.E. conducted the experiments. P.N.J. and C.W.M. conducted statistical analyses and wrote the manuscript. All authors provided feedback during the writing process and gave final approval.

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DATA ARCHIVING

Data has been fully archived in Dryad, https://doi.org/10.5061/dryad.j6k1j.

LITERATURE CITED

Abello, P., C. G. Warman, D. G. Reid, and E. Naylor. 1994. Chela loss in the shore crab *Carcinus maenas* (Crustacea: Brachyura) and its effect on mating success. Mar. Biol. 121:247–252.

Allen, B. J., and J. S. Levinton. 2007. Costs of bearing a sexually selected ornamental weapon in a fiddler crab. Funct. Ecol. 21:154–161.

Allen, P. E., and C. W. Miller. 2017. Novel host plant leads to the loss of sexual dimorphism in a sexually selected male weapon. Proc. R. Soc. Lond. B Biol Sci. 284:20171269.

Andersson, M. B. 1994. Sexual selection. Princeton Univ. Press, Princeton.

Bell, G., and V. Koufopanou. 1986. The cost of reproduction. Pp. 83–131 *in* R. Dawkins, and M. Ridley, eds. Oxford surveys in evolutionary biology. Oxford Univ. Press, Oxford.

Berzins, I. K., and R. L. Caldwell. 1983. The effect of injury on the agonistic behavior of the Stomatopod, *Gonodactylus Bredini* (manning). Mar. Behav. Physiol. 10:83–96.

Backwell, P. R., J. H. Christy, S. R. Telford, M. D. Jennions, and J. Passmore. 2000. Dishonest signalling in a fiddler crab. Proc. R. Soc. Lond. B Biol. Sci. 267:719–724.

Charnov, E. L. 1989. Phenotypic evolution under Fisher's fundamental theorem of natural selection. Heredity 62:113–116.

Cirino, L. A., and C. W. Miller. 2017. Seasonal effects on the population, morphology and reproductive behavior of *Narnia femorata* (Hemiptera: Coreidae). Insects 8:1–16.

Dial, B. E., and L. C. Fitzpatrick. 1981. The energetic costs of tail autotomy to reproduction in the lizard *Coleonyx brevis* (Sauria: Gekkonidae). Oecologia 51:310–317.

Dines, J. P., S. L. Mesnick, K. Ralls, L. May-Collado, I. Agnarsson, and M. D. Dean. 2015. A trade-off between precopulatory and postcopulatory trait investment in male cetaceans. Evolution 69:1560–1572.

Dumser, J. B., and K. G. Davey. 1974. Endocrinological and other factors influencing testis development in *Rhodnius prolixus*. Can. J. Zool. 52:1011–1022.

Eberhard, W. G. 1998. Sexual behavior of *Acanthocephala declivis guatemalana* (Hemiptera: Coreidae) and the allometric scaling of their modified hind legs. Ann. Entomol. Soc. Am. 91:863–871.

Economopoulos, A. P., and H. T. Gordon. 1971. Growth and differentiation of the testes in the large milkweed bug, *Oncopeltus fasciatus* (Dallas). J. Exp. Zool. 177:391–405.

Emberts, Z., C. M. St. Mary, and C. W. Miller. 2016. Coreidae (Insecta: Hemiptera) limb loss and autotomy. Ann. Entomol. Soc. Am. 109:678–683.

Emberts, Z., C. W. Miller, D. Kiehl, and C. M. St. Mary. 2017. Cut your losses: self-amputation of injured limbs increases survival. Behav. Ecol. 28:1047–1054.

Emlen, D. J. 1997. Alternative reproductive tactics and male-dimorphism in the horned beetle *Onthophagus acuminatus* (Coleoptera: Scarabaeidae). Behav. Ecol. Sociobio. 5:335–341.

———. 2001. Costs and the diversification of exaggerated animal structures. Science 291:1534–1536.

Fitzpatrick, J. L., M. Almbro, A. Gonzalez-Voyer, N. Kolm, and L. W. Simmons. 2012. Male contest competition and the coevolution of weaponry and testes in pinnipeds. Evolution 66:3595–3604.

Fry, C. L. 2006. Juvenile hormone mediates a trade-off between primary and secondary sexual traits in stalk-eyed flies. Evol. Dev. 8:191–201.

Futuyma, D. J. 1998. Evolutionary biology. Sinauer Associates, Inc., Sunderland, MA.

Gillespie, S. R., M. S. Tudor, A. J. Moore, and C. W. Miller. 2014. Sexual selection is influenced by both developmental and adult environments. Evolution 68:3421–3432.

Goldsmith, S. K., and J. Alcock. 1993. The mating chances of small males of the cerambycid beetle *Trachyderes mandibularis* differ in different environments (Coleoptera: Cerambycidae). J. Insect Behav. 3:351–360.

Gustafsson, L., D. Nordling, M. S. Andersson, B. C. Sheldon, and A. Qvarnstrom. 1994. Infectious diseases, reproductive effort and the cost of reproduction in birds. Phil. Trans. R. Soc. Lond. B Biol. Sci. 346:323–331.

Hosken, D. J. 2001. Sex and death: microevolutionary trade-offs between reproductive and immune investment in dung flies. Curr. Biol. 11:R379–R380.

Houston, A. I., and J. M. McNamara. 1999. Models of adaptive behaviour. Cambridge Univ. Press, Cambridge.

Kawano, K. 1997. Cost of evolving exaggerated mandibles in stag beetles (Coleoptera: Lucanidae). Ann. Entomol. Soc. Am. 90:453–461.

Kelly, C. D. 2008. Identifying a causal agent of sexual selection on weaponry in an insect. Behav. Ecol. 19:184–192.

Liu, X., F. Hayashi, L. C. Lavine, and D. Yang. 2015. Is diversification in male reproductive traits driven by evolutionary trade-offs between weapons and nuptial gifts? Proc. R. Soc. B 282:20150247.

Lüpold, S., J. L. Tomkins, L. W. Simmons, and J. L. Fitzpatrick. 2014. Female monopolization mediates the relationship between pre- and postcopulatory sexual traits. Nat. Commun. 5:3184.

McCullough, E. L., and D. J. Emlen. 2013. Evaluating the costs of a sexually selected weapon: big horns at a small price. Anim. Behav. 86:977–985.

McCullough, E. L., and B. W. Tobalske. 2013. Elaborate horns in a giant rhinoceros beetle incur negligible aerodynamic costs. Proc. R. Soc. B 280:20130197.

McCullough, E. L., C. W. Miller, and D. J. Emlen. 2016. Why sexually selected weapons are not ornaments. Trends Ecol. Evol. 31:742–751.

Miller, C. W., and E. Svensson. 2014. Sexual selection in complex environments. Annu. Rev. Entomol. 59:427–445.

Miller, C. W., G. McDonald, and A. J. Moore. 2016. The tale of the shrinking weapon: seasonal changes in nutrition affect weapon size and sexual dimorphism, but not contemporary evolution. J. Evol. Biol. 29:2266–2275.

Miyatake, T. 1997. Functional morphology of the hind legs as weapons for male contests in *Leptoglossus australis* (Heteroptera: Coreidae). J. Insect Behav. 10:727–735.

Moczek, A. P., and H. F. Nijhout. 2004. Trade-offs during the development of primary and secondary sexual traits in a horned beetle. Am. Nat. 163:184–191.

Nijhout, H. F., and D. J. Emlen. 1998. Competition among body parts in the development and evolution of insect morphology. PNAS 95:3685–3689.

Nolen, Z. I., P. E. Allen, and C. W. Miller. 2017. Seasonal resource value and male size influence male aggressive interactions in the leaf footed cactus bug, *Narnia femorata*. Behav. Processes 138:1–6.

O'Neill, D. J., and J. S. Cobb. 1979. Some factors influencing the outcome of shelter competition in lobsters (*Homarus americanus*). Mar. Behav. Physiol. 6:33–45.

Ots, I., and P. Horak. 1996. Great tits, *Parus major*, trade health for reproduction. Proc. R. Soc. Lond. B Biol. Sci. 263:1443–1447.

Parker, G. A., C. M. Lessells, and L. W. Simmons. 2013. Sperm competition games: a general model for precopulatory male–male competition. Evolution 67:95–109.

Partridge, L., and R. Sibley.1991. Constraints on the evolution of life histories. Phil. Trans. R. Soc. Lond. B Biol. Sci. 332:3–13.

Parzer, H. F., and A. P. Moczek. 2008. Rapid antagonistic coevolution between primary and secondary sexual characters in horned beetles. Evolution 62:2423–2428.

Preston, B. T., I. R. Stevenson, J. M. Pemberton, and K. Wilson. 2001. Dominant rams lose out by sperm depletion. Nature 409:681–682.

Preston, B. T., I. R. Stevenson, J. M. Pemberton, D. W. Coltman, and K. Wilson. 2003. Overt and covert competition in a promiscuous mammal: the importance of weaponry and testes size in male reproductive success. Proc. R Soc. Lond. B 270:633–640.

Procter, D. S., A. J. Moore, and C. W. Miller. 2012. The form of sexual selection arising from male–male competition depends on the presence of females in the social environment. J. Evolution Biol. 25:803–812.

Reznick, D. 1985. Costs of reproduction: an evaluation of the empirical evidence. Oikos 44:257–267.

Reznick, D., L. Nunney, and A. Tessier. 2000. Big houses, big cars, superfleas and the costs of reproduction. Trends Ecol. Evol. 15:421–425.

Roff, D. A. 1992. The evolution of life histories: Theory and analysis. Chapman and Hall, New York.

———. 2002. Life history evolution. Sinauer Associates, Sunderland, MA.

Schneider, C. A., W. S. Rasband, and K. W. Eliceiri. 2012. NIH Image to ImageJ: 25 years of image analysis. Nat. Methods 9:671–675.

Schulte-Hostedde, A. I., and J. S. Millar. 2004. Intraspecific variation of testis size and sperm length in the yellow-pine chipmunk (*Tamias amoenus*): implications for sperm competition and reproductive success. Behav. Ecol. Sociobiol. 55:272–277.

Simmons, L. W., J. L. Tomkins, and J. Hunt. 1999. Sperm competition games played by dimorphic male beetles. Proc. Biol. Sci. 266:145–150.

Simmons, L. W., M. Beveridge, and S. Krauss. 2004. Genetic analysis of parentage within experimental populations of a male dimorphic beetle, *Onthophagus taurus*, using amplified fragment length polymorphism. Behav. Ecol. Sociobiol. 2:164–173.

Simmons, L. W., and B. Roberts. 2005. Bacterial immunity traded for sperm viability in male crickets. Science 309:2031–2031.

Simmons, L. W., and D. J. Emlen. 2006. Evolutionary trade-off between weapons and testes. PNAS 103:16346–16351.

Simmons, L. W., and F. García-González. 2008. Evolutionary reduction in testes size and competitive fertilization success in response to the experimental removal of sexual selection in dung beetles. Evolution 62:2580–2591.

Simmons, L. W., S. Lupold, and J. L. Fitzpatrick. 2017. Evolutionary trade-off between secondary sexual traits and ejaculates. Trends Ecol. Evol. https://doi.org/10.1016/j.tree.2017.09.011

Sinervo, B., and D. F. DeNardo. 1996. Costs of reproduction in the wild: path analysis of natural selection and experimental tests of causation. Evolution 50:1299–1313.

Smith, L. D. 1992. The impact of limb autotomy on mate competition in blue crabs *Callinectes sapidus* Rathbun. Oecologia 89:494–501.

Somjee, U., P. E. Allen, and C. W. Miller. 2015. Different environments lead to a reversal in the expression of weapons and testes in the heliconia bug, *Leptoscelis tricolor* (Hemiptera: Coreidae). Biol. J. Linnean Soc. 115:802–809.

Somjee, U., C. W. Miller, N. J. Tatarnic, and L. W. Simmons. 2017. Experimental manipulation reveals a trade-off between weapons and testes. J. Evol. Biol. https://doi.org/10.1111/jeb.13193

Stearns, S. C. 1989. Trade-offs in life-history evolution. Funct. Ecol. 3:259–268.

———. 1992. The evolution of life histories. Oxford Univ. Press, New York.

Stephens, D. W., and J. R. Krebs. 1986. Foraging theory. Princeton Univ. Press, Princeton, NJ.

Taylor, P. W., and R. R. Jackson. 2003. Interacting effects of size and prior injury in jumping spider conflicts. Anim. Behav. 65:787–794.

Tomkins, J. L., and L. W. Simmons. 2002. Measuring relative investment: a case study of testes investment in species with alternative male reproductive tactics. Anim. Behav. 63:1009–1016.

Tomkins, J. L., J. S. Kotiaho, and N. R. LeBas. 2005. Phenotypic plasticity in the developmental integration of morphological trade-offs and secondary sexual trait compensation. Proc. R Soc. B 272:543–551.

Vessels, H. K., C. S. Bundy, and J. E. McPherson. 2013. Life history and laboratory rearing of *Narnia femorata* (Hemiptera: Heteroptera: Coreidae) with descriptions of immature stages. Ann. Entomol. Soc. Am. 106:575–585.

Von Schantz, T., M. Tufvesson, G. Göransson, M. Grahn, M. Wilhelmson, and H. Wittzell. 1995. Artificial selection for increased comb size and its effects on other sexual characters and viability in *Gallus domesticus* (the domestic chicken). Heredity 75:518–518.

Yamane, T., K. Okada, S. Nakayama, and T. Miyatake. 2010. Dispersal and ejaculatory strategies associated with exaggeration of weapon in an armed beetle. Proc. R Soc. B rspb.2009.2017.

Yasuda, C. I., and T. Koga. 2016. Do weaponless males of the hermit crab *Pagurus minutus* give up contests without escalation? Behavior of intruders that lack their major cheliped in male–male contests. J. Ethol. 34:249–254.

Zera, A. J., and R. F. Denno. 1997. Physiology and ecology of dispersal polymorphism in insects. Annu. Rev. Entomol. 42:207–231.

Zera, A. J., and L. G. Harshman. 2001. The physiology of life history trade-offs in animals. Annu. Rev. Ecol. Syst. 32:95–126.

Zuk, M. 1996. Trade-offs in parasitology, evolution and behavior. Parasitol. Today 12:46–47.

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