

How does the timing of weapon loss influence reproductive traits and trade-offs in the insect *Narnia femorata*?

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1 **ABSTRACT**

2 A longstanding goal of evolutionary biology is to understand among-individual variation in resource
3 allocation decisions and the timing of these decisions. Recent studies have shown that investment in
4 elaborate and costly weapons can result in trade-offs with investment in testes. In this study, we ask at what
5 point plasticity in resource allocation to these different structures ceases during development, if at all?
6 Furthermore, can individuals tailor their reproductive behavior to accompany structural changes? We
7 experimentally addressed these questions in the insect *Narnia femorata*, quantifying resource reallocation
8 across development for the first time, using a phenotypic engineering approach. To investigate whether
9 allocation plasticity diminishes throughout ontogeny, we induced weapon loss at a range of different
10 developmental stages and examined subsequent testes mass and reproductive behavior. We found that
11 relative testes mass increased as weapon investment decreased, implying a direct trade-off between testes
12 and weapon investment. However, autotomy post-adulthood ceased to induce larger testes mass.
13 Intriguingly, losing a weapon while young was associated with extended adult mating duration, potentially
14 enabling compensation for reduced fighting ability. Our results highlight the importance of examining the
15 ontogeny of trade-offs between reproductive traits and the flexibility of the relationship between
16 reproductive morphology and behavior.

17 INTRODUCTION

18 Male-male competition for mating opportunities has driven the evolution of elaborate sexually
19 selected weapons, ranging from the enormous antlers of stags to the exaggerated limbs of some insect
20 species (Emlen 2008). These sexually selected weapons have evolved for use in physical contests
21 between males for access to females (McCullough et al. 2016). However, the rivalry between males often
22 does not end there, continuing into the post-copulatory phase in the form of sperm competition (Parker
23 1979; Birkhead and Hunter 1990). This poses a potential challenge: producing and maintaining both pre-
24 copulatory weapons and post-copulatory traits is likely demanding. Given that animals have finite
25 resource budgets, investment in one trait may come at the cost of another, leading to a trade-off (van
26 Noordwijk and de Jong 1986; Zera and Harshman 2001). Indeed, negative correlations between
27 investment in pre-copulatory traits and primary reproductive traits (e.g. testes) have been uncovered in
28 multiple systems (e.g. Simmons and Emlen 2006; Klaus et al. 2011; Lüpold et al. 2014; Dines et al. 2015;
29 Durrant et al. 2016; Simmons et al. 2017). Experimental ablation of horn structures during development
30 in beetles leads to the growth of larger testes (Simmons and Emlen 2006), as does the removal of a hind
31 limb weapon in juvenile true bugs (Joseph et al. 2018; Somjee et al. 2018). Whilst these manipulations
32 illustrate that trade-offs exist between these traits, they also implicitly demonstrate that resource
33 allocation between pre- and post-copulatory traits is flexible and dynamic, at least during juvenile life
34 stages. Yet, our knowledge of the extent of developmental plasticity in resource allocation is incomplete.
35 Additionally, the mechanisms underlying resource reallocation across pre- and post-copulatory traits
36 remain unclear.

37 Whilst reproductive morphology and physiology may be somewhat developmentally constrained
38 in species with determinate growth, behavior is likely to be far more flexible (Bretman et al. 2011).
39 Behavioral flexibility provides individuals with a means to compensate for changes in morphology,
40 including weapon loss. Indeed, alternative reproductive strategies are characterized by divergent pre- and
41 post-copulatory investment paired with behavioral strategies that maximize the reproductive payoffs of

42 their respective phenotypes (Gage et al. 1995; Gross 1996; Puniamoorthy et al. 2012). Males that
43 prioritize investment in post-copulatory traits typically expend more in each mating event they secure to
44 increase their chances of fertilization success (Taborsky and Brockmann 2010), via extended mating
45 durations and increased sperm transfer (Okada et al. 2010; Filice & Dukas, 2019). In the other direction,
46 individuals which have been phenotypically engineered to produce less sperm display reduced mating
47 frequency (Sekii et al. 2013). If we experimentally manipulate relative investment in weapons versus
48 testes, do individuals respond behaviorally to maximize the pay-off from the combination of pre- and
49 post-copulatory traits they end up with? In particular, can individuals which have lost a weapon
50 compensate behaviorally for their limited pre-copulatory competitive ability when it comes to mating?

51 In this study we address these unanswered questions in the leaf-footed cactus bug *Narnia*
52 *femorata*, an insect in the Family Coreidae and the order of True Bugs, Hemiptera. Males of this species
53 invest in enlarged hind limbs which they use to kick and grapple with each other in contests (Nolen et al.
54 2017). Whilst these weaponized limbs are central to securing competitive and mating success, individuals
55 can readily autotomize them to evade predation attempts and entrapment (Emberts et al. 2016, 2018).
56 Leaf footed bugs are hemimetabolous, developing through five juvenile instars between hatching and
57 eclosion to adulthood, and unlike in other systems, they do not regrow their dropped limbs (Emberts et al.
58 2016). With each juvenile instar they develop increasingly large hind limbs, with broadened femurs and
59 tibial flags (Vessels et al. 2013). At eclosion to adulthood these weaponized limbs then become fixed in
60 external size. Juvenile limb autotomy (at the fourth instar) in this species is accompanied by a boost in
61 relative testes mass (Joseph et al. 2018; Miller et al. 2021), suggesting that a resource allocation trade-off
62 exists between these two metabolically expensive and condition-dependent traits (Sasson et al. 2016;
63 Greenway et al. 2020). However until now this weapon-testes trade-off has only been examined after
64 autotomy at a single juvenile time point, and the underlying mechanism behind the reallocation response
65 is unexplored.

66 We first set out to investigate how the timing of autotomy influences reallocation to testes mass
67 and begin to test the mechanistic basis of this response. If resources allocated to testes and weapon growth
68 are drawn from the same pool and directly trade off, we predicted a positive linear relationship between
69 the amount of leg mass we prevented a bug investing in and the size of their relative adult testes mass. By
70 removing the hind leg of a juvenile, we effectively blocked off investment to this body part, freeing up
71 resources which can be deployed to the development of other traits. Removing a limb later in
72 development, after many of the costs involved in producing that limb have already been expended, would
73 presumably result in a proportionally smaller quantity of available resource to reallocate to testes mass.
74 Alternatively, if autotomy acts as a trigger to switch investment to post-copulatory traits to boost sperm
75 competition, then we predicted the size of the limb removed would not correlate with relative testes mass
76 size.

77 We then asked whether autotomy is accompanied by changes in mating behavior which may
78 enable males to capitalize on the mating opportunities that may arise. Given that autotomized males have
79 reduced chances of accessing mates via male-male competition (Emberts et al. 2018), we predicted that
80 they would invest more time in each mating they engage in, extending copulations to increase the chances
81 of fertilization success.

82

83 **METHODS**

84 **Husbandry**

85 Individuals used in both experiments were laboratory reared offspring from wild caught *Narnia femorata*
86 parental pairs collected from Camp Blanding, FL. Clutches produced by the parental pairs were separated
87 and kept in small family groups (5–13 individuals per 32 oz clear plastic container) on their natural diet:
88 prickly-pear cactus (*Opuntia mesacantha subsp. lata*) with ripe fruit throughout their first three instars.
89 Once juveniles reached the 4th instar, we transferred them into their own individual 32 oz container with
90 a single potted prickly-pear cactus with ripe fruit. We randomly assigned an individual's experimental
91 type, treatment type and a unique number identifier. Parental pairs and clutches were kept in Percival
92 Scientific incubators at 28°C under a 14:10 L:D cycle. Once experimental individuals reached the 4th
93 instar, they were transferred to a rearing room lit with T5 HO fluorescent bulbs on a 14:10 L:D cycle.
94 Conditions within individual containers were kept consistent at 30–35°C and 40–60% humidity and bugs
95 were checked daily.

96

97 **Experiment 1: Effect of autotomy timing on testes investment**

98 Each *N. femorata* nymph was randomly assigned to one of five autotomy treatments upon reaching the
99 4th instar: autotomy at either the 1) fourth instar (n=35), 2) fifth instar (n=37), 3) early adulthood (2-3
100 days after emerging, n= 32), 4) 14 days post adult eclosion (i.e., at sexual maturity, n=32) or 5) an
101 unmanipulated control treatment (n=37). We induced autotomy by immobilizing the left hind femur of
102 experimental individuals with reverse-action forceps while their tarsi were in contact with a piece of
103 wood for grip and, if necessary, gently brushed them with a paintbrush to encourage them to self-
104 autotomize. Autotomized limbs were then frozen at -20°C for imaging and subsequent weighing.

105

106 **Quantifying morphology and testes mass**

107 We kept all males in individual cups provisioned with ripe fruit over the course of the experimental
108 period. Once males reached sexual maturity (14–16 days after adult enclosure) we froze, imaged and

109 measured them. We then dissected out the testes of each individual and preserved these and the remaining
110 body separately in 70% ethanol. Each male's testes and hind leg(s), plus their body and four remaining
111 legs were placed into separate pre-weighed aluminum foil boats and then dried for 72 hrs at 70°C before
112 being weighed to the nearest microgram using a Mettler Toledo XP6 microbalance.

113

114 **Experiment 2: Effects of autotomy on mating behavior**

115 Upon eclosion to fourth instar, we assigned nymphs to either a control or autotomy treatment. As in
116 Experiment 1, we induced autotomy by gripping nymphs by the left hind femur with reverse-action
117 forceps while in contact with a piece of wood, enabling their remaining limbs to gain traction during the
118 process. Control bugs were subjected to a sham procedure, in which their hind femur was gripped for
119 around a second before release following Emberts et al. (2017). All nymphs were then housed in
120 individual plastic containers containing a cactus pad and ripe cactus fruit and checked daily.

121 Once they reached adulthood, they were sexed and left to reach sexual maturity (14-21 days) before being
122 entered into behavioral trials. As mating behavior is a product of interactions between both male and
123 female phenotypes, we included both autotomized males and autotomized females alongside control
124 individuals. Behavior trial treatments consisted of the following fully factorial combinations of randomly
125 paired unrelated individuals: 1) control male-control female (n=27), 2) control male- autotomized female
126 (n=30), 3) autotomized male- control female (n=24), and 4) autotomized male- autotomized female
127 (n=25). This design enabled us to parse out male behavioral responses to their own autotomy status from
128 responses to their partner's autotomy status.

129 We continuously observed pairs in a clear plastic container with cactus and fruit for a 3-hour period, and
130 recorded the frequency of male mounting attempts on their female partners, genital contact, copulations,
131 alongside the latency to mount and mate and copulation duration. At the end of the behavior trial, we
132 separated individuals and euthanized them via freezing, before measuring their pronotum width (a proxy
133 for body size) using Mitutoyo digital calipers.

134

135 **Statistical analysis**

136 All statistical analyses were performed in R v3.5.0 (R Core Team, 2018). To test for the effects of
137 autotomy treatment on testes mass in Experiment 1, we used the *lme4* package to fit a linear model with
138 testes mass as a continuous normally distributed dependent variable and treatment category and body
139 mass as factors. Post hoc analyses were carried out using the *multcomp* package, correcting for multiple
140 testing using single-step adjusted P-values.

141 We generated a metric of hind leg mass investment each experimental insect ‘saved’ by subtracting the
142 dry mass of their autotomized limb from the dry mass of their remaining fully grown hind limb. We
143 calculated this for the 103 individuals which we had both autotomized and fully grown limb dry mass
144 measurements for across treatments, and then incorporated this value as a third predictor in subsequent
145 analysis. Put another way, this calculated metric captured the resources we prevented these individuals
146 from investing in growing a second full size hind limb, which were then theoretically freed up to reinvest
147 in the growth of other structures such as testes. In order to establish the validity of this metric, we
148 compared the fully grown single hind limb mass of all autotomized males with the mean hind limb mass
149 of intact control insects. We found no significant difference between the two, indicating that autotomy did
150 not alter growth and investment of the remaining limb (see Results). Thus, the remaining limb appears to
151 be an effective proxy for the final mass of the autotomized limb had it been able to fully develop.

152 To analyze the results of Experiment 2, we used a generalized linear model to examine predictors of
153 mating duration. We included male autotomy status and pronotum width and female autotomy status and
154 pronotum width as factors and used a quasi-Poisson distribution to account for overdispersion. To assess
155 whether mating duration constituted a stable phenotype, we calculated how repeatable it was within
156 pairings which we observed mating more than once using the *rptR* package (Schielzeth and Nakagawa
157 2013). Data were uploaded to Dryad and are available at <https://doi.org/10.5061/dryad.p8cz8w9vk>

158 RESULTS

159 Experiment 1: Influence of autotomy timing on testes investment

160 We detected a significant effect of autotomy timing on relative testes mass ($F=3.207$, d.f.= 4, $P= 0.015$,
161 Fig 1). A post hoc pairwise analysis comparing each of the treatments revealed that individuals
162 autotomized at the 5th instar had larger testes than those autotomized as sexually mature adults ($t=-3.536$,
163 $P= 0.005$) and those left unmanipulated ($t=-2.843$, $P= 0.04$). Differences between all other treatment
164 groups were non-significant. As anticipated, body mass was highly correlated with testes mass ($F= 97.88$,
165 d.f.= 1, $P< 0.001$), and was therefore included as a covariate in all subsequent analyses. To establish if
166 eclosion to adulthood constituted a critical threshold in testes reallocation ability, we then pooled
167 treatments into juvenile and adult stages. Again, the life stage at which autotomy occurred influenced
168 testes mass ($F= 6.7$, d.f.=2, $P= 0.002$); males autotomized prior to adulthood developed significantly
169 larger testes for their body size than individuals autotomized as adults ($t=-3.33$, $P= 0.0029$) or those left
170 intact as a control ($t=-2.777$, $P= 0.017$, Fig 1).

171
172 Autotomy treatment had no significant impact on the mass of the remaining adult hind limb, which did
173 not differ from the equivalent hind limb of control individuals ($n=172$, d.f.= 4, $F=1.751$, $P= 0.141$, Fig 2).
174 As such, the mass difference between the autotomized hind limb and the final adult hind limb served as
175 an effective proxy for the mass investment 'saved' via autotomy (see Methods). Using this difference as a
176 proxy for resource investment saved, we found the mass of hind leg tissue *not* grown was positively
177 correlated with testes mass ($\beta= 0.059$, $F=14.19$, $P= 0.0003$, Fig 3a). Together, body mass and the amount
178 of leg mass an individual 'saved' through autotomy explained 51% of variance in autotomized male testes
179 mass (Fig 3a). Males that were autotomized earliest in development 'saved' the largest amount of leg
180 mass investment, whereas those that were autotomized as sexually mature adults 'saved' negligible mass
181 investment. Intriguingly, the hind limbs autotomized by 2-3 day old males were significantly lighter than
182 their fully grown adult limbs (Fig 2, $F= 16.04$ d.f.=1, $P<0.001$) suggesting further investment occurs in
183 cuticle and/or muscle during this period of adult maturation.

184 Upon closer examination, the overall positive association between mass ‘saved’ and relative
185 testes mass was driven by changes induced in fourth and fifth instar individuals (fourth instar: $\beta=0.13$, $t=$
186 3.430 , $P=0.0025$, fifth instar: $\beta= 0.11$, $t= 4.483$, $P= 0.0001$, see Fig 3b). Once individuals reach
187 adulthood, we observed no significant effects of the mass of tissue ‘saved’ via autotomy on testes mass,
188 when controlling for body size (2-3 day adults: $\beta= -0.0003$, $t= -0.009$, $P= 0.99$, 14 day adults: $\beta= -0.108$,
189 $t= -0.721$, $P = 0.478$, Fig 3b).

190

191 **Experiment 2: Effects of hind-limb loss on mating behavior**

192 We found that male individuals with hind limbs autotomized as juveniles mated for significantly longer
193 with females regardless of whether females were autotomized or intact (LRT , $X^2=12.44$, $d.f.=1$, $P<0.001$,
194 Fig 4b). What’s more, this longer mating duration associated with male autotomy was significantly
195 repeatable in pairs that copulated at least twice during the 3hr trial: $R = 0.21$, $CI = [0.04, 0.357]$, $P =$
196 0.0019 . Mating duration was also positively associated with female body size (LRT , $X^2=6.22$, $d.f.=1$,
197 $P<0.0126$).

198 We found that the autotomy treatment did not appear to impact any other measured aspects of mating
199 behavior; rates of mounting and mating did not differ across the four possible pairing combinations
200 ($X^2=0.24$, $d.f.=1$, $P=0.622$, Fig 4a). Across treatments, 78 of the 106 males (73.6%) mounted the female
201 they were paired with during the mating trial and 69 of these 106 males (65.1%) subsequently succeeded
202 in mating. We found no effect of either male or female treatment (autotomy or control) on average latency
203 to first mount or average latency to first mate.

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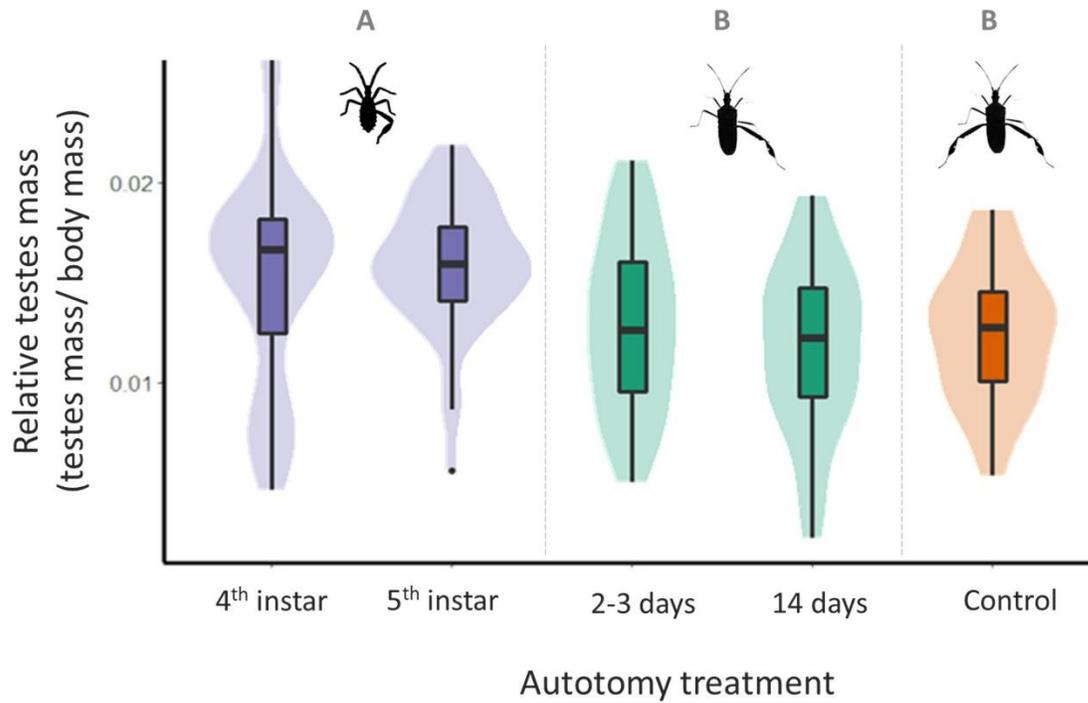


Figure 1. The relationship between autonomy timing and relative testes mass (shown here as testes mass divided by body mass for visualization purposes). Letters between groups denote statistically significant differences at the $P < 0.05$ level.

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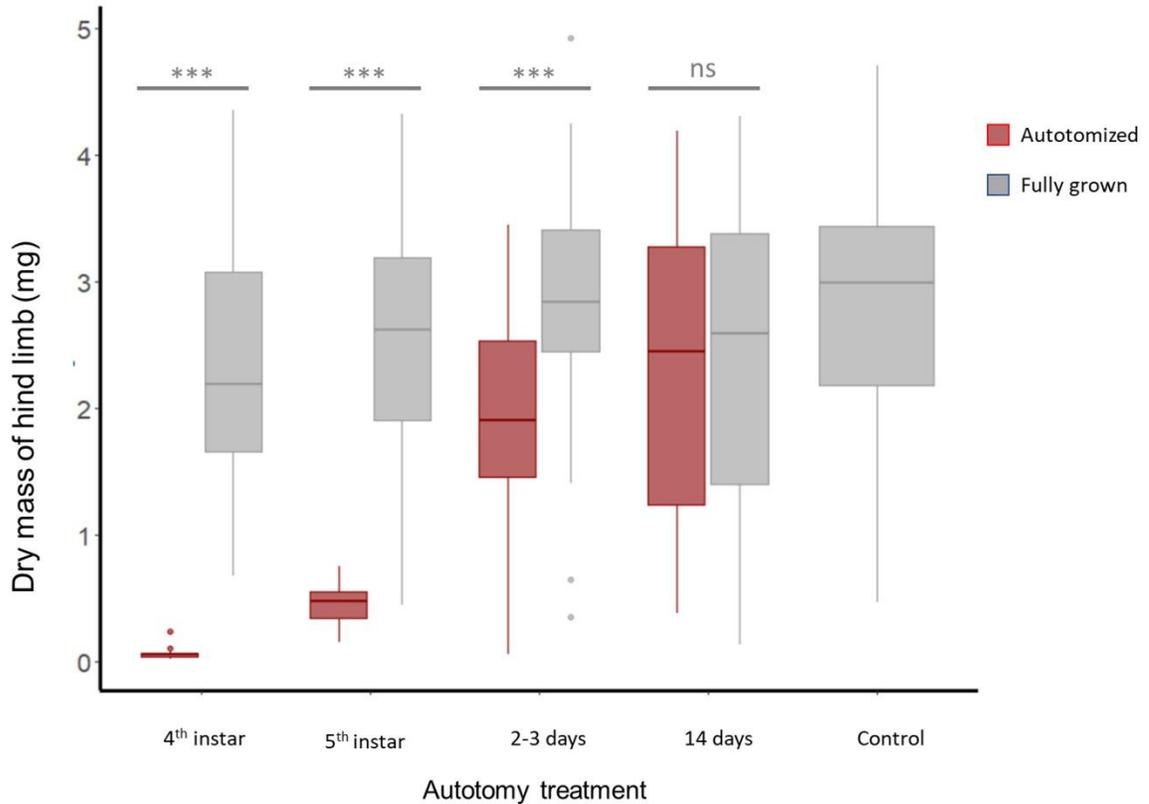
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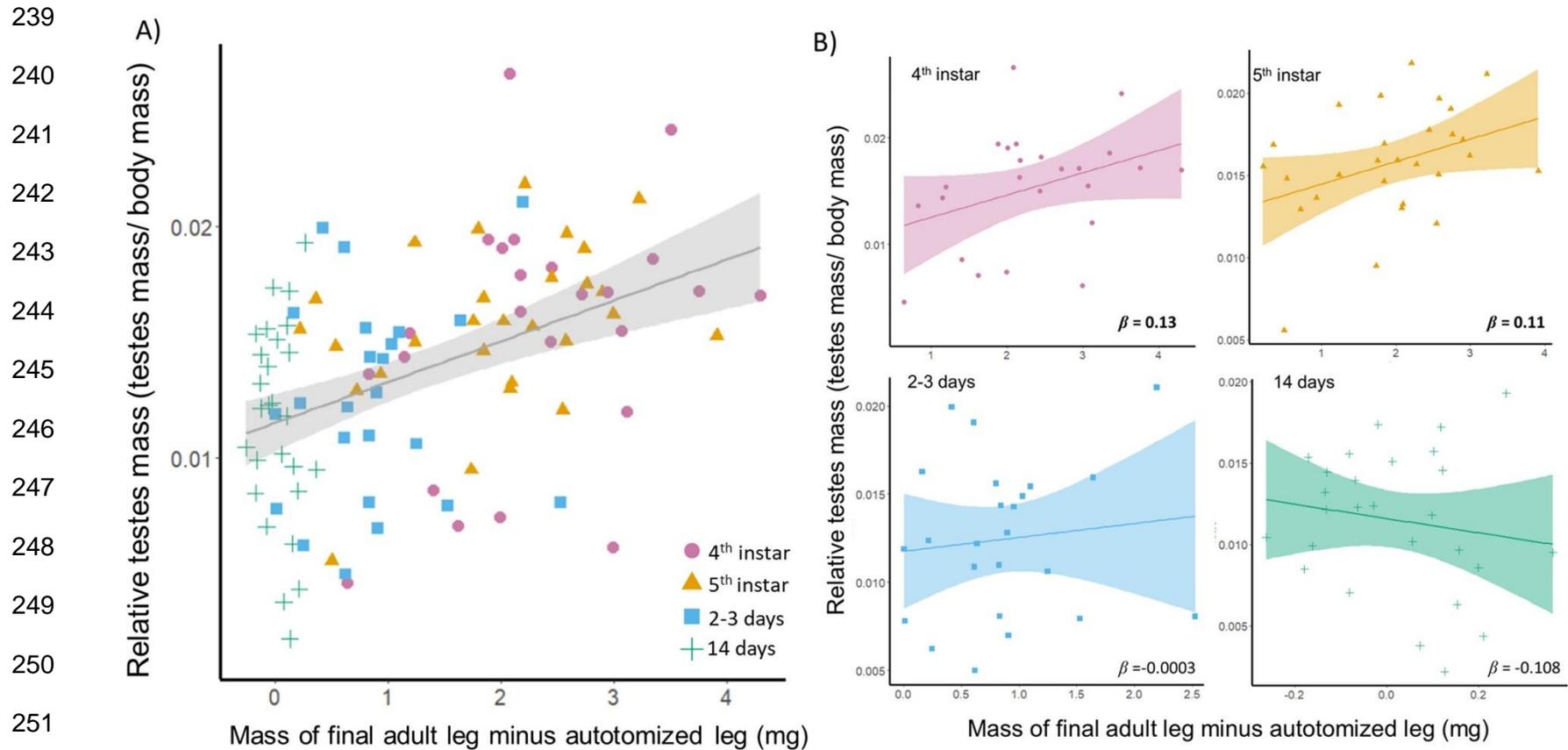
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Figure 2 Autotomy had no impact on the mass of the remaining fully grown adult hind limb (shown in grey) across all treatment groups relative to the hind limbs of intact control individuals. The mass of the autotomized leg (red) increased across instars but was significantly lower than the fully grown adult leg except when removed at 14 days (***) denotes significant pairwise differences at the $P < 0.001$ level between the autotomized and remaining adult limb mass)



253 **Figure 3. A.** The overall relationship between the mass investment ‘saved’ (i.e., mass of adult hind leg minus mass of autotomized leg) and
254 relative final testes mass, shown with linear regression and shaded 95% confidence intervals for visualization purposes. **B.** Relationships between
255 the mass investment ‘saved’ (i.e., mass of adult hind leg minus mass of autotomized leg) and relative testes mass across the four experimental

256 treatments. Slope values were calculated from linear models with testes mass as the dependent variable and body mass as a covariate. Values in
257 bold denote slope values which differ significantly from zero at the $P < 0.05$ level. Sample sizes across treatments vary between $n = 21$ and $n = 25$

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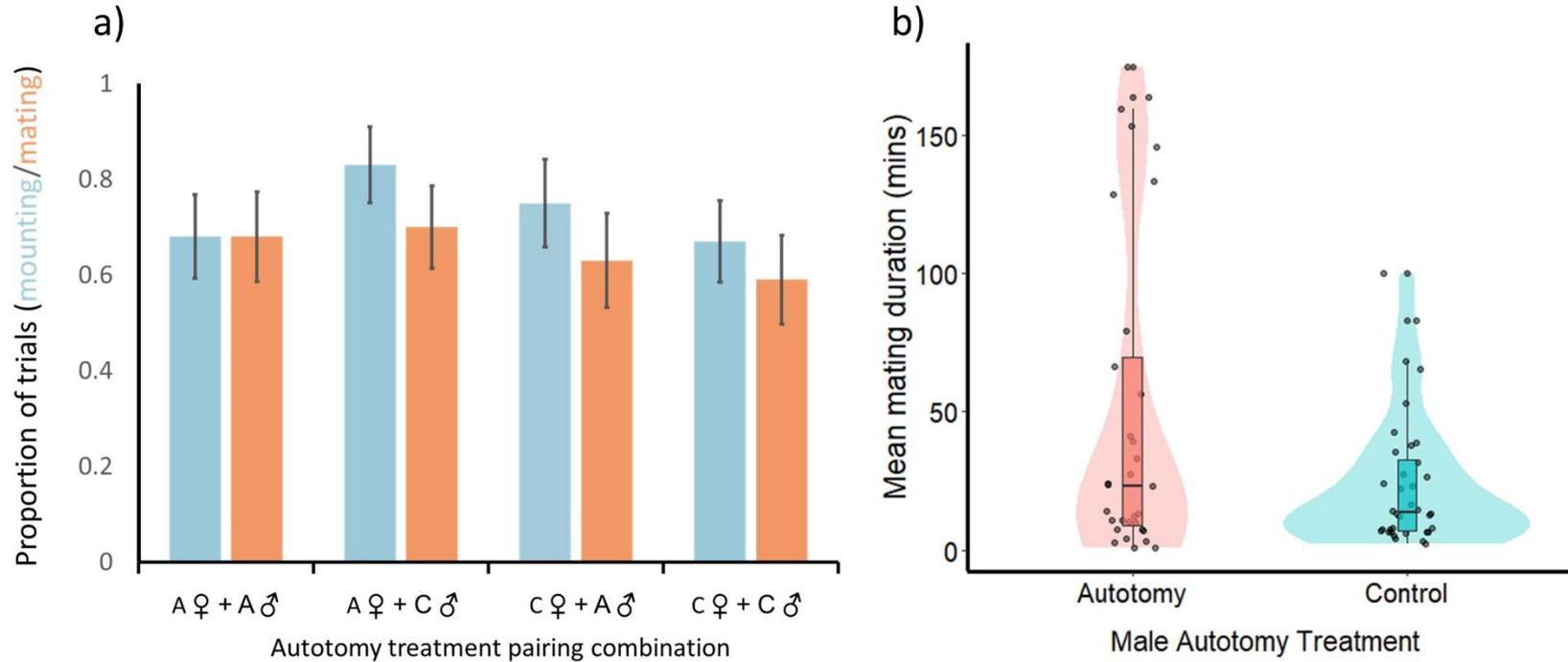
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270 **Figure 4** a) the proportion of trials in which males mounted (blue) and mated (orange) with females across four different autotomy and control

271 pair combinations +/- 1S.E. and b) the impact of male autotomy on mean mating duration

272 **DISCUSSION**

273 In this study, we demonstrate that the trade-off between these insects' precopulatory weapons and their
274 testes is developmentally time-sensitive and only manifested if limb loss occurred in the juvenile stage.
275 Induction of limb loss once individuals reached adulthood did not generate a boost in relative testes mass.
276 For the first time, to our knowledge, we were also able to directly track potential resource reallocation by
277 blocking off investment to a body part via autotomy at different time points and calculating a proxy of
278 tissue investment saved. We found a positive linear relationship between the amount of mass a bug
279 "saved" and the size of their relative adult testes mass. Intriguingly, the loss of a hind limb as a juvenile
280 was also accompanied by a behavioral change; autotomized males mated for significantly longer,
281 providing them with a potential complementary pathway to increased postcopulatory success. As
282 autotomized males' pre-copulatory chances are slim (Embets et al. 2018), investing heavily in sperm
283 production though enlarged testes may enable them to maximize their chances of siring offspring if they
284 do manage to secure a mating opportunity.

285 Autotomy may drive this morphological and behavioral plasticity by i) acting as a stimulus to switch to a
286 predominantly post-copulatory competitive strategy or ii) a closure of a resource allocation channel,
287 freeing up resources to reinvest directly elsewhere, or both. The question of why males who lose a limb
288 post-adulthood do not invest in relatively larger testes remains. This limited plasticity in response to
289 autotomy may be for one of two non-mutually exclusive reasons; a reduction in the resources available to
290 reinvest and loss of plasticity in adulthood.

291

292 **Resource availability**

293 First, investment in testes mass may be directly traded off against investment in hind leg mass. As a
294 result, experimental individuals that have invested in growing an adult limb are unlikely to make any
295 investment savings if they drop it after eclosing to their final adult form. They have already built an
296 enlarged weapon structure that will not grow larger in external size during adulthood. In support of this
297 hypothesis, we observe a significant linear relationship between hind leg weapon mass saved and relative

298 testes mass in the insects autotomized in the fourth and fifth instars. This implies the existence of a direct
299 trade-off between the two tissues, and that resources used to grow each structure are drawn from the same
300 shared pool.

301 Importantly, investment in a hind leg weapon is not limited to building the external structure. These
302 insects also incur maintenance costs and must support the ongoing metabolic activity of this enlarged hind
303 limb with its internal muscle and high density of mitochondria (Somjee et al. 2018b; Greenway et al.
304 2020). Notably, the left hind legs removed through autotomy early in adulthood were considerably lighter
305 than their fully grown right limb. This species and others lay down endocuticle for at least the first few
306 weeks of adulthood and internal tissue deposition in this weapon does not stop at eclosion (Lees, 1952;
307 Neville, 1965; Hill & Goldsworthy, 1968; Wang et al., 2018; Greenway et al. *in prep*). This suggests that
308 insects that lost a limb within the first three days of adulthood partially saved additional cuticle
309 investment as well maintenance costs for the last 10 or so days of the experiment. However, these
310 individuals did not differ significantly in their relative testes mass when compared to insects which were
311 autotomized 14 days into the experiment, having grown and maintained two full size hind limbs. Perhaps
312 the costs of tissue investment saved from adulthood onward were insufficient to generate a detectable
313 boost in relative testes mass. Alternatively, this lack of response suggests that individuals may be
314 restricted in their developmental flexibility beyond adult eclosion, rendering them unable to reallocate any
315 resources freed up via autotomy.

316

317 **Timing/ loss of plasticity**

318 Whilst the amount of tissue saved is necessarily conflated with the developmental time point at which we
319 induced autotomy (as hind limb size increases with each developmental instar), we looked for patterns in
320 relative testes size within treatments to try and parse apart these two factors. Individuals autotomized
321 during the fourth and fifth instar display positive linear relationships between mass 'saved' and relative
322 testes mass. However, adults with comparable potential mass savings to be had (with up to 2.5mg
323 difference between their autotomized and final leg) showed no such increase in testes mass, and we

324 detected no correlation between the two at this life stage. This lack of response may be attributed to
325 developmental canalization, which could restrict these insects' ability to alter investment in testes mass
326 beyond their fifth instar. Similarly, in species with minor and major male morphs, there may be a
327 threshold or switch point in development at which individuals diverge down different developmental
328 trajectories, prioritizing either pre- or postcopulatory traits (Emlen 1994; Emlen and Nijhout 1999;
329 Taborsky and Brockmann 2010). In male dung beetles, this window of sensitivity for prioritizing weapon
330 growth is a narrow period near the end of the third larval instar, during which topical hormone application
331 can induce the development of a major morph phenotype (Emlen and Nijhout 1999; Nijhout 2003). The
332 timing of allocation to weapons versus testes in hemimetabolous insects, which molt through successive
333 instars, is unclear. Soft internal tissue, such as reproductive organs, typically continues to grow and
334 develop well into adulthood when organisms hit sexual maturity (Dumser 1980; Hayes and Wall 1999).
335 However, architectural changes in testes mass (like those associated with autotomy in the fourth instar in
336 this species; Cavender et al. 2021) may not be possible past a certain developmental time point (Dumser
337 1980). As mentioned above, certain elements of the exoskeleton are fixed upon adulthood, whilst others
338 remain dynamic and continue to require investment throughout adult life. Therefore, the stages at which
339 these traits are sensitive to external environmental factors may differ, leading to complex changes in
340 directionality and timing of resource reallocation amongst these pre- and post-copulatory traits (Riska
341 1986, McDermott and Safran 2021).

342

343 **Compensatory behavior**

344 What use are larger testes without a corresponding behavioral shift? To capitalize on their increase in
345 relative testes mass, autotomized males appear to change at least one aspect of their mating behavior.

346 Males missing a hind limb increased their time spent mating with females, potentially compensating for
347 their lost weapon. The proximate mechanism for the increase in testes mass autotomized males

348 experience appears to be an increase in mitotic divisions in spermatogonia, which in turn likely increases
349 the number of spermatocytes and sperm cells produced (Cavender et al. 2021). A previous study in *N.*

350 *femorata* revealed that pairings involving autotomized males produced significantly more offspring than
351 their intact counterparts (Joseph et al. 2018). We do not yet know how mating duration relates to sperm
352 transfer, but testes mass is linked to fertilization success in this species (Greenway et al. 2020) and
353 increased mating duration may be a contributor to the boost in offspring production autotomized
354 individuals experience. In principle, mating for longer may enable autotomized males to capitalize on
355 their enhanced sperm production abilities and transfer larger ejaculates which can outcompete those of
356 intact males for access to fertilization (Parker et al. 1999; Engqvist and Sauer 2003; Pilastro et al. 2007).
357 Indeed, males also displayed longer mating durations when paired with larger females in this study,
358 supporting the interpretation that mating duration represents a form of male investment (Parker et al.
359 1999).

360 Mate guarding can be characterized by prolonged matings, thus it is possible that the increased mating
361 duration by autotomized males in this study serves to reduce the access of rival males to fertilization
362 opportunities (McClain 1989; Carroll 1991; Alcock 1994). Whilst we cannot rule out the possibility that
363 females may influence copulation duration, extended mating durations were only observed in pairings in
364 which males were autotomized and were significantly repeatable within pairings. Therefore, this mating
365 behavioral difference appears to be male associated, and may help compensate for their missing weapon.
366 By staying in copula for longer, a male can prevent a female remating before she oviposits and increase
367 the likelihood that his sperm is used to fertilize her eggs (Alcock 1994). Whilst autotomized males only
368 mate for around 15 mins longer than intact males, this could still confer a fitness benefit. For context,
369 there is limited evidence that wild adults survive beyond one month in the wild, and observed mating
370 rates under semi-natural conditions are low; males mate with between 0-4 females over a 5-day period
371 (Cirino & Miller 2017, Greenway & Miller, in prep). Given autotomized males have even lower chances
372 of securing mating opportunities due to their missing pre-copulatory weapon, extended mating durations
373 could have significant payoffs in terms of sperm transfer and reproductive success (Emberts et al. 2018).
374 Behaviors such as mate guarding, mating duration and broader mating investment are typically plastic and
375 highly context-dependent, especially when compared to developmentally constrained physiology traits

376 such as testes size (Parker 1990; Simmons 2001; Bretman et al. 2011; Shandilya et al. 2018). As such,
377 investigating the extent to which these insects adjust aspects of their mating behavior in response to
378 autotomy at different developmental time points is a logical next step of enquiry.

379

380 Overall, we found evidence of dynamic allocation between pre- and post-copulatory traits and a complex
381 interplay between morphology and behavior, which come together to shape each individual's
382 reproductive phenotype. Individuals appear to be able to redirect resources to testes growth when
383 experimentally prevented from investing in a pre-copulatory weapon, but this phenotypic plasticity
384 diminishes upon adulthood. While the proximate drivers and timing of such trade-offs have previously
385 been focused on in holometabolous insects, given their fixed resource budget and rapid development
386 phase as pupae (Nijhout and Emlen 1998; Emlen 2001; Moczek and Nijhout 2004; Tomkins et al. 2005),
387 clearly similar patterns exist in hemimetabolous insects, and potentially other animals which feed
388 continuously throughout development. Sexually selected traits represent a fertile testing ground for
389 further empirical exploration of the ontogeny of life history trade-offs (Riska 1986; Lande 2019;
390 McDermott and Safran 2021).

391

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403 EVG and CWM conceived the idea for this research and designed the methodology; EVG and EA
404 collected the data; EVG and EA analyzed the data; EVG and CWM led the writing of the manuscript. All
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406

407 *Data availability:*

408 Data is available via Dryad at: <https://doi.org/doi:10.5061/dryad.p8cz8w9vk>

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410 **References:**

- 411 Alcock, J. 1994. Postinsemination associations between males and females in insects: the mate-guarding
412 hypothesis. *Annu. Rev. Entomol.* 1–21.
- 413 Birkhead, T. R., and F. M. Hunter. 1990. Mechanisms of sperm competition. *Trends Ecol. Evol.* 5:48–52.
- 414 Bretman, A., M. J. Gage, and T. Chapman. 2011. Quick-change artists: male plastic behavioural
415 responses to rivals. *Trends Ecol Evol* 26.
- 416 Carroll, S. P. 1991. The adaptive significance of mate guarding in the soapberry bug, *Jadera haematoloma*
417 (Hemiptera: Rhopalidae). *J. Insect Behav.* 4:509–530.
- 418 Cavender, K. R., T. Ricker, M. Lyon, E. Shelby, C. W. Miller, and P. Moore. 2021. The trade-off
419 between investment in weapons and fertility is mediated through spermatogenesis in the leaf-
420 footed cactus bug *Narnia femorata*. *Ecol. Evol.*
- 421 Cirino, L. A., and C. W. Miller. 2017. Seasonal effects on the population, morphology and reproductive
422 behavior of *Narnia femorata* (Hemiptera: Coreidae). *Insects* 8(1):13
- 423 Dines, J. P., S. L. Mesnick, K. Ralls, L. May-Collado, I. Agnarsson, and M. D. Dean. 2015. A trade-off
424 between precopulatory and postcopulatory trait investment in male cetaceans. *Evolution*
425 69:1560–1572.
- 426 Dumser, J. B. 1980. The Regulation of Spermatogenesis in Insects. *Annu. Rev. Entomol.* 25:341–369.
- 427 Durrant, K. L., I. M. Skicko, C. Sturrock, and S. L. Mowles. 2016. Comparative morphological trade-offs
428 between pre- and post-copulatory sexual selection in Giant hissing cockroaches (Tribe:
429 Gromphadorhini). *Sci. Rep.* 6:36755.
- 430 Emberts, Z., C. M. S. Mary, T. J. Herrington, and C. W. Miller. 2018. Males missing their sexually
431 selected weapon have decreased fighting ability and mating success in a competitive
432 environment. *Behav. Ecol. Sociobiol.* 72:81.
- 433 Emberts, Z., C. W. Miller, D. Kiehl, and C. M. St. Mary. 2017. Cut your losses: self-amputation of
434 injured limbs increases survival. *Behav. Ecol.* 28:1047–1054.

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

437 Emlen, D. J. 2001. Costs and the diversification of exaggerated animal structures. *Science* 291:1534–
438 1536.

439 Emlen, D. J. 1994. Environmental control of horn length dimorphism in the beetle *Onthophagus*
440 *acuminatus* (Coleoptera: Scarabaeidae). *Proc. R. Soc. Lond. B Biol. Sci.* 256:131–136.

441 Emlen, D. J. 2008. The evolution of animal weapons. *Annu. Rev. Ecol. Evol. Syst.* 39:387–413.

442 Emlen, D. J., and H. F. Nijhout. 1999. Hormonal control of male horn length dimorphism in the dung
443 beetle *Onthophagus taurus* (Coleoptera: Scarabaeidae). *J. Insect Physiol.* 45:45–53.

444 Engqvist, L., and K. P. Sauer. 2003. Determinants of sperm transfer in the scorpionfly *Panorpa cognata*:
445 male variation, female condition and copulation duration. *J. Evol. Biol.* 16:1196–1204.

446 Gage, M. J. G., P. Stockley, and G. A. Parker. 1995. Effects of alternative male mating strategies on
447 characteristics of sperm production in the Atlantic salmon (*Salmo salar*): theoretical and
448 empirical investigations. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 350:391–399.

449 Greenway, E. V., L. A. Cirino, D. Wilner, U. Somjee, M.-E. Anagnostou, R. T. Hepple, and C. W. Miller.
450 2020. Extreme variation in testes size in an insect is linked to recent mating activity. *J. Evol. Biol.*
451 33:142–150.

452 Gross, M. R. 1996. Alternative reproductive strategies and tactics: diversity within sexes. *Trends Ecol.*
453 *Evol.* 11:92–98.

454 Hayes, E. J., and R. Wall. 1999. Age-grading adult insects: a review of techniques. *Physiol. Entomol.*
455 24:1–10.

456 Hill, L., and G. J. Goldsworthy. 1968. Growth, feeding activity, and the utilization of reserves in larvae of
457 *Locusta*. *J. Insect Physiol.* 14:1085–1098.

458 Joseph, P. N., Z. Emberts, D. A. Sasson, and C. W. Miller. 2018. Males that drop a sexually selected
459 weapon grow larger testes: weapons-testes tradeoff. *Evolution* 72:113–122.

460 Klaus, S. P., L. P. Fitzsimmons, T. E. Pitcher, and S. M. Bertram. 2011. Song and sperm in crickets: A
461 trade-off between pre- and post-copulatory traits or phenotype-linked fertility? *Ethology*
462 117:154–162.

463 Lande, R. 2019. Developmental integration and evolution of labile plasticity in a complex quantitative
464 character in a multiperiodic environment. *Proc. Natl. Acad. Sci.* 116:11361–11369.

465 Lees, A. D. 1952. The role of cuticle growth in the feeding process of ticks. *Proc. Zool. Soc. Lond.*
466 121:759–772.

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

469 McInain, D. 1989. Prolonged copulation as a post-insemination guarding tactic in a natural population of
470 the ragwort seed bug. *Anim. Behav.* 38:659–664.

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

473 McDermott, M. T., & Safran, R. J. (2021). Sensitive periods during the development and expression of
474 vertebrate sexual signals: A systematic review. *Ecology and Evolution*, 11, 14416– 14432. Miller,
475 C. W., P. N. Joseph, and Z. Emberts. 2021. Trade-offs between weapons and testes do not
476 manifest at high social densities. *J. Evol. Biol.* 34:726–735.

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

479 Neville, A. C. 1965. Circadian organization of chitin in some insect skeletons. *J. Cell Sci.* s3-106:315–
480 325.

481 Nijhout, H. F. 2003. Development and evolution of adaptive polyphenisms. *Evol. Dev.* 5:9–18.

482 Nijhout, H. F., and D. J. Emlen. 1998. Competition among body parts in the development and evolution
483 of insect morphology. *Proc. Natl. Acad. Sci.* 95:3685–3689.

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

486 Parker, G. A. 1979. Sexual selection and sexual conflict. In Sexual selection and reproductive competition
487 in insects, ed. Blum & Blum, pp 123-166.

488 Parker, G. A. 1990. Sperm Competition Games: Raffles and Roles. Proc. R. Soc. B Biol. Sci. 242:120–
489 126.

490 Parker, G. A., L. W. Simmons, P. Stockley, D. M. McChristie, and E. L. Charnov. 1999. Optimal copula
491 duration in yellow dung flies: effects of female size and egg content. Anim. Behav. 57:795–805.

492 Pilastro, A., M. Mandelli, C. Gasparini, M. Dadda, and A. Bisazza. 2007. Copulation duration,
493 insemination efficiency and male attractiveness in guppies. Anim. Behav. 74:321–328.

494 Puniamoorthy, N., W. U. Blanckenhorn, and M. A. Schäfer. 2012. Differential investment in pre- vs.
495 post-copulatory sexual selection reinforces a cross-continental reversal of sexual size dimorphism
496 in *Sepsis punctum* (Diptera: Sepsidae). J. Evol. Biol. 25:2253–63.

497 Riska, B. 1986. Some models for development, growth, and morphometric correlation. Evolution
498 40:1303–1311.

499 Sasson, D. A., P. R. Munoz, S. A. Gezan, and C. W. Miller. 2016. Resource quality affects weapon and
500 testis size and the ability of these traits to respond to selection in the leaf-footed cactus bug,
501 *Narnia femorata*. Ecol. Evol. 6:2098–2108.

502 Schielzeth, H., and S. Nakagawa. 2013. rptR: Repeatability for Gaussian and non-Gaussian data.

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

505 Sekii, K., D. B. Vizoso, G. Kualess, K. De Mulder, P. Ladurner, and L. Schärer. 2013. Phenotypic
506 engineering of sperm-production rate confirms evolutionary predictions of sperm competition
507 theory. Proc. R. Soc. B Biol. Sci. 280:20122711.

508 Shandilya, A., G. Mishra, and Omkar. 2018. Leg impairment affects the reproductive attributes of a
509 ladybird, *Menochilus sexmaculatus* (Fabricius). J. Asia-Pac. Entomol. 21:797–806.

510 Simmons, L. W. 2001. Sperm competition and its evolutionary consequences in the insects. Princeton
511 University Press.

512 Simmons, L. W., and D. J. Emlen. 2006. Evolutionary trade-off between weapons and testes. Proc. Natl.
513 Acad. Sci. 103:16346–16351.

514 Simmons, L. W., S. Lüpold, and J. L. Fitzpatrick. 2017. Evolutionary trade-off between secondary sexual
515 traits and ejaculates. Trends Ecol. Evol. 32:964–976.

516 Somjee, U., C. W. Miller, N. J. Tatarinic, and L. W. Simmons. 2018a. Experimental manipulation reveals
517 a trade-off between weapons and testes. J. Evol. Biol. 31:57–65.

518 Somjee, U., H. A. Woods, M. Duell, and C. W. Miller. 2018b. The hidden cost of sexually selected traits:
519 the metabolic expense of maintaining a sexually selected weapon. Proc. R. Soc. B Biol. Sci.
520 285:20181685.

521 Taborsky, M., and H. J. Brockmann. 2010. Alternative reproductive tactics and life history phenotypes.
522 Pp. 537–586 in P. Kappeler, ed. Animal Behaviour: Evolution and Mechanisms. Springer, Berlin,
523 Heidelberg.

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

527 van Noordwijk, A. J., and G. de Jong. 1986. Acquisition and Allocation of Resources: Their influence on
528 variation in life history tactics. Am. Nat. 128:137–142.

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

532 Wang, L.-Y., H. Rajabi, N. Ghoroubi, C.-P. Lin, and S. N. Gorb. 2018. Biomechanical strategies
533 underlying the robust body armour of an aposematic weevil. Front. Physiol. 9:1410

534 Zera, A. J., and L. G. Harshman. 2001. The Physiology of Life History Trade-Offs in Animals. Annu.
535 Rev. Ecol. Syst. 32:95–126.

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