

1 **Experimental manipulation reveals a trade-off between weapons**
2 **and testes**

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Abstract

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Theory predicts a trade-off between sexually-selected weapons used to secure mates and post-copulatory traits used to maximize fertilization success. However, individuals that have a greater capacity to acquire resources from the environment may invest more in both pre- and post-copulatory traits, and trade-offs may not be readily apparent. Here, we manipulate the phenotype of developing individuals to examine allocation trade-offs between weapons and testes in *Mictis profana* (Hemiptera: Coreidae), a species where the hind legs are sexually selected weapons used in contests over access to females. We experimentally prevented males from developing weapons by inducing them to autotomize their hind legs before the final molt to adulthood. We compared trait expression in this group to males where autotomy was induced in the mid legs, which are presumably not under sexual selection to the same extent. We found males without weapons invested proportionally more in testes mass than those with their mid legs removed. Males that developed to adulthood without weapons did not differ from the mid leg removal group in other traits potentially under pre-copulatory sexual selection, other post-copulatory traits, or naturally selected traits. In addition, a sample of adult males from the same population in the wild revealed a positive correlation between investment in testes and weapons. Our study presents a critical contribution to a growing body of literature suggesting the allocation of resources to pre- and post-copulatory sexual traits is influenced by a resource allocation trade-off and that this trade-off may only be revealed with experimental manipulation.

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Key-words: Allocation trade-offs, pre-copulatory competition, post-copulatory competition,

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phenotypic engineering, hemimetabolous insects

40 **Introduction**

41 Competition for mating opportunities drives the evolution of a diversity of male sexual weapons,
42 from the tusks of elephants to the exaggerated limbs of many insect species (*reviewed in* Emlen,
43 2008). However, competition among males often continues after mating and drives the evolution
44 of exaggerated post-copulatory sexual traits, from complex genitalia to sperm form and function
45 (Eberhard, 2009; Simmons & Fitzpatrick, 2012). Sexually selected weapons function in mate
46 acquisition, where males that invest more in weaponry are often more successful in
47 monopolizing mates. However, when females mate with multiple males, fertilization success
48 may also depend on a male's ability to compete with the ejaculates of other males either through
49 sperm competition (Parker, 1970; Simmons, 2001) or as females bias paternity in favor of males
50 possessing particular ejaculate traits (Eberhard, 2009). Therefore, a male's ultimate reproductive
51 success is determined by his success in both pre- and post-copulatory arenas of sexual selection.
52 Understanding the ways in which males allocate limited resources to competition for
53 reproduction before and after mating remains a fundamental question in evolutionary biology
54 (Kvarnemo & Simmons, 2013; Simmons et al. 2017).

55 Increased investment in post-copulatory traits can contribute to increased reproductive success in
56 polyandrous organisms, but these traits can also be energetically demanding. Increased
57 spermatogenesis can lead to reduced lifespan (Van Voorhies, 1992), and individuals with poor
58 nutrition may be unable to produce large testes or sustain high rates of spermatogenesis (Olsson
59 *et al.*, 1997). In addition, investment in the production of weapons should be costly. Indeed,
60 sexually selected weapons and ornaments are known to exhibit heightened condition-dependent
61 expression (Cotton *et al.*, 2004; Bonduriansky *et al.*, 2012; Miller *et al.*, 2016); generally,
62 individuals that develop sexual weapons under poor nutrient conditions have proportionally

63 smaller weapons (*e.g.* Lewis et al., 2012; Warren et al., 2013; Rosenthal & Hebets, 2015). Since
64 both pre- and post-copulatory traits can be energetically demanding, males are expected to face
65 allocation decisions when investing in one set of traits or the other. Indeed, recent theoretical
66 models of sperm competition predict that males should trade expenditure on weapons for
67 increased ejaculate expenditure as the risk and intensity of sperm competition increases (Parker
68 *et al.*, 2013) a prediction which has received support from comparative analyses within and
69 across taxa (Lüpold *et al.*, 2014; Dines *et al.*, 2015; Fitzpatrick & Lüpold, 2015; Kahrl *et al.*,
70 2015; Simmons et al. 2017). However, experimental manipulations that examine resource
71 allocation trade-offs between weapons and testes within species are rare.

72 Traits that are energetically demanding to produce may compete for limited resources in a
73 developing organism; the allocation differences between competing traits can generate reversals
74 in expression, or ‘trade-offs,’ where allocation of more resources to one trait reduces the
75 resources available to other traits (Stearns, 1989; Zera & Harshman, 2001). However, in many
76 cases negative correlations among traits are not detected. No correlations or even positive
77 correlations may occur because individuals vary in their ability to acquire resources from the
78 environment such that some individuals acquire sufficient resources to express large pre-
79 copulatory weapons and large testes, while others might compromise one or both traits (van
80 Noordwijk & de Jong, 1986; de Jong & van Noordwijk, 1992). Thus, if underlying trade-offs
81 occur, these can be masked by variation in the acquisition and allocation of available resources
82 (Reznick *et al.*, 2000; Simmons et al. 2017).

83 To reveal resource allocation trade-offs it is necessary to perform experimental studies where
84 one of the traits in question can be manipulated in isolation, to reveal effects on other traits
85 (Roff, 2002). To date exceptionally few studies have used experimental manipulations to

86 examine the relationship between pre- and post-copulatory traits; those that have employed an
87 experimental approach have reported evidence of resource allocation trade-offs between sexually
88 selected pre- and post-copulatory traits (Moczek and Nijhout 2004, Fry 2006, Simmons and
89 Emlen 2006, Joseph *et al.* accepted). The scarcity of studies is likely a result of the difficulty in
90 manipulating allocation to isolated traits, yet these experimental manipulations remain a
91 powerful tool for uncovering allocation trade-offs among life history traits (Simmons et al.
92 2017).

93 Here we test the hypothesis that males trade expenditure on pre-copulatory weapons for post-
94 copulatory ejaculate traits using the crusader bug, *Mictis profana* (Hemiptera: Coreidae). *Mictis*
95 *profana* is a sexually dimorphic insect where males invest in exaggerated hind legs used as
96 weapons over competition for mates. Hind legs can account for up to 19% of total somatic mass
97 in adult males and are used in contests, where males wrap their legs around a competitor and
98 perform a series of powerful kicks and squeezes (Tatarnic & Spence, 2013). The exaggerated
99 development of male hind legs occurs primarily during the last molt to adulthood. Sexually
100 selected weapons are often selected in concert with supportive traits that allow animals to use
101 these weapons effectively, or enhance the function of these weapons (Miyatake, 1997). The
102 compensatory role of supportive traits is hypothesized to drive correlational selection with
103 weapons, resulting in integrated development among them (Cheverud, 1996; Wagner, 1996).
104 *Mictis profana* possess sexually dimorphic tubercles that erupt from the lower abdomen of
105 males, and come into direct contact with opponents during competition. These tubercles, though
106 considerably smaller than the weapons themselves, are thought to function in concert with the
107 hind leg weapons to enhance success in male-male contests (Tatarnic & Spence, 2013).
108 Supportive traits can be less exaggerated than sexually selected weapons. However, changes in

109 weapon expression can be predicted to lead to changes in the expression of supportive traits
110 because of the close developmental integration among these traits (Cheverud, 1996; Wagner,
111 1996). We therefore looked for evidence of trade-offs between multiple sexually dimorphic traits
112 in this insect.

113 *Mictis profana* undergo limb loss, or autotomy, where they eject their hind legs in response to
114 entrapment, a feature common to many species in the family Coreidae (Emberts *et al.*, 2016).
115 Lost limbs do not grow back in *M. profana*. To test the prediction that pre- and post-copulatory
116 traits are subject to a resource allocation trade-off during development, we experimentally
117 induced weapon autotomy in our treatment group before the final molt to adulthood. If males
118 face a trade-off between allocation to weapons and testes we predict that males that have no hind
119 legs in which to invest at adulthood would have more resources to allocate to post-copulatory
120 traits (testes mass, sperm storage organ volume and sperm length) relative to other traits, such as
121 front legs or antennae. Our objective in this study was to use experimental manipulation to
122 uncover underlying trade-offs among different pre- and post-copulatory traits which might not be
123 revealed by examining phenotypic correlations from intact and unmanipulated individuals in the
124 wild.

125

126 **Materials and methods**

127 *Mictis profana* nymphs and adults were collected between Feb 21st- March 30th from wild
128 populations around metropolitan Perth, Western Australia. Insects were found feeding primarily
129 on species of *Acacia* (Fabales: Fabaceae) their native host plants (Flanagan, 1994). Nymphs
130 found in their 3rd and 4th instar were housed individually in aerated plastic containers with a

131 moist paper towel and provided with a diet of washed snowpeas and oranges, a diet previously
132 documented to be sufficient for development and reproduction (Tatarnic & Spence, 2013). We
133 kept the nymphs at a constant temperature of 29°C with a 12L:12D photoperiod. After molting
134 into their final instar before adulthood (5th instar), individuals were randomly allocated to one of
135 two treatment groups: (A) hind legs removed (no weapons) (B) mid legs removed (control for
136 leg removal). Mid legs in this species are shorter and much smaller than hind legs (proportional
137 mass at 5th instar: hind-legs = 10.4%, mid-legs = 3.9%). Their use in male-male conflict may be
138 for gripping other males and surfaces, but they do not have the striking or squeezing function of
139 the exaggerated hind limbs (Fig. 1). Thus, they are an appropriate control because they do not
140 appear to function directly in male-male competition. We induced autotomy bilaterally (A: both
141 hind legs, n= 16; B: both mid legs, n= 12) in juveniles, resulting in an adult missing those
142 autotomized limbs. For each limb removal, the leg was held firmly with reverse pressure forceps
143 to induce the insect to release the leg. We then reared the juveniles to adulthood in individual
144 containers. Adults were measured and dissected 17 days after their final molt: wild caught *M.*
145 *profana* were ready to mate at this age (Somjee *pers. obs*) and other coreids are ready to mate
146 and have fully developed sexual traits by this age (Somjee *et al.*, 2015).

147 For adults in the treatment and control groups we measured fresh weight of testes, body, and
148 hind legs (for those that had hind legs), to the nearest 0.01 mg with a digital scale (Mettler
149 AG245). We placed the left antenna and left front legs on a flat surface and photographed these
150 dorsally in standardized positions under a dissecting microscope. We extracted ventral tubercles
151 and photographed these anteriorly, to provide a clear measurement of the height of the major
152 central tubercle from the abdomen. The seminal vesicles were removed and placed on a
153 hemocytometer with a cover slip before being photographed. Seminal vesicle volume was

154 calculated by multiplying depth of the hemocytometer by the seminal vesicle area measured
155 from photographs. All photographs were taken under a Leica MZ75 dissecting microscope using
156 a Leica DFC 290 camera, and measurements were taken using ImageJ 1.46r software (Abràmoff
157 *et al.*, 2004). Sperm from seminal vesicles was diluted in insect ringer, smeared across a slide
158 and photographed under a dark-field using a Zeiss Axio-Imager compound microscope; the
159 lengths of ten sperm were measured per individual, again using ImageJ.

160 In addition to experimental manipulation, we examined correlations between pre- and post-
161 copulatory traits in a wild population to uncover the correlation among these traits in nature. We
162 collected adult males from the wild and examined the correlations between weapons mass, body
163 mass and testes mass. All measurements on wild males were taken with the same methodology
164 as experimental individuals.

165 **Statistical analysis**

166 We examined the effects of leg removal (hind or mid) on the size of eight morphological traits
167 using separate general linear models with leg removal treatment as a categorical explanatory
168 variable. Next, we constructed models to test for changes in the size of morphological traits
169 relative to body mass. We built the initial models with body mass as a continuous covariate,
170 treatment as a categorical factor, and the interaction between body mass and treatment. In all
171 instances, we calculated least square means (adjusted means) from the general linear model for
172 each treatment. Linear measurements such as antennae and front femur length were cube
173 transformed to convert their values to the same scale as mass for appropriate comparisons. All
174 data was \log_{10} transformed to improve linearity, normality and homoscedasticity. Our estimate of
175 body mass was obtained by subtracting focal traits (testes mass and hind leg mass) from body

176 mass to achieve an independent measure of body mass. This procedure helps to avoid the
177 problem of part-whole correlations (Christians, 1999). All statistical analyses were conducted in
178 R statistical software v0.99.893 (R Development Core Team, 2016). We collected 27 male
179 insects from the wild and estimated the phenotypic scaling relationship between trait size and
180 body size using OLS regression (Kilmer & Rodríguez, 2017).

181 **Results**

182 We initially examined the effects of leg removal (hind or mid) on the absolute size of eight
183 morphological traits. Only testes mass was influenced by removal of the hind legs (Table 1, Figs
184 2 and 3). The remaining traits did not differ in size between hind leg and mid leg removal
185 treatments.

186 We next examined the scaling relationships between each of the traits and body mass using
187 separate general linear models (GLMs). Our initial model included the main effects of body
188 mass, leg-removal treatment, and interaction between body mass and treatment. Statistically-
189 significant interactions of body mass with treatment would indicate that the scaling slope of body
190 mass and trait size changed depending upon whether the mid legs or hind legs were removed.
191 We did not find evidence of a change in scaling slope for any of the traits measured, thus we
192 removed the interaction and proceeded to test for a change in intercept due to treatment. We
193 found evidence that males with their hind legs removed grew disproportionately larger testes
194 compared to other measured traits (Table 2, Figs 2 and 3). We did not find evidence that any
195 other traits increased or decreased disproportionately relative to each other for males that lost
196 their hind legs.

197 For wild caught males, log testes mass and log body mass showed a positive relationship that did

198 not differ significantly from isometry (Linear regression against slope 1: $F_{1,25} = 39.4$, $p = 0.91$;
199 OLS regression slope = 0.98, SE = 0.16, $R^2 = 0.60$: Fig. 4a). Further, the relationship between log
200 weapon mass and log testes mass did not differ significantly from isometry (Linear regression
201 against slope 1: $F_{1,25} = 30.1$, $p = 0.17$; OLS regression: slope = 0.80, SE = 0.15, $R^2 = 0.53$: Fig.
202 4b).

203

204 **Discussion**

205 We found, via an experimental manipulation, that weapons and testes experience a
206 developmental trade-off in the coreid bug *M. profana*. Males that were prevented from investing
207 in pre-copulatory weapons increased investment in testes mass. No increase in investment was
208 found in any other structures measured in this study. Insects with their mid legs removed did not
209 differ in their relative allocation to testes mass. Although mid legs accounted for a lower
210 proportion of absolute mass than hind legs, males missing weapons allocated disproportionately
211 more resources towards testes than males missing mid legs. Insects that did not develop
212 weaponry showed some evidence of growing significantly larger seminal vesicles (Fig. 3), a
213 pattern that would be expected given that increased sperm production by larger testes may
214 require seminal vesicles of greater volume for storage of sperm.

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216 Insects in the family Coreidae express their weapons as a modification of hind legs that can be
217 routinely ejected in response to various forms of entrapment (Emberts *et al.* 2016). For this
218 reason, these insects provide an excellent opportunity for testing allocation trade-offs. Many
219 studies on allocation trade-offs have relied primarily on evidence of phenotypic correlations
220 among pre- and post-copulatory traits. However, phenotypic correlations can offer little support

221 for allocation trade-offs (Simmons *et al.* 2017). Positive phenotypic correlations between traits
222 are expected when individuals vary in resource acquisition and trait expression depends on
223 resource availability (van Noordwijk & de Jong, 1986; de Jong & van Noordwijk, 1992; Reznick
224 *et al.*, 2000).

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226 Phenotypic correlations from wild populations may provide an important description of the
227 natural phenotypic distribution of these traits in the wild, however, these data do not provide
228 information on within-organism resource allocation trade-offs (Simmons *et al.* 2017). Allocation
229 trade-offs can be obscured by environmental effects, and experimental manipulations that hold
230 all other variables constant are necessary to reveal allocation trade-offs among pre- and post-
231 copulatory sexual traits (Simmons & Emlen, 2006). Indeed, we found a positive correlation
232 between weapons and testes in our field collected individuals (Fig. 4), while the development of
233 weapons had a negative impact on testes in our experimental manipulation. Similar patterns
234 have been found in stalk-eyed flies, wherein eye-span and testes size are positively correlated
235 among individuals (Rogers *et al.*, 2008; Cotton *et al.*, 2010) yet experimental manipulations
236 provide direct evidence of a trade-off between these traits (Fry 2006).

237

238 Environmental conditions during development can also alter the relative expression of both
239 weapons and testes. In a related Coreid species, the heliconia bug, males reared on one host plant
240 produced larger testes but smaller weapons compared to males reared on a separate host plant
241 (Somjee *et al.*, 2015). This environmentally induced change in relative testes size was of
242 moderate but significant effect (Cohen's $d = 0.70$, Somjee *et al.*, 2015). In another study,
243 removal of a single hind leg of cactus bugs during their fourth instar resulted in adults with

244 proportionally larger testes compared to a group where a single mid-leg was removed (Cohen's d
245 = 0.39, Joseph et al. *accepted*). In comparison, in our study males that were prevented from
246 developing both hind legs had an increase in testes size approximately one standard deviation
247 larger than insects that developed without mid legs (Cohen's $d = 1.01$). In our study species,
248 weapons are much larger than mid legs and we could not determine if preventing development of
249 weapons had a proportionally larger effect on testes development per gram of tissue compared to
250 mid legs. The cost of producing weapons, relative to other somatic tissues, remains a major
251 question in studies that examine allocation trade-offs in sexually selected traits (Simmons et al.
252 2017).

253

254 In many systems, it is difficult to distinguish whether correlated traits are more strongly
255 influenced by a shared developmental structure (Klingenberg 2005), or by correlated selection
256 (Cheverud 1996). By manipulating the development of a single pre-copulatory weapon, here we
257 de-couple this relationship, and expose a resource allocation trade-off among what appear to be
258 developmentally distinct traits. Our finding that investment in male hind legs trades off with
259 investment in testes, and not with development of another close-by putative pre-copulatory
260 structure (tubercles) supports the idea that testes and weapons are developmentally integrated
261 through a history of correlated selection (Simmons & Emlen, 2006). To date few studies have
262 experimentally investigated resource allocation trade-offs among pre- and post-copulatory traits
263 (Simmons et al. 2017). In one well-studied case, onthophagine dung beetles, males
264 experimentally prevented from growing horns had increased relative investment in testicular
265 tissue (Simmons & Emlen, 2006), while males prevented from developing primary genitalia
266 increased their allocation to horn growth (Moczek & Nijhout, 2004). In our study, the behavioral

267 feature of weapon autotomy (Emberts *et al.*, 2017) provided an opportunity to prevent weapon
268 development with minimal potential injury and detrimental effects to developing insects, and
269 resulted in increased allocation of resources to testes growth.

270

271 Our investigation of a hemimetabolous insect provides additional insights into the developmental
272 links between exaggerated trait growth and allocation trade-offs. Previous studies have
273 experimentally halted trait growth in holometabolous insects such as dung beetles and stalk-eyed
274 flies. In closed developmental systems such as those of holometabolous insects, animals undergo
275 a single, relatively rapid transition between larval and adult forms, and feeding does not occur
276 during this closed transitional pupal stage. Where a developing organism must allocate a limited
277 set of resources to multiple rapidly developing traits the potential for trade-offs is high. However,
278 resource allocation trade-offs are thought less likely in open developmental systems such as
279 those of hemimetabolous insects (e.g. Tomkins *et al.*, 2005) because growth occurs in a stepwise
280 fashion, with each successive instar able to feed as it develops toward adulthood. Here we
281 prevented trait growth in such a system, where males could feed to compensate for resources lost
282 in a previous instar and potentially invest equally in all traits and yet we found evidence of a
283 trade-off between weapons and testes. There are a number of reasons that may explain this
284 observed resource allocation trade-off. The proportional investment in hind legs increases
285 significantly during the final molt to adulthood, and there might be a limit to which insects can
286 compensate for lost resources, and distribute these resources equally to all traits before they molt.
287 In our sample, the hind legs of fifth instar male *M. profana* nymphs comprised on average 10.4%
288 of total somatic tissue. As nymphs molt from 5th instar into adulthood, the proportional
289 investment in hind leg mass thus increases by up to 47.1%. This large increase in relative

290 allocation to hind legs during the final molt may provide an opportunity for disproportionate
291 allocation among traits, despite the open developmental system. Hemimetabolous insects will be
292 useful to test theoretical predictions about how distinct developmental processes might limit or
293 enhance the potential for selection to drive trade-offs among traits when resource availability
294 differs (Toubiana & Khila, 2016). Future studies might examine the ontogeny of such trade-offs
295 by examining resource allocation decisions at different discrete stages of development.

296

297 Among Coreid species, large body and weapon size in males contributes to success in territory
298 defense against rival males (Miyatake, 2002) and success in pre-mating combat (Mitchell, 1980).
299 There is also evidence that testis size contributes to post-mating reproductive success. In an
300 experimental manipulation similar to ours, Joseph *et al.* (accepted) found that males who
301 developed without a single hind leg also developed larger testes, and that females mated to these
302 males who laid viable eggs had a higher proportion of live hatchlings (Joseph *et al.* accepted),
303 suggesting a role of testes size in reproductive success. Although we found no difference in
304 sperm length between our treatments, it is possible that other ejaculate traits such as sperm
305 number, motility and seminal fluid composition might also be affected, which are likely to
306 contribute to post-copulatory competitive fertilization success (Parker, 1998; Simmons &
307 Kotiaho, 2002; Snook, 2005). Our study contributes to a small but growing body of work that
308 supports the prediction that males should face a trade-off between expenditure on gaining mates
309 and on gaining fertilizations (Parker *et al.*, 2013; Simmons *et al.* 2017). Moreover, our findings
310 illustrate the importance of experimental manipulation for detecting the presence of life-history
311 trade-offs more generally (Ketterson & Nolan Jr, 1992).

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313 **Data Accessibility**

314 All data was deposited in the Dryad repository.

315 **Authors' Contributions**

316 US, LWS, CWM and NT conceived the ideas and designed methodology; US, NT & LWS collected
317 the insects, and US performed dissections and measurements; US and CWM analyzed the data;
318 US led the writing of the manuscript. No authors in this manuscript have conflict of interest to
319 declare. All authors contributed critically to drafts and gave final approval for publication.

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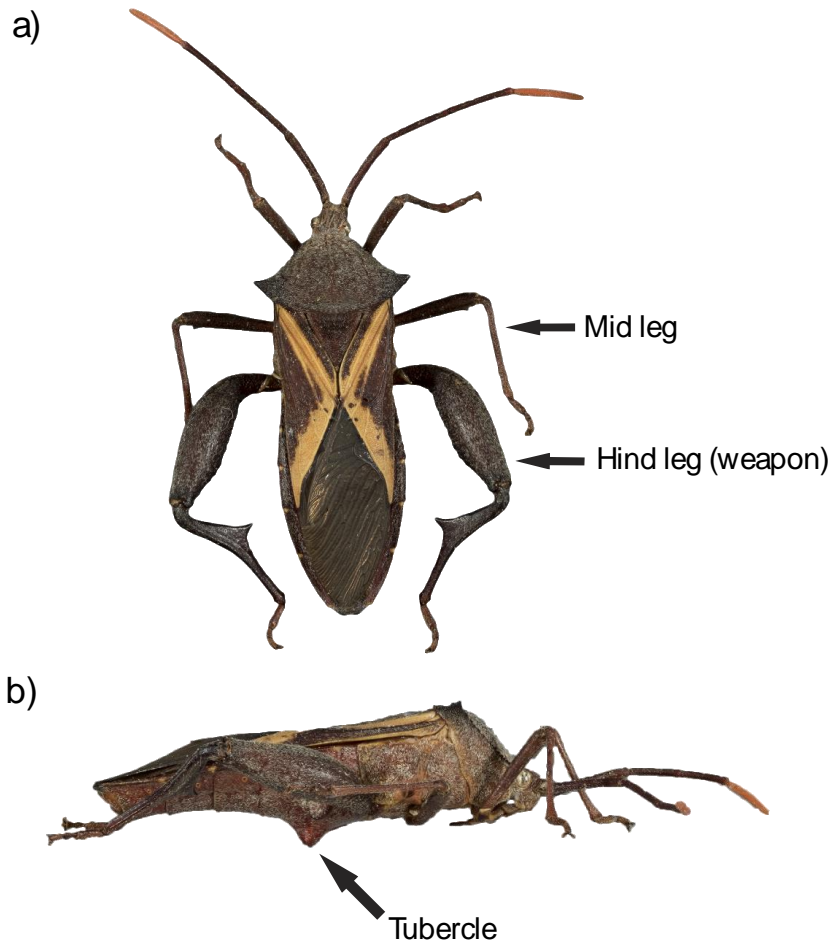
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Figures & tables

359 **Figure 1.** *Mictis profana* image



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362 **Figure 1** *Mictis profana* male with enlarged legs modified as weapons, notice enlarged femora
363 and tibial spines which contact opponents during combat (a: dorsal view). Males exhibit a large
364 central tubercle, a sclerotized projection of the abdomen which is often engaged during combat
365 with rival males (b: lateral view, photos by Nikolai Tatarnic).

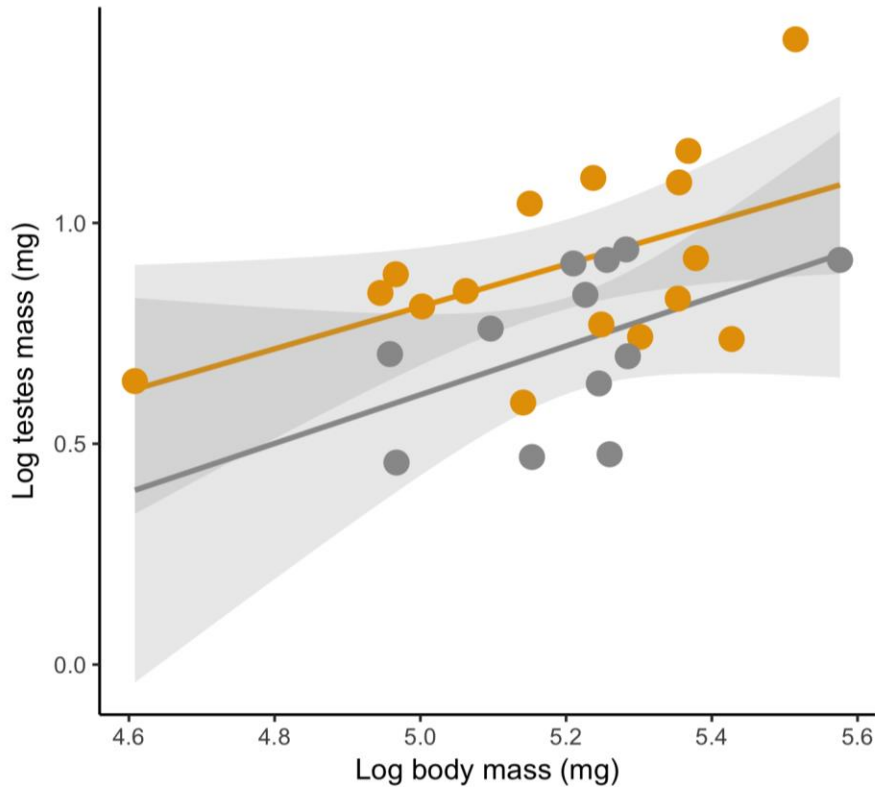
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370 **Figure 2. Testes size by treatment**



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373 **Figure 2.** Insects that developed without weapons (orange) invested disproportionately more in
374 testes mass than insects that developed without mid legs (grey). Testes weight increased with
375 body weight but treatment (hind legs removed) males developed proportionally heavier testes
376 than did control males (mid legs removed). Lines depict linear regression model predictions with
377 95% confidence interval bands.

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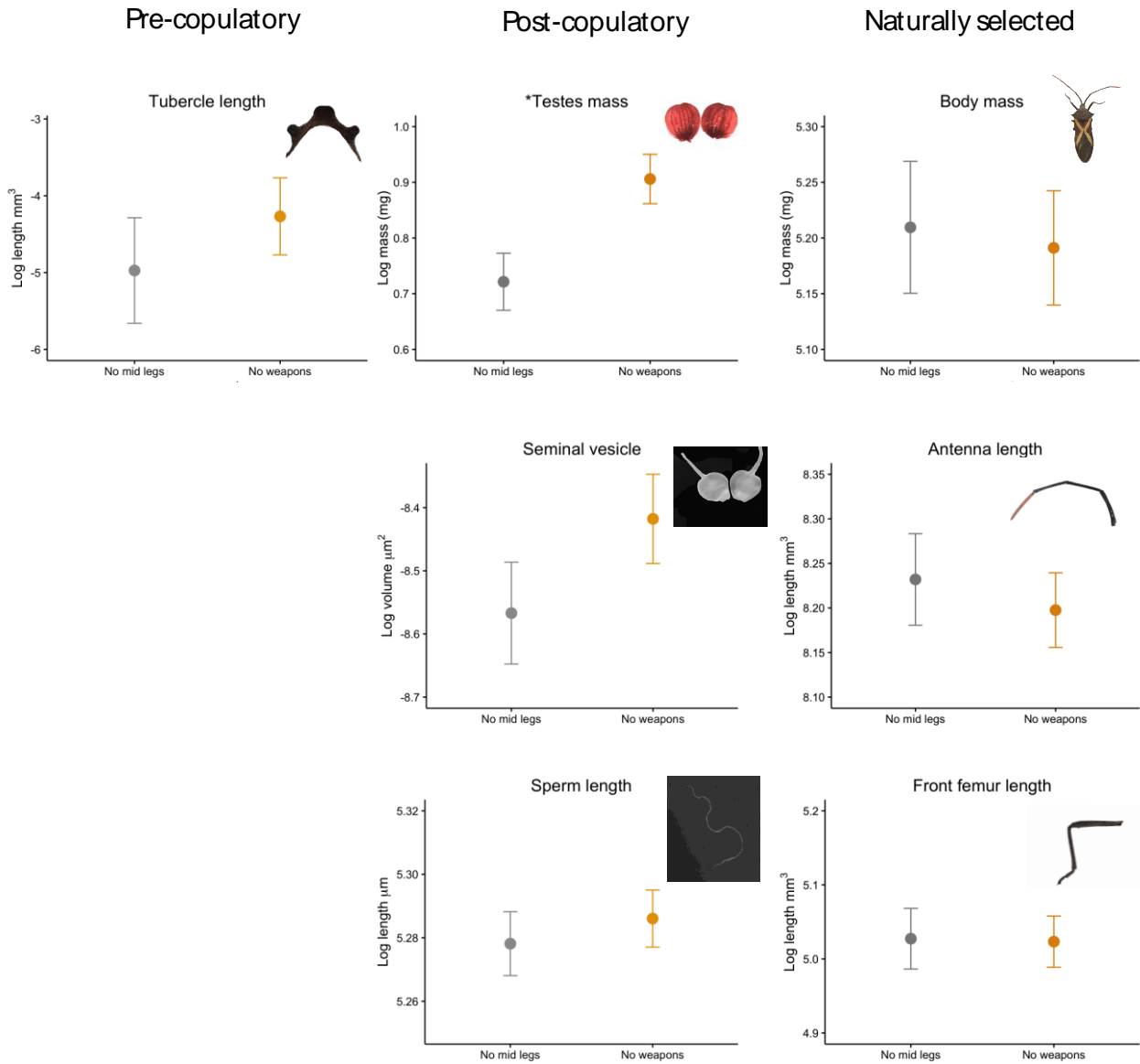
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383 **Figure 3 - Effect of weapon removal on pre-copulatory, post-copulatory and naturally**

384 **selected traits**



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386 **Figure 3:** Graphs display body mass corrected investment in pre-copulatory, post-copulatory and

387 naturally selected traits for treatments without hind legs (weapons) and without mid legs. Least

388 squared means (adjusted means) were calculated using ANCOVA to account for differences in

389 body mass for all traits (except for body mass itself). Testes mass was significantly larger in
390 males without hind legs than males without mid legs ($P < 0.05$). Seminal vesicle volume showed a
391 non-significant effect towards being larger in males without hind legs ($P = 0.11$), (*) indicates
392 significant differences among groups $p < 0.05$ (photos by Ummat Somjee).

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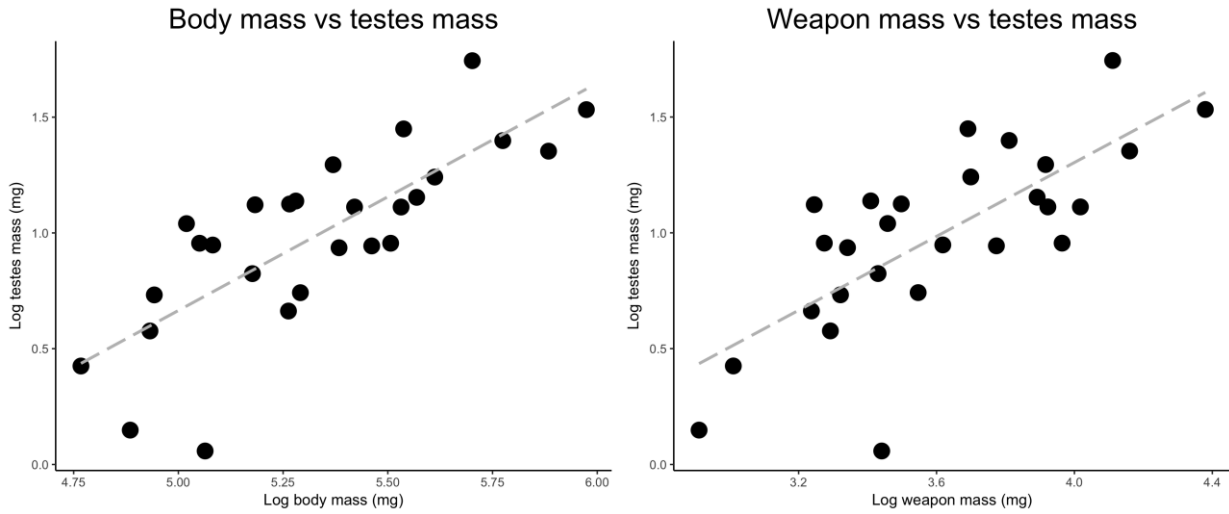
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413 **Figure 4 – Testes size in wild-caught males**

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417 **Figure 4.** In wild caught males, both testes mass (OLS regression: slope =0.98, SE = 0.16, $R^2 =$
418 0.60) and weapon mass (slope =0.80, SE = 0.15, $R^2 = 0.53$) have relationships not significantly
419 different from isometry (Linear regression against slope 1: Body mass: $F_{1,25} = 39.4$, $p = 0.91$;
420 Weapon mass: $F_{1,25} = 30.1$, $p = 0.17$). These positive correlations in wild males do not provide
421 any indication that an allocation trade-off is underlying the development of pre- and post-
422 copulatory traits in this insect.

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428 **Table 1.** Results of eight separate GLM for effects of leg removal on morphological traits in
 429 *Mictis profana* adults. Linear traits were cubed (except for sperm length), and all traits were log-
 430 transformed prior to analysis.

Trait measured	Wald Chi-Square	P
Testes mass	5.183*	0.031
Abdominal tubercle length	0.551	0.466
Seminal vesicle volume	1.718	0.204
Sperm length	0.310	0.582
Body mass	0.055	0.815
Pronotum width	0.534	0.472
Antennal length	0.495	0.489
Front femur length	0.109	0.744

431 Error d.f. = 1 for all analyses; Bold text indicates statistical significance

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438 **Table 2.** Results of separate GLM for effects of leg removal on morphological traits in *Mictis*
 439 *profana* adult males with body mass as a covariate. Linear traits were cubed prior to direct
 440 comparison with body mass. All traits were log-transformed prior to analyses. Analyses were
 441 first run including main effects and the two-way interaction to test for differences in scaling
 442 slope due to treatment and between body mass and the trait in question. In all cases, the p-values
 443 for the interaction terms were not statistically significant, were removed, and the analyses were
 444 run with only main effects to test for a shift in the allometric intercept.

Trait measured	Body Mass Wald Chi-Square	Treatment Wald Chi-Square
Testes mass	8.619***	7.406**
Abdominal tubercle length	4.087*	0.686
Seminal vesicle volume	0.496	1.917
Sperm length	0.273	0.342
Pronotum width	22.924***	0.287
Antennal length	2.271	0.267
Front femur length	9.079***	0.0056

445 Error df = 1 for all analyses. *p < .05, ** p < .01, *** p < .001. Bold text indicates traits that had
 446 a shift in their scaling relationship with body mass due to hind leg removal.

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