

Trade-offs between weapons and testes do not manifest at high social densities

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Abstract

Social conditions can alter the allocation of resources to reproductive traits. For example, an increase in social density during development is frequently associated with an increase in the testes mass of males. Sperm competition theory assumes that increased investment in testes should come at the expense of investing into precopulatory traits, such as sexually selected weaponry. However, much remains unknown about the role of the social context on the concurrent, relative investment in both testes and weapons. We found that the leaf-footed cactus bug, *Narnia femorata* (Hemiptera: Coreidae), grew nearly 20% larger testes when raised in high social densities. In addition to manipulating social density, we used autotomy (limb loss) to limit investment in their hindlimb weapon during development. At low densities, we found that those that lost a weapon during development grew larger testes by adulthood, supporting previous work demonstrating a weapons–testes trade-off. However, at high social densities, males that dropped a hindlimb did not grow larger testes, though testes were already large at this density. These results underscore the importance of the social context to resource allocation patterns within the individual.

KEYWORDS

armament, coreidae, density, group effects, leaf-footed bug, phenotypic engineering, resource allocation, sexual selection

1 | INTRODUCTION

Males in many populations must not only first locate and, in many cases, compete with other males for access to females (Andersson, 1994), but also compete for fertilization success against the sperm of other males (Parker & Pizzari, 2010). Therefore, increased pre- and post-copulatory sexual selection may promote the increased investment in traits associated with greater competitive ability, including larger weapons (Emlen, 2008) and larger testes that produce more sperm (Simmons, 2001). Though high levels of investment in weapons and testes can be important to biological fitness, these traits can be costly (e.g. Greenway et al., 2019; Somjee et al., 2018). Since both testes and weapons also have similar

developmental timings and crucial reproductive functions, they may experience life-history trade-offs (Miller et al., 2019; Simmons et al., 2017). Thus, investing in one of these traits may lead to reduced investment in the other. Trade-offs between reproductive traits are expected to shape evolutionary outcomes (Stearns, 1989), so research into the nature of trade-offs is essential.

The social context can affect reproductive investment (Ceacero et al., 2019; Conroy & Roff, 2018; Gage & Barnard, 1996; Knell, 2009; Vitousek et al., 2014; Wedell et al., 2002) and thus may influence the manifestation of trade-offs between focal traits. Importantly, testes growth is often increased when social density and sperm competition risk are high (Bailey et al., 2010; Fisher et al., 2018; Gage, 1995; He & Miyata, 1997; Johnson et al., 2017; Stockley & Seal, 2001).

Experimental studies are lacking that directly examine the effects of social density on the concurrent, relative investment in both testes and weapons.

Narnia femorata (Hemiptera: Coreidae), the leaf-footed cactus bug (Figure 1), is well suited for studies of reproductive investment. Male *N. femorata* use their enlarged hindlimbs to signal and fight over access to receptive females (Nolen et al., 2017; Procter et al., 2012). Females mate multiply and produce clutches of eggs throughout their adult lives (Allen et al., 2018; Wilner et al., 2020). The size of the hindlimb scales positively with body size, and males with larger limbs are more likely to win contests (Nolen et al., 2017; Procter et al., 2012). However, twelve per cent of adult males in the wild are missing one or more limbs, and a hindlimb is the most commonly lost (Embets et al., 2016). Males missing a hindlimb fight readily (Video S1), but they are less likely to become dominant and have reduced mating success when another male is present (Embets et al., 2018). Loss of a limb in this species and its relatives commonly occurs via autotomy, a process used to escape predation or entrapment in a bad moult (Embets et al., 2016, 2020). Autotomy in this species does not reduce laboratory survival (Joseph et al., 2018). Unlike well-studied crustaceans (Mariappan et al., 2000) and lizards (Bateman & Fleming, 2011), *N. femorata* does not regenerate autotomized limbs (Embets et al., 2017, 2019).



FIGURE 1 *Narnia femorata*. An adult male (top) and a nymph in the penultimate (4th) instar (bottom) on *Opuntia mesacantha*. Both insects shown are intact, with all six limbs. In this study, left-hindlimb autotomy was induced in a subset of our 4th instar nymphs. Autotomized juveniles develop into adults with only a single hindlimb.

Photo: C. W. Miller

Experimentally inducing autotomy on these insects can be used as a form of phenotypic engineering. Phenotypic engineering is a powerful experimental technique for investigating trait allocation patterns and trade-offs (Zera & Harshman, 2001). Researchers can, for example, prevent or reduce investment in one trait and then examine the resulting change in the expression of other traits (Moczek & Nijhout, 2004; Sekii et al., 2013). Growth in certain traits after the procedure provides evidence for a resource allocation trade-off between the manipulated trait and the trait in question (Zera & Harshman, 2001). Previous work in leaf-footed bugs has found that juvenile autotomy of a hindlimb results in increased adult testes mass and ovary mass in this species and relatives, providing evidence for a resource allocation trade-off between these traits (Joseph et al., 2018; Miller et al., 2019; Somjee et al., 2018). Two previous studies on leaf-footed bugs measured the size of a number of traits following autotomy in males, including the testes, the width of the remaining hindlimb, the trochanter (Joseph et al., 2018), the seminal vesicles, the antennae, the front femora and sperm length (Somjee, Miller, et al., 2018) with little evidence of trade-offs except between the hindlimb weapons and the testes. The hindlimb is composed of cuticle, which is part of the skeletal structure of the animal, and inside this cuticle is metabolically expensive muscle (Somjee, Woods, et al., 2018). The testes tissue has slightly lower levels of metabolic activity, but a higher maximum metabolic capacity than hindlimb muscle tissue (Greenway et al., 2019).

Here, we raised young insects in two different densities, and for half we prevented investment in a single hindlimb weapon by inducing autotomy mid-way through development. Inducing autotomy was our method of phenotypic engineering. Approximately four weeks later, we measured the size of the adult body, the testes and major components of the remaining (right) hindlimb including the muscle mass, the cuticular mass and the external width of the hind femur. We predicted that the intact males (with all limbs) raised in a higher density would grow larger testes than those raised in low density, based on findings in other species. We assumed that testes investment should increase following autotomy, based on findings in this species and relatives (Joseph et al., 2018; Somjee, Miller, et al., 2018). As a result, we predicted testes investment to be at a maximum for autotomized males raised in high density, because both factors are expected to lead to increased testes investment. We also examined the influence of experimental autotomy on cuticle and muscle mass of the remaining limb.

2 | MATERIALS AND METHODS

2.1 | Rearing, autotomy and density

We paired wild-caught adult male and female *N. femorata* collected from Starke, FL (29.9804°N, 81.9848°W) in plastic deli containers containing an *Opuntia mesacantha lata* spp. cladode and a single ripe fruit within a greenhouse (14:10 L:D cycle; 24–30°C). Paired individuals were able to mate and oviposit freely. Once nymphs hatched and developed to their second instar with their siblings, we pooled

individual nymphs from separate parents to form groups of 8 to 10 nymphs, allowing them to develop into fourth-instar juveniles in containers consisting of a single cladode and three to four ripe fruits. All plastic containers used in this study had a top diameter of 118 mm, bottom diameter of 85 mm and a height of 148 mm.

Three hundred and seventy-nine fourth-instar juveniles were randomly allocated to one of two phenotypic engineering treatments: induced autotomy of the left hindlimb (experimental treatment) or no autotomy (intact insects; control treatment). We induced autotomy during the fourth (penultimate) juvenile instar, since male hemipteran insects experience increased testis growth during the final two instars (Dumser & Davey, 1974; Economopoulos & Gordon, 1971). Following previously developed methods (Embets et al., 2016; Joseph et al., 2018), we grasped the left hindlimb near the trochanter-femoral joint and gently brushed the individual until it dropped the entrapped limb.

Wild *N. femorata* are commonly found in aggregations of variable size on their host plants (Allen et al., 2021; Cirino & Miller, 2017). We created two density treatments for the final two instars of development. We randomly placed our intact and autotomized fourth-instar individuals with either a total of (a) ten to eleven juveniles, or (b) three to four juveniles from the same autotomy treatment. We attempted to minimize any negative effects of food competition by providing groups ample food, specifically a cactus cladode with four ripe fruits. The sexes are nearly indistinguishable prior to adult eclosion, and so each container contained both sexes.

Our rearing design produced a total of 202 adult males, after approximately 85% survivorship to adulthood (consistent with previous studies; Joseph et al., 2018). Upon reaching adulthood, each male was placed in its own container for 14 days, upon which time it was frozen and eventually used for measurements. To minimize concerns of pseudo-replication and also operating with time constraints, we randomly selected one adult male from each developmental rearing cup for measurement and analyses. Our final sample size included 71 males total: 37 autotomized and 34 intact. These males were also used as a comparison group for the study reported in Miller et al., (2019).

2.2 | Testes and hindlimb measurements

In this study, we used testes mass as our gauge of testes investment. The testes of *N. femorata* are extremely variable in size, metabolically costly and positively associated with offspring production (Greenway et al., 2019). We separated the skeletal and muscular components of a sexually selected weapon because investment costs and strategies may differ due to the types of tissues involved. Investment in the distinct weapon tissues had not yet been examined in this species.

We thawed the previously frozen males, extracted their testes and placed the organs in 70% EtOH. After drying the testes for 24 hr at 60°C, we measured the mass in milligrams to the nearest microgram using a microbalance (Mettler Toledo XP6).

Induced autotomy in this study targeted the *left* hindlimb of juveniles. We removed the remaining (*right*) hindlimb from our previously frozen adult males, from both the autotomized and intact treatments, near the trochanter-femoral joint at the location of the fracture plane.

We made a horizontal slit across the femora from the trochanter-femoral to the femoral-tibial joints to facilitate eventual drying and muscle digestion. Each limb was then placed into 70% EtOH for storage. We later dried hindlimbs at 60°C for 72 hr, then weighed them in milligrams with accuracy to the nearest microgram. Next, we estimated the separate masses of the hindlimb cuticle (the exoskeleton of the hindlimb) and the hindlimb muscle of the limbs. To do so, we placed the hindlimbs in a 10% KOH solution (0.5ml at 90°C) for one hour to dissolve the soft tissue. We then re-dried the cuticle at 60°C for 72 hr and took a new measurement of dry mass. We calculated an individual's hindlimb muscle mass by subtracting the cuticular mass from the total mass. Dry muscle mass was on average 47% of the mass of the entire limb.

2.3 | Morphometric measurements

Using a Canon EOS 50D digital camera mounted to a Leica M165C dissecting microscope and Image J software v1.46r (Schneider et al., 2012), we photographed and then measured the pronotal width of each male as a proxy for overall body size. Pronotal width is a common metric of body size in insects and is highly correlated with other measures of body size in this species (Gillespie et al., 2014; Procter et al., 2012).

2.4 | Statistical analysis

We investigated the effects of developmental autotomy and social density on adult body size, dry testes mass and right hindlimb traits. Measurements were \log_e transformed to improve linearity, normality and homoscedasticity. Further, a benefit of log transformation is that the measurements become scale-independent which can allow meaningful comparison of scaling relationships (Huxley, 1924; Shingleton & Frankino, 2013).

We tested the extent to which higher density during development may lead to decreased male adult body size. Thus, we first examined the effects of autotomy, density and their interaction on body size in males using the generalized linear model (GLM) function in IBM SPSS Statistics 25, assuming a normal distribution. Next, we proceeded to use body size as a covariate in subsequent GLMs to separately examine the effects of developmental density and autotomy on relative testes mass, the remaining (*right*) hindlimb femur width, hindlimb muscle mass and hindlimb cuticular mass. Using body size as a covariate allowed us to tease apart how density affected investment in reproductive characters beyond effects simply due to larger or smaller overall body size.

Our models included body size as a continuous covariate, density and autotomy as fixed factors, and autotomy**density* and

autotomy*body size as two-way interactions. We planned three statistical contrasts (Abdi & Williams, 2010) for the measured traits, consistent with our predictions: (a) intact males raised at low versus high density, (b) intact versus autotomized males at high density and (c) intact versus autotomized males at low density.

3 | RESULTS

3.1 | Males raised at higher density became smaller adults

Male *Narnia femorata* raised at high density were 5.5% smaller in body size on average relative to those raised at low density (percentage calculated from back-transformed model output; Wald $\chi^2 = 8.381$, $p = 0.004$; Figure 2). The loss of a hindlimb during development did not have a measurable influence on mean adult body size (Wald $\chi^2 = 2.493$, $p = 0.114$; Figure 2), as expected based on previous findings (Joseph et al., 2018; Somjee, Miller, et al., 2018). The effect of density on body size did not differ if an individual experienced hindlimb loss (no statistically significant autotomy*density interaction; Wald $\chi^2 = 1.275$, $p = 0.259$). We used body size as a covariate in subsequent models, allowing us to examine the size of adult testes and hindlimbs and adjusting for differences in body size.

3.2 | Testes, but not weapons, grew larger at high versus. low density for the intact males

We first specifically examined intact males (those retaining all six limbs; black circles in Figure 3) for their testes and weapon investment at the two densities. We found that intact males had

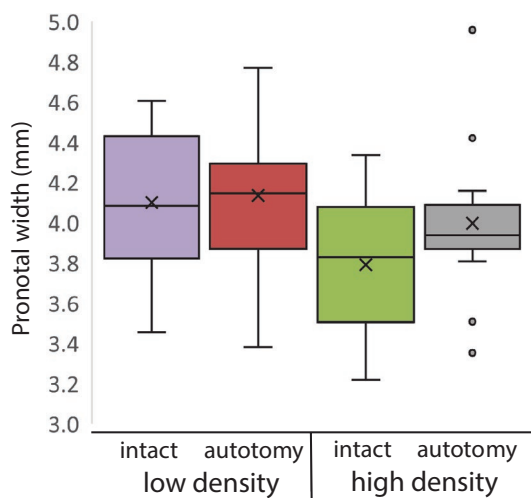


FIGURE 2 Male *Narnia femorata* raised at high density developed into adults with a 5.5% smaller mean body size relative to those raised at low density. Box plots highlight the minimum (not including outliers), first quartile, median, third quartile and maximum (not including outliers) for each set of values. Mean values are represented by an 'x' mark

on average 18.8% greater testes dry mass for their body size when they were raised at higher versus lower density (percentage calculated from back-transformed model output; contrast, $p = 0.031$; Figure 3a). Intact males exhibited no statistically significant effects of developmental density on features of the hindlimb (contrasts: hind femur width, $p = .092$; hindlimb cuticle mass, $p = 0.115$; hindlimb muscle mass, $p = 0.476$; Table 1; Figure 3b–d).

3.3 | Testes growth following autotomy only occurred when males were raised at low density

We next compared intact to autotomized males (black versus blue circles, Figure 3a) for testes dry mass in each of the rearing densities. We found that autotomized males raised at a low social density grew 39% heavier testes relative to intact males raised at this same density (percentage calculated from back-transformed model output; contrast, $p < 0.001$; Figure 3a). Intact males grew large testes when raised at a high social density (as described above). Autotomized males did not exhibit an increase in testes mass at high density relative to intact males (contrast, $p = 0.800$; Table 1; Figure 3a).

3.4 | Autotomy in low-density conditions led to increased investment in cuticle of the remaining hindlimb

Next, we compared intact to autotomized males for cuticular mass in the remaining (right) hindlimb. When males were raised in low densities, autotomized males developed 24.8% heavier hindlimb cuticle than intact males (percentage calculated from back-transformed model output; contrast, $p = 0.008$; Figure 3c). We did not see this pattern in the high-density context (contrast, $p = 0.214$; Table 1; Figure 3c). We also did not detect an effect of autotomy on the other hindlimb traits measured, hind femur width or hindlimb muscle mass, at either density (Table 1; Figure 3b,d). Although not statistically significant, the results for muscle mass echo the results from cuticular mass across autotomy and density treatments (Figure 3d). Finally, males in the high-density context showed an intriguing, but nonsignificant, trend of increased hind femur width with autotomy (Figure 3b).

3.5 | Weapon and testes growth in small males were disproportionately boosted by autotomy

We found that males of the smallest body sizes showed a disproportionate increase in both testes mass and hindlimb cuticular mass following autotomy of a hindlimb during development relative to larger males (autotomy * body size interaction; Table 1, Figure 4a,b).

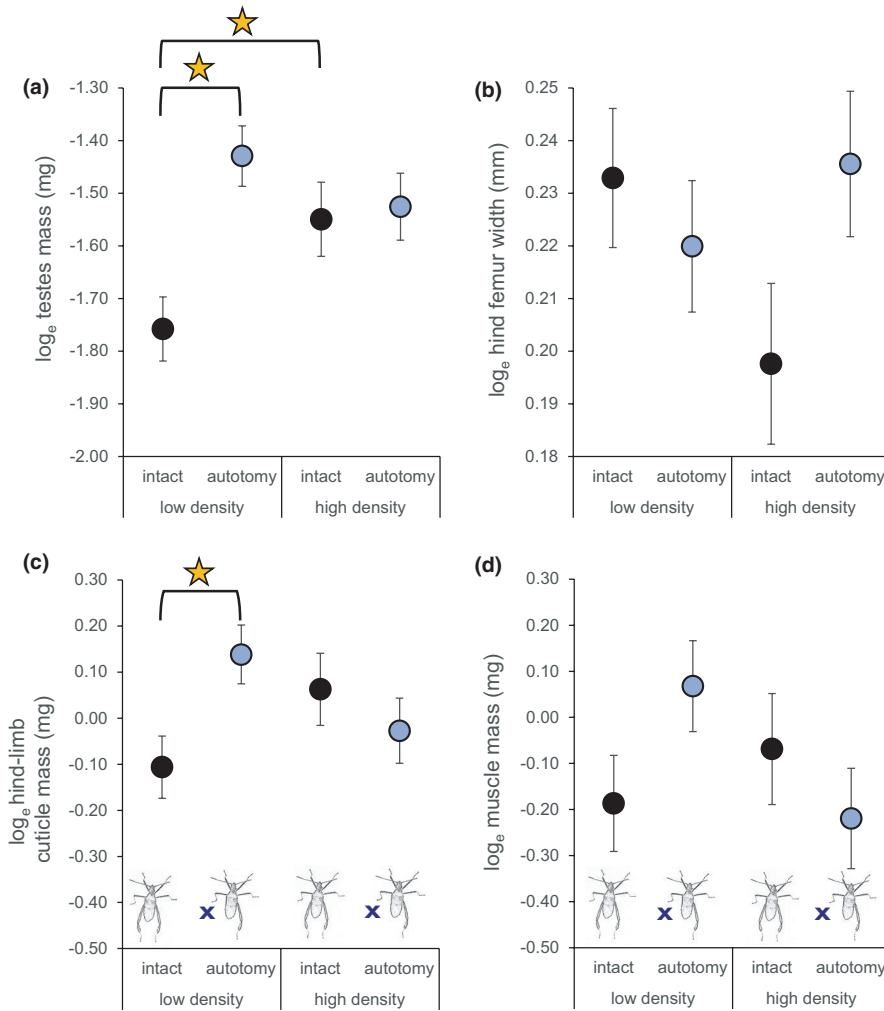


FIGURE 3 Intact males (black circles) had increased relative testes mass (a) when raised at a high density. Males raised at low density received a boost in both relative testes mass (a) and relative hindlimb cuticular mass (c) following developmental autotomy (blue circles). No detectable effect of density or autotomy was found on relative hind femur width (b) and relative muscle mass (d). Results depict investment in traits relative to body size (pronotal width), a covariate in our models. Estimated marginal means + SE. We used three planned statistical contrasts for each trait: (1) intact males raised at low versus high density, (2) intact versus autotomized males at high density, (3) intact versus autotomized males at low density. Stars and brackets indicate those planned contrasts with statistical significance ($p < 0.05$)

TABLE 1 Results of four GLM, all using the normal distribution and log-log transformation

Source	Testes mass (mg)		Hind femur width (mm)		Cuticular mass (mg)		Muscle mass (mg)	
	Wald χ^2	p	Wald χ^2	p	Wald χ^2	p	Wald χ^2	p
Body size	71.245	<0.001	247.944	<0.001	58.563	<0.001	28.606	<0.001
Autotomy	9.392	0.002	0.017	0.895	11.390	0.001	3.465	0.063
Density	0.750	0.386	0.489	0.484	0.001	0.977	0.582	0.446
Autotomy * Density	5.534	0.019	3.283	0.070	5.355	0.021	3.334	0.068
Autotomy * Body size	8.362	0.004	0.006	0.940	10.927	0.001	3.353	0.067

Note: $df = 1$.

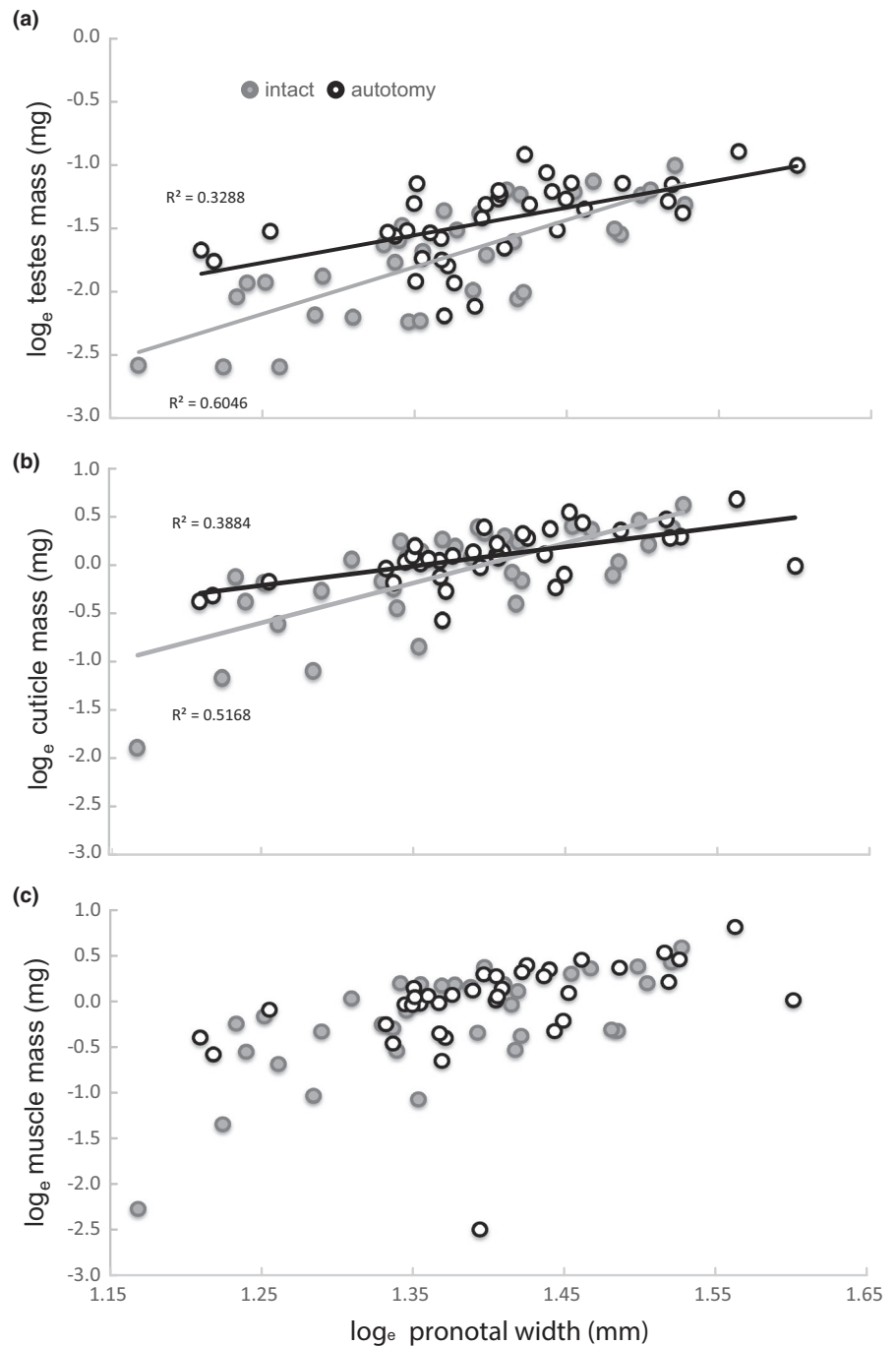
Bold text denotes statistical significance.

4 | DISCUSSION

We found pronounced effects of both social density and developmental weapon loss on reproductive allocation in male *Narnia femorata*. Males grew nearly 20% larger testes for a given body size when raised in high social densities. At low densities, we found that those that lost weapons during development grew 39% larger testes by

adulthood; a negative covariance between these traits provides evidence of a weapons–testes trade-off. At high social densities, males that dropped a hindlimb did not grow larger testes, perhaps because testes were already maximized at this density. These results underscore the importance of social density to resource allocation patterns within the individual. They also reveal that trade-offs between traits that are clearly seen in one context may not manifest in another.

FIGURE 4 Small males with developmental autotomy, relative to small males that remained intact, grew heavier testes (a) and hindlimb cuticle (b), though not hindlimb muscle mass (c) by adulthood



4.1 | Males raised at high density were smaller

Male *N. femorata* raised in high-density groups were 5.5% smaller than those raised in the low-density groups (Figure 2; 3.38 mm vs. 3.90 mm; 1.42 SD between the groups). Based on the standard deviation, this is akin to a height difference in human males of 178.4 cm versus 189.18 cm (5'10" vs. 6'3"). Insects in our study were raised either in a group of ten to eleven individuals or a group of three to four individuals, group sizes commonly seen in the wild (Allen & Miller, 2020; Cirino & Miller, 2017). The notable difference in body size suggests that access to good nutrition was limiting at the high

density even though numerous cactus fruit were provided. Some individuals may have been better able to achieve access to food and block others from regular access. These results on body size contrast with those from Allen and Miller (2020) where no mean difference in the body size of *N. femorata* was found across those that developed at different densities. However, Allen and Miller (2020) found that those late to emerge from within a group were smaller in size, an effect possibly related to feeding competition among siblings. In this study, we randomly sampled just one male per rearing container, and the effects of emergence order on testes size remain untested in this species.

4.2 | Higher density led to an increase in testes mass

According to Parker et al. (2013), male investment in testes and weapons should be related to the number of males competing for mating opportunities and the competitive advantage gained from expenditure on weapons. This model and others predict that as sperm competition risk increases, as would occur with increases in population density, relatively more resources should be allocated to testes (Parker & Pizzari, 2010). In line with these predictions, we found here that the average intact male *N. femorata* developed nearly 20% larger testes for a given body size when raised at a high density relative to low density (Table 1; Figure 3a). Other species including moths (Gage, 1995; Johnson et al., 2017) and crickets (Bailey et al., 2010) can also grow larger testes when raised in large groups or high density. Ejaculate size increases with social density across species, including crabs (Jivoff, 1997; Rondeau & Sainte-Marie, 2001), fish (Marconato & Shapiro, 1996) and rats (Bellis et al., 1990).

Although the cuticular mass of the hindlimbs trended upward with social density, we did not see this pattern with hind femur width nor muscle mass (Table 1; Figure 3b,d). Muscle inside the limbs of invertebrates is constrained in growth by the exoskeleton; thus, if the limb cavity is already completely full of muscle, an increase in muscle mass may not be possible. Importantly, the muscle measured here, primarily the muscle inside the hind femora, is not the only muscle involved in the use of the hindlimbs during physical contests. The muscle inside the femora controls primarily the flexing of the tibia-femoral joint, used when one male pinches another male between his tibia and femora, for example. Males in this species exhibit a variety of other behaviours during fights, including squeezing other males between the hind femur and the body wall, using muscles that reside within the coxa and the body cavity (Snodgrass, 1935). In this study, we attempted to measure weapon investment in a more detailed way than provided by most studies; indeed, it will be important in the future to include measurements of weapon investment that go beyond just the components of the outward, ostensible weapon (Okada et al., 2012).

4.3 | Phenotypic engineering: a trade-off between weapons and testes was not visible in high densities

By experimentally inducing some males to drop a hindlimb during development, and thus limiting weapon allocation (Embets et al., 2016), we were able to determine the extent to which the testes and other traits received increased investment under the two social densities. This method can provide inference into resource allocation trade-offs. We measured adult body size and allocation to the testes and components of the remaining hindlimb (Joseph et al., 2018). By considering both body size and social density in our statistical models, we were able to test effects of autotomy

and density when controlling for body size differences across the groups.

We found that males raised at low density were highly responsive to developmental autotomy; those with autotomy matured into adults with 39% greater relative testes dry mass than intact individuals (Table 1; Figure 3a). These results are in-line with previous studies (Joseph et al., 2018), though even more exaggerated. In contrast, males raised at high density had large testes across the board, without a detectable increase with autotomy (Figure 3a). Our findings suggest that testes allocation may already be maximized at high densities, as males raised in high-density conditions and experimentally blocked from investing in a hindlimb did not invest in their testes any further. This potential investment ceiling (Figure 3a) may reflect a developmental limit in testes size or may reveal that a further boost in testes mass is not advantageous to fitness. Regardless, the results reveal that the negative correlation between weapons and testes did not manifest for insects raised at high density.

Traits exist within the context of the whole organism, and so the presence and absence of visible trade-offs in one context or another are likely linked to increased or decreased investment in other, unmeasured traits (Zera & Harshman, 2001). A future step in this line of research would be to expand the view to see how social density affects additional life-history traits including somatic and behavioural traits related to survival and reproduction.

4.4 | Phenotypic engineering: the remaining hindlimb weapon grew more robust following autotomy, but only in low densities

Autotomy of a hindlimb might be expected to lead to increased investment in the opposite, remaining limb because of increased resource availability, similar developmental timing, and the increased dependence on this sole hindlimb for fighting and defence (Video S1). We found that autotomized males had heavier cuticle on the remaining hindlimb, though only for those males raised in a low density. We found no evidence that the other weapon characteristics measured, limb muscle mass and the width of the hind femur, increased following autotomy, though some of the nonsignificant trends were intriguing (Table 1; Figure 3b,d). Male *N. femorata* missing a hindlimb fight readily with other males, but frequently lose contests (Embets et al., 2018). Selection for increased damage resistance of the cuticle may be a result of the risk of injury that comes with engaging in fighting, but frequently losing. A heftier cuticle may protect the body and lower the risk of injury. Of course, the increased limb cuticular mass may also aid in locomotion; males missing a hindlimb may experience increased stress upon the limb that remains, leading to deposition of more cuticle. Further, because both limbs develop at the same time, the pattern we documented here may also be little more than a consequence of resources funnelling into the one remaining hindlimb following autotomy of the other. It is unclear why cuticular mass did not increase following autotomy in the high-density conditions (Figure 3c), though it may be linked to reduced resource acquisition

(Van Noordwijk & de Jong, 1986) or different developmental priorities. Additionally, cuticular mass, like testicular mass, may be maxed out for intact males at high density, unable to grow further upon autotomy.

4.5 | Phenotypic engineering: small males received a disproportionate increase in both testes mass and the skeletal component of the remaining weapon

We found that small males experienced a disproportionate increase in the testes and hindlimb cuticle following autotomy, relative to larger individuals (Table 1; Figure 4a,b). At first glance, the pattern suggests the possibility that small males may be stunted in body size to maximize their testes and hindlimb cuticle investment. Although this is possible, other results did not provide evidence of a trade-off between body size and these traits. For example, larger individuals had increased testes and hindlimb cuticle. Further, we did not find that autotomy led to smaller body size (Figure 2) even as testes increased substantially (Figure 3a). A trade-off between body size and reproductive traits may be limited to only the very smallest of individuals, if it does exist. The relatively larger boost following autotomy may instead be because the smaller males have poor overall body condition and trade-offs are expected to be more pronounced in poor-condition individuals (Van Noordwijk & de Jong, 1986). Additionally, the marginal fitness gains from an increase in testes and weapon components for tiny individuals may simply be higher than the gains experienced by larger individuals, leading to increased investment at a cost to traits not measured here.

4.6 | Results in the context of the wild animal

Leaf-footed bugs, a global family of over 2,500 species, use autotomy to escape unfavourable situations (Embets et al., 2016, 2017, 2020). Twelve per cent of adult male *N. femorata* in the wild are missing one or more limbs. The hindlimb weapon is the most commonly autotomized (Embets et al., 2016). Results from this and other species reveal that males that lose a hindlimb during development grow larger testes by adulthood (Joseph et al., 2018; Somjee, Miller, et al., 2018). Males may avoid other males by sneaking copulations (see Gross, 1996). Indeed, observations of *N. femorata* and other coreids from the wild and semi-natural enclosures reveal that dominant males patrol territories with multiple females but cannot always keep other males at bay (C. W. Miller, *personal observation*; U. Somjee, *personal observation*). Autotomized males produce more offspring than intact males in some situations (Joseph et al., 2018), and a mechanism for the increased testes mass and offspring production may be an increase in spermatogenesis (Cavender et al., *in press*). Thus, increased testes growth may be making the best of a bad, albeit common, situation. This increased testes growth following autotomy provides evidence for a pre- and post-copulatory trade-off

between investment in weapons and testes, and this conclusion is further supported by findings that larger testes increase offspring production, even in intact males, and that testes are composed of metabolically expensive tissues (Greenway et al., 2019). Costs in the context of the whole organism may be a reason that intact males limit testes growth in low-density situations. In this study, we found that intact males raised at high social densities grew larger testes that reached a possible maximum; males with autotomy at the high social densities did not increase testes mass further.

5 | CONCLUSIONS

Understanding the nature of trade-offs (Roff, 1992; Stearns, 1992), as well as their dependence on social conditions (Applebaum & Heifetz, 1999; Wilson et al., 2002), is vital to our knowledge of the evolutionary process. Indeed, trade-offs have played a central role in the development of life-history theory (Charnov & Krebs, 1974; Gadgil & Bossert, 1970; Schaffer, 1974a, 1974b). The costs of reproduction have generated great interest (Bell, 1980; Roff, 1992), and recent work has extended these ideas to testing trade-offs across pre- and post-copulatory reproductive traits in males (Simmons et al., 2017). We have learned in this study that the manifestation of trade-offs can be dependent upon social conditions during development. These results provide insights that should be broadly applicable beyond testes and weapon trade-offs. Future work on resource allocation should be mindful of the environmental context of the animal and view allocation decisions across multiple, relevant contexts whenever possible. After all, resource acquisition is well known to affect the manifestation of trade-offs (Reznick et al., 2000; Van Noordwijk & de Jong, 1986), and other factors, such as the social context, should as well.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

AUTHOR CONTRIBUTIONS

P.N.J. and C.W.M. conceived the ideas and designed methodology. P.N.J. and Z.E. collected the data. C.W.M. analysed the data. C.W.M. led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1111/jeb.13790>.

OPEN RESEARCH BADGES



This article has earned an Open Data Badge for making publicly available the digitally-shareable data necessary to reproduce the reported results. The data is available at <https://doi.org/10.5061/dryad.2z34tspm9>.

DATA AVAILABILITY STATEMENT

Data are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.2z34tspm9>

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SUPPORTING INFORMATION

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