



INVITED PAPER

Developmental Nutrition Affects the Structural Integrity of a Sexually Selected Weapon

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Synopsis Males in many species engage in physical combat over access to mates, and sexual selection has led to the evolution of weapons to enhance contest performance. The size of these often-elaborate structures is known to be exquisitely sensitive to nutrition. However, we know very little about the degree to which nutrition affects other attributes of animal weapons that can be crucial to fighting. In this study, we investigated the impact of natural dietary variation on weapon structural integrity in a fighting insect, *Narnia femorata* (Hemiptera: Coreidae). Males in this species display their enlarged, spiny hind legs to other males, and these legs serve as weapons in aggressive physical contests where they are used to strike and squeeze opponents. *N. femorata* feeds on the fruit of prickly pear cactus and sets up territories on this plant. In North Central Florida the prickly pear *Opuntia mesacantha* ssp. *lata* blooms and begins to produce fruits in April and May. *N. femorata* has multiple, overlapping generations while the green fruits slowly ripen over the next several months. We examined insects reaching adulthood at two nearby time points in this range, June and July, to test the influence of the nutrition provided by ripening green cactus fruit on weapon size and its ability to resist puncture. We also raised insects on cactus with red, ripe fruit for comparison. We found a striking effect of cactus fruit phenology on weapons. Insects raised with the more mature green fruit (those in the second cohort) had 71% larger weapon area and 4.4 times greater puncture resistance than those raised on the early green fruit (those in the first cohort). In contrast, insects raised on red, ripe fruit were moderate in size, had high puncture resistance, and they changed little phenotypically from the first to second cohort. Increased structural integrity of the hind femur weapon was associated with the increased body size that came with better nutrition. This pattern highlights that cuticle thickness increased or its material properties changed when weapons were larger. Importantly, effects of nutrition on puncture resistance also transcended size. Insects of the same size had greater structural integrity if they received superior nutrition. Sexually selected weapons are often used as visual signals to conspecifics before fights, and this work hints that the size of the weapons may be a poor signal of weapon performance when nutrition is variable.

Introduction

Sexually selected weapons have been favored by selection for their role in enhancing performance during battle, often leading to greater mating success (Andersson 1994; Emlen 2008). Iconic weapons include the branched antlers of elk, the bulky claws of fiddler crabs, and the pronounced horns of scarab beetles. Males may physically engage with weapons to push, pull, pry, strike, or squeeze opponents (Emlen

2014; Rico-Guevara and Hurme 2019). Larger weapons are often associated with greater competitive success, but success in contests is not determined by weapon size alone (Backwell et al. 2000; Lailvaux et al. 2009; McCullough et al. 2016). Many other factors contribute to competitive success (e.g., Kemp and Wiklund 2004; Judge et al. 2010; Kasumovic et al. 2010; Briffa and Lane 2017), and the ability of weapons to withstand damage may be one such factor. Indeed, physical contests

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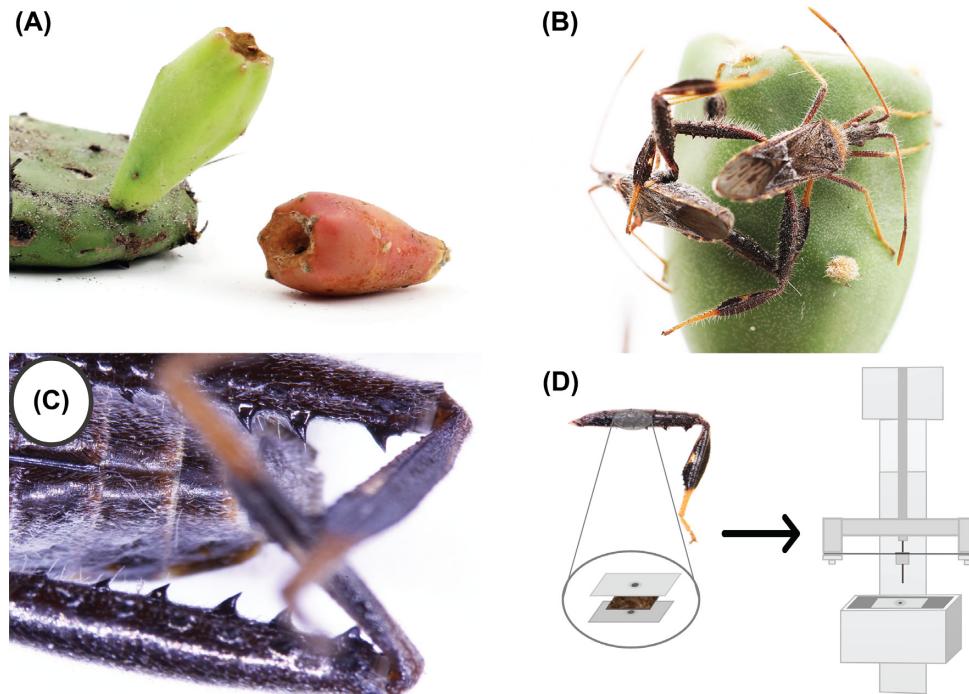


Fig. 1 (A) Seasonal differences in prickly pear cactus fruit, the food of *N. femorata*. On the left is a green, ripening fruit and on the right is a red, ripe fruit from the previous year. (B) Two males of *N. femorata* engaged in a fight. During the course of battle, males commonly press the spines of their hind femurs against the hind femurs of other males. (C) Ventral view of a male *N. femorata* highlighting the rows of sharp spines on the hind femurs. (D) Puncture resistance of the hind femur was estimated by removing a $\sim 2 \times 4$ mm rectangle of cuticle from the location shown, mounting it, and using a conically shaped pin for puncture resistance measurements. Photographs by Christine W. Miller. Illustration credits to E.V. (Ginny) Greenway.

are some of the most demanding and stressful activities performed by animals (Briffa and Sneddon 2007; Emlen 2014). These physical contests can take a toll. Up to 82% of male tule elk (Johnson et al. 2005), 50% of river shrimp (Rojas et al. 2012), and 25% of fiddler crabs (Jones 1980) show evidence of weapon damage or even complete weapon loss. Such high rates of injury are surprising because having a damaged or missing weapon can harm an individual's fighting ability and mating success (Espmark 1964; Lincoln 1972; O'Neill and Cobb 1979; Berzins and Caldwell 1983; Yasuda et al. 2011; Emberts et al. 2018). Thus, it is crucial to our understanding of weapon function and evolution to understand what impacts a weapon's susceptibility to physical injury, including its structural integrity.

Structural integrity is a term from the field of engineering that describes the ability of a structure to avoid failing due to fracture, deformation, or fatigue (Samuel and Weir 1999). One factor that likely has an impact on the structural integrity of animal weapons is nutrition. Good nutrition may allow a male to build a weapon with high structural integrity, while a diet lacking important nutrients may compromise weapon quality. Indeed, antler breakage in elk and other cervids is associated with dietary deficiencies of important min-

erals and amino acids (Johnson et al. 2007a; Landete-Castillejos et al. 2010; Ny et al. 2020). Further, nutrition has known roles in the construction of the insect cuticle (Neville 1975; Bernays 1982; Bernays and Woodhead 1982; Bernays et al. 1983) from which sexually selected weapons in insects are built (Emlen 2008). Amino acids, including phenylalanine and tyrosine, and phenols, such as gallic acid and various tannins, can be used in stabilizing the cuticle and providing rigidity (Bernays 1982; Bernays and Woodhead 1982; Bernays et al. 1983). Herbivorous insects often receive inadequate organic nitrogen from their host plants, and these effects can be exacerbated by seasonal change (Mattson 1980; Awmack and Leather 2002). Yet, virtually no experiments have addressed how nutrition impacts the ability of animal weapons to withstand damage.

Our specific objective here was to examine the influence of seasonal changes in nutrition on the structural integrity of an insect weapon. We used an herbivorous insect, the leaf-footed cactus bug, *Narnia femorata* (Hemiptera: Coreidae). Male *N. femorata* have weaponized hind legs with spiny cuticular extensions (Fig. 1). They use these robust limbs when competing with other males over access to territories on prickly pear cactus plants (Procter et al. 2012; Nolen et al. 2017).

Fighting behaviors are varied and include lunges, kicks, mounts, and end-to-end wrestling. During end-to-end wrestling, the hind legs reach posteriorly to squeeze the rival's body using quick muscular contractions, pressing the spines of the hind femur into the rival's hind femurs and ventral abdomen. Although many bodily surfaces can be contacted during an attack, our work here focused on the hind femurs because of their heavy involvement in male–male contests. Hind femurs are not only a weapon with an offensive function, but they also act as armor with a defensive role. Injuries following intensive male–male interactions can be severe and frequently include punctures, fractures, scrapes, tears, and indentations (E. V. Greenway and C. Hurd, unpublished data). Yet, engaging in battle may be worth the risks because males that win contests typically have enhanced access to reproductive females (Nolen et al. 2017; Emberts et al. 2018).

We capitalized upon natural nutrition, rather than artificial laboratory diets, to provide a real-world view of how weapon size and structural integrity can change seasonally in wild populations. Juvenile and adult *N. femorata* feed on the seasonally dynamic fruit of the prickly pear cactus (Cirino and Miller 2017). *Opuntia mesacantha* ssp. *lata* bloom within a narrow window in April and May in North Central Florida, and the green fruit ripens gradually over the next several months, finally maturing and turning deep red in the late summer and into the fall (Fig. 1). *N. femorata* have multiple overlapping generations during this time and thus each generation hatches to find fruit that is more mature (Cirino and Miller 2017). The specific nutrients that this insect requires have not been established, though studies have shown that young insects feeding upon red, ripe fruit grow into larger adults with larger testes and weapons (Miller et al. 2016; Sasson et al. 2016). These large, quantitative genetic studies took a coarse-grained approach to fruit phenology, comparing adult insect phenotypes after they fed on cactus with green, unripe fruit; red, ripe fruit; or no fruit at all. However, recent preliminary data has shown that even short-term changes in green fruit as it ripens can affect these insects (L. A. Cirino and D. A. Wilner, unpublished data). These preliminary data inspired the current study.

Here, we addressed the impact of cactus fruit ripening on weapon size and structural integrity. To accomplish this aim we measured hind femur size and puncture resistance for new, adult *N. femorata* that matured on living cactus in June, the “first cohort,” or July, the “second cohort.” During development, these insects received either a potted cactus pad with green, ripening fruit or a one with red, ripe fruit. The green fruit was attached to the cactus, allowing the fruit to mature and ripen in real time as our experiment progressed. Thus,

our second cohort of insects in this treatment experienced older green fruit that had ripened and matured relative to the early green fruit provided to the first cohort. Insects from the first and second cohorts that had not received the green fruit received red, ripe fruit instead. We anticipated the red fruit would provide a more stable resource across the cohorts because it was fully ripened and thus would provide a useful comparison.

We measured the size of the hind leg weapon and estimated structural integrity as the puncture resistance of this weapon at a biologically relevant location, one frequently contacted by the spines of opponents during fights (Fig. 1). Puncture in *N. femorata* is a severe form of structural failure, and yet it is not uncommon after intensive physical conflict (E. V. Greenway and C. Hurd, unpublished data). Our assumptions and predictions are as follows: based on preliminary data, we assumed that insects in the first cohort that received early-season green fruit would mature into the smallest adults seen in this study. We expected no phenotypic differences across cohorts for those raised on red, ripe fruit, given that these fruits were already fully matured. We predicted that insects raised on the red, ripe fruit (both cohorts) and on the older green fruit (later cohort) would have high structural integrity of their hind femurs, related to their increased size. We also predicted that we would see effects of nutrition that transcended size. In other words, with size held constant, we anticipated that those with good nutrition would achieve greater weapon structural integrity.

Materials and methods

The phenology of prickly pear cactus and *N. femorata*

Green, ripening prickly-pear fruit undergo profound internal changes that are not visible from the outside. The core of the fruit, where the seeds and pulp are located, increases rapidly in diameter starting at 50 days after flowering and continues to expand until 80 days (Barbera et al. 1992), bringing better nutrition increasingly closer to the surface of the fruit. With the core expansion is a concurrent decrease in fruit firmness, a decrease in crude fiber, a decrease in total protein, an increase in seed biomass, and an increase in sugar content (Barbera et al. 1992). *N. femorata* (Hemiptera: Coreidae) feed on prickly pear fruit throughout the year (Cirino and Miller 2017), reaching into the core of the fruit with their straw-like mouthparts (Allen and Miller 2017; Allen et al. 2021). When the fruit core is deeper within the fruit, *N. femorata* juveniles mature into adults with longer mouthparts relative to their body size (Cirino and Miller 2017). Thus, reaching the dynamic core of the fruit appears to be crucial and has

resulted in selection for mouthparts with developmental phenotypic plasticity (Allen et al. 2021). Even with the ability to grow longer or shorter mouthparts, *N. femorata* is still subject to various phenotypic effects related to the seasonal changes within cactus fruit (Miller et al. 2016; Sasson et al. 2016).

Animal and plant collection and husbandry

N. femorata in North Central Florida eats, fights, mates, and lays eggs on a prickly pear native to the area, *O. mesacantha* ssp. *lata*, from early spring through late fall. In captivity, we rear *N. femorata* on potted, growing cactus. We harvest cactus pads with or without attached fruit from nearby field sites, we place a single cactus pad within a plastic deli container with soil, and the cacti reside in the greenhouse or growth chambers as root growth begins. Ripe fruit are generally dropped from the pads during the winter months, and we store this fruit at 5°C until use. *N. femorata* reproduces readily in our rearing conditions, producing a steady stream of new individuals for observation and experimentation. The overlapping nature of generations in *N. femorata* does not result in clear cohorts in the laboratory. Thus, our use of the word “cohort” in this manuscript refers to a group of insects selected from a single time window.

Dietary manipulations

Insects for this study were drawn from a laboratory colony used for multiple experiments. We reared juveniles through their third instar in sibling groups of 5–12 individuals kept together in 32-oz clear plastic deli containers. Containers were placed in Percival incubators set at 28°C with a 14:10 light: dark cycle. These sibling groups were provided either (1) a fresh, potted cactus pad of *O. mesacantha* ssp. *lata* with an attached green, ripening fruit or (2) a fresh, potted cactus pad of *O. mesacantha* ssp. *lata* paired with a detached red, ripe fruit (Fig. 1).

When the juvenile insects reached their penultimate (fourth) instar, they were given their own container with the same nutritional treatment as assigned previously, and then moved to a greenhouse. We isolated them at this time to reduce sibling competition over feeding sites on the cactus fruit. We wished to focus on differences in nutritional quality across the treatments and minimize additional phenotypic variation known to be caused by social factors (Allen and Miller 2020; Miller et al. 2021).

Opuntia mesacantha ssp. *lata* in North Central Florida produces bright, yellow flowers in late April and early May. We noted the approximate window of cactus flowering, then selected a first cohort of new adults maturing fewer than 50 days after cactus flowering and a

second cohort maturing at least 3 weeks after the 50-day mark. To form our cohorts, we selected males randomly from available individuals. We settled upon 22 as our sample size due to the concurrent sampling needs of other projects. Our first cohort matured June 14–22 ($n = 11$), and our second cohort matured July 15–August 8 ($n = 11$). Thus, we created two cohorts (first and second) and used two distinct categories of fruit (green, ripening and red, ripe), resulting in a 2×2 experimental design.

Morphometrics and femur puncture resistance measurements

We cold-euthanized males 21–23 days after their final molt into adulthood and stored the samples at -18°C . We then removed the right hind leg from each euthanized male, gripping from the body and joint (coxa or trochanter) to prevent femur damage. To avoid thawing, this procedure was carried out with forceps inside an ice box. These hind legs were again stored at -18°C and subsequently transported to the University of Cambridge for puncture-resistance testing. Specimens were kept in a thermal freezer bag with multiple ice packs for a day (<24 h) during transportation. The other insect body parts remained at the University of Florida where we performed morphometric measurements.

We photographed each insect's body next to a micro ruler using a digital camera attached to a Leica M165 dissecting microscope. We measured body size via pronotum width, a good proxy for body size in *N. femorata* (Gillespie et al. 2014; Allen and Miller 2017) and a commonly used metric across the insects (e.g., Hunt and Simmons 2000; Rauter and Moore 2002; Martini et al. 2014). We also measured the femur area on the left hind leg as our metric of weapon size. Measurements of body size and femur area were performed with ImageJ, v.1.46 (Schneider et al. 2012). Morphological measurements were taken blind to treatment.

We examined structural integrity by simulating a leg spine from a rival male pressing against the femur of an opponent until the point of puncture. These measurements were performed using a custom-built penetrometer at the University of Cambridge. To simulate the spine, and ensure consistency across measurements, we used size 3 stainless steel Austerlitz insect pins to gauge puncture resistance. The tip of this conical pin was approximately hemispherical and comparable in tip radius to spines of *N. femorata*; we inspected each pin under a light microscope and excluded pins with irregular or damaged tips. We selected a portion of the anterior hind leg cuticle where we have often observed the spines being pressed during battle. This was also the flattest section of the hind femur, which facilitated

removal of a $\sim 2 \times 4$ mm sample. We used an aluminum sample holder with a central circular hole of 1.2 mm diameter to clamp the cuticle in place. This unit was then placed under the force tester in the penetrometer.

The penetrometer was used to estimate the force required to puncture the hind leg cuticle. This equipment included an insect pin mounted on a custom-built fiber-optic 1D-force transducer. The insect pin was held by a small set screw in a cylindrical holder 5 mm long with 1.2 mm inner diameter. The cylindrical holder was mounted in the center of a 65 mm \times 12 mm \times 0.15 mm (free length \times width \times thickness) steel beam fixed at both ends. On the middle of the back side of the beam we glued a small piece of reflective foil. We mounted a D12 fiber optic sensor (Philtex, INC., Annapolis, USA) above the beam and positioned it with a small 1-axis translation stage (Newport M-DS25). This sensor measured the distance between its tip and the reflective foil (and hence the deflection of the beam) as a voltage signal. We calibrated the sensor with weights to convert the voltage into a force in mN. The sensor operated in the linear range of its sensitive near field, with a standoff distance of 80 μ m between the sensor tip and the reflective foil target. The beam used had a spring constant of 20.9 kN/m, a resonance frequency of 530 Hz, and a noise level of ca. 0.3 mN. A linear actuator (Physik Instrumente M222.20) moved the force tester with attached insect pin into and out of the sample with a constant speed of 0.61 mm s⁻¹. A custom-built electronic control unit with a STM32F103C8T6 microcontroller was used to control actuator movement, respond to user input, and acquire force sensor signals with a 60 Hz sampling frequency. When the pin was moved into contact with the cuticle, the force transducer was deflected up to a maximum, where the cuticle fractured suddenly, and the force dropped. The movement was stopped manually when the maximum had been reached; examination of the samples after the experiments showed that the whole cuticle had been punctured. We recorded the difference between the maximum deflection and the baseline to calculate the puncture force. Puncture-resistance measurements were taken blind to treatment.

Analyses

To study the overall effects of cactus fruit phenology on pronotum width, hind femur area (weapon size) and puncture resistance, we used a Linear Model (LM). This model included the two factors “fruit category” (green, ripening vs. red, ripe), and “cohort” (first vs. second) as well as their interaction. Following significant interactions, we used independent *t*-tests to examine differences between conditions within each level of the other factor.

Next, we examined how hind femur area increased with body size (pronotum width) using standardized major axis (SMA) regression, where the four treatment conditions (2 fruit categories \times 2 cohorts) were included as single categorical factor (as required for SMA regression). SMA was used instead of ordinary least squares as both variables were measured with error; SMA is symmetric and produces the same result regardless of which variable is *X* and which is *Y*.

To study possible body size-independent effects of fruit category and cohort on hind femur area, we then performed a two-way ANCOVA with body size (pronotum width) as a covariate, and fruit category and cohort as categorical factors. For this analysis we used Type I sums of squares to consider the effect of body size before the other factors; nonsignificant interactions were removed to produce a minimal model.

We subsequently examined how puncture resistance increased with body size using SMA as before, but with hind femur area instead of pronotum width. We took this approach because insect cuticle thickness, and thus puncture resistance, is expected to increase with the size of the structure (e.g., Peeters et al. 2017), and we also wished to evaluate if the area of the hind femur serves as a reliable predictor of puncture resistance.

Finally, to test whether puncture resistance is influenced by possible size-independent effects of fruit category and cohort, we again performed an ANCOVA with hind femur area as covariate and Type I sums of squares. All morphological and force measurements were log-transformed to meet model assumptions. Analyses were conducted in R v. 4.0.1 (RCoreTeam 2020), using the *smatr* package to carry out SMA regression and statistical tests for the slope (Warton et al. 2012). Data will be deposited in DRYAD upon acceptance.

Results

We found that developmental nutrition had a pronounced effect on the attainment of adult male body size and the size of the hind femur weapon in *N. femorata* (Fig. 2A). Our 2 \times 2 design of fruit category (green, ripening or red, ripe) and cohort (first cohort or second cohort) revealed a much larger across-cohort change for those from the green, ripening fruit than for those raised on the red, ripe fruit (two-way ANOVA of pronotum width, interaction category*cohort, $F_{1,18} = 18.5$, $P < 0.001$; hind femur area, interaction category*cohort, $F_{1,18} = 30.0$, $P < 0.001$; Fig. 2A). Males raised on green, ripening fruit had a 27% larger pronotum and a 71% larger hind femur area when they emerged in the second cohort than when they emerged in the first (pronotum width: 4.43 ± 0.39 mm vs. 3.49 ± 0.25 mm, *t*-test:

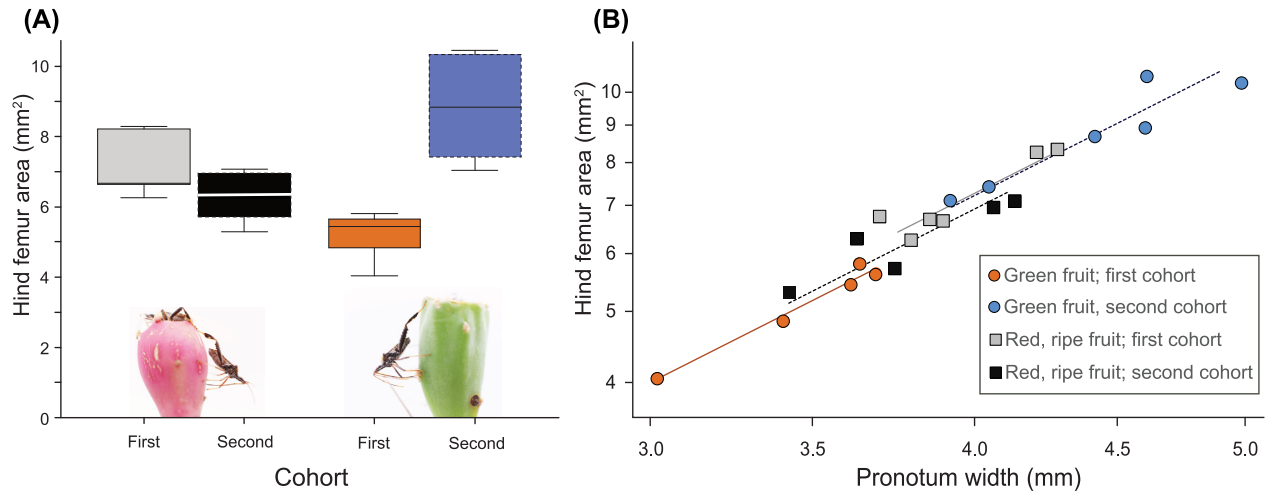


Fig. 2 (A) Male *N. femorata* emerging on green, ripening fruit in the second cohort (blue) were larger and had 71% greater hind femur area than those males that emerged in the first (orange). Males emerging on red, ripe fruit were similar in size across the cohorts. Plot shows medians (center lines), interquartile ranges (boxes), and the largest and smallest values (whiskers); we had no outliers. (B) Males emerging in the second cohort on the green, ripening fruit expressed larger femurs for a given pronotum width (body size) than those emerging in the first on the green, ripening fruit. The straight lines show SMA regression fits with a common slope. Color codings and line formats are shared in (A) and (B).

Table 1 Two-way ANCOVA with Type I sums of squares testing the effects of body size (pronotum width), cactus fruit phenology (category and cohort), and interactions on the size of the hind femur weapon. Interactions that were not statistically significant were sequentially removed from the model

	df	Sum sq	Mean sq	F-value	P-value
Log (pronotum width)	1	0.2118	0.2118	466.4	<0.001
Fruit category (red, ripe vs. green, ripening)	1	4.6e-4	4.6e-4	1.02	0.327
Cohort (first vs. second)	1	3.0e-6	3.0e-6	5.6e-3	0.941
Category*cohort	1	3.8e-3	3.8e-3	8.37	0.010
Residuals	17	7.72e-3	4.54e-4		

$t_9 = 4.63$, $P = 0.0012$; hind femur area: 8.82 ± 1.43 mm² vs. 5.15 ± 0.72 mm²; means \pm SD, t -test: $t_9 = 5.18$, $P < 0.001$; Fig. 2A). In contrast, on the red, ripe fruit the phenotypic differences between adults from different cohorts were negligible (pronotum width: $t_9 = 0.96$, $P = 0.36$; hind femur area: $t_9 = 1.69$, $P = 0.13$; Fig. 2A).

We next examined the effects of cactus fruit phenology on the size of the hind leg weapon relative to body size. Consistent with isometry within each treatment group, hind femur area scaled with the square of pronotum width across all fruit phenology treatments (no significant difference in slope, $\chi^2 = 0.68$, $df = 3$, $P = 0.88$; common SMA scaling coefficient: 1.94, 95% CI: [1.60, 2.30]; Fig. 2B). With no difference in slope, we were then able to examine changes in scaling intercept or elevation. We found that those raised on green, ripening fruit had a higher scaling intercept in the second cohort than in the first, a pattern not seen for those raised on the red, ripe fruit (two-way ANCOVA, interaction

category*cohort, $F_{1,17} = 8.37$, $P = 0.01$; Table 1). In other words, those raised in the second cohort on green, ripening fruit had larger leg weapons at any given body size; the legs were disproportionately large. Adjusted for pronotum size, the weapons of males emerged in the second cohort were 12% larger than those raised on the same diet, but that emerged in the first cohort (back-transformed adjusted means: 7.14 mm² vs. 6.37 mm²; Fig. 2B; Table 2). Males raised on red, ripe fruit varied less between cohorts and showed an opposite trend (Fig. 2B; adjusted means in Table 2).

We discovered that developmental nutrition affected the puncture resistance of the hind femur (Fig. 3A). We detected a pronounced across-cohort change for males raised on the green, ripening fruit than for those raised on the red, ripe fruit (two-way ANOVA of puncture resistance, interaction category*cohort, $F_{1,18} = 31.2$, $P < 0.001$; Fig. 3A). Overall puncture resistance for those raised on the green, ripening fruit was 4.4 times greater for those emerged in the second cohort,

Table 2 Back-transformed adjusted means of the two-way ANCOVA with Type I sums of squares testing the effects of pronotum width, fruit category, and insect cohort on hind femur area

Fruit category	Cohort	Adjusted mean (mm ²)	95% CI	Contrast
Red, ripe	First	7.06	[6.78, 7.38]	$t_{17} = 1.883, P = 0.077$
	Second	6.67	[6.37, 7.00]	
Green, ripening	First	6.37	[5.98, 6.78]	$t_{17} = 2.47, P = 0.025$
	Second	7.14	[6.76, 7.57]	

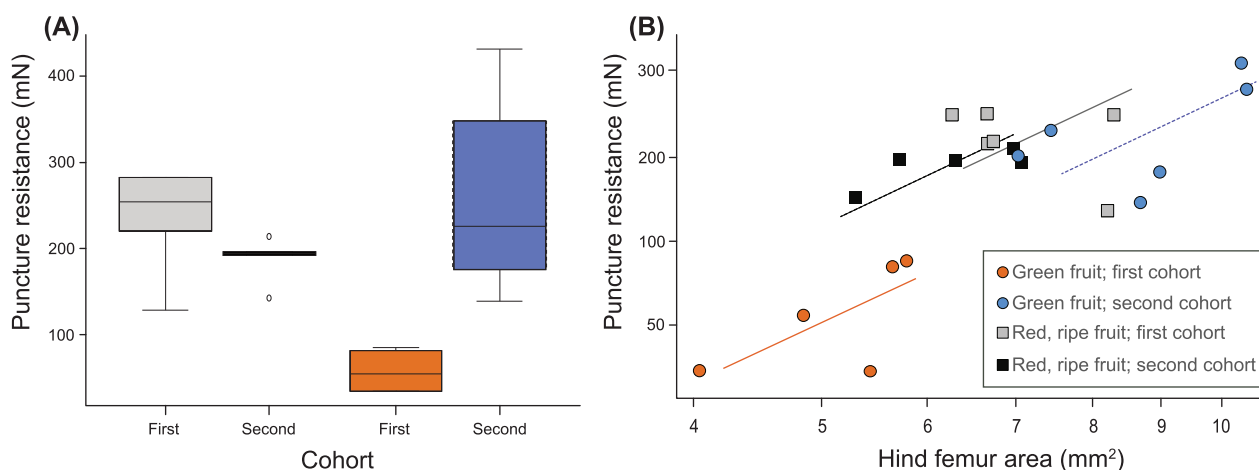


Fig. 3 (A) When raised on green, ripening fruit, males that emerged as adults in the second cohort (blue) had a 4.45 times greater puncture resistance than those emerged in the first (orange). In contrast, males raised on red, ripe fruit (grey and black) showed a slight (26%) decrease in puncture resistance from the first to second cohort. Plot shows medians (center lines), interquartile ranges (boxes), and the largest and smallest values (whiskers) that are not outliers; outliers are shown as circles. **(B)** The different line elevations reveal that the differences in puncture resistance were not fully explained by differences in structural size. Indeed, males emerging on red, ripe fruit had the greatest puncture resistance for a given hind femur size. Further, males emerging on green, ripening fruit in the second cohort had hind femurs with greater puncture resistance for their size than males emerging on green, ripening fruit in the first cohort. Puncture resistance scaled positively with hind femur area (lines show SMA regressions with a common slope on log-transformed data). Color codings and line formats are shared in **(A)** and **(B)**.

compared to those emerged in the first (raw means \pm SD: 257.8 ± 112.1 mN vs. 58.0 ± 24.6 mN, t -test: $t_9 = 3.88, P = 0.004$; Fig. 3). In contrast, the puncture resistance of new adult males fed red, ripe fruit varied less and was 21% smaller for those emerged in July compared to those emerged in June (t -test: $t_9 = 1.67, P = 0.13$; Fig. 3A).

Finally, we found that variation in puncture resistance related to nutrition were largely, but not completely, explained by variation in hind femur size. Puncture resistance strongly increased as the size of the hind femur increased (Fig. 3B; Table 3). The common SMA scaling coefficient of puncture resistance with pronotum width was 4.24 (95% CI [2.38, 7.54] with no significant difference in slope, $\chi^2 = 2.15, df = 3, P = 0.54$). This high scaling coefficient of puncture resistance suggests either a positive allometry of cuticle thickness, or (if cuticle thickness increases isometrically) that puncture force increases with cuticle thickness raised to a power > 1 .

The effects of nutrition on puncture resistance were not mediated only by size. We found that despite uniform slopes, the elevations of the SMA regressions differed significantly (Wald $\chi^2 = 18.88, df = 3; P < 0.001$; Fig. 3B). The size-corrected effect of cohort on puncture resistance was again greater for males raised on green, ripening fruit than for those raised on red, ripe fruit (two-way ANCOVA, interaction category*cohort, $F_{1,17} = 5.17, P = 0.036$; Table 3). Adjusted for size differences, the puncture resistance for those that emerged on the green, ripening fruit was 2.75 times greater for those from the second cohort, compared to those from green, ripening fruit in the first cohort (back-transformed adjusted means: 195.0 mN vs. 70.8 mN; Fig. 3; Table 4). Males raised on red, ripe fruit had greater puncture resistance and varied less between cohorts (Fig. 3B; see adjusted means in Table 4). Thus, not only is there a clear indirect effect of nutrition on puncture resistance mediated by the size of the animal, but also a direct effect that transcended size.

Table 3 Two-way ANCOVA with Type I sums of squares testing the effects of weapon size (hind femur area), fruit category, insect cohort, and the interaction category*cohort on puncture resistance. As slopes were uniform in the SMA regression (Fig. 3B, and see the text), interactions with log (hind femur area) were not included in the model

	df	Sum sq	Mean sq	F-value	P-value
Log (hind femur area)	1	1.006	1.006	46.5	<0.001
Fruit category (red, ripe vs. green, ripening)	1	0.364	0.364	16.8	<0.001
Cohort (first vs. second)	1	0.050	0.050	2.32	0.146
Category*cohort	1	0.112	0.112	5.17	0.036
Residuals	17	0.368	0.022		

Table 4 Back-transformed adjusted means of the two-way ANCOVA with Type I sums of squares testing the effects of weapon size (hind femur area) and fruit category and insect cohort on puncture resistance

Fruit category	Cohort	Adjusted mean (mN)	95% CI	Contrast
Red, ripe	First	223.9	[166.0, 302.0]	$t_{17} = 0.397, P = 0.696$
	Second	204.2	[144.5, 288.4]	
Green, ripening	First	70.8	[43.7, 117.5]	$t_{17} = 2.71, P = 0.015$
	Second	195.0	[128.8, 288.4]	

Discussion

Nutrition is a notoriously dynamic and variable environmental factor. It is well known to have a powerful influence on weapon size across taxa (Emlen and Nijhout 2000), and the size of sexually selected traits are often more sensitive to nutrition than many other traits (David et al. 2000; Bonduriansky 2007; Emlen et al. 2012). Here, we provide experimental evidence that developmental nutrition also can have a substantial impact on the structural integrity of sexually selected weapons. Male *N. femorata* emerging on green, ripening cactus fruit had femurs with 4.4 times higher puncture resistance in July compared to those from June. We also found that structural integrity was greater for larger insect weapons, suggesting an increase in cuticle thickness and/or modified construction with increased size. Importantly, we also found effects of good nutrition on structural integrity that transcended size. These results illustrate that the size of a structure is not always a clear indicator of its performance.

In this study, we raised two cohorts of *N. femorata* on living cactus. Some males were provided cactus with attached green, ripening fruit. This fruit was ripening in real time as the experiment progressed. Thus, males from the first cohort experienced younger and less-mature cactus fruit than males in the second cohort, and we compared the phenotypes of males in these cohorts. Importantly, however, many factors change seasonally, not just food. Thus, insects emerging later in the season could be phenotypically different due to other environmental factors. Thus, we designed this experi-

ment where the two cohorts of insects raised on green, ripening fruit were compared with insects raised at the same time, but with red, ripe fruit, a more stable source of nutrition. The intent of this design was to provide stronger inference on the role of dynamic nutrition in phenotypic expression. Indeed, insects raised on the green, ripening fruit revealed substantial phenotypic differences from the first to second cohort, while those raised on red, ripe fruit did not. These results reveal that even a short-term change in ripening green cactus fruit (1 month out of a 4- to 6-month ripening period) can make a substantial difference for the insects relying upon it for development.

Weapon size

Work on the leaf-footed bug family (Hemiptera: Coreidae) has shown that body and weapon size are greatly affected by nutrition (Miller and Emlen 2010; Somjee et al. 2015; Miller et al. 2016; Sasson et al. 2016; Allen and Miller 2017). Our results here further support this conclusion. We found that the body size of *N. femorata* and the two-dimensional area of their hind femur weapons were highly sensitive to developmental nutrition (Fig. 2). The largest individuals in this study were those from the second (July) cohort that emerged on the older green cactus fruit and the smallest were those from the first (June) cohort that emerged on the younger green fruit (Fig. 2A).

We also examined the effect of nutrition on the expression of weapon size relative to body size. We found that males raised in the second cohort on the more

mature green fruit had larger weapons relative to their body size than those insects raised in the first cohort on the earlier green fruit. Males raised on red, ripe fruit varied less across cohorts in relative weapon size (Fig. 2B; Table 1). These results provide support for nutrition being responsible for the degree to which males invest in weapon size relative to body size. Sexually selected traits often have heightened condition dependence and as a result they can be more responsive to nutrition than other traits (Cotton et al. 2004; Bonduriansky 2007). An elevated scaling intercept can be a result of this phenomenon, where males with excellent nutrition express larger weapons for a given body size (Miller and Emlen 2010).

It is well established that body and weapon size are important to fitness (Roff 1992; Stearns 1992; Emlen and Nijhout 2000; Emlen 2008; Emlen 2014). In *N. femorata*, increased size is linked to greater success in male–male contests (Procter et al. 2012; Nolen et al. 2017; Emberts et al. 2018) and in mating (Gillespie et al. 2014). Yet not all males can achieve large size. Seasonal changes in the quality and availability of cactus fruit, as well as spatial variation in cactus fruit availability across the landscape, translate into differences in the size of males in the wild (Cirino and Miller 2017). Here, we detected for the first time in this species that feeding upon immature green fruit from early in the year leads to small, stunted adult males while slightly older green fruit is an outstanding resource for these insects (Figs. 2 and 3). To the human eye, green, ripening cactus fruit in *O. mesacantha* does not appear to change much over time, and yet the effects on insect phenotypes tell quite a different story. Given the results seen here, it is fascinating that the insects begin breeding early in the season, leaving an entire generation to suffer reduced body and weapon size.

Weapon structural integrity

The major goal in this study was to assess the effects of nutrition on the structural integrity of a sexually selected animal weapon. We predicted that insects raised on the red, ripe fruit and on the older green fruit would have higher structural integrity of their weapons than those raised on the early green fruit. Our results confirm this prediction. Nutrition had a large impact on the structural integrity of the sexually selected weapon of *N. femorata*. In fact, we found an astonishing range in the puncture resistance of the cuticle at the location measured, from 34 mN for a June-cohort male to 433 mN for a July-cohort male, a 12.7-fold difference (Fig. 3). The difference is so pronounced that some early-cohort males feel noticeably more compressible during human handling (C. W. Miller, personal obser-

vation). On average, males emerging on green, ripening cactus fruit had femurs with 4.4 times higher puncture resistance in July compared to those from June (Fig. 3A).

Our results revealed that larger weapons have greater structural integrity (Fig. 3B). This strong, positive relationship between size and structural integrity shows one advantage that large males may have in competition—they are more damage resistant. Most likely the greater structural integrity is due to the cuticle being thicker when weapons are larger. However, it is also possible that material properties (such as strength and toughness, which are related to the composition of the cuticle and the arrangement of its components) may be enhanced for the larger insects.

Importantly, we found effects of good nutrition on structural integrity that transcended size. In other words, even though structural integrity was greater for larger animals, that does not tell the whole story. Once we controlled for size in this study, we found that males on green, ripening fruit had femurs with 2.75 times greater puncture resistance in July compared to those from June (Fig. 3B). Thus, size is not a perfect predictor of structural integrity; our results highlight that excellent nutrition can boost a male's performance above his same-size peers. These results could have serious implications. They suggest, for example, possible consequences for honest-signaling theory (Searcy and Nowicki 2005).

According to honest-signaling theory, many weapons have evolved an assessment function where they can be effective at revealing even subtle differences among males in their size, status, or physical condition (Emlen 2008). Thus, rather than immediately escalating to physical fighting, animals often begin with the use of signals. Visual signals used in male–male competition include exhibiting antlers through parallel walking in fallow and red deer (Clutton-Brock and Albon 1979; Alvarez 1993), extension of a colorful dewlap in anoles (Nicholson et al. 2007), flashing wing iridescence in dragonflies (Guillermo-Ferreira et al. 2019), and lifting and display of the hind leg in *N. femorata* (see supplementary video in Miller et al. 2021). Decades of empirical research (reviewed by Searcy and Nowicki 2005; Bradbury and Vehrencamp 2011) and pioneering theoretical work (Zahavi 1975; Enquist 1985; Grafen 1990; Smith 1991) have provided an explanation of why these signals can be evolutionary stable. While signals only need to be honest on average to be maintained (Johnstone and Grafen 1993), our work reveals that changes in nutrition can lead to size-independent effects and hence may increase the unreliability of the signal, depending upon the perceptive abilities of the receiver. The results we have shared here show that the

role of nutrition in signal honesty may be an exciting direction for future work.

Possible consequences of variation in the structural integrity of weapons

Differences in the structural integrity of male weapons may have large consequences for fitness. Fighting is one of the most physically demanding activities performed by animals (Briffa and Sneddon 2007; Emlen 2014), and it can lead to broken weapons (e.g., Johnson et al. 2005; Rojas et al. 2012; Jones et al. 2018). Once large cervid males have incurred antler damage, they become less effective at fighting, lose social rank, and are unable to secure females for breeding (Espmark 1964; Lincoln 1972; but, see Johnson et al. 2007b; Jennings et al. 2017). We do not yet know the full consequences of a weak weapon in *N. femorata*. However, leg injury may lead to the damaged leg being dropped (Embets et al. 2016; Embets et al. 2017). Males that have dropped a hind-leg weapon become subordinate in male–male interactions and have decreased mating success (Embets et al. 2018). Further, less puncture-resistant cuticle in *N. femorata* may not only result in injuries, but also in reduced offensive fighting ability. Males with a weak, flexible weapon may have an inability to produce sufficient offensive force while kicking or squeezing an opponent. Behavioral responses are also possible, where males with weak cuticle avoid contests or concede sooner to avoid damage.

An evolutionary perspective on why the structural integrity of weapons should vary

Weapon failure appears paradoxical when considering the evolutionary history of these traits. Weapons are first and foremost fighting structures with clear links to fitness (McCullough et al. 2016). Given the negative reproductive consequences of inferior weapons, sexual selection should be strong for robust and unbreakable weapons. Thus, it may seem surprising that weapons were so sensitive to routine changes in nutrition in this study and that weapon damage is readily observed across taxa. To understand this broad phenomenon, it is helpful to note that traits with a history of strong directional selection should lose genetic variation over time (Falconer and Mackay 1996). Consequently, the variation among individuals should largely reflect variation in environmental factors rather than genetic differences. Thus, it is to be expected that the expression of vital sexually selected traits should be influenced by nutrition. Further, the impacts of nutritional variation on weapons are expected to be exaggerated because fitness-related traits are expected to evolve heightened condition dependence (Bonduriansky 2007). Heightened

condition dependence is a type of developmental plasticity that couples the degree of trait expression to the quantity of metabolic resources available to the individual, optimizing the trade-off between viability and reproduction (McAlpine 1979; Andersson 1982; Nur and Hasson 1984; Andersson 1986). Indeed, the size of sexually selected traits is typically more sensitive to developmental variation in nutrition than other body parts (Cotton et al. 2004; Bonduriansky 2007). A next step in this line of research is to compare the effects of nutrition on the structural integrity of the weapon relative to other parts of the body.

The relevance for *N. femorata* in its seasonal context

In April, before the cacti flower, overwintered female *N. femorata* lay eggs and the eggs hatch approximately 2 weeks later. This insect has a 2-month generation time. Because *O. mesacantha* ssp. *lata* flower only once per year, subsequent generations develop on a resource that is different. Flowers yield green fruit that slowly matures over the next several months, becoming red, ripe cactus fruit by the summer or fall. The results here reveal that even short-term changes in the ripening of green cactus fruit leads to striking phenotypic effects on *N. femorata*. Males from the first cohort matured into small adults with disproportionately small hind leg weapons. Moreover, their weapons had low structural integrity, even for their small size. Indeed, it is possible that the sexual selection dynamics are quite different for the first generation to emerge each year. The first males to emerge are likely all small and weak, and it is unknown whether they avoid fighting or even fight readily given that they are in similar physical condition. Yet, males become progressively larger with more robust weapons over the time-period we considered. Thus, those males that are the first to emerge may only have a short window of time before they are displaced by the larger, robust males. These small males could be especially aggressive, investing heavily in reproduction early and while they can achieve it, a “live hard, die young” life-history approach. Preliminary research findings suggest that extreme physical injury can occur from fights in *N. femorata*, suggesting that small males may be taking a sizable risk in engaging with the larger males. Yet, Procter et al. (2012) found that small males occasionally become dominant over larger males. Sexual selection can fluctuate due to a myriad of environmental factors (Miller and Svensson 2014), and future studies in this species should track changes in sexual selection over time. *N. femorata* provides excellent opportunities to investigate the role of environmental dynamics in the processes and outcomes of sexual selection.

Conclusions

Within-species variation in the performance of fitness-related traits is likely ubiquitous and essential for evolutionary understanding. Recent decades of research have demonstrated that the size of sexually selected traits is exquisitely sensitive to environmental factors such as nutrition. Here, we provide some of the first experimental evidence that the structural integrity of sexually selected weapons can be influenced by nutrition, and consequentially that weapon size as a predictor of performance may become less reliable with variable nutrition. Our findings suggest that weapons that appear externally the same may perform very differently in battle.

Authors' contributions

C.W.M., Z.E., S.C., D.W., and W.F. designed the study; D.W. reared the study subjects and collected morphological information; S.C. built the penetrometer; S.C. and W.F. provided direction on the puncture-resistance assays and hosted Z.E.; Z.E. performed the assays for puncture resistance; W.F., C.W.M., S.C., Z.E., and T.E.W. analyzed the data; C.W.M. wrote the manuscript with substantial input from W.F., D.W., T.E.W., S.C., and Z.E.

Supplementary data

Supplementary data available at [ICB](#) online.

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Data availability

The data underlying this article are available as an online supplementary file.

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