

The tale of the shrinking weapon: seasonal changes in nutrition affect weapon size and sexual dimorphism, but not contemporary evolution

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Abstract

Sexually selected traits are often highly variable in size within populations due to their close link with the physical condition of individuals. Nutrition has a large impact on physical condition, and thus, any seasonal changes in nutritional quality are predicted to alter the average size of sexually selected traits as well as the degree of sexual dimorphism in populations. However, although traits affected by mate choice are well studied, we have a surprising lack of knowledge of how natural variation in nutrition affects the expression of sexually selected weapons and sexual dimorphism. Further, few studies explicitly test for differences in the heritability and mean-scaled evolvability of sexually selected traits across conditions. We studied *Narnia femorata* (Hemiptera: Coreiidae), an insect where males use their hind legs as weapons and the femurs are enlarged, to understand the extent to which weapon expression, sexual dimorphism and evolvability change across the actual range of nutrition available in the wild. We found that insects raised on a poor diet (cactus without fruit) are nearly monomorphic, whereas those raised on a high-quality diet (cactus with ripe fruit) are distinctly sexually dimorphic via the expression of large hind leg weapons in males. Contrary to our expectations, we found little evidence of a potential for evolutionary change for any trait measured. Thus, although we show weapons are highly condition dependent, and changes in weapon expression and dimorphism could alter evolutionary dynamics, our populations are unlikely to experience further evolutionary changes under current conditions.

Introduction

Males in tens of thousands of species physically engage with each other in contests over access to females, a phenomenon that has attracted human curiosity for centuries. Indeed, Darwin (1871, p. 259) wrote, 'It is certain that with almost all animals there is a struggle between the males for the possession of the female. This fact is so notorious that it would be superfluous to give instances'. In many species, males have evolved

weapons, such as antlers, spurs or fangs that function during battles (Emlen, 2014). The exaggeration and performance of these weapons influence success in male–male contests and provide a mating and reproductive advantage across diverse species (Emlen, 2008). In spite of the advantages of expressing large weapons, weapon size is highly variable within populations (Otte & Stayman, 1979; Emlen & Nijhout, 2000). The variability may be due to costs associated with growing or maintaining an exaggerated weapon (Allen & Levinton, 2007; Goyens *et al.*, 2015); only males in the very best condition may have the excess resources to invest in these costly traits. Although all traits are condition dependent to some degree, sexually selected traits are expected to evolve heightened condition dependence (McAlpine, 1979; Andersson, 1982, 1986; Nur &

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Hasson, 1984; Rowe & Houle, 1996; Punzalan *et al.*, 2008). Heightened condition dependence has been examined in sexually selected traits that influence mate choice (Cotton *et al.*, 2004), but it has not been well tested for sexually selected weapons that are used in male–male contests.

Nutrition can powerfully and fundamentally affect both the physical condition of animals and the traits that are condition dependent. Many organisms grow and develop in habitats where nutritional quality is not constant (Feeny, 1970; Awmack & Leather, 2002). Nutritional quality may be seasonal or stochastic, and in either case, the frequency of males with large weapons is predicted to change. Intriguingly, such nutritional shifts should also change the degree of sexual dimorphism in a population. Weapon homologues in females are not expected to evolve heightened condition dependence, and as a result, better nutrition should lead to greater differences between males and females (Bonduriansky, 2007a; Stillwell *et al.*, 2010; Cassidy *et al.*, 2014). Although nutrition is well known to have important phenotypic effects, surprisingly few studies have experimentally tested the condition dependence of traits for animals fed natural diets (Cotton *et al.*, 2004; Stillwell *et al.*, 2010), an approach we use and advocate for here.

Changes in weapon expression and sexual dimorphism in natural populations are exciting because they may alter the dynamics of sexual selection (Steele *et al.*, 2011; Siepielski *et al.*, 2013). For example, stabilizing selection for an optimal male phenotype can change to directional selection and back again as the mean trait size in a population shifts (Steele *et al.*, 2011). In any case, the response to selection will depend on the extent that the traits have sufficient genetic variation; that is, they are evolvable (Lynch & Walsh, 1998). The weapons of sexual competition exhibit astonishing diversity across species (Emlen, 2008), suggesting these traits may have high genetic variation. Indeed, some empirical studies, though not all, have found evidence of genetic variation in sexually selected ornaments and weapons (Houle, 1992; Pomiankowski & Møller, 1995; Prokuda & Roff, 2014). Intriguingly, traits may reveal genetic variation in some environments, but not in others. For example, raising animals on artificial diets may result in unusually high levels of genetic variation because cryptic genetic variation in a novel (artificial) environment has been revealed (Gibson & Dworkin, 2004; Ledón-Rettig *et al.*, 2014). These same traits under natural and routine conditions may have low genetic variation and low evolvability. Thus, although laboratory research incorporating artificial diets has been and will continue to be valuable in numerous ways, studies aimed at understanding ‘real-world’ levels of genetic variation, as well as the condition dependence of traits, require consideration of the conditions that animals experience in the wild.

In this study, we examine condition dependence and genetic variation for insects raised on three natural, seasonal diets. We examine how contemporary environmental and genetic influences contribute to the expression of sexually selected weapons to infer the potential for evolutionary change. Our study used the leaf-footed cactus bug, *Narnia femorata* Stal. 1862 (Hemiptera: Coreidae). Males and females in this species are dimorphic. Female *N. femorata* have larger overall body size (sexual size dimorphism), a phenomenon common in invertebrates and probably due to fecundity selection on females (Fairbairn, 1997). Males in this species have larger hind femora than females, although the dimorphism is not extreme (Gillespie *et al.*, 2014). Male *N. femorata* defend territories on cactus patches using their hind leg as weapons. Physical competitions between males can involve hind leg displays, and a variety of grappling manoeuvres (Fig. 1) including kicking, charging and end-to-end wrestling.

A priori, we predicted nutrition to influence sexual dimorphism and weapon size because *N. femorata* have a seasonally variable diet, and nutrition often has measurable effects on phenotypes. In north central Florida, these insects feed primarily on the fruit of *Opuntia mesacantha* subsp. *lata* (Small) Majure, formerly referred to as *O. humifusa*. Juveniles are common between April and December each year, with peaks in abundance to coincide with the presence of green, unripe fruit in the spring and ripe, red fruit in the late summer (L. Cirino, unpublished data). Nymphs developing in the autumn risk having fruit removed by competing herbivores before they complete development. Cactus fruit,



Fig. 1 Two male *Narnia femorata* in an escalated competition where the hind femora are used to strike and squeeze each other.

especially ripe fruit, are rapidly removed from cactus plants by herbivores such as deer, tortoises, birds and rodents (Gonzalez-Espinosa & Quintana-Ascencio, 1986; Hellgren, 1994), and cactus fruit abundance plummets in the fall (Gillespie *et al.*, 2014). As is the case in the vast majority of insects, adult body size and shape of *N. femorata* are determined during the juvenile feeding and development periods, and adults do not change size. Insects grow largest and males become more attractive when ripe fruit is available throughout development (Adesso *et al.*, 2014; Gillespie *et al.*, 2014). Females raised on cactus with ripe fruit produce more offspring. Further, when this high-quality resource is available for adults, it leads to distinctly different mating decisions with males becoming choosy and female choice changing with regard to male size and developmental diet (Gillespie *et al.*, 2014). Thus, the phenology of cactus is highly influential to development, reproductive output and sexual selection in this insect. Based on these results, we predicted that this dynamic resource would also affect weapon expression, sexual dimorphism and genetic parameters. Understanding the level of condition dependence in traits necessitates experimental manipulation of condition, the comparison of multiple traits within a sex and comparisons of these traits with homologous traits in the other sex whenever possible (Arnqvist & Thornhill, 1998; Cotton *et al.*, 2004). We are able to easily maintain cactus plants in the laboratory, allowing experimental study using realistic dietary variation.

We raised *N. femorata* on three natural diets: cactus without fruit, cactus with unripe fruit and cactus with ripe fruit, providing the range of nutritional quality (i.e. cactus without fruit < cactus with unripe fruit < cactus with ripe fruit). We predicted that the hind leg weapon (hind femora) of *N. femorata* is more sensitive to natural changes in nutrition relative to the homologous trait in females, consistent with the hypothesis that the hind femora have heightened condition dependence. We first examined the extent to which natural diets shape absolute trait expression for a range of traits in males and females. Secondly, we compared indexes of sexual dimorphism across diets. We expected that the difference in hind femora between males and females should be greatest under the most favourable conditions, when individuals have ripe, red fruit during their entire developmental period. Further, the change in sexual dimorphism in the hind femora of males and females should be greater than the relative change in homologous traits not associated with male weaponry (Bonduriansky, 2007a). We also tested the effect of natural dietary differences in generating changes in the scaling relationship between body size and the hind leg weapon, predicting that males, but not females, would produce larger hind femora relative to their body size when provided the highest quality diet (e.g. Bonduriansky & Rowe, 2005). Finally, we estimated quantitative

genetic parameters across the diets to determine whether the potential for these weapons to respond to sexual selection depended on nutritional conditions.

Materials and methods

The *N. femorata* individuals used in this study descended from approximately 40 mated females collected from the Ordway-Swisher Biological Station, Melrose, FL (29°41'N, 82°W), in September and October 2009. Insects were reared in the laboratory for three to four generations before this experiment began. We conducted a half-sib, split brood quantitative genetics rearing experiment where 33 sires were mated to four dams each ($N = 132$ dams). We raised *N. femorata* in small family groups on cacti with red, ripe fruit throughout their first three instars. We kept the newly hatched insects in groups because young juveniles in the wild frequently aggregate and have higher survivorship in groups (P. Allen, unpublished data). Using a paternal half-sib analysis, group effects are contained in the dam term, which we therefore do not analyse further and instead restrict our analyses to the sire term which lacks this confound. When the insects reached the 4th instar, we transferred them individually into plastic containers with one of three nutritional treatments: (1) a cactus pad without fruit, (2) a cactus pad with unripe green fruit or (3) a cactus pad with a ripe red fruit. The first and second treatments represent what can occur when competitive herbivores remove all ripe cactus fruit from a patch, leaving the juvenile insects stranded with only suboptimal resources. Rearing took place outside in shadehouses and glasshouses with varying temperatures. Insects were individually frozen for morphological analysis 14–30 days after becoming adults. In all, 4187 offspring were frozen, with a minimum of 83 and a maximum of 173 offspring per sire. Of the 4187 offspring, 1471 were raised in Treatment 1, 913 in Treatment 2 and 1803 in Treatment 3. Offspring from at least two dams per sire were included in the analysis (some dams did not produce sufficient numbers of offspring). The uneven distribution across categories reflects uneven distribution initially, although some survivorship differences may exist.

Morphometrics

Analysing the relative condition dependence of sexually selected traits requires that body size traits and homologous traits in females be measured for comparative purposes (Cotton *et al.*, 2004). Condition dependence was assessed by development environment (food) effects on scaling relationships and sexual dimorphism. To obtain trait measures, we photographed 2073 females and 2114 males (4187 total) using a Canon EOS 30D (Canon Inc. Tokyo, Japan) camera attached to a Leica M 165C (Leica Microsystems GmbH Wetzlar, Germany)

dissecting microscope. We measured insects from these photographs using Image J software version 1.42q (Rasband, 2009). We measured pronotum width and front femora length, traits with no obvious role in mate choice or male–male competition, and we measured the width (mm), length (mm) and area (mm²) of the hind femora which functions in kicks and squeezes during male–male competition. In some cases, insects were missing one or more legs, but other available parts were measured. Pronotum width was previously found to be an excellent proxy for body size (96% correlated with PC1 from a principal component analysis with seven morphological traits; Gillespie *et al.*, 2014) and was therefore used as our main proxy for body size here. Linear measurements were made of pronotum width (dorsal view), front femora length, hind femora length and hind femora width. We estimated the area of the hind femora and hind tibia using the ImageJ threshold function. The threshold tool interactively partitions greyscale images into features of interest and the background, allowing area measurements to be completed quickly. Leg measurements were averaged when both legs were available. Hind femora length and hind femora width are components of hind femora area and were used to explore the aspects of hind femora area that are the most dimorphic and condition dependent (Fig. 3) and for genetic estimates (Tables 3 and 4). We did not analyse the length and width of the hind femora with generalized linear model (GLM) because the analyses would have been redundant with analyses on the area of the hind femora.

Statistical analyses

We used a set of four GLMs to examine the influence of developmental diet and sex (and their interaction) on the raw measures of pronotum width, hind femora area, hind tibia area and front femora length. We then examined the effect of the developmental environment on scaling relationships between pronotum width and the other morphological traits. For the analyses of scaling relationships, we transformed (natural log) all morphological variables prior to analyses. GLMs for scaling were performed using pronotum width and developmental environment as covariates with hind femora area, hind tibia area and front tibia length as dependent variables. We conducted these analyses for each sex separately. GLMs were conducted in IBM SPSS v22 (IBM Corp., Released 2013, IBM SPSS Statistics for Windows, Armonk, NY, USA).

We calculated the index of sexual dimorphism separately for insects raised on the three diets, calculated as the mean size of females/mean size of males – 1 (Lovich & Gibbons, 1992). Negative values indicate that males have larger traits on average than females, and positive values indicate that females have larger traits on average than males.

Estimates of genetic variation must be standardized to allow comparison across traits. These estimates are typically standardized in one of two ways to infer evolvability (Hansen *et al.*, 2011): mean-scaled evolvability (the ratio of additive genetic variation to the square of the mean) and heritability (the ratio of additive genetic variation to phenotypic variation). Heritability has the advantage of familiarity, whereas mean-scaled evolvability has the advantage of measuring change in units of the mean (i.e. how much could the mean change under directional selection) which may provide more accurate measures of the amount of actual change possible (Houle, 1992; Hansen *et al.*, 2011). Both metrics are contingent on the level of additive genetic variation underlying trait expression (Houle, 1992; Blows & Hoffmann, 2005). Both are also specific for the population and environment in which they are measured. Thus, a first step in measurements of quantitative genetic parameters across environments is often to check whether the level of genetic variation is the same in both environments.

We calculated both mean-scaled evolvability I_A (Houle, 1992; Hansen *et al.*, 2011) and narrow-sense heritability h^2 (Lynch & Walsh, 1998) for all traits and for each diet separately. These were calculated from the sire variances obtained from the paternal half-sib analyses within each treatment. We used JMP Pro 11.0 (SAS Institute, Cary, NC, USA). Given the level of imbalance in our half-sib design, with unequal numbers of dams per sire that produced offspring, we used restricted maximum-likelihood analyses to generate estimates all variance components and their associated standard errors (Lynch & Walsh, 1998). Sire and dam were treated as random effects.

Results

Effects of the developmental environment on morphology

The developmental environment had clear and pronounced effects on adult body size and shape in *N. femorata* (Figs 2 and 3; Tables 1 and 2). Males and females raised on cactus with red, ripe fruit became the largest adults, followed by those raised on green, unripe fruit. Insects raised on cactus without fruit were the smallest. Insects raised on cactus with red fruit were 25% (males) to 26% (females) larger on average in body size than those of the same sex raised on cactus without fruit. Male hind femora area exhibited the largest response to developmental diet (Figs 2 and 3). Relative to the femora width of males raised on cactus without fruit, male hind femora area was 24% larger when males were raised on cactus with unripe fruit and 69% larger when males were raised on cactus with ripe fruit.

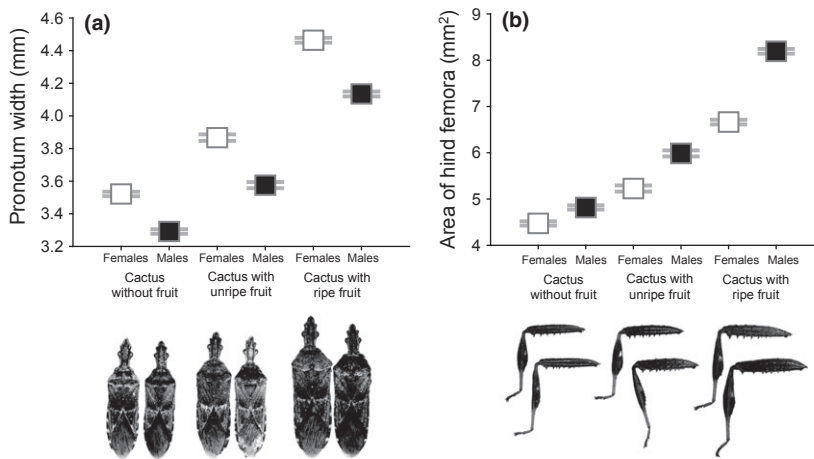


Fig. 2 Top: Effects of developmental environment on mean body size (a) and area of hind femora (b) for females and males (\pm SE). Nutrition had a larger overall effect on insect body size and hind femora than sexual dimorphism. (b) Male hind femora become disproportionately large relative to female hind femora when insects are raised on cactus with ripe fruit. Below: Insect bodies (a) and hind legs (b) for females (left) and males (right) featured at the average size and to scale for each developmental environment.

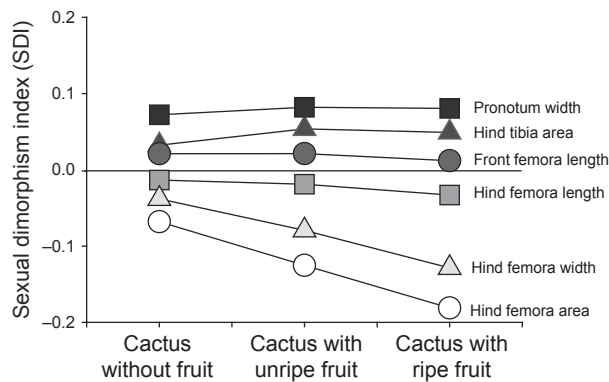


Fig. 3 Sexual dimorphism of six traits in *Narnia femorata* raised on three natural diets. Sexual dimorphism was estimated as the mean size of females/mean size of males - 1. When values are negative, they indicate that males have larger traits on average than females and vice versa when values are positive. Insects raised on cactus with red fruit have pronounced male-biased sexual dimorphism in hind femora area, an effect that appears largely due to changes in hind femora width.

Condition dependence of sexual dimorphism

Females were larger in body size (pronotum width), front femora length and tibia area than males from the same developmental environment (Fig. 2a; Table 1). Female body size increased slightly more in response to the favourable diets compared with male body size (Fig. 3; Table 1). However, the level of sexual dimorphism in these traits was fairly consistent across the developmental environments (Fig. 3), with females 7–8% larger than males in each case.

Although females were larger than males in most traits, males had larger femora than females for any given developmental environment (Fig. 2b). As predicted, the sexual dimorphism in the hind femora was not consistent across the developmental environments

Table 1 Results of four separate GLM for effects of sex and developmental diet on morphological traits in *Narnia femorata* adults.

Source	d.f.	Pronotum width <i>F</i>	Hind femora area <i>F</i>	Hind tibia area <i>F</i>	Front femora length <i>F</i>
Developmental diet	2	1868.43***	1608.70***	886.296***	1150.44***
Sex	1	462.24***	377.16***	28.763***	36.25***
Developmental diet \times Sex	2	4.58*	72.91***	1.189	1.49

Error d.f. = 4156–4183.
* $P \leq 0.05$, *** $P \leq 0.001$.

(Fig. 3; Table 1). The difference between males and females in mean area of the hind femora was 7% when insects were raised on cactus without fruit, 14% when insects are raised on cactus with unripe fruit and 23% when insects are raised on cactus with ripe fruit. Much of this difference appears to be driven by changes in the width of male hind femora across environments (Fig. 3).

Scaling with body size

The area of the hind femora of males scaled more steeply with their body size than did the area of the hind femora of females (Fig. 4). This assumes that the covariate, pronotum width, has a linear relationship common to all diet treatments and this assumption was met (Fig. 4). We found that the developmental environment affected the scaling slope between all traits and body size for both males and females as evidenced by the significant interaction between pronotum width and developmental environment (Table 2). Because of the statistically significant differences in scaling slope,

Table 2 Results of four separate GLM for effects of diet on hind femora area and hind tibia area in *Narnia femorata* adults with pronotum width (body size) as a covariate. Because the hind femora area and hind tibia area are sexually dimorphic (Table 1, Fig. 3), females and males were analysed separately. Both pronotum width and hind femora area were log-transformed prior to analyses.

Source	d.f.	Males		Females	
		Hind femora area χ^2	Hind tibia area χ^2	Hind femora area χ^2	Hind tibia area χ^2
Developmental diet	2	13.34**	118.92***	18.20***	107.83***
Pronotum width (PW)	1	17 497.87***	4777.63***	12 125.82***	3960.30***
Developmental diet \times PW	2	10.49**	113.36***	17.44***	102.94***

** $P \leq 0.01$, *** $P \leq 0.001$.

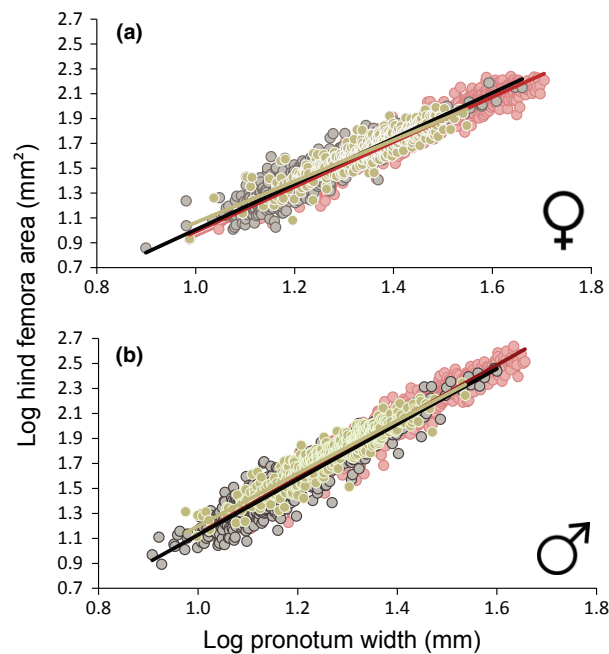


Fig. 4 (a) Females and (b) males. The scaling relationship between body size (pronotum width) on the x-axis and hind femora area on the y-axis, broken down by developmental diet (red, cactus with ripe fruit; beige, cactus with unripe fruit; black, cactus without fruit). Log-transformed values shown.

testing for differences in scaling intercept was not relevant. However, a visual inspection of Fig. 4 reveals that males in this study raised on cactus without fruit during their final two instars generally had smaller hind femora for their body size, although this effect is small, detectable only with very large sample sizes, and may not be strongly biologically significant.

Heritability and mean-scaled evolvability

We estimated h^2 and I_A separately for each sex and each developmental environment. We found that heritability was almost universally low with standard errors that overlap zero. Exceptions were only in the harsher environments, cactus without fruit and cactus with

unripe fruit. In the case of females, the largest heritability estimates were found for pronotum width (0.25) and hind tibia area (0.21) and only when insects developed on cactus with unripe fruit. In the case of males, hind femora length was found to be heritable in both the cactus without fruit (0.14) and cactus with unripe fruit (0.13) developmental environments. Hind femora area was weakly heritable in the cactus without fruit environment (0.09). Mean-scaled evolvabilities were 0 or 0.01 for all traits in all treatments for both males and females.

Our original intent was to examine the full multivariate (G) matrix of genetic covariances; however, any trait that has zero variance will have a zero covariance with any other trait. Given the sparse genetic variances, further calculations are not warranted here. However, the full dataset is available in Dryad in case there are future meta-analyses that require these data.

Discussion

Our experiment showed that the hind femora of male *N. femorata* have a heightened level of condition dependence. We found that the hind femora of males were larger than the hind femora of females (Figs 2b and 3; Table 1), even as female body size was larger (Figs 2a and 3; Table 1). In addition, the hind femora of males scaled more steeply with body size than the hind femora of females (Fig. 4). Both of these results are consistent with the hind femora of males having a history of stronger directional selection than the hind femora of females given the function of the male hind femora in battle (Voje, 2016). Yet, these results do not necessarily indicate heightened condition dependence. We found that males raised on the poorest nutrition (cactus without fruit) developed smaller hind femora relative to their body size than males raised on better nutrition, but the difference found here was small (Fig. 4). Our strongest evidence of heightened condition dependence was the dramatic change in the size of the hind femora in males relative to the size of the hind femora in females across the range of developmental diets (Figs 2b and 3; Developmental diet \times sex interaction in Table 1). The differences in most traits

between males and females in this species were modest and fairly consistent across developmental diets. However, the sexual dimorphism in the width and area of the hind femora diverged strongly between males and females for those insects reared with the best nutrition compared with those raised with poor nutrition (Fig. 3).

A major consequence of sex-specific levels of condition dependence in traits in this species is that the degree of sexual dimorphism is condition dependent. *Narnia femorata* raised on cactus without fruit are nearly monomorphic, whereas the sexes measurably diverge in hind femora area when insects experience high-quality nutrition during development (Fig. 3). The pattern of sexual dimorphism can be described as incomplete when poor condition animals approach sexual monomorphism (Bonduriansky, 2007a). Nutritionally induced variation in sexual dimorphism may be extremely common in wild populations, but it has rarely been quantified. Furthermore, extrapolating patterns of sexual dimorphism from experimental laboratory studies can be difficult unless relevant dietary differences are used. Using *N. femorata* allowed us to overcome this challenge. Cacti can be easily and frequently collected from the wild, which allows researchers to measure the effects of natural seasonal variation in cacti on patterns of sexual dimorphism.

We show that even a modest sexually selected weapon exhibits pronounced condition-dependent sexual dimorphism. These results are especially significant because much of the interest in sexually selected ornaments and weapons focuses on the evolution of traits with rare and extreme levels of sexual dimorphism (Bonduriansky, 2007b; Emlen, 2014). Further, weapons have received less empirical attention in recent years than have ornaments (Emlen, 2008). A recent study on antler flies found that the extent of sexual dimorphism in a modest weapon was positively associated with the strength of condition dependence, but dimorphism did not increase with diet quality (Oudin *et al.*, 2015). Species will undoubtedly vary in the extent and nature of condition-dependent sexual dimorphism due to the history of selection on the dimorphic traits and the viability cost of expressing the traits (Rowe & Houle, 1996; Bonduriansky, 2007a; Cassidy *et al.*, 2014). It will be important for future studies to focus on measuring condition dependence and sexual dimorphism in a wide range of sexually selected weapons, with an emphasis on weapons of a more typical size.

The patterns of heightened condition dependence found here are suggestive that the phenotypic distribution should change in the field over time. Such changes may affect the form and strength of selection and lead to intriguing evolutionary dynamics (Steele *et al.*, 2011; Miller & Svensson, 2014). Yet, despite our findings, there may not be further evolutionary consequences as evidenced by both the low mean-scaled evolvability

and sparse heritability (Tables 3 and 4). Sasson *et al.* (2016) also reported low evolvability for hind femora in *N. femorata*. The low estimates of evolvability in hind femora are an apparent paradox. The hind legs in this family of insects, the Coreidae, are highly diverse in

Table 3 Estimates of phenotypic and genetic parameters for females.

Trait	Mean	Vp	Va	h^2	SE	I_A	N (total)
Cactus without fruit							
Pronotum	3.53	0.13	0.00	0.00	0.00	0.00	882
Front femora length	3.55	0.08	0.00	0.00	0.00	0.00	874
Hind tibia area	3.59	0.72	0.01	0.01	0.09	0.00	881
Hind femora length	5.38	0.26	0.00	0.00	0.00	0.00	882
Hind femora area	4.47	0.84	0.00	0.00	0.00	0.00	881
Cactus with unripe fruit							
Pronotum	3.87	0.12	0.03	0.21	0.16	0.00	447
Front femora length	3.64	0.05	0.01	0.13	0.16	0.00	443
Hind tibia area	3.80	0.42	0.10	0.25	0.19	0.01	447
Hind femora length	5.76	0.18	0.02	0.13	0.16	0.00	447
Hind femora area	5.23	0.72	0.08	0.11	0.16	0.00	447
Cactus with ripe fruit							
Pronotum	4.46	0.27	0.00	0.00	0.00	0.00	743
Front femora length	4.07	0.16	0.01	0.06	0.12	0.00	742
Hind tibia area	5.08	1.90	0.09	0.05	0.13	0.00	742
Hind femora length	6.44	0.44	0.01	0.03	0.11	0.00	743
Hind femora area	6.66	1.95	0.05	0.02	0.12	0.00	743

All measures in mm (lengths) or mm² (area). Heritability values in bold reflect those estimates > 0.00 where the standard error (SE) does not overlap zero.

Table 4 Estimates of phenotypic and genetic parameters for males.

Trait	Mean	Vp	Va	h^2	SE	I_A	N (total)
Cactus without fruit							
Pronotum	3.29	0.13	0.01	0.08	0.09	0.00	921
Front femora length	3.47	0.10	0.00	0.04	0.10	0.00	916
Hind tibia area	3.48	0.68	0.00	0.00	0.00	0.00	920
Hind femora length	5.45	0.36	0.05	0.14	0.10	0.00	921
Hind femora area	4.82	1.72	0.16	0.09	0.09	0.01	920
Cactus with unripe fruit							
Pronotum	3.58	0.11	0.01	0.07	0.13	0.00	466
Front femora length	3.56	0.06	0.00	0.06	0.12	0.00	460
Hind tibia area	3.61	0.41	0.05	0.12	0.16	0.00	466
Hind femora length	5.86	0.25	0.03	0.13	0.13	0.00	466
Hind femora area	5.99	1.51	0.06	0.04	0.13	0.00	466
Cactus with ripe fruit							
Pronotum	4.13	0.25	0.01	0.03	0.11	0.00	727
Front femora length	4.03	0.19	0.00	0.00	0.00	0.00	724
Hind tibia area	4.86	1.75	0.00	0.00	0.00	0.00	727
Hind femora length	6.67	0.63	0.00	0.00	0.00	0.00	727
Hind femora area	8.19	4.77	0.00	0.00	0.00	0.00	727

All measures in mm (lengths) or mm² (area). Heritability values in bold reflect those estimates > 0.00 where the standard error (SE) does not overlap zero.

form and size, rivalling the diversity in the horns of the African antelopes. The great diversity suggests that these traits are highly evolvable. Moreover, condition dependence is predicted to maintain genetic variation in traits (Rowe & Houle, 1996; Kotiaho *et al.*, 2001; Tomkins *et al.*, 2004). Why are leg traits apparently not currently evolvable in this population? The fact that both sexually selected and naturally selected traits lack genetic variation may provide a clue.

As a corollary of Fisher's fundamental theorem, sexually selected traits, including both weapons and ornaments, are expected to lose genetic variation over time within populations due to the effects of directional selection (Robertson, 1955; Fisher, 1958). The action of directional selection through both male–male competition (Procter *et al.*, 2012) and mate choice (Gillespie *et al.*, 2014) has potentially eroded genetic variation for body size and correlated traits in this population of *N. femorata*. Yet, all measured traits are apparently not very evolvable here, not just those under sexual selection. Therefore, sexual selection appears not to be the complete answer. Another factor that may lead to low genetic estimates is a variable diet. We fed our insects on cacti collected from the wild. Each cactus likely differs in available nutrients, perhaps inflating the phenotypic variation in traits, and decreasing our ability to detect heritable differences among individuals (see Pemberton, 2010). Yet, our sample sizes are large relative to most evolutionary quantitative genetic studies, so we should have been able to detect even modest levels of additive genetic variation. Finally, genetic drift should be considered. Our population of insects may have experienced a genetic bottleneck, and thus the loss genetic variation, when it was introduced to Florida approximately 60 years ago on cactus nursery stock. Our laboratory colony may have also lost genetic variation over the three to four generations kept in captivity, although this is a relatively short period of time and our sample size is large. Ultimately, additional genetic work is needed on *N. femorata* to provide greater understanding of the factors influencing the genetic estimates we found here.

Our results reveal extensive phenotypic variation and low evolvability for *N. femorata* across natural diets. Environmental stress can have complex effects on genetic and phenotypic variation (Hoffmann & Merilä, 1999; Gibson & Dworkin, 2004; Tammaru & Teder, 2012; Ledón-Rettig *et al.*, 2014). Indeed, previous studies have produced a wide range of results, revealing, for example, low genetic variation across multiple treatments and higher or lower genetic variation in stressful vs. unstressful treatments (Hoffmann & Merilä, 1999; David *et al.*, 2000; Kemp & Rutowski, 2007; Delcourt & Rundle, 2011; Gosden & Chenoweth, 2011; Dmitriew & Blanckenhorn, 2014). A consensus answer to how stressful environments influence genetic variation of sexually selected traits has, thus, remained elusive.

Importantly, many such studies are conducted in laboratories on artificial diets and so the biological relevance of genetic and phenotypic estimates has been questioned. This study provides a relatively rare experimental window into genetic parameters when individuals are raised in semi-natural conditions on multiple natural diets.

In summary, we assessed the potential for dynamic patterns in weapon size, sexual dimorphism and evolvability in a population of leaf-footed cactus bug, *N. femorata*. We found striking differences in weapon expression and sexual dimorphism due to changes in developmental diet. These results are likely relevant to wild populations because we used natural, seasonal differences in available nutrition. Such changes may affect patterns of selection. However, we found virtually no evidence of evolvability in traits in this population. Thus, although sexual selection dynamics may change seasonally, they are not predicted to result in evolutionary changes. It is possible that these traits will only evolve if the environment is to shift away from current seasonal norms, revealing cryptic genetic variation that allows a response to selection. These results underscore the value of considering seasonally and environmentally varying factors when conducting research on sexually selected traits.

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