Condition dependence of male and female genital structures in the seed beetle *Callosobruchus maculatus* (Coleoptera: Bruchidae)

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Abstract

Theory predicts that costly secondary sexual traits will evolve heightened condition dependence, and many studies have reported strong condition dependence of signal and weapon traits in a variety of species. However, although genital structures often play key roles in intersexual interactions and appear to be subject to sexual or sexually antagonistic selection, few studies have examined the condition dependence of genital structures, especially in both sexes simultaneously. We investigated the responses of male and female genital structures to manipulation of larval diet quality (new versus once-used mung beans) in the bruchid seed beetle *Callosobruchus maculatus*. We quantified effects on mean relative size and static allometry of the male aedeagus, aedeagal spines, flap and paramere and the female reproductive tract and bursal spines. None of the male traits showed a significant effect of diet quality. In females, we found that longer bursal spines (relative to body size) were expressed on low-quality diet. Although the function of bursal spines is poorly understood, we suggest that greater bursal spine length in low-condition females may represent a sexually antagonistic adaptation. Overall, we found no evidence that genital traits in *C. maculatus* are expressed to a greater extent when nutrients are more abundant. This suggests that, even though some genital traits appear to function as secondary sexual traits, genital traits do not exhibit heightened condition dependence in this species. We discuss possible reasons for this finding.

Introduction

Condition dependence is a variant of developmental plasticity in which the degree of expression of a trait reflects the quantity of resources that an individual has to invest in all functions (Nur & Hasson, 1984). Condition is thought to have a genetic component, reflecting the quality of resource acquisition alleles throughout the genome, as well as an environmental component, reflecting the availability of resources in the ambient environment (Andersson, 1982; Rowe & Houle, 1996).

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Sexually selected traits have typically been thought to evolve condition-dependent expression because enhanced trait expression confers mating success benefits but also incurs viability costs (Zahavi, 1975; Andersson, 1982, 1986; Nur & Hasson, 1984; Zeh & Zeh, 1988; Grafen, 1990). Secondary sexual traits can serve as signals of quality, as well as weapons in intrasexual interactions or as mechanisms that assist copulation (such as grasping or titillating mates (Eberhard, 1985)). If male mating success is enhanced by increased trait expression, males should express the trait at the largest size that they can afford (i.e. until the mating success benefits are balanced by costs to viability).

Male genital traits are a special subset of secondary sexual traits that play a variety of roles related to sperm transfer, removal of sperm deposited by previous copulations, grasping and restraint of females, and

copulatory courtship (Eberhard, 1991, 1994; Cordoba-Aguilar, 1999; Cordoba-Aguilar *et al.*, 2003; Ramm, 2007; Peretti & Eberhard, 2009). Indeed, genital size and/or shape is subject to sexual selection in a diverse range of insect species (Bertin & Fairbairn, 2007; Simmons *et al.*, 2009; Cayetano *et al.*, 2011; House *et al.*, 2013). However, owing to the distinctive characteristics of genitalia, different predictions have been made as to whether they should be condition dependent, like other secondary sexual traits.

If they are sexually selected, genital structures may be expected to exhibit some degree of condition dependence (Hosken & Stockley, 2004). In particular, if sexual selection favours exaggeration of genital traits, and such traits are costly to express, then variation in relative size of genital traits is expected to reflect variation in individual condition (although see House *et al.*, 2013). On the other hand, in contrast to nongenital secondary sexual traits, sexual selection on genital traits may often favour functional integration and precision rather than exaggerated size (House & Simmons, 2007; Werner & Simmons, 2008). Individual variation in condition may therefore be manifested in variation in the fine structure of genital traits rather than their size. Indeed, genital traits often exhibit negative static allometries and relatively little variation in size (Eberhard *et al.*, 1998), suggesting that genitalia are strongly canalized rather than selected for size exaggeration. Moreover, just as with precopulatory courtship (Schlinger *et al.*, 2013), the nervous and muscular systems used to deploy the genitalia may be as important as genital morphology in determining the outcomes of sexual interactions, particularly if the genitalia are used in a stimulatory rather than coercive manner. Therefore, although condition may play an important role in genital form and function, the sizes of genital structures may not be condition dependent.

Weak condition dependence is also predicted by the lock-and-key hypothesis (Arnqvist, 1997), which posits that male and female genitalia are selected to achieve a precise match to reduce the risk of interspecies matings (Dufour, 1844; Mayr, 1963). This hypothesis predicts that genital traits should be canalized to resist developmental perturbation or stress. Despite some empirical support (Sota & Kubota, 1998), this hypothesis has been rejected in most studies (Eberhard, 1985, 2005; Arnqvist & Thornhill, 1998; Eberhard & Ramirez, 2004; House & Simmons, 2005; Mutanen *et al.*, 2006; Gilligan & Wenzel, 2008; Andrade *et al.*, 2009; Crews, 2009; Eberhard *et al.*, 2009). A related idea is the 'one-size-fits-all' hypothesis, which postulates that selection favours male genitalia that facilitate successful interaction with females spanning a range of sizes (House & Simmons, 2007; Eberhard *et al.*, 2009). According to both the lock-and-key and one-size-fits-all hypotheses, genital size should scale weakly with body size and condition.

Surprisingly few investigations have attempted to directly gauge the presence of condition dependence in genitalia via diet manipulation (and none, to our knowledge, have examined the effects of variation in genetic quality). Of the studies that have been conducted, a mixed picture emerges. Some studies have reported sensitivity of genital size and/or shape to variation in nutrient abundance (Arnqvist & Thornhill, 1998; Soto *et al.*, 2007; Cothran & Jeyasingh, 2010) or other factors, such as temperature, that may be expected to influence condition (Andrade *et al.*, 2005), whereas other studies have detected no response in genital trait expression to variation in nutrient abundance (Schulte-Hostedde & Alarie, 2006; House & Simmons, 2007; Rodriguez & Al-Wathiqui, 2011). A limitation of most of these studies is the omission of female genitalia, reflecting a persistent research bias (Ah-King *et al.*, 2014).

In the bruchid seed beetle *Callosobruchus maculatus*, the expression of some male genital traits (notably, the spines of the male intromittent organ) has been shown to affect male competitive fertilization success (Hotzy & Arnqvist, 2009; Hotzy *et al.*, 2012) and to impose substantial fitness costs on females (Crudgington & Siva-Jothy, 2000; Edvardsson & Tregenza, 2005; Rönn *et al.*, 2007). The male aedeagal spines have also been shown to respond via evolution of the static allometry slope to manipulation of sexual selection opportunity (Cayetano *et al.*, 2011). Although past research has focused mainly on the aedeagal spines, the genital complex of *C. maculatus* males includes a series of other appendages. Likewise, *C. maculatus* females possess a complex genital tract that also includes spines in the bursa copulatrix. Although the function of bursal spines is unclear, their marked enlargement in another *Callosobruchus* species (Kingsolver, 1979) suggests the possibility of a sexually antagonistic role (e.g. prevention or limitation of intromission). The condition dependence of these structures has not been investigated previously.

Given the range of hypotheses outlined above, the overall degree of condition dependence of the male and female genitalia, as well as the condition dependence of specific components of the genital tract, is difficult to predict. However, because the aedeagal spines of *C. maculatus* are a sexually antagonistic trait that facilitates sperm competition (Hotzy & Arnqvist, 2009), spine length allometry responds to manipulation of sexual selection (Cayetano *et al.*, 2011), and the removal of the longest spines results in a marked reduction in fertilization success (Hotzy *et al.*, 2012), we predicted that the aedeagal spines would exhibit condition-dependent expression, with high-condition males expressing longer spines than low-condition males. Condition dependence might be detected as a response in mean trait size, but it may also be expressed as an allometric response: for example, high condition may enable the

largest males to express disproportionately larger traits (Bonduriansky, 2007).

We manipulated condition by rearing *C. maculatus* larvae on fresh or previously used mung beans and examined responses in several male and female genital traits. Our results provide little evidence of condition dependence of genital structures in either sex. Interestingly, however, we observed a response in the female bursal spines, which were relatively longer in low-condition females.

Materials and methods

Study system

Callosobruchus maculatus live among and breed in legume grain stocks and have likely had an association with these food sources for millennia (Messina, 1991). Eggs are attached by females to the surfaces of beans. Larvae burrow into and develop within eggs, emerging approximately 4 weeks later as sexually mature adults. A single copulation can result in up to 90 eggs being fertilized, and these eggs can be deposited over a period of about 3 days. Individual males can mate with dozens of females (Ofuya, 1995). Successful reproduction does not typically require adults to ingest additional nutrients after emergence.

Acquisition of beetle stock

Callosobruchus maculatus were acquired from a stock maintained at the Department of Primary Industries and Fisheries (DPIF), Queensland, originating from a population in Kingaroy, Australia in 2003. The laboratory population originated with 357 individuals and was reared thereafter with 250–300 individuals per generation for approximately 20 generations on mung beans (*Vigna radiata*). For the current experiment, a batch of 600 beetles were obtained from this population and continued in the laboratory with approximately 500 individuals per 200 g of mung beans under a 14-h:10-h day:night photoperiod (Maklakov *et al.*, 2009) at 70% relative humidity and 30°C.

Experimental diet treatments

Previous studies have shown that larval competition is an important determinant of food medium quality (Guedes *et al.*, 2007) and that *C. maculatus* female prefer to oviposit on hitherto unused beans (Mitchell, 1975; Messina & Mitchell, 1989). We therefore used beans that had previously harboured a single larva as a low-quality medium and previously unused beans as a high-quality medium. A pilot study confirmed that used beans resulted in substantially smaller adults than new beans. Very small beans, which are frequently unable to support more than one larva (Mitchell, 1975), were not used.

Experimental procedures

Twenty male–female pairs, derived from two different laboratory cultures (to ensure outbreeding) maintained under conditions of polygamy, were allowed to mate in large Petri dishes containing mung beans. Beans that came to harbour a single egg were later placed individually in glass shell vials. The eclosed individuals became the parents in this study. These individuals were randomly paired and observed to mate once, then separated. Ten of the dams were first exposed to a high-quality larval medium (50 fresh beans) in a large Petri dish for 24 h, after which the beans were inspected to confirm that a satisfactory number of eggs had been distributed across them. A few beans harboured multiple eggs and were removed. The dams were then transferred to Petri dishes containing a low-quality larval medium (50 once-used beans) for 24 h, after which their eggs were similarly inspected and beans harbouring more than one egg removed. Another ten dams were first offered a low-quality larval medium and then a high-quality larval medium. The eggs were stored in their Petri dishes in an environment chamber at 27°C until eclosion. After approximately 3 weeks, the eclosed individuals were frozen at −18°C for later measurement of morphology. Ten individuals of each sex from each family (*n* = 400 individuals in total) were randomly chosen for measurement (but a few individuals could not be measured: see Results for actual sample sizes).

Traits examined

Six male genital traits were measured: the length and width of the aedeagus (the intromittent organ of the male); aedeagal spine length (estimated as the mean length of the three longest spines on this organ); the mean length of the two elongate, basally fused prongs of the paramere that run laterally along either side of the aedeagus; the total spinal area, representing the combined areas of the lateral and dorsal sclerotized portions of the aedeagus; and the length of a triangular flap-like structure ('triangular flap') extending from the end of the aedeagus. Three female genital traits were also measured: the combined length of the bursa copulatrix and copulatory tract ('tract length'); the mean length of the bursal spines; and the number of bursal spines (see Cayetano *et al.*, 2011 for details and illustration). The use of length measurements is consistent with theory about condition dependence being mainly about trait size and reflects the fact that the traits measured are mainly elongated structures, with length measurements capturing most of the variation in the sizes of these structures.

For both sexes, the proxy for body size, used as a covariate in the analysis of genital traits, was the mean length of the two elytra. This was the most highly repeatable continuous measurement (Table 1) and

Table 1 Effect coefficients from linear mixed model (using restricted maximum likelihood) of effects of diet and order of presentation on mean body sizes across both sexes with elytra length as a proxy. Family is a categorical, random predictor nested within order of presentation. For random effects (Family, Family * Diet and Family * Sex), the proportion of total variance explained by each variance component is shown.

Source Fixed factors	Numerator d.f.	Denominator d.f.	Effect coefficient
Sex	1	18	−166.4047***
Diet	1	18	−86.8045***
Order	1	18	4.4003
Sex * Diet	1	338	38.7975***
Sex * Order	1	18	−8.1559
Diet * Order	1	18	0.0951
Sex * Diet * Order	1	338	12.9589
Family and interactions			Prop. of total variance
Family			0.0932
Family × Sex			0.0423
Family × Diet			0.1849

***$P < 0.001$.

captured much of the variation in body size, loading most strongly on the first principal component of the correlation matrix for each sex (analysis not shown).

Dissection and measurement procedures

Individuals were dissected in a drop of glycerol on a glass microscope slide, and their genitalia were removed using a pair of fine forceps and then photographed. The aedeagus was photographed both on its lateral side (exposing its curvature to allow its length and lateral spinal area to be measured) and on its dorsal side (to measure dorsal spinal area). The paramere and triangular flap were detached from the aedeagus with a graphite micro-blade and imaged separately. The female bursa was dissected out, and the bursal spines were separated with a graphite blade. Aedeagal spines, triangular flaps and bursal spines were all pressed under a cover slip to more accurately gauge their lengths along their maximum extent.

Each structure was photographed using a Leica DFC420 digital camera attached to either a Zeiss Axioskop 40 compound microscope or a Leica MZ16A stereo-microscope. Images were then measured with IMAGE J software (Rasband, 1997–2014). Male triangular flaps, parameres, and aedeagal spines, and female bursal spines were measured using a straight-line function from the distal tip to the approximate edge of sclerotization of the respective organs. Aedeagal length was measured with a segmented line function along the extent of the organ between the distal end of the

aedeagus and the beginning of the sclerotized spinal area. Aedeagal spinal area was found by drawing a boundary around the dorsal and lateral sclerotized spinal areas (for further details, see Cayetano *et al.*, 2011).

Measurement repeatabilities, represented as intraclass correlations (Lessells & Boag, 1987), were obtained for all genital traits and for elytra and were high (0.8–1.0) for most traits, but moderate (0.59) for flap length (0.59) (Cayetano *et al.*, 2011).

Statistical analysis

The analysis is based on 20 families comprising 400 individuals (ten individuals of each sex per family). Analysis of body size was performed using restricted maximum likelihood (REML) with larval diet (high or low quality) and order of presentation of high- and low-quality beans (high–low or low–high) as fixed effects, and family as a categorical, random effect nested within order of presentation. For all other traits except bursal spine number, analyses were carried out on log-transformed values. A linear mixed model was fitted separately for each trait using REML, with larval diet (high or low quality) and order of presentation (high–low or low–high) as fixed effects, and log-transformed mean elytra length (our proxy for body size) as covariate. We first tested for treatment effects on allometric slope, indicated by a significant interaction between a fixed factor and elytra length. If no treatment effect on allometric slope was detected, the model was re-fitted after removing interactions with elytra length, and these simplified models were used to test for treatment effects on mean relative trait size (i.e. trait size in relation to body size).

Number of bursal spines is a discontinuous (count) variable. This trait was therefore analysed using a generalized linear mixed model with a Poisson distribution and a log-link function, with larval diet and order of presentations included as fixed effects and family (nested in order of presentations) included as a random effect. Individual identity was included as an additional random factor in the model to correct for overdispersion (Harrison, 2014). Treatment effects on allometric slope were not tested for this trait because it is not continuously distributed and the range of values is small.

Analyses were performed using R (version 3.1.0). The package lme4 and functions *lmer* and *glmer* were used to fit general and generalized linear mixed effects models, respectively (see Tables). The function ANOVA was used to test the significance of each fixed effect by comparing the amount of variance explained by a model that included this effect with the amount of variance explained by a model that excluded this effect. Specifically, models were re-fitted using maximum likelihood and compared using a likelihood-ratio test based on a chi-squared statistic with degrees of freedom corresponding to the difference in number of parameters

between the two models (Bolker, 2008; Crawley, 2013).

Results

Effects of diet manipulation on body size

Diet had a strong effect on body size: both sexes were larger in the high-quality diet treatment (Table 1; Fig. 1). There was a significant Sex × Diet interaction, with females showing a greater response to diet manipulation than males, resulting in a greater sexual size
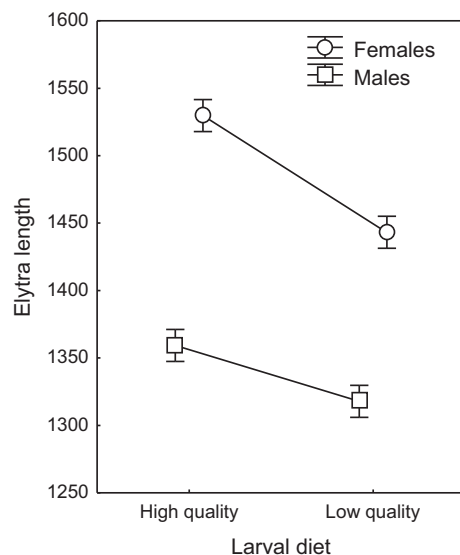

Fig. 1 Body size (in pixels) of males and females across diet treatments. Bars indicate standard errors of least square means, based on analysis using raw data of mean elytra length.

dimorphism on the high-quality diet. Females were larger than males in both treatments. The effect of order of presentation of new and used beans was not significant. The Family × Diet variance component accounted for > 18% of residual variance, indicating substantial variation among families in response to larval diet quality.

Effects of diet manipulation on allometric slopes of genital traits

There was little evidence of treatment effects on the static allometries of any male or female trait (Table 2). All interactions of fixed effects with elytra length (EL) were nonsignificant, with the exception of a marginally significant Order × EL interaction for total aedeagal spine area. All models were therefore simplified by removing interactions with EL and re-fitted to test for treatment effects on mean relative trait sizes.

Effects of diet manipulation on mean relative sizes of genital traits

All traits except aedeagus length, bursa length and number of bursal spines scaled significantly and positively with elytra length (Table 3). Larval diet had no significant effects on mean relative sizes of any male traits, but female mean relative bursal spine length was greater in the low-quality diet treatment than in the high-quality treatment (Fig. 2). Order of presentation affected male aedeagus width (greater in new-used than in used-new) and female reproductive tract length (greater in used-new than in new-used). We did not detect any significant Diet × Order interactions. Family accounted for a substantial proportion of total variance for male aedeagal spine length, aedeagus width, flap length and paramere length, and for female bursal

Table 2 Effect coefficients from linear mixed models of male and female genital traits testing for effects on allometric slopes. Diet (fresh or once-used beans) and order of presentation ('Order') were fitted as categorical, fixed predictors and family was fitted as a categorical, random predictor nested within order of presentation. Mean log-transformed elytra length (EL) was fitted as a covariate. For random effects (Family and Family * Diet), the proportion of total variance explained by each variance component is shown.

	Male traits						Female traits	
	Mean aedeagal spine length	Total aedeagal spine area	Aedeagus width	Aedeagus length	Flap length	Paramere length	Tract length	Mean bursal spine length
EL	0.2193***	0.5367***	0.3358***	0.3489	0.3148	0.4543***	−0.3608	0.9709*
Diet	−1.2569	0.3068	−0.8131*	0.6489	−0.8128	−0.7002	−2.8663	−0.2577*
Order	−1.0690	1.9113	−1.3766**	1.5247	0.6549	0.6399	−3.1876*	1.3527
Diet * Order	0.6562	−1.7166	2.7802	−2.5605	0.9242	0.0945	3.5756	−2.6495
Diet * EL	0.4026	−0.0989	0.2595	−0.2084	0.2587	0.2245	0.9051	0.0892
Order * EL	0.3413	−0.6113*	0.4344	−0.4888	−0.2116	−0.2030	1.0094	−0.4340
Diet * Order * EL	−0.2088	0.5481	−0.8900	0.8199	−0.2947	−0.0309	−1.1279	0.8434
Family	0.1764	<0.0001	0.1851	<0.0001	0.1880	0.2773	0.0100	0.2290
Family * Diet	0.0290	0.1814	0.3317	0.1361	0.0391	0.0166	0.1984	0.0403

*P < 0.05; **P < 0.01; ***P < 0.001.

Table 3 Effect coefficients from linear mixed models of male and female genital traits, testing for effects on mean relative trait sizes. Diet (fresh or once-used beans) and order of presentation ('order') were fitted as categorical, fixed predictors and family was fitted as a categorical, random predictor nested within order of presentation. Log of mean elytra length (EL) was fitted as a covariate. For random effects (Family and Family * Diet), the proportion of total variance explained by each variance component is shown. For number of bursal spines, a generalized linear model (Poisson distribution with log-link function) was used on raw (untransformed) values with raw EL as covariate and individual identity as an additional random effect (not shown).

|	Male traits						Female traits		
	Mean aedeagal spine length	Total aedeagal spine area	Aedeagus width	Aedeagus length	Flap length	Paramere length	Tract length	Mean bursal spine length	Number of spines
EL	0.5209***	0.3333***	0.4441***	0.2287	0.2598*	0.4698***	0.3872	1.1211**	0.0001
Diet	0.0031	−0.0049	−0.0023	−0.0027	−0.0070	<−0.0001	0.0113	0.0277*	0.0240
Order	0.0004	−0.0038	−0.0157**	−0.0065	−0.0079	0.0040	0.0266*	−0.0298	0.0608
Diet * Order	0.0006	0.0017	−0.0023	0.0041	0.0080	0.0011	−0.0127	0.0265	−0.0740
Family	0.1878	<0.0001	0.1794	<0.0001	0.1841	0.2950	0.0191	0.2352	<0.0001
Family * Diet	0.0199	0.1342	0.3653	0.1423	0.0666	0.0094	0.2175	0.0363	<0.0001

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
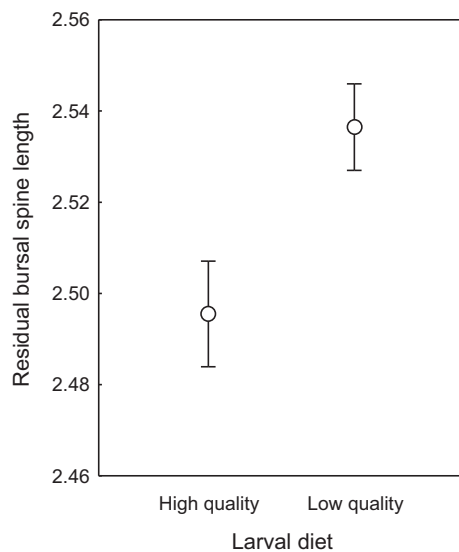
Fig. 2 Mean relative size (in pixels) of bursa spines across diet treatments. Bars indicate standard errors of least square means, based on analysis using log-transformed trait values and log-transformed mean elytra length as covariate.

spine length. The Family × Diet interaction accounted for a substantial proportion of variance for total spine area, aedeagus width and length, and female reproductive tract length.

Discussion

The results of this study suggest that condition has little effect on the sizes of genital traits of *C. maculatus*. No male genital traits were found to be condition dependent. It was in females that the only clear evidence of a response to diet manipulation *per se* was observed, with relative mean bursal spine length being greater in individuals reared on a low-quality diet. Although this shows that bursal spine length is sensitive to condition, the response is in the opposite direction to the expectation for condition-dependent traits (which are expected to be relatively larger in high-condition individuals). In addition, the order of presentation of the two larval diets had a significant effect on some male and female traits, suggesting that beetles may use environmental cues as proxies for manipulating the phenotype of their offspring via parental effects. However, because few significant effects were detected, and these effects were not predicted a priori, we cannot exclude the possibility that these effects are false positives.

As expected, individuals reared in a low-quality diet medium were smaller than those reared in a high-quality diet medium. Females showed a stronger response, consistent with other work showing that females express greater phenotypic variation across different developmental environments (Teder & Tammaru, 2005). In *C. maculatus*, the greater body size of females may translate into greater sensitivity to variation in diet quality because a larger portion of a bean's nutritional resources is consumed by a female larva than by a male larva. Responses to diet quality variation may also be mediated by maternal effects: previous studies have shown that females emerge earlier and lay relatively small eggs when they are grown in a high population density (Fox & Savalli, 1998), and both female and male condition affect the body size of offspring (Hallsson *et al.*, 2012).

Responses of genital traits to larval diet quality were generally equivalent to the response of overall body size (quantified as elytra length), such that no treatment effects were observed on the relative sizes of most traits. In the context of recent work on this system, it is interesting that no diet quality effect was observed on

the male genital spines. The lack of a dietary response in the relative length of the male genital spines would suggest that these structures are either not energetically costly to grow or, alternatively, not subject to directional sexual selection for length exaggeration. The beetles used in the current experiment were descended from laboratory populations maintained for many generations under polygamy, and a previous study showed that enforced monogamy led to the evolution of a shallower static allometry for the male genital spines (Cayetano *et al.*, 2011). This evolutionary response in monogamous populations suggests the presence of directional sexual selection on spine length in large males in polygamous populations. Thus, the most plausible explanation for the lack of heightened condition dependence of male genital spine length or its static allometry is that the development of the male genital spines does not impose a high demand on the developing embryo's resources, such that even males reared on a low-quality larval diet are able to produce genital spines of optimal length.

Among both male and female genital traits, only bursal spine length showed an overall response to diet quality treatment. Mean relative bursal spine length was greater under the low-quality diet treatment. The function of the bursal spines is poorly understood, but it has been suggested that these spines may function to break open spermatophores (van Lieshout *et al.*, 2014) or to limit the depth of intromission of the male aedeagus and thus potentially reduce damage caused by male aedeagal spines (Cayetano *et al.*, 2011). The possibility of a sexually antagonistic function for these spines also suggests a potential adaptive explanation for their response to diet quality. Reduced nutritional content of used beans might serve as a proxy for a high-density environment where sexual conflict is strong. Moreover, on a low-quality larval diet, females' body size advantage relative to males tends to be reduced, perhaps making females less adept at resisting male advances. If the bursal spines serve as a post-copulatory resistance trait, it may be advantageous for females that develop on a low-quality larval diet to express these spines at a greater relative size, thus compensating to some extent for their reduced ability to resist intromission.

For most of the male and female traits, family and/or the family × diet interaction accounted for a substantial proportion of variance. This is consistent with the presence of genetic variation in body size and relative sizes of genital structures, although maternal and common environment effects cannot be excluded because all offspring within a family share the same mother and were reared in the same Petri dish.

Order of presentation affected male aedeagus width and female reproductive tract length. These effects suggest maternal effects on genital traits (see Dowling *et al.* (2007) and Gay *et al.* (2009) for evidence of maternal effects on sperm length, as well as Hallsson *et al.* (2012)

for evidence of such an effect on body size in this species). Females can also modulate their egg-laying rate in response to relevant environmental cues, such as the availability of ovipositional medium (Messina & Slade, 1999). The functional significance (if any) of maternal adjustment of offspring genital traits in response to variation in bean quality remains unclear although, as noted above, bean quality may serve as a cue of the social environment that offspring are likely to experience.

Conclusions

The lack of a response to diet manipulation observed for most traits in this study suggests that these genital traits are not very costly to express, such that individuals in low condition can attain the same phenotypic mean as individuals in high condition. Given their small size in relation to the total size of the body, genital traits may typically represent a relatively small investment of nutrients and energy, and most species may therefore experience little selection for condition-dependent expression of such traits. It is also possible that male performance during copulation may depend more on the neuromuscular machinery involved in the deployment of genitalia than on the sizes of genital traits. Intriguingly, although we found no effect of diet on male aedeagal spines, we detected a significant negative effect of condition on the expression of female bursal spines. Our finding of weak condition dependence in genital traits is consistent with the results of several previous studies on other species (Schulte-Hostedde & Alarie, 2006; House & Simmons, 2007; Rodriguez & Al-Wathiqui, 2011). In contrast, other studies have detected significant effects of condition on the expression of genital traits in other arthropods (Arnqvist & Thornhill, 1998; Andrade *et al.*, 2005; Soto *et al.*, 2007; Cothran & Jeyasingh, 2010). A better understanding of the functions and expression costs of genital traits is required to resolve the causes of such variation, both among species and between traits within species.

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