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# The relative importance of genetic and nongenetic inheritance in relation to trait plasticity in *Callosobruchus maculatus*

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## Abstract

A trait's response to natural selection will reflect the nature of the inheritance mechanisms that mediate the transmission of variation across generations. The relative importance of genetic and nongenetic mechanisms of inheritance is predicted to be related to the degree of trait plasticity, with nongenetic inheritance playing a greater role in the cross-generational transmission of more plastic traits. However, this prediction has never been tested. We investigated the influence of genetic effects and nongenetic parental effects in two morphological traits differing in degree of plasticity by manipulating larval diet quality within a cross-generational split-brood experiment using the seed beetle *Callososbuchus maculatus*. In line with predictions, we found that the more plastic trait (elytron length) is strongly influenced by both maternal and paternal effects whereas genetic variance is undetectable. In contrast, the less plastic trait (first abdominal sternite length) is not influenced by parental effects but exhibits abundant genetic variance. Our findings support the hypothesis that environment-dependent parental effects may play a particularly important role in highly plastic traits and thereby affect the evolutionary response of such traits.

## Introduction

Both genetic and nongenetic inheritance may contribute to intergenerational change in phenotype and fitness. Although genetic inheritance involves the transmission of DNA-sequence variation, nongenetic inheritance encompasses a diverse array of proximate mechanisms that can mediate the transmission of environmental variation across generations (Bonduriansky & Day, 2009). Such environment-dependent parental effects operate in parallel with genetic inheritance. Theory predicts that the dynamics of intergenerational changes in phenotype and fitness and even the outcome of evolution may differ depending on the mechanisms of inheritance involved (Jablonka & Lamb, 2005; Danchin *et al.*, 2011; Day & Bonduriansky, 2011). Understanding the contributions of genetic and nongenetic mechanisms to a trait's inheritance is therefore of central importance for understanding its evolutionary history, as well as predicting its response to current natural selection. The relative importance of genetic and nongenetic inheritance mechanisms is likely to vary among different phenotypic traits within a population (Jablonka & Lamb, 2005). However, very little is known about the nature of such variation.

An important form of variation among traits that may be related to variation in the relative importance of genetic and nongenetic mechanisms of inheritance is developmental plasticity (Bonduriansky & Day, 2009). By definition, a larger fraction of total phenotypic variance is explained by environmental variation in highly plastic traits than in less plastic traits (Houle, 1992; Falconer & Mackay, 1996; Lynch & Walsh, 1998). The relative importance of nongenetic inheritance (e.g. parental effects) is likely to reflect the degree of trait plasticity for two reasons. First, whereas a nonplastic trait is subject exclusively to genetic effects, a plastic trait can be influenced by the environment, including

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the developmental environment provided by parental phenotypes. Second, in highly plastic traits, additive genetic variance may be masked by high environmental variation and therefore difficult to detect (Houle, 1992; Lynch & Walsh, 1998). Highly plastic traits may therefore be expected to exhibit abundant maternal and/or paternal effect variance, but little or no genetic variance, whereas the opposite pattern may be expected for traits with lower degree of plasticity.

It is well established that traits can differ in their degree of plasticity. For example, behavioural traits, life history traits and secondary sexual traits are known to be highly plastic in comparison with nonsexual (metric) morphological traits (Houle, 1992; Cotton *et al.*, 2004; Griffith & Ejima, 2009). In insects and many other animals, the resource availability in the environment is often a particularly important determinant of individual trait expression (Arnqvist & Thornhill, 1998; David *et al.*, 2000; West-Eberhard, 2003; Bonduriansky & Rowe, 2005). Some traits are greatly affected by resource abundance, whereas other traits appear to be buffered/canalized against environmental heterogeneity, resulting in similar expression across a range of environments (Waddington, 1942; Schmalhausen, 1949; De Visser *et al.*, 2003). However, in many cases, the quality of the environment is not only important for the individual that is exposed to it, but also for its progeny. Acquired environmental condition can be transferred across generations via parental effects (Fox & Savalli, 1998; Qvarnström & Price, 2001; Bonduriansky & Head, 2007). Thus, apart from its own external environment, an individual can also be affected by the environment experienced by its parents. Environment-dependent parental effects have been reported in many species (Mousseau & Fox, 1998; Bonduriansky & Head, 2007; Badyaev & Uller, 2009), including the seed beetle *Callosobruchus maculatus* (Fox, 1994; Fox & Savalli, 1998; Fox *et al.*, 2004).

A greater contribution of nongenetic mechanisms of inheritance to phenotypic variation in highly plastic traits will alter (and, indeed, greatly complicate) evolutionary predictions for such traits. Nongenetic mechanisms of inheritance that mediate the transmission across generations of environmentally induced (acquired) traits may influence the dynamics and course of evolution for several reasons (Jablonka & Lamb, 1995, 2005; Bonduriansky & Day, 2009; Danchin *et al.*, 2011; Day & Bonduriansky, 2011). In particular, nongenetically inherited factors can affect the phenotypic mean and variance of the population and thereby alter the pattern of selection on genetic variation. Because nongenetic inheritance can decouple change across generations in the population-mean phenotype from change in allele frequencies, nongenetic inheritance mechanisms can also have highly complex effects on evolutionary dynamics. Furthermore, the effects of nongenetic inheritance may interact with within-generation plasticity to affect evolutionary dynamics, and/or plasticity itself can influence evolution by speeding it up (Price *et al.*, 2003; West-Eberhard, 2003; DeWitt & Scheiner, 2004) or such that, for example, high environmental variation in the phenotype could reduce the additive genetic covariance between phenotype and fitness and thus reduce the response to selection in highly plastic traits (Kruuk *et al.*, 2002).

The rate of trait evolution will, of course, also critically depend on its additive genetic variance (Falconer & Mackay, 1996), as well as the structure of genetic covariances/correlations with other traits, which can facilitate or impede the response to selection (Lande, 1979; Walsh & Blows, 2009). Genetic correlations can be measured for the same trait across environments, but also between sexes for homologous traits (Lande & Arnold, 1983). A genetic correlation of less than one suggests partial independence of traits, which implies that independent evolution of traits/sexes is possible. The relation between additive genetic variance and plasticity is complex (Houle, 1992), whereas the relation between genetic variances/covariances and nongenetic inheritance is unknown.

Thus, both nongenetic and genetic mechanisms of inheritance could have important consequences for a trait's response to environmental change, and their relative importance might be dependent on trait plasticity. If this is true, understanding the evolution of such traits will require models that integrate the hypothesized evolutionary consequences of both trait plasticity and nongenetic inheritance.

To our knowledge, no study has as yet investigated the relative importance of genetic and nongenetic mechanisms of inheritance in traits of varying degrees of plasticity within a species. We predicted that highly plastic traits would be amenable to effects of parental condition, but genetic variation/covariance would be difficult to detect. In contrast, we expected that traits with a lower degree of plasticity would be less affected by parental condition, but genetic variation/covariance would be easier to detect. Although more plastic traits are, by definition, more susceptible to environmental effects, it is not inevitable that this sensitivity should extend to the parental environment (i.e. susceptibility to parental effects). Indeed, the opposite might be true if greater sensitivity to the ambient environment swamps out any prior effects of parental environment. To test our predictions, we conducted a split-brood, cross-generational experiment using the seed beetle *C. maculatus*. We manipulated an environmental factor (bean quality) of both parents and their offspring and examined the contributions of genetic and nongenetic parental effects to phenotypic variation in elytron length (a highly plastic trait) and the length of the first abdominal sternite (a much less plastic trait).

## Material and methods

### Study organism

We used the seed beetle *Callosobruchus maculatus* as a study organism. *Callosobruchus maculatus* is a cosmopolitan pest of stored legumes (Fabaceae). Mated female seed beetles cement their eggs to the surface of the host bean (Messina, 1993), and newly hatched larvae burrow into the seed. The larval development and pupation are completed entirely within a single host seed. Adults emerging from the bean are well adapted to storage conditions, requiring neither food nor water to reproduce. They live for an average of 10 days (without food or water supply); their entire life cycle from egg to egg is completed in 22–28 days at 28 °C (Messina, 1993). The seed beetles used were obtained from the Department of Primary Industries and Fisheries, Queensland, from an Australian population collected in Kingaroy in 2003. The population was initiated with 357 individuals grown on mung beans (*Vigna radiata*) and continued at 250–300 individuals per generation since that time. A sample of 600 beetles was obtained from this population and continued in the laboratory with approximately 500 individuals per 200 g of organic mung beans per generation. Beetles were kept at room temperature for approximately 18 generations prior to the experiment.

### Experimental set-up

We used a cross-generational split-brood design where bean quality was manipulated over two generations. Once-used mung beans served as the 'low-quality' larval diet treatment, and fresh mung beans were the 'high-quality' larval diet treatment. *Callosobruchus maculatus* females prefer unused beans as an oviposition substrate (Mitchell, 1975), and previous studies have shown that beetles reared on once-used beans emerge at a substantially smaller adult body size (Cayetano, 2010), suggesting that used beans provide a low-quality environment for *C. maculatus* larvae and produce low-condition adults. Correspondingly, our study showed that once-used beans yielded adult beetles of smaller body size (see Results). A pilot study revealed that elytron length (Fig. 1a) is a highly plastic trait (i.e. affected greatly by bean quality), whereas the length of the first abdominal sternite (Fig. 1b) is a trait with a low degree of plasticity (i.e. weakly affected by bean quality). We therefore selected these traits for our comparison of traits with high and low degree of plasticity.

We formed ten virgin male–female pairs ($F_0$ families) and allowed them to mate and lay eggs on 6 g low- and 6 g high-quality beans (Fig. 2). Both males and females were provided with each type of bean in randomized order; each bean type was presented for 24 h after which it was switched for each female. Six grams of beans is a sufficient amount to assure that females lay only one egg per bean. The beans with attached eggs were incubated at room temperature (23 °C). This resulted in a split-brood design, with full siblings from each family raised in contrasting environments. Emerging $F_1$ individuals were collected daily during their emergence period, the sexes were separated and a subset of the $F_1$ individuals emerging from each type of bean was likewise paired and provided with fresh and used beans to create $F_2$ progeny reared in contrasting environments.

$F_1$ individuals are referred to as 'parents', whereas $F_2$ individuals are referred to as 'offspring'. $F_1$ individuals are characterized by their sex and environmental condition, based on whether they were raised on fresh or used beans. We refer to each of the parental sex (male, female) and condition (high, low) combinations with the following terms: Parent male high, parent male low, parent female high and parent female low.

From each $F_0$ family, six randomly chosen, virgin $F_1$ males and females were mated to a randomly chosen individual of the opposite sex (random female; random male) each in order to create the $F_2$ generation. Virgin mates were randomly sampled from the stock population. By mating each low-/high-quality male/female to a randomly assigned mate from the stock population, we eliminate a potential effect of an interaction of parental conditions on the resulting offspring. Again each male–female pair was allowed to mate and lay eggs on both 6 g low- and high-quality beans; each

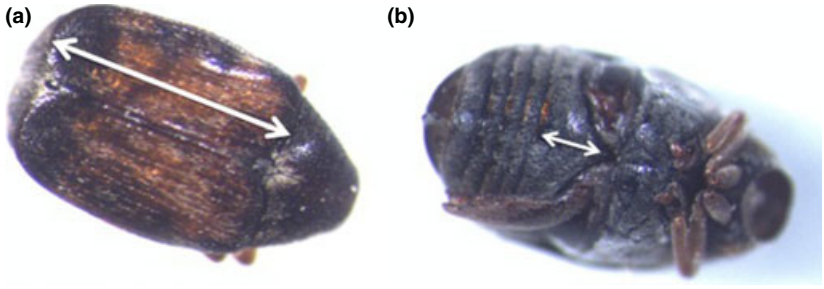


**Fig. 1** (a) Dorsal and (b) Ventral surface of *Callosobruchus maculatus*. Arrows are indicating the parts (elytron length and first abdominal sternite length) measured.

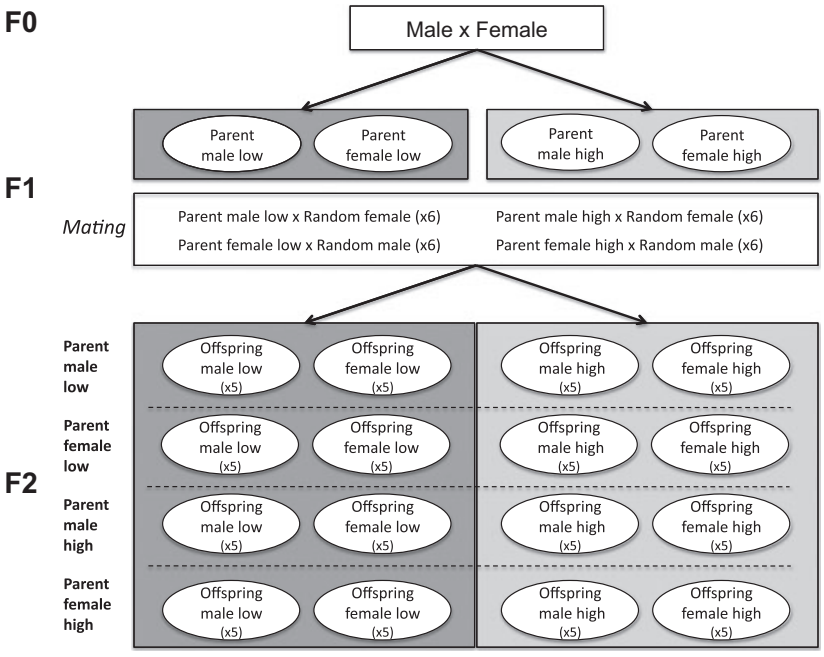


**Fig. 2** Schematic illustration of the cross-generational split-brood experiment. Shown for one of in total ten $F_0$ families.

source was presented for 24 h, order randomized. This set-up resulted in 24 mating pairs (4 parental sex × condition combinations, 6 replicates per combination), each laying eggs on new and used beans ($N = 48$ Petri dishes), from each $F_0$ family, and in a total of 240 mating pairs and 480 Petri dishes for the ten $F_0$ families. Thus, each Petri dish contained a unique combination of family, sex and condition of parent and quality of bean. Beans were incubated at room temperature (23 °C) and emerging offspring ($F_2$ generation) counted, sexed and frozen. $F_2$ individuals are characterized by sex and condition of parent, their own sex and environmental condition they were raised in. We refer to each of the offspring–sex–condition combinations using the following terms: offspring male high, offspring male low, offspring female high and offspring female low.

Individuals of generation $F_2$ (five males and females per Petri dish) were dried in an oven at 50 °C for 48 h, imaged and measured. To quantify lengths of the elytron and the first abdominal sternite, each beetle's dorsal and ventral surface was photographed at 16 × magnification with a Leica DFC420 digital camera (Leica Microsystems, Heerbrugg, Switzerland) mounted on a Leica MS5 stereoscope (Leica Microsystems). From images, the respective lengths were measured using image analysis software (IMAGEJ 1.34s; National Institutes of Health, Bethesda, MD, USA).

## Statistical analysis

The analysis is based on data for $F_2$ individuals, with the two traits, elytron length and first abdominal sternite length, analysed in separate univariate models. Because of the complexity of the design, as well as missing data (resulting from emergence failure or highly biased sex ratio in some replicates), we were unable to test all effects within one model. We therefore used a combination of two different analyses.

First, to test for effects of $F_1$ (i.e. parental) sex and diet and $F_2$ (i.e. offspring) sex and diet on $F_2$ (i.e. offspring) phenotype, we carried out analysis of variance on means for $F_2$ offspring within each $F_0$ family, with $F_1$ sex, $F_1$ diet, $F_2$ sex and $F_2$ diet as within-subjects factors (Table 1). This analysis was thus based on up to 16 $F_2$ means (2 $F_1$ sexes × 2 $F_1$ diets × 2 $F_2$ sexes × 2 $F_2$ diets) for each $F_0$ family (some cells were missing for some families – see Results for actual degrees of freedom). We also performed separate analyses for offspring of $F_1$ females and males. Data were standardized within $F_0$ families to minimize family effects, and a separate analysis was carried out for each trait (Table 1). These analyses were performed in STATISTICA 7.0 (StatSoft Inc., Tulsa, OK, USA).

Second, to estimate quantitative genetic parameters for elytron length and first abdominal sternite length within each $F_1$ (i.e. parental) sex and diet combination, we fitted four linear mixed effects models for each trait (Table 1). For each trait, separate models were fitted for each combination of parental sex and diet (i.e. Parent male high, Parent male low, Parent female high and Parent female low). Environmental condition of offspring (low vs. high) and offspring sex were fitted as fixed effect predictors, and $F_0$ family and $F_1$ replicate identity (nested within family) were included as random effects. As we were also interested in the

**Table 1** Statistical models fitted to the $F_2$ individuals; elytron length and first abdominal sternite length were analysed in separate univariate models.

| | Single-trait model | Predictor and response variables |
|---|---|---|
| Repeated measures ANOVA | Y ~ | Response variable; Y = $F_2$ mean Elytron length or $F_2$ mean first abdominal sternite length ($F_2$ mean is the trait mean of $F_2$ within each $F_0$ family) |
| Full model | $F_1$ diet * $F_1$ sex * $F_2$ diet * $F_2$ sex | Within-subjects factors |
| For separate $F_1$ sexes | $F_1$ diet * $F_2$ diet * $F_2$ sex | Within-subjects factors |
| MCMCglmm | Y ~ | Response variable; Y = $F_2$ Elytron length or $F_2$ first abdominal sternite length |
| (linear mixed | $F_2$ diet * $F_2$ sex + | Fixed effects |
| effects model; | us ($F_2$ diet * $F_2$ sex): $F_0$ Family + us | Random effects and their interaction with fixed effects‡ |
| Bayesian framework)† | ($F_2$ diet * $F_2$ sex): $F_1$ Replicate + | |
| | idh ($F_2$ diet * $F_2$ sex):units | Heterogeneous residual variance§ |

†Fitted for each $F_1$ (i.e. parental) diet and sex combination (i.e. for four combinations: Parent male high, Parent male low, Parent female high, Parent female low).
‡us-matrix: both variances and covariances are estimated; $F_1$ Replicate = $F_1$ Replicate is nested within $F_0$ family.
§idh-matrix: only variances are estimated, covariances are set to zero.

covariances between sexes and between environments, we fitted the interactions of offspring sex and offspring condition with the two random effects (random slopes). For the family identity and the replicate identity random effects, we estimated unstructured variance–covariance matrices, that is, one variance for each offspring condition and offspring sex combination (four variances) and all covariances between them (six covariances). We fitted heterogeneous residual variances; in the residual variance–covariance matrix, all covariances were fixed to zero, because each individual was measured in only one environment, and hence, there is no replication to estimate a residual covariance.

These analyses were performed using the MCMCglmm package (Hadfield, 2010) in R 2.13.0 (R Development Core Team, 2011). We used uninformative proper priors for both fixed and random effects. Fixed effect priors were normally distributed with expected value (mean) zero [mu = rep (0, 4)] and degree of belief (variance) of $10^8$ [V = diag (4)*$10^8$]. Random effects priors (one for each random effect and its interactions with fixed effects) were inverse Wishart distributed with expected (co)variances [V = diag (4)] and degree of belief parameter (nu = 3.002). We allowed the Markov chain a burn-in period of 10 000 iterations, after which we ran 60 000 iterations and sampled every 50th iteration from the posterior distribution, resulting 1200 stored values per chain. These settings resulted in appropriate conversion of the chain. Conversion was assessed by checking for potential autocorrelations of consecutive values in the chain and via visual inspection of potential trends in the chain as well as of the shape of the posterior density distribution of fixed and random effects, respectively. Autocorrelation between consecutive values was low (< 0.04), there were no trends in the chain and posterior distributions were not skewed. Fixed effects were deemed significant if their 95% credible interval (95% CI) does not include zero. We tested for significance of random effects using model comparison based on the deviance information criterion (DIC) (Hadfield, 2010; Wilson *et al.*, 2010). The posterior genetic variance–covariance matrix did not differ between different parental condition–sex combinations within each trait; thus, we pooled the data for all parental condition and sex combinations and calculated the relevant genetic variances and correlations based on the resulting posterior genetic variance covariance matrix. We were interested in genetic correlations between sexes ($r_{MF}$) for each $F_2$ environment and between environments ($r_{LH}$) for each sex. Genetic correlations are assessed by dividing the genetic covariance between traits/sexes by the square root of the product of genetic variances for each of the traits/sexes. Genetic correlations are significantly different from zero if their 95% CI does not overlap zero (Hadfield, 2010).

## Results

### Elytron length (highly plastic trait)

We found strong effects of offspring diet and offspring sex across the entire data set (repeated measures ANOVA, Offspring diet: $F_{1,4} = 51.01$, $P = 0.0020$; Offspring sex: $F_{1,4} = 10779.93$, $P < 0.0001$), as well as within each of the parental sex and diet combinations (Table 2). Elytron length was significantly reduced on lower offspring diet, and offspring males had significantly shorter elytron length compared with offspring females (Fig. 3). There was no interaction between offspring diet and offspring sex (Table 2).

We found a significant overall effect of parental diet on offspring elytron length (repeated measures ANOVA, $F_{1,4} = 7.92$, $P = 0.0481$). However, this effect reflected a parental diet × parental sex × offspring sex interaction ($F_{1,4} = 36.66$, $P = 0.0037$): the paternal diet effect

**Table 2** Effects of offspring diet and offspring sex and effects of family and replicate identity on elytron length and first abdominal sternite length.

| | Parent male high | Parent male low | Parent female high | Parent female low |
|---|---|---|---|---|
| *Fixed effects*[1] | | | | |
| | 95% CI | 95% CI | 95% CI | 95% CI |
| Elytron length | | | | |
| Offspring diet | −16.18; −0.84**‡ | −29.70; −5.57**‡ | −24.62; −6.748*** | −23.16; −5.58*** |
| Offsrping sex | −82.60; −66.51*** | −82.38; −64.18*** | −82.68; −70.49*** | −76.47; −63.97*** |
| Offspring diet × Offspring sex | −16.74; 8.15 | −16.39; 17.64 | −8.61; 15.13 | −8.04; 15.52 |
| First abdominal sternite | | | | |
| Offspring diet | −9.31; 2.35 | −9.74; 1.96 | −9.61; 0.30† | −8.81; 0.79† |
| Offspring sex | −57.42; −45.19*** | −56.39; −42.22*** | −58.80; −46.80*** | −52.56; −42.98*** |
| Offspring diet × Offspring sex | −6.06; 12.38 | −7.29; 9.65 | −0.76; 15.51†‡ | −2.28; 10.92 |
| *Random effects*[2] | | | | |
| | DIC | DIC | DIC | DIC |
| Elytron length | | | | |
| Full model | 6010.53 | 3311.29 | 5719.14 | 5816.43 |
| Family | 6024.23 | 3312.58 | 5717.57 | 5814.79 |
| Replicate | 6020.01 | 3312.97 | 5741.30 | 5834.94 |
| First abdominal sternite | | | | |
| Full model | 5418.28 | 2889.91 | 5124.49 | 5014.92 |
| Family | 5430.16 | 2902.57 | 5123.11 | 5016.59 |
| Replicate | 5431.88 | 2889.45 | 5145.07 | 5033.21 |

A mixed effects random slope model was fitted for each parental sex and condition (Parent male in high condition; parent male in low condition; parent female in high condition; parent female in low condition). Fixed effects are presented with 95% credible intervals (95% CI). Deviance information criterion (DIC) is presented for full and reduced models.
[1]Significance codes: for fixed effects 0 '***' 0.001 '**' 0.01 '*' 0.05 '†' 0.1 '‡' 1.
[2]Significance of random effects was accessed using model comparison based on the deviance information criterion (DIC).

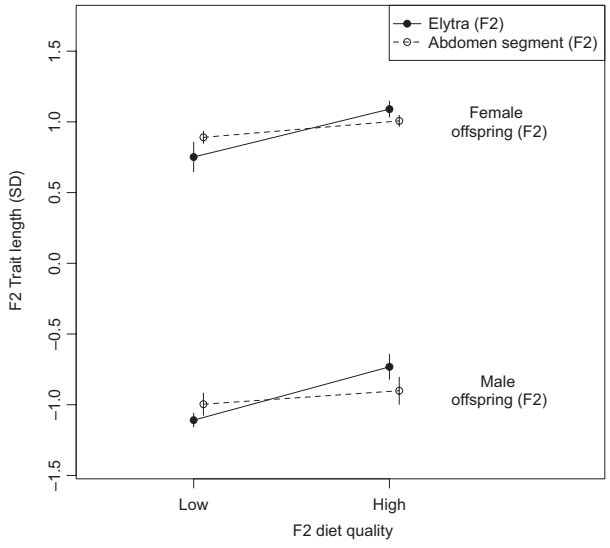


**Fig. 3** Effects of offspring sex and offspring diet on elytron length and first abdominal sternite length.

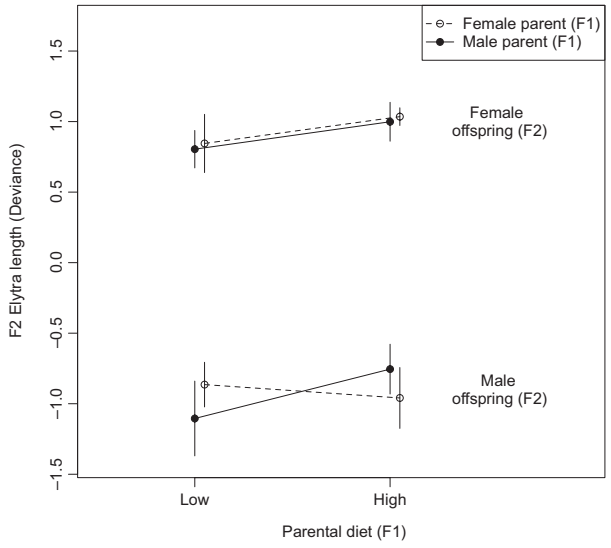


**Fig. 4** Effects of maternal and paternal condition on offspring elytron length. Daughters but not sons are affected positively by maternal condition.

was positive for offspring of both sexes (i.e. fathers reared on high-quality beans produced larger offspring), whereas the maternal effect was positive for daughters but weakly negative for sons (Fig. 4). Similar effects were obtained in separate analyses for offspring of $F_1$ males and females: we observed a positive paternal diet effect for $F_2$ offspring of both sexes (repeated measures ANOVA, $F_{1,4} = 13.99$, $P = 0.0201$), whereas the maternal diet effect was not significant overall (repeated measure

**Table 3** Sex (males, females) and diet (low, high)–specific genetic variances, correlations between sexes ($r_{MF}$) within diet and correlation of trait across diets ($r_{LH}$) within sex for elytron length and first abdominal sternite length.

| | Genetic variances | | | | Genetic correlation | | | |
|---|---|---|---|---|---|---|---|---|
| | Male | | Female | | $r_{MF}$ | | $r_{LH}$ | |
| | Low | High | Low | High | Low | High | Male | Female |
| Elytron length | | | | | | | | |
| Posterior mode | 2.127 | 3.280 | 1.731 | 1.986 | 0.912 | 0.895 | 0.933 | 0.859 |
| 95% – CI | 0.367; 79.429 | 0.390; 30.652 | 0.175; 30.156 | 0.261; 24.856 | −0.797; 0.996 | −0.667; 0.989 | −0.391; 0.996 | −0.827; 0.983 |
| First abdominal sternite length | | | | | | | | |
| Posterior mode | 17.171 | 22.805 | 15.112 | 4.122 | **0.964** | **0.952** | **0.982** | **0.929** |
| 95% – CI | 3.953; 76.918 | 4.073; 85.656 | 3.104; 63.476 | 0.579; 32.927 | 0.588; 0.996 | 0.129; 0.993 | 0.747; 0.996 | 0.018; 0.994 |

Presented with 95% credible intervals (95% CI). Significant difference from zero is indicated in boldface.

ANOVA, $F_{1,8} = 0.14$, $P = 0.72$), but interacted significantly with offspring sex (repeated measure ANOVA, $F_{1,8} = 5.92$, $P = 0.0409$).

For the MCMC results, we found that except for males reared on high diet, the effect of family was not significant (Table 2) and genetic variances were generally low and hard to detect for elytron length (Table 3). Both the within-sex between environment (diet) genetic correlations and the genetic correlations between sexes (within diets) were not different from zero (Table 3).

### First abdominal sternite length (trait with low degree of plasticity)

We found a strong overall effect of offspring sex on offspring first abdominal sternite length (repeated measures ANOVA, Offspring sex: $F_{1,4} = 65315.72$, $P < 0.0001$) and a weaker effect of offspring diet (repeated measures ANOVA, Offspring diet: $F_{1,4} = 8.29$, $P = 0.0450$) (Fig. 3). The effect of offspring sex was highly significant in separate analyses for offspring of $F_1$ males and females (repeated measures ANOVAs, Offspring sex: $F_{1,4\text{-}8} > 5000$, $P < 0.0001$), but the effect of offspring diet was not (repeated measures ANOVAs, Offspring diet: $F_{1,4\text{-}8} < 3$, $P > 0.13$). The effect of offspring sex was also significant within each parental sex and diet combination (Table 2). There was no interaction between offspring diet and offspring sex, except a marginally significant interaction for offspring of mothers reared on high diet (Table 2).

We could not detect any overall effect of parental diet on offspring first abdominal sternite length (repeated measure ANOVA, $F_{1,4} = 1.13$, $P = 0.3469$), nor any interaction of offspring sex or environment with parental diet (repeated measures ANOVA, $F_{1,4} < 0.3$, $P > 0.5$). Similar results (not shown) were obtained in separate analyses for offspring of $F_1$ males and females.

However, the MCMC results revealed that the effect of family was significant in all parental sex by diet combinations except for offspring of mothers reared on high diet (Table 2), and we were able to detect significant genetic variances; genetic correlations across environments (for males and females) and the genetic correlations between sexes (within diets) were high (> 0.90) and significantly different from zero (Table 3).

## Discussion

We performed a cross-generational split-brood experiment, manipulating the quality of the environment (i.e. bean quality) over two generations to test the prediction that a highly plastic trait will be more heavily influenced by parental effects and genetic variances will be more difficult to detect compared to a trait with lower degree of plasticity. We found that elytron length (highly plastic trait) differed between sexes and was influenced both by diet and by parental effects, whereas genetic variance could not be detected for this trait. Further, genetic correlations between sexes and environments were low and did not differ from zero. In contrast, first abdominal sternite length (less plastic trait) was found to differ between sexes, was less strongly affected by diet and was not affected by parental effects. We were able to detect significant genetic variance for this trait, and genetic correlations between sexes and environments (diets) were significantly greater than zero and generally high (> 0.90).

The observed pattern is consistent with the expectation that highly plastic traits will be more amenable to parental effects, but additive genetic variation and genetic correlations will be difficult to detect. Parental effects are expected to be more important for highly plastic traits because the expression of such traits is sensitive to the ambient conditions, including the environment provided by parental phenotypes (Bondurianskey & Day, 2009). Morphological traits vary markedly

in their degree of plasticity, with genitalic traits typically exhibiting very low plasticity and secondary sexual traits often showing extreme plasticity in response to nutrient availability (Cotton *et al.*, 2004; House & Simmons, 2007; Ramm *et al.*, 2010; Rodriguez & Al-Wathiqui, 2011). In insects, a developmental mechanism capable of contributing to this variation has recently been identified: differential sensitivity of different adult tissue precursors to signalling via the insulin/insulin-line growth factor pathway (Emlen, *et al.* 2012). Support for our predictions in terms of both maternal and paternal effects lends credence to our conclusions. The difficulty in detecting genetic variances/covariances for highly plastic traits reflects the fact that a great amount of environmental variation is present in the phenotypic variance, and this environmental variance could 'mask' the genetic variance. In contrast, traits with lower degree of plasticity are likely to be relatively insensitive to the developmental environment and parental influences on this environment, whereas genetic variance will comprise a larger proportion of phenotypic variance for such traits. Importantly, the additive genetic variance for highly plastic traits could potentially be as high as for less plastic traits if estimated in a perfectly controlled environment. Indeed, significant additive genetic variance for elytron length in *C. maculatus* has been detected in a previous study (Messina, 1993), possibly reflecting greater genetic variation in the study population or a difference in the ambient environment.

### Parental effects

We detected effects of both maternal and paternal larval diet quality – that is, parental condition – in the highly plastic trait (elytron length). Effects were generally positive, that is, offspring of parents in high condition were larger than offspring of parents in low condition. The exception was the apparent absence of a maternal effect on sons. This suggests that individuals reared on a high-quality diet may pass their acquired condition to their progeny, thus potentially enhancing offspring fitness (Mousseau & Fox, 1998; Qvarnström & Price, 2001).

Environmentally induced maternal effects have been shown to be important in this species (Fox & Savalli, 1998; Fox *et al.*, 2004). For instance, Fox & Savalli (1998) showed that an environmentally induced reduction in body size is transmitted to the progeny by a nongenetic maternal effect. Our results are consistent with these findings. However, surprisingly, we found the maternal effect to be sex specific – positive for daughters but slightly negative or nil for sons (Fig. 4). This could be due to mothers investing their acquired resources differentially in offspring of different sexes, as observed in birds, lizards and mites (Nagelkerke & Sabelis, 1998; Cordero *et al.*, 2000; Radder *et al.*, 2009). It is also possible that male and female offspring are affected differently by condition-dependent factors transferred to eggs by their mothers.

We also found significant paternal effects on offspring body size. Paternal effects on offspring viability have been reported in this species (Savalli & Fox, 1998; Bilde *et al.*, 2008), but this is to our knowledge the first study showing that acquired paternal condition affects offspring body size in *C. maculatus*. Paternal effects in *C. maculatus* are most likely mediated by seminal fluids in the large ejaculate (approximately 8% of male body weight) (Savalli & Fox, 1998), which have been shown to positively affect female egg production (Savalli & Fox, 1998; Fox *et al.*, 2006). Nutrients in the male ejaculate have also been shown to be incorporated into female reproductive and somatic tissue (Boucher & Huignard, 1987). Fox *et al.* (2006) showed that larval diet has significant effects on ejaculate size in *C. maculatus*. This suggests that bean quality could have an effect on male ejaculate size which in turn could affect the female and the offspring. However, it remains unclear what specific components (if any) of the ejaculate (i.e. nutrients, water) actually mediate the observed paternal effect.

### Evolutionary implications

Our findings have important implications for the study of evolutionary consequences of genetic and parental effects as well as their relative importance in shaping adaptive evolution of traits that differ in their degree of plasticity. Our results suggest that the relative importance of nongenetic inheritance, here found in the form of trans-generational transmission of environmental variation in condition (Bonduriansky & Day, 2009), is more important in a highly plastic trait. This implies that in a trait with high plasticity, phenotypic change is decoupled from genotypic change to a greater extent compared with a trait that is less plastic. By this decoupling, some limitations associated with genetic inheritance are removed, allowing the phenotypic distribution of a population to change in ways that might not be possible otherwise (Bonduriansky & Day, 2009). The phenotypic mean and variance in turn can influence selection on genes, which implies that the presence of trans-generational environment-dependent paternal effects could impact the evolution of the trait. The greater role of nongenetic inheritance could thereby provide an additional mechanism whereby a trait's plasticity could influence its evolution.

The more plastic a trait is, the more room there is for effects of an individual's own environment and its parents' environment to influence the trait's expression. The response of such a trait is likely to be different from a trait with lower degree of plasticity, for several reasons. First, its heritability might be affected and potentially different due to environment-dependent

parental effects (Jablonka & Lamb, 1995, 2005; Qvarnström & Price, 2001; Bonduriansky & Head, 2007). This suggests that such effects can contribute to the pool of heritable variation and thus influence the potential to respond to selection and therewith impact evolution (Bonduriansky & Day, 2009). Second, plasticity itself can influence the dynamics and course of evolution, because it allows for responses that cannot be achieved by genes alone: rapid response to environmental change (Baldwin, 1896; Lande, 2009), rapid amplification of phenotypic variation in novel environments (West-Eberhard, 2003) and facultative responses to environmental variation involving fine-tuning of the phenotype (Hinton & Nowlan, 1987). Environmentally induced phenotypes can also become genetically assimilated (Waddington, 1953; West-Eberhard, 2003). A highly plastic trait therefore has various possibilities – besides a response that is purely based on the genetic variance – to respond to a change in the environment compared to a trait with lower degree of plasticity.

It remains to be determined whether the relative importance of genetic and nongenetic inheritance is related to the degree of trait plasticity in a linear/proportional way. This relationship could be established by investigating more than two traits with different degree of plasticity. Also, it would be interesting to ascertain the extent to which trait plasticity is associated with the importance of different mechanisms of nongenetic inheritance (e.g. transmission of epigenetic marks, nutrients or hormones). Finally, it is important to verify the generality of our findings by testing for an association between plasticity, nongenetic inheritance and genetic variation in other species, and using other types of environmental variation.

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