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Genetic and nutritional effects on male traits and reproductive performance in *Tribolium* flour beetles

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Abstract

In *Tribolium* flour beetles and other organisms, individuals migrate between heterogeneous environments where they often encounter markedly different nutritional conditions. Under these circumstances, theory suggests that genotype-by-environment interactions (GEI) may be important in facilitating adaptation to new environments and maintaining genetic variation for male traits subject to directional selection. Here, we used a nested half-sib breeding design with *Tribolium castaneum* to partition the separate and joint effects of male genotype and nutritional environment on phenotypic variation in a comprehensive suite of life-history traits, reproductive performance measures across three sequential sexual selection episodes, and fitness. When male genotypes were tested across three nutritional environments, considerable phenotypic plasticity was found for male mating and insemination success, longevity and traits related to larval development. Our results also revealed significant additive genetic variation for male mating rate, sperm offence ability (P_2), longevity and total fitness and for several traits reflecting both larval and adult resource use. In addition, we found evidence supporting GEI for sperm defence ability (P_1), adult longevity and larval development; thus, no single male genotype outperforms others in every nutritional environment. These results provide insight into the potential roles of phenotypic plasticity and GEI in facilitating *Tribolium* adaptation to new environments in ecological and evolutionary time.

Introduction

A fundamental goal in evolutionary ecology is understanding how organisms adapt to heterogeneous environments, which in turn requires knowing how genetic and environmental factors act jointly to mediate organismal trait expression. Phenotypic plasticity occurs when a single genotype exhibits altered phenotypes within different environments, and is described by a genotype's reaction norm. Adaptive phenotypic plasticity may facilitate the colonization of new environments (Schlichting & Pigliucci, 1998; West-Eberhard, 2003; Ghalambor *et al.*, 2007). Genotype-by-environment interactions (GEI) are of considerable evolutionary interest because they indicate the variation between genotypes in their reaction norms, and thus can influence a population's potential to adapt to new environments.

In addition, GEI have often been invoked as a possible mechanism maintaining the high genetic variation that is observed in sexually selected traits (e.g. Pomiankowski & Møller, 1995; Bussière *et al.*, 2008; Ingleby *et al.*, 2010). The presence of such genetic variation is unexpected because it should be depleted by directional selection acting via mate choice or competition (Kirkpatrick & Ryan, 1991; Rowe & Houle, 1996; Hine *et al.*, 2004). However, GEI can potentially cause some male genotypes to perform optimally in certain environments, whereas different genotypes excel in other environments (Gillespie & Turelli, 1989; Rodriguez & Greenfield, 2003; Hunt *et al.*, 2004; Danielson-François *et al.*, 2006). GEI are especially relevant to populations where individuals

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migrate between environments with spatially variable selection, or when populations have overlapping generations that experience temporal changes in selection (Kokko & Heubel, 2008). When such interactions change the relative performance rankings of genotypes across environments (ecological crossover), genetic variation could be maintained even in the face of consistent selection acting within environments.

In spite of this, most classical studies of sexual selection have been conducted under a single set of environmental conditions (Andersson, 1994). Although these studies have greatly expanded our knowledge concerning the targets of selection, they do not account for the fact that the environments in which reproductive traits and performance evolve will vary both spatially and temporally. Recently, an increasing number of empirical studies have examined GEI for sexual traits (reviewed by Bussière *et al.*, 2008; Ingleby *et al.*, 2010). For example, male ultrasonic signals in the lesser waxmoth *Achroia grisella* exhibit GEI under environments that differ with respect to larval food quality, density and temperature (Jia *et al.*, 2000). Crossover GEI are also present for males' attractiveness based on these signals (Danielson-François *et al.*, 2006). Additionally, Rodriguez & Greenfield (2003) documented GEI for female preferences across different temperature environments. Thus, these studies provide evidence that GEI can help maintain genetic variation in a male signal that is subject to directional intersexual selection.

One gap in our knowledge is based on the fact that these and other empirical studies addressing the role of GEI in sexual selection have focused nearly exclusively on traits related to mating success, such as male courtship signals (see table 1 in Ingleby *et al.*, 2010). Importantly, the vast majority of taxa have polyandrous females that mate with multiple males (Jennions & Petrie, 2000; Simmons, 2001). Thus, male reproductive success is often determined by processes such as sperm competition and cryptic female choice that operate during and after mating (Parker, 1970; Eberhard, 1996, 2009; Simmons, 2001; Birkhead & Pizzari, 2002). In spite of this, remarkably few studies have examined GEI for any male traits or performance measures related to these important post-copulatory episodes of sexual selection (but see, e.g., Ward, 1998; Engqvist, 2008; Morrow *et al.*, 2008).

Finally, although most selection studies have focused on morphological traits, selection is also predicted to act on whole-organism performance such as foraging ability, running speed and mating rate (Arnold, 1983; Kingsolver & Huey, 2003; Irschick *et al.*, 2007). A more complete understanding of GEI and sexual selection will require testing whether GEI exist for composite measures of reproductive performance, including mating rates, insemination success and sperm competitive ability.

The flour beetle *Tribolium* (Coleoptera: Tenebrionidae) has become a model study organism for understanding sequential episodes of sexual selection (reviewed by Fedina & Lewis, 2008). *Tribolium* are worldwide stored-product pests that consume many different food products stored by humans (Good, 1936; Sokoloff, 1974; Levinson & Levinson, 1994); thus, *Tribolium* beetles inhabit spatially discrete food patches that vary widely in their nutritional quality (Baker & Loschiavo, 1987). Numerous studies have provided insight into pheromones used by *Tribolium* males to attract females (Suzuki, 1981; Arnaud *et al.*, 2002; Ming & Lewis, 2010), as well as mating behaviour of both sexes (Lewis & Iannini, 1995; Edvardsson & Arnqvist, 2000; Bloch Qazi, 2003). Females of the red flour beetle, *Tribolium castaneum* Herbst, are highly promiscuous, and they store and subsequently utilize sperm from multiple males (Lewis & Jutkiewicz, 1998; Lewis, 2004; Lewis *et al.*, 2005; Pai *et al.*, 2005, 2007). As a result, a male's reproductive success depends to a large extent on his gaining paternity share through post-mating processes (Lewis & Austad, 1990; Haubruge *et al.*, 1997; Bernasconi & Keller, 2001; Nilsson *et al.*, 2003; Pai & Yan, 2003; Fedina & Lewis, 2004, 2006, 2007; Fedina, 2007). Previous studies in *T. castaneum* found genetic strain × environment interactions across different flour types for the life-history traits of 14-day larval weight (Hardin *et al.*, 1967), larval development time and pupal weight (Via, 1991). In addition, Via & Conner (1995) documented within-strain GEI for pupal weight and development time across different flour types. Thus, although GEI have been shown for traits related to larval resource acquisition, it remains to be investigated whether *T. castaneum* exhibit GEI for adult resource use and reproductive performance across different nutritional environments.

In this study, we conducted a quantitative genetic analysis to investigate the separate and joint effects of genotype and environment in *T. castaneum* using a nested half-sibling design in which families were split across different nutritional environments. We experimentally manipulated diet nutritional quality across a representative range that might be encountered by natural *Tribolium* populations by mixing wheat flour with non-nutritive cellulose. For sons from each family reared on three levels of diet quality, we measured life-history traits (larval growth rate, development time and pupal weight) and traits reflecting adult resource use (starvation resistance and resource reacquisition). We also measured male performance during sequential episodes of sexual selection: precopulatory (mate attraction), pericopulatory (mating rate and insemination success) and post-copulatory (sperm offence and sperm defence) reproductive performance. Finally, we measured adult longevity and assessed males' relative reproductive success in a realistic population context to estimate males' total fitness. By examining genetic and nutritional effects across this comprehensive suite of male traits, this study aimed to gain insight into the potential roles of phenotypic plasticity and GEI in facilitating *Tribolium* adaptation to new environments in ecological and evolutionary time.

Methods

Synthetic population

Tribolium castaneum for this experiment were derived from an outbred strain that we created specifically to restore whatever genetic variability might have been previously depleted by selection. This synthetic strain was created by systematically crossing a laboratory strain with two strains recently collected from field populations (H-1 and P-1 strains provided by Dr James Campbell, USDA Agricultural Research Service). Parental crosses consisted of seven replicates for each of the six possible male–female crosses among these three strains. Offspring from each parental cross were equally represented and combined into a single large population (> 500 individuals) that was maintained for four generations on King Arthur enriched wheat flour under standard conditions (29 °C and 70% RH in a dark incubator) before starting the breeding experiment described below. Throughout the study, beetles were maintained at 29 °C and 70% RH in a dark incubator.

Manipulating diet quality

Like other stored-product pests, *Tribolium* exist in discrete populations and often inhabit food patches that differ widely in their nutritional quality (Good, 1936; Sokoloff, 1974). In this study, we designed a highly repeatable manipulation of diet quality by adding to wheat flour various ratios of microcrystalline cellulose (Lattice NT200; FMC Biopolymer, Philadelphia, PA, USA), a non-nutritive and nontoxic filler that matches the particle size of wheat flour. Previous work has shown that these diets closely resemble other nutritionally poor diets in their effects on pupal mass (Fedina & Lewis, 2007). The major advantage of this approach is that it provides a repeatable, quantitative alteration in nutritional content that is unconfounded by qualitative differences in nutrient composition among different grain types.

We created three diets that differed in nutritional quality using King Arthur unbleached enriched wheat flour mixed with Lattice NT200 to produce three treatments: low (1 flour : 4 filler), medium (1 : 2) and high (4 : 1) nutritional quality. Larval cannibalism was eliminated as a possible confounding factor by keeping larvae and adults individually in 0.5-mL microfuge tubes with their respective diets.

Breeding design

To estimate genetic, diet and GEI effects, this study used a nested half-sib, split brood design in which 12 randomly selected sires were each mated to three randomly selected dams, and eggs collected from each maternal family were distributed and reared in each of the three levels of diet quality. This paternal half-sibling design allowed us to partition the additive from nonadditive genetic variation for male traits and performance (Roff, 1997; Lynch & Walsh, 1998; Conner & Hartl, 2004). Traits, reproductive performance and fitness were measured as described below for 3–10 focal males from each diet/family combination. Based on the large number of sons measured (~340) and the time-consuming nature of several behavioural measurements, we conducted this experiment in three consecutive time blocks.

Male life-history traits

For each family and diet combination, we measured larval growth rates (weight gain to larval day 14), development time (days from egg to pupa) and pupal weight. Pupae and day 14 larvae were weighed to the nearest 0.01 mg (Mettler AT261 balance; Mettler Toledo, Columbus, OH, USA). Using an average egg weight of 0.03 mg for *T. castaneum* (Howe 1968), we calculated relative growth rate (von Bertalanffy, 1960) as:

$$\text{Relative growth rate (RGR)} = \frac{(\text{Ln 14 days mass}) - (\text{Ln 0.03 mg})}{14\ \text{days}}$$

Beetles were sexed at the pupal stage based on their genital lobe morphology, and focal males for adult measurements were randomly selected from each diet/family combination. An index of adult body condition (Jakob *et al.*, 1996) for each focal male was calculated as adult body mass (measured in mg at 3 weeks post-eclosion) divided by pronotal width (in mm).

Adult resource use

Tribolium are income breeders (*sensu* Stearns, 1992), with adult food intake used to fuel reproductive activities. Because they may experience wide fluctuations in resource availability within and between food patches (Fedina & Lewis, 2008), an individual's ability to resist starvation and then subsequently regain body mass is likely to be an important aspect of organismal performance connected to resource use. We measured two aspects of resource use for focal males from each family/diet combination when they were 3 week post-eclosion. Starvation resistance was measured by monitoring the weight lost by males held for 4 days without food, expressed as a percentage of initial body mass. This 4-day period allowed us to measure how each male responded to short-term food absence without causing any mortality. Resource reacquisition ability was measured by giving these 4-days starved males access to 100% wheat flour for 16 h and measuring their subsequent weight gain expressed as a percentage of post-starvation mass.

Reproductive performance measures

Male olfactory attractiveness

In several *Tribolium* species, adult males secrete 4,8-dimethyldecanal (DMD), an aggregation pheromone that also functions to attract females from a distance (Suzuki, 1980, 1981; Arnaud *et al.*, 2002). Male olfactory cues influence female mate choice and male sperm precedence in *T. castaneum* (Boake & Wade, 1984; Boake, 1985, 1986; Lewis & Austad, 1994). *Tribolium castaneum* males reared on low-quality diet showed reduced production of DMD (Ming & Lewis, 2010).

In this study, we measured how attractive focal males were to virgin females based on their olfactory cues. Males (2 months old) were kept individually in closed 0.5-mL microfuge tubes filled with diet for 2 weeks to condition the flour medium with male-produced chemical cues. Female choice assays were conducted by releasing 20 virgin females into a 35-mm plastic arena containing a small circle of 100 mg male-conditioned medium and an equal amount of control medium corresponding to that male's diet quality treatment (either low-, medium- or high-quality diet). Arenas were covered and kept in the dark at 24 °C for 15 min, after which the number of females attracted to each cue was counted. Male olfactory attractiveness was calculated as the proportion of females found in medium containing focal male cues out of the total number of females found in either medium.

Mating and insemination rates

Tribolium castaneum is characterized by high mating rates for both sexes, but not all copulations result in successful sperm transfer (Fedina & Lewis, 2008). Focal males' ability to mate and inseminate multiple females was measured by placing individual males (~2.5 months old) in a 3.5-cm-diameter mating arena with five virgin females. Arenas were kept at 24 °C and copulations (scored as described by Lewis & Iannini, 1995) were counted during 15-min observation periods. Immediately following copulation, each mated female was removed via suction aspiration and replaced with another virgin female; this maintained constant female density during and between trials. Mated females were kept individually and allowed to oviposit for 2 weeks (females typically lay ~15 eggs day^{-1}), and successful insemination was scored by the presence of larvae that would have developed from any fertilized eggs. Male insemination success was calculated as the proportion of mated females that were inseminated.

Paternity share in competitive matings

In polygamous species, sexual selection continues after mating as males will be selected to increase their paternity share (also called sperm precedence) relative to other males mating with the same female. Two commonly used measures of post-copulatory reproductive performance (Simmons, 2001) are sperm defence (P_1) and sperm offence (P_2). Sperm defence refers to male's ability to maintain paternity share when a female subsequently remates, whereas sperm offence refers to male's ability to gain paternity share at the expense of previous mates. Following numerous previous studies of *T. castaneum* sperm precedence (e.g. Schlager, 1960; Lewis & Austad, 1990, 1994; Edvardsson & Arnqvist, 2000, Bernasconi & Keller, 2001; Fedina & Lewis, 2006, 2005, 2008), we used Chicago black, an autosomal semidominant body colour allele (Sokoloff *et al.*, 1960) as genetic paternity marker. Homozygous black males and females were used as background genotype to measure competitive paternity share of wild-type focal males (~3 months post-eclosion).

We measured sperm defence (P_1) by allowing a virgin black female (b/b) to first mate *ad libitum* with a focal wild-type (+/+) male in 2 g flour for 24 h; successful insemination by this first male was later confirmed by the presence of progeny developed from fertilized eggs laid by the female in these intermating vials. Females were isolated for 24 h to standardize sperm storage and then allowed to mate *ad libitum* with three Chicago black males for 24 h. Following the last mating period, females were placed in new containers with 10 g flour to oviposit for 1 week, and progeny were reared to adulthood. Sperm offence (P_2) of focal males was measured similarly, except that virgin black females were first mated *ad libitum* with three Chicago black males, followed by the wild-type focal males.

After 45 days, body colour phenotype of all adult progeny was scored, and focal male sperm defence and sperm offence were calculated as the proportion of focal male progeny (+/b). To avoid confounding paternity share of 0 or 1 with insemination failures during copulation by a female's first or last mates, we only included in our analysis those females that had positive evidence of insemination by males of both genotypes.

Fitness components

Reproductive success in a population context

We measured focal males' reproductive success within a population context (RSPC) using a reproductive performance assay modified from Boake (1985) that was conducted under seminatural conditions. By assessing relative reproductive success of focal males within a population of conspecifics, this RSPC measure combines males' premating, perimating and post-mating reproductive success. RSPC was measured by housing each focal (+/+) male with five virgin homozygous black (b/b) females and four virgin homozygous black males together for 1 week in 10 g flour (we used 100% wheat flour to standardize conditions for all experimental males). Eggs laid during this time were reared to adults, and RSPC for each focal wild-type male was calculated as the percentage of total progeny sired by that focal male (% +/b

progeny). This technique, which provides a point estimate of relative male reproductive success at a particular age, was measured twice during each male's lifetime: at 3 months post-eclosion (early reproductive success, RSPC1) and 4.5 months (later reproductive success, RSPC2).

Longevity

Longevity is an important component of *Tribolium* fitness because it determines lifetime mating opportunities as well as how many colonizing opportunities an individual has. *Tribolium castaneum* adults can live from ~4 months to over 3 years and are capable of reproducing throughout their adult lives (Good, 1936; Sokoloff, 1974). We maintained focal males individually in 0.5-mL microfuge tubes and checked their survival and renewed the flour medium biweekly until death. Under these conditions, median male longevity was 460 days (minimum 65 days and maximum 887 days, $n = 335$ males): it was necessary to truncate longevity values for 44 males that still remained alive at 707–886 days old at the end of the experiment.

Total fitness

Many suggestions for how best to estimate total fitness have been made, and each method has its own advantages and disadvantages (Hunt *et al.*, 2004). For organisms such as *Tribolium* beetles that mate repeatedly over their long adult lifespan, a reasonable estimate of total fitness can be based on the total number of eggs sired by each focal male over his lifetime. To estimate this for each focal male, we combined male reproductive success (% progeny sired) with longevity (days lifetime^{-1}) as measured above with an average value for female fecundity of 17 eggs day^{-1} (Park & Frank, 1948) to estimate the number of progeny per lifetime that were sired by each male as:

$$\begin{aligned}\text{Total fitness} = 17\,\text{Progeny/day} * [&(\text{RSPC1\%} * 120\,\text{days}) \\ &+ \text{RSPC2\%} * (\text{longevity} - 120\,\text{days})].\end{aligned}$$

Statistical analysis

Plasticity and GEI

All statistical analyses were conducted using SAS 9.1 (SAS Institute, Cary, NC, USA). To measure the effects of diet, genotype and GEI, we calculated Type IV sums of squares from mixed-model ANOVAS (Freund *et al.*, 1986; Rawson & Hilbish, 1991, Via & Conner, 1995) for each of the life-history traits, reproductive performance measures and fitness components: these were run in Proc GLM, and each model included block, diet, sire nested within block, dams nested within sires, sire × diet and dam × diet interactions. In these models, sire and dam (both randomly selected) were treated as random effects, as was diet because we aimed to estimate the overall effect of diet quality. We assessed potential GEI for each trait by examining the sire × diet interaction effects (Fry, 1992; Lynch & Walsh, 1998). Additionally, we estimated the proportion of total phenotypic variance contributed by each effect using the restricted maximum-likelihood method (REML) in Proc MIXED (Fry, 2004). Total fitness was square-root-transformed prior to analysis to meet normality assumptions. We analysed two measures of reproductive performance, P_1 and P_2, using a generalized linear model (Proc GENMOD), with a binomial error distribution and a logit link function (Arnqvist & Danielsson, 1999, Fedina & Lewis, 2004); variance components for these two traits were estimated using Proc VARCOMP. We included dam effects in each model, but because our goal was to estimate only additive genetic variance, components of nonadditive genetic variation (due to maternal effects, dominance and epistasis) were not further considered here.

Although male longevity was not normally distributed and no suitable transformations could be found, we calculated Type IV sums of squares and variance components as described above, because other studies have found these to approximate unbiased estimates for non-normal yet balanced data (Messina & Fry, 2003). We also analysed longevity using a Cox proportional hazards model (JMP 9) to test for differences due to diet, sire genotype and diet × sire interaction.

To investigate GEI in more detail, we estimated cross-environment genetic correlations for each trait expressed in different nutritional environments by calculating genetic correlations within traits measured across pairs of environments (1 and 2) as: $r_{A12} = \text{COV}_{A12}/\sqrt{V_{A1}}*V_{A2}$ (Falconer, 1952; Lynch & Walsh, 1998), where V_{A1} and V_{A2} are additive genetic variances of the trait measured in environments 1 and 2 and COV_{A12} is the trait covariance between the two environments. These terms were estimated using REML methods (MIXED in SAS) and used to test the null hypothesis that $r_{A12} = 1$ (Messina & Fry, 2003; Fry, 2004). Genetic correlations of traits across environments that are significantly less than +1 provide additional support for GEI and suggest the potential for independent trait evolution in different nutritional environments.

Assessing ecological crossover

For those traits showing evidence of GEI, we tested for ecological crossover interactions (Lynch & Walsh, 1998) by calculating Spearman's rank correlations between the adjusted sire means for each trait under two different nutritional environments. We tested each correlation coefficient against a null hypothesis that $r_s = 0$; accepting the null hypothesis of no correlation between family trait means in the two environments indicates a crossover GEI interaction.

Estimating trait heritabilities

Within each environment, we calculated narrow-sense heritabilities (h^2) as four times the sire variance

component divided by total phenotypic variance (Falconer & Mackay, 1996). Variances were estimated using the REML method (MIXED in SAS). In some cases, estimated variance components were negative, an outcome commonly attributed to sampling error and nested mating designs (Bridges & Knapp, 1987). We calculated 95% confidence intervals on these heritability estimates using a bootstrapping procedure in R2.13. Additionally, we estimated the coefficient of additive genetic variance (CV_A, or evolvability) for each trait, calculated as the square-root of the additive genetic variance divided by the trait mean (Houle, 1992).

Descriptive statistics for phenotypic traits are reported as $\bar{X}$ ± 1 SE, unless otherwise specified.

Results

Life-history traits

Overall, *Tribolium castaneum* males showed relative larval growth rates of 0.24 ± 0.002 mg day^{-1}. Both development time and pupal weight were highly variable, with development from egg to pupa taking 18–40 days and pupal weight ranging from 1.3 to 2.8 mg. For each of these life-history traits, differences in diet nutritional quality accounted for the single largest proportion of observed variation (Table 1a), representing 28.9% of total phenotypic variance for larval growth rate, 54.1% for development time and 42.6% for pupal weight. As expected, higher diet quality was associated with faster larval growth rates (Fig. 1a), shorter development times (Fig. 1b) and higher pupal weight. In addition, significant sire effects were found for both development time and pupal weight (Table 1a), indicating additive genetic variance for both traits. We found significant GEI for both larval growth rate and development time (Table 1a diet × sire terms, Fig. 1a,b).

Adult body condition of focal males ranged from 1.09 to 2.13 mg mm^{-1} (1.67 ± 0.008), with significant variation due to diet (Table 1a, Fig. 1d), which accounted for 36.8% of the total phenotypic variance. Male body condition also showed significant additive genetic

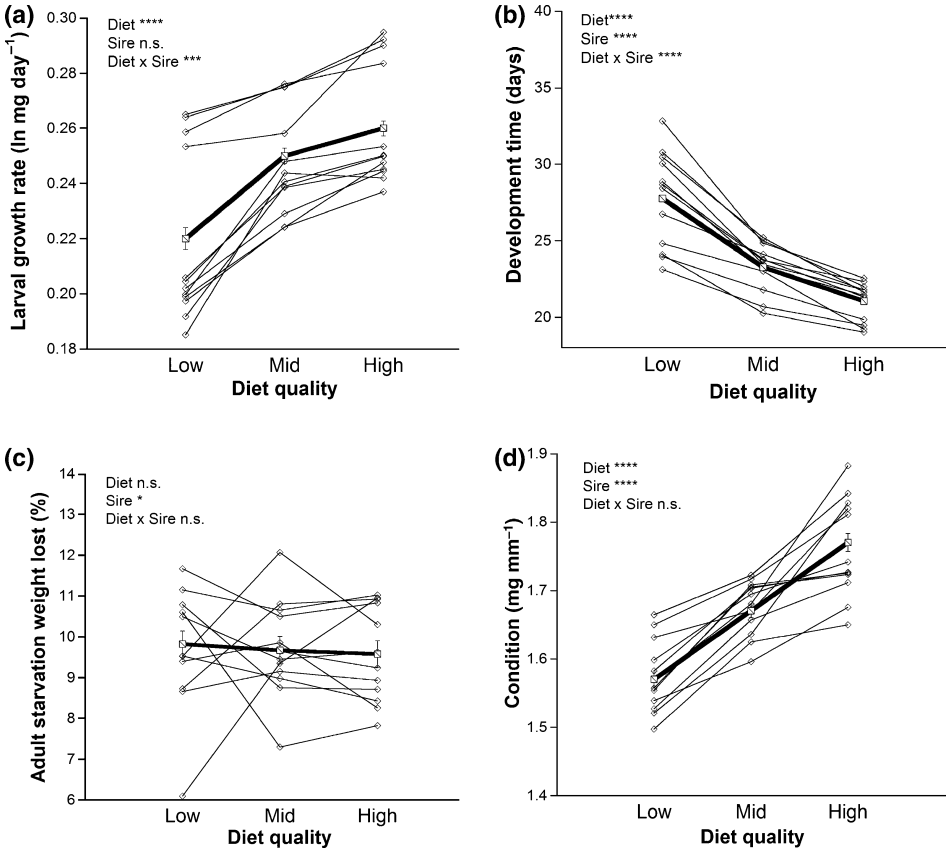

Fig. 1 Genetic and nutritional effects on male *Tribolium castaneum* life-history and resource acquisition traits: (a) larval growth rate (to day 14), (b) pupal mass, (c) adult starvation weight loss (% adult mass lost during 4-day starvation) and (d) adult body condition (adult mass/pronotal width). Overall effects of diet quality are indicated by reaction norms connecting trait means in each nutritional environment (thick black lines, ±1 SE shown). Thin grey lines represent adjusted least-square means across full/half-sib families reared in each nutritional environment.

Table 1 Summary of statistical analysis conducted on a nested half-sib breeding experiment in which *Tribolium castaneum* males from each family were split across low, medium and high quality diets. Tests of significance (*P*-values from SAS GLM and GENMOD procedures) and variance components (proportion of the total phenotypic variance from SAS MIXED and VARCOMP procedures) of model terms for (a) life history and resource acquisition traits, (b) reproductive performance, and (c) fitness components. Variance components are reported as zero when negative estimates were obtained, and as missing (–) when estimates could not be obtained because models failed to converge.

	(a) Life history and resource acquisition traits											
	Larval growth rate		Development time		Pupal mass		Starvation resistance		Resource reacquisition		Condition index	
	P	Var. (%)	*P*	Var. (%)	*P*	Var. (%)	*P*	Var. (%)	*P*	Var. (%)	*P*	Var. (%)
Block	< 0.0001	44.10	< 0.0001	18.93	< 0.0001	3.61	0.19	0.76	0.001	3.68	0.0001	8.28
Diet	< 0.0001	28.90	< 0.0001	54.10	< 0.0001	42.56	0.89	0	0.65	0	< 0.0001	36.82
Sire	0.09	0.00	< 0.0001	0.66	< 0.0001	5.17	0.03	0	0.03	1.78	0.0002	2.65
Dam (sire)	< 0.001	5.30	< 0.005	1.42	0.0003	6.40	0.03	4.77	0.51	4.14	0.0002	10.28
Diet × sire	< 0.0001	1.90	< 0.0001	4.16	0.16	0.95	0.15	0.00	0.1	0	0.26	0.90
Diet × dam	0.04	2.90	0.16	1.71	0.45	0.48	0.43	3.22	0.67	0	0.43	0.66
Error		17.00		19.01		40.83		91.25		90.40		45.64

	(b) Reproductive performance									
	Olfactory attraction		Mating rate		Insemination success		Sperm defence (P_1)		Sperm offence (P_2)	
	P	Var. (%)	*P*	Var. (%)	*P*	Var. (%)	*P*	Var. (%)	*P*	Var. (%)
Block	0.001	8.43	0.004	0.56	0.0009	3.53	0.29	0	0.56	0
Diet	0.09	1.50	0.04	1.50	0.002	5.27	0.33	0	0.11	0
Sire	0.18	0.00	0.0002	4.79	0.13	0	0.76	0	0.005	6.6
Dam (sire)	0.17	3.67	0.003	6.52	0.002	6.99	0.0003	0	< 0.0001	9.91
Diet × sire	0.41	0	0.07	0.97	0.206	0.45	0.002	0.13	0.23	0
Diet × dam	0.86	0	0.06	10.12	0.15	6.54	–	–	–	–
Error		86.40		75.53		77.22		99.87		83.49

	(c) Fitness components							
	Early reproductive success		Late reproductive success		Longevity		Total fitness	
	P	Var. (%)	*P*	Var. (%)	*P*	Var. (%)	*P*	Var. (%)
Block	< 0.0001	4.55	0.61	0	< 0.0001	7.54	0.01	2.84
Diet	0.12	0.57	0.067	2.70	< 0.0001	31.14	< 0.0001	29.78
Sire	0.0009	4.27	0.46	0.00	0.0001	0.67	0.0009	4.62
Dam (sire)	0.00002	5.89	0.39	2.12	0.01	2.00	0.04	3.74
Diet × sire	0.13	0	0.49	0	< 0.0001	7.76	0.17	0
Diet × dam	0.007	11.55	0.86	0	0.02	6.99	0.17	7.35
Error	0	73.17	0	95.18	0	43.92	0	51.67

variance (Table 1a, sire effect, Fig. 1d), accounting for 2.7% of the total variance.

Adult resource use

During 4 days of starvation, adult males lost 2–19% (9.5 ± 0.2) of their total body weight. When the same males were subsequently provided with food, there was considerable variation in males' ability to recover this body weight over 16 h; some males recovered none, whereas others gained up to 20% over their post-starvation weight (7.36 ± 0.23%). Significant sire effects indicated additive genetic variance for both of these traits related to adult resource use, but there were no significant diet or GEI effects (Table 1a).

Reproductive performance

We measured several aspects of reproductive performance for focal *Tribolium* males, including olfactory attractiveness to females, mating rate, insemination success and sperm offence and defence in competitive matings. Male olfactory attractiveness showed no significant variation due to diet quality, sire or GEI (Table 1b). When supplied with virgin females *ad libitum* for 15 min, male mating rate averaged 0.38 ± 0.01

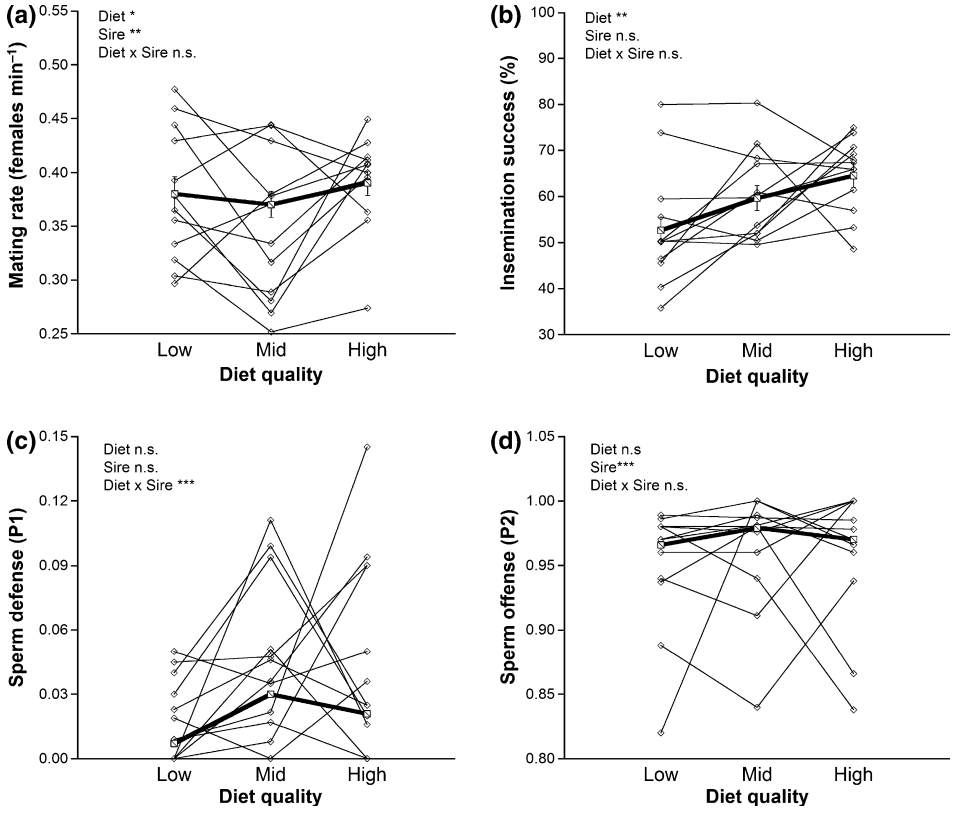

Fig. 2 Genetic and nutritional effects on reproductive performance of *Tribolium castaneum* males: (a) mating rate (with *ad libitum* access to virgin females), (b) insemination success (% of copulations that resulted in successful sperm transfer), (c) sperm defence (P_1, proportion of offspring sired when females subsequently remate with other males) and (d) sperm offence (P_2, proportion of offspring sired when males mated with previously mated females). Overall effects of diet quality are indicated by reaction norms (thick black lines) connecting either trait means ±1 SE (for mating rate and insemination success) or medians (for P_1 and P_2). Thin grey lines represent adjusted least-square means (medians for P_1 and P_2) across half/full-sib families reared in each nutritional environment.

females min^{-1} and showed significant diet and sire effects that accounted, respectively, for 1.5% and 4.8% of the total variation (Table 1b, Fig. 2a); the latter indicates significant additive genetic variance for male mating rate.

Not all copulations led to successful insemination of females, however, with males having an average insemination success of only 59.6 ± 1.5%. Insemination success was influenced by diet, which accounted for 5.3% of the total phenotypic variation (Table 1b); males from high-quality diet showed the highest insemination success (Fig. 2b).

There was no effect of diet quality on the paternity share of focal males measured in competitive matings (Table 1b, Fig. 2c,d). Significant additive genetic variance was detected for male sperm offence (P_2), with a sire effect that accounted for 6.6% of the total phenotypic variance (Table 1b, Fig. 2d). In addition, there was significant GEI for male sperm defence (P_1), indicating that the reproductive performance of male genotypes during competitive mating conditions was altered when they encountered different nutritional environments (Table 1b diet × sire, Fig. 2c).

Fitness components

We measured relative reproductive success of focal males within a realistic population context by housing each focal male with four males and five females homozygous for black body colour. Under these conditions, the experimental *Tribolium* males sired an average of 37% of all offspring produced when they were tested at 3 months post-eclosion (early reproductive success, Fig. 3a) and 48% when tested 1.5 months later (later reproductive success, Fig. 3b). Early reproductive success showed significant additive genetic variation (Table 1c, sire effect).

Longevity of adult *T. castaneum* males ranged from 65 to 887 days, with a median longevity across all diets of 460 days (IQR = 536 days). Diet quality accounted for 31.1% of total phenotypic variance for longevity

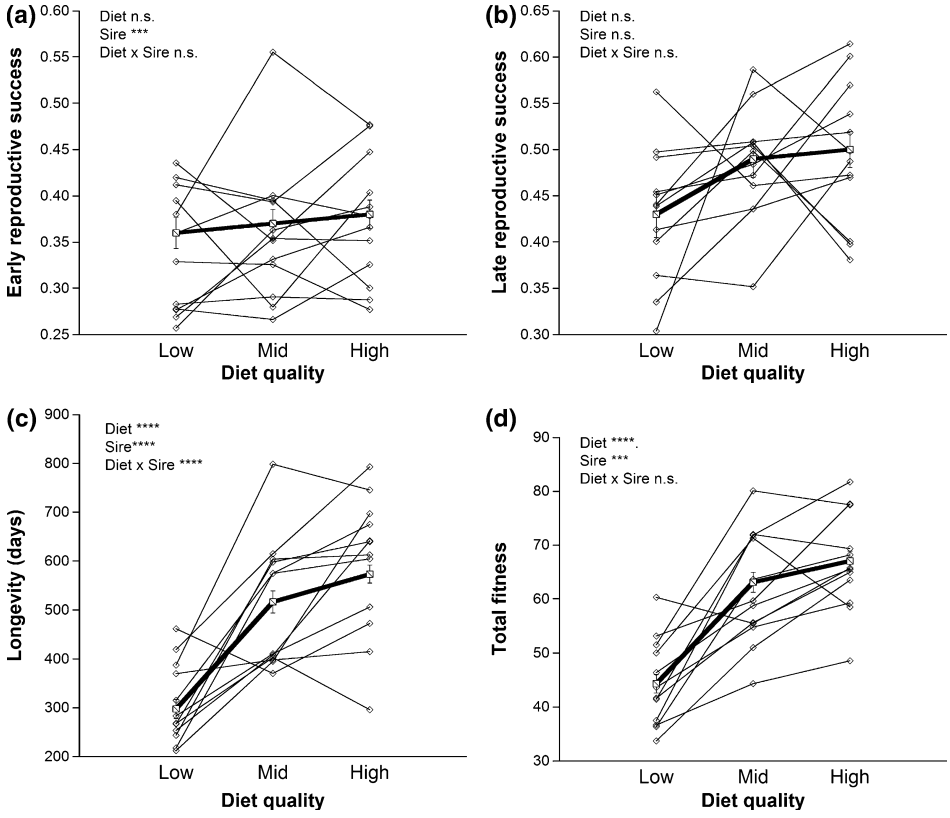

Fig. 3 Genetic and nutritional effects on male *Tribolium castaneum* fitness components: (a) Early reproductive success (proportion of progeny sired by focal male, measured at 3 months post-eclosion), (b) late reproductive success (measured at 4.5 months post-eclosion), (c) adult longevity (days from pupation until death) and (d) total fitness (as square-root of the estimated number of progeny sired over each male's lifetime). Overall effects of diet quality are indicated by reaction norms connecting trait means in each nutritional environment (thick black lines, ±1 SE shown). Thin grey lines represent adjusted least-square means across full/half-sib families reared in each nutritional environment.

(Table 1c, Fig. 3c), which also showed significant sire effects (2%) as well as significant GEI (7.8%). Survival analysis conducted using a Cox proportional hazards model yielded similar results, with significant effects of diet (likelihood ratio $\chi^2 = 72.1$, $P < 0.0001$), sire (likelihood ratio $\chi^2 = 17.6$, $P = 0.04$) and GEI (likelihood ratio $\chi^2 = 51.5$, $P = 0.0004$).

Total fitness for each male, estimated as the number of progeny sired per lifetime, ranged from 51 to 10 672 (median = 3729, IQR = 3751). Total fitness showed significant variation due to both diet quality and sire (Fig. 3d), accounting, respectively, for 29.8% and 4.6% of total phenotypic variation (Table 1c).

To further examine genotype–environment interactions, we also calculated genetic correlations between different pairs of environments for the subset of traits that exhibited significant GEI based on the GLM analysis. For male longevity in low- vs. high-quality diet, the cross-environment correlation was = −0.01, which was significantly less than +1 ($P = 0.002$). The remaining traits (larval growth rate, development time and sperm defence) all had cross-environment genetic correlations that were ≥ 0.5, and none of these were significantly less than +1. When we examined ecological crossover between nutritional environments for these traits, we found significant alterations in the rank order of sire means for male longevity as well as for sperm defence (Table 2).

Table 2 Tests of ecological crossover genotype-by-environment interactions (GEI) between pairs of environments differing in diet quality for *Tribolium castaneum* based on Spearman's rank correlations between family means in each environment. Significance levels are given for tests of H_0: $r_s = 0$, with acceptance of null hypothesis supporting crossover GEI.

	Diet quality		r_s	P
Development time	Low	High	0.75	0.005
	Medium	High	0.76	0.004
	Medium	Low	0.82	0.001
Sperm defence (P_1)	Low	High	0.06	0.84
	Medium	High	−0.40	0.19
	Medium	Low	0.10	0.75
Longevity	Low	High	−0.17	0.60
	Medium	High	0.59	0.04
	Medium	Low	0.06	0.86

Estimates of heritability and CV_A showed patterns roughly consistent with the ANOVA results, as most traits with significant sire effects had moderate additive genetic variation in at least one nutritional environment (Table S1). Several traits including development time, pupal mass and total fitness showed higher heritabilities under low- compared with high-quality diet. A few traits, including male mating rate and sperm offence ability (P_2), showed the opposite trend as heritability estimates were higher under high-quality diet.

Discussion

Tribolium flour beetles have evolved over several thousand years to colonize and exploit an ecological niche consisting of various grains and other foods stored by humans (Levinson & Levinson, 1994). Beetles are transported by commerce or fly between spatially discrete habitat patches that vary widely in their nutritional quality (Good, 1936; Sokoloff, 1974; Baker & Loschiavo, 1987). This study provides several new insights into the potential roles of genetic, nutritional and GEI effects in the evolutionary dynamics of organisms that grow up and live in such heterogeneous environments. In this study, we experimentally manipulated *Tribolium* diet quality, a highly relevant environmental dimension for these insects. Using a nested half-sib breeding design, we were able to partition the nutritional, genetic and GEI contributions to total phenotypic variation in a comprehensive suite of male life-history traits, reproductive performance measures and fitness components. When tested across nutritional environments, male genotypes showed highly plastic responses for mating rates, insemination success, longevity and total fitness and for larval growth and development. This study also revealed significant additive genetic variation for several traits reflecting both larval and adult resource use, as well as for male mating rate, sperm offence, longevity and total fitness. Finally, we found evidence supporting GEI among *Tribolium* males for larval traits, as well as for sperm defence and male longevity. In the following sections, we discuss each of these sources of variation in turn, highlighting several particularly intriguing results.

Phenotypic plasticity across nutritional environments

Several larval, reproductive performance and fitness-related traits showed considerable phenotypic plasticity when male genotypes were tested across different nutritional environments. As expected based on previous studies of *Tribolium* growth (Good, 1936; Sokoloff, 1974; Via & Conner, 1995), we found strong effects of diet quality on larval traits (growth rate and development time), body size (pupal weight) and condition (Fig. 1, Table 1a). Our study also revealed a strong effect of nutritional environment on male reproductive performance: when males were given *ad libitum* access to virgin females, those on low-quality diets had lower mating rates (no. of females mated within 15 min), as well as a lower likelihood of successfully inseminating the female during each mating (Table 1b, Fig. 2b). During copulation, *T. castaneum* males transfer sperm contained within a spermatophore manufactured by reproductive accessory glands (Bloch Qazi *et al.*, 1996). Our previous work (Fedina & Lewis, 2006) has shown that adult starvation reduces the size of these male glands as well as the number of sperm transferred during mating. Thus, reduced mating and insemination rates seen on low-quality diet could be due to the direct effects of poor nutrition on males' rates of spermatophore or sperm production. Alternatively, this reduction in male mating and insemination ability might reflect cryptic female choice favouring males on high-quality diet, as *T. casteneum* females can selectively prevent spermatophore transfer during copulation (Fedina & Lewis, 2006). In contrast to a study by Amitin & Pitnick (2007) that showed sperm competitive ability in *Drosophila melanogaster* was influenced by rearing environment, we found no effect of male *Tribolium* nutritional environment on either sperm offence or sperm defence. High-quality diet also significantly increased total fitness, mainly through effects on male longevity (Table 1c, Fig. 3c,d).

Male genetic variation

Our results also demonstrated substantial genetic variation for several traits related to *Tribolium* larval development, adult resource use and male reproductive performance. We found significant additive genetic variation in adult body condition index (Table 1a), in addition to confirming previous studies that have shown genetic variation for egg-to-pupa development time (Via & Conner, 1995) and pupal weight (reviewed by King & Dawson, 1972; Sokoloff, 1974). Significant sire effects were also detected for the two aspects of adult resource use that we measured: adult weight loss during 4 days of starvation and subsequent weight gain. Taken together, these results suggest a strong genetic basis in *Tribolium* for the ability to acquire and assimilate food resources by both larvae and adults.

These results also contribute to our understanding of genetic variation in male reproductive performance, as few quantitative genetic studies in any taxa have comprehensively assessed pericopulatory (during mating) and post-copulatory reproductive performance. In this study, male genotype had a strong influence on male mating rate (Table 1b, Fig. 2a), suggesting that some genotypes may have higher rates of spermatophore or sperm production. Our finding that male genotype also influenced sperm offence ability (P_2; Table 1b, Fig. 2d) adds to a growing list of studies demonstrating additive genetic variation in male sperm competition and related traits across many arthropod taxa (e.g. Clark *et al.*,1995; Hughes, 1997; Radwan, 1998; Hosken *et al.*, 2001;

Simmons & Kotiaho, 2002; Friberg *et al.*, 2005; Konior *et al.*, 2005; Fiumera *et al.*, 2005; Engqvist, 2008). Previous work on the repeatability and heritability of sperm competitive ability in *T. castaneum* (Tregenza *et al.*, 2009) found no evidence for heritable variation in P_2 using father–son regression; in that study, males were restricted to have only a single (observed) mating. In our study, we measured P_2 by allowing males to mate with previously mated females during a 24-h period, which represents more realistic conditions as males have been shown to frequently engage in repeated copulations with the same female (Lewis, 2004). However, this methodological difference is unlikely to account for observed genetic variation in P_2 because differences in the number of repeated copulations do not affect male paternity success (Lewis, 2004). Alternatively, it is possible that these differences reflect real differences in genetic variation between our synthetic *Tribolium* strain and the GA-1 laboratory strain studied by Tregenza *et al.* (2009), as long-term laboratory culture has the potential to deplete genetic variation.

We also found surprisingly strong effects of male genotype on several male fitness components, based on significant sire effects for the proportion of progeny sired by focal males (early reproductive success), adult longevity and total fitness (Table 1c, Fig. 3). Although selection is expected to deplete such variation, these results are consistent with many previous studies that have also documented substantial additive genetic variation in traits that are closely related to fitness (reviewed by Houle, 1992) or subject to directional sexual selection (reviewed by Pomiankowski & Møller, 1995). As discussed below, GEI for longevity may be responsible for maintaining variation in this fitness-related trait. Many of the traits showing significant sire effects showed only moderate heritability estimates, although these estimates carry large standard errors associated with low sample sizes. Several of these traits showed highest heritabilities under low-quality diet, consistent with patterns shown across different flour types for *Tribolium* pupal weight (Via & Conner, 1995).

Genotype-by-environment interactions

Genotype-by-environment interactions are of considerable evolutionary interest because they indicate variation among genotypes in their reaction norms and thus can influence a population's potential to adapt to new environments. GEI may also help maintain additive genetic variation in traits subject to directional selection, as they could prevent the fixation of a single genotype when organisms migrate between spatially variable environments (Gillespie & Turelli, 1989; Kokko & Heubel, 2008).

Remarkably few studies have examined GEI for male traits or performance measures related to post-copulatory episodes of sexual selection. We found significant GEI for males' sperm defence ability (P_1), indicating genetic variation in reaction norms for male ability to protect his paternity share when a female subsequently remates with other males (Table 1b, Fig. 2c). This finding that the paternity success of male genotypes in competitive matings depends on diet quality is, to our knowledge, a novel result. Furthermore, *Tribolium* male sperm defence ability also showed ecological crossover interactions (Table 2), based on failure to reject the null hypothesis of no correlation between genotype rankings across environments. However, these correlation results should be interpreted cautiously based on the low statistical power associated with testing only 12 sires. Sperm defence represents an influential component of male post-copulatory reproductive performance because, like many other creatures, *Tribolium* females mate with multiple males (Fedina & Lewis, 2008). Thus, significant GEI for *Tribolium* sperm defence ability indicate that no single male genotype will reproductively outperform others in every nutritional environment. As a result, genetic variants will remain in the population provided that there is sufficient migration among habitat patches that vary in nutritional quality. Only a few previous studies have looked at GEI for any aspects of male post-copulatory reproductive success. Another study documented GEI for sperm length of *D. melanogaster* males reared at two different larval densities (Morrow *et al.*, 2008). Engqvist (2008) found significant GEI for the rate of sperm transfer (related to paternity success) by male scorpionflies, *Panorpa cognata*, that were reared on two larval diets differing in prey availability. Thus, it seems likely that future studies may reveal GEI in many aspects of male post-copulatory reproductive performance.

This study also revealed strong GEI in adult longevity of *Tribolium* males (Table 1c, Fig. 3c), with evidence of ecological crossover interactions (Table 2) and significant additive genetic variation (Table 1c). One caveat is that this study measured longevity for isolated males that were provided with *ad libitum* food, thus controlling for possible differences in competitive ability. However, these results indicate genetic differences in adult longevity as well as in genotypes' reaction norms for longevity across different nutritional conditions. When populations inhabiting heterogeneous environments are connected by migration, such ecological crossover can lead to the preservation of genetic variation in lifespan within a species (Gillespie & Turelli, 1989).

Finally, our results confirm the presence of GEI among *T. castaneum* males for larval growth rate and egg-to-pupa development time (Table 1, Fig. 1). This is consistent with considerable previous work in *T. castaneum* documenting among-strain GEI for these life-history traits (reviewed by King & Dawson, 1972; Via, 1991) as well as within-strain GEI for development time measured across corn, rice, oats and wheat flour (Via & Conner, 1995). Ours was the first study to look at *Tribolium* adult resource use, where we found no evidence for GEI. These results suggest that *Tribolium*

exhibit greater genetic variation in how resource use by larvae, compared with adults, changes across different nutritional environments.

In summary, this study used *Tribolium* flour beetles to partition the genetic, nutritional and GEI variance contributions for a comprehensive suite of life-history traits, male reproductive performance measures and fitness components. Male genotypes exhibited considerable phenotypic plasticity across different nutritional environments in their male mating and insemination success, longevity and several larval traits. Our results also revealed significant additive genetic variation for several traits reflecting both larval and adult resource acquisition, as well as for male mating rate, sperm offence ability, longevity and total fitness. In addition, we found evidence supporting GEI among male genotypes for sperm defence ability and longevity and for larval traits. This work suggests that plasticity in *Tribolium* male reproductive performance as well as genetic variation in reaction norms may be important in facilitating these beetles' adaptation to new nutritional environments in ecological and evolutionary time.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 Estimates of genetic variation (narrow-sense heritabilities and coefficients of additive genetic variation) for life history traits, reproductive performance measures, and fitness components for *Tribolium castaneum* males across nutritional environments consisting of different ratios of wheat flour mixed with cellulose filler (low diet quality = 1 flour : 4 filler, medium = 1 : 2, high = 4 : 1).

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