Direct and correlated responses to artificial selection on sexual size dimorphism in the flour beetle, *Tribolium castaneum*

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Abstract

Sexual size dimorphism (SSD) is a conspicuous yet poorly understood pattern across many organisms. Although artificial selection is an important tool for studying the evolution of SSD, previous studies have applied selection to only a single sex or to both sexes in the same direction. In nature, however, SSD likely arises through sex-specific selection on body size. Here, we use *Tribolium castaneum* flour beetles to investigate the evolution of SSD by subjecting males and females to sexually antagonistic selection on body size (sexes selected in opposite directions). Additionally, we examined correlated responses to body size selection in larval growth rates and development time. After seven generations, SSD remained unchanged in all selected lines; this observed lack of response to short-term selection may be attributed to evolutionary constraints arising from between-sex body size correlations. Developmental traits showed complex correlated responses under different selection treatments. These results suggest that sex-specific larval development patterns may facilitate the evolution of SSD.

Introduction

Sexual size dimorphism (SSD) is one of the most conspicuous and general patterns of phenotypic variation occurring in animal species (Fairbairn *et al.*, 2007; Stillwell *et al.*, 2010). SSD may evolve when both sexes are selected for different expression of body size because of niche dimorphism, sexual selection or divergent reproductive roles (Hedrick & Temeles, 1989; Fairbairn *et al.*, 2007). However, the evolution of SSD is complex because the response of body size to sex-specific selection might be constrained by between-sex genetic correlations (Lande, 1980, 1987; Chippindale *et al.*, 2001; Fairbairn *et al.*, 2007). Because males and females share autosomal genes, high genetic correlations of homologous traits between the sexes are commonly observed (Lande, 1980; Roff, 1997; Poissant *et al.*, 2010). As a consequence, selection in one sex can influence body size in the other sex, which would prevent each sex from attaining its optimal body size (Lande, 1980; Bedhomme & Chippindale, 2007).

Recently, there has been an increase in research aimed at elucidating the evolution of SSD and investigating its constraints. Some of these studies include comparative

analysis (e.g. Meagher, 1992; Blanckenhorn *et al.*, 2007), quantitative genetics analysis (e.g. McDaniel, 2005; Rolff *et al.*, 2005), genetic manipulations (Chippindale *et al.*, 2001) and artificial selection experiments (e.g. Wilkinson, 1993; Reeve & Fairbairn, 1996; Delph *et al.*, 2004; Messina, 2004; Fox *et al.*, 2007). Artificial selection experiments are a powerful tool for studying SSD because contrary to other approaches that can only yield a snapshot of the evolutionary process by studying SSD on current populations, artificial selection has the potential to recreate how SSD might change over time. Previous studies using artificial selection to investigate the evolution of SSD (e.g. Reeve & Fairbairn, 1996; Delph *et al.*, 2004) looked for a change in SSD by either applying selection on body size in only one sex (e.g. Reeve & Fairbairn, 1996; Delph *et al.*, 2004) or simultaneously selecting for larger body sizes in males and females (e.g Alicchio & Palenzona, 1971; Reeve & Fairbairn, 1996). In these experiments, even though body size quickly responded to artificial selection, only a weak or no change in SSD was observed.

To date, artificial selection has not yet been used to explore a third important selective scenario for the evolution of SSD, which is selection acting antagonistically on body size between the sexes. In natural populations, selection frequently acts simultaneously but in opposite directions on body size in males and females (see Cox & Calsbeek, 2009). For example, in the house

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finch, *Carpodacus mexicanus*, where males are typically larger than females, Badyaev & Martin (2000) found that fecundity selection acted to increase male body size but reduced female body size. In the water strider *Aquarius remigis*, where females are larger than males, Preziosi & Fairbairn (2000) and Fairbairn (2007) found that fecundity selection favoured larger females (with large abdomens), whereas selection favoured smaller males (with small abdomen and thorax). By applying artificial selection simultaneously but in opposite directions to males and females, it might therefore be possible to disrupt the between-sex genetic correlations and alter SSD.

Additionally, adult body size is correlated with various developmental traits such as development time and growth rate (Stearns, 1992). In insects, an increase in body size is typically genetically correlated with increased development time and/or increased juvenile growth rate (Roff, 2000). Although various studies have shown that artificial selection on body size causes correlated responses in developmental traits (e.g. Partridge & Fowler, 1993; Partridge *et al.*, 1999; Cortese *et al.*, 2002; Teuschl *et al.*, 2007), to our knowledge, no previous studies have looked for sex-specific responses in correlated traits.

In this experiment, we examined how SSD in the red flour beetle, *Tribolium castaneum*, changed when we applied short-term sexually antagonistic selection on body size to males and females. Like many insects (Stillwell *et al.*, 2010), *Tribolium* females are larger than males and this SSD is evident in both pupal and adult stages (Sokoloff, 1977). We used pupal mass as a measure of body size, as this trait has been shown to have high heritability (Conner & Via, 1992; Via & Conner, 1995; Koncagül & Berger, 2006) and to be genetically correlated between the sexes (Via & Conner, 1995). We established three selection regimes in which we simultaneously selected male and female pupal mass in different directions: Increase size ($\uparrow$F $\uparrow$M), Increase SSD ($\uparrow$F $\downarrow$M) and Reverse SSD ($\downarrow$F $\uparrow$M). On the basis of previous works (Lande, 1980; Reeve & Fairbairn, 1996; Fairbairn, 1997; Fairbairn *et al.*, 2007), we predicted that if SSD responded to artificial selection it would increase in both the Increase Size and the Increase SSD treatments, while we expected SSD to decrease and eventually reverse direction in the Reverse SSD treatment. Also, we expected that selection on body size would produce correlated changes in development time and larval growth rate and that such changes might differ between the sexes.

Materials and methods

Beetles used in this experiment were derived from a synthetic strain we created to increase genetic variation available for 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). Throughout the experiment, beetles were maintained on enriched wheat flour (King Arthur, Norwich, VT, USA) and kept in a dark incubator at 29 °C, 70% relative humidity. We measured body size using 4- to 6-day pupal mass; during this time, pupal mass is relatively constant (N. Tigreros and S.M. Lewis, unpublished data). Pupae were sexed under 20× magnification based on their genital lobe morphology. Pupae were weighed to the nearest 0.01 mg (Mettler AT261 balance) and then kept individually in 0.5-mL microfuge tubes with flour until they became reproductive adults.

To examine how males and females respond to sexually antagonistic artificial selection on body size, we simultaneously selected both sexes for seven generations. We created four different selection treatments. We applied sexually antagonistic selection in two treatments: in one of these, we selected for increasing SSD (Increase SSD treatment; denoted $\uparrow$F $\downarrow$M), whereas in the second treatment we selected for reversed SSD (Reverse SSD treatment; $\downarrow$F $\uparrow$M). We also created a treatment to examine changes in SSD when both sexes were selected for higher pupal mass (Increase Size treatment; $\uparrow$F $\uparrow$M). As a control, a fourth treatment was created where parents for each generation were not selected on the basis of body size, but instead were randomly chosen. We maintained two replicate lines for each selection treatment, for a total of eight lines. We used an individual selection design, measuring pupal mass for 80 males and 80 females within each line (a total of 1280 pupae per generation), and then choosing 15 males and 15 females with the most extreme phenotypes as specified by their selection treatment. This design yielded an estimated intensity of selection of 1.4, representing the mean deviation of selected individuals in phenotypic standard deviation units (Falconer & Mackay, 1996).

Matings were set up between selected adults when they were 10–21 day posteclosion. To avoid the possibility of assortative mating or sperm competition, 15 pairs were established by randomly assigning each male to mate with a randomly chosen female within each selected line. In the two control lines, individuals were chosen randomly to form 15 mating pairs. After a 48-h mating period, the 15 females were placed together in a jar with approximately 100 g of flour to lay eggs for 5 days. Females were then removed, and larvae were allowed to develop with excess food until pupation.

In each generation, SSD was calculated as the difference between mean male and mean female pupal mass. We used control lines to adjust for changes unrelated to the selection regimes (e.g. environmental changes; data for control males and females is shown in Fig. S1). For each selected line, male and female pupal mass and SSD were corrected by calculating the difference between treatment and control means for each generation (Falconer & Mackay, 1996; Conner & Hartl, 2004).

After completing seven generations of artificial selection, we measured how male pupal mass, female pupal mass and SSD responded to selection. We analysed the average response to selection by testing the slope of a regression line fitted to the generation means (testing the null hypothesis that the regression slope = 0; Falconer & Mackay, 1996). Slopes were calculated separately for each of the two replicates within each treatment, as well as for the two replicates pooled. We detected a slight increase in pupal mass of both sexes within the control lines (for males, regression slope $b = 0.03$ mg/generation, $P = 0.03$; for females, $b = 0.03$ mg/generation, $P = 0.02$). Such changes may have resulted from unexpected changes in the environment, such as food quality or general management (Falconer & Mackay, 1996), and were taken into account in our corrected response to selection as explained above.

To look at phenotypic body size correlations, we calculated Pearson correlations between mean male and mean female pupal mass within the two replicate lines for each selection treatment for the seven generations. These statistical analyses were conducted using SPSS software (SPSS Base 8.0 for Windows, SPSS Inc., Chicago, IL, USA).

Correlated responses

After seven generations of selection, we tested whether artificial selection on male and female pupal mass had produced correlated responses in developmental traits by measuring larval growth rate and development time for males and females. To measure correlated responses, we used F_1 progeny of crosses between the two replicate lines of each selection treatment; combining replicates in this way reduces potential inbreeding effects (Partridge & Fowler, 1993). For each selection treatment, we randomly chose ten males and ten females from each replicate, for a total of 20 pairs; we allowed them to mate and collected approximately four eggs from each female (all eggs were laid within a 12–15 h period). These F_1 progeny were kept individually in vials with excess flour and used to measure larval development time and growth rate. Development time was calculated as days from egg laying to pupation. On the basis of the differences in larval growth rates that have been observed in *T. castaneum* (N. Tigreros and S.M. Lewis, unpublished data) and other insects (Gotthard *et al.*, 1994), we separately estimated early and late larval growth rates. Using the *T. castaneum* average egg weight of 0.03 mg (Howe, 1968), along with individual larval mass at 14 days and pupal mass measured as described above, we calculated relative growth rates as follows:

$$\text{Early relative growth rate (early RGR)} = \frac{(Ln\, 14\ days\ mass) - (Ln\ 0.03\ mg)}{14\ days}$$

$$\text{Late RGR} = \frac{(Ln\ pupal\ mass) - (Ln\ 14\ days\ mass)}{\#\ days}$$

To test whether these developmental traits showed correlated responses to artificial selection on pupal mass, we used a mixed model approach (Proc Mixed in SAS 9.1.3; SAS Institute, Cary, NC, USA). For each of the three dependent variables (development time, early RGR or late RGR), we examined the overall effect of selection treatment and sex, both included as fixed factors. Because some individuals were from the same family (same mother and father), we also included a random term that accounted for this within-family correlation. We used preplanned linear contrasts to compare the control with each of the three selection treatments (Control vs. Increase size, Increase SSD and Reverse SSD).

Results

Response to selection

Response to artificial selection on pupal mass of *T. castaneum* was rapid in both sexes. After seven generations of selection in the Increase Size treatment, mean pupal mass of males and females had increased by 70% (Fig. 1a), with both sexes showing significant responses to selection (Table 1). However, in the two treatments where we applied sexually antagonistic selection, pupal mass did not show the expected response. In the Increase SSD treatment ($\uparrow$F $\downarrow$M), we observed no consistent change in pupal mass for either sex over seven generations (Fig. 1b); pooling the two replicate lines, females showed a slightly negative slope (Table 1). In contrast, in the Reverse SSD treatment ($\downarrow$F $\uparrow$M), both sexes showed a marked decrease in mean pupal mass (Fig. 1c); after seven generations of selection, males and females were 44% and 50% lighter, respectively, with both sexes in both replicates exhibiting a significant decline in response to selection (Table 1).

Sexual size dimorphism showed little change in all of the selection treatments (Table 1; regression of SSD across generations, all $P > 0.3$). Across seven selected generations, changes in average pupal mass of males closely matched those of females, and mean pupal mass of the two sexes showed significant positive correlations within each selection treatment (Fig. 2).

Correlated responses to selection

All three developmental traits showed significant differences among the selection treatments after seven generations (Early growth rate: $F_{3,\ 214} = 23.58$, $P < 0.0001$; Late growth rate: $F_{3,\ 212} = 6.21$, $P < 0.0005$; Development time: $F_{3,\ 215} = 20.55$, $P < 0.0001$). In the Increase Size treatment, both sexes showed significantly higher early growth rates compared to controls after seven
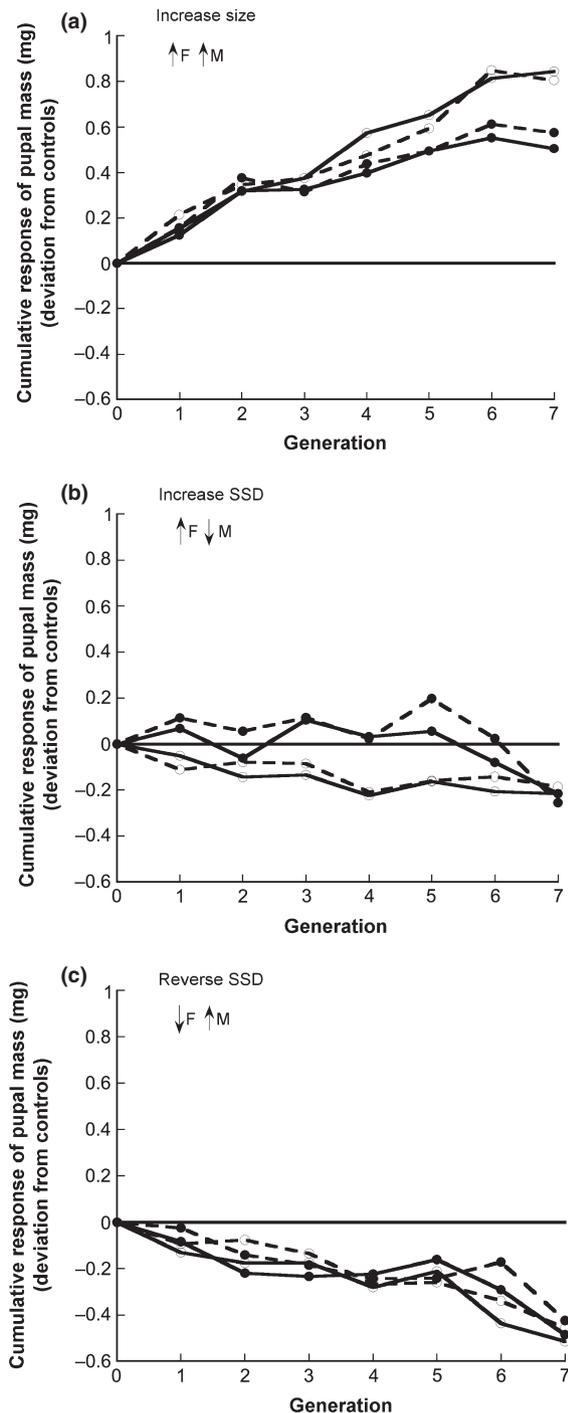
(a) Increase size
↑F ↑M

(b) Increase SSD
↑F ↓M

(c) Reverse SSD
↓F ↑M

Fig. 1 Cumulative responses to artificial selection on male (dashed lines) and female pupal mass (solid lines) in the flour beetle *Tribolium castaneum* relative to control (unselected) treatment. Four selection regimes were used: (a) Increase Size of both sexes (↑F ↑M). (b) Increase sexual size dimorphism (SSD) (↑F ↓M), and (c) Reverse SSD (↓F ↑M). Responses shown are calculated as the deviation of each selected line from the control means (unselected individuals). There were two replicates for each selection treatment (represented by open and closed symbols).

Table 1 Linear regression slopes describing changes of cumulative response, over seven generations, of mean pupal mass for females, males and sexual size dimorphism (SSD) (calculated as $\bar{X}_f - \bar{X}_m$) for three different selection treatments: Increase male and female size (↑F ↑M), increase SSD (↑F ↓M) and reverse SSD (↓F ↑M). Test of a H_o of regression slope = 0. Regression test significant at *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$.

Selection treatments	Replicate	Response (slopes)		
		Females	Males	SSD
Increase size (↑F ↑M)	1	0.12***	0.115***	0.009
	2	0.075***	0.081***	−0.006
	Avg.	0.099***	0.098***	0.002
Increase SSD (↑F ↓M)	1	−0.029**	−0.022*	−0.007
	2	−0.023	−0.023	−0.001
	Avg.	−0.026*	−0.022	−0.004
Reverse SSD (↓F ↑M)	1	−0.064**	−0.061***	−0.003
	2	−0.051**	−0.049**	−0.002
	Avg.	−0.057**	−0.055***	−0.002

selected generations (Fig. 3a; $t = 7.31$, $P < 0.0001$). In this selection treatment, no correlated responses were detected in either late growth rate (Fig. 3b; $t = -1.88$, $P = 0.06$) or development time (Fig. 3c; $t = 1.85$, $P = 0.07$). In contrast, in the Increase SSD treatment (↑F ↓M), there was no change in early (Fig. 3a; $t = 0.53$, $P = 0.59$) or late (Fig. 3b; $t = -0.09$, $P = 0.92$) growth rates; however, there was a significant decrease in development time (Fig. 3c; $t = -2.09$, $P = 0.04$). In the reverse SSD treatment (↓F ↑M), there was no change in early growth rate (Fig. 3a; $t = 1.57$, $P = 0.117$), although there was a significant increase in late growth rate (Fig. 3b; $t = 2.31$, $P = 0.02$), and a significant decrease in development time (Fig. 3c; $t = -5.38$, $P < 0.0001$). The faster late growth rate seems to be mostly driven by the females, although no sex-by-treatment interaction was detected (Fig. 3b; $F_{3, 212} = 1.21$, $P = 0.31$).

Discussion

This experimental study provides insight into SSD using a novel approach in which artificial selection on body size was simultaneously applied in opposite directions to males and females in *Tribolium* flour beetles. In nature, SSD is thought to evolve when sex-specific selection produces different body size optima for males and females (Fairbairn *et al.*, 2007). However, when we attempted to alter SSD using short-term sexually antagonistic artificial selection, we found no change in either the Increase SSD (↑F ↓M) or Reverse SSD (↓F ↑M) treatments across seven selected generations. When both sexes were selected simultaneously for increased body size (↑F ↑M), although beetles became larger we also found no change in SSD. Within-line mean male and female body size remained highly correlated in every selection treatment. Finally, we found that selection
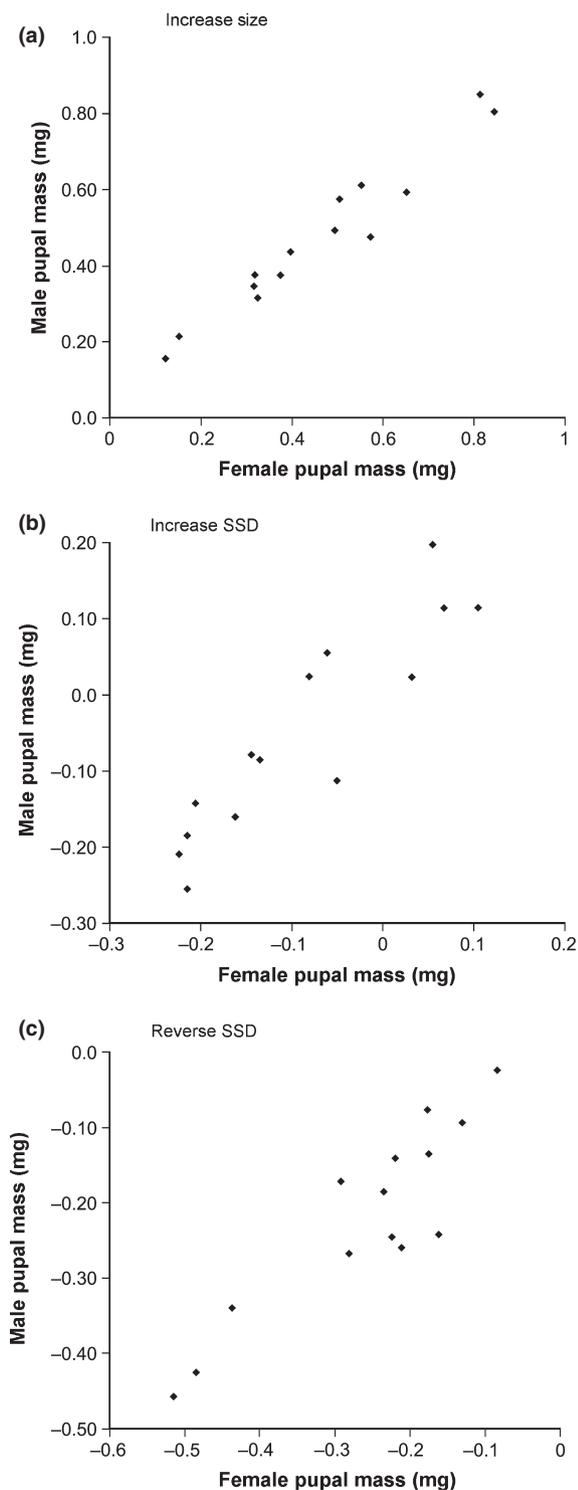
Fig. 2 Relationship between mean male and mean female pupal mass (means were corrected by calculating their deviation from the controls) during seven generations. Pearson correlations were calculated for Increase Size ($r = 0.97$, $P < 0.0001$, $N = 14$), Increase Sexual size dimorphism (SSD) ($r = 0.91$, $P < 0.0001$, $N = 14$) and Reverse SSD ($r = 0.90$, $P < 0.0001$, $N = 14$) treatments.

treatments produced correlated changes in different *Tribolium* developmental traits.

Previous artificial selection experiments studying SSD have applied directional body size selection to only a single sex (e.g. Reeve & Fairbairn, 1996; Delph *et al.*, 2004) or have simultaneously selected both sexes in the same direction (e.g. Reeve & Fairbairn, 1996). Artificial selection experiments looking at changes in SSD in *Drosophila* have found either no change or an unexpected decrease in SSD; for example, SSD decreased even though selection was applied to increase male size or increase size in both sexes (Zeleny, 1921; Alicchio & Palenzona, 1971; Partridge & Fowler, 1993; Reeve & Fairbairn, 1996; Teuschl *et al.*, 2007). Delph *et al.* (2004) performed artificial selection in the dioecious plant, *Silene latifolia*, to determine how between-sex genetic correlations influence sexual dimorphism in flower size. In lines where male plants with larger flowers were selected for four generations, female flowers also became larger; similarly, when smaller female flower size was selected, males also had smaller flowers. This study documented high between-sex genetic correlations in flower size that appeared to limit any change in size dimorphism of *S. latifolia* flowers. In our study of *Tribolium beetles*, we also found no change in the degree of SSD even when we applied sexually antagonistic artificial selection on body size. Thus, although Reeve & Fairbairn (2001) genetic simulation model suggests that significant changes in SSD can occur over relatively short time scales, experimental results to date indicate that high degrees of sexual dimorphism are unlikely to evolve under short-term selection on body size.

Our *Tribolium* results showing constant SSD even in the face of sexually antagonistic selection support previous artificial selection experiments indicating that between-sex body size correlations constrain the evolution of high SSD. In the Reverse SSD ($\downarrow$F $\uparrow$M) treatment, both sexes exhibited reduced pupal mass; even though males were subjected to direct selection for increased size, their pupal mass declined as a correlated response to selection on females. In the Increase SSD ($\uparrow$F $\downarrow$M) treatment, neither sex responded to selection, suggesting that the effect of direct selection in one sex was counterbalanced by opposing selection in the other. Even with the sexually antagonistic selection that we applied in these treatments, the phenotypic correlation between male and female mean body sizes within each line remained high across seven generations. In a previous study (Via & Conner, 1995), moderately positive genetic correlations in body size were found between the sexes in *T. castaneum*, although measures varied between two strains and across five environments, with estimates ranging from $r_A = 0.08$ to 0.653. Although genetic correlations were not measured in our study, this evidence is consistent with the finding by Delph *et al.* (2004) that such correlations act to constrain the independent evolution of sex-specific body sizes.
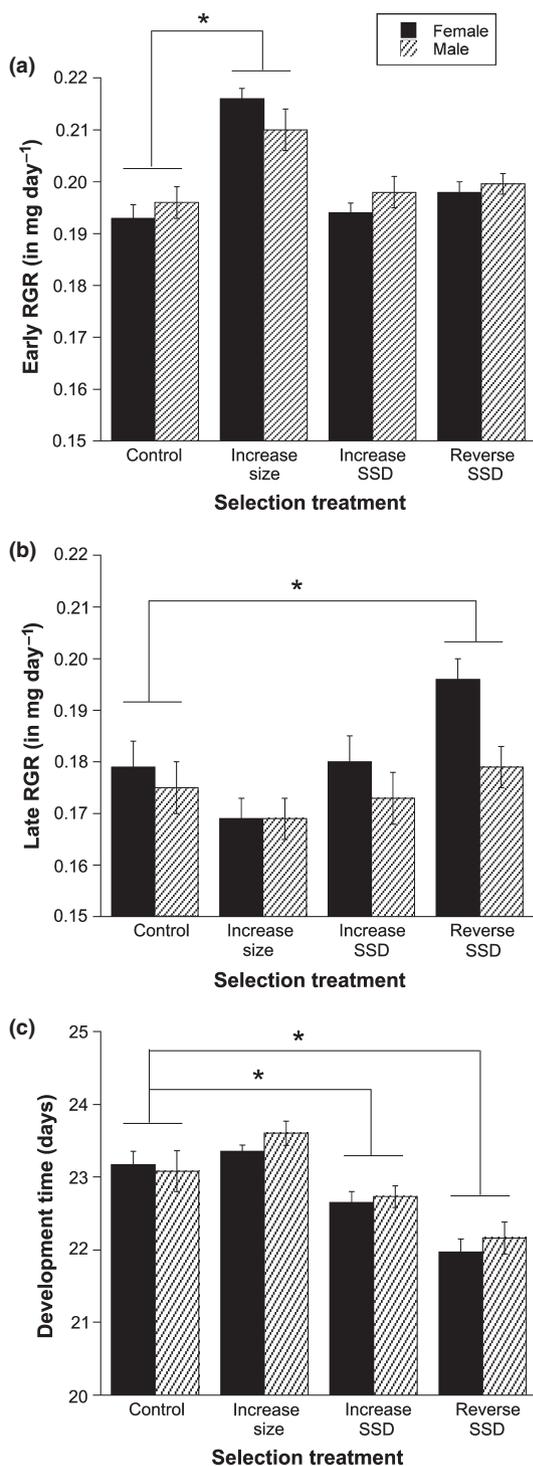
Fig. 3 Correlated responses of developmental traits when *Tribolium castaneum* pupae were selected for Increase Size (↑F ↑M), Increase Sexual size dimorphism (SSD) (↑F ↓M) and Reverse SSD (↓F ↑M). Panels show (a) early relative growth rate (RGR), (b) late RGR and (c) development time. Asterisks indicate treatments that differ significantly (following Bonferroni corrections) from the unselected control treatment.

Our results also provide insight into the physiological mechanisms generating the changes in body size we observed under different artificial selection regimes. We found several significant correlated changes in *Tribolium* beetles' developmental traits when we applied artificial selection on pupal mass. In the Increase Size treatment, larger body size for both sexes was associated with a significant increase in growth rates during early larval development (hatching to day 14) relative to controls; there was no change in development time or late growth rate. In the Reverse SSD treatment, smaller body sizes for both sexes were associated with significantly shorter development times but increased late growth rates. Similar correlated responses were found by Englert & Bell (1969), who observed that *Tribolium* beetles selected for large pupal mass showed increased early growth rates (represented as 13-day larval mass) whereas beetles selected for small pupal mass showed shorter development times. These results indicate that changes in *Tribolium* body size are mediated by distinct physiological mechanisms depending on whether size is increasing or decreasing. It also supports Nijhout *et al.* (2010) mathematical model showing that the correlation between body size and development time is context-dependent and that as a consequence body size and development time can change independently of each other.

Body size is a complex polygenic trait and, even in the face of high between-sex correlations, the evolution of SSD may be facilitated by sex-biased expression of shared genes affecting the physiological mechanisms controlling growth and development (Badyaev, 2002; Mank, 2009; Stillwell & Davidowitz, 2010). Growth in holometabolous insects is restricted to juvenile stages; thus, adult body size is determined by the size attained during the final larval stage. The physiological mechanisms that determine insect size at metamorphosis have been particularly well studied in the tobacco hornworm, *Manduca sexta* (reviewed by Davidowitz *et al.*, 2005; Nijhout *et al.*, 2006; Shingleton *et al.*, 2007), where three factors have been shown to regulate body size: larval growth rate, a critical size threshold and the duration of a terminal growth phase. Thus, changes in body size can arise through alterations during several stages in the growth process. Recent work has shown that sex differences in the genetic architecture and physiological mechanisms that control larval size contribute to the maintenance of SSD in *M. sexta* (Stillwell & Davidowitz, 2010). Our results also provide support for the idea that SSD involves the evolution of sex-specific modifiers acting on shared developmental pathways (Badyaev, 2002; Stillwell & Davidowitz, 2010). In the reverse SSD treatment, females that were directly selected for reduced size showed 9% higher late growth rates compared to males from the same selected line, whose body sizes also decreased. Although this difference was not statistically significant, it does suggest the existence of a sex-specific

compensatory mechanism to maintain body size in females.

In conclusion, artificial selection experiments provide a powerful tool that may contribute to understanding how selection acts on sex-specific patterns of growth and development. Additional work elucidating sex differences in growth and development will provide greater insight into how SSD is maintained and responds to selection.

Acknowledgments

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Supporting information

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

Figure S1 Changes in mean pupal weight of *Tribolium castaneum* for control (unselected) females (solid lines) and males (dashed lines) across seven generations.

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