PROCEEDINGS OF THE ROYAL SOCIETY B

Proc. R. Soc. B (2010) **277**, 3819–3826
doi:10.1098/rspb.2010.0895
Published online 7 July 2010

Sex differences in phenotypic plasticity of a mechanism that controls body size: implications for sexual size dimorphism

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The degree and/or direction of sexual size dimorphism (SSD) varies considerably among species and among populations within species. Although this variation is in part genetically based, much of it is probably due to the sexes exhibiting differences in body size plasticity. Here, we use the hawkmoth, *Manduca sexta*, to test the hypothesis that moths reared on different diet qualities and at different temperatures will exhibit sex-specific body size plasticity. In addition, we explore the proximate mechanisms that potentially create sex-specific plasticity by examining three physiological variables known to regulate body size in this insect: the growth rate, the critical weight (which measures the cessation of juvenile hormone secretion from the corpora allata) and the interval to cessation of growth (ICG; which measures the time interval between the critical weight and the secretion of the ecdysteroids that regulate pupation and metamorphosis). We found that peak larval mass of males and females did not exhibit sex-specific plasticity in response to diet or temperature. However, the sexes did exhibit sex-specific plasticity in the mechanism that controls size; males and females exhibited sex-specific plasticity in the growth rate and the critical weight in response to both diet and temperature, whereas the ICG only exhibited sex-specific plasticity in response to diet. Our results suggest it is important for the sexes to maintain the same degree of SSD across environments and that this is accomplished by the sexes exhibiting differential sensitivity of the physiological factors that determine body size to environmental variation.

Keywords: sexual dimorphism; body size; physiology; development; plasticity; *Manduca sexta*

1. INTRODUCTION

Males and females of most animals differ in their size, a phenomenon known as sexual size dimorphism (SSD; Fairbairn 1997, 2007). The direction and magnitude of SSD varies considerably among the major taxa and among species due to variation in the sources of selection acting in concert to create SSD: variation among taxa/species in the magnitude of sexual selection favouring large size in males (owing to male–male competition or female choice), variation in fecundity selection favouring large size in females (larger females produce more eggs) and variation in a variety of sources of selection favouring small size in both sexes (Stillwell *et al.* 2010). In addition, recent studies have shown that the magnitude of SSD, but often not the direction, changes considerably among populations within species (Blanckenhorn *et al.* 2006, 2007; Stillwell *et al.* 2007*a*). Although much of this intraspecific variation in SSD is partly genetically based and hence due to selection, some of this variation is also probably due to a sex difference in phenotypic plasticity in body size (Fairbairn 2005; Stillwell *et al.* 2010). However, how such sex differences in body size plasticity are generated is puzzling because males and females share the same genes that control growth and development (Badyaev 2002). Consequently, how the sexes grow to different sizes and how the sexes exhibit sex-specific plasticity in response to environmental variability is poorly understood, particularly in invertebrates such as insects (Stillwell *et al.* 2010).

Although many environmental and ecological variables induce plasticity in body size and other traits of ectothermic animals (Stillwell *et al.* 2007*a*; Teder *et al.* 2008; Blanckenhorn 2009), two are particularly important in inducing plasticity in growth and life-history traits: diet quantity/quality and temperature (Davidowitz *et al.* 2004; Stillwell *et al.* 2007*b*). Insects typically mature at larger sizes when raised at lower temperatures and when raised on higher quality diets (Atkinson 1994; Berrigan & Charnov 1994; Davidowitz *et al.* 2004; Stillwell & Fox 2005; Stillwell *et al.* 2007*b*; Kingsolver & Huey 2008). Insects also generally exhibit sex-specific plasticity in body mass in response to diet quality/quantity (Stillwell *et al.* 2010). For example, Bonduriansky (2007) found that in the Australian fly, *Telostylinus angusticollis*, males were generally more sensitive to rearing diet (low-quality versus high-quality diet) than were females; on the low-quality diet, males and females were nearly identical in size, whereas males were considerably larger than females on the high-quality diet. However, studies that have investigated whether temperature creates sex-specific plasticity in size are inconsistent (Stillwell *et al.* 2010); for instance, Stillwell & Fox (2007) found that males of the seed-feeding beetle, *Callosobruchus maculatus*, were generally more sensitive to rearing temperature than were females, creating temperature-induced variation in SSD. However, other studies have found that temperature produced no sex-specific plasticity in body size (Stillwell *et al.* 2010; this study). Despite a recent increase in interest on

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Received 27 April 2010
Accepted 14 June 2010

studying sex differences in body size plasticity, the mechanisms that produce these sex differences in plasticity are still poorly understood. Understanding both the ultimate (evolutionary/ecological) and proximate (developmental/physiological) mechanisms that generate these patterns are essential to understanding the evolution of intraspecific variation in SSD in animals.

Although the proximate mechanisms responsible for sex-specific plasticity in body size remain largely unknown, there are only four ways the sexes could differ in their plasticity in size: males and females must exhibit differences in plasticity in (i) size at hatching, (ii) growth rate, (iii) the duration of the growth period and/or (iv) size-dependent survival (Blanckenhorn 1997; Badyaev 2002; Esperk *et al.* 2007; Stillwell & Fox 2007; Stillwell *et al.* 2010). Few studies have examined these variables in the context of sex-specific plasticity, but of those that have, the results are not consistent (Stillwell *et al.* 2010). Alternatively, such sex differences in body size plasticity could be generated through sex differences in physiological mechanisms. The regulation of body size and plasticity in body size are known to be under physiological control in insects (Stern & Emlen 1999; Davidowitz & Nijhout 2004; Davidowitz *et al.* 2004; Nijhout & Davidowitz 2009). However, the physiological mechanisms that potentially generate sex-specific plasticity in body size have not previously been explored.

Here we use the hawkmoth, *Manduca sexta* (Lepidoptera: Sphingidae), a model system in insect physiology, to investigate sex differences in plasticity of the underlying physiological mechanisms that potentially create sex-specific norms of reaction in body size. In insects, growth is typically exponential, such that most growth occurs in the last larval instar (Nijhout *et al.* 2006). In *M. sexta*, 90 per cent of the accumulation of mass occurs in the final (fifth) instar (Davidowitz *et al.* 2004). During the last instar, a complex physiological process is set in motion that ultimately terminates growth and hence regulates adult body size in *M. sexta*. This process is controlled by three factors: the growth rate, the critical weight (which measures the cessation of juvenile hormone (JH) secretion from the corpora allata) and the interval to the cessation of growth (ICG; which measures the time interval between the critical weight and the secretion of the ecdysteroids that regulate pupation and metamorphosis). These three variables interact nonlinearly to explain 95 per cent of the variation in body size and plasticity in body size (D'Amico *et al.* 2001; Davidowitz *et al.* 2003, 2004, 2005; Davidowitz & Nijhout 2004; Nijhout *et al.* 2006, 2010).

In this study, we test for sex differences in body size plasticity in *M. sexta*, which exhibits the typical female-biased (females larger) SSD observed in most insects (Davidowitz *et al.* 2004; Stillwell *et al.* 2010). We also test whether any sex differences in plasticity in size are produced through sex differences in plasticity in the growth rate, the critical weight and the ICG. We do this by raising moths on different qualities of diet and at different temperatures. We first examined the peak size of last (fifth) instar *M. sexta* to evaluate whether there were any sex differences in plasticity in size. We then measured the growth rate, the critical weight and the ICG to see whether and how sex-specific plasticity in these variables is translated into plasticity in larval size. Finally, we measured pupal mass to test whether any diet-induced and temperature-induced sex-specific plasticity in the last larval instar translates into sex-specific plasticity in pupal mass.

2. MATERIAL AND METHODS

(a) *Natural history of* Manduca sexta

The hawkmoth, *M. sexta* (Linnaeus), is distributed from South America to southern Canada (Rothschild & Jordan 1903; Davidowitz *et al.* 2004). Throughout this geographical range, it feeds almost exclusively on solanaceous plants (Madden & Chamberlin 1945; Yamamoto & Jenkins 1972), but also feeds on one species of the genus *Proboscidea* (Martyniaceae) in central Arizona, USA (Mechaber & Hildebrand 2000). Its life cycle is intimately tied to its host plant. Adults lay eggs singly on the undersides of leaves. The eggs hatch and larvae feed on the foliage for approximately three to four weeks before burrowing into the soil to pupate.

(b) *Study populations*

The colonies used in our experiments originate from colonies that have been raised in the laboratory on artificial diet for more than 250 generations (D'Amico *et al.* 2001; Kingsolver 2007). The colony we used to initiate the population used in the diet experiment was created by crossing laboratory colonies from the University of Washington, the University of Arizona and North Carolina State University. This new colony was maintained at 16 L : 8 D, at 25°C for eight generations prior to the experiment. The colony used to initiate the population used in the temperature experiment was created by crossing colonies from the University of Washington, Duke University and the University of Arizona and was also reared for eight generations prior to the experiments.

(c) *Experimental design*

We performed two separate experiments (one experiment to examine diet-induced plasticity and one experiment to examine temperature-induced plasticity) to explore sex differences in plasticity in peak larval mass, pupal mass, growth rate, the critical weight and the ICG.

(i) *Diet experiment*

Hatching larvae were raised singly in individual cups (Solo P100, approx. 30 cm^3 volume) on the standard 100 per cent artificial diet at 25°C (16 L : 8 D) until they reached the fifth instar. Larvae moulting to the fifth instar were removed each day and transferred to their experimental treatments (note that 90% of the mass is accumulated in the fifth instar, see §1). We randomly assigned individuals to three different qualities of artificial diet (100, 60 and 40%; all at 25°C; 16 L : 8 D; Davidowitz *et al.* 2003, 2004). The nutrients in these diets were reduced by the appropriate proportions. For example, the 60 per cent diet contained 60 per cent of the nutrients per gram compared with the standard 100 per cent diet (the standard diet used to raise the colonies). A non-nutritive bulk (Alphacel, ICN, Aurora, OH, USA) was added to the diet to make up the remaining portion, such that the quantity of food that larvae received in each treatment was identical. The vitamin, antibacterial and antifungal components were the same for all three diets. The formula for these diets can be found in Davidowitz *et al.* (2003). In larvae reared on the 40 per cent diet, the critical weight and ICG were not detectable (Davidowitz *et al.* 2003). Therefore, the data for the 40 per cent diet are not presented.

Larvae were weighed once daily throughout the fifth instar on an electronic balance. All fifth instar larvae were raised individually in clear plastic cups (Solo TP9, approx. 266 cm^3 in volume). Larvae were checked once daily until they secreted ecdysone, which is detected by the deposition of pink ommochrome pigments along the dorsum, the exposure of the dorsal vessel and the onset of wandering behaviour (Nijhout & Williams 1974). At this point, larvae were placed into wooden blocks (5 × 10 cm), drilled with 2.5 cm diameter holes to mimic the underground pupation cell. The individuals in these wooden blocks were placed at 25°C (16 L : 8 D) to pupate. One week later, the pupae were removed, sexed and weighed on an electronic balance.

(ii) *Temperature experiment*

We used the same protocol as in the diet experiment, with the following changes: larvae were raised in groups (larvae were supplied with ample food and were kept at low densities to minimize larval competition) on the standard 100 per cent artificial diet at 25°C (16 L : 8 D) from hatching to the fourth instar. Fifth instar larvae were randomly transferred to one of three temperatures (20, 25 or 30°C; 16 L : 8 D). All larvae were raised on the standard 100 per cent diet at all three temperature treatments. After secretion of ecdysone, larvae from all three temperature treatments were transferred to 25°C (16 L : 8 D) and allowed to pupate.

In total, we raised 874 larvae for the diet experiment and 333 larvae for the temperature experiment.

(d) *Measurement of physiological variables*

The critical weight, operationally defined as the mass at which further growth and feeding are no longer necessary for a normal time course to pupation, can be measured directly only at the population level, because a large number of individuals (more than 300; Davidowitz *et al.* 2003) are required. Therefore, it is not possible to measure the critical weight directly in individuals. However, knowing the growth rate of the larva, its peak mass and the population (colony) level ICG allows us to estimate the critical weight for each individual (Davidowitz *et al.* 2003). We thus calculated the individual critical weight as,

$$cw_i = pm_i - (icg_p * gr_i),$$

where cw_i is the individual critical weight, pm_i is the individual peak mass, icg_p is the ICG of the population and gr_i is the individual growth rate. Growth rate was calculated as the next to last 2 days of growth (last mass measurement − 1 day) − (last mass measurement − 2 days)/1 day. This measurement ensured that the growth rate was measured during the linear phase of growth (Nijhout *et al.* 2006). Finally, we calculated the individual ICG as,

$$icg_i = \frac{pm_i - cw_p}{gr_i},$$

where icg_i is the individual ICG and the cw_p is the population (colony) critical weight. Note that all of these equations accurately predict empirical data from diverse genetic strains of *M. sexta* (Nijhout *et al.* 2006).

(e) *Statistical analyses*

All statistical analyses were done in SAS v. 9.2 (SAS institute, Cary, NC, USA) using ANOVA (type III sums of squares). All data were approximately normally distributed, so no transformation was necessary. Temperature, diet and sex were treated as fixed factors.

We tested for sex differences in plasticity by examining the temperature-by-sex and diet-by-sex interactions in the ANOVAs. However, interactions produced by ANOVA can be misleading when the *proportional* or *relative* effects (i.e. ratios such as female/male size) are of interest because ANOVA tests for interactions by measuring linear differences between treatment means (Stanton & Thiede 2005; Stillwell *et al.* 2007*b*; Fraker & Peacor 2008). In other words, when there is a large effect of one variable on the overall mean, linear differences do not translate into proportional changes. For example, the linear difference between the mean ICG of females and males changed considerably with temperature, as indicated by a highly significant temperature-by-sex interaction in ANOVA ($F_{2,327} = 11.0$, $p < 0.0001$; figure 4*b*). However, there was no change in the *relative* difference between male and female ICG; the ICG of females is 1.36 times longer than that of males at 20°C, while it is 1.41 times longer at 30°C ($F_{2,327} = 0.98$, $p = 0.38$; figure 4*d*). This example shows that an ANOVA on the original data would produce a significant interaction, but this is not a test for whether the *relative* difference between females and males is changing with diet or temperature, which is a direct measure of the magnitude of SSD and the main focus of this study. Therefore, we performed our analysis as a two-step process. We first examined main effects (temperature, diet and sex; panels *a* and *b* in figures 1–5). We then tested for diet-by-sex and temperature-by-sex interactions for all traits using relative trait values (individual trait value/mean within each temperature or diet treatment (averaged across sexes)) to remove the large scaling effects of the diet and temperature treatments (Stanton & Thiede 2005; Stillwell *et al.* 2007*b*; panels *c* and *d* in figures 1–5). These relative trait values were normally distributed and thus did not violate assumptions of ANOVA. Log transformation would also remove scale effects, but log transformation can often impair biological interpretation, because statistical interactions do not necessarily translate directly to biological interactions (Grissom 2000; Stanton & Thiede 2005; Fraker & Peacor 2008).

3. RESULTS

(a) *Main effects of diet, temperature and sex*

As expected, fifth instar larvae of *M. sexta* were largest on the high (100%) quality diet and increased in size with decreasing temperature (diet experiment: $F_{1,870} = 422$, $p < 0.0001$; temperature experiment: $F_{2,327} = 88.2$, $p < 0.0001$; figure 1*a*,*b*). The growth rate of larvae was also significantly higher on the high-quality diet and decreased substantially with decreasing temperature (diet: $F_{1,870} = 2383$, $p < 0.0001$; temperature: $F_{2,327} = 274$, $p < 0.0001$; figure 2*a*,*b*), consistent with the general patterns found in other insects (Atkinson 1994; Atkinson & Sibly 1997). In addition, the critical weight was considerably higher on the high-quality diet ($F_{1,870} = 60.7$, $p < 0.0001$; figure 3*a*). Though the critical weight changed substantially with temperature ($F_{2,327} = 6.14$, $p = 0.002$; figure 3*b*), this was largely because of a lower average critical weight of males at 20°C (see below). Davidowitz *et al.* (2004) found that the critical weight does not change with temperature (as is the case of females at all three temperatures and males at 25 and 30°C in this study). However, the critical weight was measured in Davidowitz *et al.* (2004) at the population level, which is much less

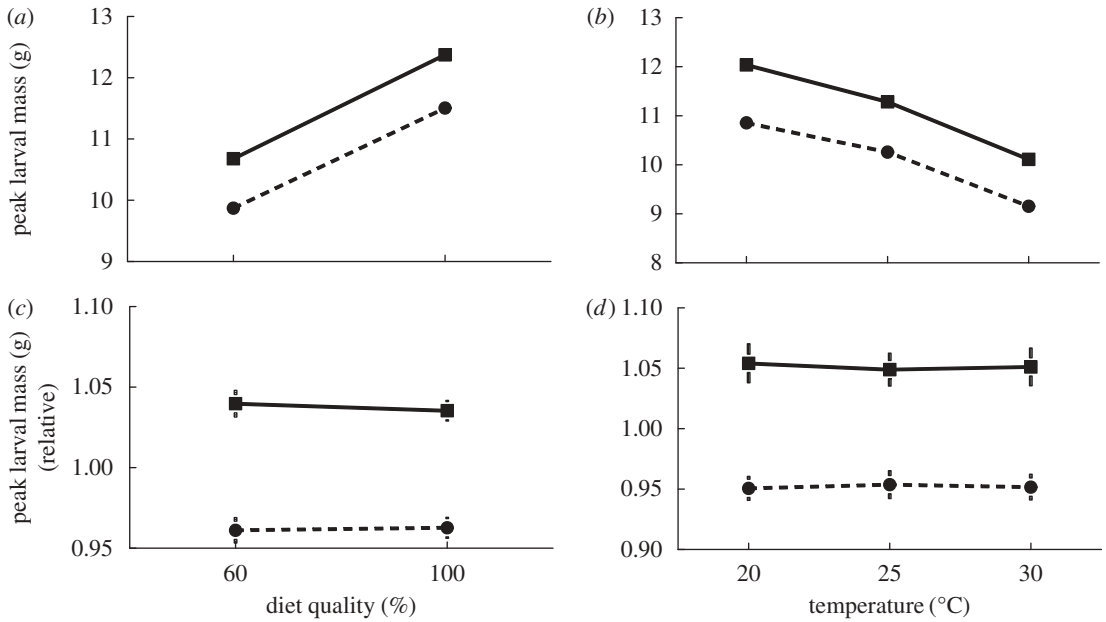

Figure 1. Peak larval mass of fifth instar *M. sexta* raised on two different qualities of diet (60 and 100%; *a*,*c*) and at three different temperatures (20, 25 and 30°C; *b*,*d*). Relative peak larval mass (*c*,*d*) are the means after removing the large effect of diet and temperature (individual trait/mean trait for each treatment, following Stanton & Thiede (2005) and Stillwell *et al.* (2007*b*)). Standard error bars are included, but are smaller than the symbols for some experimental treatments. Solid lines, females; dashed lines, males.

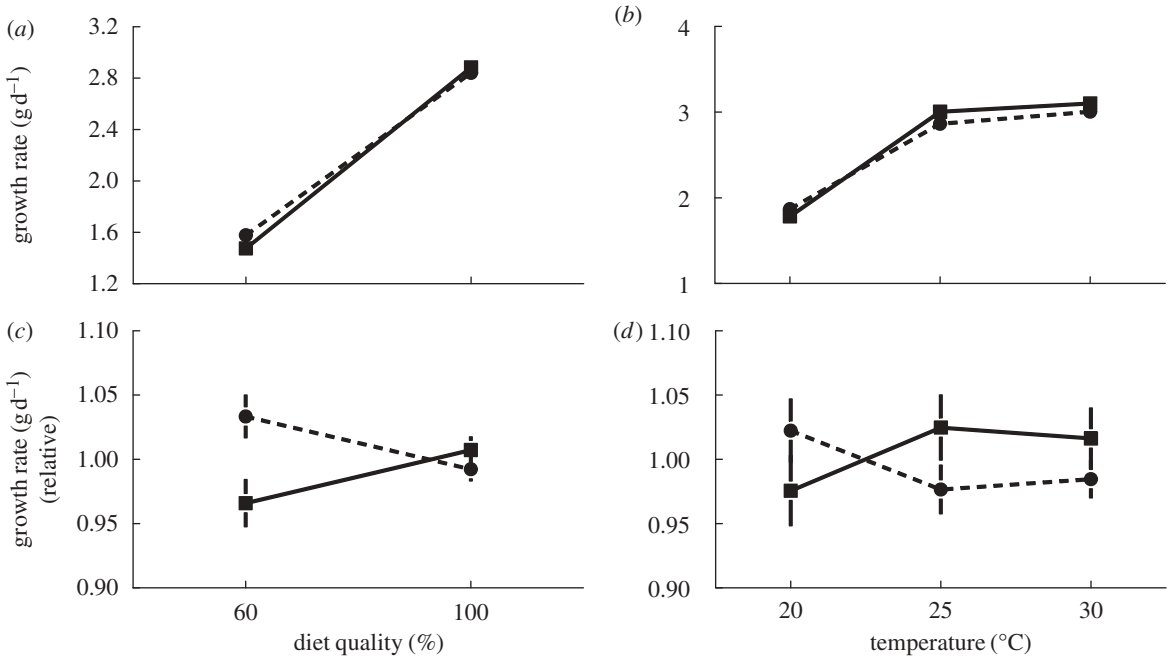

Figure 2. Growth rate of fifth instar *M. sexta* raised on two different qualities of diet (60 and 100%; *a*,*c*) and at three different temperatures (20, 25 and 30°C; *b*,*d*). Relative growth rate (*c*,*d*) are the means after removing the large effect of diet and temperature (individual trait/mean trait for each treatment, following Stanton & Thiede (2005) and Stillwell *et al.* (2007*b*)). Standard error bars are included, but are smaller than the symbols for some experimental treatments. Solid lines, females; dashed lines, males.

sensitive to individual growth trajectories than is the measure of individual critical weight (cw_i) used in this study.

The ICG increased substantially on the low-quality diet and as temperature decreased (diet: $F_{1,870} = 316$, $p < 0.0001$; temperature: $F_{2,327} = 448$, $p < 0.0001$; figure 4*a*,*b*). At the population level, Davidowitz *et al.* (2004) found that the ICG does not change with diet. In this study, the ICG was measured at a more sensitive individual level.

Pupal mass was considerably larger on the high-quality diet ($F_{1,870} = 1059$, $p < 0.0001$; figure 5*a*), congruent with the general pattern found in other insects (Berrigan & Charnov 1994). Pupal mass also increased substantially

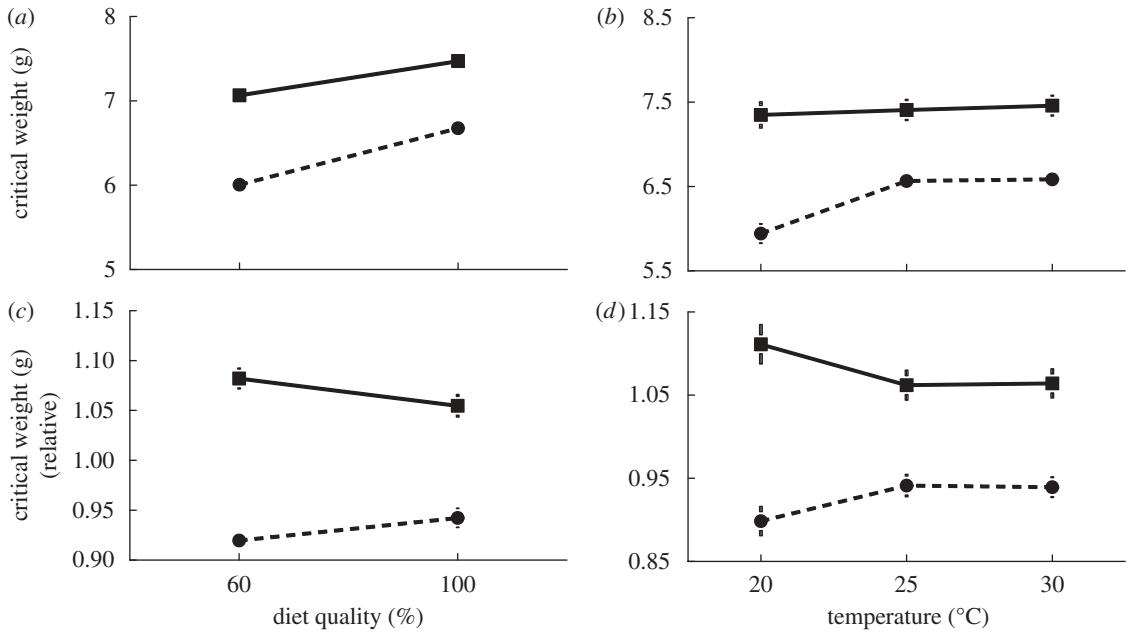

Figure 3. The critical weight of fifth instar *M. sexta* raised on two different qualities of diet (60 and 100%; *a*,*c*) and at three different temperatures (20, 25 and 30°C; *b*,*d*). Relative critical weight (*c*,*d*) are the means after removing the large effect of diet and temperature (individual trait/mean trait for each treatment, following Stanton & Thiede (2005) and Stillwell *et al.* (2007*b*)). Standard error bars are included, but are smaller than the symbols for some experimental treatments. Solid lines, females; dashed lines, males.

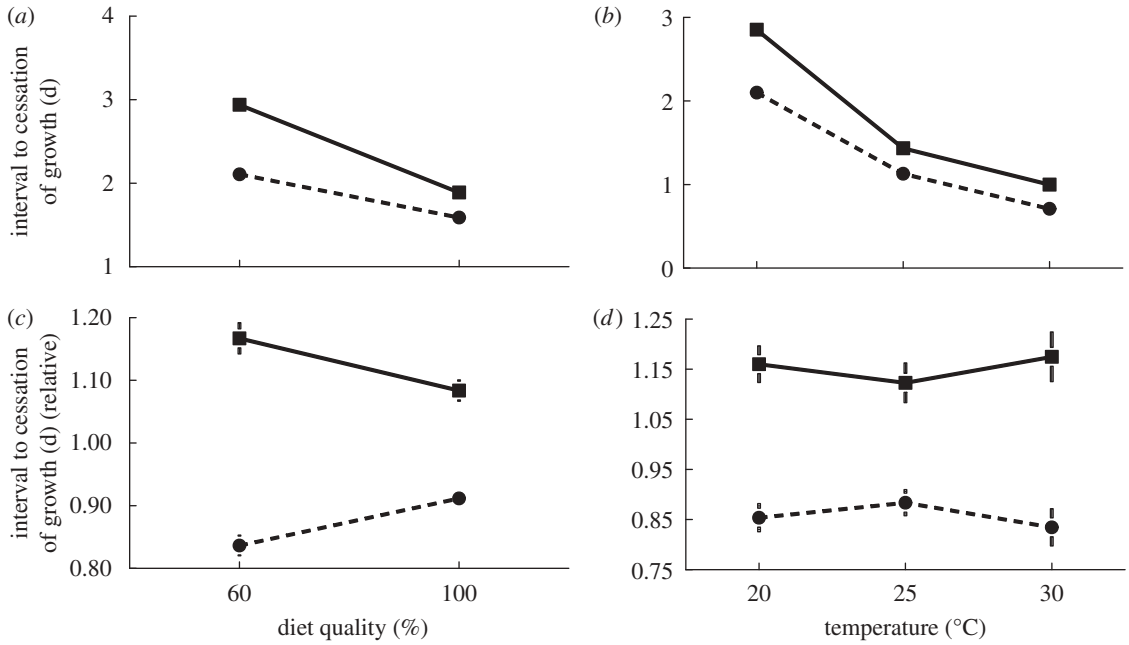

Figure 4. The interval to cessation of growth (ICG) of fifth instar *M. sexta* raised on two different qualities of diet (60 and 100%; *a*,*c*) and at three different temperatures (20, 25 and 30°C; *b*,*d*). Relative ICG (*c*,*d*) are the means after removing the large effect of diet and temperature (individual trait/mean trait for each treatment, following Stanton & Thiede (2005) and Stillwell *et al.* (2007*b*)). Standard error bars are included, but are smaller than the symbols for some experimental treatments. Solid lines, females; dashed lines, males.

with declining temperature ($F_{2,327} = 59.9$, $p < 0.0001$; figure 5*b*), consistent with the temperature–size rule of ectotherms (Atkinson 1994).

The sexes differed in a number of traits measured in both experiments. Female larvae were 8 per cent larger on average than males in both experiments (sex effect in diet experiment: $F_{1,870} = 107$, $p < 0.0001$; sex effect in temperature experiment: $F_{2,327} = 87.5$, $p < 0.0001$; figure 1*a*,*b*), congruent with the general female-biased SSD observed in insects (Stillwell *et al.* 2010). Though the sexes did not differ in growth rate in either experiment ($F \leq 1.21$, $p \geq 0.27$ for both diet and temperature experiments), the critical weight of females was substantially higher than that of males (diet: $F_{1,870} = 180$, $p < 0.0001$; temperature: $F_{2,327} = 116$, $p < 0.0001$; figure 3*a*,*b*), indicating that the initiation of the cessation

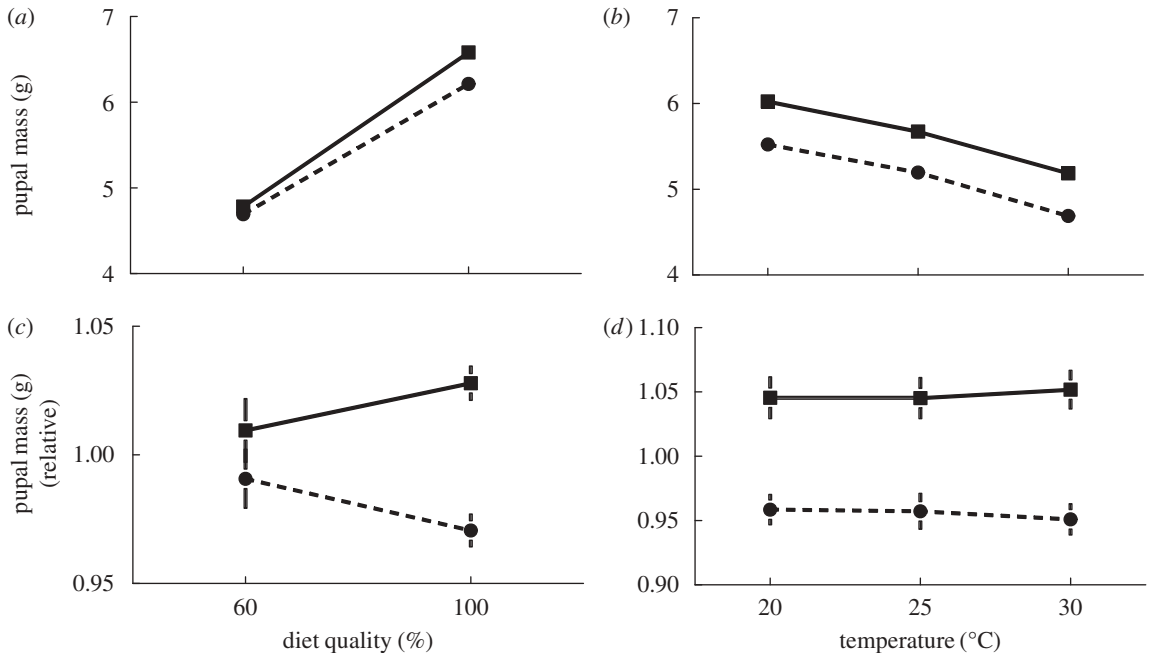

Figure 5. Pupal mass of fifth instar *M. sexta* raised on different qualities of diet (60 and 100%; *a*,*c*) and at different temperatures (20, 25 and 30°C; *b*,*d*). Relative pupal mass (*c*,*d*) are the means after removing the large effect of diet and temperature (individual trait/mean trait for each treatment, following Stanton & Thiede (2005) and Stillwell *et al.* (2007*b*)). Standard error bars are included, but are smaller than the symbols for some experimental treatments. Solid lines, females; dashed lines, males.

of JH synthesis occurs earlier in males (Stillwell & Davidowitz 2010). In addition, the ICG was considerably longer in females (diet: $F_{1,870} = 165$, $p < 0.0001$; temperature: $F_{2,327} = 96.1$, $p < 0.0001$; figure 4*a*,*b*), suggesting that it takes longer for females to clear JH from their haemolymph than males (Stillwell & Davidowitz 2010). Also, female pupae were, on average, substantially larger than male pupae (diet: $F_{1,870} = 20.0$, $p < 0.0001$; temperature: $F_{2,327} = 61.7$, $p < 0.0001$; figure 5*a*,*b*).

(b) *Diet-by-sex and temperature-by-sex interactions*

Because sex-specific plasticity in body size is common in insects (Stillwell *et al.* 2010), we had predicted that we would find sex-specific plasticity in size in response to diet and temperature in *M. sexta*, and that these sex differences in plasticity in size would be created through sex differences in one or more of the three physiological variables that control size. However, relative peak larval mass of males and females did not respond differently to either diet (diet-by-sex interaction: $F_{1,870} = 0.16$, $p = 0.69$; figure 1*c*) or temperature (temperature-by-sex interaction: $F_{2,327} = 0.05$, $p = 0.95$; figure 1*d*), inconsistent with the general pattern of sex-specific plasticity found in most insects (Stillwell *et al.* 2010). Interestingly, there were sex differences in the relative plasticity of several of the physiological variables. For example, males grew relatively faster than females on the low-quality diet, whereas females grew relatively faster than males on the high-quality diet ($F_{1,870} = 7.81$, $p = 0.005$; figure 2*c*). Similarly, males grew relatively faster than females at 20°C, but females tended to grow faster than males at 25 and 30°C; however, the effect was small and not statistically significant ($F_{2,327} = 2.50$, $p = 0.08$; figure 2*d*). Though the critical weight was higher in females on all qualities of diet and at all temperatures, the magnitude of this relative difference between sexes was greatest when raised on the low-quality diet ($F_{1,870} = 6.05$, $p = 0.01$; figure 3*c*) and at the lowest temperature ($F_{2,327} = 4.46$, $p = 0.01$; figure 3*d*). Likewise, though the ICG is relatively longer in females on all diets and at all temperatures, the degree of this difference was greatest on the low-quality diet ($F_{1,870} = 17.3$, $p < 0.0001$; figure 4*c*). However, there were no relative sex differences in temperature-induced plasticity of the ICG ($F_{2,327} = 0.98$, $p = 0.38$; figure 4*d*).

Interestingly, although we detected no sex differences in plasticity of larval size in response to both diet quality and temperature, we did find a sex difference in diet-induced plasticity in pupal mass; the relative degree of female-biased SSD in *M. sexta* was smallest on the low-quality diet, but the effect was only marginally significant ($F_{1,870} = 3.68$, $p = 0.06$; figure 5*c*). This is consistent with the sex difference in plasticity in size found in a majority of insects (Stillwell *et al.* 2010). However, we did not find a relative sex difference in temperature-induced plasticity in pupal mass ($F_{2,327} = 0.15$, $p = 0.86$; figure 5*d*).

4. DISCUSSION

The proximate mechanisms responsible for producing SSD in animals remain largely unknown, particularly the mechanisms that produce sex differences in body size plasticity (Badyaev 2002; Stillwell *et al.* 2010). Here we showed that, in contrast to the general pattern of sex-specific plasticity found in most insects (Stillwell *et al.* 2010), the sexes do not exhibit differences in relative plasticity in larval size. However, the sexes did exhibit relative differences in plasticity in the physiological variables known to control body size in *M. sexta*; the growth rate, the critical weight and the ICG of the sexes

responded differently to diet, whereas the growth rate (note this was only marginally significant) and the critical weight of the sexes responded differently to temperature. These results are contrary to our predictions that we would find sex differences in body size plasticity in response to both diet and temperature, and that a sex difference in plasticity of one or more of the three physiological variables that regulate size would produce sex-specific plasticity in size. However, we did find a relative sex difference in pupal mass plasticity in the diet experiment; the sexes were nearly monomorphic on the low-quality diet, but were dimorphic on the high-quality diet. In a similar experiment with these same treatments (100 and 60% diet quality), we found considerable sex-specific plasticity in adults, indicating that sex differences in adult plasticity are largely due to differences that arise during the larval–pupal and pupal–adult transitions and not because of differences that accrue during larval growth (R. C. Stillwell & G. Davidowitz 2009, unpublished data).

The sex differences in the plasticity of the physiological variables suggest that the endocrine systems of males and females respond differently to environmental variation. Though the sexes exhibited sex-specific plasticity in several of the physiological variables that control size, there were no overall sex differences in relative size plasticity in larvae. The crossing pattern of relative growth rate in both diet and temperature (figure 2*c*,*d*) negates the differential response of relative critical weight and ICG (figures 3*c*,*d* and 4*c*,*d*), resulting in no overall sex difference in plasticity in larval size. It appears that females compensate for their reduced growth rate on the low-quality diet and low temperature by increasing their critical weight and ICG relative to males. It is interesting to note that the biggest sex difference in the physiological variables occurs at low temperature and low-quality diet, which are more stressful (Davidowitz *et al.* 2004). This suggests that the compensatory adjustment of the physiological factors is intensified under more stressful conditions.

One major caveat of our study is that plasticity in size and other traits is probably rarely generated by single environmental variables in nature. Rather, plasticity in growth and development is more likely to be generated via interactions between multiple environmental factors. A majority of plasticity studies and studies that have tested for sex differences in plasticity in body size have examined environmental variables independently of one another. Nevertheless, recent studies have shown that multiple environmental variables can interact, producing complex patterns of plasticity (Sultan *et al.* 1998; Sultan 2001; Relyea 2004; Relyea & Auld 2005; Stillwell *et al.* 2007*b*; Diamond & Kingsolver 2010). In addition, interactions between environmental variables can have different effects on the sexes, producing complexity in sex differences in plasticity in size and hence affecting patterns of SSD (De Block & Stoks 2003). We examined the effects of diet and temperature on SSD in isolation of each other in this study owing to the prohibitively large sample sizes that would be required of a factorial design using *M. sexta* (two diet treatments × three temperature treatments × two sexes = 12 treatments). However, it is possible (even likely) that diet and temperature interact to produce complex patterns of sex differences in plasticity. Future studies should thus incorporate multiple environmental variables into their experimental designs when practical to assess whether interactions among environmental variables affect sex differences in plasticity in size.

In conclusion, we showed in this study that sex differences in relative plasticity of physiological variables that control body size in *M. sexta* do not translate into sex differences in relative peak larval size because a change in one of these variables is counteracted by a change in one or more of the other physiological variables. Our results reveal the complex way in which males and females respond to environmental variation differently and the consequences this has for SSD. Understanding the proximate mechanisms that generate SSD and sex-specific plasticity in size will be critical in future studies to elucidate the ultimate mechanisms (ecological and evolutionary) that produce variation in SSD in animals.

We thank Sarah Diamond, Bryan Helm, Joel Kingsolver, Kristen Potter and an anonymous reviewer for comments on previous versions of this manuscript. G.D. thanks Rivka Eisner, Morgan Lange, Sasha Schlicher, Angie Berg and Christopher Shreeve for assistance in data collection and colony maintenance. This work was supported by NSF grants to G.D. (IBN 9975168 and IBN 0212621) and by a Postdoctoral Excellence in Research and Teaching fellowship to R.C.S. through NIH Training grant no. 1 K12 GM00708 to the Center for Insect Science, University of Arizona.

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