Sexual Dimorphism of Body Size Is Controlled by Dosage of the X-Chromosomal Gene Myc and by the Sex-Determining Gene tra in Drosophila

Kristina Wehr Mathews,1 Margrith Cavegn, and Monica Zwicky
Institute of Molecular Life Sciences, University of Zurich, 8057, Switzerland
ORCID ID: 0000-0002-3655-1088 (K.W.M.)

ABSTRACT Drosophila females are larger than males. In this article, we describe how X-chromosome dosage drives sexual dimorphism of body size through two means: first, through unbalanced expression of a key X-linked growth-regulating gene, and second, through female-specific activation of the sex-determination pathway. X-chromosome dosage determines phenotypic sex by regulating the genes of the sex-determining pathway. In the presence of two sets of X-chromosome signal elements (XSEs), Sex-lethal (Sxl) is activated in female (XX) but not male (XY) animals. Sxl activates transformer (tra), a gene that encodes a splicing factor essential for female-specific development. It has previously been shown that null mutations in the tra gene result in only a partial reduction of body size of XX animals, which shows that other factors must contribute to size determination. We tested whether X dosage directly affects animal size by analyzing males with duplications of X-chromosomal segments. Upon tiling across the X chromosome, we found four duplications that increase male size by >9%. Within these, we identified several genes that promote growth as a result of duplication. Only one of these, Myc, was found not to be dosage compensated. Together, our results indicate that both Myc dosage and tra expression play crucial roles in determining sex-specific size in Drosophila larvae and adult tissue. Since Myc also acts as an XSE that contributes to tra activation in early development, a double dose of Myc in females serves at least twice in development to promote sexual size dimorphism.

KEYWORDS Drosophila melanogaster; Myc; tra; dosage compensation; sexual dimorphism

Most animals display striking sexual dimorphism that evolved for success in sexual reproduction (Hedrick and Temeles 1989; Cline and Meyer 1996; Fairbairn 1997). This involves not only the development of sex-specific structures like genitalia, but also distinct behaviors, pigmentation, and body sizes. In the fruit fly Drosophila melanogaster, as in most species of insects, females attain larger body sizes than males (Darwin 1871; Shine 1990; Stillwell and Davidowitz 2010). Although this aspect of sexual dimorphism in Drosophila has long been appreciated, how the sex-specific difference in body size is genetically controlled is still unknown. In principle, there are two possibilities: genes of the sex-determining pathway might control the sex-specific difference in body size, or the different chromosomal constitution of males (XY) and females (XX) could initiate a process that leads to sex-specific size irrespective of the genes of the sex-determining pathway.

In Drosophila, Sex-lethal (Sxl) is the master switch of the sex-determination pathway, a cascade of sex-determining genes that includes transformer (tra) and doublesex (dsx) (McKeown et al. 1988; Nagoshi et al. 1988; Burtis and Baker 1989; Cline and Meyer 1996). Although Sxl is transcribed in both sexes, only XX animals produce a protein product with defined roles (Bell et al. 1988; Cline 1988; Duffy and Gergen 1991; Torres and Sanchez 1991; Kappes et al. 2011). Sxl activates tra, which ensures that dsx is expressed in the female mode. XX flies lacking tra expression are physically and behaviorally transformed into males, with the exception of their body size. They are larger than their XY brothers (Sturtevant 1945), having adult sex-specific traits that are intermediate in size between male and female traits (Brown and King 1961). Recently it has been reported that XX pupae
that are homozygous mutants for \textit{tra} are 10% larger than \textit{XY} pupae (Rideout et al. 2015). Control \textit{XX} females, however, are 30% larger than males. This indicates that \textit{tra} contributes to male-female differences in body size, but also that \textit{tra} is not the sole regulator of this trait.

In agreement with the observation that \textit{XX} animals lacking \textit{tra} are intermediate in size, \textit{XX} animals carrying hypomorphic alleles of \textit{Sxl} are phenotypically male and are smaller than wild-type females (Cline 1984; Evans and Cline 2013). In addition to activating \textit{tra}, \textit{Sxl} also controls dosage compensation; a process by which \textit{X}-chromosomal transcription is equalized in animals where females have two \textit{X} chromosomes and males have only one (Lucchesi and Kuroda 2015). Due to this essential function, females with homozygous null mutations in \textit{Sxl} are lethal, which explains the use of viable hypomorphic \textit{Sxl} alleles that do not completely remove the gene function. Due to this difficulty, it has not yet been possible to distinguish between two alternatives: \textit{Sxl} might be the sole regulator of sex-specific size acting through multiple genes, one of which is \textit{tra}. Alternatively, factors that are not controlled by \textit{Sxl} might contribute to regulation of sex-specific size in concert with \textit{Sxl} acting through \textit{tra}.

We wondered whether genes outside the sex-determining pathway participate in regulating sex-specific body size. In particular, we were curious whether \textit{X}-chromosome gene dosage plays a direct role in determining body size. This idea was based on the fact that, while penetrant, the process of dosage compensation in \textit{Drosophila} is not absolute, with a substantial fraction of \textit{X}-linked genes being under- or overexpressed in males compared to females (Smith and Lucchesi 1969; Roberts and Evans-Roberts 1979; Legube et al. 2006; Chang et al. 2011; Lott et al. 2011; Sun et al. 2013). Our rationale was that \textit{XX} animals might be larger than \textit{XY} animals because they express two copies of a growth gene that is not, or is only partly, dosage compensated. If such a gene existed, it should fulfill four criteria: (1) A duplication of this gene should lead to an increase in body size in males, (2) the increase in size seen with a duplication should be eliminated in a male carrying a mutation of the gene, (3) females with only one functional copy of the gene should be smaller than control females with two functional copies, and (4) females should express the gene at higher levels than males during the critical phase of female-specific growth.

Through a combination of genetic and molecular analyses, we identified several \textit{X}-linked genes that lead to an increase in male size upon duplication. However, only one of our candidates, the \textit{Myc} gene, fulfilled all our criteria. Mutations in \textit{Myc} restored males with a \textit{Myc}-containing duplication to normal male size, and heterozygous females carrying one mutation of \textit{Myc} were smaller than control females. Furthermore, \textit{Myc} displays a sex-specific difference in expression in the third larval instar stage. Our experiments identify \textit{Myc} as a potent regulator of sex-specific body size in \textit{Drosophila}, both in larvae and in adult tissue.

**Materials and Methods**

**Drosophila stocks**

\textit{Oregon R} (OR), \textit{white}^{1118} (\textit{w}^{1118}) and \textit{white}^{1} (\textit{w}^{1}) lines were used as controls. \textit{Dp(1;Y)} and \textit{Dp(1;3)} duplication lines, including \textit{Df(1)Exel6233} (stock 7707), \textit{Cg-Gal4} (stock 7011) and \textit{UAS-tra} (stock 4590), were obtained from the Blooming-\textit{ton} Stock Center; \textit{CanA^{DRO}} (stock 109-862) and \textit{SL1RP1^{GS1189}} (stock 200-175) lines from the \textit{Drosophila} Genetic Resource Center (Kyoto, Japan). \textit{Myc}^{P0} (Johnston et al. 1999), \textit{Mnt}^{1} (Loo et al. 2005), \textit{cytD}^{1} (Emmerich et al. 2004), \textit{CanA-14}^{P0} (Nakai et al. 2011), \textit{shi4C} (Kasprowicz et al. 2014), \textit{tra}^{1} (Sturtevant 1945), \textit{UAS-Desat1} (Kohler et al. 2009), \textit{da-Gal4} (Wodarz et al. 1995), and \textit{genomicSmc3-3a} (Heidmann et al. 2004) were as described. \textit{UAS-lacZ86} and \textit{UAS-lacZ75c} flies were a gift from Johannes Bischof. Flies were raised at 25 ± 1°C and 60–70% humidity with a 12:12 light:dark photoperiod on standard medium (100 g/l yeast, 75 g/l dextrose, 55 g/l cornmeal, 10 g/l flour, 8 g/l agar, 7.5 ml/l nipagin).

**Experimental design**

All crosses were performed at 25°C in bottles containing 45 ml standard medium. In the first round of screening, \textit{Dp(1;Y)} males were backcrossed to \textit{OR} females twice and \textit{F}_2-sibling wandering larvae were weighed individually. For fine-scale mapping using \textit{Dp(1;3)} duplication lines, \textit{Dp(1;3)} males were crossed with \textit{w}^{1118} females. Heterozygous \textit{Dp(1;3)} males were then crossed to \textit{w}^{1118} females and wandering-male sibling larvae were weighed individually.

**Larval measurements**

Wandering third instar larvae were rinsed with water, blotted dry, and weighed on a Mettler Toledo MX5 Microbalance. Next, for the \textit{Dp(1;3)} screen, individual larvae were placed into apple juice-agar-containing wells of 64-well nucleon dishes, sealed with punctured Parafilm M, and maintained until adulthood at which point genotype was assessed by eye color. At least 20 wandering larvae of each genotype from each cross were analyzed in this manner, except for larvae with \textit{Dp(1;3)DC271} for which only 10 male larvae were tested due to weak viability. For \textit{tra} experiments, \textit{tra}^{1} females were first mated with \textit{Df(3L)st-E52} males for 48 hr and then cocultured with \textit{OR} females, which serve as internal controls. Offspring third instar wandering larvae were weighed and maintained as described above. Gonads from adult \textit{tra}^{1}/\textit{Df(3L)st-E52} flies were dissected to determine genotype. Testes develop fully in \textit{XY};\textit{tra}^{1}/\textit{Df(3L)st-E52} flies, whereas only rudimentary testes form in \textit{XX};\textit{tra}^{1}/\textit{Df(3L)st-E52} flies.

**Adult trait measurements**

Between 10 and 32 flies were measured for each genotype, dependent upon availability due to genotype fitness. A minimum of two data sets was collected for each genotype. For interocular distance (IOD) measurements, flies were positioned on apple agar plates and photographed using a Leica MZ FLIII Stereomicroscope in combination with a
Moticam 2300 camera and Motic Images Plus software. ImageJ 2.0.0 provided by Fiji was used for manual measurement of IODs, defined by the distance from eye edge to eye edge across the anterior orbital bristles.

**Quantitative PCR analysis**

We assessed RNA levels in feeding larvae in their third instar stage using reverse transcriptase-quantitative PCR (RT-qPCR). Total RNA was isolated from OR larvae dissected 68–72 hr after egg laying using TRI Reagent (Sigma Chemical, St. Louis, MO). Dnase I digestion (Ambion) was carried out twice for 30 min at 37°. Reverse transcription was performed using a Transcriptor High Fidelity Complementary DNA (cDNA) Synthesis Kit on 150 ng total RNA using oligo(dT)16, as described by the manufacturer (Roche). For each sex, at least 10 larvae were dissected. A minimum of three biological replicates was used for each experimental point. cDNA generated from 150-ng RNA was used as the template for amplification, using validated primers in MESA Green qPCR Mastermix for SYBR Assay (Eurogentec). Gene expression was normalized to the expression of two reference genes: *tubulin binding protein* and *α-tubulin*. Primers used for qPCR analysis were as follows: *Myc*, 5′-GAG TAG GGA AAC TAT GTT-3′/5′-GTA AAG CAC CAT TGC CAT TA-3′; *Mnt*, 5′-CCA CAA TGG AAC CAC CAC ATT TC-3′/5′-CGG TTG TTG CTC CTC CAT TAG TT-3′; *Max*, 5′-CAA GGA GAG CIT CAC CAA CC-3′/5′-TCT GTA TGC ATT CCG TGG TC-3′; *rpl1*, 5′-CAA CAA CCG ACA GGC CTA C-3′/5′-GAA TAC GGA AAC TAT GTT-3′; *rpl135*, 5′-AGA CCA TTC CAG TGC TGA CC-3′/5′-TCT GTC TGG CTT TTT GCG GTA CAC AT-3′; *cyclD*, 5′-CAC GGA CAA CAG CAT CTA CAA-3′/5′-GGA AGT CCA AGG GAG GAG TCA CA-3′; *CanA/Pp2B*, 5′-CGA CTT CCT GCA CAA CA-3′/5′-GTC TGG CTT TTT GCG GTA CAT-3′; and *Desat1*, 5′-AGG TAA CCT GGC TGG TGA AC-3′/5′-TAT GCC ATC CCT CTC CAA AG-3′.

**Data availability**

The authors state that all data necessary for confirming the conclusions presented in the article are represented fully within the article. All supporting data and reagents used in this work will be made available upon request.

**Results**

**At the third instar larval stage, females consistently weigh 30% more than males**

We started with the hypothesis that sex-specific differences in body size might be controlled, not only by genes of the sex-determining pathway, but also by other genes whose dosage could regulate growth. We sought to identify factors that contribute to the growth difference between males and females. To assess sex-specific size, we chose to measure body size of larvae in their wandering stage, because weight differences between the sexes have reached a maximum in this phase lasting several hours, with females weighing 30% more than males (Bakker 1959; Okamoto et al. 2009). By comparison, adult traits are more complex and female traits are, on average, a mere 10.5% larger than those of adult males, making smaller changes in adult size between sexes more laborious to detect. Further complicating adult measurements, the degree of sexual-size dimorphism is not uniform across traits, reflecting the autonomous and specialized nature of growth regulation in imaginal disks (Cowley and Atchley 1988; David et al. 2003).

Growth in *Drosophila* is extremely sensitive to environmental conditions including nutrition, temperature, oxygen levels, and humidity (Nijhout et al. 2014). We raised all flies in a climate-controlled room on normal food to reduce environmental effects. Furthermore, we considered only weight ratios of flies cocultured within the same vial for our screen. Whereas a weight ratio of 1.0 would be expected if females and males were of equal size, in *Drosophila* larvae the weight ratio of wild-type females to males is 1.3. To examine possible crowding effects on sexual size dimorphism, we examined a range of population densities for control *w*¹¹⁸ and wild-type OR fly strains used in our screens. We found that the female: male weight ratio was consistently within a range of 1.26–1.32 and 1.29–1.32 for *w*¹¹⁸ and wild-type OR flies, respectively, across a wide range of densities (Supplemental Material, Table S1).

**X-chromosome screen for dose-dependent modifiers of body size**

We were interested in determining what, other than the sex-determining gene *tra*, could account for size differences between sexes in *Drosophila*. To test whether *X* dosage plays a role, we analyzed the effect of duplicating *X*-chromosome segments on male size. We chose to analyze males because they express neither *Sxl* nor *tra*, and would therefore allow us to identify growth effects regulated by genes that do not act through the sex-determining pathway.

For our initial assessment, we selected and analyzed 22 isogenic stocks with *Y*-linked *X* duplications that together cover >91% of the *X* chromosome (Cook et al. 2010). Due to severely reduced viability and fertility caused by two duplications, we were unable to generate sufficient offspring to assess the effects of these duplications on growth, reducing our coverage of the *X* chromosome to 81%. The remaining 19% of the *X* chromosome not covered in this initial screen was distributed across five gaps, which we will discuss later.

Of the 20 duplications we examined, 4 had a positive effect (Table 1 and Table S2). A striking increase in growth was observed, as these four duplication lines yielded *XX/YY*, *DpX* weight ratios of 1.20 or less. In each of these cases, males carrying a duplication experienced a growth increase of at least 8% compared to control males. We chose to focus on these for further study.

**Narrowing down the four *X*-chromosomal regions that increase male size in a dose-dependent manner**

Our regions of interest defined by duplications *Dp(1;Y)BSC74, Dp(1;Y)BSC47, Dp(1;Y)BSC223*, and *Dp(1;Y)BSC240* (hereafter referred to as region A, B, C, and D, respectively) range
between 681 kbp and 1.7 Mbp in size. To narrow our regions of interest and confirm our results, we analyzed a second set of smaller Y-linked X-chromosomal duplications specific to regions A–D in a similar manner as described above (Table 2 and Table S3).

Region A, defined by Dp(1;Y)BSC74, was analyzed further using two small duplications, Dp(1;Y)BSC76 and Dp(1;Y)BSC82, which map within the region (Figure S1A). We found that Dp(1;Y)BSC76 reproduced results found, with the original duplication giving rise to a ratio of XX/XY,DpX of 1.19, which corresponds to an 8% increase in growth of test males compared to wild-type males. Dp(1;Y)BSC82, a smaller duplication that corresponds to the proximal end of Dp(1;Y)BSC74 and Dp(1;Y)BSC76, had a far stronger impact. The weight ratio of wild-type females to males with Dp(1;Y)BSC82 was 1.15, indicating a 13% weight increase in males compared to wild type (Figure S1A and Table 2). Our attempt to narrow down our 1.7 Mbp region of interest [Dp(1;Y)BSC74] thus led us to identify a candidate within 1.0 Mbp defined by Dp(1;Y)BSC82.

Four additional duplications in region B, Dp(1;Y)BSC49, Dp(1;Y)BSC52, Dp(1;Y)BSC55, and Dp(1;Y)BSC105, were used to narrow our region of interest from 681 to 219 kbp. Region B was newly defined by the proximal ends of Dp(1;Y)BSC52, which replicates the male growth increase observed with Dp(1;Y)BSC47, and Dp(1;Y)BSC55, which does not (Figure S1B and Table 2).

Duplications that define regions C and D overlap one another, making it unclear whether a single growth-promoting sequence resides in their shared sequence or whether multiple sequences generate the observed effects. We employed four Dp(1;Y) lines whose X-chromosome duplications together tile across regions C and D. A large region within C was refractory to this method of analysis due to weak viability and sterility in

---

**Table 1 Dp(1;Y) duplications spanning the X chromosome used in the first round of screening**

| Duplication | First replicate | Second replicate | Third replicate | XX/XY,DpX |
|-------------|----------------|-----------------|----------------|-----------|
| BSC296      | ♀ 1.93 ± 0.02  | ♀ 2.02 ± 0.02   | ♀ 2.13 ± 0.02  | 1.33      |
|             | ♀ 1.45 ± 0.02  | ♀ 1.70 ± 0.01   | ♀ 1.78 ± 0.01  | **1.19**  |
| BSC74       | ♀ 1.96 ± 0.02  | ♀ 2.07 ± 0.02   | ♀ 2.08 ± 0.02  | 1.31      |
|             | ♀ 1.66 ± 0.02  | ♀ 1.61 ± 0.01   | ♀ 1.95 ± 0.02  | 1.26      |
| BSC158      | ♀ 1.95 ± 0.02  | ♀ 1.61 ± 0.01   | ♀ 1.49 ± 0.02  | 1.27      |
| BSC91       | ♀ 2.01 ± 0.02  | ♀ 2.18 ± 0.04   | ♀ 1.58 ± 0.02  | 1.29      |
| BSC277      | ♀ 2.18 ± 0.04  | ♀ 1.56 ± 0.02   | ♀ 1.99 ± 0.02  | 1.30      |
| BSC178      | ♀ 1.90 ± 0.02  | ♀ 1.98 ± 0.02   | ♀ 1.51 ± 0.01  | 1.34      |
| BSC33       | ♀ 1.90 ± 0.02  | ♀ 1.98 ± 0.02   | ♀ 1.47 ± 0.01  | 1.33      |
| BSC170      | ♀ 1.99 ± 0.02  | ♀ 1.54 ± 0.02   | ♀ 1.49 ± 0.01  | 1.30      |
| BSC58       | ♀ 2.03 ± 0.01  | ♀ 2.04 ± 0.02   | ♀ 1.51 ± 0.03  | 1.29      |
| BSC47       | ♀ 2.07 ± 0.02  | ♀ 2.06 ± 0.02   | ♀ 1.75 ± 0.02  | 1.18      |
|             | ♀ 1.72 ± 0.03  | ♀ 1.72 ± 0.03   | ♀ 2.06 ± 0.02  | 1.18      |
| BSC185      | ♀ 2.06 ± 0.01  | ♀ 1.55 ± 0.02   | ♀ 1.97 ± 0.02  | 1.29      |
| BSC231      | ♀ 1.53 ± 0.01  | ♀ 1.67 ± 0.02   | ♀ 1.53 ± 0.01  | 1.25      |
| BSC223      | ♀ 2.05 ± 0.02  | ♀ 1.98 ± 0.02   | ♀ 1.66 ± 0.02  | 1.20      |
|             | ♀ 1.73 ± 0.02  | ♀ 1.66 ± 0.02   | ♀ 1.94 ± 0.02  | **1.19**  |
| BSC240      | ♀ 2.00 ± 0.02  | ♀ 1.56 ± 0.03   | ♀ 1.71 ± 0.01  | 1.29      |
| BSC200      | ♀ 2.04 ± 0.02  | ♀ 2.05 ± 0.04   | ♀ 1.58 ± 0.01  | 1.30      |
| BSC67       | ♀ 2.09 ± 0.02  | ♀ 1.60 ± 0.03   | ♀ 2.09 ± 0.02  | 1.33      |
| BSC14       | ♀ 2.05 ± 0.02  | ♀ 1.58 ± 0.01   | ♀ 2.05 ± 0.02  | 1.33      |
| BSC129      | ♀ 1.90 ± 0.01  | ♀ 1.42 ± 0.03   | ♀ 1.58 ± 0.01  | 1.37      |
| BSC329      | ♀ 2.12 ± 0.03  | ♀ 2.06 ± 0.02   | ♀ 2.12 ± 0.02  | 1.27      |
| BSC276      | ♀ 2.12 ± 0.02  | ♀ 2.16 ± 0.03   | ♀ 2.16 ± 0.03  | 1.18      |

Mean weights ± SE of Dp(1;Y) male larvae (XY,DpX) and sibling females (XX). Duplications yielding XX/XY, DpX ratios of 1.20 or less are in boldface font. ♀, female; ♂, male.
males expressing \(Dp(1;Y)BSC225\) (Figure S1C and Table 2). The complete region C duplication, \(Dp(1;Y)BSC223\), must therefore contain one or more suppressors to the observed male sterility and fertility. Each of the duplications tested across region D produced larger males, but neither had as strong an effect on growth as \(Dp(1;Y)BSC240\). These results suggest that multiple dosage-sensitive regions exist within region D (Figure S1D and Table 2). Thus, we next performed a finer-tuned analysis of our regions of interest using smaller duplications to individuate growth-promoting and possible growth-suppressing sequences.

**Fine-scale mapping and gene identification**

We selected 54 duplication lines that each have a small segment of the X chromosome translocated to the third chromosome and collectively cover the entirety of the narrowed regions A–D (Figure 1A and Table S4). An additional 50 duplication lines were selected to cover gaps 1–5, for which no large duplications were either available or amenable to screening (Figure 1B and Table S5). The selected duplication lines are part of an X-chromosome duplication kit prepared by insertion of BAC clones marked with the dominant mini-white eye marker into polytene band 65B2 on the third chromosome (Venken et al. 2006, 2010). Using these lines, we designed a genetic mating scheme to generate male larvae carrying duplications alongside sibling males that do not. Since all \(Dp(1;3)\) lines were prepared in a \(w^{1118}\) background, we used \(w^{1118}\) males as controls in this set of experiments.

Of the duplications we analyzed, the vast majority (91%) provided male larvae a slight growth advantage over their \(w^{1118}\) siblings. We noticed that both male and female wandering larvae mutant for a \(w\) allele are strikingly smaller (8 and 11%, respectively) (Figure S2) than OR controls and asked if the mini-white marker in \(Dp(1;3)\) duplication lines contributed to larval size. Two transgenic lines carrying an insertion comprised of a UAS-lacZ transgene and the mini-white marker were a mere 1–2% larger than \(w^{1118}\) controls, leading us to conclude that the contribution of mini-white to size is modest at best.

In region A, 2 of the 22 duplications tested, \(Dp(1;3)DC059\) and \(Dp(1;3)DC060\), were exceptionally potent in promoting growth. Males harboring either of these were on average 12–14% larger than \(w^{1118}\) siblings lacking the duplication. A single gene locus, the *Drosophila* gene diminutive (Myc), is found in the large overlap of the duplications. We conclude that introduction of an extra copy of *Myc* is sufficient to substantially increase larval growth in males (Figure 1A, Figure 2A, and Table S4). Interestingly, the proximal-most duplication tested in region A repressed male growth, giving rise to males that were 5 ± 1% smaller than \(w^{1118}\) controls. We strongly suspected that expression of Mnt, a transcription factor known to antagonize Myc (Loo et al. 2005) which is also contained in this duplication, caused the observed reduced growth. Indeed, a null mutation in *Mnt* relieves the negative effect on growth imposed by the duplication \(Dp(1;3)DC463\) (Figure 3A). *Mnt* and *Myc* dosage are thus both essential for regulation of growth in larvae.

Of the 12 duplications tested in region B, several were able to elicit modest increases in male size, but none could replicate the 10 ± 1% weight increase resulting from duplication of the complete region B (Figure 1A). We conclude that the size increase observed in our initial screen by duplication of the entire region is a cumulative effect of multiple loci.

A total of 20 \(Dp(1;3)\) duplications were selected to tile across regions C and D. Of these, two duplications [\(Dp(1;3)DC133\) and \(Dp(1;3)DC316\)] mimicked the increases in male size (9 ± 1% and 10 ± 1%, respectively) observed upon duplication of complete region C or D (Figure 1A and Table S4). We narrowed our regions of interest by analyzing neighboring duplications, which partially overlap \(Dp(1;3)DC133\) in region C and \(Dp(1;3)DC316\) in region D. Five candidate genes for region C were thus identified: *tay bridge* (*tya*), membrane steroid binding protein (*MSBP*), *CG15916*, *shibire* (*shi*), and *cycID* (*cycD*) (Figure 2B and Table 3). Likewise, using this approach, we identified five candidates within region D: *Calcineurin A at 14F* (*CanA-14F*), *CG13014*, structural maintenance of chromosome 3 (*SMC3*), ubiquitin conjugating enzyme 7, and *CG9784* (Figure 2C and Table 3).

Of the duplications tested within the five gap regions, \(Dp(1;3)RC026\) which covers part of gap 3, resulted in the most striking increase (9.5%) in male size compared to \(w^{1118}\) male siblings (Figure 1B and Table S5). A mere three candidate genes were identified upon subtracting away flanking regions without growth effects: *CG15211*, *Ant2*, and *sesb* (Figure 2D and Table 3). Finally, there remain two small regions, one in gap 1 and one in gap 3 that precluded analysis due to weak fertility and viability of duplications \(Dp(1;3)DC068\) and \(Dp(1;3)DC232\).

---

**Table 2 \(Dp(1;Y)\) duplications within regions of interest identified in the primary screen**

| Duplication | Region | First replicate | Second replicate | XXXXY, DpX |
|-------------|--------|----------------|------------------|------------|
| BSC76       | A      | \(\varphi\) 2.03 ± 0.03 | \(\varphi\) 1.70 ± 0.03 | 1.19       |
| BSC82       | A      | \(\varphi\) 1.98 ± 0.03 | \(\varphi\) 1.72 ± 0.03 | 1.15       |
| BSC49       | B      | \(\varphi\) 2.13 ± 0.02 | \(\varphi\) 1.99 ± 0.02 | 1.23       |
| BSC52       | B      | \(\varphi\) 2.01 ± 0.03 | \(\varphi\) 1.63 ± 0.02 | 1.22       |
| BSC55       | B      | \(\varphi\) 2.05 ± 0.02 | \(\varphi\) 1.54 ± 0.02 | 1.34       |
| BSC105      | B      | \(\varphi\) 2.07 ± 0.02 | \(\varphi\) 1.51 ± 0.02 | 1.37       |
| BSC225      | C      | 0.03 1.15 | 0.02 1.24 | 1.26       |
| BSC225      | C/D    | \(\varphi\) 2.09 ± 0.03 | \(\varphi\) 1.70 ± 0.02 | 1.23       |
| BSC232      | D      | \(\varphi\) 2.07 ± 0.03 | \(\varphi\) 1.70 ± 0.02 | 1.22       |
| BSC244      | D      | \(\varphi\) 2.20 ± 0.03 | \(\varphi\) 1.77 ± 0.02 | 1.24       |

Mean weights ± SE of \(Dp(1;Y)\) male larvae (XY, DpX) and sibling females (XX). \(\varphi\), female; \(\varphi\), male.
In conclusion, through a second round of screening, we succeeded in substantially trimming three (regions A, C, and D) of our four regions of interest (Figure 1A). More specifically, in region A we identify Myc and Mnt as dose-dependent modifiers of growth (Figure 1A and Figure 2A). We narrowed region C and region D so that five candidate genes remained in each (Figure 1A; Figure 2, B and C; and Table 3). Additionally, we detected a new region of interest in gap 3, one of the five gap regions not covered in the first round of screening (Figure 1B). In gap 3, we identify a short list of only three candidate genes (Figure 2D and Table 3).

**Genetic analysis of candidate genes**

We reasoned that if the additional copy of Myc provided by region A duplications [Dp(1;3)DC059 and Dp(1;3)DC060] was responsible for the observed growth increase in males, then reducing Myc dosage in flies with one of these duplications should return them to wild-type size. Indeed, reducing Myc levels through introduction of the hypomorphic Myc^{50} allele (Johnston et al. 1999) in males expunged the positive growth effects provided by Dp(1;3)DC060 (Figure 3A).

None of the small Dp(1;3) duplications tested across region B reproduced the growth increase observed in our original screen. Of note is that the region B-defining, Y-linked duplication chromosome harbors a segment of the distal X chromosome (19F4-h29) in addition to the one described in our fine-scale mapping. We had originally omitted the distal X sequence for further analysis because the entirety of this region was also represented in the terminal Y-linked duplication [Dp(1;Y)BSC276], a duplication that only modestly affected growth. We examined one candidate, SLIRP1, in this terminal region at 19F4 due to its described role in larval feeding behavior (Ryuda et al. 2011). Duplication of the SLIRP1 sequence did not increase growth in larvae (Figure S3A), leaving the question of what the growth promoter in region B is open.

Of the five candidates in region C (tay, MSBP, CG15916, shi, and cycD), we suspected that duplication of cycD was causing the observed larval-growth phenotype. We crossed Dp(1;3)DC133 flies into a cycD heterozygous null background and found a strong reduction (5 ± 1%) in growth (Figure 3A). The growth phenotype of the duplication is only partially suppressed, so it stands to reason that, while copy number of cycD can clearly play a role in male growth, a second factor expressed by the duplication contributes to the observed growth increase in region-C duplication males. Introduction of a genomic transgene expressing shi did not increase male weight (Figure S3B). Further analysis of candidates should uncover other growth promoter(s) in this region. Identification of these was beyond the scope of this article.

In region D, we were also left with five candidate genes: CanA-14F, CG13014, SMC3, Ubc7, and CG9784. We first assessed the impact of genomic SMC3 transgenes on size but found no significant weight changes (Figure S3C). Next, we made use of an available null mutation in CanA-14F and found that Dp(1;3)DC316 males lacking a copy of endogenous CanA-14F were ~3% smaller than flies with the duplication alone (Figure 3A). Given that this duplication also contains Pp2B-14D, a second of the three calcineurin catalytic subunits found in Drosophila, we assessed the weights of larvae lacking a copy of each CanA-14F and Pp2B-14D genes (CanA^{BKO}) in duplication males. Knocking down both aforementioned calcineurin catalytic subunits from Dp(1;3)DC316 males completely eliminated the duplication’s positive growth effect (Figure 3A). We deduce that duplication of CanA-14F and Pp2B-14D genes together are likely the root cause of growth increase in males with Dp(1;3)DC316.
Effects of Myc, cycD, and calcineurin subunit gene dosage on female size

We reasoned that if gene dosage plays a role in sexual dimorphism of body size, then reducing the dosage of our candidate genes in females should render them smaller than their wild-type counterparts. Indeed, we found that female larvae heterozygous for the strong Myc hypomorph Myc<sup>99</sup> or the Myc deficiency Df(1)Exel6233 weighed ~15% and 10% less than controls, respectively (Figure 3B). Reduction of cycD dosage by half, on the other hand, did not significantly affect female growth (Figure 3B). Finally, neither CanA-14F<sup>KO</sup> nor CanA<sup>ΔK</sup> heterozygous female larvae were significantly smaller than <sup>1118</sup> controls (Figure 3B). These results suggest that, while Myc, cycD, and calcineurin expression levels have the potential to affect growth of Drosophila larvae, only Myc is likely to play a role in wild-type sexual dimorphism of body size.

Quantitative analysis of candidate gene expression

Our finding that Myc, cycD, and calcineurin dosage can affect larval growth made us curious if transcript levels differ between sexes of wild-type larvae. We performed qPCR analysis to determine transcript levels of our candidates in feeding third instar OR larvae. Synchronized male and female sibling larvae were collected between 68 and 72 hr after egg laying, a time period when females grow more rapidly than males (Okamoto et al. 2009). Given the central role attributed to the fat body in regulating organismal growth (Colombani et al. 2005; Delanoue et al. 2010; Parisi et al. 2013), we assessed transcript levels from isolated fat-body tissue in addition to determining whole larval levels. We found Myc transcript levels to be 1.7 times higher in both female fat body and whole preparations as compared to sibling males (Figure 4A). Furthermore, introduction of a Myc duplication into males resulted in Myc transcript levels that were 1.6 times higher than wild-type males, while females heterozygous for a Myc deficiency accumulated less than half of what was detected in control females (Figure S4, A and B). On the other hand, no significant gender-specific differences in cycD or CanA-14F/Pp2B-14D transcript levels were detected in either whole or fat body tissues (Figure 4A). These results support our idea that Myc transcript levels play a key role in regulating sex-specific size differences in Drosophila larvae.

Examination of Myc cofactors and effectors

Myc is part of the conserved Myc-Max-Mnt network of transcription factors, which have been implicated in regulation of proliferation in vertebrates and Drosophila alike (Grandori et al. 2000; Bellosta and Gallant 2010). Max forms heterodimers with Myc, which together bind E-box sequences to activate transcription of nearby genes. Mnt, on the other hand, antagonizes Myc by heterodimerizing with Max and binding similar sequences and repressing transcription (Orian et al. 2003; Bellosta and Gallant 2010). We were curious whether Max or Mnt transcript levels were also sex-specifically regulated, but found no significant differences between female and male larvae (Figure 4B). Sex-specific Myc-Max-Mnt target gene expression therefore likely results from gender-specific imbalance in Myc transcript levels.

Ecdysone signaling in the larval fat body downregulates Myc expression as well as Myc target genes rpl11 or rpl135 (Grewal et al. 2005; Delanoue et al. 2010). Surprisingly, we
found no upregulation of either rpl1 or rpl13s in whole female extracts, indicating that other Myc targets mediate body size dimorphism in Drosophila (Figure 4B).

The lipid desaturase Stearoyl-CoA desaturase (Desat1) is the Drosophila ortholog of vertebrate Stearoyl-CoA desaturase-1/SCD1 (Brock et al. 2007; Kohler et al. 2009). In mice, mutations in scdl result in leaner and smaller animals (Ntambi et al. 2002). Similarly, reducing Desat1 in the Drosophila fat body results in smaller pupae and adults (Parisi et al. 2013). Furthermore, it was shown that Desat1 expression in the fat body is essential for fat body Myc-induced systemic growth. We asked whether Desat1 might be targeted by Myc to drive sex-specific differences in body size. Surprisingly, we found that Desat1 transcript levels were elevated in the male fat body compared to the female fat body, making it unlikely that Myc regulation of body size is mediated by Desat1 levels (Figure 4C). Finally, we overexpressed Desat1 in the fat body and found no significant difference in either female or male body size (Figure 4D). We conclude that Myc must regulate sex-specific growth via factors other than Desat1.

**Myc and tra together promote larval and adult growth**

It has been demonstrated that mutations in the tra gene hinder growth of XX animals, resulting in pupae and adults that are of an intermediate size between control female and male animals (Brown and King 1961; Rideout et al. 2015). We assessed the mass difference between male and female third instar larvae and tested the effect of tra mutations at this stage. In consonance with previous findings mentioned above, we found that XX larvae lacking tra were 12 ± 0.04% smaller than wild-type females, accounting for roughly half of the observed mass difference between control males and females. Lack of tra in XY larvae had no impact on size (Figure S5A).

Experiments presented up to this point describe two elements that ensure that female larvae weigh more than their male siblings. This sex-specific feature is regulated by the dosage of Myc and by female-specific expression of tra. We wondered whether these two elements together are sufficient to promote growth of males in such a way that they achieve the size of control females. Duplicating Myc in XY males by adding Dp(1;3)DC060 led to larvae that weighed 12% more than control larvae (Table 4). We expressed Tra ubiquitously in larvae using a da-Gal4 driver and obtained wandering XY larvae that attained ~18% more mass than cocultured w1118 male controls. A second copy of Myc in addition to da-Gal4 and UAS-tra expression further increased the size of larvae, resulting in XY larvae that were 25% larger than control males, or in other words, not distinguishable from the size of control w1118 females having XX chromosomes (Table 4).
In a complementary experiment, we measured the larval mass of XX animals that were heterozygous for the hypomorphic allele Myc\textsuperscript{P0} and homozygous for null mutations in \textit{tra}. These animals were strikingly reduced in weight. Wild-type female larvae were $1.21 \pm 0.02$ larger than sibling Myc\textsuperscript{P0}/+; tra\textsuperscript{2}/tra\textsuperscript{2} larvae, nearing the $1.26 \pm 0.01$ weight ratio determined for wild-type females:males cultured in the same bottle. Put another way, Myc\textsuperscript{P0}/+; tra\textsuperscript{2}/tra\textsuperscript{2} larvae were 20% smaller than wild-type females, a significant reduction from the 12% difference measured between XX tra null larvae and wild-type females (Figure S5B).

We wondered whether the two genes, \textit{Myc} and \textit{tra}, also regulate sex-specific size of adult structures. We chose to measure the distance between eyes (IOD) because this trait has been demonstrated to robustly reflect adult body size (Vonesch et al. 2016). In males expressing an extra copy of \textit{Myc} and \textit{da} driven \textit{tra}, we found that IODs were increased to nearly the size of IODs of females. Individually, each element only led to a partial increase of the IOD (Table 4).

In conclusion, our results show that \textit{Myc} dosage and \textit{tra} are essential for the regulation of sexual size dimorphism in \textit{Drosophila} larvae and adults.

**Discussion**

**\textit{Sxl} and \textit{tra} expression in females contribute to size difference between sexes**

Sex-specific differences in \textit{Drosophila} body size result from an acceleration in female larval growth rate compared to that of males (Blanckenhorn 2007). The observation that hypomorphic mutations in \textit{Sxl} hinder growth of XX animals led to the widely accepted model that, in addition to determining sex in dimorphic structures like the genitalia, expression of full-length Sxl protein is also responsible for the larger size of females (Cline 1984).

Results presented in this article concur with previous findings that \textit{tra} is required in females to ensure that normal body size is attained (Brown and King 1961; Rideout et al. 2015). Since \textit{tra} expression in the female is dependent on Sxl, these results support the model that Sxl plays a role in body size dimorphism in \textit{Drosophila}. However, \textit{tra} is only responsible for about half of the sex-specific size difference, begging the question of what else could contribute to regulating the differing sizes in males and females.

Several independent lines of study point to Sxl functions outside of the canonical sex-determination pathway (Horabin 2005; Penn and Schedl 2007; Chau et al. 2012; Evans and Cline 2013; Li et al. 2013). Given the abundance of putative Sxl binding sites in the genome, it is likely that still more targets of Sxl exist, with promoters of growth potentially among them (Kelley and Kuroda 1995; Robida et al. 2007).

There are, however, arguments against seeing Sxl as the only regulator of sexual dimorphism. The role of Sxl in regulation of \textit{tra} expression in \textit{Drosophila} is an evolutionarily recent adaptation (Bopp et al. 1996; Serna et al. 2004; Siera and Cline 2008). In most insects, sex is not determined by \textit{Sxl} activity; rather it is directly regulated through sexually dimorphic expression of \textit{tra} (Verhulst et al. 2010). And although it is well established that females are in general larger than males, to what extent \textit{tra} regulates growth in other insects is not known. This leaves open the question of whether a Sxl-independent mechanism is responsible for the sexual dimorphism of body size.

**Myc dosage contributes to sexual dimorphism of shape and size**

In addition to regulating \textit{tra} splicing, Sxl blocks splicing and represses translation of \textit{male specific lethal-2 (msl-2)} messenger RNA in females (Bopp et al. 1991; Kelley et al. 1997; Gebauer et al. 1998). As an essential component of the dosage compensation complex, Msl-2 mediates the roughly two-fold upregulation of gene expression from the single male X chromosome. This process relieves the inherent aneuploidy between sex chromosomes in \textit{Drosophila} and thereby ensures viability of males. Interestingly, a number of genes including \textit{Lipid serum protein1-alpha}, \textit{extradenticle}, \textit{pys1}, l(1)G0193, and CG11750 escape dosage compensation (Roberts and Evans-Roberts 1979; Legube et al. 2006). Furthermore, whole transcriptome analysis has revealed sexually dimorphic expression of a large number of X-linked genes both upstream and downstream of \textit{tra} (Chang et al. 2011).

These findings led us to investigate whether X-chromosome dosage contributes to body size differences between sexes. Indeed, we find that \textit{Myc} appears to escape dosage compensation in males, putting them at a growth disadvantage compared to sibling females. Interestingly, \textit{Myc} serves as one of several XSEs, albeit a weak one, whose early zygotic expression cues the establishment of sustained expression of full-length Sxl protein in females (Erickson and Quintero 2007; Kappes et al. 2011). \textit{Myc} therefore helps to drive sexual dimorphism by (a) assisting in the activation of \textit{Sxl} transcription in females, and (b) evading dosage compensation in males, a process activated in absence of the full-length Sxl protein.

**Table 3 Candidates identified in duplication screen**

| Candidates | Region |
|-----------|--------|
| Myc       | A      |
| CG15211   | Gap 3  |
| Ant2      | Gap 3  |
| sesB      | Gap 3  |
| Tay       | C      |
| MSBP      | C      |
| CG15916   | C      |
| Shi       | C      |
| CycD      | C      |
| CanA-14F  | D      |
| CG13014   | D      |
| SMC3      | D      |
| Ubc7      | D      |
| CG9784    | D      |

Fourteen candidate genes were identified as a result of fine-scale mapping of regions of interest A-D and gap regions 1-5 using X-chromosome duplications translocated to the third chromosome. Myc, a known promoter of cell growth, was identified as the sole candidate in region A.
Aneuploidy and position

The first screen for the effects of aneuploidy on Drosophila development was performed 40 years ago in a tremendous effort by Lindsley and Sandler et al. (Lindsley et al. 1972; Wolfner 2016). Upon generating translocations of second- and third-chromosome segments to the Y chromosome through X irradiation, the authors mapped breakpoints of the translocations and selected a collection of stocks with breakpoints spaced evenly across the autosomes. When two translocation stocks were crossed together, the progeny were either heterozygous for a deleted autosomal region or triploid for the same region. Of the 555 translocation crosses performed, 57 showed morphological phenotypes, only 3 of which showed a triploid phenotype. In our study of X duplications in males, we similarly find that the vast majority of genes are well tolerated when an additional copy is present. Only two regions of the X severely reduced male viability and fertility upon duplication.

Since relatively few regions showed a phenotype in Lindsley and Sandler et al.’s autosomal screen, it was concluded that additive effects of aneuploidy for a number of genes must underlie the observed disruptive phenotypes. In only one of the four growth-promoting regions we identified in our initial screen using 1;Y duplications, were we able to identify a single gene, Myc, whose duplication alone reproduced the phenotype of the 1;Y duplication. In two regions we were able to identify smaller duplications that partially phenocopied the growth effect of the large duplications. In the fourth region, we found no other duplications within that region that could reproduce the growth effects of the region-defining duplication. One caveat of our study is thus that individual positive or negative regulators of growth will have eluded our detection due to additive or synergistic effects of duplicating multiple genes at once. A second caveat to our screen is that gene expression from duplications used in this study might differ from expression of corresponding genes in their endogenous locations. Well-described position effects as well as regulation through feedback loops almost certainly impact effective expression levels of genes on the duplications used in this study (Birchler 2010).

Ploidy and growth

Accelerated tissue growth in females can be explained by increased rate of cell division, increased cell growth, or both. An often-used strategy in development in situations where growth is rapid or energy sources are limited is to increase cell...
volume rather than cell number (Kondorosi et al. 2000). Increasing DNA content is correlated with increased cell size and presents an effective means to promote growth (Maines et al. 2004). Drosophila larval tissues are highly polyploid, which strongly suggests that their growth results in large part from endoreplication. Myc has been shown to be required for endoreplication and cell growth, with ectopic expression leading to higher ploidy and larger cells (Pierce et al. 2004; Demontis and Perrimon 2009).

In this study, we find that Myc is more highly expressed in female than male larvae. It is tempting to speculate that sex-specific differences in Myc expression affect ploidy, possibly in a tissue-specific manner, and thereby contribute to sexual dimorphism of body size. Interestingly, it has been shown that Myc expression in the fat body alone results in similar increases in cell and larval size as found with ubiquitously expressed Myc (Johnston et al. 1999; Pierce et al. 2004). Increasing fat-body cell size through other means, however, such as local overexpression of CycD/Cdk4 or Rheb, does not impact larval size (Parisi et al. 2013). Therefore, Myc must act through other targets to promote organismic growth.

Parisi et al. (2013) reported further that expression of Myc in the fat body alone also drives growth of adult traits through increases in cell size and cell number, indicating that growth is stimulated not only in endoreplicative tissues but also in imaginal tissues of the larva. Our examination of IODs in adults shows that copy number of Myc likewise affects growth not only of larval tissue, but adult tissue as well.

**The insulin signaling pathway: a common target for Myc and tra?**

Myc plays an evolutionarily conserved role in promoting growth in Drosophila, as does insulin signaling (de la Cova and Johnston 2006). Partial loss-of-function mutations in components of the insulin pathway result in small but well-proportioned flies, while overexpression of insulin-like proteins lead to larger body sizes due to increased cell size and cell number (Brogiolo et al. 2001; Oldham et al. 2002; Rulifson et al. 2002). Similarly, hypomorphic mutations in Myc also yield small but normally proportioned flies (Johnston et al. 1999). Unlike insulin signaling, however, general overexpression of Myc accelerates growth of cell size but not cell division (Johnston et al. 1999; Grifoni and Bellosta 2015). Myc and the insulin signaling pathway therefore have distinct targets through which they regulate growth. In larval muscle tissue, both Myc and the insulin signaling pathway are required to autonomously increase cell size and to systemically promote organismal growth by affecting feeding behavior (Demontis and Perrimon 2009). Organismal growth resulting from Myc expression in the fat body, on the other hand, is mediated through humoral factors from the fat body that induce Drosophila insulin-like peptide 2 (Dilp2) release from the brain (Geminard et al. 2009; Parisi et al. 2013).

Rideout et al. (2015) presented evidence that tra expression in the female fat body is also instrumental to Dilp2 release from larval brain. Interestingly, two known targets of tra, dsx and fru, affect sexual dimorphism of body shape and behavior, respectively, but not size (Billeter et al. 2006; Camara et al. 2008). A novel branch of the sex-determination pathway, acting through an as yet unidentified target of tra, therefore contributes to dimorphism of body size in Drosophila. These results provide a clue as to how the sex-determination pathway and sex-specific growth regulation by Myc may be linked, possibly via the insulin signaling pathway.

Finally, in a recent study Hudry et al. (2016) reveal an unexpected role for tra in expression, rather than splicing, of genes involved in sexually dimorphic proliferation of adult intestinal stem cells. With this effect in mind, it seems likely that tra might regulate the expression of genes that drive sexual dimorphism during early development as well. The idea that Myc expression levels could be directly affected by tra opens the way to intriguing speculations that deserve further investigation.

**Conclusion**

In this study, we present two means by which sex-specific gene expression leads to sexual dimorphism of body size. As others have already shown, we find that body-size dimorphism depends in part on tra expression in the female (Brown and King 1961; Rideout et al. 2015). By screening the X chromosome for dose-dependent modifiers of growth, we identified a second factor that regulates growth differently in males and females. We find that a simple twofold difference in the copy number of Myc has a potent impact on growth. Furthermore, we find that we can increase male size further by expressing tra in addition to increasing Myc dosage. Females are thus larger than males not only because they express tra, but also because the growth-promoting gene Myc escapes dosage compensation. Since in early stages of embryogenesis Myc acts as an X-chromosome signal element that helps to activate Sxl and tra in females, we conclude that Myc acts twice in development to regulate dimorphism of body size. It acts first in the female embryo, where it contributes to the counting mechanism essential for activation of the sex-determining pathway. Second, the elevated expression of Myc in females directly promotes growth during larval stages of XX animals. Interestingly, the largest positive effect on growth is observed when Tra and elevated levels of Myc are present together in the larvae. Conversely, eliminating tra expression while simultaneously reducing Myc dosage in females results in XX

---

**Table 4 Larval weight and IOD changes due to increase in Myc dosage and ectopic expression of Tra in males**

| Genotype     | Larval weight | IOD       |
|--------------|---------------|-----------|
| XX XY        | 1.28 ± 0.02   | 1.10 ± 0.02|
| XY, Dp Myc:XY| 1.12 ± 0.01   | 1.06 ± 0.00|
| XY, da-Gal4, UAS-tra:XY | 1.18 ± 0.01 | 1.06 ± 0.02|
| XY, da-Gal4, UAS-tra, Dp Myc:XY | 1.25 ± 0.03 | 1.09 ± 0.01|

Ratios ± SD of mean weights of wandering third instar larvae and of mean IODs of 1- to 5-D-old adults with respect to cocultured w1118 males.
animals of nearly XY animal size. It will be interesting to learn if tra and Myc act together through a single pathway or independently through distinct pathways to promote organismal growth.

Acknowledgments

We thank Toshiro Aigaki, Konrad Basler, Johannes Bischof, Ernst Hafen, Christian Lehner, and Patrik Verstreken, as well as the Bloomington Drosophila Stock Center and the Drosophila Genetic Resource Center Kyoto for providing stocks. We are grateful to Martin Moser and Eliane Escher of the sequencing and robotics facility who executed the quantitative PCR analysis to determine transcript levels. Daniel Bopp kindly allowed us to use his microscope for adult fly measurements. We thank the Zurich fly community for numerous interactions and feedback in talks and presentations. We are especially grateful to Konrad Basler and Tripti Gupta for critically reading our manuscript. Our work was supported by the Swiss National Science Foundation grant number 3100-65333.01 to M.Z., a grant from the Swiss Bundesprogramm Chancengleichheit to M.Z., the Julius Klaus Foundation 3100-65333.01 to M.Z., and the Zurich Chancengleichheit to M.Z., the Julius Klaus Foundation.

We thank Toshiro Aigaki, Konrad Basler, Johannes Bischof, Ernst Hafen, Christian Lehner, and Patrik Verstreken, as well as the Bloomington Drosophila Stock Center and the Drosophila Genetic Resource Center Kyoto for providing stocks. We are grateful to Martin Moser and Eliane Escher of the sequencing and robotics facility who executed the quantitative PCR analysis to determine transcript levels. Daniel Bopp kindly allowed us to use his microscope for adult fly measurements. We thank the Zurich fly community for numerous interactions and feedback in talks and presentations. We are especially grateful to Konrad Basler and Tripti Gupta for critically reading our manuscript. Our work was supported by the Swiss National Science Foundation grant number 3100-65333.01 to M.Z., a grant from the Swiss Bundesprogramm Chancengleichheit to M.Z., the Julius Klaus Foundation, and the University of Zurich.

Literature Cited

Bakker, K., 1959 Feeding period, growth, and pupation in larvae of Drosophila melanogaster. Entomol. Exp. Appl. 2: 171–186.
Bell, L. R., E. M. Maine, P. Schedl, and T. W. Cline, 1988 Sex-lethal, a Drosophila sex determination switch gene, exhibits sex-specific RNA splicing and sequence similarity to RNA binding proteins. Cell 55: 1037–1046.
Bellosta, P., and P. Gallant, 2010 Myc function in Drosophila. Genes Cancer 1: 542–546.
Billeter, J. C., E. J. Rideout, A. J. Dornan, and S. F. Goodwin, 2006 Control of male sexual behavior in Drosophila by the sex determination pathway. Curr. Biol. 16: R766–R776.
Birchler, J. A., 2010 Reflections on studies of gene expression in aneuploids. Biochem. J. 426: 119–123.
Blanckenhorn, W. U., A. F. Dixon, D. J. Fairbairn, M. W. Foellmer, P. Gilbert et al., 2007 Proximate causes of Rensch’s rule: does sexual size dimorphism in arthropods result from sex differences in development time? Am. Nat. 169: 245–257.
Bopp, D., L. R. Bell, T. W. Cline, and P. Schedl, 1991 Developmental distribution of female-specific Sex-lethal proteins in Drosophila melanogaster. Genes Dev. 5: 403–415.
Bopp, D., G. Calhoun, J. I. Horabin, M. Samuels, and P. Schedl, 1996 Sex-specific control of Sex-lethal is a conserved mechanism for sex determination in the genus Drosophila. Development 122: 971–982.
Brock, T. J., J. Browse, and J. L. Watts, 2007 Fatty acid desaturation and the regulation of adiposity in Caenorhabditis elegans. Genetics 176: 865–875.
Brogliolo, W., H. Stocker, T. Ikeya, F. Rintelen, R. Fernandez et al., 2001 An evolutionarily conserved function of the Drosophila insulin receptor and insulin-like peptides in growth control. Curr. Biol. 11: 213–221.
Brown, E. H., and R. C. King, 1961 Studies on the expression of the transformer gene of Drosophila melanogaster. Genetics 46: 143–156.
Burtis, K. C., and B. S. Baker, 1989 Drosophila doublesex gene controls somatic sexual differentiation by producing alternat-
gene sex-lethal by its target transformer. Genetics 180: 1963–1981.
Smith, P. D., and J. C. Lucchesi, 1969 The role of sexuality in dosage compensation in Drosophila. Genetics 61: 607–618.
Stillwell, R. C., and G. Davidowitz, 2010 Sex differences in phenotypic plasticity of a mechanism that controls body size: implications for sexual size dimorphism. Proc. Biol. Sci. 277: 3819–3826.
Sturtevant, A. H., 1945 A gene in Drosophila melanogaster that transforms females into males. Genetics 30: 297–299.
Sun, L., A. F. Johnson, R. C. Donohue, J. Li, J. Cheng et al., 2013 Dosage compensation and inverse effects in triple X metafemales of Drosophila. Proc. Natl. Acad. Sci. USA 110: 7383–7388.
Torres, M., and L. Sanchez, 1991 The sisterless-b function of the Drosophila gene scute is restricted to the stage when the X:A ratio determines the activity of Sex-lethal. Development 113: 715–722.

Venken, K. J., Y. He, R. A. Hoskins, and H. J. Bellen, 2006 P[acman]: a BAC transgenic platform for targeted insertion of large DNA fragments in D. melanogaster. Science 314: 1747–1751.
Venken, K. J., E. Popodi, S. L. Holtzman, K. L. Schulze, S. Park et al., 2010 A molecularly defined duplication set for the X chromosome of Drosophila melanogaster. Genetics 186: 1111–1125.
Verhulst, E. C., L. van de Zande, and L. W. Beukeboom, 2010 Insect sex determination: it all evolves around transformer. Curr. Opin. Genet. Dev. 20: 376–383.
Vonesch, S. C., D. Lamparter, T. F. Mackay, S. Bergmann, and E. Hafen, 2016 Genome-wide analysis reveals novel regulators of growth in Drosophila melanogaster. PLoS Genet. 12: e1005616.
Wodarz, A., U. Hinz, M. Engelbert, and E. Knust, 1995 Expression of crumbs confers apical character on plasma membrane domains of ectodermal epithelia of Drosophila. Cell 82: 67–76.
Wolfner, M. F., 2016 Lindsley and Sandler et al. on gene dosage and the Drosophila genome. Genetics 202: 1247–1249.

Communicating editor: H. J. Bellen