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

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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 *tra* are 10% larger than *XY* pupae (Rideout *et al.* 2015). Control *XX* females, however, are 30% larger than males. This indicates that *tra* contributes to male-female differences in body size, but also that *tra* is not the sole regulator of this trait.

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

We wondered whether genes outside the sex-determining pathway participate in regulating sex-specific body size. In particular, we were curious whether *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 *Drosophila* is not absolute, with a substantial fraction of *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 *XX* animals might be larger than *XY* animals because they express two copies of a growth gene that is not, or is only partly, dosage compensated. If such a gene exists, 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 *X*-linked genes that lead to an increase in male size upon duplication. However, only one of our candidates, the *Myc* gene, fulfilled all our criteria. Mutations in *Myc* restored males with a *Myc*-containing duplication to normal male size, and heterozygous females carrying one mutation of *Myc* were smaller than control females. Furthermore, *Myc* displays a sex-specific difference in expression in the third larval instar stage. Our experiments identify *Myc* as a potent regulator of sex-specific body size in *Drosophila*, both in larvae and in adult tissue.

Materials and Methods

Drosophila stocks

Oregon R (*OR*), *white^1118* (*w^1118*), and *white^1* (*w^1*) lines were used as controls. *Dp(1;Y)* and *Dp(1;3)* duplication lines, including *Df(1)Exel6233* (stock 7707), *Cg-Gal4* (stock 7011) and *UAS-tra* (stock 4590), were obtained from the Bloomington Stock Center; *CanA^dKO* (stock 109-862) and *SLIRP1^GS1189* (stock 200-175) lines from the *Drosophila* Genetic Resource Center (Kyoto, Japan). *Myc^P0* (Johnston *et al.* 1999), *Mnt^1* (Loo *et al.* 2005), *cycD^1* (Emmerich *et al.* 2004), *CanA-14F^KO* (Nakai *et al.* 2011), *shi4C* (Kasprowicz *et al.* 2014), *tra^1* (Sturtevant 1945),*UAS-Desat1* (Kohler *et al.* 2009), *da-Gal4* (Wodarz *et al.* 1995), and *genomicSmc3-ha* (Heidmann *et al.* 2004) were as described. *UAS-lacZ86* and *UAS-lacZ75c* flies were a gift from Johannes Bischof. Flies were raised at 25 ± 1° 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° in bottles containing 45 ml standard medium. In the first round of screening, *Dp(1;Y)* males were backcrossed to OR females twice and F_2-sibling wandering larvae were weighed individually. For fine-scale mapping using *Dp(1;3)* duplication lines, *Dp(1;3)* males were crossed with *w^1118* females. Heterozygous *Dp(1;3)* males were then crossed to *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 *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 *Dp(1;3)DC271* for which only 10 male larvae were tested due to weak viability. For *tra* experiments, *tra^1* females were first mated with *Df(3L)st-E52* males for 48 hr and then cocultured with OR females, which serve as internal controls. Offspring third instar wandering larvae were weighed and maintained as described above. Gonads from adult *tra^1/Df(3L)st-E52* flies were dissected to determine genotype. Testes develop fully in *XY;tra^1/Df(3L)st-E52* flies, whereas only rudimentary testes form in *XX;tra^1/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′-GAC GGA TAC GGA AAC TAT GTT-3′/5′-GTA AAG GGC CAT TGC GAT TA-3′; *Mnt*, 5′-CCA CAA TGG AAC CAC AAT TC-3′/5′-CGG TTG CTC CTC CAT TAG TT-3′; *Max*, 5′-CAA GGA GAG CTT CAC CAA CC-3′/5′-TCT GTA TGC ATT CGG TGG TC-3′; *rpl1*, 5′-CAA CAA CCG ACA GGC CTA C-3′/5′-GAA TAC GGG CCA CAG CAC-3′; *rpl135*, 5′-AGA CCA TTC CAG TGC TGA CC-3′/5′-CTC GTC GAA GGA ATC CAC AT-3′; *cycD*, 5′-CAC GGA CAA CAG CAT CTA CAA-3′/5′-GGA AGT CCA AGG GAG TCA CA-3′; *CanA/Pp2B*, 5′-CGA CTT CCT GCA GAA CAA CA-3′/5′-GTC TGG CTT TTT GCG GTA CAT-3′; and *Desat1*, 5′-ACG 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 *w¹¹¹⁸* 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 *w¹¹¹⁸* 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/XY, 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

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 ♂ 1.45 ± 0.02			1.33
BSC74	♀ 1.96 ± 0.02 ♂ 1.66 ± 0.02	♀ 2.02 ± 0.02 ♂ 1.70 ± 0.01	♀ 2.13 ± 0.02 ♂ 1.78 ± 0.01	**1.19**
BSC158	♀ 1.95 ± 0.02 ♂ 1.49 ± 0.02			1.31
BSC91	♀ 2.08 ± 0.02 ♂ 1.69 ± 0.02	♀ 2.07 ± 0.02 ♂ 1.61 ± 0.01		1.26
BSC277	♀ 2.01 ± 0.02 ♂ 1.58 ± 0.02			1.27
BSC178	♀ 2.18 ± 0.04 ♂ 1.69 ± 0.02			1.29
BSC33	♀ 1.90 ± 0.02 ♂ 1.47 ± 0.01	♀ 1.98 ± 0.02 ♂ 1.51 ± 0.01		1.30
BSC170	♀ 1.99 ± 0.02 ♂ 1.49 ± 0.01			1.34
BSC58	♀ 2.03 ± 0.01 ♂ 1.51 ± 0.03	♀ 2.04 ± 0.02 ♂ 1.54 ± 0.02		1.33
BSC47	♀ 2.07 ± 0.02 ♂ 1.75 ± 0.02	♀ 2.06 ± 0.02 ♂ 1.72 ± 0.03	♀ 2.16 ± 0.03 ♂ 1.81 ± 0.03	**1.18**
BSC185	♀ 2.06 ± 0.01 ♂ 1.55 ± 0.02			1.33
BSC231	♀1.97 ± 0.02 ♂ 1.53 ± 0.01			1.29
BSC223	♀ 2.05 ± 0.02 ♂ 1.73 ± 0.02	♀ 1.98 ± 0.02 ♂ 1.66 ± 0.02	♀ 2.12 ± 0.02 ♂ 1.71 ± 0.02	**1.20**
BSC240	♀ 2.00 ± 0.02 ♂ 1.71 ± 0.01	♀ 1.94 ± 0.02 ♂ 1.56 ± 0.03	♀ 2.04 ± 0.02 ♂ 1.78 ± 0.02	**1.19**
BSC200	♀ 2.04 ± 0.02 ♂ 1.58 ± 0.01	♀ 2.05 ± 0.04 ♂ 1.60 ± 0.03		1.29
BSC67	♀ 2.09 ± 0.02 ♂ 1.67 ± 0.02			1.25
BSC14	♀ 2.05 ± 0.02 ♂ 1.58 ± 0.01			1.30
BSC129	♀ 1.90 ± 0.01 ♂ 1.42 ± 0.03			1.33
BSC329	♀ 2.12 ± 0.03 ♂ 1.55 ± 0.02			1.37
BSC276	♀ 2.12 ± 0.02 ♂ 1.63 ± 0.02	♀ 2.06 ± 0.02 ♂ 1.66 ± 0.02		1.27

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.

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

Duplication	Region	First replicate	Second replicate	XX/XY, DpX
BSC76	A	♀ 2.03 ± 0.03		1.19
		♂ 1.70 ± 0.03		
BSC82	A	♀ 1.98 ± 0.03		1.15
		♂ 1.72 ± 0.03		
BSC49	B	♀ 2.13 ± 0.02	♀ 1.99 ± 0.02	1.23
		♂ 1.73 ± 0.02	♂ 1.63 ± 0.02	
BSC52	B	♀ 2.01 ± 0.03	♀ 1.97 ± 0.03	1.22
		♂ 1.63 ± 0.04	♂ 1.63 ± 0.02	
BSC55	B	♀ 2.05 ± 0.02		1.33
		♂ 1.54 ± 0.02		
BSC105	B	♀ 2.07 ± 0.02		1.37
		♂ 1.51 ± 0.02		
BSC225	C	—		—
BSC226	C/D	♀ 2.09 ± 0.03		1.23
		♂ 1.70 ± 0.02		
BSC232	D	♀ 2.07 ± 0.03		1.22
		♂ 1.70 ± 0.02		
BSC244	D	♀ 2.20 ± 0.03		1.24
		♂ 1.77 ± 0.02		

Mean weights ± SE of Dp(1;Y) male larvae (XY,DpX) and sibling females (XX). ♀, 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* (*tay*), *membrane steroid binding protein* (*MSBP*), *CG15916*, *shibire* (*shi*), and *cyclinD* (*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*.
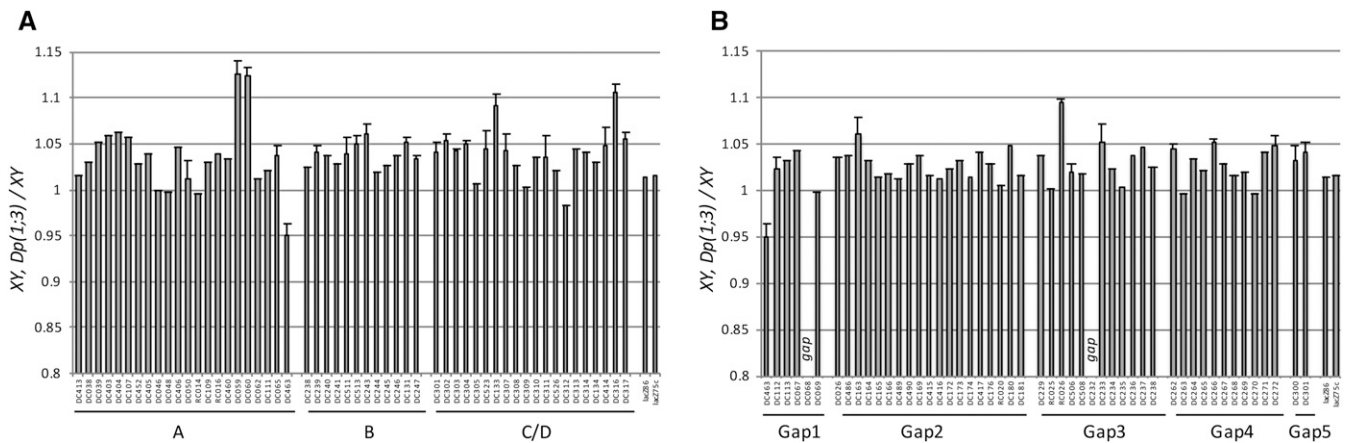
Figure 1 Regions of interest (regions A–D) and gaps (gap 1–5) not covered in *Dp(1;Y)* screen were examined using smaller *X* duplications, *Dp(1;3)*, translocated to the third chromosome. (A) Males with *X* duplications that span regions A–D were crossed with *w* females (allele *w^1118^*), sons with the duplication were again crossed with *w* females and F$_2$ sibling males were analyzed. Bars represent weight ratios of third instar wandering *w* males with a duplication to *w* males with no duplication. Control *lacZ* transgene-expressing lines were examined in the same manner. (B) Males with *X* duplications spanning gaps 1–5 were examined as described for regions A–D above. The absence of bars for duplication lines *Dp(1;3)DC068* and *Dp(1;3)DC232* reflect persistent gaps in coverage, resulting from reduced viability and fertility in males with these duplications. Error bars indicate where experiments were repeated.

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^P0^* 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^dKO^*) 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*.
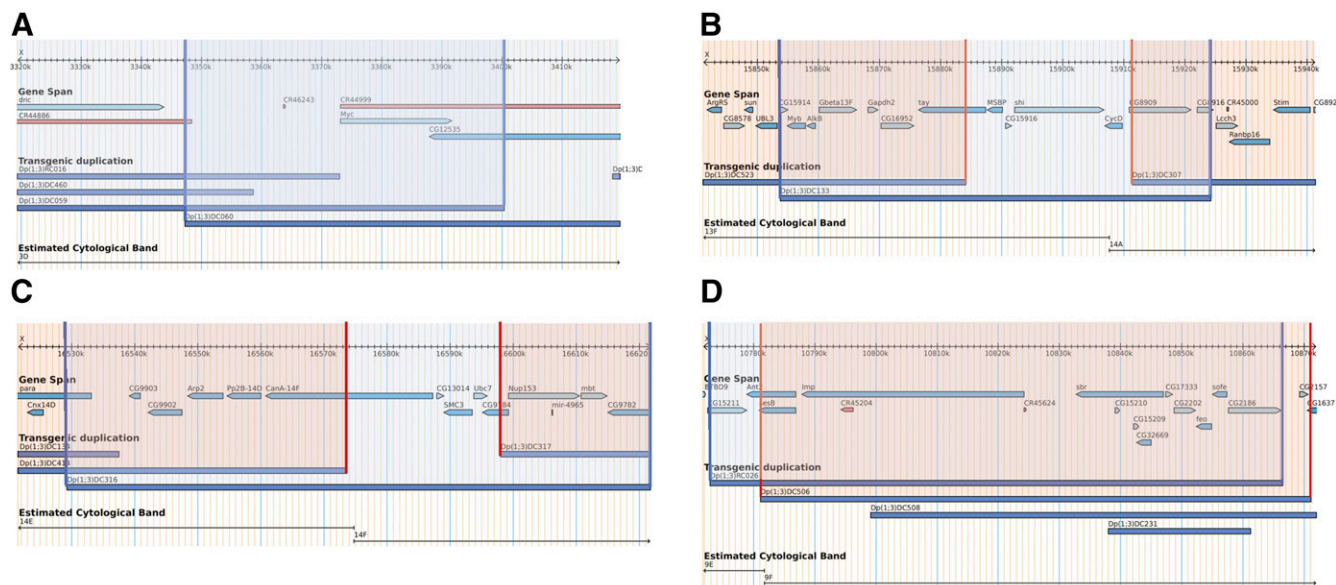
Figure 2 Fine-scale mapping of duplication-sensitive regions using overlapping *Dp(1;3)* lines. Strong growth-promoting regions are highlighted in blue, regions with minor or no growth effects are shaded in pink. (A) Only the *Myc* locus is fully contained in growth-promoting duplications *Dp(1;3)DC059* and *Dp(1;3)DC060*. (B) Candidates in region C narrowed to *tay*, *MSBP*, *CG15916*, *shi*, and *cycD*. (C) In region D, four candidates remain: *CanA-14F*, *SMC3*, *Ubc7*, and *CG9784*. (D) Three genes in *Dp(1;3)RC026* are candidates for dose-sensitive growth promoters: *CG15211*, *Ant2*, and *sesB*. Maps were derived from GBrowse representations in FlyBase.

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^{P0}* or the *Myc* deficiency *Df(1)Exel6233* weighed ~15% and 10% less than cocultured *w^{1118}* 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^{KO}* nor *CanA^{dKO}* heterozygous female larvae were significantly smaller than *w^{1118}* 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 larvae 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 *rpl1* or *rpl135* (Grewal *et al.* 2005; Delanoue *et al.* 2010). Surprisingly, we
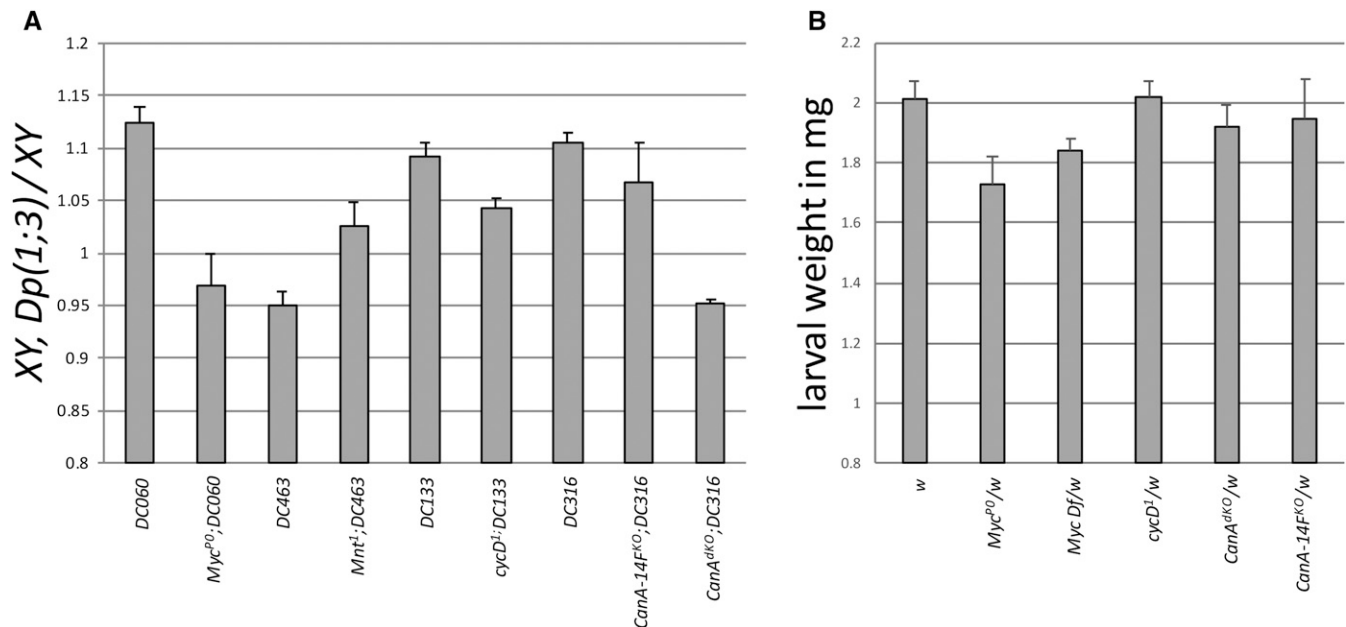
Figure 3 (A) Effect of mutations in a candidate gene on male larvae with a duplication that induces or restricts growth. In each case, animals were heterozygous for the mutations described. The strong hypomorphic *Myc* mutation, *Myc^P0^*, obliterates the growth promoting effects of *Dp(1;3)DC060*, indicating that duplication of *Myc* underlies the weight increase in *Dp(1;3)DC060* males. The converse negative effect on growth imposed by *Dp(1;3) DC463* is alleviated through a mutation of *Mnt*, implicating this gene as the growth suppressor. A partial suppression of the *Dp(1;3)DC133*'s growth promotion was observed by the null mutation *cycD^1^*. A second factor in *Dp(1;3)DC133* likely contributes to the observed increase in male size. A partial suppression of the growth induced by *Dp(1;3)DC316* was also found through elimination of *CanA-14F (CanA-14F^KO^)*. Combined removal of *CanA-14F* and the neighboring gene calcineurin *Pp2B-14D (CanA^dKO^)* resulted in complete eradication of the duplication's effects. (B) The effects of dosage of candidate genes were studied in females. Wandering female larvae heterozygous for mutations in candidate genes in a *w* background (allele *w^1118^*) were weighed. A significant weight reduction compared to *w* was only observed in females heterozygous for the hypomorphic *Myc* mutation, *Myc^P0^*, or for a deficiency in *Myc*.

found no upregulation of either *rpl1* or *rpl135* 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 *scd1* 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 *w^1118^* 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 *w^1118^* females having *XX* chromosomes (Table 4).

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.

In a complementary experiment, we measured the larval mass of *XX* animals that were heterozygous for the hypomorphic allele *Myc^{PO}* and homozygous for null mutations in *tra*. These animals were strikingly reduced in weight. Wild-type female larvae were 1.21 ± 0.02 larger than sibling *Myc^{PO}/+*; *tra⁻/tra⁻* larvae, nearing the 1.26 ± 0.01 weight ratio determined for wild-type females:males cultured in the same bottle. Put another way, *Myc^{PO}/+*; *tra⁻/tra⁻* 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, *Myc* and *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 *Myc* and *da*-driven *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 *Myc* dosage and *tra* are essential for the regulation of sexual size dimorphism in *Drosophila* larvae and adults.

Discussion

Sxl and tra expression in females contribute to size difference between sexes

Sex-specific differences in *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 *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 *tra* is required in females to ensure that normal body size is attained (Brown and King 1961; Rideout *et al.* 2015). Since *tra* expression in the female is dependent on Sxl, these results support the model that Sxl plays a role in body size dimorphism in *Drosophila*. However, *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 *tra* expression in *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 *Sxl* activity; rather it is directly regulated through sexually dimorphic expression of *tra* (Verhulst *et al.* 2010). And although it is well established that females are in general larger than males, to what extent *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 *tra* splicing, Sxl blocks splicing and represses translation of *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 *Drosophila* and thereby ensures viability of males. Interestingly, a number of genes including *Lipid serum protein1-alpha*, *extradenticle*, *pvf1*, *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 *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 *Myc* appears to escape dosage compensation in males, putting them at a growth disadvantage compared to sibling females. Interestingly, *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). *Myc* therefore helps to drive sexual dimorphism by (a) assisting in the activation of *Sxl* transcription in females, and (b) evading dosage compensation in males, a process activated in absence of the full-length Sxl protein.
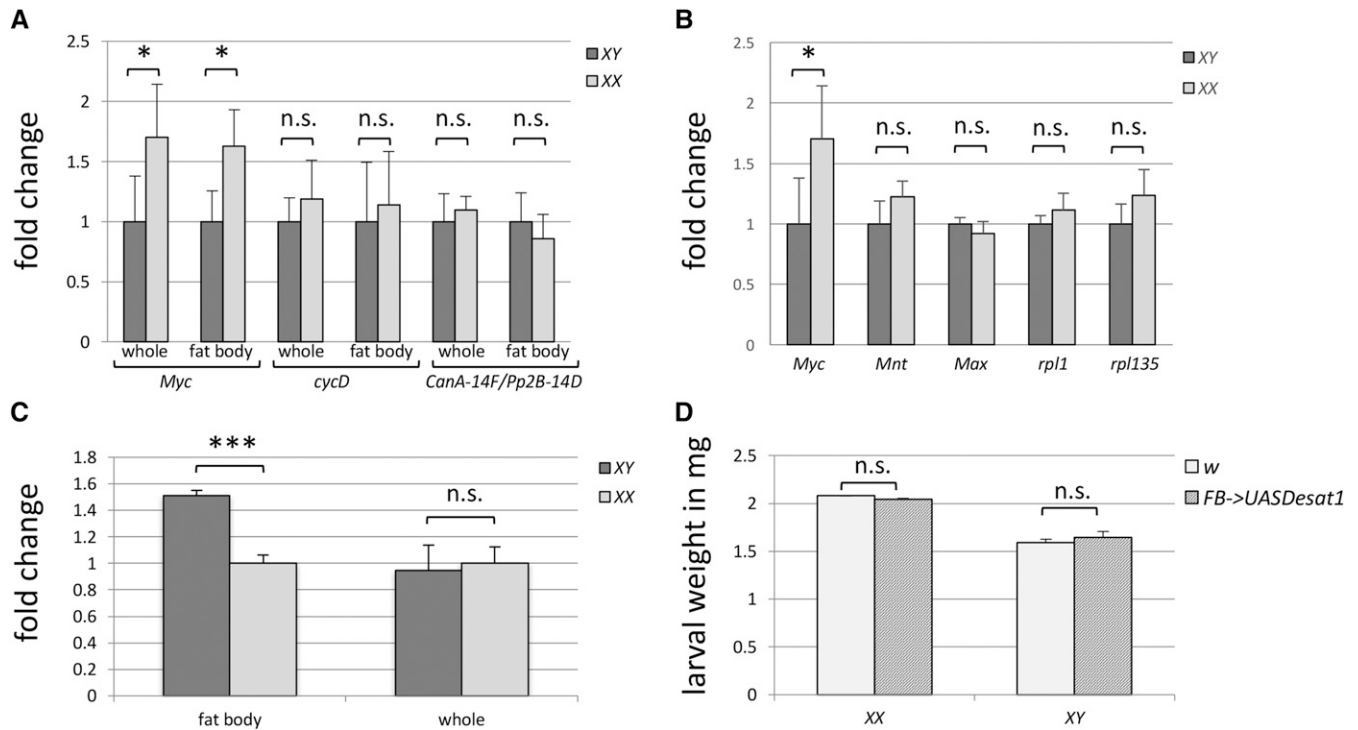
Figure 4 Transcript levels of *Myc* and related genes were assessed by qRT-PCR in feeding third instar larvae. (A) *Myc* transcript levels in whole larvae, as well as in isolated fat body tissue, are 1.7 times higher in female than in sibling male larvae. No significant differences in *cycD* or *CanA14-F/Pp2B-14D* transcript levels were observed between the sexes. (B) Transcripts of neither *Mnt* nor *Max*, the two other components of the Myc-Mnt-Max interactome, amass in a sexually dimorphic manner. Transcripts for the ribosomal proteins *Rpl1* and *Rpl135*, known targets of Myc, are expressed equally in males and females as well. (C) Overall levels of *Desat1* RNA are the same in both sexes but transcript levels are 1.5 times higher in male fat-body tissue compared to that of sibling females. (D) Male and female larvae with *Cg-Gal4*-driven expression of *Desat1* in fat-body tissue are not significantly different in size than cocultured w^{1118} control animals. * $P < 0.02$, *** $P < 0.001$; *P*-values were calculated using Student's *t*-test. Error bars represent the standard deviation. n.s., not significant.

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. We, too, conclude that in most cases the growth phenotypes we observed are the composite effects of genes within a 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

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 *w^1118* males.

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 organismal 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 over-expression 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 dis-

tinct 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*

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.

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