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# Synergistic interaction between particular X-chromosome deletions and *Sex-lethal* causes female lethality in *Drosophila melanogaster*

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**Abstract.** We studied the effect on female viability of *trans*-heterozygous combinations of X-chromosome deficiencies and $Sxl^{f1}$, a null allele of *Sex-lethal*. Twentyfive deficiencies, which together covered 80% of the X chromosome, were tested. Seven of these *trans*-heterozygous combinations caused significant levels of female lethality. Two of the seven interacting deficiencies include the previously known sex determination genes *sans fille* and *sisterless-a*. Four of the remaining uncover X-chromosomal regions that were not hitherto known to contain sex determination genes. These newly identified regions are defined by deficiencies *Df(1)RA2* (7D10; 8A4-5), *Df(1)KA14* (7F1-2; 8C6), *Df(1)C52* (8E; 9C-D) and *Df(1)N19* (17A1; 18A2). These four deficiencies were characterized further to determine whether it was the maternal or zygotic dosage that was primarily responsible for the observed lethality of female embryos. *daughterless* and *extra macrochaetae*, two known regulators of *Sxl*, influence the interaction of these deficiencies with *Sxl*.

**Keywords.** *Drosophila melanogaster*; sex determination; *Sex-lethal*.

## 1. Introduction

In *Drosophila melanogaster*, the primary signal for sex determination is the ratio of the number of X chromosomes to the number of sets of autosomes (the X : A ratio) (Bridges 1921; reviewed by Cline 1993). The X : A ratio is measured very early in development and the signal is conveyed to an X-linked master regulatory gene, *Sex-lethal* (*Sxl*) (Cline 1978). An X : A ratio of 1 results in the transcriptional activation of the early-acting, female-specific promoter of *Sxl* and this is essential for female development (Keyes *et al.* 1992). Once *Sxl* has been activated, the primary signal is not necessary for maintenance of *Sxl* activity during subsequent development because *Sxl* activity is autoregulated via alternative RNA splicing (Bell *et al.* 1991). When the X : A ratio equals 1/2, the early promoter of *Sxl* is not activated and male development ensues. Activation of *Sxl* in females leads to appropriate regulation of downstream genes concerned with somatic sex determination, germline sex determination, and dosage compensation (reviewed by Baker 1989; Steinmann-Zwicky *et al.* 1990; McKeown 1992; Pauli and Mahowald 1990; Steinmann-Zwicky 1992; Kuroda *et al.* 1993).

Three X-linked genes, *sisterless-a* (*sis-a*), *sisterless-b* (*sis-b*) and *runt*, have been identified as numerator components of the X : A signal. All three genes are positive regulators of *Sxl* and mutations in them show dose-dependent, female-specific

lethality in *trans*-heterozygous combination with $Sxl^{fl}$, a null mutation of *Sxl* (Cline 1986, 1988; Torres and Sanchéz 1989, 1992; Duffy and Gergen 1991). It is thought that heterozygosity for *sis-a*, *sis-b* or *runt* in *trans* combination with $Sxl^{fl}$ lowers *Sxl* product levels, resulting in female lethality.

If there are other X-chromosomal genes or regions that contain elements involved in the regulation of *Sxl*, it may be possible to detect them by similarly examining their interaction with $Sxl^{fl}$. Analysis of the interaction of $Sxl^{fl}$ with relatively large deficiencies of the X chromosome might therefore be an appropriate method for rapid screening of chromosomal segments for additional elements involved in *Sxl* regulation. We have attempted both mutational and deletion analysis of the X chromosome. Results of the deletion analysis are reported here.

## 2. Materials and methods

The deficiency stocks used were obtained from the Umeà (Sweden), Bloomington (USA) and TIFR (Bombay, India) stock centres. The $Sxl^{fl}$ mutation was kindly provided by Dr Thomas Cline, *sis-b* ($sc^{10\text{-}1}$) by Dr Lucas Sanchéz, the $Dp(1;2)sn^{+72d}$ (7A8;8A5;32C;58E) (Lefevre 1981) stock and *da* by Dr Anthony Mahowald, the *emc* allele by Dr James Posakony, and *Df(1)D2*, $Df(1)fu^{B10}$, $Df(1)os^{UE19}$ and $Df(1)os^{1A}$ by Dr Norbert Perrimon (Eberl *et al.* 1992). The break points of the deletion chromosomes used are given in table 1. See Lindsley and Zimm (1992) for complete descriptions of these stocks. Flies were raised at 25°C on standard corn meal–sucrose–yeast medium in half-pint bottles under uncrowded conditions.

Twentyfive deficiencies, which together uncover about 80% of the X chromosome, were tested. In each case, females heterozygous for the deficiency were crossed to $cm\ Sxl^{fl}\ ct^{6}$ males. In this cross three classes of progeny—females of the genotypes $Df(1)/Sxl^{fl}$ and $FM7/Sxl^{fl}$, and *FM7* males—are expected to emerge; these are designated A, B and C respectively. In control crosses, females heterozygous for the deficiency were crossed to $cm\ Sxl^{+}\ ct^{6}$ males. In order to keep nonspecific variation to a minimum, the same balancer X chromosome, *FM7c* ($w^{a}\ sn^{X2}\ v^{Of}\ g^{4}\ B$), was used in all crosses. A maternal role for the elements uncovered by the deficiency is likely if, in the progeny, females of both classes, i.e. $Df(1)/Sxl^{fl}$ and $FM7/Sxl^{fl}$, show reduced viability. This is represented in the text and in table 1 as two ratios: the sex ratio [(A + B)/C], i.e. the ratio of the total number of females to that of males; and the A/B ratio, i.e. the ratio of the doubly heterozygous $Df(1)/Sxl^{fl}$ females to $FM7/Sxl^{fl}$ females. These ratios were computed for both experimental and control crosses. Since the viability of balancer males can fluctuate owing to nonspecific effects, including the genetic background, and lead to variation in the sex ratio, we decided to focus on deficiencies that show a large, i.e. at least six-fold, reduction in the sex ratio in experimental crosses. A reduction in the number of $Df(1)/Sxl^{fl}$ females compared to $FM7/Sxl^{fl}$ females is indicative of a zygotic role for the deleted segment; the interaction was considered significant if the deficiency caused at least a three-fold reduction in viability of $Df(1)/Sxl^{fl}$ females.

## 3. Results

Of the 25 deletions tested, seven—*HC244*, *RA2*, *KA14*, *C52*, *RA37*, *C246* and

*N19*—caused significant levels of female lethality in combination with $Sxl^{fl}$. The results of the crosses involving each of these deficiencies are described below. Four of the deficiencies—*RA2*, *KA14*, *C52* and *C246*—resulted in more than six-fold reduction in the sex ratio [(A + B)/C]. Three—*HC244*, *RA37* and *N19*—showed synergistic interactions with $Sxl^{fl}$ and significantly reduced the viability of doubly heterozygous females in comparison with females heterozygous for $Sxl^{fl}$ alone (table 1).

**Table 1.** Effect of interaction between particular X-chromosome deletions and *Sxl* on survival.

| Deficiency *Df(1)* (cytology) | | A/B ratio* | No. of class B females | Sex ratio** |
|---|---|---|---|---|
| 1. S39 | C | 1·09 | 487 | 1·66 |
| (1E1-2;2B5-6) | E | 0·88 | 244 | 2·01 |
| 2. *Pgd35* | C | 1·22 | 1014 | 2·44 |
| (2C2-4;2E2-F1/F5) | E | 1·22 | 1348 | 2·98 |
| 3. *JC19* | C | 0·88 | 1218 | 2·95 |
| (2F6;3C5) | E | 0·77 | 1196 | 2·47 |
| **4. *HC244*[a]** | **C** | **0·83** | **187** | **0·75** |
| **(3E8;4F11)** | **E** | **0·16** | **275** | **0·37** |
| 5. *dm75e19* | C | 1·14 | 516 | 2·65 |
| (3C11;3E4) | E | 1·30 | 641 | 3·22 |
| 6. *C149* | C | 0·92 | 416 | 4·60 |
| (5A8-9;5C5-6) | E | 1·12 | 599 | 6·60 |
| 7. *N73* | C | 1·30 | 200 | 4·33 |
| (5C2;5D5-6) | E | 1·92 | 371 | 2·28 |
| 8. *HA32,Sxl*$^{-}$ | C | 1.01 | 328 | 2·28 |
| (6E4-5;7A6) | E | 0 | 760 | 1·88 |
| **9. $RA2^{d1}$** | **C** | **1·01** | **452** | **3·39** |
| **(7D10;8A4-5)** | **E** | **0·94** | **88** | **0·15** |
| **10. $KA14^{d2}$** | **C** | **1·05** | **394** | **5·49** |
| **(7F1-2;8C6)** | **E** | **1·22** | **252** | **0·28** |
| **11. $C52^{d3}$** | **C** | **0·81** | **275** | **1·42** |
| **(8E;9C-D)** | **E** | **0·77** | **98** | **0·19** |
| 12. *HC133* | C | 0·99 | 438 | 1·74 |
| (9B9-10;9E-F) | E | 1·05 | 542 | 1·95 |
| **13. *RA37*[b]** | **C** | **0·85** | **387** | **1·53** |
| **(10A6;10B15)** | **E** | **0·03** | **1019** | **1·36** |
| 14. *HA85* | C | 1·10 | 753 | 1·53 |
| (10D;11A3-5) | E | 0·88 | 481 | 1·81 |
| 15. *M13* | C | 1·20 | 288 | 1·19 |
| (10D;11A3-5) | E | 0·74 | 246 | 1·36 |
| 16. *KA6* | C | 0·81 | 532 | 1·43 |
| (10E1;11A7-8) | E | 0·94 | 438 | 4·08 |
| 17. *N105* | C | 0·85 | 857 | 2·32 |
| (10F1;10F9-10) | E | 1·01 | 958 | 2·60 |

(*contd*)

**Table 1** (*contd*)

| Deficiency *Df(1)* (cytology) | | A/B ratio* | No. of class B females | Sex ratio** |
|---|---|---|---|---|
| 18. *RA47* | C | 1·11 | 350 | 8·19 |
| (10F1;10F9-10) | E | 1·42 | 469 | 4·59 |
| 19. *JA26* | C | 1·05 | 721 | 2·08 |
| (11A1;11D-E) | E | 0·73 | 480 | 0·83 |
| 20. *N12* | C | 1·15 | 429 | 3·50 |
| (11D1-2;11F7-8) | E | 1·24 | 436 | 1·86 |
| **21. *C246*[c]** | **C** | **0·87** | **373** | **3·31** |
| **(11D3;12A1-2)** | **E** | **0·25** | **68** | **0·13** |
| 22. *HA92* | C | 0·91 | 907 | 3·22 |
| (12A6-7;12D3) | E | 1·11 | 1114 | 3·60 |
| **23. *N19*[d4]** | **C** | **1·05** | **1114** | **3·42** |
| **(17A1;18A2)** | **E** | **0·25** | **1355** | **1·52** |
| 24. *fu*[B10] | C | 0·85 | 730 | 3·14 |
| (17C5-D1;18A4-7) | E | 0·15 | 899 | 1·13 |
| 25. *JA27* | C | 0·80 | 1478 | 4·66 |
| (18A5;20A) | E | 2·20 | 1321 | 1·77 |
| 26. *DCB-1-35b* | C | 1·04 | 646 | 3·64 |
| (19F1-2;20E-F) | E | 2·39 | 457 | 4·26 |

E, experimental cross, *Df(1)/FM7* × *Sxl*[fl]/Y; C, control cross, *Df(1)/FM7* × *Sxl*[+]/Y

*Ratio of the *Df(1)/Sxl*[fl] vs *FM7/Sxl*[fl] and *Df(1)/Sxl*[+] vs *FM7/Sxl*[+] females obtained in the progeny of *Df(1)FM7* × *Sxl*[fl]/Y and *Df(1)/FM7* × *Sxl*[+]/Y crosses respectively

**(A + B)/C, Ratio of total females (classes A and B) to total males (class C)

a, Steinmann-Zwicky and Nothiger 1985; b, Cline 1986; c, Belote *et al.* 1985; d1, d2, d3, d4, this study

### 3.1. *Df(1)RA2*

In the cross involving *Df(1)RA2* and *Sxl*[fl], the sex ratio was 0·15 (171 females and 1113 males). *Df(1)RA2* is deleted from 7D10 to 8A4–5. In the control cross, the sex ratio was 3·39 (933 females and 275 males). Although the number of females was reduced drastically in the experimental cross, the ratio of *Df(1)/Sxl*[fl] to *FM7/Sxl*[fl] females (referred to as the A/B ratio in table 1) was within normal limits (1·01 in control and 0·94 in experimental cross), leaving open the possibility that the element(s) deleted could be acting maternally. The following cross was performed to test for maternal effect: *FM7/Sxl*[fl] females were mated with *Df(1)RA2/Y*; *Dp(1;2)sn*[+72d]*/CyO* males. The compensating duplication in the latter stock allows the deficiency chromosome to be passed through males. In this cross the sex ratio was 1·10 (511 females and 490 males), indicating that the female-specific lethality seen in the cross [*Df(1)RA2/FM7* × *Sxl*[fl]/Y] was most likely due to a maternal effect.

### 3.2 *Df(1)KA14*

In the experimental cross involving *Df(1)KA14*, the sex ratio was 0·28 (559 females

and 2021 males); in the control cross, the sex ratio was 5·49 (807 females and 147 males). *Df(1)KA14* (7F1-2; 8C6) and *Df(1)RA2* (7D10; 8A4-5) are overlapping deletions, which suggests that the observed interactions could be due to the region common to both, i.e. chromosomal bands 7F1-2 to 8A4-5. Here again, the A/B ratio was within normal limits (1·05 in control and 1·22 in experimental), suggesting that the effect of interacting elements is likely to be maternal.

### 3.3 *Df(1)C52*

The region defined by *Df(1)C52* uncovers bands 8E to 9C-D. A partially overlapping deletion, *Df(1)HC133*, which lacks the region from 9B9-10 to 9E-F, did not show any interaction (see table 1). Thus the region interacting with $Sxl^{fl}$ can be narrowed down to 8E to 9B9-10. In this case also, the A/B ratio was normal, but a significant reduction in the total number of females was seen (173 females and 929 males). This too is consistent with a maternal role for the region uncovered by *Df(1)C52*, but reciprocal crosses to confirm this possibility could not be carried out because duplications covering this region were not available.

### 3.4 *Df(1)C246*

A sex ratio of 0·13 (85 females and 675 males) was seen in the cross involving *Df(1)C246* and $Sxl^{fl}$. *Df(1)C246* uncovers bands 11D3 to 12A1-2. In the control cross, the sex ratio was 3·31 (698 females and 211 males). In addition to a reduction in the overall number of females, the *Df(1)/*$Sxl^{fl}$ class of females were less viable than *FM7/*$Sxl^{fl}$ females (17 *Df(1)/*$Sxl^{fl}$ and 68 *FM7/*$Sxl^{fl}$; A/B ratio = 0·25). The A/B ratio in the control cross was 0·87 (325 *Df(1)/*$Sxl^{+}$ and 373 *FM7/*$Sxl^{+}$ females). A partially overlapping deletion *Df(1)N12* (table 1), in which bands 11D1-2 to 11F7-8 are deleted, showed almost no interaction with $Sxl^{fl}$ (sex ratio 3·50 in control and 1·86 in experimental cross, A/B ratio 1·15 in control and 1·24 in experimental cross). Therefore the interacting region can be narrowed down to bands 11F7-8 to 12A1-2.

### 3.5 *Df(1)HC244*

This deficiency uncovers chromosomal bands 3E8 to 4F11. In the cross involving *Df(1)HC244* and $Sxl^{fl}$, the sex ratio was 0·37 (318 females and 862 males). There was also significant reduction in viability of *Df(1)/*$Sxl^{fl}$ females compared to *FM7/*$Sxl^{fl}$ females (43 *Df(1)/*$Sxl^{fl}$ and 275 *FM7/*$Sxl^{fl}$, A/B ratio 0·16). In the control cross, the sex and A/B ratios were 0·75 (342 females and 453 males) and 0·83 (155 *Df(1)/*$Sxl^{+}$ and 187 *FM7/*$Sxl^{+}$) respectively.

### 3.6 *Df(1)RA37*

Chromosomal bands 10A6 to 10B15 are deleted in *Df(1)RA37*. In the progeny of the cross *Df(1)RA37* × $Sxl^{fl}$, a substantial reduction in the number of *Df(1)/*$Sxl^{fl}$ females was seen. The A/B ratios were 0·03 (30 *Df(1)/*$Sxl^{fl}$ and 1019 *FM7/*$Sxl^{fl}$) in

the experimental cross and 0·85 (328 *Df(1)/Sxl*$^+$ and 387 *FM7/Sxl*$^+$) in the control cross.

### 3.7 *Df(1)N19*

The region defined by *Df(1)N19*, which uncovers chromosomal bands 17A1 to 18A2, also caused female lethality in *trans* combination with *Sxl*$^{fl}$. Females doubly heterozygous for *Df(1)N19* and *Sxl*$^{fl}$ were four-fold less viable than *FM7/Sxl*$^{fl}$ females (344 *Df(1)/Sxl*$^{fl}$ and 1355 *FM7/Sxl*$^{fl}$). This observation is consistent with zygotic interaction of one or more elements in the deleted segment with *Sxl*$^{fl}$. Out of four overlapping deficiencies (*D2*, *fu*$^{B10}$, *os*$^{UE19}$, *os*$^{1A}$) tested for interaction with *Sxl*$^{fl}$, only *Df(1)fu*$^{B10}$ (17C5-D1; 18A4-7) caused a significant reduction in the number of doubly heterozygous females (137 *Df(1)fu*$^{B10}$*/Sxl*$^{fl}$ and 899 *FM7/Sxl*$^{fl}$) [see table 1; data not shown for others]. Hence the interacting region is within chromosomal bands 17C5-D1 to 18A2.

As noted earlier, it is possible to distinguish among effects that are maternal, zygotic, or both by examining whether female viability is reduced (i) equally in both classes of females (*Df(1)/Sxl*$^{fl}$ and *FM7/Sxl*$^{fl}$), (ii) only in *Df(1)/Sxl*$^{fl}$ females, or (iii) in both, but more severely in *Df(1)/Sxl*$^{fl}$ females. On this basis the effects of *RA2*, *KA14* and *C52* appear to be maternal, and those of *RA37* and *N19* zygotic. The remaining deficiencies, *HC244* and *C246*, appear to have both maternal and zygotic effects. It should be noted that deficiencies *HC244*, *RA37* and *C246* cover regions of the X chromosome known to contain previously identified sex determination genes. These deficiencies show the same pattern of interaction as in previous studies (see Discussion). Therefore they were not studied further.

### 3.8 *Female-lethal interaction between four of the deletions and Sxl*$^{fl}$ *is enhanced by daughterless and decreased by extra macrochaetae*

To test if the four new interacting deficiencies identified in this screen interact with *daughterless* (*da*) and *extra macrochaetae* (*emc*), two known regulators of *Sxl*, we analysed the influence of mutations in these genes on female lethality in crosses between *Sxl*$^{fl}$ and *RA2*, *KA14*, *C52* or *N19*. In control crosses, females of the same genotype were crossed to *Sxl*$^+$ males (table 2). The autosomal gene *da*$^+$ codes for a maternal product that is essential for the proper activation of *Sxl* (Cline 1978). Whereas daughters of *da/da* mothers do not survive, sons are unaffected. Among the female progeny of *da/+* mothers, those that are heterozygous for a null allele of *Sxl* (*Sxl*$^+$*/Sxl*$^-$) are less viable than their *Sxl*$^+$*/Sxl*$^+$ sisters, suggesting that *da* and *Sxl* interact in a dose-dependent manner (Cline 1980). The gene *emc*$^+$ has recently been shown to be a maternally acting negative regulator of *Sxl*. Sex ratio of the progeny of mothers heterozygous for *emc* is normal or nearly so. Males with reduced maternal *emc*$^+$ activity and imbalance of *sis-b*$^+$ to *dpn*$^+$ (*deadpan*$^+$, an autosomal regulator of *Sxl*) dosage, i.e. with two copies of *sis-b*$^+$ and one copy of *dpn*$^+$, show reduced viability (Younger-Shepherd *et al.* 1992).

The effect of *da* and *emc* on the interaction between *Sxl* and the deletions *RA2*,

**Table 2.** Interactions among *da*, *emc*, *Sxl* and the deficiencies.

| | Paternal genotype** | |
|---|---|---|
| | $Sxl^+$ | $Sxl^{fl}$ |
| Maternal genotype* | Numbers of surviving females/males (sex ratio) | |
| 1. +/+; $da^1$/+ | 546/511 (1·07) | 496/738 (0·67) |
| 2. +/+; $emc^{ML}$/+ | 561/509 (1·10) | 1089/843 (1·29) |
| 3. RA2/+; $da^1$/+ | 1181/1166 (1·01) | 17/1152 (0·01) |
| 4. RA2/+; $emc^{ML}$/+ | 1166/775 (1·50) | 884/653 (1·35) |
| 5. KA14/+; $da^1$/+ | 1182/1018 (1·16) | 31/1239 (0·03) |
| 6. KA14/+; $emc^{ML}$/+ | 1156/721 (1·60) | 753/539 (1·40) |
| 7. C52/+; $da^1$/+ | 513/520 (0·99) | 9/414 (0·02) |
| 8. C52/+; $emc^{ML}$/+ | 409/268 (1·53) | 539/552 (0·98) |
| 9. N19/+; $da^1$/+ | 1182/703 (1·68) | 588/1166 (0·50) |
| 10. N19/+; $emc^{ML}$/+ | 1264/576 (2·19) | 1156/568 (2·04) |

*The $da^1$ mutation employed in these crosses is a weak allele, and $emc^{ML}$ a strong allele

**The relevant genotypes were *cm* $Sxl^+$ $ct^6$ and *cm* $Sxl^{fl}$ $ct^6$

*KA14*, *C52* and *N19* was studied by crossing $Sxl^{fl}$ males with females doubly heterozygous for the deficiency and $da^1$ (a hypomorphic allele) or $emc^{ML}$ (a strong allele). Viability of females was significantly lower in the progeny of *Df(1)*/+; *da*/+ mothers than in those of singly heterozygous mothers (table 2; see also table 1). For instance, in the cross *RA2*/+;*da*/+ × $Sxl^{fl}$/Y, 1152 males and 17 females were obtained, suggesting a strong maternal influence of the genes uncovered by *Df(1)RA2*. However, female viability was restored when mothers carrying *RA2*, *KA14*, *C52* or *N19* were also heterozygous for $emc^{ML}$ (table 2). For example, *RA2*/+; $emc^{ML}$/+ heterozygotes when crossed to $Sxl^{fl}$ males gave rise to 884 females and 653 males. Similarly, a strong maternal influence was observed in the case of *KA14* and *C52*, whereas the elements uncovered by *N19* seem to interact with $Sxl^{fl}$ in a zygotic fashion. These results provide further evidence that additional genetic elements involved in the regulation of *Sxl* may be present in the regions uncovered by *RA2*, *KA14*, *C52* and *N19*. The extent of female lethality (almost 100%) observed for interactions between $Sxl^{fl}$ on the one hand and *RA2*, *KA14* or *C52* and *da* on the other could therefore serve as a robust assay to isolate interacting genes.

## 4. Discussion

Genes involved in a particular developmental pathway may be identifiable on the basis of their failure to complement mutant genes at other loci. Such an approach has been successfully used to identify genes involved in sex determination in the region uncovered by *Df(1)C246*. It was observed that in XX flies heterozygous for both *transformer* (*tra*) and *transformer-2* (*tra-2*), two genes involved in somatic sex determination, *Df(1)C246* led to the development of intersexes in a significant proportion of the progeny (Belote *et al.* 1985). Such genetic screens have been successful in analysing other developmental pathways as well. For instance, this approach enabled Tricoire (1988) to identify X-chromosomal regions interacting with *Krüppel*, *hunchback* and *hairy*, three genes involved in segmentation. Simon *et al.* (1991) screened for mutations that decrease the effectiveness of signalling by a protein kinase, the product of the *sevenless* gene, and isolated seven mutations whose wild-type counterparts code for products essential for signalling by the product of the *sevenless* gene.

In the present study, female-specific lethality was observed in seven out of the twentyfive X-chromosome deletions tested. These are *HC244*, *RA2*, *KA14*, *C52*, *RA37*, *C246* and *N19*. Deficiencies *HC244* and *RA37* have been investigated by other workers and the following genes involved in the sex determination pathway have been identified: *snf* (Oliver *et al.* 1988; Flickinger and Salz 1994) [also named *fs(1)A1621* by Gans *et al.* 1975, and *liz* by Steinmann-Zwicky 1988] in the region defined by *Df(1)HC244*, and *sis-a* in the region defined by *Df(1)RA37* (Cline 1986). The independent identification of these regions in the present study adds to the validity of this type of genetic approach. The gene *sisterless-c* (*sis-c*) has recently been identified as an additional numerator component of the X:A ratio (Cline 1993). *Df(1)N19*, one of seven deficiencies found to interact with $Sxl^{fl}$ (table 1), uncovers *sis-c*. It seems likely that *sis-c* is also uncovered by $Df(1)fu^{B10}$, which partially overlaps with *Df(1)N19* (table 1).

*runt* is a positive regulator of *Sxl* (Duffy and Gergen 1991; Torres and Sanchéz 1992). In *trans*-heterozygous combination with $Sxl^{fl}$, *runt* causes a reduction in the number of female offspring. However, in the present study, deletion *Df(1)JA27*, which uncovers *runt*, did not cause female-specific lethality when combined with $Sxl^{fl}$. This may be because of differences in genetic background in the two sets of experiments. Interaction between *runt* and $Sxl^{fl}$ is not as strong as that between $Sxl^{fl}$ and *sis-a* or *sis-b*. Double heterozygotes for $Sxl^{fl}$ and *runt* show only a 50% reduction in female viability ($runt/Sxl^{fl}$ = 206, $FM6/Sxl^{fl}$ = 416; Duffy and Gergen 1991). In the light of this observation, and the fact that the strength of the interactions among sex determination genes can vary widely depending on genetic background (Cline 1988), it is not surprising that female lethality was not observed in our experiments involving $Sxl^{fl}$ and *Df(1)JA27*.

Female lethality resulting from interaction between *Sxl* and deficiencies *RA2*, *KA14*, *C52* and *N19* is enhanced by *da* and decreased by *emc*, whose wild-type alleles are both early regulators of *Sxl*. Early activation of *Sxl* is mediated by a set of genes all of which except two (*sis-a* and *runt*) encode helix-loop-helix (HLH) proteins. While the amounts of maternally provided $da^+$ and $emc^+$ products are expected to be the same in both male and female embryos, a two-fold difference is expected in the product levels of the zygotically active numerator genes *sis-a*,

*sis-b* and *runt*. It appears that the probability of activation of *Sxl* depends on limiting concentrations of products of numerator genes. Our observation that the interactions between $Sxl^{fl}$ and deficiencies *RA2*, *KA14*, *C52* and *N19* are influenced by *da* and *emc* suggests that the genes uncovered by these deficiencies may also code for HLH transcription regulators.

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