Genetic Evidence That the *sans fille* Locus Is Involved in Drosophila Sex Determination

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ABSTRACT

Females homozygous for *sans fille*^{1621} (= *fs(1)1621*) have an abnormal germ line. Instead of producing eggs, the germ-line cells proliferate forming ovarian tumors or excessive numbers of nurse cells. The *Sex-lethal* gene product(s) regulate the branch point of the dosage compensation and sex determination pathways in the soma. The role of *Sex-lethal* in the germ line is not clear but the germ line of females homozygous for female sterile *Sex-lethal* alleles or germ-line clones of loss-of-function alleles are characterized by ovarian tumors. Females heterozygous for *sans fille*^{1621} or *Sex-lethal* are phenotypically wild type with respect to viability and fertility but females *trans*-heterozygous for *sans fille*^{1621} and *Sex-lethal* show ovarian tumors, somatic sexual transformations, and greatly reduced viability.

ADULT fruit flies show extensive sexual dimorphism. A number of genes have been identified that are specifically required for morphological aspects of sexual identity (BAKER and BELOTE 1983; CLINE 1985; BAKER, NAGOSHI and BURTIS 1987) and/or sex-specific regulation of *X*-linked gene expression (LUCCHESI and MANNING 1987). The development of a female (diplo-*X*) somatic cell appears to be controlled by a cascade of regulatory gene products. In diplo-*X* cells *Sxl*$^+$ is "on" resulting in the activation of *transformer*$^+$ (*tra*) and *transformer-2*$^+$ (*tra-2*). *tra*$^+$ and *tra-2*$^+$ are required for expression of female specific *doublesex*$^+$ (*dsx*) gene product(s) that, along with *intersex*$^+$ gene product(s), repress male differentiation. *Sxl*$^+$ activity is also believed to repress the expression of dosage compensation genes. The absence of *Sxl*$^+$ expression in diplo-*X* flies probably leads to heightened levels of *X*-linked gene expression and a resultant lethal imbalance of gene products (LUCCHESI and SKRIPSKY 1981). While failure to initiate *Sxl*$^+$ expression is lethal to diplo-*X* flies, failure to maintain *Sxl*$^+$ expression in diplo-*X* somatic cells results in male morphology (SÁNCHEZ and NÖTHIGER 1982; CLINE 1985).

Sex determination and dosage compensation in the germ line is different than in the soma. The absence of *Sxl*$^+$ expression does not kill diplo-*X* germ-line cells but does block oogenesis (SCHÜPBACH 1985) resulting in the accumulation of large numbers of small germ-line cells in the ovary (ovarian tumor phenotype). None of the other genes of the somatic sex determination pathway are required in the female germ line (MARSH and WIESCHAUS 1978; SCHÜPBACH 1982; BELOTE and BAKER 1983) and only one sex determination gene, *tra-2* (BAKER and RIDGE 1980; BELOTE and BAKER 1983), and one dosage compensation gene, *maleless* (BACHILLER and SÁNCHEZ 1986), are known to be required in the male germ line. It is also known that germ line cells transplanted into hosts of the wrong chromosomal sex do not function (VAN DUESEN 1976) and that haplo-*X* germ cells do not proceed (if they even survive) as far in oogenesis as *Sxl*$^+$ diplo-*X* germ cells in a diplo-*X* soma (SCHÜPBACH 1985).

If there are more undiscovered or unrecognized germ-line sex determination genes one approach to finding those genes lies in the analysis of female sterile mutations characterized by "Sxl-like" ovarian tumors or mutations resulting in other early "blocks" in oogenesis (LEUTHOLD 1986). We have examined the interaction of *sans fille* (*snf*) (= *fs(1)1621*) (GANS, AUDIT and MASSON 1975; GOLLIN and KING 1981; PERRIMON *et al.*, 1986) with *Sxl* and find that mutations in these genes interact to produce a variety of defects in both the soma and the germ line of diplo-*X* flies.

MATERIALS AND METHODS

Stocks: Visible mutants and balancers are described in LINDSLEY and GRELL (1968). The following stocks were obtained from the Bowling Green stock center: *y cho, rb, peb v, rg, y cv v f, y f, sc ec cv ct g f, bi ct*^{6} *g*^{2}. The recessive female sterile mutations *fs(1)575, fs(1)107, fs(1)456 and fs(1)snf*^{1621} (= *fs(1)1621*) are marked with *v*^{24} and balanced with *FM3* (GANS, AUDIT and MASSON 1975). *fs(1)*ovo^{D1rS1} is maintained in *FM3* or *C(1)DX, y f/Y* stocks (OLIVER, PERRIMON and MAHOWALD 1987a). *Sxl*$^{fs\#1}$, *fs(1)ovo*^{rM1} (= *fs(1)M1*), *fs(1)ovo*^{rM2} (= *fs(1)M38*), *fs(1)M40* and *fs(1)M66* are marked with *y cv v f* and were maintained in *FM0* or *FM3* stocks

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TABLE 1

Rearrangement cytology

Name	Cytology	Reference
Df(1)bi^{DL3}	Df(1)3C7-12;4E1-2	BANGA *et al.* (1986)
Df(1)bi^{DL5}	Df(1)3C7-12;4E1-2	BANGA *et al.* (1986)
Df(1)GA102	Df(1)3D5;3F7-8	CRAYMER and ROY (1980)
Df(1)HF366	Df(1)3E8;5A7-8	CRAYMER and ROY (1980)
Df(1)HC244	Df(1)3E8;4F11-12	CRAYMER and ROY (1980)
Df(1)rb^{33}	Df(1)3F3-4;4C15-16	BANGA *et al.* (1986)
T(1;2)rb^{+71g}	T(1;2)3F3;5E8;23A15	CRAYMER and ROY (1980)
Df(1)rb^{47}	Df(1)4A1-2;4D1-2	BANGA *et al.* (1986)
Df(1)rb^{1}	Df(1)3F6-4A;4C7-8	BANGA *et al.* (1986)
Df(1)rb^{46}	Df(1)4A3-6;4C6-7	BANGA *et al.* (1986)
Df(1)RC40	Df(1)4B1;4F1	CRAYMER and ROY (1980)
Df(1)rb^{30}	Df(1)4B1-2;4F2-4	BANGA et al. (1986)
Df(1)bi^{D2}	Df(1)4B6;4D7-E1	BANGA *et al.* (1986)
Df(1)rb^{13}	Df(1)4C5-6;4D3-E1	BANGA *et al.* (1986)
Df(1)ovo^{D1rG7}	Df(1)4C5-6;4E2-3	OLIVER, PERRIMON and MAHOWALD (1987a)
Df(1)GA56	Df(1)4C5-6;4D1	CRAYMER and ROY (1980)
Df(1)ovo^{D1rG6}	Df(1)4C5-6;4E2-3	OLIVER, PERRIMON and MAHOWALD (1987a)
Df(1)JC70	Df(1)4C15-16;5A1-2	CRAYMER and ROY (1980)
In(1)ovo^{D1rG5}	In(1)4E1-2;5A1-6	OLIVER, PERRIMON and MAHOWALD (1987a)
Df(1)C149	Df(1)5A8-9;5C5	CRAYMER and ROY (1980)
Df(1)N73	Df(1)5C2;5D5-6	CRAYMER and ROY (1980)

(MOHLER 1977; OLIVER, PERRIMON and MAHOWALD 1987a). *cm Sxl*^{fm1} *ct* and *y cm Sxl*^{7BO} were maintained in *FM3* or *C(1)DX, yf/Y* stocks (SALZ, CLINE and SCHEDL 1987). *Sxl*^{fs1} (MOHLER 1977; PERRIMON *et al.* 1986) is a female sterile allele, *Sxl*^{fm1} is a loss-of-function allele, and *Sxl*^{7BO} is a male viable deletion of the entire *Sxl* locus and an undetermined number of flanking genes (SALZ, CLINE and SCHEDL 1987). The *w v l(1)44*^{ts}*/FM3/B*^{s}*Y* stock was used for collecting virgins (KOMITOPOULOU *et al.* 1983; OLIVER, PERRIMON and MAHOWALD 1987a). Refer to Table 1 for the cytology of rearranged chromosomes.

Complementation and growth conditions: Flies were grown on standard cornmeal molasses media seeded with live yeast, under uncrowded conditions. Each recessive female sterile or visible mutation was tested for complementation with the deletions. To enhance expressivity, progeny were grown at 29° in complementation tests involving *bi* or *peb*. All other mapped mutations were tested at 25°. The *hnt* (WIESCHAUS, NÜSSLEIN-VOLHARD and JÜRGENS 1984; EBERL and HILLIKER 1988) and *svb* loci (WIESCHAUS, NÜSSLEIN-VOLHARD and JÜRGENS 1984; OLIVER, PERRIMON and MAHOWALD 1987a; EBERL and HILLIKER 1988) were mapped by examining the cuticles of deletion-bearing males. It is assumed that if the deletion-bearing males exhibit the same phenotype as *hnt* and/or *svb* then the corresponding wild-type copy is removed by the deletion. The *snf* locus was mapped both by complementation and by interaction with *Sxl* (see below).

The mapping of the diplolethal region is as follows. The parent males were generated by mating *T(1;2)rb*$^{+71g}$ males to virgin *C(1)DX, y f/Y; bw*^{D}*/bw*^{D} females. The resulting male progeny are *Df(1)rb*^{71g} *ct*^{6} *v/Y; Dp(1;2)rb*$^{+71g}$*/bw*^{D}. These males were mated to females heterozygous for deletions. All of these deletions result in embryonic lethality in hemizygous males such that the eclosion of deletion-bearing males indicates that the diplolethal region is removed by the deletion. For example, *Df(1)rb*^{1} males always die as embryos because they lack a large region of the *X* chromosome. For *Df(1)rb*^{1}*/Y* males to live, the deleted portion of the chromosome must be represented on the duplication inserted on the second chromosome, but the diplolethal region must be present in only one dose. Since the diplolethal region is present on the duplication, the *Df(1)rb*^{1} deletion must remove the diplolethal region. The same protocol was followed for testing the suppression of dilplolethality by *Sxl* alleles. The criterion for viability was eclosion. For diplolethal testing, females were mated overnight at 25° and moved to either 18° ± 0.5°, 20° ± 0.5°, 25° ± 0.5°, or 29° ± 0.5°.

Interaction of *snf*^{1621} and *Sxl*: To reduce differences in genetic background all female parents used in testing for interaction of *snf*^{1621} with *Sxl* were generated by crossing males to virgin *w v l(1)44*^{ts}*/FM3* females. The progeny were shifted to 29° during late larval or early pupal stages to kill *w v l(1)44*^{ts}*/Y* males. *FM3/Y* males also die, such that only females eclosed. The resulting class of balanced virgin female progeny were *snf*^{1621} *v*^{24}*/FM3* or *y cm Sxl*^{7BO}*/FM3*. *snf*^{1621} *v*^{24}*/FM3* females were mated with *y cm Sxl*^{7BO}*/Y*, *cm Sxl*^{fm1} *ct/Y*, or *y f/Y* males. *y cm Sxl*^{7BO}*/FM3* females were mated to *snf*^{1621} *v*^{24}*/Y* or *y f/Y* males. Virgin *y cm Sxl*^{7BO}*/ snf*^{1621} *v*^{24} females from these crosses were mated to *y f/Y* males or allowed to mate with their brothers. For experiments involving the interaction of deletions of *snf* with *Sxl*, genetic background was reduced by crossing females heterozygous for the deletions and *FM7* or *FM6* to *y f/Y* males. Virgin females heterozygous for the deletion chromosome and *y f* were mated to either *y cm Sxl*^{7BO}*/Y*, *cm Sxl*^{fm1} *ct/Y* or *y f/Y* males. Genotypes were assigned based on B, y, and/or v phenotypes. We did not correct for recombination in unbalanced crosses. Females were mated overnight at 25° and moved to either 18° ± 0.5°, 20° ± 0.5°, 25° ± 0.5°, or 29° ± 0.5°. The criterion for viability was eclosion. For crosses of *snf/+* or *Sxl/+* females to experimental males (*Sxl/Y* or *snf*^{1621}*/Y*) and control males (*y f/Y*) relative viability was calculated as follows: (number of experimental females ÷ number of experimental males) × (number of control males ÷ number of control females). The 95% confidence interval was calculated according to the delta method

(BISHOP, FIENBERG and HOLLAND 1975). For crosses of males to snf^{1621}/Sxl^{7BO} females relative viability equals the number of females ÷ the number of snf^{1621}/Y males.

Phenotypic examination: Cuticle phenotypes were evaluated by collecting and mounting embryos according to VAN DER MEER (1977). These embryo whole mounts were microscopically examined under phase contrast and dark field illumination. Ovaries and testes were dissected in phosphate buffered saline solution and examined by phase contrast microscopy. Forelegs were removed and mounted in either Euparal or ethanol following an overnight digestion of soft tissues in 10% KOH. The presence of sex combs on legs was determined by microscopic examination.

RESULTS

Flies heterozygous for two or more sex determination genes often show a mutant phenotype even though flies heterozygous for a single sex determination gene are phenotypically wild-type (BAKER and RIDGE 1980; CLINE 1980, 1986; CRONMILLER and CLINE 1987; SCOTT 1987). Because snf^{1621} and Sxl^{-} have similar female germ line phenotypes possible dominant interactions between *snf* and *Sxl* mutations were investigated. Three phenotypic manifestations of the interaction were examined: relative viability of females compared to males, somatic sexual morphology and fertility.

Female viability: If the interaction between *snf* and *Sxl* mutations results in defects in Sxl^{+} expression prior to settling the sex-specific level of *X*-linked gene expression, females might be expected to die due to the expression of male-specific dosage compensation genes. Female progeny *trans*-heterozygous for Sxl^{7BO} (a hemizygous viable deletion) and snf^{1621} or deletions removing *snf* (snf^{-} = *Df(1)HC244* or $Df(1)rb^{30}$) were less than 20% as viable as control females (Figure 1A) suggesting that a single wild-type dose of each of these loci lowers female viability. The viability of $snf^{1621}/Sxl^{f\#1}$ and $snf^{-}/Sxl^{f\#1}$ females was similarly reduced (not shown). The female progeny heterozygous for the snf^{+} deletion, $Df(1)ovo^{D1rG7}$, and Sxl^{7BO} were as viable as controls. Low viability was also seen among the $+/Sxl^{7BO}$ progeny (Figure 1B) from heterozygous snf^{1621}, *Df(1)HC244*, or $Df(1)rb^{30}$ female parents. When female parents were heterozygous for snf^{-}, the viability of $+/Sxl^{7BO}$ progeny was nearly as low, less than 20%, as that of the snf^{-}/Sxl^{7BO} siblings. The viability of the $+/Sxl^{7BO}$ progeny of heterozygous snf^{1621} female parents and the $+/snf^{1621}$ progeny of heterozygous Sxl^{7BO} females were about 35% as viable as controls. The $+/Sxl^{7BO}$ progeny of heterozygous $Df(1)ovo^{D1G7}$ parents were as viable as controls. These data suggest that the maternal doses of snf^{+} and Sxl^{+} are important for the survival of *Sxl* or *snf* heterozygotes.

The female lethality due to *snf* and *Sxl* interaction depends on temperature. At higher temperatures both classes of heterozygous Sxl^{7BO} female progeny derived from $snf^{1621}/+$ female parents were more viable than at lower temperatures (Table 2, columns 1 and 2). The cold-sensitivity of the interaction is likely to be due to wild-type gene products since deletions of *snf* also show a cold-sensitive interaction with Sxl^{7BO} or $Sxl^{f\#1}$ (not shown). The two classes of heterozygous snf^{1621} females derived from heterozygous Sxl^{7BO} female parents were also more likely to live at higher temperatures (Table 2, columns 3 and 4).

The viability of snf^{1621}/Sxl^{7BO} females also depends on the parental origin of the chromosomes. At 25°, the viability of snf^{1621}/Sxl^{7BO} females derived from $Sxl^{7BO}/+$ female parents is 71% but the viability of the same class of females derived from $snf^{1621}/+$ female parents is 13% suggesting that snf^{1621} has a stronger maternal effect than Sxl^{7BO}. If both *snf* and *Sxl* maternal gene products are important for the viability of female offspring then female parents heterozygous for both snf^{1621} and Sxl^{7BO} might bear few female progeny. The viability of heterozygous Sxl^{7BO} female progeny from snf^{1621}/Sxl^{7BO} female parents was reduced (Table 2, columns 5 and 7) but the viability of heterozygous snf^{1621} female progeny was not (Table 2, columns 6 and 8). While the high frequency of snf^{1621}/Sxl^{7BO} lethality seen when the female parents were of the same genotype (Table 2, column 5) may indicate that both *snf* and *Sxl* have a maternal function, the wild-type viability of the $snf^{1621}/+$ and snf^{1621}/snf^{1621} classes of progeny from these parents suggests that Sxl^{7BO} does not have a maternal effect.

The interaction between *snf* and *Sxl* mutations reduces the viability of females heterozygous for *Sxl*. The dominant maternal effect of the snf^{-} deletions, $Df(1)rb^{30}$ and *Df(1)HC244*, on the viability of *Sxl* heterozygotes could be due to other loci removed by the deletions, however, maternal heterozygosity for two additional deletions of *snf*, *Df(1)HF366* and *Df(1)JC70*, also reduces the viability of *Sxl* heterozygotes whereas a set of snf^{+} deletions, *Df(1)GA102*, $Df(1)rb^{1}$, *Df(1)RC40*, $Df(1)rb^{46}$, $Df(1)bi^{D2}$, $Df(1)ovo^{D1rG7}$ and $Df(1)ovo^{D1rG6}$, do not reduce the viability of *Sxl* heterozygotes. These overlapping deletions that do not interact with *Sxl* collectively remove all the regions removed by the deletions that do interact with *Sxl*, with the exception of the 4F region, which contains *snf*.

Sexual identity: Abnormal Sxl^{+} expression results in intersex characteristics in addition to lethality (CLINE 1985). When diplo-*X* flies lose *Sxl* function via mitotic recombination Sxl^{-} patches of phenotypically male tissues appear among the female tissues of diplo-*X* flies (SÁNCHEZ and NÖTHIGER 1982; CLINE 1985). Similar mosaic intersexes are seen when diplo-*X* flies are heterozygous for *Sxl* and homozygous for dosage compensation mutations (SKRIPSKY and LUCCHESI

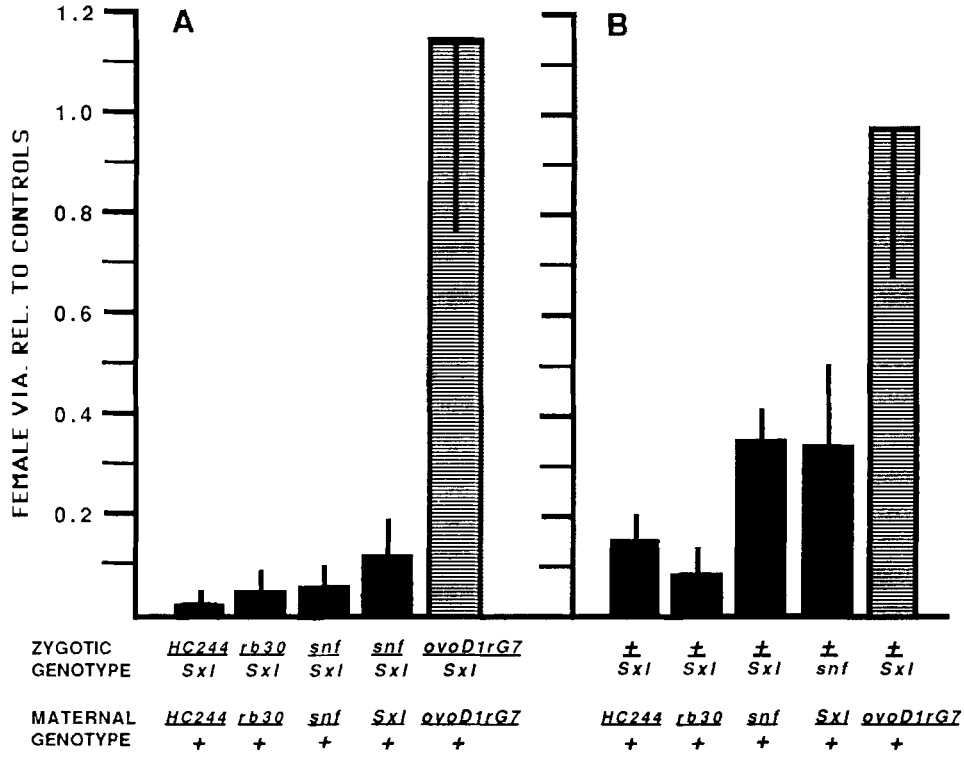

FIGURE 1.—*snf* and *Sxl* mutations interact to reduce female viability. The bars show the viability of experimental females relative to control females. Black bars show the interaction between *snf* and *Sxl*. The striped bars represent a second control. The zygotic and maternal genotypes of experimental females with respect to *snf* and *Sxl* are shown at the bottom of the figure. *HC244* = *Df(1)HC244*, *rb30* = *Df(1)rv*^{30}, *snf* = *snf*^{1621} *v*^{24}, *Sxl* = *y cm Sxl*^{7BO}, *ovoD1rG7* = *Df(1)ovo*^{D1rG7}, + = *y f*, or *FM3*. *Df(1)HC244* and *Df(1)rb*^{30} fail to complement *snf*^{1621}. *Df(1)ovo*^{D1rG7} complements *snf*^{1621}. *snf*^{1621} and *Sxl*^{7BO} parental females were balanced with *FM3*, deletions were carried over *FM6* or *FM7* and out crossed to *y f* prior to mating with experimental or control males. Females of the given maternal genotype were crossed to *Sxl*^{7BO}/*Y*, *snf*^{1621}/*Y* or *y f*/*Y* males. In each case the viability of female progeny from the cross to *Sxl*^{7BO}/*Y* or *snf*^{1621}/*Y* males is compared to the viability of female progeny from the cross to *y f*/*Y* males. (A) Females progeny receiving *Df(1)HC244*, *Df(1)rb*^{30}, *snf*^{1621}, *Sxl*^{7BO}, or *Df(1)ovo*^{D1rG7} from the female parent and either *snf*^{1621} or *Sxl*^{7BO} from the male parent. (B) The sibling female progeny of those shown in panel A. Error bars show either the upper or lower limits of the 95% confidence interval. Flies were grown at 20°.

TABLE 2

Interaction leading to female lethality is cold sensitive

Temperature (°C)	MAT[b] ZYG[c]	Relative viability of females[a]							
		snf/+ *snf/Sxl*	*snf/+* *+/Sxl*	*Sxl/+* *snf/Sxl*	*Sxl/+* *snf/+*	*snf/Sxl* *snf/Sxl*	*snf/Sxl* *snf/+*[d]	*snf/Sxl* *Sxl/+*[d]	*snf/Sxl* *snf/snf*
20		0.08	0.38	0.12	0.35	0.005	1.6	0.41	ND[e]
25		0.13	0.59	0.71	0.63	0.04	1.1	0.57	0.99
29		0.49	0.73	0.77	0.74	0.38	1.3	0.83	ND

[a] The relative viability of female progeny from *snf/+* and *Sxl/+* females is in relation to the viability of female progeny from control crosses. For the female progeny derived from *snf/Sxl* female parents viability is relative to the viability of male siblings. For each cross, 300–1800 progeny were scored.
[b] (*snf* = *snf*^{1621} *v*^{24}) (*Sxl* = *y cm Sxl*^{7BO}) (+ = *FM3*).
[c] Zygotic genotypes are inferred from genetic markers.
[d] (+ = *y f*).
[e] Not done.

1982). This is in contrast to the "true" intersex phenotype where individual cells appear have both male and female characteristics. True intersexes are seen, for example, in the absence of the later acting *dsx* gene (BAKER and RIDGE 1980; BELOTE and BAKER 1983). There are a number of sex-specific cuticle structures that can be used to identify the sex of small clones of cells. We looked for male-specific bristles, sex combs, on the forelegs of *snf*^{1621}/*Sxl*$^{-}$ flies escaping the lethal interaction at 20°. Mosaic intersex forelegs, with one or more normal appearing male sex combs among the normal female bristles (Figure 2), were seen on about 25% of the legs of diplo-*X* flies heterozygous for *snf*^{1621} and either *Sxl*^{7BO} or *Sxl*$^{f\#1}$ (Table 3). In only two cases did forelegs of diplo-*X* flies homozygous for *Sxl*$^{+}$ show male sex combs. Both of these

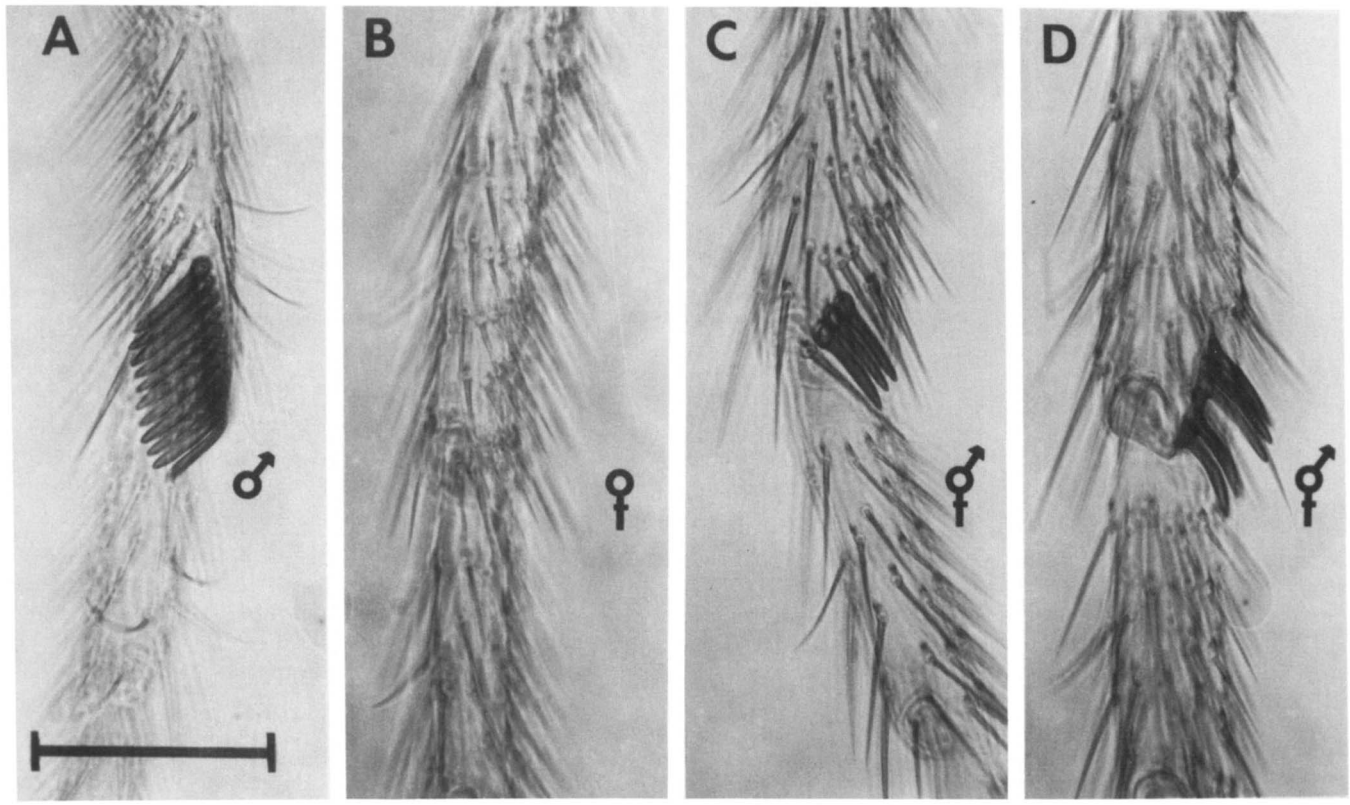

FIGURE 2.—*snf*^{1621}/*Sxl* females have mosaic intersex forelegs. (A) Foreleg of a wild-type male (Ore R strain). The dark thickened bristles are the male sex combs. (B) Foreleg of a wild-type female (Ore R strain). Note the absence of male sex combs. (C, and D) Forelegs of *snf*^{1621} *v*^{24}/*y cm Sxl*^{7BO} females grown at 20°. Some bristles have been transformed into male sex combs while others retain female morphology. Legs were taken from females presented in Figure 1 (bar = 100 μm).

TABLE 3

Interactions leading to intersexes

Maternal genotype[a]	Zygotic genotype[a]	Legs scored	Intersex legs[b]
snf/+	+/*Sxl*[c]	259	0
snf/+	*snf*/*Sxl*[c]	73	23 (2.4)
snf/+	+/+	115	0
snf/+	*snf*/+	132	0
Sxl/+	*snf*/+	239	2 (1.5)
Sxl/+	*snf*/*Sxl*	156	36 (1.7)
Sxl/+	+/+	192	0
Sxl/+	*Sxl*/+	353	0

[a] The parents and progeny are the same as presented in Figure 1.

[b] The mean number of male sex comb bristles on diplo-*X* intersexes is shown in parentheses.

[c] *Sxl* = *Sxl*^{7BO} or *Sxl*$^{f\#1}$.

mosaic intersex forelegs were found among the *snf*^{1621}/+ female progeny of heterozygous *Sxl*^{7BO} female parents, again suggesting that *Sxl* may have a maternal effect. No male sex combs were found on the +/*Sxl*$^{-}$ females derived from heterozygous *snf*^{1621} mothers [even though the viability of those females was markedly decreased (*cf.* Figure 1B)] or on any of the control diplo-*X* flies. Mosaic intersex forelegs were also seen on *snf*$^{-}$/*Sxl*$^{-}$ diplo-*X* flies (STEINMANN-ZWICKY and NÖTHIGER 1985; this study, not shown). The mosaicism exhibited by flies heterozygous for *snf*^{1621} or *snf*$^{-}$ and *Sxl*^{7BO} or *Sxl*$^{f\#1}$ suggests that the transformation of diplo-*X* tissues to male morphology is due to interaction of *snf* and *Sxl* in the zygote.

The presence of male sex combs on diplo-*X* flies is consistent with failure to maintain *Sxl*$^{+}$ expression in at least some somatic cells. Evidence for defects in dosage compensation was also gathered. When small groups of cells are killed during the blastoderm stage the adult structures normally derived from that portion of the fate map are deleted (LOHS-SCHARDIN *et al.* 1979). About 6% (42 of 751) of the *snf*^{1621}/*Sxl*$^{-}$ females were missing eyes, legs or wings. These defects were highly variable and almost always unilateral suggesting that a stochastic interaction results in the death of imaginal precursors. Like the sexual transformations, absence of adult body parts did not depend on the maternal genotype. Heterozygous *Sxl* female progeny receiving two zygotic copies of *snf*$^{+}$ rarely had such defects (3 of 730).

Fertility: Expression of *Sxl*$^{+}$ is required in the germ line of females flies for fertility (SCHÜPBACH 1985). Female progeny heterozygous for *snf*^{1621} and either *Sxl*^{7BO} or *Sxl*$^{f\#1}$ grown at either 18° or 20° were always sterile (84 females scored) regardless of maternal genotype. Only 11% of the *snf*^{1621}/*Sxl*$^{-}$ females were

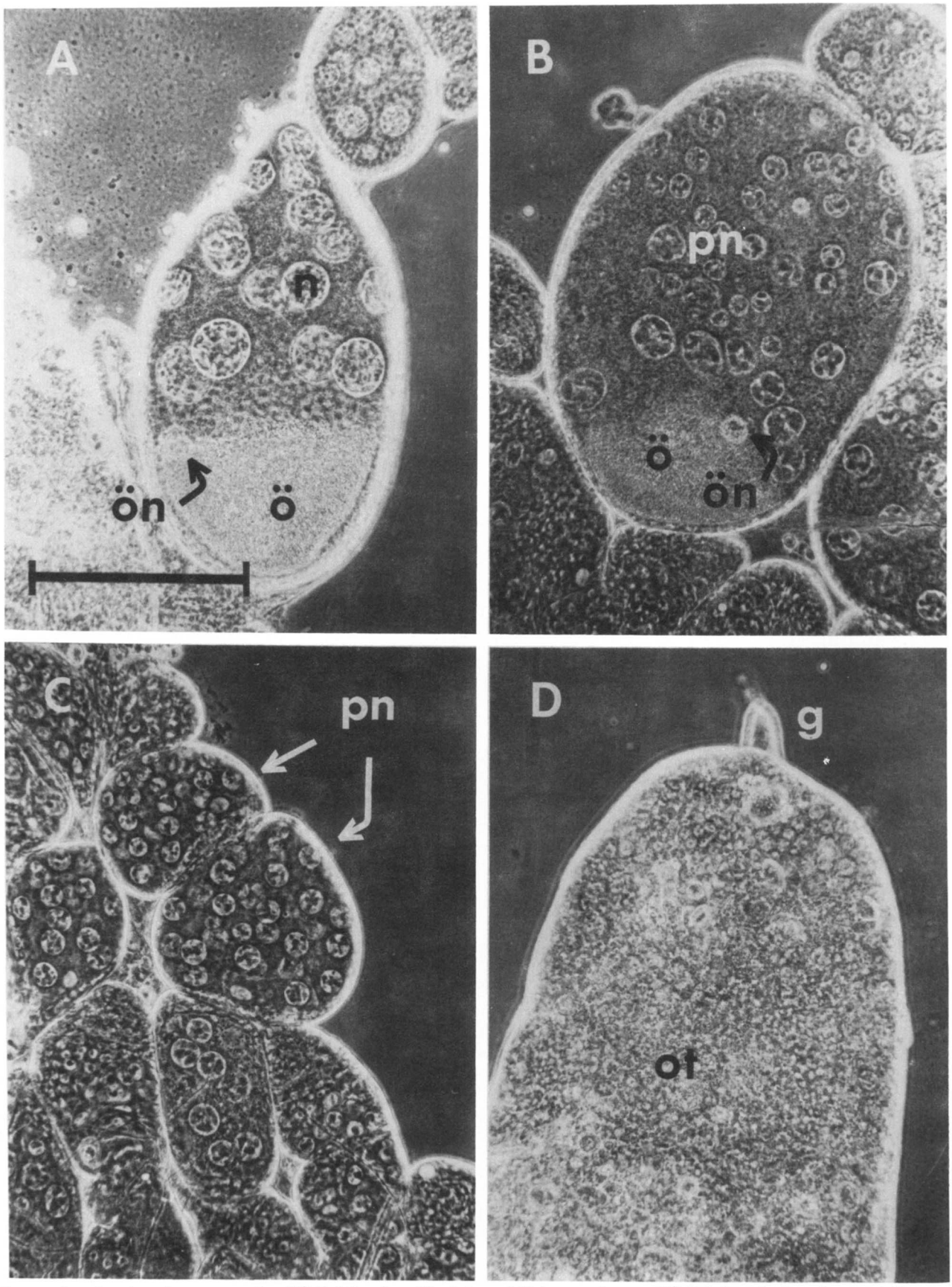

FIGURE 3.—Ovarian phenotypes of snf^{1621}/Sxl females. All of these follicles are from snf^{1621}/Sxl^{-} females but show variable ovarian phenotypes. (A) A normal appearing follicle with 15 nurse cell nuclei (n) visible in the anterior (top) and a single oocyte (ö) in the posterior of the egg chamber (bottom). The oocyte has begun to accumulate yolk. The oocyte nucleus is faintly visible (ön). The ovary is from a

sterile at 25° (123 females scored), but, at 29°, a temperature which had the least extreme effect on viability, 80% of the snf^{1621}/Sxl^{-} females were sterile (103 females scored). As with the unilateral defects in the adult cuticle and presence of intersex forelegs, the female sterility phenotype is restricted to those flies heterozygous for both snf^{1621} and *Sxl* (not shown). The cold-sensitive interaction results in somatic and germ-line defects, but the heat-sensitive interaction appears to affect only the germ line.

The wild-type ovary is composed of about 20 ovarioles containing, in sequence from anterior to posterior, stem cells, multicellular cysts resulting from karyokinesis and incomplete cytokinesis, cysts with 15 polyploid nurse cells and an oocyte, and mature oocytes (KING 1970; MAHOWALD and KAMBYELLIS 1980). Nurse cells and oocytes are not seen in Sxl^{-} germ-line clones (SCHÜPBACH 1985) or in the ovaries of any of three female sterile alleles of *Sxl* (D. MOHLER, personal communication; this study, not shown). The diplo-*X* germ-line phenotype is the production of ovarian tumors. The snf^{1621} diplo-*X* germ line is characterized by large ovarian tumors, excessive numbers of nurse-like cells, and occasional normal mature oocytes (GOLLIN and KING 1981; WIESCHAUS, AUDIT and MASSON 1981; PERRIMON and GANS 1983). The nurse cell phenotype predominates at the expense of the ovarian tumor phenotype when adult flies are shifted from 29° to 25° (GOLLIN and KING 1981). At 29° the ovaries of snf^{1621}/Sxl^{-} females contained very large ovarian tumors (Figure 3D), egg chambers with excessive numbers of nurse cells, or normal egg chambers (Figure 3A). When adult females were shifted to 25° the ovarian tumors were less frequent, ovarioles with excessive numbers of nurse cells were more common (Figure 3, B and C) and more females laid eggs. The ovarian tumors of snf^{1621}/Sxl^{-} females grown at 20° were smaller, and no nurse-like cells, or normal egg chambers were seen. We did not examine snf^{-}/Sxl^{-} fertility at low temperatures because of the high frequency of lethality exhibited by those females, but we did examine snf^{-}/Sxl^{-} ($Df(1)JC70/Sxl^{7BO}$) ovaries at 29° and found that 6 of 15 contained large ovarian tumors. In summary, at 29° snf^{1621}/Sxl^{-} ovaries are snf-like and at 20° the ovaries are Sxl-like. At the light microscopic level cells contained within the ovarian tumors appear similar to the spermatocytes of wild-type males (Figure 4) but spermatids, sperm, or the cytoplasmic crystals characteristic of *X/O* germ-line cells were never observed.

The *snf* locus is not a counting element: The *snf* locus is removed by four cytologically visible deletions we examined, $Df(1)HC244$, $Df(1)rb^{30}$, $Df(1)HF366$ and $Df(1)JC70$. Two of these deletions, $Df(1)HF366$ and $Df(1)HC244$, were previously shown to cause cold-sensitive lethality and somatic sexual transformations in *trans* to $Sxl^{f\#1}$ (STEINMANN-ZWICKY and NÖTHIGER 1985). However, no maternal effect of these deletions was reported. The strictly zygotic effect along with the fact that males carrying a duplication of this region die (STEWART and MERRIAM 1980; BUSSON *et al.* 1983; STEINMANN-ZWICKY and NÖTHIGER 1985; OLIVER, PERRIMON and MAHOWALD 1987a, b; CLINE 1987) led STEINMANN-ZWICKY and NÖTHIGER (1985) to suggest that the region is important for "counting" *X*-chromosomes. If counting elements were reduced in number by deletions the apparent *X:A* ratio would approach 0.5 in diplo-*X* flies and the apparent *X:A* ratio would approach 1.0 in haplo-*X* flies bearing duplications of the region. The consequence of these "miscounted" *X:A* ratios would be lower levels of Sxl^{+} in diplo-*X* flies and higher levels of Sxl^{+} expression in haplo-*X* flies, leading to inappropriate *X*-linked transcription rates. Strong evidence to support this idea was the finding that males bearing duplications of this region survive if they have no functional *Sxl* gene to activate.

The "reading" of the *X:A* ratio is believed to be a zygotic function but $Df(1)HC244$, $Df(1)rb^{30}$ (*cf.* Figure 1), $Df(1)HF366$, and $Df(1)JC70$ (not shown) have maternal effects on the viability of *Sxl* heterozygotes. Because of this we reexamined the diplolethal region to determine if males with no *Sxl* gene to activate survive in the presence of the duplication and to determine if the diplolethal region and the *snf* locus map to the same location (see also CLINE 1987; OLIVER, PERRIMON and MAHOWALD 1987b). Males bearing a translocation including the diplolethal region, and a female haploinsufficient region (BUSSON *et al.* 1983) were crossed to females heterozygous for various alleles of *Sxl*. The viability of male progeny from these crosses was examined to determine if males carrying one Sxl^{+} allele were less viable than males with defective or deleted *Sxl* genes. Duplication bearing males were not recovered, at 25° or 29°, regardless of *Sxl* genotype (Table 4). Diplolethality is heat-

snf^{1621}/Sxl^{7BO} female derived from a $Sxl^{7BO}/FM3$ female parent which developed from egg to 0–2-day adult at 29° and was then shifted to 25° for 11 days. (B) A follicle with an oocyte and oocyte nucleus and a large number of nuclei showing a polyploid nurse cell morphology (pn). (C) Follicles with greater than 15 nuclei showing nurse cell morphology and no oocyte. Ovaries shown in B and C are from $snf^{1621}/Sxl^{f\#1}$ female, derived from a $snf^{1621}/FM3$ female parent, which developed from egg to 0–2-day adult at 29° and was then shifted to 25° for 11 days. (D) An ovarian tumor egg chamber (ot) showing no signs of either oocytes or nurse cells. This large mass of small cells occupied most of the ovariole from the germarium (g) to the oviduct (off the photograph). The female bearing this tumor was a snf^{1621}/Sxl^{7BO} sibling of the snf^{1621}/Sxl^{7BO} female bearing the normal egg chamber in panel A. This female was kept at 29°. $Sxl^{f\#1}$ is marked with *cm* and *ct*. See Figure 1 for remaining genotypes. Bar = 160 μm.

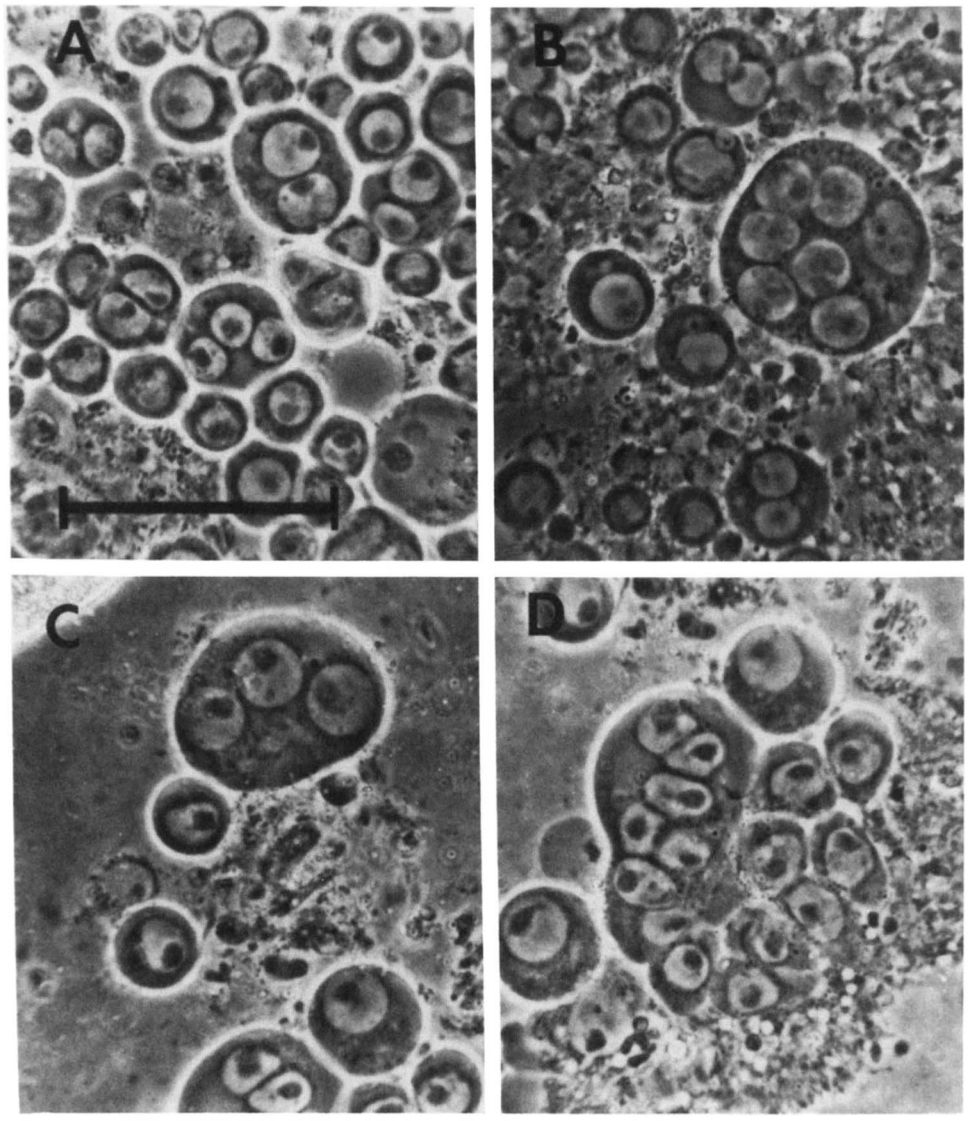

FIGURE 4.—snf^{1621}/Sxl ovarian tumor cells are similar to normal male germ cells. (A) Ovarian tumor cells from a snf^{1621}/Sxl^{fm1} female derived from a $snf^{1621}/FM3$ female parent. This female was grown and maintained at 29° for 10–12 days following eclosion. (B) Ovarian tumor cells from a snf^{1621}/Sxl^{7BO} female derived from a $Sxl^{7BO}/FM3$ female parent. This female was also grown and maintained at 29° for 10–12 days following eclosion. (C and D) Cells from the anterior tip of the testis of two wild-type males (Ore R strain). See Figures 1 and 2 for full genotypes (bar = 40 μm).

sensitive: at 20°, duplication bearing males were occasionally recovered (7–16% viability compared to females), but survival is not dependent on *Sxl* genotype. These males were usually sterile. Thus, we were unable to repeat the earlier results.

The diplolethal region was mapped by mating translocation bearing males with females heterozygous for various deletions. $Df(1)HC244/Y$, $Df(1)rb^{47}$/Y, $Df(1)rb^{1}/Y$ (Table 5) males bearing the duplication survive at 25° but only $Df(1)HC244$ also removes *snf*, indicating that *snf* and the diplolethal region can be separated. Additionally, the snf^{-} deletion $Df(1)rb^{30}$ does not remove the diplolethal region. All males surviving at 25° or 29° were sterile, but males surviving at 20° were occasionally fertile. Males bearing deletions removing the diplolethal region are viable at all temperatures, but are more viable at 20° and less viable at 29° (not shown). The residual temperature-sensitivity lethality and lack of fertility suggests that there may be other dosage sensitive loci in the duplicated region. Deletion mapping of diplolethal and *snf* region indicates that at least 14 complementation groups fall between the diplolethal region and *snf* (Figure 5). This is certainly an underestimate, since lethal loci could not be mapped by complementation due to the diplolethality. Cytologically, the diplolethal region (3F; 4A) and *snf* (4F1-11) are separated by about 40 polytene chromosome bands.

DISCUSSION

Many of the key genes required exclusively for assigning somatic sexual identity and/or regulating dosage compensation in *Drosophila* have been identified and arranged into a reasonable model of interacting genes (BAKER and BELOTE 1983; CLINE 1985; BAKER, NAGOSHI and BURTIS 1987; LUCCHESI and MANNING 1987). The expression of Sxl^{+} is regulated zygotically by the *X:A* ratio. Maternally contributed

TABLE 4

***Sxl* does not rescue diplolethality**

X =[a]	Temperature (°C)	X/Df;Dp[b]	X/Df[c]	X/Y;Dp[d]	X/Y[e]
Sxl^+	20	390	0	22	323
	25	824	0	0	704
	29	187	0	0	138
$Sxl^{f\#1}$	20	125	0	15	96
	25	553	0	1	565
	29	213	0	0	240
Sxl^{7BO}	20	548	0	10	337
	25	776	0	0	680
	29	390	0	0	309
$Sxl^{fs\#1}$	20	219	0	9	155
	25	580	0	0	399
	29	80	0	0	75

[a] Sxl^+ = *y f.* $Sxl^{f\#1}$ = *cm* $Sxl^{f\#1}$ *ct.* Sxl^{7BO} = *y cm* Sxl^{7BO}. $Sxl^{fs\#1}$ = *y cv* $Sxl^{fs\#1}$ *v f.*
[b] $X/Df;Dp = X/Df(1)rb^{71g}\ g\ ct^6\ v$; $Dp(1;2)rb^{+71g}/+$.
[c] $X/Df = X/Df(1)rb^{71g}\ g\ ct^6\ v$; $+/bw^D$.
[d] $X/Y = X/Y$; $Dp(1;2)rb^{+71g}/+$.
[e] $X/Y = X/Y$; $+/bw^D$.

TABLE 5

Deletion mapping of the diplolethal region

X =[a]	Temperature (°C)	X/Df;Dp[b]	X/Df[b]	X/Y;Dp[b]	X/Y[b]
HC244	25	88	0	57	0
rb^{47}	25	410	0	329	0
rb^1	25	1029	0	222	0
rb^{30}	25	92	0	0	0

[a] See Table 1 for cytology.
[b] See Table 4 for genotypes.

gene products, such as *daughterless*$^+$ (*da*) (CLINE 1980, 1984; CRONMILLER and CLINE 1987) and possibly those of an unidentified gene(s) in the 11D; 12A1-2 region (BELOTE *et al.* 1985; SCOTT 1987) are also required for activation of Sxl^+. Another positive regulator of Sxl^+ is Sxl^+ itself (CLINE 1984). A homozygous female-lethal derivative of a constitutive *Sxl* mutant is able to *trans*-activate a wild-type copy of *Sxl* even when the maternal level of da^+ is insufficient for normal Sxl^+ activation.

Is snf^+ a positive regulator of Sxl^+? The Sxl^+ gene product(s) is believed to be required for: (1) transcription of *X*-linked genes at a level to allow for diplo-*X* viability, (2) activation of genes required for somatic sexual identity, and (3), the completion of diplo-*X* oogenesis. The interaction between *snf* and *Sxl* results in: (1) diplo-*X* lethality at both the organismal level and at the level of groups of cells, (2) the sexual transformation of diplo-*X* cells, and (3) failure to complete oogenesis.

Because the organismal lethal interaction has both maternal and zygotic components, we suggest that maternal and early zygotic snf^+ gene products serve to activate or maintain early Sxl^+ expression. In some of the heterozygous *Sxl* progeny of $snf^-/+$ females the single dose of maternal snf^+ may be insufficient for the activation or maintenance of Sxl^+ expression. Those $+/Sxl^-$ progeny escaping this early defect in Sxl^+ expression may be able to proceed through development without further consequences because of zygotic snf^+ expression and Sxl^+ autoregulation, while the somatic cells of snf^-/Sxl^- females have a continuing probability of losing Sxl^+ function. This would explain why there is no maternal effect resulting in altered somatic sexual identity. The zygotic interaction in the soma may be due to the heritable loss of *Sxl* expression in a group of cells derived from single progenitor cells. A few of the surviving snf^-/Sxl^- females show the relics of cell death in the form of missing adult structures and many more show small patches of male cells in the foreleg. We suggest that the "clonal" lethality is due to early loss of Sxl^+ function prior to repression of dosage compensation functions and that later loss of Sxl^+ function causes the mosaic intersex phenotype.

Mutations in *snf* and *Sxl* interact to produce severe defects in female development, but we do not know if *snf* interacts directly with *Sxl*. There is evidence suggesting that snf^+ is not a general regulator of all somatic sex determination genes. Diplo-*X* flies heterozygous for both *tra* and *tra-2* are phenotypic females but *X/X*; *tra/+*; *tra-2/+* flies sometimes develop as intersexes if they are also heterozygous for an additional sex determination gene (BAKER and RIDGE 1980). $snf^-/+$; *tra/+*; *tra-2/+* ($snf^- = Df(1)JC70$) flies do not develop as intersexes (SCOTT 1987).

Female sterility may be due to defects in snf^+ and/or Sxl^+ expression: The zygotic interaction of *snf* and *Sxl* leads to both cold-sensitive and heat-sensitive female sterility but the sterility has slightly different phenotypic manifestations at high *vs.* low temperatures. The cold-sensitive sterility of snf^{1621}/Sxl^{7BO} or $snf^{1621}/Sxl^{f\#1}$ females is fully penetrant and results in small ovarian tumors which is consistent with defects in Sxl^+ expression (SCHÜPBACH 1985; D. MOHLER, personal communication). The sterility of snf^{1621}/Sxl^- females grown at 29°, a temperature with negligible effects on the soma, is due to excessive numbers of nurse cells in addition to large ovarian tumors and is not fully penetrant. These heat-sensitive phenotypes are the same as reported for the ovaries of snf^{1621} homozygotes (GOLLIN and KING 1981). The cold-sensitive and heat-sensitive sterility may be due to defects in the expression of germ-line specific Sxl^+ functions, but defects in snf^+ expression due to reduced Sxl^+ gene product might cause the same phenotype.

As previously suggested (BAKER and BELOTE 1983; SCHÜPBACH 1985), it is tempting to speculate that ovarian tumors resulting from defects in Sxl^+ ex-

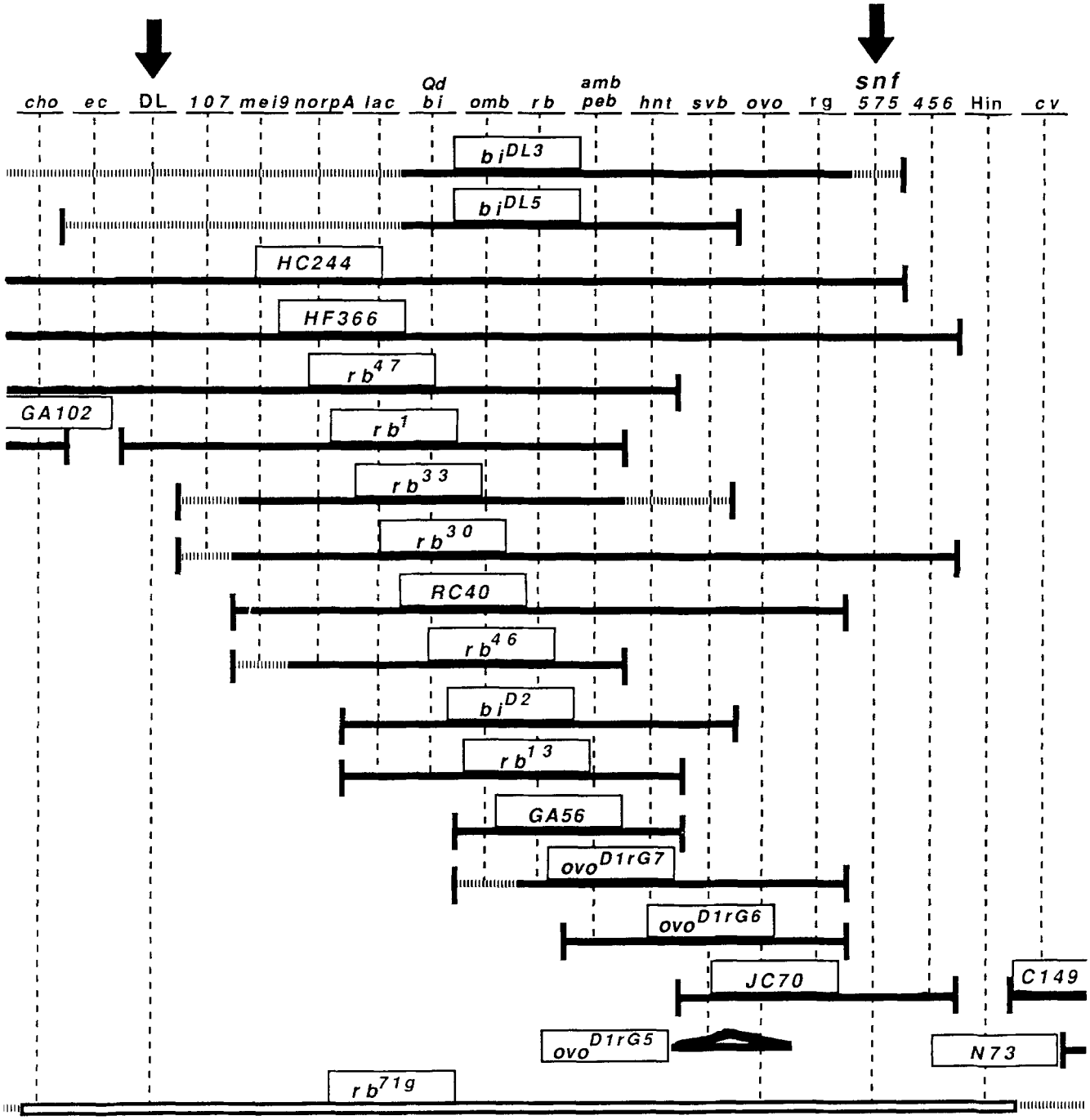

FIGURE 5.—The diplolethal region and *snf* map to different locations. A complementation map of the *snf* region. Genetic loci are shown at the top of the figure. The bold arrows show the locations of the diplolethal region and the *snf* locus. Rearrangements are shown below. With the exceptions of the inversion, *In(1)ovo*^{D1rG5} (triangle), and the translocation, *T(1;2)rb*^{71g} (open bar), all the rearrangements are deletions (closed bars). The names of the rearrangements (without prefixes) are shown in the boxes associated with each representative symbol. The junction of the dashed vertical lines from each locus(i) with the rearrangement symbol indicates that the rearrangement removes the wild-type copy of the gene. Dashed lines at either end of a rearrangement indicate that complementation was not determined. Bold vertical lines at the ends of the rearrangements indicate that the next locus is complemented by the rearrangement. Cytological breakpoints are given in Table 1. Genetic loci and references are as follows: *cho = chocolate; ec = echinus*; DL = male diplolethal region; *107 = fs(1)107; mei9 = meiotic 9; norpA = no optic receptor potential A; lac = lacquer; Qd = Quadroon; bi = bifid; rb = ruby; amb = amber; peb = pebbled; hnt = l(1)hindsight; svb = l(1)shavenbaby; ovo = fs(1)ovo; rg = rugose; snf = sans fille* [mapped by both complementation and based on the lethal interaction of rearrangements with *Sxl* (see Figure 1 and text)]; *575 = fs(1)*575; *456 = fs(1)456*; Hin = Female haplolethal region (see Table 3); *cv = crossveinless. mei9, norpA, lac, Qd, omb* and *amb* were mapped by BANGA *et al.* (1986). *bi* and *rb* were mapped by Banga *et al.* (1986) and in this study. We did not map *amb* but this locus should be removed by *Df(1)ovo*^{D1rG6} based on cytology. See LINDSLEY and GRELL (1968); GANS, AUDIT and MASSON (1975); MOHLER (1977); GOLLIN and KING (1981); WIESCHAUS, NÜSSLEIN-VOLHARD and JÜRGENS (1984); EBERL and HILLIKER (1988); PERRIMON *et al.* (1986); BANGA *et al.* (1986) and OLIVER, PERRIMON and MAHOWALD (1987a) for phenotypic descriptions of the mutant alleles.

pression or inappropriate *X:A* ratios (SCHÜPBACH, WIESCHAUS and NÖTHIGER 1978; SCHÜPBACH 1985) are the result of germ-line sexual transformation. The ovarian tumors due to mutations at a number of loci have been examined (SMITH and KING 1957; JOHNSON and KING 1972; GOLLIN and KING 1981; KING and RILEY 1982), and these workers suggest that ovarian tumors are the result of over proliferation of female germ line cells. Determining if any or all ovarian tumors are the result of germ-line sexual transformations will be important.

Why does snf^{1621} have no effect on the soma in females wild-type for *Sxl*? The snf^{1621} mutation acts as a strong partial loss-of-function allele based on the female sterility phenotype of snf^{1621} homozygotes compared to females *trans*-heterozygous for snf^{1621} and deletions of the locus (GOLLIN and KING 1981), and the lethal interaction of snf^{1621} with *Sxl* compared to the lethal interaction with deletions of *snf*. It is surprising that snf^{1621} has such a strong dominant effect on *Sxl* yet shows no recessive effects on female viability or somatic sex determination (GOLLIN and KING 1981; this study). Further, the mutational focus of snf^{1621} sterility appears to depend on the germ-line genotype (WIESCHAUS, AUDIT and MASSON 1981; PERRIMON and GANS 1983). The maternal influence of *snf* mutations on *Sxl* heterozygotes is consistent with germ-line expression of snf^+ but the zygotic effect of snf^{1621} and snf^- on *Sxl* heterozygotes suggests that snf^+ is active in the soma. Mutations have been identified in *Caenorhabditis elegans* that show no mutant phenotype unless the organism is also mutant at an additional locus (*cf.* FERGUSON, STERNBERG and HORVITZ 1987); snf^+ may have a primary role in the female germ line and only exhibit a phenotype in the soma in conjunction with depressed Sxl^+ function. Since only one nondeletion mutant allele of *snf* is known and since many female sterile mutations represented by only one mutant allele are partial defects in essential genes (PERRIMON *et al.* 1986), it is possible that loss-of-function *snf* alleles may result in homozygous zygotic lethality. It will be important to isolate additional alleles of *snf* in order to determine if snf^+ has an indispensable role in diplo-*X* somatic cells.

What causes diplo-lethality? Diploid flies with a single *X*-chromosome are males while those with two *X*-chromosomes are females (BRIDGES 1921). The *X:A* ratio can be viewed as a "titration" of *X*-chromosomes by autosomally produced factors. It is thought that the loci or sites on the *X*-chromosome, counted by autosomal factors, are dispersed along the entire length of the *X*-chromosome (DOBZHANSKY and SHULTZ 1934), although at least one gene or site, *sisterless-a*, has discrete counting element activity (CLINE 1985, 1986).

The diplolethal region and *snf* map to different locations and removing Sxl^+ function does not rescue males from the lethal effect of duplications of the region (OLIVER, PERRIMON and MAHOWALD 1987b; CLINE 1987; T. CLINE, personal communication; this study) indicating that diplolethality in males is not due to *snf* or counting element activity. Since diplolethality is dependent on temperature, other environmental or genetic variables may explain the rescue of diplolethality, by loss of *Sxl*, reported by STEINMANN-ZWICKY and NÖTHIGER (1985). It seems likely that the diplolethality is due to over expression of a gene or genes in that region, resulting in genetic unbalance as suggested by CLINE (1987). The diplolethality may depend on multigenic determinants since residual heat-sensitive male lethality and sterility were observed in the deletion mapping experiments. In support of this argument, we have been unable to generate sustainable stocks of new duplications of either rb^+ or svb^+, or remove the 25° diplolethality associated with the translocation used in this study, despite extensive screening (N. PERRIMON, B. OLIVER and A. P. MAHOWALD, unpublished data).

Perspectives. Recent molecular data indicate that gene required for sex determination are regulated, at least in part, by differential pre-mRNA processing (MAINE *et al.* 1985; BAKER, NAGOSHI and BURTIS 1987; BOGGS *et al.* 1987). It will be important to analyze the species of transcripts present in ovarian tumors and snf^-/Sxl^- embryos to determine if snf^+ activity is important for Sxl^+ splicing or transcription. It will also be important to attempt to separate the lethal effects of the *snf, Sxl* interaction from the sexual transformation phenotype by, for example, studying combinations of *snf, Sxl*, and dosage compensation mutations. If the snf^-/Sxl^- lethality, but not the sexual transformation and sterility phenotypes, is supressed by defects in dosage compensation, the case for snf^+ acting specifically on the regulation of Sxl^+ would be greatly strengthened. Finally, the surprising effects of an ovarian tumor mutation on both the soma and germ line of *Sxl* heterozygotes suggests that other ovarian tumor mutations warrant a closer examination.

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Note added in proof: A recent paper published by CLINE (1988) in GENETICS also indicates that the *snf* and diplo-lethal regions can be genetically separated.

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