Genetics of 51D-52A, a Region Containing Several Maternal-Effect Genes and Two Maternal-Specific Transcripts in Drosophila

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ABSTRACT

Two genomic clones exhibiting a maternal-specific pattern of expression map to cytological region 52A. To elucidate the function of these clones we have undertaken a mutagenesis of the cytological region 51D-52A. This paper presents the results of this screen and the preliminary analysis of female-sterile and lethal mutations isolated. A total of twelve complementation groups have been identified, four of which are defined exclusively by female-sterile alleles. Only one visible mutation was isolated, a recessive temperature-sensitive allele of *Thickened-arista* (Ta^{rts}). Several of the seven lethal loci display an embryonic lethal phase. Three of the four female-sterile loci affect chorion structure with one resulting in underamplification of the chorion genes, and two (possibly three) of the four female-steriles affect nuclear division/DNA replication. Thus it appears that this is a "developmentally important" region, possibly representing a clustering of genes involved in either DNA replication or nuclear division.

MUCH of the Drosophila genome has been well studied at the genetic, cytological and molecular levels (*e.g.*, CAVENER, OTTESON and KAUFMAN 1986; DAVIS and MACINTYRE 1988; Gausz *et al.* 1981, 1986; HILLIKER *et al.* 1980; HOCHMAN 1976; JUDD, SHEN and KAUFMAN 1972; KAUFMAN, LEWIS and WAKIMOTO 1980; KOTARSKI, PICKERT and MACINTYRE 1983; LEFEVRE 1981; LEICHT and BONNER 1988; PERRIMON, ENGSTROM and MAHOWALD 1984, 1985; ROBERTS *et al.* 1985; WOODRUFF and ASHBURNER 1979), but there remain many regions about which little is known. The region around 52A on the second chromosome is an example of such a region. Results of a molecular screen for maternally expressed genes indicated the presence of two maternal-specific sequences mapping at 52A (UNDERWOOD and LENGYEL 1988), suggesting that the region might be important in early development. Yet, at the time, no maternal-effect genes had been mapped to the region. Subsequently, in a screen for second chromosomal female-sterile mutations SCHÜPBACH and WIESCHAUS (1989) found three maternal-effect mutations which map within *Df(2R)XTE18*, which uncovers the region between 51E and 52C. In an initial attempt to define the genetics of the region in which these maternal-specific clones are located, we have undertaken a genetic screen to saturate the region around 52A, screening for both lethal and female-sterile mutations.

Since there is little or no detectable transcription from the zygotic genome prior to 1.5 hr (ANDERSON and LENGYEL 1979; EDGAR and SCHUBIGER 1986; MCKNIGHT and MILLER 1976; ZALOKAR and ERK 1976), the early embryo must rely upon transcripts made by the mother during oogenesis and stored in the oocyte. Although most of the genes required for correct embryogenesis are undoubtedly housekeeping in nature, and hence required at other stages in the life cycle, several processes are specific to this time period (*e.g.*, fertilization, rapid nuclear division, establishment of axis polarity, etc.). Mutations in these specific genes should result in a specific form of female-sterility, maternal-effect lethality.

Maternal-effect genes are those in which the aberrant phenotype is expressed in the offspring of mutant females. In some maternal-effect genes, the genotype of the embryo can alter the phenotype, *i.e.*, there can be paternal rescue. For strict (nonrescuable) maternal-effect genes the phenotype is determined by the genotype of the mother alone. Many such mutations have been isolated in screens for female-sterile mutations (*e.g.*, GANS, AUDIT and MASSON 1975; MOHLER 1977; RICE and GAREN 1975; SCHÜPBACH and WIESCHAUS 1989). There are three major classes of maternal-effect mutation: maternal-effect female-sterile mutations, in which, because of structural defects in the female reproductive tract, mutant females either do not lay eggs or the eggs are not fertilized (*e.g.*, *ovaless*, MORGAN, BRIDGES and STURTEVANT 1925); maternal-effect lethal mutations, where females do not produce viable offspring (*e.g.*, the female-steriles described by SCHÜPBACH and WIESCHAUS 1989); and maternal-effect grandchildless, where offspring produced by mu-

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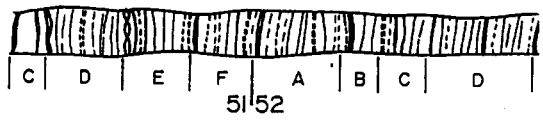
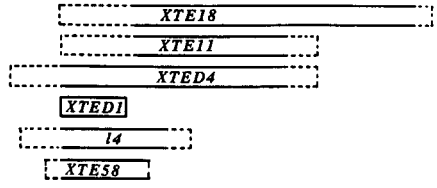
A. Chromosomal Region



B. Deficiencies



C. Cytological breakpoints of 2R deficiencies

Chromosome Designation	Cytological Breakpoint	Source of Deletion
Df(2R)XTE18	51D3 - E3-4; 52C9-D1	Davis & MacIntyre (1988)
Df(2R)XTE11	51D3 - E3-4; 52A6-10	Davis & MacIntyre (1988)
Df(2R)XTED4	51C; 52A6-10	Deletion of TE 19[a]
Df(2R)XTED1	51D3; 51D12	Deletion of TE 19[a]
Df(2R)l4	51C3-7; 51E7-11	γ-ray[a]
Df(2R)XTE58	51D1-2; 51E5	Deletion of TE 19[b]

[a] This study
[b] MacIntyre, personal communication

FIGURE 1.—Cytogenetic map of the 51C-52D region of chromosome 2. Extent of deficiencies are indicated below (B) the cytological map (A), with regions of uncertainty indicated by stippling. (C) Cytological breakpoints of deficiencies used and generated in this study.

tant females are sterile (*e.g.*, *tudor*, BOSWELL and MAHOWALD 1985).

This paper presents the results of a mutational analysis of part of the second chromosome, region 51D-52A (the region uncovered by *Df(2R)XTE11*), and a preliminary characterization of the mutants within the region. The screen selected for visible, lethal, and female-sterile mutations. We have identified a total of twelve complementation groups within this region, four of which are female-sterile. Phenotypic analysis of the female steriles suggests a possible clustering of genes involved in either DNA replication or nuclear division.

MATERIALS AND METHODS

Culture conditions and fly stocks: Flies were grown on standard cornmeal-molasses-agar medium and kept at 25° unless otherwise noted. Descriptions of all visible mutations can be found in either LINDSLEY and GRELL (1968) or LINDSLEY and ZIMM (1985; 1987). The parental chromosomes for mutagenesis (Table 1) were: *cn bw* (screen I, III and IVa), *cn bw sp* (screen IVb) and *TE19* (screen II, stock *1(TE19)* from G. ISING (ISING and BLOCK 1984). Balancer chromosomes used: *CyO* = *In(2LR)O, Cy dp*^{1vl} *pr cn*^{2}, and *Gla CyO* = *In(2LR)Gla, In(2LR)O, Cy dp*^{1vl}. Deficiencies used are listed in Figure 1C with cytological breakpoint and source. *Df(2R)XTE11* and *Df(2R)XTE18* (DAVIS and MACINTYRE 1988) were used in the mutational screens. *Df(2R)XTE58* was also generated by MacIntyre. *T(2;3)Ta*^{L} and *1(2)crc* came from the Bloomington stock center. Three female-sterile mutants were originally isolated by T. SCHÜPBACH: *mat(2)synPL63*, *mat(2)cellQL46* (*QL46*) (SCHÜPBACH and WIESCHAUS 1989) and *fs(2)PA77*. These will be abbreviated as *PL63*, *QL46* and *PA77*.

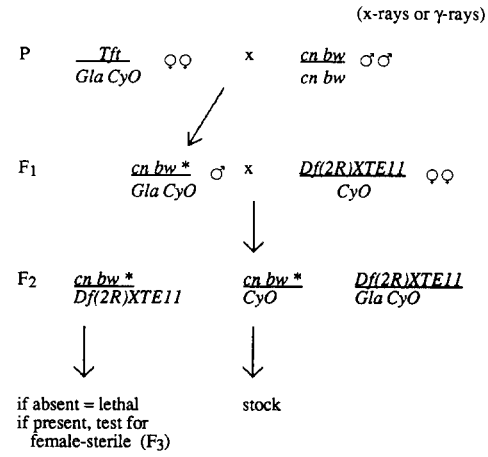


FIGURE 2.—Mutagenesis screen to induce and recover lethal and female-sterile mutants. Isogenized *cn bw* males were mated *en mass* to *Tft/Gla Cyo* females. Single F_1 males were then mated to *Df(2R)XTE11* virgin females. If lethal mutations were induced that were uncovered by this deficiency all F_2 progeny of the individual matings would be phenotypically Curly. In crosses where a lethal was not present straight-wing females (*cn bw* */Df(2R)XTE11*) were then tested for fertility.

Isolation of female-sterile and recessive lethal mutations in the 51D-52A region: The intention of the mutagenesis was to saturate the region surrounding 52A with mutations and to screen for both female-sterile and recessive lethal mutations. Figure 1 shows the cytogenetic map with the deficiencies and cytological breakpoints for strains used and generated in this study. Table 1 summarizes the results of the screens. The first step involved screening through a collection of ethylmethane sulfonate (EMS)-induced, second chromosome female-sterile mutations generated by SCHÜPBACH and WIESCHAUS (1989), testing for female-sterility or lethality when hemizygous with the deficiency (either *Df(2R)XTE11* or *Df(2R)XTE18*, Table 1, screen I). In the next step, additional deficiencies were generated by deletion of the transposable element *TE19*, located at 51E$_{1-2}$ (ISING and BLOCK 1984), as described by DAVIS and MACINTYRE (1988), using 4000 rad of X-irradiation (Table 1, screen II). Putative deficiencies were identified by the loss of the w^{+} allele contained within *TE19*, then were verified cytologically.

The primary screen to identify induced lethal mutations and female-steriles in the 51D-52A region is outlined in Figure 2. Males of an isogenized *cn bw* (or *cn bw sp*) stock were irradiated with 4000 rad of either X- or γ- (from a ^{60}Co source) rays, and mated to *Tft/Gla CyO* virgin females *en mass* for 4 days. Single F_1 males that were phenotypically Glazed eye and Curly wing (*cn bw* */Gla CyO*) were mated to virgin females heterozygous for either *Df(2R)XTE11/CyO* (screen IV) or *Df(2R)XTE18/CyO* (screen III). The presence of a lethal was detected if there were only heterozygous Cy progeny in the F_2, and if these lethals bred true in subsequent crosses. If hemizygous Cy^{+} (*cn bw* */Df(2R)XTE11*) were present, these females (3–5) were mated to their Cy sibs to test for fertility. The absence of an F_3 indicated female sterility. When either a lethal or female-sterile was found within the deficiency, stocks were established using Cy Gla^{+} (*cn bw* */CyO*) virgin females and males from the F_2. X- and γ-rays were used as mutagen in the hopes of generating DNA alterations which would be detectable on Southern blots.

Genetic complementation analysis: All lethal and female-sterile mutations were mated to the five XTE deficiency stocks. The progeny were screened for (1) the presence of hemizygous Cy^+ individuals (absence indicating lethality) and (2) fertility of hemizygous Cy^+ females. At least 100 progeny were scored for each cross. Deficiency mapping defined seven segments (Figures 3 and 4). All mutants mapping within a given segment were crossed to all other mutants mapping within that segment and to representative alleles of complementation groups in adjacent segments to establish allelism. The proximal-distal order of complementation groups within each segment is, for the most part, arbitrary. Some lethal loci exhibit female-sterility when in trans with certain deficiencies. In these cases the loci were placed near the edge of these deficiencies whenever possible. Within each segment loci with similar phenotypes were grouped together.

Lethal phase analysis: For lethal mutations, lethal phase analysis was performed for both homozygous (mut/*CyO* × mut/*CyO* = mut/mut) F_1 and hemizygous (mut/*CyO* × *XTE11*/*CyO* = mut/*XTE11*) F_1. For female-sterile mutations, lethal phase analysis was performed on both the F_1 (the generation in which homozygotes and hemizygotes were produced) and the F_2 (eggs laid by homozygous or hemizygous females). Because it was not possible to identify the genotype of eggs laid, control crosses utilizing the parental chromosome in place of the mutant were set up to control for lethality resulting from the balancer and/or deficiency chromosomes. Lethal phase thus becomes defined as that period when there is an increase in lethality over the control cross. Flies were placed in collecting bottles and allowed to lay eggs on molasses-agar plates for 4-hr intervals. A total of 500 embryos per cross was harvested and placed on 15 mm × 60 mm plastic petri dishes containing standard media, 100 embryos per dish. After 36 hr, unhatched eggs were collected, counted, and stained with Hoechst (see below) to check for fertilization. Number of pupal cases was counted, as were the number and genotype of adults. Larval lethality was defined as the difference between the number of hatched eggs and the total number of adults and dead pupae recovered.

Cytological analysis: Heterozygous (*cn mut bw*/*CyO*) virgin females were mated to *cn bw* males and grown at 25°. Every two days adults were transferred to fresh vials and the vials containing eggs and larvae were transferred to 18°. Larvae were fed fresh yeast daily. Salivary glands from late third instar larvae with white Malphigian tubules (indicating *cn mut bw*/*cn bw*) dissected in Drosophila ringers solution (EPHRUSSI and BEADLE 1936) were stained in lacto-acetic orcein and squashed according to the method of YOON, RICHARDSON and WHEELER (1973). Chromosome squashes were viewed under phase contrast and bright field microscopy on either a Zeiss Axiophot or an Olympus microscope.

Chromosomes for *in situ* hybridization were prepared as described by Underwood and Lengyel (1988). Digoxygenin-labeled DNA was prepared according to manufacturers directions-(Boehringer Mannheim, Genius kit). Squashes were heat-sealed (2 × SSC, 65°, 30 min), denatured (0.7 M NaOH, 3 min), hybridized overnight (in 50% formamide, 5 × SSC, 100 μg/ml salmon sperm DNA, 100 μg/ml tRNA, 5 ng digoxygenin-DNA in total volume 10 μl, 45°), washed (2 × SSC, 42°, twice; PBS, room temperature, three times), and the signal detected with alkaline phosphatase [blocked with 2% BSA, 3.5 mg/ml heparin for 1 hr; incubated with antidigoxygenin (1:1000) 1–2 hr; washed (3 ×, 100 mM Tris, pH 7.5, 150 mM NaCl; 3 ×, 100 mM Tris-HCl, pH 9.5, 100 mM NaCl, 50 mM $MgCl_2$); incubated in chromogenic substrate solution (4.5 μl/ml NBT (nitroblue-tetrazolium), 3.5 μl/ml X-phosphate in PBS) for 10 min to several hours, checked periodically by phase contrast microscopy; stopped by immersing slide in TE (10 mM Tris, 1 mM EDTA)]. This is a modification (suggested by Pignoni and Lengyel) of the method devised by Engels and co-workers (1986) for use with biotinylated probes.

Phenotypic descriptions: *Hoechst-staining*: Staged embryos were dechorionated in commercial bleach (active ingredient 5.25% sodium hypochlorite), rinsed in distilled water, fixed in formaldehyde, and the vitelline membranes were removed by methanol, using a modification of the method of DEQUIN, SAUMWEBER and SEDAT (1984). All steps were scaled down by a factor of 10 for use in an Eppendorf tube. Embryos were stained in Hoechst 33258 (Polysciences) 1 μg/ml at room temperature for 10 min (ZUSSMAN and WIESCHAUS 1987) and mounted in Mowiol 4.88 (Calbiochem).

Larval cutical preps: In order to observe cuticular patterns in late embryos, embryos were mounted in polyvinyl lactophenol (PVL, Bio/medical Specialities) and cleared by heating overnight at 60° according to the method of MERRILL, TURNER and KAUFMAN (1987).

Adult cuticular structures: Adults were boiled in KOH according to the method of SZABAD (1978) and mounted in PVL.

Transmission electron microscopy: Stage 14 oocytes and/or embryos were fixed for ultrastructural analysis of the chorion by a modification of the method of KALT and TANDLER (1971). All fixation and embedding times were lengthened. Embryos were embedded in Spurrs (Electron Microscopy Sciences) medium, sectioned on an LKB Microtome III, and viewed on a Zeiss EM10.

Slot blot analysis of chorion genes: DNA was isolated from stage 13 egg chambers according to the procedure of DE CICCO and SPRADLING (1984). Samples were slot blotted onto nitrocellulose using a Schleicher and Schuell Minifold II apparatus, according to the manufacturer's instructions. The probes for Southern hybridizations were third chromosome chorion gene cluster subclone p302.77 and X chromosome chorion gene cluster subclone p104.41 (SPRADLING 1981). The rDNA probe was subclone pDmrY22 (DAWID, WELLAUER and LONG 1978) which served as a probe for nonamplifying DNA. Hybridization was visualized by autoradiography and quantitated by excising the bands and measuring radioactivity by scintillation counting. Degree of amplification for a given sample was determined by dividing the ratio of chorion:rDNA signals by the ratio of chorion:rDNA signals from an unamplified control (the embryo DNA). The absolute level of amplification for a non-mutant strain such as $\pi 2$ varied as much as 2–3 fold, from 40× (Figure 5A) to 120× (not shown). However when amplification levels for a mutation affecting amplification are expressed as percentage of amplification in wild type (SNYDER, GALANOPOULOS and KAFATOS 1986) or heterozygotes, the value obtained is more consistent from one experiment to another.

RESULTS

Isolation of recessive lethal and female-sterile mutations in the 51D-52A subinterval. *Df(2R)XTE11*, extending from 51D3-E_{3-4} to 52A_{6-10} (Figure 1), was used in a series of standard F_2/F_3 screens for recessive lethal/female-sterile mutations (see Materials and Methods for screen descriptions). Table 1 summarizes the four screens. The first screen tested a collection of second chromosome homozygous female-sterile mutations generated by SCHÜP-

TABLE 1

Summary of screens for recessive lethal and female-sterile mutations in the 51D-52A region

Screen I.	Results of EMS screen for female-sterile mutations (SCHÜPBACH and WIESCHAUS 1989)	
	Total no. chromosomes screened	7,351
	No. female-sterile over *Df(2R)XTE18* (or *11*)	3
Screen II.	Results of X-ray screen for deletions of *TE19*[a]	
	Total no. chromosomes screened	3,552
	No deletions	3
	[[a] Three additional XTE deficiencies used, *XTE11*, *18*, and *58* were generated by DAVIS and MACINTYRE (1988)]	
Screen III.	Results of X-ray screen	
	Total no. chromosomes screened	1,876
	No. of lethal phenotypes over *Df(2R)XTE18*	3
	No. of female-sterile over *Df(2R)XTE18*	1
Screen IV.	Results of γ-ray screen	
	Total no. chromosomes screened	7,634
	No. of lethal phenotypes over *Df(2R)XTE11*	19
	No. of female sterile over *Df(2R)XTE11*	4
	No. visibles	1

BACH and WIESCHAUS (1989) on a *cn bw* chromosome using EMS as a mutagen. Three female-sterile mutations (*PA77*, *PL63*, and *QL46*) were found to lie within both *Df(2R)XTE18* and *Df(2R)XTE11*. In the second screen we generated 3 additional deficiencies (*Df(2R)XTED1*, *Df(2R)XTED2*, *Df(2R)XTED4*) within the region, through loss of a w^+-bearing transposable element *TE19* (located in $51E_{1,2}$), followed by cytological verification.

The third and fourth screens are outlined in Figure 2. Screen III utilized X-rays and the larger deficiency *Df(2R)XTE18* (Figure 1). Three lethals (*l12*, *l6*, *l16*) and one female-sterile (*fs8*) were recovered. Screen IV, utilizing γ-rays, was conducted in two parts. The first half (3979 chromosomes) utilized the *cn bw sp* chromosome and yielded 11 lethals, two of which are deficiencies (*Df(2R)l4* and *l13*), one visible (Ta^{rts}), and one female-sterile (*fs27*). Some of the test crosses were set up at 18° to screen for temperature-sensitive mutations, and two were found ($l17^{ts}$ and Ta^{rts}). The second part of screen IV returned to the *cn bw* chromosome and yielded nine lethals and two female-steriles (*fsA5*, *fsA3*).

Deficiency mapping and complementation analysis: All mutants were mapped with respect to the five major deficiencies and later the additional deficiencies generated in screen IV. Results from these crosses are presented in Figure 3. With only one exception (*fs14*), all alleles of each complementation group gave identical results. The mutation $fs14^{ts}$ is defined as an allele of *l(2)51Ea* based on producing female sterility when heterozygous with either *l7*, *l19*, or *l25* at 18°. It complements all other alleles of the locus, and thus is probably a weak hypomorph. There is a female-sterile interaction between $fs14^{ts}$ and *Df(2R)XTED1* at 18°. This is probably the result of a position effect because (1) the other alleles of the locus fully complement *Df(2R)XTED1* and (2) there is no interaction between $fs14^{ts}$ and *Df(2R)XTE58*, yet *Df(2R)XTED1* is entirely contained within *Df(2R)XTE58* (based on cytological data).

Several interesting observations can be made from the data in Figure 3. Although lethal mutations were isolated as being lethal in combination with *Df(2R)XTE11*, some combinations of lethal mutation and deficiency resulted in female-sterility rather than lethality. This is true for both of the temperature sensitive mutations. Three of the lethal mutants yield female-sterility when in combination with the deficiency *Df(2R)l4* (two of these are temperature sensitive), suggesting that it is something about the *Df(2R)l4* lesion which results in female-sterility rather than lethality when in combination with certain lethal alleles (representing four loci) but not with others (representing four loci). Three of the eight female-sterile mutants are homozygous semilethal, but this may be due to additional changes outside the region of interest on the mutated chromosome. The mutation *PA77* (allele of *fs(2)51Fa*) is homozygous female-sterile, but lethal when hemizygous with all of the deficiencies with which it interacts, suggesting that it is a hypomorph.

Figure 4 summarizes the mapping results. The region can be broken into seven segments based on deficiency mapping, with the order within each region being arbitrary. *l13* is defined as a deficiency based on noncomplementarity with two complementing loci (*fs(2)51Fa*, allele *PA77* and *l(2)51Ec*, allele *l 5*), but it is not cytologically visible. *l5* fails to complement both *l13* and *Df(2R)l4*, but *l13* and *Df(2R)l4* complement, suggesting that each deficiency removes a different part of *l5*. Only one locus, *l(2)51Ea*, contains both lethal and female-sterile alleles. All other female-sterile alleles (seven alleles representing four loci) lie at one end of the region (segments V and VI), suggesting a clustering of genes possibly involved in a similar function (see DISCUSSION).

In a region containing approximately 30 cytological bands we have isolated 30 mutations, lethal and female-sterile, which fall into 12 complementation groups. Seven loci are represented by only one allele (including two of the four loci containing only female-sterile alleles), one by two, two by three, one by four, and one by eight. The average number of alleles is 2.25. Because of the overabundance of alleles at the *l(2)51Ea* locus (eight, see Figure 4) and the high number of loci with single alleles (seven), it is not possible to meet the statistical assumptions for a good-

DEFICIENCY CHROMOSOMES

Complementation Group	Allele	*XTE18*	*XTE11*	*XTED4*	*l13*	*l4*	*XTED1*	*XTE58*	Homozygous[a]
l(2)51Da	$l17^{ts}$ 25°	l	l	l	+	fs	l	l	l
	18°	l	l	l	+	+	l	l	l
l(2)51Db	3 alleles	l	l	l	+	l	l	l	l
l(2)51Dc	*lJ2*	l	l	l	+	l	fs	fs	+
l(2)Ta	Ta^{rts} 25°	+	+	+	+	fs	+	+	l
	18°	Ta	Ta	fl	+	fs	+	Ta	l
l(2)51Ea	$fs14^{ts}$ 25°	l	fs	fs	+	sl, fs	+	+	l
	18°	l	sl, fs	fs	+	sl, fs	fs	+	l
l(2)51Ea	7 alleles	l	l	l	+	l	+	+	l
l(2)51Eb	4 alleles	l	l	l	+	l	+	+	l
l(2)51Ec	*l5*	l	l	l	l	fs	+	+	l
fs(2)51Fa	*PA77*	l	l	l	l	+	+	+	fs
fs(2)51Fb	*QL46*	fs	fs	fs	+	+	+	+	sl
	fsA3	fs	fs	fs	+	+	+	+	sl
fs(2)abc	*PL63*	fs	fs	fs	+	+	+	+	fs
	fs27	sl, fs	fs	fs	+	+	+	+	fs
	fsA5	sl, fs	sl, fs	fs	+	+	+	+	sl, fs
fs(2)sun	*fs8*	fs	fs	fs	+	+	+	+	sl, fs
l(2)51F	*lK5*	l	l	l	+	+	+	+	sl
l(2)52AD	2 alleles	l	+	+	+	nd	nd	+	l

FIGURE 3.—Complementation behaviors of 51D-52A chromosomal deficiencies. All hemizygous combinations of mutant and deficiency were scored for visible, lethal and/or female-sterile interactions. Crosses were performed at 25° unless otherwise noted. All alleles of a given locus demonstrated identical deficiency interactions with the exception of $fs14^{ts}$ (see text) which is therefore listed separately. Abbreviations: fl = female-lethal; fs = female-sterile; l = lethal (0/>100 flies counted); nd = not determined; sl = semilethal (<10% of total progeny); Ta = thickened arista; + = viable and fertile. [a] Possibly due to additional mutation(s) on the chromosome.

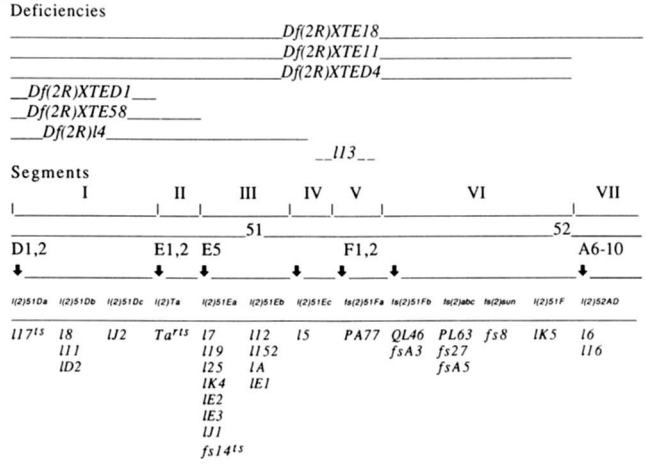


FIGURE 4.—Summary of complementation and mapping data. Lines at the top of the figure represent regions missing in the deficiencies. The endpoints of the deficiencies are used to define the subsegments given in the center of the figure. These are numbered in order (I through VII) from proximal to distal on the chromosome. Approximate cytological positions are indicated by D1,2, E1,2, F1,2 and A6,10 and the corresponding down arrows (↓) above and between the locus designations. These arrows indicate positions where breakpoints allow left-right orientation of the flanking genes. The locus name is given above the line with the alleles listed below. Loci that mutate to lethality are named by their location on the chromosome, *e.g.*, *l(2)51E*. The lower-case letter following this designation is given alphabetically from proximal to distal within each lettered region. Female-steriles are similarly named. *Ta^{rts}: Thickened arista recessive temperature-sensitive; abc: abnormal chromatin; sun: supernova.*

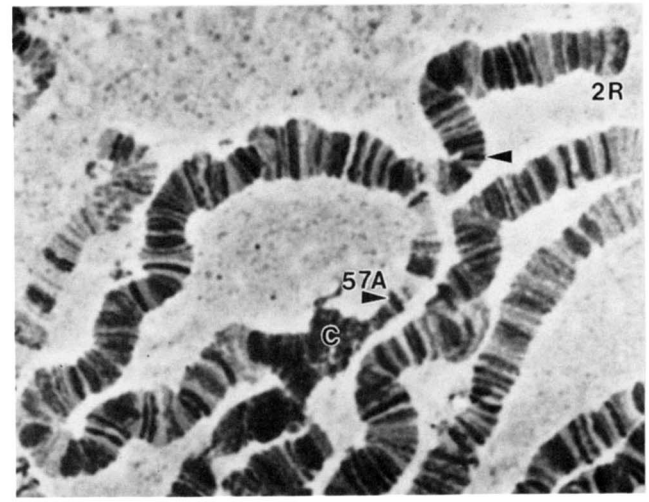


FIGURE 5.—Cytology of Ta^{rts}. Salivary chromosome squash of Ta^{rts}/cn bw, showing second chromosomal region 57A (arrowheads) inserting into centromeric heterochromatin (C).

ness-of-fit χ^2 to a truncated Poisson distribution (BARRETT 1980). As a result of this we cannot test for mutational saturation of this region. However, the surplus of single-allele loci suggests that saturation of the region has not been reached. The relatively large number of alleles at *l(2)51Ea* may indicate that this is a mutational hot spot for γ-radiation.

Cytology: Most of the lethal and female-sterile mutations isolated in this study are cytologically normal. As mentioned previously *Df(2R)l4* and *l13* were identified as deficiencies based on genetic data. *l13* is not cytologically visible and *Df(2R)l4* removes the region $51C_{3-7};51E_{7-11}$. Two additional mutations have chromosomal alterations which lie outside the region uncovered by the deficiencies used in the mutagenic screen. $l17^{ts}$ exhibits a deficiency for the region 57D-58B and Ta^{rts} has a chromosomal alteration which places centromeric heterochromatin adjacent to cytological region 57A (Figure 5). Their interactions

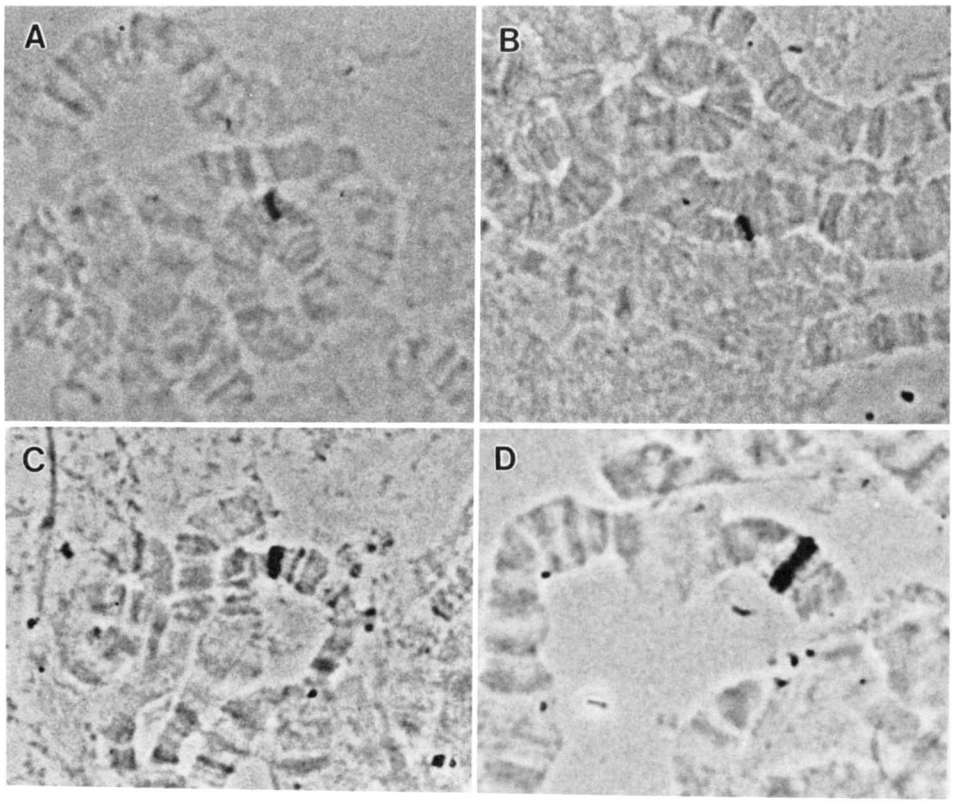


FIGURE 6.—*In situ* hybridization to deficiency chromosomes with maternal-specific clones IM137 and IM144. Salivary gland chromosomes from *Df(2R)XTE11* (A and B) or *Df(2R)l4* (C and D) were probed with digoxygenin-labeled IM137 (A and C) or IM144 (B and D). *Note*: with both probes, label only goes halfway across chromosome arm in *Df(2R)XTE11*, but across entire arm in *Df(2R)l4*.

could be explained either by position effects or by additional lethal mutations which lie within the deficiency region (see discussion).

Maternal-specific clones at 52A: Two genomic clones, IM137 and IM144, were isolated in a molecular screen for sequences which encode maternal-specific RNAs (UNDERWOOD and LENGYEL 1988). These were defined as maternal-specific because their transcripts are at least 150-fold more abundant in maternal RNA populations (0–1-hr embryos, prior to the onset of zygotic transcription) than any other stage of development except the adult, where their presence was presumed to be due to presence in the ovary. *In situ* hybridization to salivary gland chromosomes placed IM137 at $52A_{3,5}$ and IM144 at $52A_{1,2}$ (UNDERWOOD and LENGYEL 1988). The purpose of this study was to describe the region in which these sequences map genetically. To confirm that both cloned sequences lie within the deficiency utilized in this screen (*Df(2R)XTE11*), and to further define the genetic/deficiency region in which they reside, *in situ* hybridization to deficiency chromosomes was performed. Both IM137 and IM144 are contained within the region deleted by *Df(2R)XTE11* (Figure 6, A and B; note the hybridization only across the looped-out, non-deficiency homologue), but not within the region uncovered by *Df(2R)l4* (Figure 6, C and D; note the hybridization across both halves of chromosome, immediately adjacent to the folded, deficiency region). This places the two cloned sequences in the "female-sterile" region (segment V and VI, Figure 4).

Lethal phase analysis: Lethal phase analyses were performed on representative alleles of all lethal loci and on all female-sterile alleles, both as homozygotes and as hemizygotes with *Df(2R)XTE11* or *Df(2R)XTE18*. Figure 7 presents the results for the lethal mutations and Figure 8 for female-sterile mutations. Because most of the female-sterile mutations had some associated lethality, lethal phase analyses were performed on both the F_1 (detecting recessive lethality associated with the mutation) and F_2 (detecting death resulting from a maternal effect) generations.

Several interesting observations can be made from the data presented in Figures 7 and 8. With the dose of radiation employed in these screens it would not have been unexpected to have produced more than one mutation/chromosome. Thus, homozygous phenotypes most likely are due to background effects and the hemizygous phenotype represents the loss-of-function phenotype. Many of the lethals show primarily embryonic lethality. Several loci (including hemizygous *fs(2)51Fa* (allele *PA77*) and most of the alleles of *l(2)51Eb*) result in pupal lethality, exhibiting a range of phenotypes from no apparent development following pupariation to production of an aberrant pharate adult (data not shown). Most of the lethals do not exhibit distinctive phenotypes, at least at the level of analysis performed here, with one notable exception, Ta^{rts}. Almost all of the female-sterile mutations result in embryonic lethality in the F_2. As detailed analyses of the female-steriles will be presented elsewhere, only a brief description will be presented here.

Phenotypic descriptions: Flies heterozygous for Ta^{rts} and a deficiency (either *Df(2R)XTE11,*

Complementation Group	Mutant Line	Mutagen	Lethal Period			
			Embryonic	Larval	Pupal	Adult[a]
l(2)51Da	*l17[ts]*	γ-ray	●	○		
l(2)51Db	*l8*	γ-ray	● ○	●		
	l11	γ-ray	● ○	●		
	lD2	γ-ray	● ○			
l(2)51Dc	*lJ2*	γ-ray		● ○	● ○	● ○
l(2)Ta	*Ta[rts]*	γ-ray	● ○			○
l(2)51Ea	*l7*	γ-ray	● ○		○	
	l19	γ-ray	● ○		○	
	l25	γ-ray	● ○			
	lE2	γ-ray	●	○	○	
	lE3	γ-ray		● ○	○	
	lJ1	γ-ray	● ○			
	lK4	γ-ray		● ○	○	
	fs14[ts]	γ-ray	● ○	●	●	
l(2)51Eb	*l12*	x-ray	●	● ○	● ○	
	l152	γ-ray	●		● ○	
	lA	x-ray		● ○	● ○	
	lE1	γ-ray		●	● ○	
l(2)51Ec	*l5*	γ-ray	●	● ○		
l(2)51F	*lK5*	γ-ray		○	● ○	● ○
l(2)52AD	*l6*	x-ray	● ○	○		
	l16	x-ray	● ○			

FIGURE 7.—Summary of lethal phase analysis of lethal mutations in the 51D-52A region. All lethal phase determinations were done at 25°. Lethal periods of mutant homozygotes are indicated by black circles. Lethal periods of mutants hemizygous over the deficiency *Df(2R)XTE11* are indicated by open circles. [a] Symbols under adult represent survival to adulthood but at reduced levels.

Complementation Group	Mutant Line	Mutagen	Lethal Period							
			F_1				F_2			
			Embryonic	Larval	Pupal	Adult[a]	Embryonic	Larval	Pupal	Adult[a]
fs(2)51Fa	*PA77*	EMS		● ○	○	● l	■			
fs(2)51Fb	*QL46*	EMS		● ○	●	sl ○	■ □	□		□
	fsA3	γ-ray	● ○			sl ○	□	□		□
fs(2)abc	*PL63*	EMS		●		● ○	■ □			
	fs27	γ-ray	○		● ○	● ○	■ □			
	fsA5	γ-ray			● ○	sl ○	■ □			
fs(2)sun	*fs8*	γ-ray	● ○	●		sl ○	□			

FIGURE 8.—Summary of lethal phase analysis of female-sterile mutations in the 51D-52A region. All lethal phase determinations were done at 25°. Lethal period for both the F_1 and the F_2 generations. Symbols used: (●) homozygous lethal (*m/CyO* × *m/CyO* → F_1 *m/m*); (○) hemizygous lethal (*m/CyO* × *Df(2R)XTE11/CyO* → F_1 *m/Df(2R)XTE11*); (■) homozygous F_2 lethal (*m/m* ♀ × *OR* ♂ → F_2); (□) hemizygous F_2 lethal (*m/Df(2R)XTE11* ♀ × OR ♂ → F_2). [a] All female-sterile lines showed some lethality prior to adulthood. When survival to adult is severely affected this is indicated by *sl* (≤10% of progeny) and *l* (≤2% of progeny) in the adult column. These cannot be tested in the F_2 generation.

Df(2R)XTE18 or *Df(2R)XTE58*) exhibit reduced viability (approximately one-half expected) and a thickening at the base of the arista at 18° which resembles the dominant effect of $T(2;3)Ta^L$ (CAVENER, OTTESON and KAUFMAN 1986; KAUFMAN, LEWIS and WAKIMOTO 1980; compare Figure 9, C and E). When Ta^{rts} is heterozygous with a balancer chromosome (Figure 9B) the aristae are normal (compare to Figure 9A); there is no dominant effect. When Ta^{rts} is hemizygous with a deficiency (*i.e*, *Df(2R)XTE18*) at 25° (Figure 9D) the aristae are also normal, but at 18° (Figure 9E) the base of the arista is thickened and the bracted

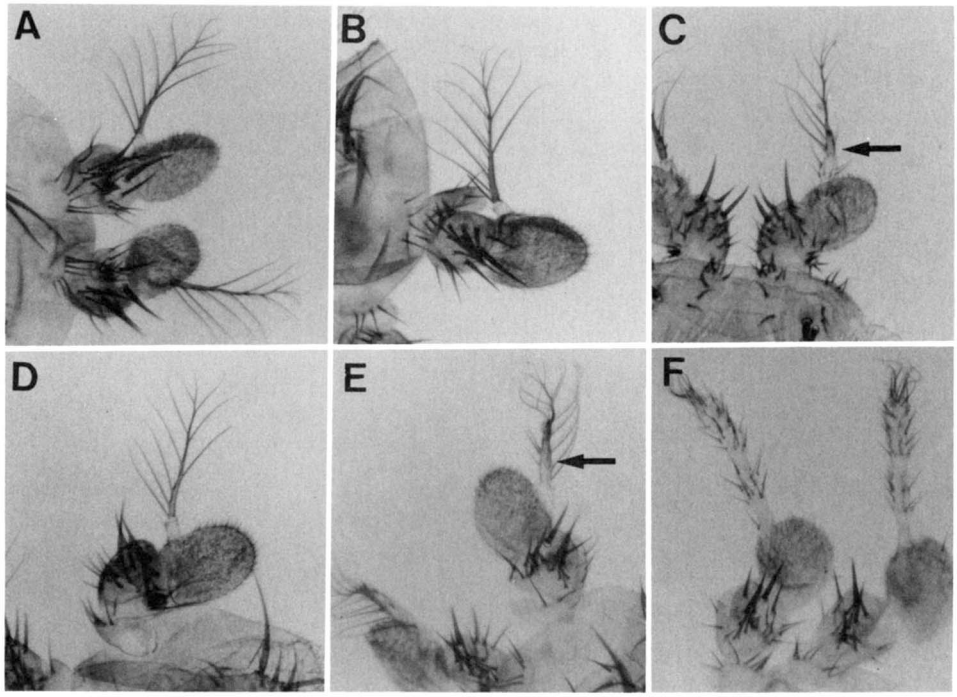


FIGURE 9.—Ta^{rts}: a temperature-sensitive recessive allele of *Thickened arista* ($T(2;3)Ta^{L}$). (A) Control (*cn bw sp*) arista, 18°. (B) Ta^{rts}/Cyo, 18°; arista normal. (C) $T(2;3)Ta^{L}$/TM3, 25°; note thickening at base of arista. (D) $Ta^{rts}/Df(2R)XTE18$, 25°; arista normal. (E) $Ta^{rts}/Df(2R)XTE18$, 18°; note thickening at base of arista and leg-like bracted bristles (arrow). (F) Ta^{rts}/Ta^{L}, 18°; note increased severity of arista-to-leg transformation when compared to either C or E.

bristles typical of the leg are present, indicating temperature sensitivity. Figure 9C shows the thickened arista phenotype associated with $T(2;3)Ta^{L}$. When Ta^{rts} is heterozygous with Ta^{L} the characteristic transformation of arista into leg-like structures is enhanced at 18° (Figure 9F) but not at 25° (data not shown). Thus Ta^{rts} is a recessive, temperature-sensitive allele of $T(2;3)Ta^{L}$. The second chromosome breakpoint for $T(2;3)Ta^{L}$ (51E) lies within our deficiency and Ta^{L} is lethal when hemizygous with *Df(2R)XTE11*, *Df(2R)XTE18* and *Df(2R)XTED4*; semilethal with a strong thickened-arista phenotype when hemizygous with *Df(2R)l4*; and viable with an enhanced thickened-arista phenotype when hemizygous with *Df(2R)XTED1* and *Df(2R)XTE58*.

We initially attempted to classify the female-sterile defects as affecting either egg morphology or early embryogenesis, but have found considerable overlap. At least two of the four female-steriles affect nuclear division, and a third is hemizygous lethal with a phenotype involving nuclear division/migration. Figure 10A shows the normal pattern of nuclei found at the blastema stage (approximately 1.5 hr). Mutant embryos of a similar or slightly older age are shown in figures 10B-C. Hemizygous *fs(2)supernova* (*sun*) allele *fs8* ($fs(2)sun^{fs8}/Df(2R)XTE11$) females lay eggs in which nuclear division stops at or before the fifth division, with a majority of the embryos having only eight nuclei (Figure 10C). Increased nuclear staining with increased embryo age suggests that DNA replication continues in the absence of nuclear division. Embryos laid by hemizygous *fs(2)abnormal chromatin* (*abc*) allele *PL63* females at a similar age exhibit abnormal chromatin clumps, unusual nuclear distribution and some nuclear bridges (Figure 10B; K. VESSEY, R. LUDWICZAK, A. BRIOT, and E. UNDERWOOD, manuscript in preparation). Other alleles of the *fs(2)abc* locus exhibit arrest following three (*fsA5*) or four (*fs27*) rounds of nuclear division and abnormal nuclear distribution. *fs(2)51Fa* allele *PA77* is hemizygous lethal resulting in embryos exhibiting asynchronous mitosis at the blastoderm stage when reared at 18° (data not shown). At 25° hemizygous *PA77* is pupal-lethal. As homozygotes, *PA77* females produce eggs with defective chorions (Figure 11B, see below) that are either unfertilized (~50%) or fail to undergo pronuclear fusion (E. UNDERWOOD, unpublished observation).

Mutants in three of the four female-sterile loci show defects in chorion structure, in both the endochorion and exochorion (*fs(2)abc, fs(2)51Fa, fs(2)51Fb*). The structure of the normal chorion, as found in *cn bw* stage 14 oocytes (mature eggs ready to be laid), is shown in Figure 11A. The outer or exochorion is a homogeneous layer. The inner or endochorion contains a continuous roof, floor with small breaks, and solid pillars joining the two at regular intervals. In stage 14 oocytes produced by homozygous *fs(2)52Fa* allele *PA77* females the endochorion contains breaks in the roof, very few pillars, and the exochorion is amorphous (Figure 11B). Homozygous *fs(2)51Fb* allele *QL46* females produce stage 14 oocytes whose endochorions have breaks in the roof. In addition, debris can be seen in the space between the roof and the floor, and there are virtually no pillars. The exochorion appears to be more compact than normal (Figure 11C). The latter defect is more pronounced in oocytes produced by hemizygous females, which

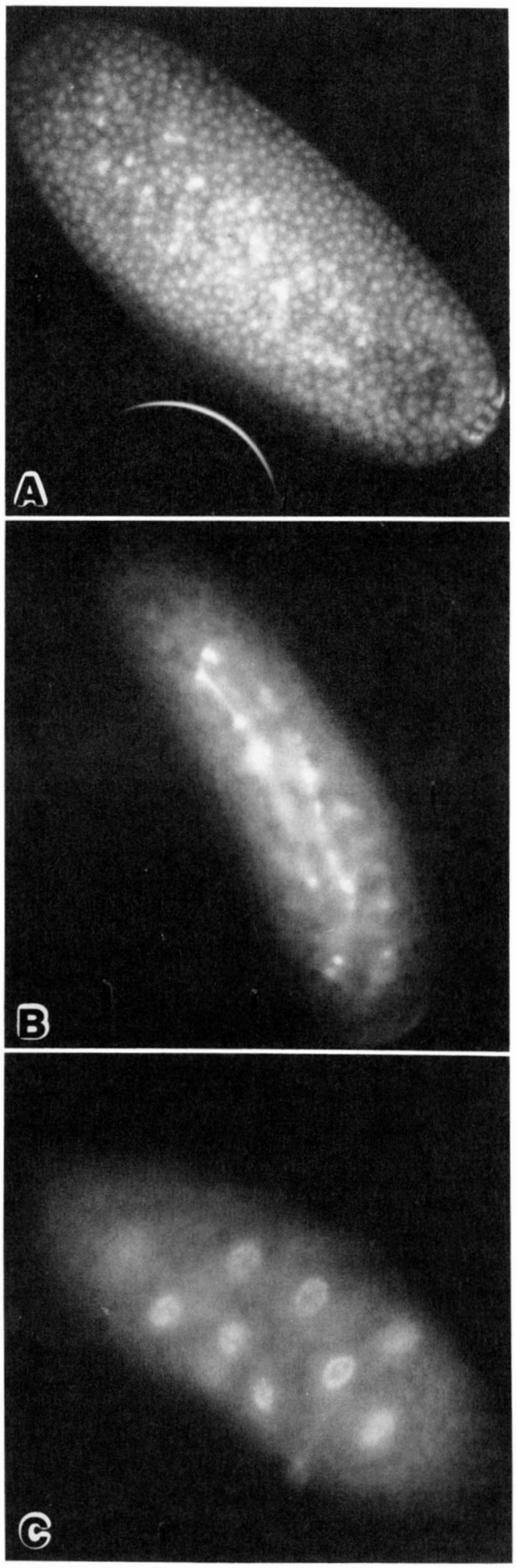


FIGURE 10.—Maternal-effect, female-sterile mutations affecting nuclear division. Embryos laid by hemizygous females mated to wild-type males were stained with the DNA-specific stain Hoechst 33258. (A) Control (*cn bw/Df(2R)XTE11* ♀ × *OR* ♂). (B) *PL63/Df(2R)XTE11* ♀ × *OR* ♂. (C) *fs8/Df(2R)XTE11* ♀ × *OR* ♂.

have little or no exochorion (data not shown). Additionally, some eggs laid by either hemizygous or homozygous females still have follicle cell debris attached (data not shown), something never seen in wild type. Stage 14 oocytes produced by hemizygous *fs(2)abc* allele *fsA5* females also exhibit an amorphous exochorion. The endochorion roof is continuous, there are no pillars and the floor is clumpy (Figure 11D).

Of the mutants affecting chorion ultrastructure only *PA77* (allele of *fs(2)51Fa*) results from underamplification of the chorion genes. Figure 12 shows an autoradiograph of a slot blot comparing DNA purified from stage 13 oocytes of *π2* (control, non-female sterile, Figure 12A), *PA77* heterozygotes (Figure 12B), *PA77* homozygotes (Figure 12C), and from *π2* embryos (Figure 12D) probed with rDNA, a non-amplified control (Column 1), and a third chromosome chorion gene cluster probe (column 2). In this experiment, *PA77* homozygotes (Figure 12C) amplified at 15% the level of *PA77* heterozygotes (Figure 12B). In a duplicate experiment, the level was 11% (see MATERIALS AND METHODS for how fold amplification was calculated). *PA77* also reduces amplification of the X chromosome chorion gene cluster (data not shown), however the percent decrease is less than the decrease in third chromosome amplification, as has been previously observed with other mutations affecting chorion gene amplification (ORR, KOMITOPOULOU and KAFATOS 1984; SNYDER, GALANOPOULOS and KAFATOS 1986). The other female-sterile mutations in this region do not affect amplification of either chorion gene cluster (data not shown).

DISCUSSION

We have screened the region uncovered by *Df(2R)XTE11* looking for both female-sterile and lethal mutations. The region contains several genes affecting early development, having 4/12 loci exhibiting female sterility and several of the lethal loci exhibiting female sterility in combination with certain deficiencies. Additionally, several of the lethal mutations have an embryonic lethal phase. Two maternal-specific transcripts have also been mapped to this region.

It is difficult to say if the mutagenic screens reported here resulted in saturation of this genetic interval, as the unusual allelic distribution recovered makes it impossible to conduct statistic analyses (BARRETT 1980). We have achieved an average allelic frequency of 2.25. The surplus of single-allele loci include a number of female-sterile loci (2/7). SCHÜPBACH and WIESCHAUS (1989) saw a similar phenomenon in their screen for female-sterile loci on the second chromosome. They interpreted the surplus to be due to female steriles representing hypomorphic alleles of loci that would be lethal as amorphs. This cannot

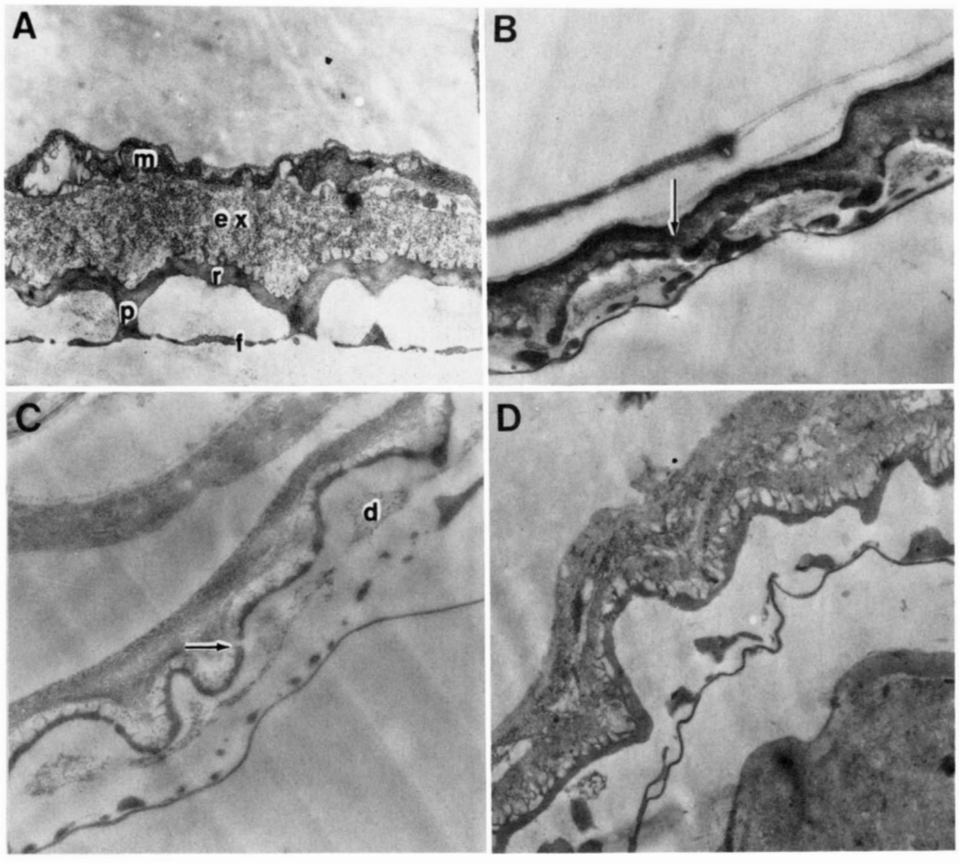


FIGURE 11.—Chorion defect in stage 14 oocytes produced by female-sterile mutants. (A) *cn bw* (normal chorion structure). (B) *fs(2)51Fa* allele *PA77*, (C) *fs(2)51Fb* allele *OL46*, (D) *fs(2)abe*^{pA5}*/XTE11*. Abbreviations: d, debris; ex, exochorion; f, floor of endochorion; m, muscle layer; p, pillars; r, roof of endochorion; arrow, break in roof. Magnification 12,500.

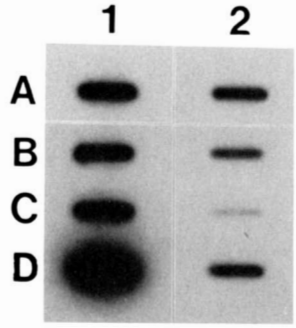


FIGURE 12.—*PA77* (allele of *fs(2)51Fa*) causes underamplification of the chorion genes. DNA was purified from ~50 stage 13 egg chambers isolated from strain $\pi 2$ females (A), *PA77* heterozygotes (B), *PA77* homozygotes (C), and from $\pi 2$ embryos (D). One half of each sample was transferred to nitrocellulose by slot blotting and hybridized with radiolabeled rDNA probe (column 1) and with a third chromosome chorion locus probe (column 2). Hybridization was visualized by autoradiography.

explain our data, since we screened for both lethals and female steriles and only one locus was found to have both types of allele. Differential sensitivity of loci to different mutagens may be part of the problem. *l(2)51Ea*, having eight γ-ray-induced alleles, is obviously sensitive to this form of mutagenesis. Although mutagenic screen conditions were chosen to avoid inducing premeiotic clusters (mutagenized males were only mated for 4 days), there is a slight possibility that the initial four alleles may represent a cluster of identical mutational events arising from a spontaneous premeiotic mutation. This seems unlikely because, except for *l7* and *l19*, all have different lethal phases.

Only one visible mutation was uncovered in this screen. Ta^{rts} is a recessive temperature-sensitive mutation which exhibits a thickened arista phenotype when hemizygous with certain deficiencies at 18°. Ta^{rts} is also female lethal when hemizygous with *Df(2R)XTED4*, but males of this genotype (Ta^{rts}/*Df(2R)XTED4*) do not display the antenna-to-leg transformation. Ta^{rts} enhances the effect of $T(2;3)Ta^{L}$, a translocation with a breakpoint which lies within the XTE deficiencies utilized in this screen. Both Ta^{L} and Ta^{rts} exhibit chromosomal rearrangements; Ta^{L}, a translocation between $51E_{1-2}$ and $84B_{1-2}$ (CAVENER, OTTESON and KAUFMAN 1986; KAUFMAN, LEWIS and WAKIMOTO 1980); and Ta^{rts}, placing heterochromatin adjacent to 57A. They do share a common breakpoint, and the breakpoint associated with Ta^{rts} is not within the region screened. The thickened arista phenotype could result in both cases from a position effect caused by moving heterochromatin adjacent to this region of the second chromosome. Alternatively the thickened arista phenotype associated with Ta^{rts} could be unrelated to the chromosomal rearrangement, but rather the result of an additional mutation within the deficiency region that is allelic to the breakpoint of Ta^{L}. Breakpoints in heterochromatin which induce position effect variegation of adjacent genes have been shown to be temperature sensitive, with the mutant

effects more severe at lower developmental temperatures (SPOFFORD 1976). The thickened arista phenotype of Ta^{rts} is only apparent at lower temperatures and thus is probably a position effect.

Most of the female-sterile mutations cluster at one end of the region under study. Some have been shown to affect chorion morphology, while others affect DNA replication/early nuclear division.

fs(2)51Fa allele *PA77* is a temperature-sensitive hypomorphic mutation, hemizygotes being more severely affected (lethal) than homozygotes (female-sterile). Hemizygous *PA77* individuals yield different lethal phases depending upon temperature. At 25° they are pupal lethal, exhibiting a range of phenotypes from no apparent development following pupariation to production of an aberrant pharate adult. Included in this range are individuals which resemble *l(2)crc* (*cryptocephal*) in that discs evert but fail to evaginate so that the head structures are found inside the thorax. In *crc* this is due to the integument's containing more glucosamine than normal, resulting in increased rigidity (FRISTROM 1965). However, *crc* is not allelic to *PA77* nor does it lie within *Df(2R)XTE18*. At 18° *PA77* hemizygotes exhibit asynchronous mitoses with regions of the blastoderm devoid of nuclei and necrotic ectoderm at germ band extension when they die. Eggs laid by homozygous *PA77* females are either unfertilized or fail to undergo pronuclear fusion. These display a chorion defect due to underamplification of the chorion genes. All of which suggests a defect in DNA replication/nuclear division.

Eggs produced by both homozygous and hemizygous *QL46* (allele at the *fs(2)52Fb* locus) females also have defective chorions, as do those laid by females hemizygous for alleles at the *fs(2)abc* locus (*PL63*, *fs27* and *fsA5*), but all of these have normal chorion gene amplification. Additionally, embryos produced by hemizygous *fs(2)abc* females exhibit abnormal chromatin clumping and nuclear bridges with development arresting in most embryos between the third and sixth nuclear division. Embryos produced by hemizygous *fs(2)sun* allele *fs8* females also show defective nuclear division, with division stopping around the forth or fifth cycle, while DNA replication continues (as judged by increased nuclear staining). Thus, among the four loci clustered at the distal end of *Df(2R)XTE11*, at least three affect some aspect of DNA replication/nuclear division (*fs(2)sun*, *fs(2)abc* and *fs(2)51Fa*) and at least three affect chorion structure (*fs(2)51Fa*, *fs(2)51Fb* and *fs(2)abc*), one resulting from improper DNA replication (*fs(2)51Fa*).

The region uncovered by *Df(2R)XTE11* appears to be divided into two segments, with the female-sterile mutations "clustering" in the distal end of the region. It may be that this is a region in which a number of genes affecting DNA replication/nuclear division are clustered, or it may be that we are dealing with a single complex locus having several hypomorphic alleles capable of complementing *inter se*. Only a detailed molecular analysis of this region will allow us to resolve this issue. Mapping within this female-sterile region (sections V and VI, Figure 4) are two genomic clones whose pattern of expression (highly abundant in ovaries and preblastoderm embryos, and absent or in very low amounts at other times in development) suggests that they encode a maternal gene product which functions prior to completion of the blastoderm. Embryonic development of eggs laid by both *fs(2)sun* and *fs(2)abc* females is arrested preblastoderm. Southern analysis has not allowed us to link these cloned sequences with any of the female-sterile mutations (data not shown), so we are in the process of "walking" in the region to help define exactly how this region is functioning to help produce a developmentally competent egg.

We wish to thank G. ISING for providing the *TE19* stock; and T. SCHÜPBACH for providing *Df(2R)XTE11*, *Df(2R)XTE18* and *Df(2R)XTE58*, and for allowing us to screen through her collection of second chromosome female-sterile mutations. We thank MICHAEL SHORT and JACKIE INSLEY for help with the mutagenesis. R. WOODRUFF, M. E. NEWPORT and T. SCHÜPBACH provided valuable suggestions on the manuscript. This work was supported by research grants from the National Science Foundation (DCB-8718307), and the OBOR Selective Excellence Programs for Research Challenge and Academic Challenge. J. T. was supported by a Helen Hay Whitney Memorial Foundation postdoctoral fellowship.

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