Identification of Regions Interacting With *ovo*^{D} Mutations: Potential New Genes Involved in Germline Sex Determination or Differentiation in *Drosophila melanogaster*

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ABSTRACT

Only a few *Drosophila melanogaster* germline sex determination genes are known, and there have been no systematic screens to identify new genes involved in this important biological process. The ovarian phenotypes produced by females mutant for dominant alleles of the *ovo* gene are modified in flies with altered doses of other loci involved in germline sex determination in Drosophila (*Sex-lethal*$^{+}$, *sans fille*$^{+}$ and *ovarian tumor*$^{+}$). This observation constitutes the basis for a screen to identify additional genes required for proper establishment of germline sexual identity. We tested 300 deletions, which together cover ~58% of the euchromatic portion of the genome, for genetic interactions with *ovo*^{D}. Hemizygosity for more than a dozen small regions show interactions that either partially suppress or enhance the ovarian phenotypes of females mutant for one or more of the three dominant *ovo* mutations. These regions probably contain genes whose products act in developmental hierarchies that include ovo^{+} protein.

An essential step in the production of gametes is the choice by germ cells between the male and the female fate. In *Drosophila melanogaster,* germline sex determination is regulated by cell-autonomous and nonautonomous factors (reviewed by PAULI and MAHOWALD 1990; STEINMANN-ZWICKY 1992; BURTIS 1993). The cell-autonomous level of control is dependent on the chromosomal constitution of the germ cell. In diploid flies the presence of a single *X* chromosome (1 *X*:2A, *X*/A ratio = 0.5) leads to male differentiation, whereas two *X* chromosomes (2 *X*:2A, *X*/A ratio = 1) leads to female development. Experiments in which germ cells of one chromosomal constitution were transplanted into organisms of the opposite sex (either in terms of chromosomes or somatic phenotype) revealed a second nonautonomous level of regulation; the sex of the soma influences the differentiation of the germ cells (SCHÜPBACH 1985; STEINMANN-ZWICKY *et al.* 1989; STEINMANN-ZWICKY 1994). Except during the larval stages, 1 *X*:2A germ cells are essentially insensitive to the sex of the surrounding soma, but their differentiation arrests early during spermatogenesis in a female soma. In contrast the sexual identity of 2 *X*:2A germ cells correlates with the sex of the surrounding soma. Similar conclusions can be reached with the analysis of various mutants. Germline autonomous mutations exist that cause the accumulation of spermatocytes in 2 *X*:2A females (OLIVER *et al.* 1988, 1993; BOPP *et al.* 1993; ALBRECHT and SALZ 1993; PAULI *et al.* 1993; WEI *et al.* 1994), and somatic line dependent sex determination mutations cause the accumulation of primary spermatocytes (and more advanced stages) in 2 *X*:2A flies fully or partially transformed into males (CLINE 1984; NÖTHIGER *et al.* 1989; OLIVER *et al.* 1993).

A few genes, *Sex-lethal*$^{+}$ (*Sxl*$^{+}$), *sans fille*$^{+}$ (*snf*$^{+}$), *female lethal (2)d*$^{+}$ (*fl(2)d*$^{+}$), *ovarian tumor*$^{+}$ (*otu*$^{+}$) and *ovo*$^{+}$, have been shown to be important for cell-autonomous germline sex determination (WIESCHAUS *et al.* 1981; SCHÜPBACH 1985; PERRIMON *et al.* 1986; OLIVER *et al.* 1988, 1990, 1993; STEINMANN-ZWICKY 1988; STEINMANN-ZWICKY *et al.* 1989; GRANADINO *et al.* 1992; BOPP *et al.* 1993; PAULI *et al.* 1993). Mutations in these genes produce two classes of phenotypes. The first group, represented by the *ovo* locus, which encodes a putative zinc finger polypeptide (MÉVEL-NINO *et al.* 1991; GARFINKEL *et al.* 1994), is characterized by the death of female germ cells homozygous for strong alleles of the locus (OLIVER *et al.* 1987, 1990, 1994). Reminiscent of somatic sex determination, this lethality might be the result of inappropriate dosage compensation (see LUCCHESI and MANNING 1987; PAULI and MAHOWALD 1990; OLIVER *et al.* 1987, 1993). Females mutant for genes of the second class of germline sex determination genes show an ovarian tumor pheno-

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type: ovarioles contain numerous undifferentiated germ cells resembling early spermatocytes, rather than egg chambers composed of an oocyte and 15 nurse cells. This class is examplified by the *snf* gene (also known as *fs(1)G1621* or *liz*) (OLIVER *et al.* 1988, 1990; STEINMANN-ZWICKY 1988; SALZ 1992), which encodes a U1A snRNP protein (FLICKINGER and SALZ 1994). The *otu* locus belongs to both classes depending on the allele considered: strong loss-of-function leads to the absence of female germ cells (STORTO and KING 1988), whereas partial loss-of-function allows the differentiation of 2 *X*:2A germ cells toward maleness (PAULI *et al.* 1993; BAE *et al.* 1994).

This study describes a systematic search for additional genes involved in germline sex determination. Genetic interactions between the dominant female sterile allele ovo^{D2} and mutations deficient for somatic activity of Sxl^{+} suggest a role of ovo^{+} in the reception or in the implementation of a signal from the soma (OLIVER *et al.* 1990; PAULI and MAHOWALD 1990). The ovo^{D} mutants also show dominant genetic interactions with either snf^{1621} (OLIVER *et al.* 1990) or otu^{-} mutations (PAULI *et al.* 1993), which result in enhanced mutant phenotypes. Thus, it is possible to identify both suppressors and enhancers of ovo^{D} ovarian phenotypes. Using the same scheme, we have analyzed the effect of hemizygosity of various euchromatic regions on the ovarian phenotypes of flies carrying ovo^{D}. We have studied cytologically visible deficiencies covering ~58% of the *D. melanogaster* genome.

MATERIALS AND METHODS

Deficiency stocks were mainly obtained from the Indiana Stock Center (Bloomington, IN) and the Mid-America Stock Center (Bowling Green, OH). Flies were grown under uncrowded conditions on standard Drosophila medium at 25° unless otherwise indicated. Six to eight 1–4-day-old virgin females were mated to five to six ovo^{D} males. The progeny were collected daily and aged for 7 days. Females were dissected in phosphate-buffered saline (PBS) and their ovaries were squashed and observed under a compound microscope. Refer to LINDSLEY and ZIMM (1992) and FLYBASE (1994) for description of mutations, chromosomes and cytology. See SPRADLING (1993) for a general description of ovarian differentiation and mutant phenotypes.

In otherwise wild-type backgrounds, most egg chambers of $ovo^{D2}/+$ heterozygous ovaries arrest around oogenic stage 6 and very few vitellogenic oocytes are found (BUSSON *et al.* 1983; OLIVER *et al.* 1990). To quantify the effect of a deficiency on the ovo^{D2} ovarian phenotype, the number of oocytes at vitellogenic stage 10 or older per ovary was scored. These numbers were organized in the following categories: no vitellogenic oocyte, 1 or 2 oocytes, 3–4, 5–7, 8–10, 11–15, 16–20 and so on. Usually, 50–70 ovaries were scored for each progeny class. The oocytes/ovary distributions were analyzed using the nonparametric Smirnov test (CONNOVER 1980). In this test the cumulative distribution of frequencies of eggs/ovary in females double heterozygous for ovo^{D2} and a deficiency (ovo^{D2}/Df or $ovo^{D2}/+;Df/+$) was compared to the cumulative distribution of frequencies in sibling females ($ovo^{D2}/+$, flies with the balancer chromosome). The maximal distance $T1$ between the two distributions was calculated. $T1$ varies from 0.0, when the distributions are identical, to 1.0, when the distributions do not overlap. The latter case occurs if the number of oocytes/ovary in any experimental fly is always smaller or larger than the number of eggs/ovary in any control female. The P significance levels are reached when $T1$ is larger than the product of 1.52 ($P < 0.05$) or 1.63 ($P < 0.01$) by the square root of $(n1 + n2)/(n1n2)$, where $n1$ and $n2$ are the number of ovaries scored for experimental and control flies respectively.

Given the sensitivity of ovo^{D2} to genetic background, statistically significant departures from randomness are not uncommon. We have therefore ranked the mean number of advanced egg chambers/ovary seen in individual experiments with the mean numbers of all the crosses involving the same chromosome. A mean number falling within the lower range (10th percentile) or upper range (90th percentile) is indicative of a particularly strong genetic interaction.

We have controlled for unwanted background effects by outcrossing. In several cases the genetic background of deficiency stocks was changed by outcrossing them for at least four generations with particular balancer stocks. As noted in the APPENDICES, these new backgrounds are indicated by a star after the balancer name. For instance, all the stocks with the balancer noted *FM6** have a similar background except for the deficiency chromosomes (average difference for the autosomes smaller than $^{1}/_{16}$).

RESULTS

We have shown that the ovarian phenotypes of $ovo^{D}/+$ females can be modified by mutations in three genes involved in sex determination (OLIVER *et al.* 1990; PAULI *et al.* 1993). Partial suppression, that is the production of more vitellogenic eggs, was observed in females heterozygous for both ovo^{D2} and any of several Sxl^{-} alleles. By using mutations defective in subsets of Sxl^{+} function, suppression was attributed to the reduced gene dosage of Sxl^{+} in the somatic cells, suggesting a possible role of ovo^{+} in the reception or implementation of somatic sex determining signal(s). An opposite interaction, described as enhancement of the ovarian phenotype, was found in the presence of the snf^{1621} mutation: in females heterozygous for both snf^{1621} and either ovo^{D2} or ovo^{D3}, no vitellogenic stages were observed. Furthermore, synergistic interaction was observed between ovo^{D1} and snf^{1621} leading to the production of ovarian tumors that contained cells resembling early spermatocytes. Mutations in *otu* show interactions with ovo^{D} that are similar to those shown by snf^{1621} (PAULI *et al.* 1993). These interactions suggest that the doses of ovo^{+}, otu^{+} and snf^{+} are important for female germline sexual identity.

The observations outlined above prompted us to search for other genes interacting with ovo^{D} mutations. We systematically tested the *D. melanogaster* genome using cytologically visible deletions. The ovo^{D2} allele was extensively used in these experiments due to its intermediate phenotype and its sensitivity to genetic background. Using 300 different deficiencies, we have ana-

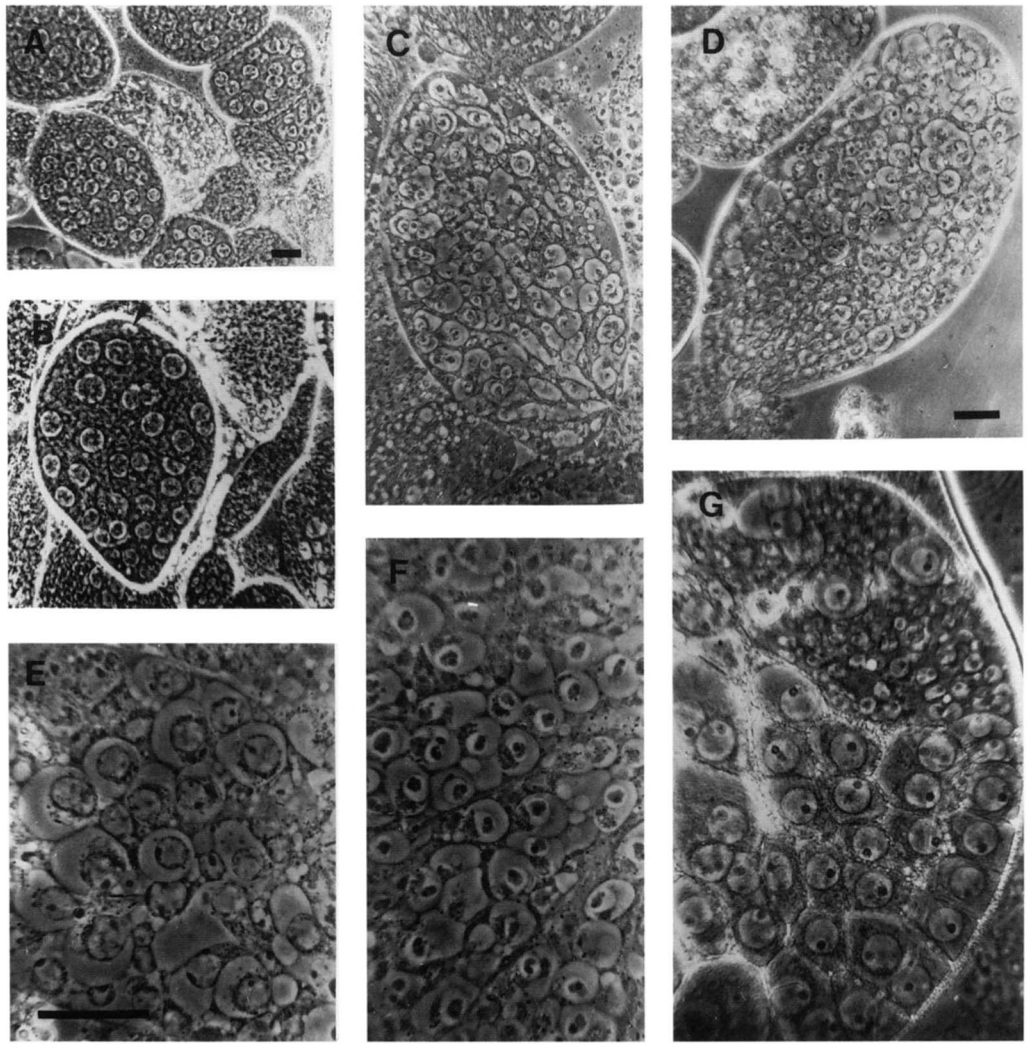

FIGURE 1.—Abnormal egg chambers found in ovo^{D2} heterozygotes. (A and B) Two examples of supernumerary nurse cells. The egg chamber shown in B contained 30 or 31 nurse cells and one oocyte (arrow head). Genotype: ovo^{+}/ovo^{D2}; $Df(3L)BK10/+$ (A) and $ovo^{+}Df(1)HA32/ovo^{D2}+$ (B). (C and D) Low magnification of two egg chambers that contain poorly differentiated germ cells. Genotype: ovo^{+}/ovo^{D2}; $Df(3L)st7/+$ (C) and (D) *FM7*, ovo^{+}/ovo^{D2}. (E and F) Higher magnification of squashed egg chambers similar to those shown in C and D. Note that the morphology of these germ cells is different from that of male germ cells shown in G. Genotype of E and F: ovo^{+}/ovo^{D2}; $Df(3L)st7/+$. (G) Testis (*FM7a*/Y). Same magnification as D. Bars, 20 μm.

lyzed ~58% of the euchromatic genome, divided as follows: 80% of the *X* chromosome (92 deficiencies), 55% of the second chromosome (111 deficiencies), 51% of the third chromosome (94 deficiencies) and 40% of the fourth chromosome (3 deficiencies). The Tables give the list of the deficiencies that were tested, their cytology and their interaction with ovo^{D2}.

The ovo^{D} phenotypes: Three dominant antimorphic alleles of *ovo* have been isolated (BUSSON *et al.* 1983). As heterozygotes, the strongest mutation, ovo^{D1}, reduces viability of female germ cells and arrests oogenesis around stage 4 (PERRIMON 1984; OLIVER *et al.* 1990), although more advanced previtellogenic stages can occasionally be observed. In $ovo^{D2}/+$ females oogenesis mainly stops at stage 6, although a few defective vitellogenic oocytes are produced. Two types of abnormal egg chambers were also observed (Figure 1). The first type, called pseudonurse cell chambers, consists of egg chambers containing more than 15 nurse cells. The number of extra nurse cells usually does not exceed 25, and the egg chambers contain zero to two oocyte nuclei. Occasional egg chambers with two oocytes and 30 nurse cells have been observed. The second type consists of egg chambers full of undifferentiated germ cells. In contrast to snf^{1621}, Sxl^{-} or otu^{-} ovarian tumors, which show clear male character, it is not possible to determine the sex of these $ovo^{D2}/+$ germ cells based on morphology (see OLIVER *et al.* 1988, 1990; PAULI *et al.* 1993; WEI *et al.* 1994). The frequency of these two types of abnormal egg chambers is usually <20% of the total number (50–150 per ovary) of egg chambers. The ability of $ovo^{D2}/+$ oocytes to proceed into vitellogenesis is

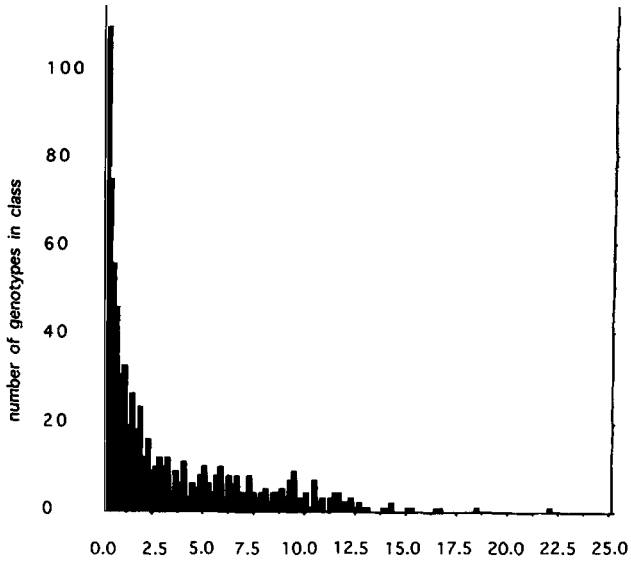

FIGURE 2.—The range of $ovo^{D2}/+$ phenotypes in 748 backgrounds. The mean numbers of advanced egg chambers per ovary for the different backgrounds (generally over 50 ovaries were used to generate each mean value) have been plotted against the number of occurrences. Bar width is 0.2 advanced chambers/ovary.

sensitive to genetic background (OLIVER *et al.* 1990; PAULI *et al.* 1993). We previously analyzed 15 different wild-type backgrounds and found that the number of vitellogenic oocytes per ovary varied between 0.2 and <4 (with a single exception at 8.4) (OLIVER *et al.* 1990). We have examined many more backgrounds in this paper (748 different classes of female progeny) and found that the distribution of mean numbers of vitellogenic eggs per ovary usually falls within the 0.2 to 4 range (Figure 2). The weakest dominant allele, $ovo^{D3}/+$, is the closest to wild-type, producing many eggs that look almost normal except for the permeability of their vitelline membrane and the frequent fusion of their dorsal appendages (OLIVER *et al.* 1990).

Data handling: Before discussing the effect of hemizygosity of some regions on the ovo^{D2} ovarian phenotype in more detail, some general comments are warranted. To quantify the effect of a deletion on the development of $ovo^{D2}/+$ ovaries, we counted the number of vitellogenic oocytes between stage 10 and maturity per ovary. The number of vitellogenic oocytes per ovary in females heterozygous for both ovo^{D2} and a given deficiency was compared to the number observed in sibling females heterozygous for ovo^{D2} and a balancer chromosome (*T*1 value, see MATERIALS AND METHODS). If a statistically significant difference was found, we utilized additional criteria to determine if the interaction was specific for the tested chromosome segment and to identify those regions showing the strongest interactions. First, we determined if the number of advanced egg chambers per ovary was different from other $ovo^{D2}/+$ females tested in this study. We used as external controls all the female progeny tested with a given chromosome (for example, we scored the rank of a given *X* chromosome among all tested *X* chromosomes and *X* balancers). The top and bottom 10th percentile ranks for each of the chromosomes are indicated in the APPENDICES. This criterion is useful to decide whether a significant *T*1 value is due to the balancer chromosome rather than the deficiency chromosome. We considered that a high *T*1 value was biologically significant only when the deficiency lay in the lower 10th or upper 90th percentile. This test also helps to identify regions that may have a maternal effect on the ovo^{D} phenotype (not significant *T*1, but both the deficiency and the balancer chromosome in the 10th or 90th percentile). Second, the balancer chromosome as well as the rest of the genetic background was changed by outcrossing some deficiencies with a given balancer stock for at least four generations, resulting in a >93% change in the background on nontested chromosomes. Deficiency-specific interactions are expected to be independent of the other chromosomes. Third, if several overlapping deletions showed similar interactions, this probably indicates that the interaction is real even if one of the deficiencies fell within the wild-type percentile range. The third criterion is certainly the best because it rules out the possibility that the observed interaction is due to an undetected mutation present on the deficiency chromosome but outside the deleted region.

Using the above criteria, we have selected a number of regions for full description. Detailed results for regions of interest are given in APPENDICES A–E. The full set of data can be obtained from D. PAULI and will be submitted to Drosophila Information Services. Selected deletions, especially those resulting in an enhanced phenotype, were also analyzed with ovo^{D3} (APPENDIX F).

X chromosome regions interacting with ovo^{D}: About 80% of the euchromatin of the *X* chromosome has been tested, using 92 deficiencies (Table 1, Figure 3). Two regions on the *X* chromosome (removing either ovo^{+} and snf^{+}, or Sxl^{+}) have been previously shown to interact (OLIVER *et al.* 1990) and will not be discussed here. Data for other regions of interest are presented in APPENDIX A.

Region 1F-2B: Four overlapping deletions near the tip of the *X* [*Df(1)sta, Df(1)S39, Df(1)A94* and *Df(1)RA19*] showed strong suppression of ovo^{D2}; they all allowed the production of large numbers of vitellogenic oocytes that were generally less flaccid and with better developed dorsal appendages than control $ovo^{D2}/+$ eggs. The suppression has been found in several different backgrounds. The effect of the gene dose of this region has a dramatic effect on the development of $ovo^{D2}/+$ ovaries. $ovo^{D2}/Su(ovo^{D})1F\text{-}2B^{-}$ females have between 8 and 22 advanced oogenic stages per ovary when any of the four deletions were used, and this value was consis-

TABLE 1

X chromosome deficiencies tested

No.	Name	Cytology	Interaction with ovo^{D2}	No.	Name	Cytology	Interaction with ovo^{D2}
1	*ac*	tip of *X*	N	48	*HC133*	9B9-10;9E-F	E
2	*260-1*	1A1;1B4-6	N	49	*sbr1*	9B9-10;9F13-A1	E
3	*y74k24*	1A1;1B5-6	N	50	*v-L11*	9C4;10A1-2	E
4	*su83*	1B10;1D6-E1	N	51	*v-M1*	9D3;10A1-2	E
5	*sta*	1D3-E1;2B3-4	S	52	*ras59*	9E1;9F10-11	E
6	*S39*	1E1-2;2B5-6	S	53	*ras203*	9E1-2;9F13	E
7	*A94*	1E3-4;2B9-10	S	54	*ras-P14*	9E1-2;9F3-4	N
8	*RA19*	1E3-4;2B9-10	S	55	*v-L3*	9F10;10A7-8	N
9	*dor2T*	2B6;2E1-2	N	56	*v-L2*	9F13;10A1	N
10	*Pgd35*	2C2-4;2E2-F1	N	57	*RA37*	10A6;10B15-17	N
11	*Pgd-kz*	2D3-4;2F5	N	58	*KA7*	10A9;10F6-7	N
12	*64c18*	2E1-2;3C2	N	59	*N71*	10B2-8;10D3-8	N
13	*2F1-3A4*	2F1;3A4	N	60	*HA85*	10C1-2;11A1-2	N
14	*X12*	2F5-3A1;3B5-C1	N	61	*m259-4*	10C2-3;10E1-2	N
15	*JC19*	2F6;3C5	N	62	*M-13*	10D;11A3-5	N
16	*HC194*	3A1;3C3-4	N	63	*KA6*	10E1;11A7-8	N
17	*N-8*	3C2-3;3E3-4	N	64	*RA47*	10F1;10F9-10	N
18	*N-71h*	3C4;3D5	N	65	*N105*	10F7;11D1	N
19	*N-69h9*	3C6;3D1 or D4	N	66	*KA10*	11A1;11A7-8	N
20	*biDL5*	3C7-12;4E1-2	N	67	*JA26*	11A1;11D-E	N
21	*dm75e19*	3C11;3E4	N	68	*HF368*	11A2;11B9	N
22	*GA102*	3D4-5;3F7-8	N	69	*wy26*	11B17-C1;11E9-10	E
23	*A113*	3D6-E1;4F5	E	70	*N12*	11D1-2;11F1-2	N
24	*rb33*	3F4;4C15	N	71	*C246*	11D-E;12A1-2	S
25	*rb1*	3F6-4A1;4C7-8	N	72	*g-l*	11F10;12F1	N
26	*rb46*	4A3-6;4C6-7	N	73	*KA9*	12E2-3;12F5-13A1	N
27	*RC40*	4B1;4F1	E	74	*RK3*	12E2-6;13A6-11	N
28	*biD2*	4B6-C1;4D7-E1	N	75	*RK5*	12E9-11;13A9-B1	N
29	*GA56*	4C5-6;4D1	N	76	*RK4*	12F5-6;13A9-B1	N
30	*rb13*	4C5-6;4D3-E1	N	77	*sd72b*	13F1;14B1	N
31	*C149*	5A8-9;5C5-6	N	78	*l9*	13F;14E-F	E
32	*N73*	5C2;5D5-6	N	79	*r-D1*	14B6;15A2 or 14C2-4;15B2	N
33	*JF5*	5E3-5;5E8	N	80	*B*	15F9;16A7	N
34	*G4e[L]H24i[R]*	5E3-8;6B	N	81	*N19*	17A1;18A2	N
35	*Sxl-bt*	6E2;7A6	S	82	*E160.2*	17B2-C1;18A	N
36	*HA32*	6E4-5;7A6	N	83	*E128*	17C;18A	N
37	*ct-J4*	7A2-3;7C1	N	84	*JA27*	18A5;20A	N
38	*ct 268-42*	7A5-6;7B8-C1	N	85	*HF396*	18E1-2;20	N
39	*ct 4b1*	7B2-4;7C3-4	N	86	*mal3*	19A1-2;20E-F	N
40	*C128*	7D1;7D5-6	N	87	*16-3-22*	19D1;20A2	N
41	*HA11*	7D13-14;7D22	N	88	*B57*	19E1-2;19F1	N
42	*RA2*	7D10;8A4-5	E	89	*GA37*	19E2;19F6	N
43	*KA14*	7F1-2;8C6	E	90	*JA21*	19E5-6;20	N
44	*C52*	8E;9C-D	N	91	*DCB1-35b*	19F1-2;20E-F	N
45	*ras217*	9A;9E7-8	E	92	*JC4*	20A1;20E-F	N
46	*v-L15*	9B1-2;10A1-2	N				
47	*N110*	9B3-4;9D1-2	N				

N, no interaction; S, suppression; E, enhancement.

tent when the backgrounds were changed by outcrossing. For example, using three different stocks, ovo^{D2}/*Df(1)A94* females had 22.1, 12.8 and 8.5 advanced chambers per ovary, whereas the ovo^{D2}/*Balancer* internal-control females had respectively 5.8, 1.9 and 2.0 advanced chambers per ovary. The value of 22.1 advanced egg chambers per ovary for ovo^{D2}/*Df(1)A94* females is the highest found in this study of 300 deficiencies. There was no evidence for a maternal effect, because the *T*1 values (between 0.192 and 0.8) were highly significant (with one exception). Thus, there was generally little overlap between the oocyte/ovary distributions of experimental and of control female progeny of these crosses. The region of overlap between

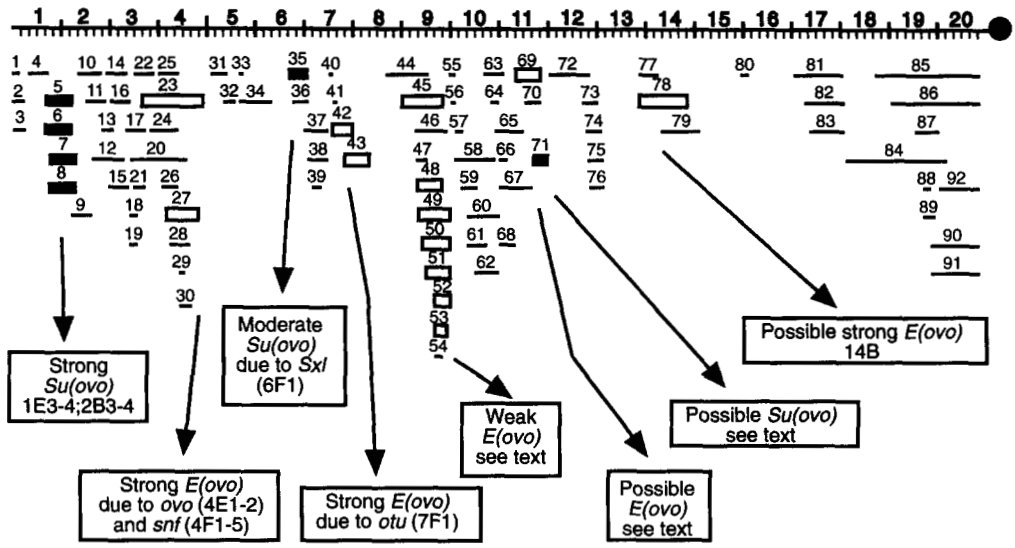

FIGURE 3.—Summary of genetic interactions between ovo^D and X-chromosome deficiencies. The numbered divisions of the polytene chromosomes are represented above a bold line representing the chromosome. Letter divisions are represented by tics below the chromosome line. Segments of the chromosome removed by deficiencies used in this study are represented by lines or boxes. Thin lines are used when the given deficiency chromosome did not show an interaction with ovo^D. ■, the deficiency chromosome suppressed the ovo^D/+ phenotype. □, the deficiency chromosome enhanced the ovo^D/+ phenotype. Text boxes direct attention to regions that are discussed in RESULTS. See Table 1 for the correspondance between the numbers above the lines or boxes and the names of the deficiencies and their cytology.

Df(1)sta, Df(1)S39, Df(1)A94 and *Df(1)RA19* is 1E3-4 to 2B3-4, suggesting that the $Su(ovo^D)1F\text{-}2B^+$ locus maps within this interval.

Region 7F-8A: Two overlapping deficiencies [*Df(1)RA2* and *Df(1)KA14*] showed strong enhancement of ovo^D. Females heterozygous for one of these two deficiencies and ovo^{D2} or ovo^{D3} had no or very few vitellogenic oocytes. The number of previtellogenic egg chambers was also somewhat reduced compared to control siblings. These two deletions also interacted with ovo^{D1}, producing some germ cells that look like spermatocytes. A similar interaction has been described between ovo^{D1} and snf^{1621}, although the ovo^{D1}/snf^{1621} interaction produced a more penetrant phenotype (OLIVER *et al.* 1990). These experiments prompted a more careful study of this region demonstrating that the interactions with 7F-8A deletions are due to reduced otu^+ dose (PAULI *et al.* 1993).

Region 9E-F: The majority of deletions overlapping at 9E-F enhanced the ovo^{D2}/+ mutant phenotype. For example, females of genotypes $ovo^{D2}/Df(1)ras217$, $ovo^{D2}/Df(1)sbr1$, $ovo^{D2}/Df(1)ras59$ or $ovo^{D2}/Df(1)ras203$ averaged less than one advanced egg chamber per ovary and fell within the 10th percentile for X chromosomes tested, whereas the internal control females had many more. In six out of eight crosses using these deletions, *T*1 values were >0.5 and as high as 0.935 (indicating that phenotypic overlap was only 6.5%). Several other deletions enhanced the ovo^{D2}/+ mutant phenotype as shown by high *T*1 values. Even though the internal control references indicated that the experimental females were quite different from the sibling females, some experimental females were within the normal wild-type range seen in ovo^{D2}/+ females. There were some cases of statistical nonsignificance (in progeny of crosses of ovo^{D2}/Y males to either *Df(1)ras-P14/FM7a* or *Df(1)ras203/FM7a* females). Given the number of overlapping deficiencies showing an interaction, we suggest that an enhancer is localized in this region, but the effect of the dose of this enhancer on ovo^{D2}/+ females is mild compared to the effect of the dose of snf^+ or otu^+.

Region 11: Two chromosomes deleting segments in this region showed opposite effects on the ovo^{D2}/+ phenotype. Females of genotype $ovo^{D2}/Df(1)wy26$ averaged only 0.1 advanced egg chambers per ovary. This number is in the 10th percentile for the X chromosomes, and there was almost no overlap between these ovaries and those of the internal control $ovo^{D2}/FM7$ females (*T*1 = 0.94).

The ovo^{D2}/Df and the ovo^{D2}/+ progeny from *Df(1)C246/FM6* females averaged 12.7 and 15.3 vitellogenic egg chambers per ovary, respectively. Both mean numbers are in the 90th percentile for X chromosomes, but the value of *T*1 is not significant, raising the prospect of a maternal effect. Thus, there may also be a suppressor of ovo^D in segment 11 of the X chromosome.

Interestingly, reduced dose of the 11D-F region results in synergistic mutant phenotypes with a number of somatic sex determination genes (BELOTE *et al.* 1985). Given that the regulation of pre-mRNA splicing as a mechanism of control is used in both germline and

TABLE 2

Deficiencies of the left arm of chromosome *2*

No.	Name	Cytology	Interaction with ovo^{D2}	No.	Name	Cytology	Interaction with ovo^{D2}
93	*TE75w+*	tip;21B4-6	N	125	*64j*	34D1-2;35B9-C1	N
94	*al*	21B8-C1;21C8-D1	N	126	*TE35A-5*	34D2;35C1	N
95	*S2*	21C6-D1;22A6-B1	N	127	*b75*	34D4-6;35E5-6	S
96	*ast1*	21C7-8;23A1-2	N	128	*el80f1*	34E3;35D7	N
97	*ast-2*	21D1-2;22B2-3	N	129	*75C*	35A1-2;35D4-7	N
98	*S3*	21D2-3;21F2-22A1	N	130	*C75RL*	35A2;35B3	N
99	*dpp59*	22A;23A(?)	N	131	*W*	35A2-3;35B3-5	N
100	*DTD2*	22D4-5;22E2-4	N	132	*do1*	35B1-2;35D1-2	N
101	*edSZ*	24A3-4;24D3-4	N	133	*A446*	35B1-3;35E6-F2	N
102	*ed-dp-h1*	24C1,2-3;25A1-4	N	134	*osp29*	35B2-3;35E6	N
103	*dp-h28*	24D8;24F6-7	N	135	*H20*	36A8-9;36E1-2	N
104	*dp-h25*	24E2-4;25B2-5	N	136	*TW137*	36C2-4;37B9-C1	N
105	*M-zB*	24E2-F1;24F6-7	N	137	*TW50*	36E4-F1;38A6-7	N
106	*dp-h19*	24F1-2;24F6-7	N	138	*E71*	36F2-6;37C6-D1	E
107	*dp-h24*	24F4;25A1-4	N	139	*TW158*	37B2-8;37E2-F4	N
108	*tkvSz-2*	25D2-4;25D6-E1	S	140	*pr-A16*	37B2-12;38D2-5	N
109	*cl-h3*	25D2-4;25F1-2	N	141	*TW130*	37B9-C1;37D1-2	E
110	*cl-h2*	25D6;25E4-5	N	142	*VA16*	37B9-C1;37F5-38A1	E
111	*cl1*	25D7-E1;25E6-F3	E	143	*VA12*	37C2-5;38B2-C1	N
112	*cl7*	25D7-E1;26A7-8	N	144	*Sd77*	37D1-2;38C1-2	S
113	*GpdhA*	25D7-E1;26A8-9	N	145	*pr76*	37D;38E	S
114	*2802*	25F2-3;25F4-26A1	S	146	*E55*	37D2-E1;37F5-38A1	S
115	*spdX4*	27E;28C	N	147	*TW2*	37D2-E1;38E6-9	N
116	*wgCX3*	28A?-?	N	148	*TW9*	37E2-F4;38A6-C1	S
117	*30A;C*	30A;30C	N	149	*TW150*	37F5-38A1;38B2-C1	S
118	*J-der 2*	31B;32A1-2	N	150	*TW84*	37F5-38A1;39D3-E1	S
119	*J-der 27*	31D;31F3	N	151	*TW65*	37F5-38A1;39E2-F1	N
120	*Prl*	32F1-3;33F1-2	N	152	*TW161*	38A6-B1;40A4-B1	S
121	*escP3-0*	33A1-2;33B1-2	N	153	*TW1*	38A7-B1;39C2-3	N
122	*escP2-0*	33A1-2;33E	N	154	*DS6*	38F5;39E7-F1	N
123	*esc10*	33A8-B1;33B2-3	N	155	*PR31*	2L heterochromatin	N
124	*prd1.7*	33B3-7;34A1-2	N				

somatic sex determination (BOPP *et al.* 1993; OLIVER *et al.* 1993); regions that interact in sensitive screens in both hierarchies are not unexpected.

Region 14: One deficiency in this region showed a very strong interaction with ovo^{D} mutations. Females of genotype $ovo^{D2}/Df(1)l9$ produced no vitellogenic oocytes, and we noted some reduction in the total number of egg chambers. Interactions as strong as this are very rare, but in the absence of overlapping deletions producing the same interaction, the localization of an enhancer in this region is unsure. Complementation tests have been used to show that the *Df(1)l9* chromosome did not fortuitously carry snf^{-} or otu^{-} mutations.

We have tested *Df(1)l9* with the other dominant alleles of *ovo*. Females of genotype $ovo^{D3}/Df(1)l9$ produced almost no vitellogenic oocytes (the average was 0.01), which is well outside the phenotypic range of $ovo^{D3}/+$ or even $ovo^{D2}/+$. The internal $ovo^{D3}/+$ controls averaged 8.8 advanced egg chambers per ovary. This result is statistically highly significant ($T1$ = 0.891). These data provide strong evidence that the *Df(1)l9* chromosome bears an $E(ovo^{D})$. Unlike in the cases of snf^{-} or otu^{-} (OLIVER *et al.* 1990; PAULI *et al.* 1993), *Df(1)l9* had no dominant effect on the ovo^{D1} phenotype. Overlapping but noninteracting deficiencies limit the putative enhancing region to 14B.

Other regions on the X chromosome: While testing lethal mutations in the otu^{+} region (data not shown), we found a *FM7* chromosome producing no vitellogenic oocytes with either ovo^{D2} or ovo^{D3}. Obviously, this putative strong enhancer cannot be easily mapped. Because this *FM7* balancer fully complements snf^{1621}, otu^{-} and *Df(1)l9*, it suggests the existence of a fourth strong *X*-linked enhancer of ovo^{D} (probably localized in the 20% of the *X* chromosome for which no deletions are presently available).

Regions on the left arm of the second chromosome interacting with ovo^{D}: Fifty-seven percent of 2L (63 deletions) has been analyzed (Table 2, Figure 4).

Region 37C-38A: Nineteen deficiencies uncovering re-

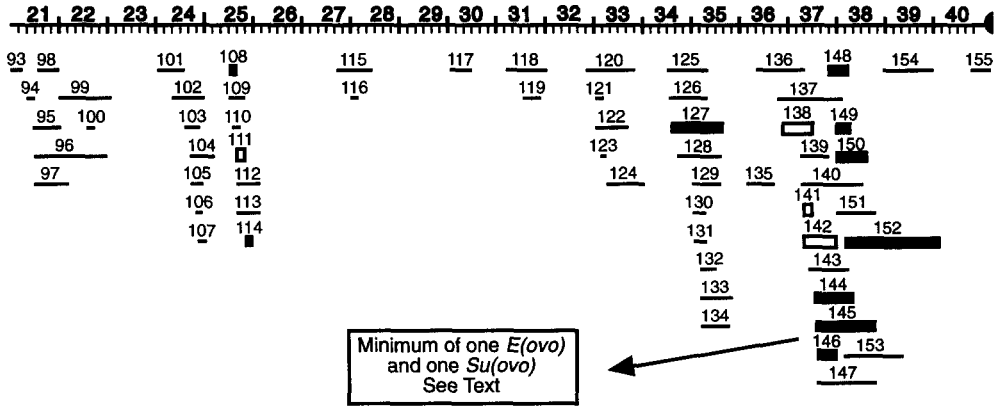

FIGURE 4.—Summary of genetic interactions between ovo^D and left arm second-chromosome deficiencies. Same format as Figure 3. See Table 2 for the list of deficiencies.

gions 36C2-4 to 40A4-B1 have been tested for their effect on the $ovo^{D2}/+$ female phenotype (APPENDIX B). Three of these deficiencies behaved as enhancers of ovo^{D2} [*Df(2L)E71*, *Df(2L)TW130* and *Df(2L)VA16*], seven as suppressors [*Df(2L)Sd77*, *Df(2L)pr76*, *Df(2L)E55*, *Df(2L)TW9*, *Df(2L)TW150*, *Df(2L)TW84* and *Df(2L)-TW161*], whereas the others showed no (or weak) interaction. Our interpretation of these observations requires the presence of at least one enhancer and one suppressor. The localization of the enhancer would be 37C and the suppressor would be at the border between 37F and 38A. Some of the deletions (*Df(2L)TW50*, *Df(2L)TW158* and *Df(2L)pr-A16*) that showed no interaction would uncover both interacting loci. Besides these two regions of interaction, we cannot rule out the existence of additional weak enhancers and suppressors more proximally.

The enhanced phenotype seen in $ovo^{D2}/+$; *Df(2L)-E71*/+, $ovo^{D2}/+$; *Df(2L)TW130*/+ or $ovo^{D2}/+$; *Df-(2L)VA16*/+ is moderate as the averaged number of advanced egg chambers per ovary varied between 0.0 and 0.4. The complete absence of advanced egg chambers seen in two cases would normally be interpreted as strong enhancement, but the *T*1 values in both cases where $ovo^{D2}/+$; *Df(2L)TW130*/+ females had no advanced egg chambers were only 0.198 and 0.089. Although not statistically significant in terms of either T1 or rank, the $ovo^{D2}/+$ progeny of heterozygous *Df(2L)TW50* mothers showed consistently fewer than expected vitellogenic egg chambers. The reduction in the number of advanced egg chambers in both classes of female progeny consistently seen with these deficiencies suggests a maternal effect. However, there is also one result indicating that the effect is zygotic (T1 = 0.619 in a cross using the first *Df(2L)TW130/CyO* stock), making any conclusions about maternal *vs.* zygotic action tentative.

The suppressed phenotype seen in $ovo^{D2}/+$; *Df(2L)-Sd77*/+, $ovo^{D2}/+$; *Df(2L)pr76*/+, $ovo^{D2}/+$; *Df(2L)-E55*/+, $ovo^{D2}/+$; *Df(2L)TW9*/+, $ovo^{D2}/+$; *Df(2L)-TW150*/+, $ovo^{D2}/+$; *Df(2L)TW84*/+ and $ovo^{D2}/+$; *Df(2L)TW161*/+ females was strong when compared to either internal controls or the normal phenotypic range of $ovo^{D2}/+$. The experimental females averaged between seven and nine advanced egg chambers per ovary. These values were in the 90th percentile for second chromosomes. The values of *T*1 were frequently quite impressive; four crosses involving *Df(2L)pr76*, *Df(2L)TW9*, *Df(2L)TW150*, or *Df(2L)TW84* resulted in *T*1 values >0.9. The distribution of the number of vitellogenic egg chambers found in $ovo^{D2}/+$; *Df(2L)-*

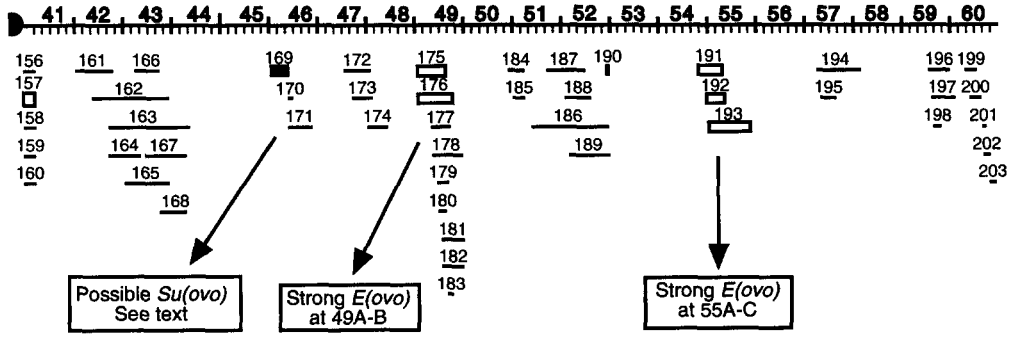

FIGURE 5.—Summary of genetic interactions between ovo^D and right arm second-chromosome deficiencies. Same format as Figure 3. See Table 3 for the list of deficiencies.

TABLE 3

Deficiencies of the right arm of chromosome *2*

No.	Name	Cytology	Interaction with ovo^{D2}
156	*M-S2-4*	41A	N
157	*M-S2-8*	41A	E
158	*M-S2-10*	41A	N
159	*rl10a*	41A	N
160	*rl10b*	41A	N
161	*cn88b*	42A;42E	N
162	*pk78s*	42C1-7;43F5-8	N
163	*cn9*	42E;44C	N
164	*pk78k*	42E3;43C3	N
165	*P32*	43A3;43F6	N
166	*ST1*	43B3-5;43E1-8	N
167	*cn83c*	43C5-D1;44B6-C1	N
168	*CA53*	43E6;44B6	N
169	*B5*	46A;46C	S
170	*eve1.27*	46C3-4;46C9-11	N
171	*X1*	46C;46E-F	N
172	*en-A*	47D3;48A5-6	N
173	*en-B*	47E3-6;48A4-B2	N
174	*en30*	48A3-4;48C6-8	N
175	*vg135*	49A;49D-E	E
176	*vgC*	49A4-13;49E7-F1	E
177	*vgD*	49C1-2;49E2-6	N
178	*vg104*	49C4;49F13	N
179	*vg107*	<49Da-49Ea	N
180	*vg133*	<49Da-49Dc	N
181	*vg33*	49D;50A	N
182	*vgB*	49D3-4;49F15-50A3	N
183	*vg136*	vg-49Ea	N
184	*L-R+48*	50F-51A1;51B	N
185	*trix*	51A1-2;51B6	N
186	*JP1*	51C3;52F5-9	N
187	*XTE18*	51E3;52C9-D1	N
188	*WMG*	52A;52D	N
189	*JP5*	52A13-B3;52F10-11	N
190	*JP8*	52F5-9;52F10-53A1	S
191	*Pcl7B*	54E8-F1;55B9-C1	E
192	*Pcl11B*	54F6-55A1;55C1-3	E
193	*Pc4*	55A;55F	E
194	*PuD17*	57B5;58B1-2	N
195	*Pl3*	57B20;57D8-9	N
196	*bwD23*	59D4-5;60A1-2	N
197	*bwS46*	59D8-11;60A7	N
198	*bw5*	59D10-E1;59E4-F1	N
199	*Px*	60B8-10;60D1-2	N
200	*Px2*	60C5-6;60D9-10	N
201	*D11-MP*	60E1-2;60E5-6	N
202	*M-c33a*	60E2-3;60E11-12	N
203	*Kr10*	60E10-11;60F5	N

pr76/+ ovaries showed only 2% overlap with the control sibling females. The interactions remained significant when deletion stocks with different genetic backgrounds where constructed and tested. The consistency of the suppression clearly suggests the existence of a *Su(ovo^D)* at 37F-38A.

Regions on the right arm of the second chromosome interacting with ovo^D: Fifty-two percent of 2R (48 deletions) has been analyzed. The results are summarized in Table 3 and in Figure 5. Detailed data for the three interacting regions are presented in APPENDIX C.

Region 46: A single deficiency (*Df(2R)B5*) removing the 46AC segment acted as suppressor of the ovo^{D2}/+ phenotype. The double heterozygotes had nearly 10 advanced egg chambers per ovary, placing these flies in the 90th percentile rank, and the value for *Tl* was very high (0.886). Given the absence of overlapping deficiencies, we cannot rule out the possibility that the location of the responsible suppressor is elsewhere on the chromosome.

Region 49A-B: Nine deficiencies of region 49 have been analyzed. Two of them, *Df(2R)vg135* and *Df(2R)vgC,* acted as very strong enhancers of ovo^{D2}, whereas the others did not interact. These results indicate the presence of an enhancer at 49A-B. Females of genotypes ovo^{D2}/+; *Df(2R)vg135*/+ or ovo^{D2}/+; *Df(2R)vgC*/+ never produced advanced egg chambers, whereas the sibling control females showed a typical ovo^{D2}/+ phenotype. This interaction results in a more enhanced phenotype than is seen in ovo^{D2}/otu^- females and rivals that seen in ovo^{D2}/snf^{1621} females. The *Df(2R)vg135* deletion also acted as a very strong enhancer of ovo^{D3}. Females of genotypes ovo^{D3}/+; *Df(2R)vg135*/+ (from either of two stocks) did not have advanced egg chambers, whereas the control siblings produced on average 5.2 and 14.7 well differentiated oocytes. The *Tl* values were very high (0.93 and 1.0), clearly indicating that the enhancement seen in this experiment is highly significant. We have initiated the molecular characterization of a female sterile locus with a phenotype similar to otu^- (G. PENNETTA and D. PAULI, unpublished results).

Region 55A-C: Another strong enhancer of ovo^D has been revealed by three overlapping deletions showing similar strong enhancement of ovo^{D2} and ovo^{D3}. Females of genotypes ovo^{D2}/+; *Df(2R)Pcl7B*/+ or ovo^{D2}/+; *Df(2R)Pcl11B*/+ showed complete arrest of differentiation before vitellogenic stages and even a reduced number of early egg chambers in a fraction of the ovaries. A third deletion *Df(2R)Pc4* resulted in similar enhancement of the ovo^{D2}/+ phenotype, but occasional advanced egg chambers were observed (0.02 oocytes per ovary in one cross and none in another). The overlap in the above deleted regions localizes an enhancer in 55A-C. The *Tl* values were moderate to high (0.202–0.897), suggesting that a maternal effect of the $E(ovo^D)55A\text{-}C^+$ gene dose is unlikely. Work aimed at identifying this enhancer has been initiated.

Regions on the left arm of the third chromosome interacting with ovo^D: Fifty-seven percent of 3L (44 deletions) has been analyzed. The results are summarized in Table 4 and in Figure 6. Detailed data concern-

TABLE 4

Deficiencies of the left arm of chromosome *3*

No.	Name	Cytology	Interaction with ovo^{D2}
204	*emcE12*	61A;61D3-4	E
205	*Ar12-1*	61C;61F3	E
206	*Ar14*	61C3-4;62A	E
207	*RG5*	62A10-13;63C3-5	N
208	*RG7*	62B2-8;62F2-5	N
209	*R*	62B7;62B12	N
210	*GN19*	63E6-9;64B2-4	N
211	*X37*	63E6-9;64B14-17	N
212	*ems13*	64B2-4;64E	N
213	*V65c*	64E;65C-D	N
214	*h-i22*	66D10-11;66E1-2	N
215	*29A6*	66F5;67B1	S
216	*AC1*	67A;67D	N
217	*vin2*	67F2-3;68D6	E
218	*vin5*	68A2-3;69A1-3	N
219	*vin4*	68B1-3;68F3-6	S
220	*vin6*	68C8-11;69A4-5	S
221	*vin7*	68C8-11;69B4-5	N
222	*BK9*	68E;69A1	N
223	*fzGF3b*	70B?;70D6	N
224	*fzGS1a*	70C6-15;70E4-6	N
225	*fzM21*	70D2-3;71E4-5	N
226	*fzD21*	70D;71F	N
227	*st-f13*	71B1-2;73A3-4	N
228	*BK10*	71C;71F	N
229	*th102*	72B1;72D12	N
230	*st8P*	72E4;73B4	N
231	*st4*	72E5-F1;73B5-7	N
232	*st7*	72F3-4;74C3-4	N
233	*81K19*	73A3;74F	N
234	*W10*	75B3-6;75C1-2	N
235	*w[+R4]*	75B8-11;75C5-7	S
236	*Cat*	75C1-2;75F1	N
237	*VW3*	76A3;76B2	N
238	*in61*	76F;77D	N
239	*rdgC*	77A1;77D1	N
240	*ri79C*	77B-C;77F-78A	N
241	*Pc-MK*	78A3;79E1-2	S
242	*Pc*	78D1-2;79A4-C1	S
243	*Pc23937-30A*	78D	S
244	*Pc-Cp1*	78D3-6;78E-F	S
245	*Pc-T7*	78E1-2;79E4	N
246	*1-16*	80Fa-g	N
247	*10-26*	80FfG + 81Fa	N

ing the regions discussed below are presented in APPENDIX D.

Region 61C-D: Three deficiencies [*Df(3L)emcE12*, *Df(3L)Ar12-1* and *Df(3L)Ar14*] showed similar interactions with ovo^{D2}. Females of the genotypes $ovo^{D2}/+$; *Df(3L)emcE12*/+, $ovo^{D2}/+$; *Df(3L)Ar12-1*/+ or $ovo^{D2}/+$; *Df(3L)Ar14*/+ had either no advanced egg chambers or very few (0.03 per ovary), suggesting that they delete a moderate to strong enhancer of ovo^{D}. The effect of two of these deletions (*Df(3L)emcE12* and *Df(3L)Ar14*) was not significantly different from that of the balancers in our quantitative test of vitellogenic stages ($T1 = 0.01$ and 0.02, respectively), and the third was significant only at the P 0.05 level ($T1 = 0.209$). However, the very small number of vitellogenic oocytes observed and the strong reduction in the number of early egg chambers observed (10–25 per ovary compared to the controls with 50–150) are good evidence for the localization of an enhancer between 61C3-4 and 61D3-4. The low $T1$ values are indications that *E(ovo^{D})61C-D* has a maternal effect, an idea supported by the experiments using ovo^{D3}.

Females of genotype $ovo^{D3}/+$; *Df(3L)emcE12*/+ or $ovo^{D3}/+$; *Df(3L)Ar14*/+ averaged only 0.3 and 0.1 advanced egg chambers per ovary, respectively. This represents a dramatic enhancement of the $ovo^{D3}/+$ phenotype. As in the case of ovo^{D2} experiments, there is a suggestion of a maternal effect. The internal control females for the *Df(3L)emcE12* cross showed considerable overlap with the experimental class ($T1 = 0.016$), and the control females for *Df(3L)Ar14* were also in the lower range of ovo^{D3} crosses.

This region contains a gene known to be involved in somatic sex determination. The emc^{+} gene plays a maternal role in the activation of Sxl^{+} transcription in the soma (YOUNGER-SHEPARD *et al.* 1992). The possible role of emc^{+} in germline sex determination would be worth investigating. It should be noted that neither *da* (CRONMILLER and CLINE 1987) nor *sis-b* (GRANADINO *et al.* 1993; STEINMANN-ZWICKY 1993) are required in the germline. The emc protein would therefore have to interact with other bHLH transcription factors.

Region 67: A possible enhancer of ovo^{D2} has been tentatively placed in region 67F-68A based on interactions with *Df(3L)vin2*. The finding that $ovo^{D3}/+$; *Df(3L)vin2*/+ females had fewer eggs than controls indicates that the enhancer localized on the *Df(3L)vin2* chromosome also interacts with ovo^{D3}. Overlapping deletions in this region would be necessary to confirm the existence of this enhancer.

Region 78D: Five deficiencies removing portions of the 78–79 region were analyzed. Four of them [*Df(3L)Pc-MK*, *Df(3L)Pc*, *Df(3L)Pc23937-30A* and *Df(3L)Pc-Cp1*] improved the $ovo^{D2}/+$ mutant phenotype, allowing the differentiation of many oocytes that were usually not as flaccid as control eggs and without fusion of their dorsal appendages. The fifth deletion, *Df(3L)Pc-T7*, showed no interaction. The suppressor therefore maps to 78D and is quite strong. Females of genotypes $ovo^{D2}/+$; *Df(3L)Pc-MK*/+, $ovo^{D2}/+$; *Df(3L)Pc*/+, $ovo^{D2}/+$; *Df(3L)Pc23937-30A*/+ or $ovo^{D2}/+$; *Df(3L)Pc-Cp1*/+ averaged at least 10 advanced egg chambers per ovary. Of all the crosses reported in this manuscript, only $ovo^{D2}/Su(ovo^{D})1F\text{-}2B^{-}$ females (Figure 3) have shown higher average numbers of eggs. Additionally, the overlap between experimental females

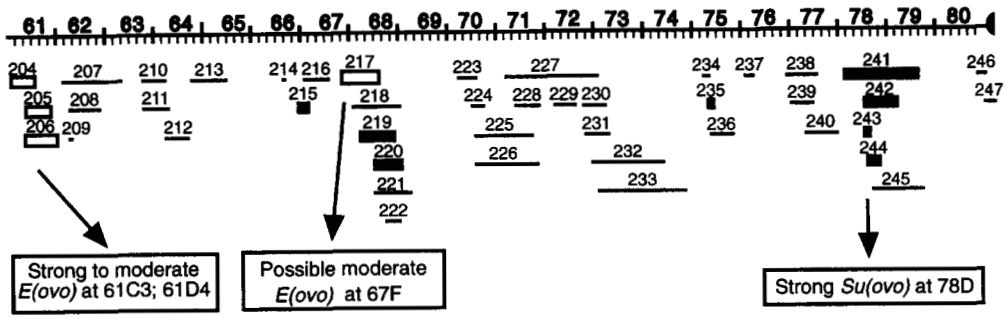

FIGURE 6.—Summary of genetic interactions between ovo^D and left arm third-chromosome deficiencies. Same format as Figure 3. See Table 4 for the list of deficiencies.

and control siblings was minimal or absent. In experiments using *Df(3L)Pc23937-30A/TM3* or *Df(3L)Pc-Cp1/TM3* female parents, *T*1 = 1.0. The complete lack of phenotypic overlap, the strong degree of suppression and the consistent nature of the interaction using chromosomes deleting overlapping regions unambiguously localize a strong suppressor of ovo^D at 78D. A number of *Pc* alleles were found to interact with ovo^{D2}, suggesting that the suppression is due to a reduced dose of Pc^+ (B. OLIVER, unpublished results).

Regions on the right arm of the third chromosome and the fourth chromosome interacting with ovo^D: Forty-five percent of 3R (50 deletions) and 40% of the fourth chromosome (3 deletions) have been analyzed. These results are summarized in Table 5, Figure 7 and APPENDIX E for the regions discussed below.

Region 82F: Two deletions [*Df(3R)6-7* and *Df(3R)-3-4*] suggest the presence of a suppressor between 82F1-2 and 82F3-6. The number of advanced egg chambers per ovary (5.7 and 11.7, respectively) fell in the 90th percentile for third chromosomes, and the values of *T*1 were very high (0.775 and 1.000, respectively), suggesting that this zygotic suppression is highly significant.

Region 85D: Two deficiencies in region 85 [*Df(3R)-by10* and *Df(3R)by416*] strongly enhanced ovo^{D2}, whereas two others showed no interaction [*Df(3R)-by62*and *Df(3R)GB104*]. This would localize an enhancer at 85D10-11. Females of genotypes $ovo^{D2}/+$; *Df(3R)by10/+* or $ovo^{D2}/+$; *Df(3R)by416/+* averaged no or very few advanced egg chambers per ovary (0.0 and 0.01, respectively), which constitutes a strong enhancement of the $ovo^{D2}/+$ phenotype. Additionally, these females showed reduced numbers of early egg chambers. The numbers of vitellogenic egg chambers per ovary in the internal controls were also reduced yielding low values for *T*1. We believe the observed enhancement to be significant, based on further experiments using ovo^{D3}.

Females of genotypes $ovo^{D3}/+$; *Df(3R)by10/+* or $ovo^{D3}/+$; *Df(3R)by416/+* averaged only 0.01 or 0.03 advanced egg chambers per ovary, respectively. This is a very strong enhancement for the $ovo^{D3}/+$ phenotype. The egg chamber distributions for $ovo^{D3}/+$; *Df(3R)-by10/+* and $ovo^{D3}/+$; *Df(3R)by416/+* were significantly different from the internal controls, although the average numbers of vitellogenic egg chambers per ovary among the controls (1.2 and 2.9, respectively) were also in the lower range for ovo^{D3}. We therefore do not rule out a maternal effect.

Region 88-90: There may be one or more enhancers and suppressors of ovo^{D2} in this region, but because of the small number of deletions available, it was not possible to unambiguously map them. Specific chromosomes showing interactions are described below.

Df(3R)su(Hw)7 (88A9;88B2) strongly interacted with ovo^{D2}, leading to the complete absence of vitellogenic stages. However, other deletions uncovering this deficiency showed no clear interaction at all. The value for *T*1 in this cross was also low. The *Df(3R)su(Hw)7* chromosome also enhanced the ovo^{D3} phenotype.

The *Df(3R)red1* (88B1;88D3-4) chromosome also resulted in very few advanced egg chambers in $ovo^{D2}/+$ females (0.04), but again the value of T1 was low (0.086). We do not have overlapping deletions for the 88C2-3;88D2-3 interval to confirm the existence of the enhancer detected with *Df(3R)red1*.

None of the female progeny from a cross of *Df(3R)sbd105/T(2;3)apXa* females to ovo^{D2}/Y males showed advanced egg chambers. The complete absence of advanced egg chambers in both classes of female progeny did not occur in any other experiments reported in this paper or in any of our previous experiments (OLIVER *et al.* 1990; PAULI *et al.* 1993). Both the deficiency chromosome (*Df(3R)sbd105*) and the balancer (*T(2;3)apXa*) also behaved as strong enhancers of ovo^{D3}. This effect was most probably not due to the *T(2;3)apXa* chromosome, which has been used in other experiments. Unfortunately, there is no overlapping set of deficiencies to confirm the identification of an enhancer of ovo^D in the 88F9-89A1;89B5 interval. Given the strength of the interaction as well as the possible maternal effect, testing additional deficiency chromosomes deleting this region is a high priority.

TABLE 5

Deficiencies of the right arm of chromosome *3* or chromosome *4*

No.	Name	Cytology	Interaction with ovo^{D2}
248	*Z*	82A;82E3-4	N
249	*110*	82C;82F	N
250	*6-7*	82D3-8;82F3-6	S
251	*3-4*	82F1-2;82F10-11	S
252	*Tp110,Dp(3;3) Dfd[rvX1]*	83C1-2;84B1 and 83D3-4;84A4-5	N
253	*Tp16*	83D1-2;84A4-5	N
254	*Win11*	83E1-2;84A4-5	N
255	*Dfd13*	83E3;84A4-5	N
256	*9A99*	83F2-84A1;84B1-2	S
257	*Scr*	84A1-2;84B1-2	N
258	*MAP11*	84A1-2;84B1-2	N
259	*MAP2*	84A1-2;84A3	N
260	*pbX2*	84A4-5;84B1-2	N
261	*Antp3*	84A4-5;84C2-3	N
262	*Hu*	84A6-B1;84D4-5	N
263	*Antp17*	84B1-2;84D11-12 or 84A6;84D14	N
264	*A41*	84B1-2;84D1-2	N
265	*D6*	84D2-3;84F13-16	N
266	*dsx28*	84D13-E1;85A4-5	N
267	*dsx5*	84E1-2;84F11-12	N
268	*p40*	84E8-9;85B6	N
269	*by10*	85D8-12;85E7-F1	E
270	*by416*	85D10-12;85E1-3	E
271	*by62*	85D11-14;85F6	N
272	*GB104*	85D12;85E10	N
273	*M-Kx1*	86C1;87B1-5	N
274	*cu40*	86C1-2;86D8	N
275	*TE32*	86E2-4;87C6-7	N
276	*TE10*	86F1-2;87C5-7	N
277	*kar1W*	87A6-7;87D12-13	N
278	*ry615*	87B11-13;87E8-11	N
279	*ry27*	87D1-2;87F1-2	N
280	*red3l*	87F12-14;88C1-3	N
281	*su(Hw)7*	88A9;88B2	E
282	*red-P93*	88A10-B1;88C2-3	N
283	*red1*	88B1;88D3-4	E
284	*sbd105*	88F9-89A1;89B9-10	E
285	*sbd104*	89B5;89C	N
286	*bxd100*	89B5-6;89E2-3	N
287	*P10*	89C1-2;89E1-2	N
288	*P2*	89D9-E1;89E2-3	N
289	*C4*	89E;90A	S
290	*P14*	90C2-D1;91A1-2	S
291	*ChaM7*	91A;91F5	N
292	*DlBX12*	91F1-2;92D2-6	N
293	*e-N19*	93B;94	N
294	*eBS2*	93C3;93F	N
295	*Tl-P*	97A;98A1-2	N
296	*Tl-X*	97B;97D1-2	N
297	*tllG*	99F1-2;100B4-5	N
298	*Df(4)M62f*	101E;102B10-17	N
299	*Df(4)M63a*	101F2-102A1;102A2-5	N
300	*Df(4)G*	102E2; tip	N

Other regions: The *Df(3R)C4* chromosome (89E;90A) and the *Df(3R)P14* chromosomes (90C2-D1;90A1-2) showed significant suppression of $ovo^{D2}/+$, in terms of both *Tl* and percentile rank. We have not tested overlapping deleted segments in this two regions. Two *TM3* balancer chromosomes (from stocks *Df(3R)dsx5* and *Df(3R)kar1W*) strongly enhanced ovo^{D2}. We do not know where the putative interacting loci are localized.

DISCUSSION

The establishment of the sexual identify of the germ cells in *D. melanogaster* appears to be a complex process that involves inductive signal(s) from surrounding somatic cells (NÖTHIGER *et al.* 1989; STEINMANN-ZWICKY *et al.* 1989; OLIVER *et al.* 1993; STEINMANN-ZWICKY 1994) as well as intrinsic factors that are dependent on the number of *X* chromosomes compared to the number of autosomes (SCHÜPBACH 1985; STEINMANN-ZWICKY *et al.* 1989; OLIVER *et al.* 1994). Nothing is known concerning the nature of the somatic signals. Only five loci have been shown to be involved in the germ cells for determination of their sexual identity: Sxl^+, snf^+, $fl(2)d^+$, otu^+ and ovo^+ (OLIVER *et al.* 1988, 1990, 1993; STEINMANN-ZWICKY 1988; STEINMANN-ZWICKY *et al.* 1989; WEI *et al.* 1991, 1994; GRANADINO *et al.* 1992; BOPP *et al.* 1993; PAULI *et al.* 1993). None of these genes have been demonstrated to act as receptors of the somatic signals or as counting elements of the *X*:A ratio, although the level of expression of the *ovo*::*lacZ* reporter gene depends on the number of *X* chromosomes in the germ cells (OLIVER *et al.* 1994). Obviously, the understanding of germline sex determination requires the identification of other key loci.

One problem in this task is the difficulty of linking specific phenotypes to defects in sex determination. For instance, many female sterile mutations are broadly described as ovarian tumors on the ground of apparent overproliferation of cystocytes. These abnormal egg chambers contain numerous small poorly differentiated germ cells. In some cases the resemblance between these abnormal germ cells and the wild-type spermatogonia or young spermatocytes of males have been supported by molecular studies that showed the expression of male-specific genes or reporters in these germ cells (BOPP *et al.* 1993; OLIVER *et al.* 1993; PAULI *et al.* 1993; BAE *et al.* 1994; WEI *et al.* 1994). This observation as well as various experiments using genetic interactions provide strong evidence that the ovarian tumors produced by Sxl^-, snf^- and otu^- genes are due to transformation of the female germ cells toward maleness and that the corresponding wild-type alleles are essential in the female germline for the establishment of its sexual identity (OLIVER *et al.* 1988, 1990, 1993; STEINMANN-ZWICKY 1988; PAULI *et al.* 1993). Given that we find interactions between these genes and ovo^D, it is possible

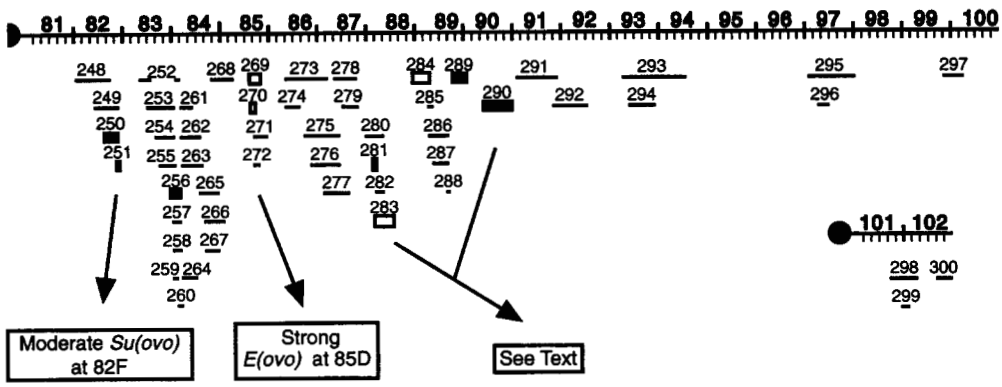

FIGURE 7.—Summary of genetic interactions between ovo^D and right arm third-chromosome and fourth-chromosome deficiencies. Same format as Figure 3. See Table 5 for the list of deficiencies.

that interactions between sex determination genes may be a more powerful criterion than the homozygous phenotype for what is or is not a germline sex determination gene. Additionally, it is very likely that several elements of the signaling pathway required for germline sex determination are also used in cellular communication during other steps of Drosophila development. Mutations in many of the genes involved in the production, reception and implementation of the somatic signals might therefore lead to nonsex-specific lethality or other phenotypes that cannot easily be linked to sex determination of the germline.

The broad screen we report here does not rely on any assumptions about the homozygous mutant phenotype and is therefore of great help in the identification of new genes necessary for establishment of germ cell sexual identity and female differentiation. Using 300 deletions, we have tested the effect of hemizygosity of some 58% of the *D. melanogaster* genome on the $ovo^{D2}/+$ ovarian phenotype. We have identified at least four regions that strongly suppress the $ovo^{D2}/+$ phenotype (in intervals 1–2, 37, 78 and 82) and six regions that strongly enhance both the $ovo^{D2}/+$ and $ovo^{D3}/+$ phenotypes (in intervals 7–8, 37, 49, 55, 61 and 85). The existence of at least three other strong enhancers could also be inferred, but they could not be localized because the interacting chromosomes were balancers. In addition, several weaker modifying regions have also been identified. Altogether we suggest that there are at least 10 $E(ovo^D)$ and 8–10 $Su(ovo^D)$ loci in the *D. melanogaster* genome. We anticipate that this rather large number of modifiers of ovo^D includes genes required in sex determination, oogenesis and for other vital processes. Identification and characterization of these 20 or so genes would make a valuable contribution to our understanding of germline sex determination and female germline differentiation in Drosophila.

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LITERATURE CITED

ALBRECHT, E. B., and H. K. SALZ, 1993 The Drosophila sex determination gene *snf* is utilized to establish the female-specific splicing of *Sex-lethal*. Genetics **134:** 801–807.

BAE, E., K. R. COOK, P. K. GEYER and R. N. NAGOSHI, 1994 Molecular characterization of ovarian tumors in Drosophila. Mech. Dev. **47:** 151–164.

BELOTE, J. M., M. B. MCKEOWN, D. J. ANDREW, T. N. SCOTT, M. F. WOLFNER *et al.*, 1985 Control of sex differentiation in *Drosophila melanogaster*. Cold Spring Harbor Symp. Quant. Biol. **52:** 605–614.

BOPP, D., J. I. HORABIN, R. A. LERSCH, T. W. CLINE and P. SCHEDL, 1993 Expression of the *Sex-lethal* gene is controlled at multiple levels during *Drosophila* oogenesis. Development **118:** 797–812.

BURTIS, K. C., 1993 The regulation of sex determination and sexually dimorphic differentiation in *Drosophila*. Curr. Opin. Cell Biol. **5:** 1006–1014.

BUSSON, B., M. GANS, K. KOMITOPOULOU and M. MASSON, 1983 Genetic analysis of three dominant female sterile mutations located on the *X* chromosome of *Drosophila melanogaster*. Genetics **105:** 309–325.

CLINE, T. W., 1984 Autoregulatory functioning of a Drosophila gene product that establishes and maintains the sexually determined state. Genetics **107:** 231–277.

CONNOVER, W. J., 1980 *Practical Non-parametric Statistics*. John Wiley & Sons, New York.

CRONMILLER, C., and T. W. CLINE, 1987 The *Drosophila* sex determination gene *daughterless* has different functions in the germ line versus the soma. Cell **48:** 479–487.

FLICKINGER, T. W., and H. K. SALZ, 1994 The *Drosophila* sex determination gene *snf* encodes a nuclear protein with sequence and functional similarity to the mammalian U1A snRNP protein. Genes Dev. **8:** 914–925.

FLYBASE, 1994 The Drosophila genetic database. *ftp.bio.indiana.edu*.

GARFINKEL, M. D., J. WANG, Y. LIANG and A. P. MAHOWALD, 1994 Multiple products from the *shavenbaby-ovo* gene region of *Drosophila melanogaster*—relationship to genetic complexity. Mol. Cell. Biol. **14:** 6809–6818.

GRANADINO, B., A. SÁN JUAN, P. SANTAMARIA and L. SÁNCHEZ, 1992 Evidence of a dual function in *fl(2)d* a gene needed for *Sex-lethal* expression in *Drosophila melanogaster*. Genetics **130:** 597–612.

GRANADINO, B., P. SANTAMARIA and L. SÁNCHEZ, 1993 Sex determi-

nation in the germ line of *Drosophila melanogaster*. Activation of the gene *Sex-lethal*. Development **118:** 813–816.

LINDSLEY, D. L., and G. ZIMM, 1992 *The genome of Drosophila melanogaster*. Academic Press, San Diego.

LUCCHESI, J. C., and J. E. MANNING, 1987 Gene dosage compensation in *Drosophila melanogaster*. Adv. Genet. **24:** 371–429.

MÉVEL-NINIO, M., R. TERRACOL and F. C. KAFATOS, 1991 The *ovo* gene of Drosophila encodes a zinc finger protein required for female germ line development. EMBO J. **10:** 2259–2266.

NÖTHIGER, R., M. JONGLEZ, M. LEUTHOLD, P. MEIER-GERSCHWILLER and T. WEBER, 1989 Sex determination in the germ line of Drosophila depends on genetic signals and inductive somatic factors. Development **107:** 505–518.

OLIVER, B., N. PERRIMON and A. P. MAHOWALD, 1987 The *ovo* locus is required for sex-specific germ line maintenance in Drosophila. Genes Dev. **1:** 913–923.

OLIVER, B., N. PERRIMON and A. P. MAHOWALD, 1988 Genetic evidence that the *sans fille* locus is involved in Drosophila sex determination. Genetics **120:** 159–171.

OLIVER, B., D. PAULI and A. P. MAHOWALD, 1990 Genetic evidence that the *ovo* locus is involved in Drosophila germ-line sex determination. Genetics **125:** 535–550. (Corrigendum **126:** 477).

OLIVER, B., Y.-J. KIM and B. S. BAKER, 1993 *Sex-lethal*, master and slave: A hierarchy of germ-line sex determination in Drosophila. Development **119:** 897–908.

OLIVER, B., J. SINGER, V. LAGET, G. PENNETTA and D. PAULI, 1994 Function of Drosophila ovo^+ in germ-line sex determination depends on *X* chromosome number. Development **120:** 3185–3195.

PAULI, D., and A. P. MAHOWALD, 1990 Germ line sex determination in Drosophila. Trends Genet. **6:** 259–264.

PAULI, D., B. OLIVER and A. P. MAHOWALD, 1993 The role of the *ovarian tumor* locus in *Drosophila melanogaster* germline sex determination. Development **119:** 123–134.

PERRIMON, N. 1984 Clonal analysis of dominant female-sterile germline-dependent mutations in *Drosophila melanogaster*. Genetics **108:** 927–939.

PERRIMON, N., J. D. MOHLER, L. ENGSTROM and A. P. MAHOWALD, 1986 X-linked female sterile loci in *Drosophila melanogaster*. Genetics **113:** 695–712.

SALZ, H. K., 1992 The genetic analysis of *snf*: a *Drosophila* sex determination gene required for activation of *Sex-lethal* in both the germline and the soma. Genetics **130:** 547–554.

SCHÜPBACH, T. 1985 Normal female germ cell differentiation requires the female *X* chromosome to autosome ratio and expression of *Sex-lethal* in *Drosophila melanogaster*. Genetics **109:** 529–548.

SPRADLING, A. C., 1993 Developmental genetics of oogenesis, pp. 1–70 in *The Development of Drosophila*, edited by M. BATE and A. M. ARIAS. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

STEINMANN-ZWICKY, M., 1988 Sex determination in *Drosophila:* the *X* chromosomal gene *liz* is required for *Sxl* activity. EMBO J. **7:** 3889–3898.

STEINMANN-ZWICKY, M., 1992 How do germ cells choose their sex? *Drosophila* as a paradigm. BioEssay **14:** 513–518.

STEINMANN-ZWICKY, M., 1993 Sex determination in *Drosophila: sis-b*, a major numerator element of the X:A ratio in the soma, does not contribute to the X:A ratio in the germ line. Development **117:** 763–767.

STEINMANN-ZWICKY, M., 1994 Sex determination of the *Drosophila* germ line: *tra* and *dsx* control somatic inductive signals. Development **120:** 707–716.

STEINMANN-ZWICKY, M., H. SCHMID and R. NÖTHIGER, 1989 Cell-autonomous and inductive signals can determine the sex of the germline in Drosophila by regulating the gene *Sex-lethal*. Cell **57:** 157–166.

STORTO, P. D., and R. C. KING, 1988 Multiplicity of functions for the *otu* gene products during *Drosophila* oogenesis. Dev. Genet. **9:** 91–120.

WEI, G., B. OLIVER and A. P. MAHOWALD, 1991 Gonadal dysgenesis reveals sexual dimorphism in the embryonic germline of Drosophila. Genetics **129:** 203–210.

WEI, G., B. OLIVER, D. PAULI and A. P. MAHOWALD, 1994 Evidence for sex transformation of germline cells in ovarian tumor mutants of Drosophila. Dev. Biol. **161:** 318–320.

WIESCHAUS, E., C. AUDIT and M. MASSON, 1981 A clonal analysis of the roles of somatic cells and germ line during oogenesis in Drosophila. Dev. Biol. **88:** 92–103.

YOUNGER-SHEPARD, S., H. VAESSIN, E. BIER, L. Y. JAN and Y. N. JAN, 1992 *deadpan*, an essential pan-neural gene encoding an HLH protein, acts as a denominator in Drosophila sex determination. Cell **70:** 911–922.

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APPENDIX A

ovo^{D2} heterozygotes with reduced doses of regions on chromosome *1*

No.	Deficiency/balancer[a]	Cytology	Progeny	Oocytes/ovary[b]		Statistics[c]		Percentile[d]
				Mean	No.	*T*1	*P*	
5	*sta/FM6*	1D3-E1;2B3-4	*ovoD2/Df*	9.0	89	0.192	NS	wtr
			ovoD2/Bal	6.7	86			wtr
	*sta/FM7a**		*ovoD2/Df*	12.4	83	0.511	<0.01	90%
			ovoD2/Bal	6.2	65			wtr
6	*S39/FM6*	1E1-2;2B5-6	*ovoD2/Df*	18.6	40	0.704	<0.01	90%
			ovoD2/Bal	5.8	48			wtr
	*S39/FM7a**		*ovoD2/Df*	11.3	80	0.800	<0.01	90%
			ovoD2/Bal	1.9	80			wtr
	*S39/FM7c**		*ovoD2/Df*	7.3	70	0.608	<0.01	wtr
			ovoD2/Bal	2.0	65			wtr
7	*A94/FM6*	1E3-4;2B9-10	*ovoD2/Df*	22.1	43	0.629	<0.01	90%
			ovoD2/Bal	9.4	66			wtr
	*A94/FM7a**		*ovoD2/Df*	12.8	68	0.690	<0.01	90%
			ovoD2/Bal	4.4	72			wtr
	*A94/FM7c**		*ovoD2/Df*	8.5	61	0.529	<0.01	wtr
			ovoD2/Bal	2.3	59			wtr
8	*RA19/FM6*	1E3-4;2B9-10	*ovoD2/Df*	8.1	48	0.333	<0.01	wtr
			ovoD2/Bal	4.8	64			wtr
	*RA19/FM7a**		*ovoD2/Df*	11.3	84	0.661	<0.01	90%
			ovoD2/Bal	3.1	82			wtr
42	*RA2/FM7c*	7D10;8A4-5	*ovoD2/Df*	0.0	112	0.393	<0.01	10%
			ovoD2/Bal	0.9	107			wtr
43	*KA14/FM7c*	7F1-2;8C6	*ovoD2/Df*	0.03	89	0.945	<0.01	10%
			ovoD2/Bal	9.9	91			wtr
	*KA14/FM6**		*ovoD2/Df*	0.0	60	0.900	<0.01	10%
			ovoD2/Bal	7.7	50			wtr
44	*C52/FM6*	8E;9C-D	*ovoD2/Df*	9.6	30	0.199	NS	wtr
			ovoD2/Bal	12.3	64			90%
45	*ras217/FM6*	9A;9E7-8	*ovoD2/Df*	0.2	69	0.855	<0.01	10%
			ovoD2/Bal	10.1	69			wtr
	*ras217/FM7a**		*ovoD2/Df*	0.2	88	0.501	<0.01	10%
			ovoD2/Bal	3.5	78			wtr
46	*v-L15/FM6*	9B1-2;10A1-2	*ovoD2/Df*	4.9	102	0.385	<0.01	wtr
			ovoD2/Bal	9.8	119			wtr
47	*N110/FM6*	9B3-4;9D1-2	*ovoD2/Df*	7.5	62	0.210	NS	wtr
			ovoD2/Bal	9.6	62			wtr
48	*HC133/FM7c*	9B9-10;9E-F	*ovoD2/Df*	3.8	79	0.590	<0.01	wtr
			ovoD2/Bal	9.4	72			wtr
49	*sbr1/FM6*	9B9-10;9F13-A1	*ovoD2/Df*	0.4	60	0.935	<0.01	10%
			ovoD2/Bal	13.9	62			90%
	*sbr1/FM7a**		*ovoD2/Df*	0.3	87	0.374	<0.01	10%
			ovoD2/Bal	1.9	84			wtr
50	*v-L11/? B*	9C4;10A1-2	*ovoD2/Df*	1.0	44	0.724	<0.01	wtr
			ovoD2/Bal	6.9	43			wtr
51	*v-M1/FM6*	9D3;10A1-2	*ovoD2/Df*	0.5	54	0.711	<0.01	wtr
			ovoD2/Bal	6.7	59			wtr
52	*ras59/FM6*	9E1;9F10-11	*ovoD2/Df*	0.0	66	0.780	<0.01	10%
			ovoD2/Bal	3.6	50			wtr
	*ras59/FM7a**		*ovoD2/Df*	0.9	66	0.301	<0.01	wtr
			ovoD2/Bal	2.6	64			wtr
53	*ras203/FM6*	9E1-2;9F13	*ovoD2/Df*	0.3	61	0.899	<0.01	10%
			ovoD2/Bal	8.9	59			wtr
	*ras203/FM7a**		*ovoD2/Df*	0.5	82	0.147	NS	wtr
			ovoD2/Bal	0.8	69			wtr
54	*ras-P14/FM6*	9E1-2;9F3-4	*ovoD2/Df*	5.5	62	0.541	<0.01	wtr
			ovoD2/Bal	11.7	60			90%
	*ras-P14/FM7a**		*ovoD2/Df*	1.0	64	0.117	NS	wtr
			ovoD2/Bal	0.9	71			wtr

APPENDIX A

Continued

No.	Deficiency/balancer[a]	Cytology	Progeny	Oocytes/ovary[b] Mean	Oocytes/ovary[b] No.	Statistics[c] T1	Statistics[c] P	Percentile[d]
55	*v-L3/FM7a**	9F10;10A7-8	*ovoD2/Df*	5.3	205	0.099	NS	wtr
			ovoD2/Bal	4.4	156			wtr
56	*v-L2/FM7a**	9F13;10A1	*ovoD2/Df*	5.2	62	0.129	NS	wtr
			ovoD2/Bal	5.6	62			wtr
65	*N105/FM6*	10F7;11D1	*ovoD2/Df*	2.0	131	0.732	<0.01	wtr
			ovoD2/Bal	9.8	129			wtr
66	*KA10/FM7c*	11A1;11A7-8	*ovoD2/Df*	5.8	69	0.270	<0.05	wtr
			ovoD2/Bal	7.9	68			wtr
67	*JA26/FM7c*	11A1;11D-E	*ovoD2/Df*	3.0	50	0.329	<0.01	wtr
			ovoD2/Bal	0.9	55			wtr
68	*HF368/FM7c*	11A2;11B9	*ovoD2/Df*	8.3	79	0.519	<0.01	wtr
			ovoD2/Bal	4.1	82			wtr
	*HF368/FM7a**		*ovoD2/Df*	6.9	68	0.580	<0.01	wtr
			ovoD2/Bal	2.3	66			wtr
69	*wy26/FM7*	11B17-C1; 11E9-10	*ovoD2/Df*	0.1	84	0.940	<0.01	10%
			ovoD2/Bal	8.6	84			wtr
70	*N12/FM6*	11D1-2;11F1-2	*ovoD2/Df*	6.7	77	0.115	NS	wtr
			ovoD2/Bal	7.2	70			wtr
71	*C246/FM6*	11D-E;12A1-2	*ovoD2/Df*	12.7	40	0.223	NS	90%
			ovoD2/Bal	15.3	93			90%
77	*sd72b/FM7c*	13F1;14B1	*ovoD2/Df*	2.7	48	0.097	NS	wtr
			ovoD2/Bal	2.6	53			wtr
78	*l9/FM7a**	13F;14E-F	*ovoD2/Df*	0.0	100	0.371	<0.01	10%
			ovoD2/Bal	0.9	70			wtr
79	*r-D1/FM7a**	14B6;15A2 or 14C2-4;15B2	*ovoD2/Df*	6.0	70	0.095	NS	wtr
			ovoD2/Bal	5.1	88			wtr

[a] Females of the indicated genotypes were crossed to $ovo^{D2}v^{24}/Y$ males. Asterisk (or double asterisk) after a given balancer chromosome indicates stocks with similar backgrounds obtained by outcrosses for at least four generations.

[b] Number of ovaries scored and mean number of egg chambers/ovary at stage 10 or more mature.

[c] *T1*, maximal distance between the cumulative distribution of egg chambers/ovary for the deficiency females *vs.* the balancer females; *P*, level of significance; NS, not significant.

[d] Rank of the particular *X*-chromosome compared to all the *X* chromosomes tested. Wild-type range (wtr) for the *X* chromosome is between 0.4 and 11.3 oocytes/ovary.

APPENDIX B

ovo^{D2} heterozygotes with reduced doses of regions on the left arm of chromosome 2

No.	Deficiency/balancer	Cytology	Progeny	Oocytes/ovary		Statistics		Percentile
				Mean	No.	*Tl*	*P*	
136	*TW137/Cy*	36C2-4;37B9-C1	*ovoD2/+;Df/+*	3.8	86	0.286	<0.01	wtr
			ovoD2/+;Bal/+	5.2	96			wtr
137	*TW50/Cy*	36E4-F1;38A6-7	*ovoD2/+;Df/+*	0.6	47	0.354	<0.01	wtr
			ovoD2/+;Bal/+	0.1	60			10%
	*TW50/CyO***		*ovoD2/+;Df/+*	0.5	64	0.228	NS	wtr
			ovoD2/+;Bal/+	0.1	95			10%
	*TW50/Gla**		*ovoD2/+;Df/+*	0.7	88	0.076	NS	wtr
			ovoD2/+;Bal/+	1.1	73			wtr
138	*E71/CyO*	36F2-6;37C6-D1	*ovoD2/+;Df/+*	0.1	102	0.559	<0.01	10%
			ovoD2/+;Bal/+	1.8	130			wtr
139	*TW158/CyO*	37B2-8;37E2-F4	*ovoD2/+;Df/+*	0.4	64	0.248	NS	wtr
			ovoD2/+;Bal/+	1.5	69			wtr
140	*pr-A16/CyO*	37B2-12;38D2-5	*ovoD2/+;Df/+*	2.3	66	0.079	NS	wtr
			ovoD2/+;Bal/+	1.6	68			wtr
141	*TW130/CyO*	37B9-C1;37D1-2	*ovoD2/+;Df/+*	0.4	60	0.619	<0.01	wtr
			ovoD2/+;Bal/+	3.3	61			wtr
	*TW130/CyO**		*ovoD2/+;Df/+*	0.0	88	0.198	NS	10%
			ovoD2/+;Bal/+	0.4	86			wtr
	*TW130/Gla**		*ovoD2/+;Df/+*	0.0	120	0.089	NS	10%
			ovoD2/+;Bal/+	0.2	101			wtr
142	*VA16/CyO*	37B9-C1;37F5-38A1	*ovoD2/+;Df/+*	0.1	80	0.313	<0.01	10%
			ovoD2/+;Bal/+	0.7	80			wtr
143	*VA12/CyO*	37C2-5;38B2-C1	*ovoD2/+;Df/+*	1.7	60	0.333	<0.01	wtr
			ovoD2/+;Bal/+	0.3	60			wtr
144	*Sd77/CyO*	37D1-2;38C1-2	*ovoD2/+;Df/+*	7.0	60	0.698	<0.01	90%
			ovoD2/+;Bal/+	0.6	58			wtr
145	*pr76/CyO*	37D;38E	*ovoD2/+;Df/+*	12.1	60	0.983	<0.01	90%
			ovoD2/+;Bal/+	0.4	60			wtr
146	*E55/Cy*	37D2-E1;37F5-38A1	*ovoD2/+;Df/+*	12.0	67	0.821	<0.01	90%
			ovoD2/+;Bal/+	2.7	73			wtr
	*E55/In(2LR)Cy**		*ovoD2/+;Df/+*	9.6	60	0.402	<0.01	90%
			ovoD2/+;Bal/+	5.6	52			wtr
	*E55/CyO***		*ovoD2/+;Df/+*	2.9	60	0.650	<0.01	wtr
			ovoD2/+;Bal/+	0.1	60			wtr
147	*TW2/Cy*	37D2-E1;38E6-9	*ovoD2/+;Df/+*	0.1	61	0.005	NS	10%
			ovoD2/+;Bal/+	0.1	80			wtr
148	*TW9/CyO*	37E2-F4;38A6-C1	*ovoD2/+;Df/+*	9.5	62	0.907	<0.01	90%
			ovoD2/+;Bal/+	1.0	71			wtr
	*TW9/CyO**		*ovoD2/+;Df/+*	8.9	36	0.849	<0.01	90%
			ovoD2/+;Bal/+	0.2	82			wtr
	*TW9/Gla**		*ovoD2/+;Df/+*	3.6	83	0.608	<0.01	wtr
			ovoD2/+;Bal/+	0.3	80			wtr
149	*TW150/CyO*	37F5-38A1;38B2-C1	*ovoD2/+;Df/+*	9.3	66	0.923	<0.01	90%
			ovoD2/+;Bal/+	0.6	65			wtr
	*TW150/Gla**		*ovoD2/+;Df/+*	9.2	77	0.548	<0.01	90%
			ovoD2/+;Bal/+	2.0	72			wtr
	*TW150/CyO**		*ovoD2/+;Df/+*	4.0	87	0.639	<0.01	wtr
			ovoD2/+;Bal/+	0.2	65			wtr
150	*TW84/CyO*	37F5-38A1;39D3-E1	*ovoD2/+;Df/+*	6.6	65	0.923	<0.01	wtr
			ovoD2/+;Bal/+	0.1	79			wtr
	*TW84/Gla**		*ovoD2/+;Df/+*	7.4	66	0.804	<0.01	90%
			ovoD2/+;Bal/+	0.2	70			wtr
	*TW84/CyO**		*ovoD2/+;Df/+*	2.7	89	0.434	<0.01	wtr
			ovoD2/+;Bal/+	0.5	75			wtr
151	*TW65/Cy*	37F5-38A1;39E2-F1	*ovoD2/+;Df/+*	4.2	63	0.508	<0.01	wtr
			ovoD2/+;Bal/+	1.9	58			wtr
152	*TW161/CyO*	38A6-B1;40A4-B1	*ovoD2/+;Df/+*	7.9	114	0.757	<0.01	90%
			ovoD2/+;Bal/+	1.1	107			wtr
153	*TW1/CyO*	38A7-B1;39C2-3	*ovoD2/+;Df/+*	0.8	132	0.369	NS	wtr
			ovoD2/+;Bal/+	3.3	125			wtr
154	*DS6/CyO or SM6a*	38F5;39E7-F1	*ovoD2/+;Df/+*	3.4	50	0.146	NS	wtr
			ovoD2/+;Bal/+	2.3	45			wtr

See APPENDIX A for explanations. Wild-type range (wtr) for chromosome 2 is between 0.1 and 6.6 oocytes per ovary.

APPENDIX C

ovo^{D2} heterozygotes with reduced doses of regions on the right arm of chromosome *2*

No.	Deficiency/balancer	Cytology	Progeny	Oocytes/ovary		Statistics		Percentile
				Mean	No.	*T*1	*P*	
169	*B5/CyO*	46A;46C	*ovoD2/+;Df/+*	9.6	70	0.886	<0.01	90%
			ovoD2/+;Bal/+	0.4	70			wtr
175	*vg135/CyO*	49A;49D-E	*ovoD2/+;Df/+*	0.0	88	0.387	<0.01	10%
			ovoD2/+;Bal/+	0.7	62			wtr
176	*vgC/SM5*	49A4-13;49E7-F1	*ovoD2/+;Df/+*	0.0	80	0.667	<0.01	10%
			ovoD2/+;Bal/+	2.0	51			wtr
177	*vgD/Cy*	49C1-2;49E2-6	*ovoD2/+;Df/+*	1.4	86	0.608	<0.01	wtr
			ovoD2/+;Bal/+	6.4	62			wtr
	*vgD/CyO***		*ovoD2/+;Df/+*	1.6	50	0.320	<0.05	wtr
			ovoD2/+;Bal/+	0.5	50			wtr
178	*vg104/SM5*	49C4;49F13	*ovoD2/+;Df/+*	0.4	65	0.265	NS	wtr
			ovoD2/+;Bal/+	1.7	59			wtr
179	*vg107/SM5*	<49Da-49Ea	*ovoD2/+;Df/+*	0.2	70	0.586	<0.01	wtr
			ovoD2/+;Bal/+	1.9	70			wtr
180	*vg133/SM5*	<49Da-49Dc	*ovoD2/+;Df/+*	0.8	60	0.020	NS	wtr
			ovoD2/+;Bal/+	0.8	39			wtr
181	*vg33/SM5*	49D;50A	*ovoD2/+;Df/+*	1.1	59	0.103	NS	wtr
			ovoD2/+;Bal/+	1.7	64			wtr
182	*vgB/SM5*	49D3-4;49F15-50A3	*ovoD2/+;Df/+*	1.2	97	0.235	NS	wtr
			ovoD2/+;Bal/+	1.7	44			wtr
183	*vg136/SM5*	vg-49Ea	*ovoD2/+;Df/+*	0.8	66	0.153	NS	wtr
			ovoD2/+;Bal/+	0.8	63			wtr
191	*Pcl7B/CyO*	54E8-F1;55B9-C1	*ovoD2/+;Df/+*	0.0	90	0.897	<0.01	10%
			ovoD2/+;Bal/+	4.1	58			wtr
192	*Pcl11B/CyO*	54F6-55A1;55C1-3	*ovoD2/+;Df/+*	0.0	73	0.278	<0.01	10%
			ovoD2/+;Bal/+	0.4	72			wtr
193	*Pc4/CyO*	55A;55F	*ovoD2/+;Df/+*	0.02	144	0.202	<0.01	10%
			ovoD2/+;Bal/+	0.4	134			wtr
	*Pc4/In(2LR)Cy**		*ovoD2/+;Df/+*	0.0	78	0.783	<0.01	10%
			ovoD2/+;Bal/+	6.6	60			90%

See APPENDIX A for explanations. Wild-type range (wtr) for chromosome *2* is between 0.1 and 6.6 oocytes per ovary.

APPENDIX D

ovo^{D2} heterozygotes with reduced doses of regions on the left arm of chromosome *3*

No.	Deficiency/balancer	Cytology	Progeny	Oocytes/ovary		Statistics		Percentile
				Mean	No.	*T*1	*P*	
204	*emcE12/TM2*	61A;61D3-4	*ovoD2/+;Df/+*	0.03	96	0.010	NS	10%
			ovoD2/+;Bal/+	0.05	96			10%
205	*Ar12-1/TM2*	61C;61F3	*ovoD2/+;Df/+*	0.03	100	0.209	<0.05	10%
			ovoD2/+;Bal/+	0.4	118			wtr
206	*Ar14/TM2*	61C3-4;62A	*ovoD2/+;Df/+*	0.0	116	0.020	NS	10%
			ovoD2/+;Bal/+	0.03	102			10%
217	*vin2/TM3*	67F2-3;68D6	*ovoD2/+;Df/+*	0.0	48	0.318	NS	10%
			ovoD2/+;Bal/+	0.6	44			wtr
218	*vin5/TM3*	68A2-3;69A1-3	*ovoD2/+;Df/+*	0.2	54	0.616	<0.01	wtr
			ovoD2/+;Bal/+	2.3	55			wtr
219	*vin4/TM3*	68B1-3;68F3-6	*ovoD2/+;Df/+*	6.0	74	0.269	<0.01	90%
			ovoD2/+;Bal/+	8.8	76			90%
220	*vin6/TM3*	68C8-11;69A4-5	*ovoD2/+;Df/+*	8.1	128	0.182	NS	90%
			ovoD2/+;Bal/+	6.7	145			90%
221	*vin7/TM3*	68C8-11;69B4-5	*ovoD2/+;Df/+*	0.8	60	0.050	NS	wtr
			ovoD2/+;Bal/+	0.8	63			wtr

APPENDIX D

Continued

No.	Deficiency/balancer	Cytology	Progeny	Oocytes/ovary Mean	Oocytes/ovary No.	Statistics *T1*	Statistics *P*	Percentile
241	*Pc-MK/TM3*	78A3;79E1-2	*ovoD2/+;Df/+*	10.1	97	0.584	<0.01	90%
			ovoD2/+;Bal/+	3.1	108			wtr
242	*Pc/TM3*	78D1-2;79A4-C1	*ovoD2/+;Df/+*	16.6	73	0.501	<0.01	90%
			ovoD2/+;Bal/+	10.6	70			90%
	Pc/TM3 (other stock)		*ovoD2/+;Df/+*	14.2	88	0.960	<0.01	90%
			ovoD2/+;Bal/+	1.1	58			wtr
243	*Pc23937-30A/TM3*	78D	*ovoD2/+;Df/+*	16.5	70	1.000	<0.01	90%
			ovoD2/+;Bal/+	0.2	70			wtr
244	*Pc-Cp1/TM3*	78D3-6;78E-F	*ovoD2/+;Df/+*	13.0	68	1.000	<0.01	90%
			ovoD2/+;Bal/+	0.5	74			wtr
245	*Pc-T7/TM3*	78E1-2;79E4	*ovoD2/+;Df/+*	0.5	71	0.082	NS	wtr
			ovoD2/+;Bal/+	0.3	70			wtr

See APPENDIX A for explanations. Wild-type range (wtr) for chromosomes *3* and *4* is between 0.1 and 5.7 oocytes per ovary.

APPENDIX E

ovo^{D2} heterozygotes with reduced dose of regions on the right arm of chromosome *3*

No.	Deficiency/balancer	Cytology	Progeny	Oocytes/ovary Mean	Oocytes/ovary No.	Statistics *T1*	Statistics *P*	Percentile
248	*Z/TM3*	82A;82E3-4	*ovoD2/+;Df/+*	1.9	65	0.317	<0.01	wtr
			ovoD2/+;Bal/+	0.4	76			wtr
249	*110/TM3*	82C;82F	*ovoD2/+;Df/+*	0.4	58	0.426	<0.01	wtr
			ovoD2/+;Bal/+	2.6	86			wtr
250	*6-7/TM3*	82D3-8;82F3-6	*ovoD2/+;Df/+*	5.7	68	0.775	<0.01	90%
			ovoD2/+;Bal/+	0.7	54			wtr
251	*3-4/TM3*	82F1-2;82F10-11	*ovoD2/+;Df/+*	11.7	66	1.000	<0.01	90%
			ovoD2/+;Bal/+	0.3	66			wtr
269	*by10/TM3*	85D8-12;85E7-F1	*ovoD2/+;Df/+*	0.0	55	0.278	<0.05	10%
			ovoD2/+;Bal/+	0.6	90			wtr
270	*by416/TM3*	85D10-12;85E1-3	*ovoD2/+;Df/+*	0.01	148	0.193	NS	10%
			ovoD2/+;Bal/+	0.5	100			wtr
271	*by62, T(2;3)by62/TM1*	85D11-14;85F6	*ovoD2/+;Df/+*	0.9	67	0.221	NS	wtr
			ovoD2/+;Bal/+	2.0	57			wtr
272	*GB104/TM3*	85D12;85E10	*ovoD2/+;Df/+*	0.3	62	0.258	NS	wtr
			ovoD2/+;Bal/+	1.2	62			wtr
280	*red3l/MKRS*	87F12-14;88C1-3	*ovoD2/+;Df/+*	0.2	42	0.254	NS	wtr
			ovoD2/+;Bal/+	0.7	67			wtr
	red3l/TM3		*ovoD2/+;Df/+*	0.3	78	0.283	<0.01	wtr
			ovoD2/+;Bal/+	1.0	80			wtr
281	*su(Hw)7/TM6B*	88A9;88B2	*ovoD2/+;Df/+*	0.0	189	0.092	NS	10%
			ovoD2/+;Bal/+	0.2	130			wtr
282	*red-P93/In(3L)p In(3R)P18*	88A10-B1;88C2-3	*ovoD2/+;Df/+*	2.1	111	0.292	NS	wtr
			ovoD2/+;Bal/+	0.3	13			wtr
283	*red1/TM1*	88B1;88D3-4	*ovoD2/+;Df/+*	0.04	108	0.086	NS	10%
			ovoD2/+;Bal/+	0.2	70			wtr
284	*sbd105/T(2;3)apXa*	88F9-89A1;89B9-10	*ovoD2/+;Df/+*	0.0	50	0.000	NS	10%
			ovoD2/+;Bal/+	0.0	63			10%
289	*C4/Dp(3;3)P5*	89E;90A	*ovoD2/+;Df/+*	6.8	65	0.795	<0.01	90%
			ovoD2/+;Bal/+	0.8	62			wtr
290	*P14/T(2;3)apXa*	90C2-D1;91A1-2	*ovoD2/+;Df/+*	6.6	69	0.813	<0.01	90%
			ovoD2/+;Bal/+	0.4	72			wtr

See APPENDIX A for explanations. Wild-type range (wtr) for chromosomes *3* and *4* is between 0.1 and 5.7 oocytes per ovary.

APPENDIX F

Strong enhancers of *ovo^{D2}* also enhance *ovo^{D3}*

No.	Deficiency/balancer	Progeny	Oocytes/ovary		Statistics	
			Mean	No.	*T*1	*P*
78	*Df(1)l9/FM7a*	*ovoD3/Df*	0.01	120	0.891	<0.01
		ovoD3/FM7a	8.8	69		
175	*Df(2R)vg135/CyO*	*ovoD3/+;Df/+*	0.0	90	0.930	<0.01
		ovoD3/+;CyO/+	5.2	100		
	Df(2R)vg135/Gla	*ovoD3/+;Df/+*	0.0	80	1.000	<0.01
		ovoD3/+;Gla/+	14.7	89		
204	*Df(3L)emcE12/TM2*	*ovoD3/+;Df/+*	0.3	100	0.016	NS
		ovoD3/+;TM2/+	0.3	86		
206	*Df(3L)Ar14/TM2*	*ovoD3/+;Df/+*	0.0	100	0.470	<0.01
		ovoD3/+;TM2/+	1.6	100		
217	*Df(3L)vin2/TM3*	*ovoD3/+;Df/+*	0.2	94	0.851	<0.01
		ovoD3/+;TM3/+	5.8	63		
262	*Df(3R)Hu/TM3*	*ovoD3/+;Df/+*	0.2	64	0.598	<0.01
		ovoD3/+;TM3/+	2.2	61		
269	*Df(3R)by10/TM3*	*ovoD3/+;Df/+*	0.01	116	0.501	<0.01
		ovoD3/+;TM3/+	1.2	78		
270	*Df(3R)by416/TM3*	*ovoD3/+;Df/+*	0.03	100	0.706	<0.01
		ovoD3/+;TM3/+	2.9	84		
281	*Df(3R)su(Hw)7/TM6B*	*ovoD3/+;Df/+*	0.1	89	0.587	<0.01
		ovoD3/+;TM6B/+	1.2	58		

See APPENDIX A for explanations.