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Identification of Chromosomal Regions Involved in *decapentaplegic* Function in Drosophila

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ABSTRACT

Signaling molecules of the transforming growth factor β (TGF-β) family contribute to numerous developmental processes in a variety of organisms. However, our understanding of the mechanisms which regulate the activity of and mediate the response to TGF-β family members remains incomplete. The product of the Drosophila *decapentaplegic* (*dpp*) locus is a well-characterized member of this family. We have taken a genetic approach to identify factors required for TGF-β function in Drosophila by testing for genetic interactions between mutant alleles of *dpp* and a collection of chromosomal deficiencies. Our survey identified two deficiencies that act as maternal enhancers of recessive embryonic lethal alleles of *dpp.* The enhanced individuals die with weakly ventralized phenotypes. These phenotypes are consistent with a mechanism whereby the deficiencies deplete two maternally provided factors required for *dpp*'s role in embryonic dorsal-ventral pattern formation. One of these deficiencies also appears to delete a factor required for *dpp* function in wing vein formation. These deficiencies remove material from the 54F-55A and 66B-66C polytene chromosomal regions, respectively. As neither of these regions has been previously implicated in *dpp* function, we propose that each of the deficiencies removes a novel factor or factors required for *dpp* function.

SIGNALING molecules of the transforming growth factor β (TGF-β) superfamily are present in numerous organisms where they elicit a variety of cellular responses in a number of different tissues. The best genetically characterized member of this superfamily is the product of the *decapentaplegic* (*dpp*) gene in *Drosophila melanogaster* (Padgett *et al.* 1987). DPP participates in several events during Drosophila development including oogenesis (Twombly *et al.* 1996), dorsal-ventral patterning (Irish and Gelbart 1987), patterning of the mesoderm (Frasch 1995; Staehling-Hampton *et al.* 1994), morphogenesis of the larval midgut (Immergluck *et al.* 1990; Panganiban *et al.* 1990), adult appendage development (Spencer *et al.* 1982), morphogenetic furrow progression in the developing eye (Heberlein *et al.* 1993), and wing vein formation (Segal and Gelbart 1985; Yu *et al.* 1996). We have taken a genetic approach to identify factors required for *dpp* function in Drosophila with the expectation that their identification will shed light on the molecular mechanisms underlying *dpp*'s diverse roles and those of other TGF-β superfamily members.

Recent work has significantly advanced our knowledge of the molecular mechanisms underlying the functions of various TGF-β family members, but our understanding of these complex processes remains incomplete. TGF-β family members are initially synthesized as proproteins which undergo cleavage to yield mature ligands which consist of both homo- and heterodimeric forms (reviewed in Massague 1990). Several proteins are known to function extracellularly to antagonize TGF-β activities (reviewed by Sasai and De Robertis 1997; Sive and Bradley 1996). One such molecule has been identified in Drosophila as the product of the *short gastrulation* (*sog*) gene (Francois *et al.* 1994). The inhibitory action of SOG is in turn antagonized by the product of the *tolloid* (*tld*) gene which has been shown to proteolytically cleave SOG in complexes containing DPP (Marques *et al.* 1997). TGF-β family members initiate responses in target cells by binding to heteromeric complexes of transmembrane serine/threonine kinase receptors (reviewed by Attisano and Wrana 1996; Massague 1996). Two Drosophila genes, *thick veins* (*tkv*) and *saxophone* (*sax*) encode type I receptors for DPP (Brummel *et al.* 1994; Nellen *et al.* 1994; Penton *et al.* 1994; Xie *et al.* 1994). A third Drosophila gene, *punt*, encodes a type II receptor for DPP (Letsou *et al.* 1995; Ruberte *et al.* 1995). A family of related molecules function downstream of the receptor complex as elements of the signal transduction pathway (Derynck and Zhang 1996; Massague 1996; Wrana and Attisano 1996; Newfeld *et al.* 1997). The founding member of this family is the product of the Drosophila gene *Mothers against dpp* (*Mad*) (Sekelsky *et al.* 1995). Finally, CrebB-17A (Eresh *et al.* 1997) and the products of the *schnurri* (*shn*) (Arora *et al.* 1995; Grieder *et al.* 1995; Staehling-Hampton *et al.* 1995) *extradenticle* (*exd*) (Mann and

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Abu-Shaar 1996) and *vrille* (*vri*) (George and Terracol 1997) genes have been suggested as candidates for DPP-responsive transcription factors in Drosophila.

Mutations in many of the genes mentioned above interact genetically with *dpp* mutations. *Mad* and *Med* were first identified in a screen for mutations which act as dominant maternal enhancers of recessive embryonic lethal *dpp* mutations (Raftery *et al.* 1995). This screen took advantage of two properties of *dpp* function during early dorsal-ventral patterning. First, while *dpp* expression at this stage is strictly zygotic, many of the other factors in the *dpp* dorsal-ventral patterning pathway are expressed maternally. Second, specification of cell fates along the dorsal-ventral axis is exquisitely sensitive to levels of *dpp* activity (Ferguson and Anderson 1992a; Wharton *et al.* 1993). The strategy, therefore, was to look for mutations which reduced dpp function by decreasing the level of a maternally provided product required for *dpp*'s role in embryonic dorsal-ventral patterning. When such a mutation is placed in combination with an appropriate *dpp* mutation, there is insufficient *dpp* function to specify sufficient numbers of the most dorsal cell fates, and lethality results.

The screen which identified *Mad* and *Med* used ethyl methane sulfonate to generate lesions which were then screened for the ability to maternally enhance recessive embryonic lethal *dpp* mutations. While this screen identified multiple alleles of both of these loci, the limited number of mutagenized chromosomes tested suggested the possibility that additional loci remained which could be identified using this approach. We therefore employed a similar strategy to identify additional chromosomal regions containing genes required for *dpp* function. We surveyed a collection of deficiencies for the ability to maternally enhance dpp^{hr4}. We identified two regions not previously implicated in *dpp* function, 54F-55A and 66B-66C. We have found that these interactions are dependent on both the deficiency and the *dpp* mutation, and that the lethality correlates with a defect in dorsal-ventral patterning. We also show that deficiencies of the 54F-55A region can interact with *dpp* at at least one other stage of development.

MATERIALS AND METHODS

Stocks and culture conditions: All crosses were carried out at 25° on cornmeal-agar medium. The *dpp* rescue construct, *P{dpp-Sal20}332.19*, is as described in Hursh *et al.* (1993) and the *dpp* transgene bearing balancer, *CyO23*, is as described in Wharton *et al.* (1993). Additional balancers used are as follows: *CyO*, Cy^{1} dp^{lv1} pr^{1} cn^{2}, *TM3* Sb^{1} e^{1} Ser^{1}, and *SM6a*, al^{2} Cy^{1} dp^{lv1} cn^{2P} bw^{k1} sp^{2}. All other strains are as described in FlyBase (FlyBase 1996). Deficiency-bearing stocks were obtained from the Bloomington Indiana Stock Center with the exception of *Df(1)wy26* which was obtained from D. Pauli (Geneva, Switzerland) and deficiencies in the 78 cytological region which were obtained from A. Carpenter (Cambridge, UK). The *Tp(3;3)P47*, bx^{34e}- and *Tp(3;3)bxd100*-bearing stocks were obtained from E. Lewis (Pasadena, CA). A collection of ethyl methane sulfonate-induced lethal mutations were obtained from S. Bray (Cambridge, UK) and are as described in Bray and Kafatos (1991).

Deficiency screen: Females bearing deficiencies on the third and *X* chromosomes were crossed to *net* dpp^{hr4}/*CyO*. In order to distinguish all classes of progeny, females bearing second chromosome deficiencies were crossed to either net dpp^{hr4}/*Pin* or *net* dpp^{hr4}/*Tft* males. Several of the second chromosome deficiencies were also outcrossed to a common *CyO* balancer prior to testing for maternal enhancement. Multiple broods of each cross were scored such that a minimum of 100 progeny were counted for any given cross. For each cross, the recovery of mutant progeny relative to expectations was calculated as the ratio of the number of individuals recovered for each of two *dpp* mutant classes to the number of individuals recovered for a non-*dpp* mutant control class. For deficiencies of the *X* chromosome, the number of females carrying the deficiency and dpp^{hr4} was compared to the number of females carrying the deficiency and *CyO*. The number of females carrying the *X* chromosome balancer and dpp^{hr4} was compared to this same control class. Likewise, for deficiencies of the second chromosome, the number of progeny carrying the deficiency and dpp^{hr4} was compared to the number of progeny carrying the deficiency and *CyO*, and the number of progeny carrying the second chromosome balancer and dpp^{hr4} was compared to this same control class. For deficiencies of the third chromosome, the number of progeny carrying both the deficiency and dpp^{hr4} was compared to the number of progeny carrying both the deficiency and *CyO*, and the number of progeny carrying the third chromosome balancer and dpp^{hr4} was compared to this same control class.

Tests of overlapping deficiencies in the 78A-78C interval for maternal enhancement activity: Females bearing the following deficiencies were crossed to dpp^{hr4}-bearing males: *Df(3L)D-5rv6*, *Df(3L)ME14*, *Df(3L)Pc-9a*, *Df(3L)Pc-12h*, *Df(3L)Pc-14d*, *Df(3L)Pc-101*, *Df(3L)Pc-810*, *Df(3L)Pc-2q*, *Df(3L)ME1325.* Enhancement activity was assessed as described above for other third chromosome deficiencies.

Recombinational mapping: Recombinants for *Df(2R)Pcl-11B* were generated by first crossing to y^{1} w^{67c23}. Individual recombinant chromosomes were recovered and balanced by crossing to y^{1} w^{67c23}; Bc^{1} $Egfr^{E1}$/*CyO*. Recombinants for *Df(3L)66C-G28* and *Df(3L)Pc-MK* were generated by crossing to Canton S and individual recombinant chromosomes were recovered and balanced by crossing to y^{1} w^{67c23}; D^{3} gl^{3}/*TM3*. All recombinant lines were assayed for enhancement activity by crossing either to z^{1} w^{11E4}; dpp^{hr4} *TE52/CyO* males for the second chromosome deficiency, or to *net* dpp^{hr4}/*CyO* males for the third chromosome deficiencies. The presence of the deficiency was determined for the *Df(2L)Pcl-11B*-derived lines by crossing to cn^{1} thr^{1} bw^{1} sp^{1}/*CyO* and assaying the viability of the recombinant chromosome over thr^{1}. The presence of the deficiency in each of the remaining recombinant lines was similarly determined by crossing the *Df(3L)66C-G28*-derived lines to *Df(3L)66C-I65/TM3* and the *Df(3L)Pc-MK*-derived lines to $P\{ry^{+t7.2} = PZ\}$ $l(3)04063^{04063}$ ry^{506}/*TM3*.

Rescue of enhancement by an additional copy of *dpp*$^{+}$: Females of the general genotype, y^{1} w^{67c23}; *Df*/+ were crossed to y^{1} w^{67c23}; dpp^{hr4} *TE52*/+; *P{dpp-Sal20}332.19*/Brd^{1} males.

Tests for maternal enhancement of other *dpp* alleles: Unbalanced, deficiency-bearing individuals were crossed to either males or females of the following genotypes: dpp^{hr56} cn^{1} bw^{1}/*CyO*, Cy^{1} dp^{lv1} pr^{1}, net^{1} dpp^{hr4}/*CyO*, or z^{1} w^{11E4}; net^{1} dpp^{hr27} ed^{1}/*CyO*.

Tests for interactions with *dpp* during imaginal disc development: Females of each of the four following genotypes: y^{1} w^{67c23}; $In(2L)dpp^{s4}$, ast^{1} dpp^{s4} $dpp^{d\text{-}ho}$ ed^{1} dp^{ov1} cl^{1}/*SM6a*, y^{1} w^{67c23}; ast^{1} dpp^{s6} $dpp^{d\text{-}ho}$ ed^{1} dp^{ov1} cl^{1}/*SM6a*, y^{1} w^{67c23}; dpp^{d5}/*SM6a*, y^{1} w^{67c23}; $In(2L)dpp^{d6}$, dpp^{d6}/*SM6a*, were crossed to males of each of the three follow-

ing genotypes: *z*^{1} *w*^{11E4}; *dpp*^{hr4} *TE52/+; Df(3L)66C-G28 Brd*^{1}*/+*, *net*^{1} *dpp*^{hr4} *Df(2R)Pcl-11B/CyO23*, and *net*^{1} *dpp*^{hr4} *Df(2R)Pcl-7B/CyO23.* Additionally, females of the genotype *net*^{1} *dpp*^{hr4}*/CyO* were crossed to males of each of two genotypes, *ast*^{1} *dpp*^{s6} *Df(2R)Pcl-11B dp*^{ov1} *b pr*^{1}*/SM6a* and *ast*^{1} *dpp*^{s6} *Df(2R)Pcl-7B/SM6a.*

Cuticle and wing preparations: Differentiated embryos were collected from crosses between *Df/+* females and *y*^{1} *w*^{67c23}; *net*^{1} *dpp*^{hr27} *ed*^{1}*/CyO-wg P{lacZ}* males. Cuticles were prepared as described in Ashburner (1989). Wings were mounted in Euparal (ASCO Laboratories, Gordon, UK). All photomicrographs were made with bright-field optics on an Olympus BHS microscope (Olympus Corp., Lake Success, NY). Images were assembled into figures using Adobe Photoshop (Adobe Systems Inc., San Jose, CA) and Canvas (Deneba Software Inc., Miami, FL).

Tests of existing mutations in the 54F-55A and 66B-C intervals for maternal enhancement activity: The following mutants reported to be in the region of the enhancing deficiency, *Df(2R)Pcl-11B*, were tested for maternal enhancement by crossing females bearing these mutations to *dpp*^{hr4}-bearing males: *stau*^{1}, *P{PZ}l(2)06850, P{PZ}l(2)04548, P{PZ}l(2)03091.* A collection of ethyl methane sulfonate-induced lethal mutations in the 54F-55A cytological region (Bray and Kafatos 1991) were also tested for maternal enhancement activity with respect to *dpp*^{hr4}. This collection included multiple alleles of five lethal complementation groups plus an additional seven lethal mutants which failed to complement both *Df(2R)Pcl7B* and *Df(2R)Pcl11B* (S. Lazar and S. Bray, personal communication).

The following mutants reported to be in the region of the enhancing deficiency, *Df(3L)66C-G28*, were tested for maternal enhancement activity with respect to *dpp*^{hr4}: *P{hsneo}105, P{lacw}l(3)0139, T(2;3)WT(3;4)Antp, P{PZ}l(3)04111, P{lacW}l(3)j1c7, In(3LR)269, In(3LR)283, l(3)SG10*^{m27}, *l(3)SG11*^{5m33}, *l(3)SG12*^{j51}, *l(3)SG13*^{e20}, *P{PZ}l(3)01323, P{PZ}l(3)02067, P{PZ}l(3)03928, P{PZ}l(3)07217, P{PZ}l(3)08223, Tp(3;3)P47-bx*^{34e}, *Tp(3;3)bxd100*, and *Df(3L)66C-I65, T(2;3)TE35B-SR401, P{w*$^{+}$ *= *}30, T(2;3)E(da).*

RESULTS

Survey of deficiencies for enhancement activity: We tested 129 deficiency-bearing stocks for the ability to maternally enhance the recessive embryonic lethal mutation *dpp*^{hr4}. Together the deficiencies contained in these stocks uncover approximately 55% of the euchromatic genome and provide an efficient means to systematically search for regions containing genes which are required for *dpp* function. The *dpp* allele we used reduces *dpp* activity yet still confers sufficient activity to support normal development in heterozygous individuals (Wharton *et al.* 1993). The strategy we employed was designed to identify deficiencies that acted maternally to reduce *dpp* function in these heterozygous animals to a level which was no longer sufficient to support the development of viable individuals.

Maternally enhancing deficiencies were identified as those that generate a substantially reduced number of *dpp* mutant progeny relative to their non-*dpp*-mutant siblings in crosses of deficiency-bearing females to *dpp* mutant males. This was determined by comparing the number of individuals in each of the two *dpp* mutant classes to the number of individuals in a nonmutant class from such a cross.

Maternally acting deficiencies could be distinguished from those which act strictly zygotically or those which require both maternal and zygotic activities. Maternally acting deficiencies result in a diminution of both classes of *dpp* mutant progeny in crosses of deficiency-bearing females to *dpp* mutant males. In contrast, zygotically acting deficiencies or deficiencies that require both maternal and zygotic activities for enhancement result in a reduction only in the class of *dpp* mutant progeny that also carry the deficiency.

The deficiency-bearing stocks we used represent a wide variety of genetic backgrounds and this, we believe, is the basis for considerable variability in the relative survival of *dpp* mutant progeny that we observe in these crosses. For this reason we chose to pursue only those stocks which showed clear maternal enhancement; namely those deficiencies that could support the development of *dpp* mutant progeny at a frequency less than 25% of their nonmutant siblings.

Our search for deficiencies that maternally enhanced *dpp*^{hr4} initially identified five candidate cytogenetic intervals. The results of these crosses are summarized in Figure 1and details are provided in the appendix. Two of the enhancing deficiencies, *Df(2R)Pcl-11B* and *Df(3L)66C-G28*, appear to identify novel loci involved in *dpp* function. One deficiency, *Df(3R)Scr*, appears to interact due to the deletion of a previously identified gene involved in dorsal-ventral patterning. The maternal enhancement activity in two of the deficiency-bearing lines, *Df(1)JA26* and *Df(3L)Pc-MK*, did not appear to result from disruption of a discrete element within the deficiency.

***Df(1)JA26* and *Df(3L)Pc-MK*:** The enhancement activity in both of these stocks segregated with the deficiency-bearing chromosome. However, in neither of the stocks does it does appear to result from disruption of a single locus within the deficiency. Four other deficiencies together span the entire region deleted by *Df(1)JA26* and none of these show maternal enhancement activity with respect to *dpp* (see Figure 1 and appendix). The source of the enhancement activity in this stock must therefore reside on the deficiency-bearing chromosome outside the deficiency, or require deletion of multiple loci within *D1(1)JA26*, which are not together deleted in one of the overlapping deficiencies.

Similarly none of a collection of deficiencies, which together span the entire region deleted by *Df(3R)Pc-MK* showed any maternal enhancement activity with respect to *dpp* (see materials and methods and data not shown). Additionally, the enhancement activity in this stock appeared to be recombinationally separable from the deficiency (Table 1). This evidence suggests that all or part of the enhancement activity present on this chromosome lies outside the region affected by the deficiency.

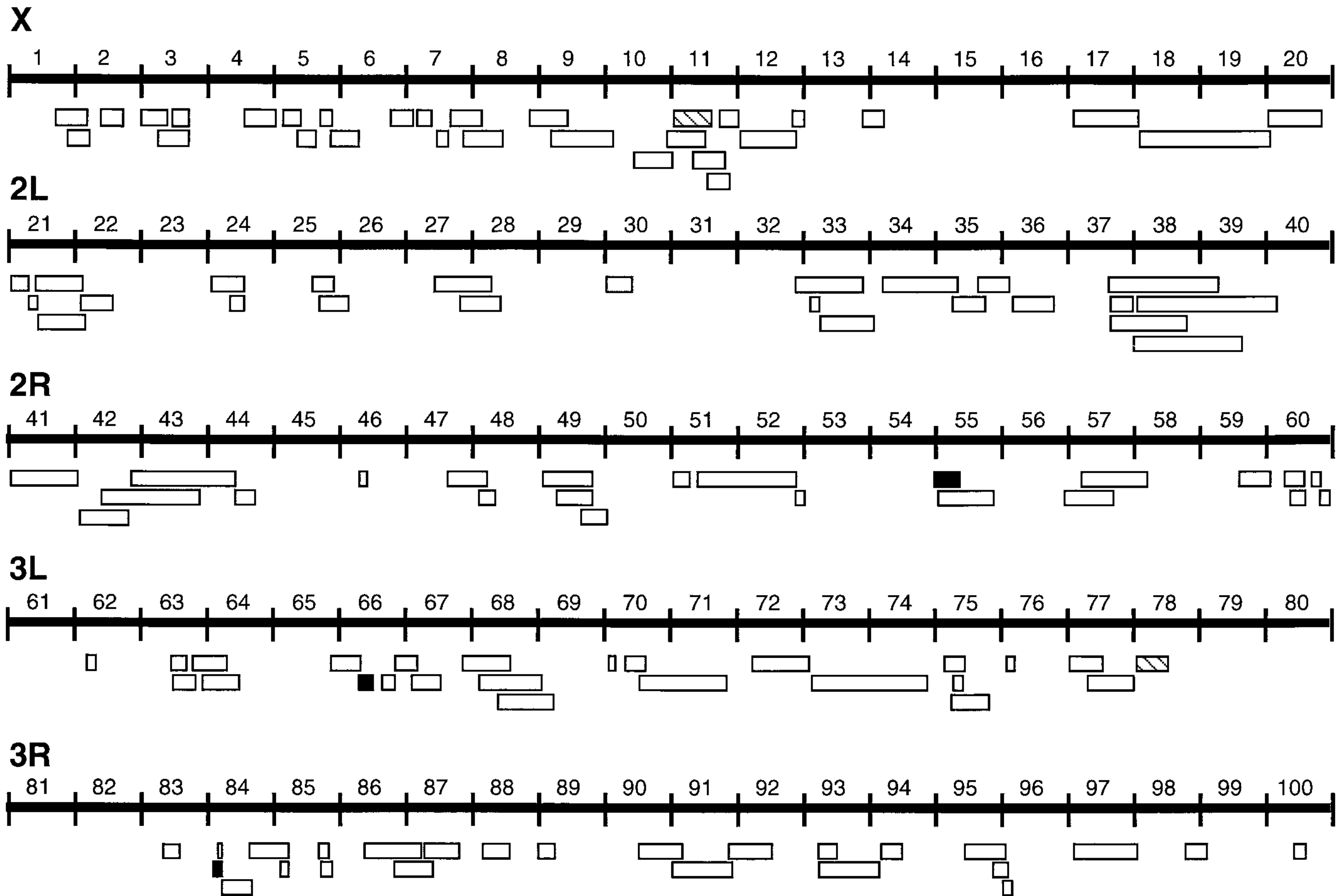

Figure 1.—Results of a deficiency survey for maternal enhancement of *dpp*. Each of the five major chromosome arms is represented with numbered cytological divisions. The approximate extent of each of the deficiencies tested is represented as a box below the appropriate chromosome arm. □, deficiencies that showed no enhancement activity. ■, deficiencies that showed enhancement activity with respect to dpp^{hr4}. ▧, deficiencies that initially showed enhancement activity but were later ruled out based on criteria described in the text.

Df(3R)Scr: This deficiency acts not as a maternal enhancer, but as a zygotic enhancer of *dpp* (see appendix), and appears to do so due to deletion of the previously identified gene *zerknüllt* (*zen*). Maintenance of *zen* expression during dorsal-ventral patterning has been shown to be regulated by *dpp* (Ray *et al.* 1991). Furthermore, hyperploidy for dpp^+ has been shown to rescue the lethality of hypomorphic *zen* mutations (Ferguson and Anderson 1992b). Additionally, we have observed that zen^7 can interact zygotically with dpp^{hr4} in a manner similar to this deficiency (data not shown). We suggest that the deletion of *zen* in this stock is at least in part responsible for the activity we observe; however, *Df(3R)Scr* also removes the gene *labial* (*lab*) whose expression in the developing midgut is regulated by *dpp* (Immergluck *et al.* 1990; Panganiban *et al.* 1990). Absence of *lab* or perhaps an additional gene within the deficiency could also be contributing to the interaction between *Df(3R)Scr* and *dpp*.

Df(2R)Pcl-11B: The stock bearing *Df(2R)Pcl-11B* shows

TABLE 1

Cosegregation of deficiency and enhancement activity

Parental deficiency	Deficiency-bearing recombinants: Enhancement	Deficiency-bearing recombinants: No enhancement	Recombinants lacking deficiency: Enhancement	Recombinants lacking deficiency: No enhancement
Df(2R)Pcl-11B	51	0	0	43
Df(3L)66C-G28	36	0	0	41
Df(3L)Pc-MK	13	1	0	63

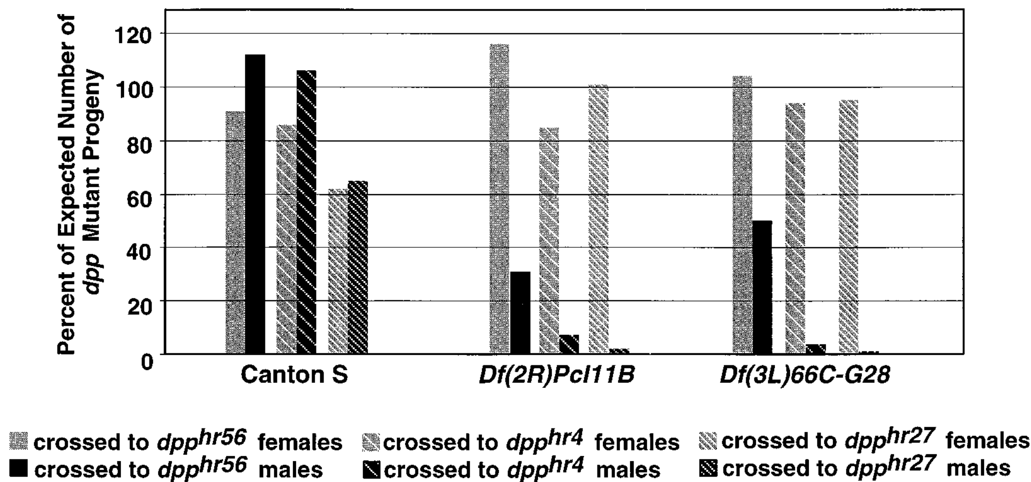

Figure 2.—Deficiencies maternally enhance other recessive embryonic lethal *dpp* alleles in accordance with the known severity of those alleles.

substantial reductions in the relative numbers of *dpp* mutant progeny recovered in crosses of females bearing this deficiency to dpp^{hr4} mutant males (see appendix). We have carried out a number of experiments in an attempt to test this initial observation and to explore the dependence of this interaction on both the deficiency and the *dpp* mutation.

We attempted to recombinationally separate the deficiency from the enhancement activity present on the *Df(2R)Pcl-11B*-bearing chromosome. However, all 94 recombinant lines showed cosegregation of the enhancement activity and the deficiency (Table 1). This result is consistent with the deficiency being the source of the enhancement activity, although a tightly linked independent enhancer cannot be ruled out. Furthermore, *Df(2R)Pcl-7B*, which has limits similar to *Df(2R)Pcl-11B* (54E8-F1;55B9-C1 for *Df(2R)Pcl-7B* as compared to 54F6-55A1;55C1-3 for *Df(2R)Pcl-11B*), also shows maternal enhancement activity with respect to dpp^{hr4}. Compared to controls, 14% of the expected number of dpp^{hr4} mutant progeny are recovered from crosses of *Df(2R)Pcl-7B* females to dpp^{hr4} males, whereas 84% of the expected number of dpp^{hr4} mutant progeny are recovered from crosses of *Df(2R)Pcl-7B* males to dpp^{hr4} females.

We took two approaches to test the dependence of the interaction on the *dpp* mutation. First we looked for similar interactions between *Df(2R)Pcl-11B* and other recessive embryonic lethal *dpp* mutations that were in different genetic backgrounds. We found that this deficiency enhanced other recessive embryonic lethal *dpp* mutations in accordance with the known severity of those *dpp* mutations (Figure 2) (Wharton *et al.* 1993). If the original interaction between the deficiency and the dpp^{hr4} bearing chromosome were due to something other than the *dpp* mutation on that chromosome, it is unlikely that the *Pcl-11B* deficiency would show a similar interaction with other *dpp* mutations in other genetic backgrounds. It is even less likely that these interactions would correlate with the severity of the *dpp* allele. In all cases these interactions are dependent on the deficiency being present maternally. When deficiency-bearing males are crossed to *dpp* mutant females no enhancement is observed (Figure 2). We also observe all of these same phenomena when *Df(2R)Pcl-7B*-bearing individuals are crossed to these *dpp* mutations (data not shown).

Second, if the interaction between *Df(2R)Pcl-11B* and dpp^{hr4} is dependent on the *dpp* mutation, then it should be possible to rescue the enhanced *dpp* mutant progeny with an additional dpp^+ present on another chromosome. We first confirmed that the dpp^+ transgene we were using for this purpose could function in this manner using the well-characterized maternal enhancer of *dpp*, *Mad* (Table 2). When we included this transgene in crosses of *Df(2R)Pcl-11B*-bearing females to dpp^{hr4} mutant males, we recovered *dpp* mutant progeny at a frequency comparable to that of their nonmutant siblings only when the *dpp* mutant progeny also carried the dpp^+ transgene. Mutant progeny which did not also carry the dpp^+ transgene were recovered at a much lower frequency comparable to that seen previously for crosses between deficiency-bearing females and *dpp* mutant

TABLE 2

Rescue of maternal enhancement by *dpp* rescue construct

	Percent of expected *dpp* mutant progeny[a]	
Maternal enhancer	dpp^+ transgene present[a]	No dpp^+ transgene present[b]
Df(2R)Pcl 11B	113	25
Df(3L)66C-G28	124	17
Mad^{12}	92	0

[a] The percent of expected for the rescued *dpp* mutant progeny is a comparison of the number of progeny that carry both the *dpp* rescue construct and the *dpp* mutation to the number of progeny that carry the *dpp* rescue construct and no *dpp* mutation.

[b] The percent of expected for the nonrescued *dpp* mutant progeny is a comparison of the number of progeny that carry the *dpp* mutation and no *dpp* rescue construct to the number of progeny that carry neither the *dpp* rescue construct nor the *dpp* mutation.

TABLE 3

Frequency of cuticular defects in enhanced progeny

Cross[a]	Percent of embryos with herniated head	Number scored
+ ♀ × *dpp*^{hr27} ♂	14	578
Df(2R)Pcl-11B ♀ × *dpp*^{hr27} ♂	33	442
Df(3L)66C-G28 ♀ × *dpp*^{hr27} ♂	48	521

[a] Only relevant aspects of the cross are shown here; see materials and methods for details.

males (Table 2). The ability of an additional wild-type copy of *dpp* to rescue the enhanced *dpp* mutant progeny provides further support for the assertion that the interaction between *Df(2R)Pcl-11B* and *dpp*^{hr4} is dependent on the *dpp* mutation and is not the result of another element or elements on that *dpp*^{hr4}-bearing chromosome.

If the reduction in the number of *dpp* mutant progeny that we observe in crosses between *dpp* mutant males and *Df(2R)Pcl-11B*-bearing females is the result of an increase in the severity of a defect caused by the *dpp* mutation, then it should be possible to identify lethal phenotypes among the enhanced progeny which are similar to those observed for more severe *dpp* alleles. For crosses involving *dpp*^{hr4} males and *Df(2R)Pcl-11B*-bearing females, the existence of such a correlation remains unclear. We find that enhanced individuals from these crosses die during both embryonic and larval development, and inspection of the cuticles of enhanced embryos reveals no consistent gross abnormalities (data not shown). These observations are consistent with enhancement activity that interferes with *dpp*'s role in dorsal-ventral patterning, but causes subtle defects that manifest themselves in lethality spread over an extended developmental period. Alternatively, these observations are consistent with enhancement activity that interferes with several of *dpp*'s roles, and this interference at multiple stages is the basis for the broad phase of lethality.

We do observe phenotypes consistent with an increase in the severity of the *dpp* mutation among the *dpp* mutant progeny from crosses of *Df(2R)Pcl-11B*-bearing females to *dpp*^{hr27} males. The *dpp*^{hr27} allele, on its own, results in a relatively low level of dominant lethality (Wharton *et al.* 1993) and correspondingly few heterozygous individuals with herniated head skeletons (Table 3). In crosses of *Df(2R)Pcl-11B*-bearing females to *dpp*^{hr27} males we observe a substantial increase in the fraction of progeny with herniated head skeletons (Table 3; see also Figure 3 for an example of phenotype). The phenotypes of these individuals are very similar to the phenotypes observed in individuals heterozygous for null alleles of *dpp* (Wharton *et al.* 1993) and are consistent with a mechanism whereby *Df(2R)Pcl-11B* causes synthetic lethality in combination with *dpp*^{hr27} by further reducing *dpp* function during dorsal-ventral patterning.

We tested the ability of *Df(2R)Pcl-11B* to interact with *dpp* during other stages of development (see materials and methods). The mutation *dpp*^{s6} disrupts *dpp* function during wing vein formation (Segal and Gelbart 1985; Yu *et al.* 1996). The transheterozygous combination of *dpp*^{s6} and *dpp*^{hr4} further reduces *dpp* function at this stage but results in relatively few wings with abnormal venation (Figure 4). The addition of either *Df(2R)Pcl-11B* (Figure 4) or *Df(2R)Pcl-7B* (data not shown) in this background results in an increased percentage of wings with abnormal venation (84% as compared to 29% for controls). The ability of these deficiencies to interfere with *dpp* function during wing vein formation is consistent with the gene or genes that they delete being involved with *dpp* function during multiple stages of development.

Finally, we tested for interactions between *Df(2R)Pcl-11B* and alleles of several loci involved in *dpp* signaling. We found that *Df(2R)Pcl-11B* acted as a strong maternal

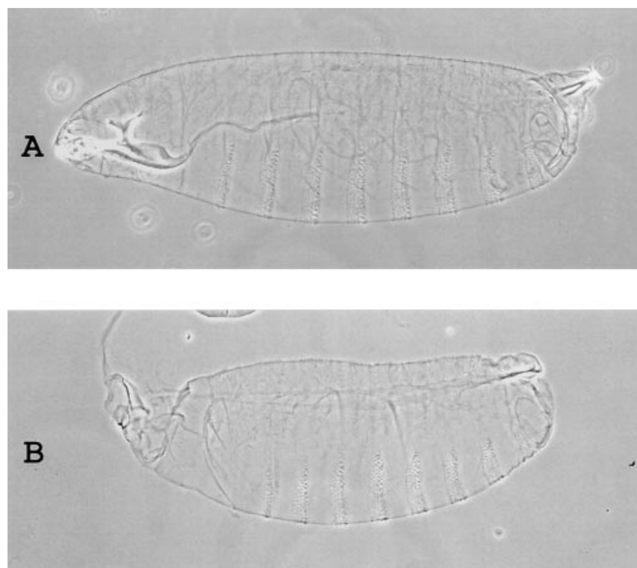

Figure 3.—Cuticular defects observed among maternally enhanced progeny. (A) Phenotypically wild-type cuticle of a first instar larva. (B) An example of the herniated head phenotype observed in the progeny of crosses between *Df(3L)66C-G28* females and *dpp*^{hr27} males. This phenotype is also observed among the progeny of crosses between *Df(2R)Pcl-11B* females and *dpp*^{hr27} males.

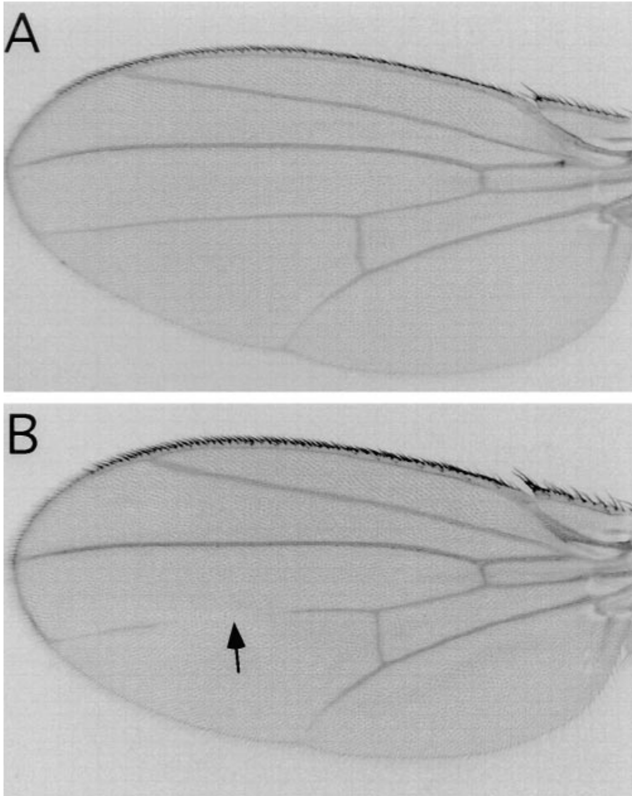

Figure 4.—*Df(2R)Pcl-11B* enhances *dpp* wing vein phenotypes. (A) dpp^{s6}/dpp^{hr4} individuals exhibit a largely normal pattern of adult wing veins, with only 29% (n = 44) of wings exhibiting any interruption of the fourth longitudinal wing vein, whereas (B) 84% (n = 91) of dpp^{s6} *Df(2R)Pcl-11B*/dpp^{hr4} individuals exhibit interruptions of the fourth longitudinal wing vein (arrow).

enhancer of a gain-of-function allele of *screw*, scw^{E1}. Two percent of the expected number of scw^{E1} progeny are recovered from crosses of *Df(2R)Pcl-11B*-bearing females to scw^{E1}-bearing males. This interaction is consistent with the fact that scw^{E1} itself acts as a strong zygotic enhancer of recessive embryonic lethal alleles of *dpp* (Raftery *et al.* 1995). The fact that *Df(2R)Pcl-11B* does not enhance scw^{E1R1}, a loss-of-function allele that does not act as an enhancer of *dpp*, supports the assertion that this interaction is the result of the combined activities of *Df(2R)Pcl-11B* and scw^{E1}, each of which independently decreases the activity of a *dpp*-dependent pathway. We also tested for interactions between *Df(2R)Pcl-11B* and mutants in *tolloid*, *Mad*, *Med*, *shrew*, and *60A* and found none.

***Df(3L)66C-G28*:** The stock bearing *Df(3L)66C-G28* also shows substantial reductions in the relative numbers of *dpp* mutant progeny when females bearing this deficiency are crossed to dpp^{hr4} mutant males (see appendix). We carried out a number of experiments similar to those described for *Df(2R)Pcl-11B* in an attempt to support this initial observation and to demonstrate its dependence on both the deficiency and the *dpp* mutation.

We tested 77 recombinant lines for segregation of enhancement activity and the *Df(3L)66C-G28* deficiency, and complete cosegregation was observed (Table 1). As with *Df(2R)Pcl-11B*, these data are consistent with the deficiency *Df(3L)66C-G28* being the source of the enhancement activity in this stock.

We also established that the interaction between *Df(3L)66C-G28* and dpp^{hr4} was dependent on the *dpp* mutation. Crosses between *Df(3L)66C-G28* and other recessive embryonic lethal *dpp* mutations showed that *Df(3L)66C-G28* could interact with other *dpp* mutations in other genetic backgrounds. Furthermore, these interactions showed the same correlation with the severity of the *dpp* mutation, and the same maternal dependence that we observed for the interactions between *Df(2R)Pcl-11B* and these alleles (Figure 2). The interaction between *Df(3L)66C-G28* and dpp^{hr4} was also rescuable with an additional wild-type copy of *dpp*, as was the case for the Mad^{12}-dpp^{hr4} and *Df(2R)Pcl-11B*-dpp^{hr4} interactions (Table 2). The observations that *Df(3L)66C-G28* interacts with other recessive embryonic lethal *dpp* mutations in other genetic backgrounds and that its interaction with dpp^{hr4} is rescuable with an additional wild-type copy of dpp^{+} are both consistent with the *dpp* dependence of this interaction.

The phenotype of *dpp* mutant progeny that are maternally enhanced by *Df(3L)66C-G28* is similar to that of progeny enhanced by *Df(2R)Pcl-11B*. When *Df(3L)66C-G28*-bearing females are crossed to dpp^{hr4} males, no consistent gross cuticular defects are observed among the enhanced progeny, and lethality occurs during both embryonic and larval stages (data not shown). However, when *Df(3L)66C-G28*-bearing females are crossed to dpp^{hr27} males, we observe a substantial increase in the number of progeny with herniated head skeletons as compared to controls (Table 3 and Figure 3). The increased number of individuals with herniated head skeletons among the progeny of this cross suggests that *Df(3L)66C-G28* is acting in combination with the *dpp* mutation to decrease the level of *dpp* activity below the threshold required for viable dorsal-ventral pattern formation in the enhanced embryos.

We tested several mutant combinations for interactions between *Df(3L)66C-G28* and *dpp* during other stages of development (see materials and methods). We were unable to identify an increase in the severity of a *dpp*-like phenotype in the presence of the deficiency in any of the mutant combinations we tested. While it is possible that the *dpp* mutant combinations we tested were not sufficiently sensitive to detect such an interaction, it is also possible that this deficiency only affects *dpp* function during dorsal-ventral patterning.

As with *Df(2R)Pcl-11B*, we found that *Df(3L)66C-G28* acted as a strong maternal enhancer of scw^{E1}. No scw^{E1} progeny were recovered from crosses of *Df(3L)66C-G28* females to scw^{E1} males. We also failed to observe interac-

tions between *Df(3L)66C-G28* and mutants in *tolloid*, *Mad*, *Med*, *srw*, and *60A*.

DISCUSSION

Our survey of deficiencies identified three regions that contain putative dominant enhancers of recessive embryonic lethal alleles of *dpp*. One of these deficiencies deletes *zen*, a gene previously shown to be regulated by *dpp* in dorsal-ventral pattern formation (Ray *et al.* 1991). The other two deficiencies, *Df(2R)Pcl-11B* and *Df(3L)66C-G28*, delete regions not previously implicated in dorsal-ventral pattern formation or *dpp* function. Our screen did not identify loci previously implicated in *dpp* function. As has been observed for loci which act as dominant modifiers of *ras* (Karmin *et al.* 1996), many of the loci which interact with *dpp* do so only as antimorphic gain-of-function alleles, *e.g.*, *tld* (Childs and O'Connor 1994; Finelli *et al.* 1994), *sog* (Ferguson and Anderson 1992b; Francois *et al.* 1994), *sax* (Brummel *et al.* 1994; Nellen *et al.* 1994; Xie *et al.* 1994), *tkv* (Nellen *et al.* 1994; Penton *et al.* 1994), *put* (Letsou *et al.* 1995; Ruberte *et al.* 1995), *shn* (Arora *et al.* 1995; Grieder *et al.* 1995; Staehling-Hampton *et al.* 1995), and *vri* (George and Terracol 1997). As would be expected for loss-of-function alleles of these loci, no interactions were observed in cases where one of these loci were deleted by a tested deficiency. The two loci that do show strong interactions with *dpp* as nulls, *Mad* (Raftery *et al.* 1995; Sekelsky *et al.* 1995) and *Med* (Raftery *et al.* 1995), were not deleted by any of the tested deficiencies.

We have shown that the interactions between both *Df(2R)Pcl-11B* and *dpp*, and *Df(3L)66C-G28* and *dpp* require the *dpp* mutation and are not the result of interactions with other lesions on the *dpp* mutant chromosome. We have demonstrated that the enhancement activity present in the *Df(2R)Pcl-11B* and *Df(3L)66C-G28* stocks lies within 1.1 and 1.3 map units of the respective deficiencies. We have also provided phenotypic evidence that, in both cases, these mutant combinations disrupt normal dorsal-ventral pattern formation. This evidence is consistent with the lethality in each case resulting from a decrease in the level of a maternally provided product required for *dpp* function at this stage.

Several possibilities exist for the molecular identities of factors which would be required for *dpp* function during dorsal-ventral patterning. All of the elements of the *dpp* signaling pathway identified to date are required for *dpp* function during multiple stages of development (Brummel *et al.* 1994; Nellen *et al.* 1994; Penton *et al.* 1994; Xie *et al.* 1994; Arora *et al.* 1995; Grieder *et al.* 1995; Letsou *et al.* 1995; Ruberte *et al.* 1995; Sekelsky *et al.* 1995; Staehling-Hampton *et al.* 1995). We have demonstrated that two overlapping deficiencies [*Df(2R)Pcl-11B* and *Df(2R)Pcl-7B*] of the 54F-55A cytological region can interact with *dpp* at least one other developmental stage. This observation is consistent with these deficiencies deleting a gene fundamentally involved in *dpp* function at multiple stages.

We did not observe any evidence for an interaction between *Df(3R)66C-G28* and *dpp* at later stages of development. It is possible that the particular mutant combinations we tested were not sufficiently sensitive to detect such an interaction and that *Df(3R)66C-G28* does, in fact, interact with *dpp* at multiple stages. It is also possible that *Df(3R)66C-G28* and *dpp* only interact during dorsal-ventral pattern formation and that the relevant factor or factors deleted in *Df(3R)66C-G28* are only required for *dpp* function at that stage. If this were the case, such a factor would be a candidate for one which mediates the specificity of the response to, or the activity of, *dpp* at this stage.

We chose to use deficiencies to identify regions that contain maternal enhancers because this approach had several advantages over other types of screens. Deficiencies provided a means of rapidly surveying a large portion of the genome. The relatively small number of stocks involved allowed us to be quantitative in our analysis of their enhancement activities. Our assay was therefore more sensitive than those in previous screens where such analysis is impractical, and all but the strongest enhancers would be discarded. The enhancing deficiencies we isolated yield a significant number of escapers, and this may be one reason they were not identified in previous screens. Our deficiency screen also immediately suggested a cytological location for the source of the enhancement activity in the interacting stocks, and therefore greatly reduced the amount of recombinational mapping required to localize the activity. With this approach, we could also target our search to new regions and thus avoid continued reisolation of previously identified enhancer loci. Finally, the use of deficiencies in this assay suggests that null mutations in identified loci are capable of interacting with *dpp*. A deficiency screen therefore potentially allowed us to avoid recovering spuriously interacting gain-of-function mutations in genes that are not normally involved in *dpp* function.

While our analysis of the interactions between *dpp* and the deficiencies was complicated by the fact that the deficiencies were generated on a number of different backgrounds, the primary disadvantage of our approach is the fact that the deficiencies disrupt multiple complementation groups. To further pursue the function and molecular identity of these maternal enhancers, we must first identify genetic lesions which selectively disrupt them. To this end, we have tested all available mutants in the 54F-55A and 66B-66C cytological intervals and found none that act as maternal enhancers of *dpp* (see materials and methods).

A number of plausible explanations exist for why the source of the enhancement activity is not represented among the previously generated mutants in these re-

gions. First, a complete collection of noncomplementing mutations is unavailable for either deficiency, and so it is possible that the sources of enhancement activity were simply not represented in the collection tested. This is a possibility for both *Df(2R)Pcl-11B* for which an extensive collection of ethyl methane sulfonate-generated noncomplementing mutations exist (Bray and Kafatos 1991) and *Df(3L)66C-G28* for which far fewer genetic reagents are available. It is also possible that the source of the enhancement activity in one or both cases could be a non-vital gene that would not have been isolated in many of the strategies used to generate the mutations we tested. Yet another possibility is that, in one or both cases, the source of the enhancement activity is multigenic, requiring the simultaneous loss of multiple loci within the deficiency. If this is the case, it may not be possible to recreate the enhancement activity with a mutation in only a single locus. A precedent for identifying a deficiency containing multiple genes all involved in a particular process exists for the apoptotic genes *reaper*, *head involution defective*, and *grim* which are all deleted by *Df(3L)WR10* (reviewed in McCall and Steller 1997). These genes were identified in a deficiency screen for apoptotic mutants not unlike the one described here used to identify enhancers of *dpp* (White *et al.* 1994).

In order to understand the basis of the interactions we describe, the source of the enhancement activity within *Df(2R)Pcl-11B* and *Df(3L)66C-G28* will require further characterization both genetically and molecularly. Future work will be directed at providing this characterization. In this paper, we have presented evidence that strongly supports the contention that factors required for *dpp* function are encoded within the 54F-55A and 66B-66C cytological intervals. Until the sources of the enhancement activities are specifically identified, we propose to refer to the enhancer uncovered by *Df(2R)Pcl-11B* as *E(dpp)55A* and the enhancer ucovered by *Df(3L)66C-G28* as *E(dpp)66C.* It is our hope that continued characterization of these loci will provide the foundation for the discovery of additional components of pathways regulating *dpp* or mediating *dpp* signaling and that their identification will further our understanding of the molecular mechanisms underlying *dpp* function specifically and TGF-β function in general.

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APPENDIX

Deficiencies surveyed for maternal enhancement activity of *dpp*^{hr4}

Deficiency	Cytology[a]	Percentage of expected: *dpp*^{hr4}, *Df*-bearing progeny	Percentage of expected: *dpp*^{hr4}-bearing progeny
Df(1)S39	1E1-2;2B5-6	80	77
Df(1)A94	1E3-4;B9-10	111	132
Df(1)Pdg35	2C2-4;2E2-F5	93	88
Df(1)JC19	2F6;3C5	67	56
Df(1)N-8	3C2-3;3E3-4	123	111
In(1)dm75e19	3C1-2;3E4	87	91
Df(1)JC70	4C15-16;5A1-2	77	75
Df(1)C149	5A8-9;5C5-6	123	100
Df(1)N73	5C2;5D5-6	113	119
Df(1)JF5	5E3-5;5E8	104	89
In(1)G4e^{L}H24i^{R}	5E3-8;6B	79	70
Df(1)HA32	6E4-5;7A6	83	76
Df(1)ct4b1	7B2-4;7C3-4	86	81
Df(1)C128	7D1;7D5-6	89	117
Df(1)RA2	7D10;8A4-5	105	103
Df(1)KA14	7F1-2;8C6	97	87
Df(1)C52	8E;9C-D	103	139
Df(1)v-L15	9B1-2;10A1-2	75	128
Df(1)HA85	10C1-2;11A1-2	63	39
Df(1)N105	10F7;11D1	63	90
Df(1)JA26	**11A;11D-E**	**17**	**28**
Df(1)wy26	11B17-C1;11E9-10	93	75
Df(1)N12	11D1-2;11F1-2	50	68
Df(1)C246	11D-E;12A1-2	96	116
Df(1)g	12A3-10;12E8	86	100
Df(1)KA9	12E2-3;12F5-13A1	115	102
Df(1)sd72b	13F1;14B1	77	95
Df(1)N19	17A1;18A2	56	118
Df(1)JA27	18A5;20A	97	100
Df(1)JC4	20A1;20E-F	93	84
Df(2L)net-PMF	21A1;21B7-8	83	123
Df(2L)al	21B8-C1;21C8-D1	94	100
Df(2L)ast2	21D1-2;22B2-3	62	39
Df(2L)dp-79b	22A3;22D5-E1	102	54
Df(2L)ed1	24A3-4;24D3-4	66	56
Df(2L)sc19-8[b]	24C2-8;24D4	67	90
Df(2L)cl-h3	25D2-4;25F1-2	163	258
Df(2L)GpdhA	25D7-E1;26A8-9	97	97
Df(2L)J-H	27C7-9;28B3-4	107	139
Df(2L)spd	27E1-8;28C1-6	100	115
Df(2L)30A-C	30A3-6;30C5	92	104
Df(2L)Prl	32F1-3;33F1-2	35	60
Df(2L)esc10	33A8-B1;33B2-3	89	91
Df(2L)prd1.7[c]	33B2-3;34A1-2	47	
Df(2L)b87e25[c]	34B12-C1;35B10-C1	33	
Df(2L)osp29	35B1-3;35E6	96	83
Df(2L)r10	35D1-2;36A6-7	32	44
Df(2L)H20	36A8-9;36F1	92	136
Df(2L)TW50[d]	36E4-F1;38A6-7	25	71
Df(2L)pr-A14	37D2-5;39B1-C4	38	133
Df(2L)E55	37D2;38A1	121	70
Df(2L)pr76	37D1-7;38E5	80	59
Df(2L)TW84	37F5-38A1;39D3-E1	120	72
Df(2L)TW161	38A6-B1;40A4-B1	102	110

(*continued*)

APPENDIX (*continued*)

Deficiencies surveyed for maternal enhancement activity of *ddp^{hr4}*

Deficiency	Cytology[a]	Percentage of expected	
		dpp^{hr4}, *Df*-bearing progeny	*dpp^{hr4}*-bearing progeny
Df(2R)M41A4	h44-46;42A1-2	90	110
Df(2R)cn88b	442A;42E	108	102
In(2R)pk78s	42C1-7;43F5-8	53	69
Df(2R)cn9	42E2-5;44C1-2	49	70
Df(2R)44CE	44C1-2;44E2-4	129	96
Df(2R)eve	46C3-4;46C9-11	44	107
Df(2R)en-A	47D3;48B2	86	58
Df(2R)en30	48A3-4;48C6-8	103	52
Df(2R)vg135	49A1-13;49E1-2	107	98
Df(2R)vg-C	49B2-3;49E2	55	34
Df(2R)vg-B	49D3-4;49F15-50A3	47	65
Df(2R)trix	51A1-2;51B6	58	58
Df(2R)Jp1	51C3;52F8-9	77	107
Df(2R)Jp8	52F5-9;52F10-53A1	51	44
Df(2R)Pc1-11B	**54F6-55A1;55C1-3**	**16**	**23**
Df(2R)Pc4	55A1;55F1-3	84	34
Df(2R)AA21	56F9-17;57D11-12	75	38
Df(2R)Pu-D17	57B4;58B	83	113
Df(2R)bw-S46	59D8-11;60A7	83	107
In(2LR)Px4	60C5-6;60D1	82	202
Df(2R)Px2	60C5-6;60D11	127	164
Df(2R)M-c33a	60E5-9;60E11-12	35	175
Df(2R)Kr10	60F1;60F5	98	113
Df(3L)R	62B7;62B12	84	162
Df(3L)HR232	63C1;63D3	131	102
Df(3L)HR119	63C2;63F7	105	92
Df(3L)GN50[c]	63E1-2;64B17	77	
Df(3L)GN24	63F5-7;64C13-15	100	114
Df(3L)pbl-X1	65F3;66B10	101	97
Df(3L)66C-G28	**66B8-9;66C9-10**	**6**	**8**
Df(3L)h-i22	66D10-11;66F4-5	69	150
Df(3L)29A6	66F5;67B1	156	149
Df(3L)AC1	67A2;67D11-13	57	63
Df(3L)vin2	67F2-3;68D6	84	80
Df(3L)vin5	68A2-3;69A1-2	100	103
Df(3L)vin7	68C8-11;69B4-5	82	96
Df(3L)Ly	70A2-3;70A5-6	77	137
Df(3L)fz-GF3b	70C2;70D6	118	106
Df(3L)fz-D21	70D2;71E8	98	113
Df(3L)st-f13	71C1;73A3-4	60	61
Df(3L)81K19	73A3;74F1-4	94	145
Df(3L)W10	75A6-7;75C1	80	55
Df(3L)W4	75B10;75C5-6	72	92
Df(3L)Cat[c]	75B8;75F1	83	
Df(3L)VW3	76A3;76B2	63	74
Df(3L)rdgC-co2	77A1;77D1	103	65
Df(3L)ri-79C	77C1;77F1-5	93	72
Df(3L)Pc-MK	**78A2;78C9**	**4**	**5**
Df(3R)TPl10[e]	83C1-2;83D4-5 and 84A4-5;84B1-2	82	105
Df(3R)Scr	**84A1-2;84B1-2**	**16**	**108**
Df(3R)Antp17	84A6-B2;84D11-14	64	88
Df(3R)p712	84D4-6;85B6	93	146
Df(3R)p819	85A3;85B6	81	82

(*continued*)

APPENDIX (*continued*)

Deficiencies surveyed for maternal enhancement activity of *ddp*^{hr4}

Deficiency	Cytology[a]	Percentage of expected	
		dpp^{hr4}, *Df*-bearing progeny	*dpp*^{hr4}-bearing progeny
Df(3R)by10	85D8-12;85E10-13	49	62
Df(3R)by62	85D11-14;85F6	74	63
Df(3R)M-Kx1	86C1;87B1-5	95	73
Df(3R)T-32	86E2-4;87C6-7	31	76
Df(3R)ry615	87B11-13;87E8-11	75	79
Df(3R)red-P1	88B1;88D3-4	78	71
Df(3R)sbd105	88F9-89A1;89B9-10	87	—
Df(3R)P14	90C2;91B1-2	94	126
Df(3R)Cha7	90F1-4;91F5	80	78
Df(3R)Dl-BX12	91F1-2;92D3-6	93	110
Df(3R)e-R1	93B6;93D2-4	59	71
Df(3R)e-N19	93B2-13;94A3-12	104	106
Df(3R)hhE23	94A1-16;94D1-4	94	68
Df(3R)crb87-4	95D1-2;96A2	120	117
Df(3R)crb87-5	95F7;96A17-18	49	40
In(3LR)Ubx^{7LL}*ats*^{R}	96A1-7;96A21-25	81	82
Df(3R)T1-P	97A1-10;98A1-2	92	78
Df(3R)3450	98E3;99A6-8	97	97
Df(3R)awd-KRB	100C6-7;100D3-4	101	72

[a] Only the extent of the deficiency is given. Additional inversion or translocation breakpoints which may exist on the deficiency-bearing chromosome are omitted.

[b] This stock carries both *Df(2L)sc19-8*, which extends from 24C2-8 to 25C8-9 and *Dp(2;1)B19*, which extends from 24D4 to 25F2.

[c] It was not possible to distinguish between the deficiency-bearing and nondeficiency-bearing progeny of these crosses.

[d] This stock carries both *Df(2L)TW50*, which extends from 36E4-F1 to 38A6-7 and *Dp(2;2)M(2)m[+]*.

[e] This stock carries both *Df(3R)TP110*, which extends from 83C1-2 to 84B1-2 and *Dp(3;3)Df[drvX1]*, which extends from 83D4-5 to 84A4-5.