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Characterization of *Tp(3;3)ME61* and genetic observations on chromosomal regions 96 and 97 of *Drosophila melanogaster*.

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Tp(3;3)ME61 was induced by X-rays on a *Df(3L)Pc-101, mwh*^{1} *Eip78C*^{101} *red*^{1} *e*^{4} chromosome in a screen for genetic modifiers of *Eip78C*. Although the *Tp(3;3)ME61* chromosome does not interact with *Eip78C*, it was retained because it was thought to be a simple deficiency of 96F to 97D. Subsequently, it was found to complement *Df(3R)Tl-X* (97B;97D1-2), and further cytological analyses showed that the deleted chromosomal segment had been inserted into centric heterochromatin (probably 3Lh). The transposed segment often showed reverse self-synapsis, suggesting it contains an ancestral tandem duplication centered in 97B. The orientation of the transposed segment could not be determined. By cytological criteria, it was impossible to tell whether the deleted chromosomal segment was 96F9-10;97C5-D1 or 96F11-12;97D2-3. The analysis was complicated by the apparent ability of 96F8,9 and 97D1,2 to synapse, which suggests homology between these bands. The complementation tests detailed below indicate that the deletion breakpoints are most likely 96F11-12;97D2-3.

The *Df(3R)ME61* component was separated from the *Dp(3;3)ME61* component by a *H-Pr* crossover in *Tp(3;3)ME61/H*^{1} *Pr*^{1} *Bsb*^{1} females. The complementation tests shown in Table 1 were undertaken to define the endpoints of the aberration genetically. The results are depicted graphically in Figure 1. The cytological breakpoints of aberrations used in this study are listed in Table 2.

Cytologically, the proximal euchromatic breakpoint of *Df(3R)ME61* lies between the *In(3LR)257* breakpoint at 96E2-4 and the proximal breakpoint of *Df(3R)Tl-P* at 97A2-7 and within *Df(3R)Espl3*. The *gro* locus has been mapped to 96F11-14 by *in situ* hybridization (Hartley *et al*., 1988), and the complementation of *Df(3L)ME61* and *gro*^{1} and *gro*^{C105} suggests that 96F11-12 is more accurate as a proximal breakpoint for *Df(3L)ME61* than 96F9-10. While the noncomplementation of *Df(3R)ME61* and the deficient inversion *In(3R)Na* might have allowed the 96F breakpoint of *Tp(3;3)ME61* to be localized more precisely, *In(3R)Na/Df(3R)Espl3* flies were viable. In fact, the absence of bands in proximal 97A was verified cytologically in polytene preparations from *In(3R)Na/Df(3R)Espl3* larvae. This indicates that either the deletion of proximal 97A has no overt phenotypic effect and *In(3R)Na* and *Df(3R)ME61* share a cryptic lethal, or the *Df(3R)Espl3* stock carries a transposition of 97A.

Tp(3;3)ME61 in combination with *Df(3R)Espl3* was lethal. This suggests that the 96F breakpoint of *Tp(3;3)ME61* disrupts a vital locus, that one or more vital loci are subject to position effect suppression, or both.

The distal euchromatic breakpoint of *Tp(3;3)ME61* lies between *Tl* and *His2Av*, because *Df(3R)ME61* failed to complement *Tl* loss-of-function alleles, but complemented *His2Av*^{05146}. *Tl* was mapped to 97D1-2 by *in situ* hybridization (Ranz *et al*., 2001) and from the breakpoints of revertants of *Tl* dominant alleles (Tearle and Nusslein-Volhard, 1987). *His2Av* was mapped to 97D3-6 by *in situ*

hybridization (Spradling *et al*., 1999). This suggests that 97D2-3 is the more accurate cytology for the distal *Tp(3;3)ME61* breakpoint.

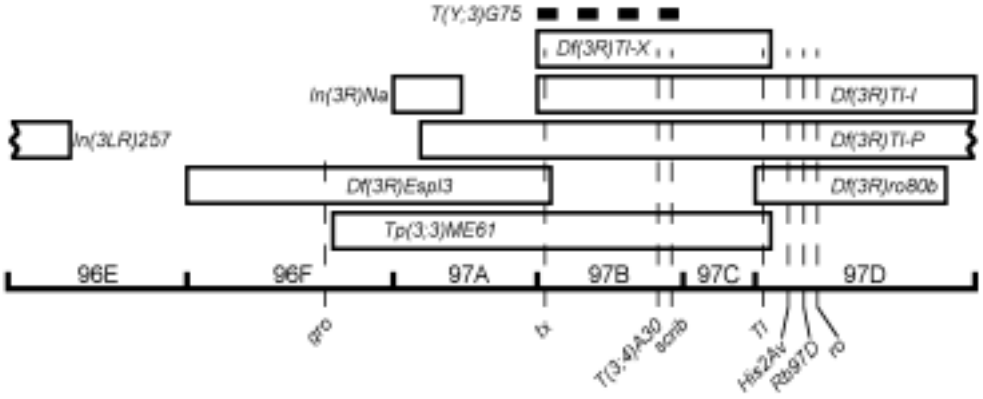

Figure 1. *Tp(3;3)ME61* and the surrounding chromosomal region. Our genetic results strongly suggest that *Tp(3;3)ME61* is a euploid aberration, so we have used the *Tp(3;3)ME61* box to represent both the intact *Tp(3;3)ME61* chromosome and the separated *Df(3R)ME61* component of the transposition. The thick dashed line indicates the approximate position of the *T(Y;3)G75* breakpoint.

Table 1. Complementation data.

	Df(3R)ME61	*Tp(3;3)ME61*	*Df(3R)Espl3*	*Df(3R)Tl-P*	*Df(3R)Tl-X*	*Df(3R)Tl-I*	*Df(3R)ro80b*
In(3LR)257	C	C	C	C	C	C	
Df(3R)Espl3	NC	NC	NC	NC	NC (v)	NC (v)	
gro^{1}	C (v)		NC (v)	C (v)	C (v)	C (v)	
gro^{C105}	C (v)		NC	C (v)	C (v)	C (v)	
Df(3R)ME61	NC		--	NC	NC	NC	--
Tp(3;3)ME61		NC	--	NC	C	NC	--
In(3R)Na	NC	C	C	C	C	C	
T(Y;3)R87	C						
T(Y;3)R71	C						
T(Y;3)B158	C						
tx^{1}	NC (v)	C (v)	NC (v)	NC (v)	NC (v)	NC (v)	
T(Y;3)G75	NC (v)	C (v)	NC (v)	NC (v)	NC (v)	NC (v)	
T(3;4)A30	NC	C	C	NC	NC	NC	
scrib^{7B3}	NC	C	C	NC	NC	NC	
Df(3R)ro80b	NC	NC			NC		NC
Tl^{k344}	NC (f)			NC (f)	NC (f)	NC (f)	NC*
Tl^{3}	NC (f)	C (f)			NC (f)		NC (f)
Tl^{4}	NC (f)	C (f)			NC (f)		NC (f)
His2Av^{05146}	C				C		NC
Rb97D^{1}	C (m)			NC (m)	C (m)	NC (m)	NC (m)
ro^{1}	C (v)						

Complementation (C) or noncomplementation (NC) for visible (v), male sterile (m) or female sterile (f) phenotypes; otherwise, C or NC for lethality. (--) redundant. **Tl*^{k344} and *Df(3R)ro80b* chromosomes share secondary lethals.

Table 2. Aberrations used in this study.

Aberration	Cytology	Reference for breakpoints
Tp(3;3)ME61	96F11-12;97D2-3;3het	This study
Df(3R)Espl3	96F1;97B1	Preiss *et al.,* 1988
Df(3R)ro80b	97C5-D1;97D13	Knibb *et al.*, 1993
Df(3R)Tl-I	97B;97E	Anderson *et al.*, 1985
Df(3R)Tl-P	97A2-7;98A1-3	This study
Df(3R)Tl-X	97B;97D1-2	Karsch-Mizrachi and Haynes, 1993
In(3LR)257	79D2-E1;96E2-4	This study
In(3R)Na	96F11-97A1;97A2-5;86F1-3	Carfagna and Nicoletti, 1963
T(Y;3)B158	h1-h17;97B	Seattle-La Jolla Drosophila Labs, 1971
T(Y;3)G75	h18-h25;97B	Seattle-La Jolla Drosophila Labs, 1971
T(Y;3)R87	h18-h25;97A	Seattle-La Jolla Drosophila Labs, 1971
T(Y;3)R71	h1-h2;97B	Gatti and Pimpinelli, 1983
T(3;4)A30	97B9-C1;h59-61	This study

The transposed chromosomal segment includes an intact *Tl* locus, suggesting that it is not grossly deleted at its distal end. *Tp(3;3)ME61* complemented *Df(3R)Tl-X*, placing the breakpoint of the transposition distal to the breakpoint of *Df(3R)Tl-X* within the *Tl-His2Av* interval. Likewise, the *Tl* locus must be intact, because females bearing either Tl^{r3} or Tl^{r4} and *Tp(3;3)ME61* produced abundant adult progeny. Nevertheless, they produced approximately 10% dead brown-colored embryos, suggesting that the *Tl* locus is subject to position effect variegation.

The current genome annotation predicts the existence of six genes between *Tl* and *His2Av* (Adams *et al.*, 2000). *Tp(3;3)ME61/Df(3R)ro80b* larvae died attempting to pupariate. Pier Paolo d'Avino, Cambridge University, examined these pseudopupae and reported they had nonexistent or rudimentary imaginal disks and small salivary glands (personal communication). These phenotypes are consistent with disruption, deletion or position effect suppression of genes in the *Tl-His2Av* interval. Of particular interest are the putative insulin-like growth factor receptors *CG6396* and *CG6390*, and the potential mitotic serine/threonine protein kinase *BcDNA:LD09009*. Our complementation results between *Df(3R)Tl-X*, *Df(3R)Tl-P* and *Df(3R)ro80b* and mutations in 97D are consistent with the results of Kidd *et al.* (1999) and Knibb *et al*. (1993).

Our studies mapped the *taxi (tx)* locus more precisely and provided information about its mutant phenotype and the strength of mutant alleles. *tx* maps to the region of overlap between the proximal ends of *Df(3R)Tl-I* and *Df(3R)Tl-X* and the distal end of *Df(3R)Espl3* in proximal 97B. The phenotypes of tx^1/Df individuals were more severe than the phenotypes of tx^1 homozygotes, indicating that tx^1 is hypomorphic. tx^1 homozygotes frequently held only one wing out and rarely showed other wing phenotypes. tx^1/Df individuals had strongly outheld wings that were thickened, slightly cupped and occasionally blistered. *Df(3R)Espl3/Df(3R)Tl-X* and *Df(3R)Espl3/Df(3R)Tl-I* exhibited a phenotype which probably represents the *tx* null phenotype. The thickened and opaque wings were always held out and somewhat crumpled when they were not filled with melanized fluid. Nevertheless, viability was only slightly reduced and females were fertile.

T(Y;3)G75, with a breakpoint in 97B, exhibited position effect suppression of both *tx* and *scribbled (scrib)*. *T(Y;3)G75* in combination with *Df(3R)Espl3*, *Df(3R)Tl-X*, *Df(3R)Tl-P*, *Df(3R)Tl-I* or *Df(3R)ME61* showed a *taxi* phenotype slightly more severe than that of tx^1/Df; most had strongly outspread wings with clear blisters. *T(1;Y)G75/P{lacW}*$scrib^{j7B3}$ showed a weak *scrib* phenotype: females laid eggs that did not hatch. Other *T(Y;3)* chromosomes with similar third chromosome breakpoints *(T(Y;3)R87, T(Y;3)R71* and *T(Y;3)B158)* gave viable and visibly normal phenotypes in combination with *Df(3R)ME61*.

Our cytology of *T(3;4)A30* places its third chromosome breakpoint at 97B9-C1. *T(3;4)A30* is lethal in combination with deletions of 97BC. According to David Bilder, University of California at Berkeley (personal communication), *T(3;4)A30* complemented *scrib* loss-of-function alleles, and rescuable maternal effect phenotypes of *scrib* were not rescued by paternal contribution of *Ts(3Lt;4Rt)A30*, *i.e.* the translocation segregant missing the distal portion of 3R could not provide *scrib*$^{+}$ function to rescue the abnormal cuticular phenotypes of embryos derived from homozygous *scrib* maternal germ line clones when it was contributed by the male parent. This places the *T(3;4)A30* breakpoint proximal to *scrib*. We found that *T(Y;3)G75* may have position effects on the lethal gene(s) disrupted by *T(3;4)A30*, since *T(Y;3)G75/T(3;4)A30* flies had reduced viability.

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References: Anderson, K.V. *et al.*, 1985, Cell 42: 779-789; Adams, M.D. *et al.*, 2000, Science 287: 2185-2195; Carfagna, M., and B. Nicoletti 1963, Dros. Inf. Serv. 38: 32; Gatti, M., and S. Pimpinelli 1983, Chromosoma 88: 349-373; Hartley, D.A. *et al.*, 1988, Cell 55: 785-795; Karsch-Mizrachi, I., and S.R. Haynes 1993, Nucleic Acids Res. 21: 2229-2235; Kidd *et al.*, 1999, Dros. Inf. Serv. 82: 114-115; Knibb, W.R. *et al.*, 1993, Molec. Gen. Genet. 239: 109-114; Preiss, A. *et al.*, 1988, EMBO J. 7: 3917-3927; Ranz, J.M. *et al.*, 2001, Genome Res. 11: 230-239; Seattle-La Jolla Drosophila Laboratories, 1971, Dros. Inf. Serv. 47 (Suppl.); Spradling, A.C. *et al.*, 1999, Genetics 153: 135-177; Tearle and Nusslein-Volhard, 1987, Dros. Inf. Serv. 66: 209-269.