Map Position and Expression of the Genes in the 38 Region of Drosophila

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ABSTRACT

With the completion of the Drosophila genome sequence, an important next step is to extract its biological information by systematic functional analysis of genes. We have produced a high-resolution genetic map of cytological region 38 of Drosophila using 41 deficiency stocks that provide a total of 54 breakpoints within the region. Of a total of 45 independent *P*-element lines that mapped by *in situ* hybridization to the region, 14 targeted 7 complementation groups within the 38 region. Additional EMS, X-ray, and spontaneous mutations define a total of 17 complementation groups. Because these two pools partially overlap, the completed analysis revealed 21 distinct complementation groups defined by point mutations. Seven additional functions were defined by *trans*-heterozygous combinations of deficiencies, resulting in a total of 28 distinct functions. We further produced a developmental expression profile for the 760 kb from 38B to 38E. Of 135 transcription units predicted by GENSCAN, 22 have at least partial homology to mobile genetic elements such as transposons and retroviruses and 17 correspond to previously characterized genes. We analyzed the developmental expression pattern of the remaining genes using poly(A)$^+$ RNA from ovaries, early and late embryos, larvae, males, and females. We discuss the correlation between GENSCAN predictions and experimentally confirmed transcription units, the high number of male-specific transcripts, and the alignment of the genetic and physical maps in cytological region 38.

DROSOPHILA is an outstanding model system for the study of gene activity in higher eukaryotes, and much of what we know about genetic pathways and how they function to build a complex organism rests upon work carried out in flies. Its utility is rooted in the experimental genetics that has attained an extraordinarily high level of sophistication over nearly a century of continuous development. Recently, a milestone was reached with the sequencing of the Drosophila genome (Adams *et al.* 2000), which removes the need for cloning and sequencing individual genes. Biological information must now be extracted from the genome sequence by systematic functional analysis of genes. Computer analysis alone can reveal only some of these functions. The large majority of genes have either no obvious function that can be predicted from their sequence or only a very general one, such as RNA binding, which gives no insight into a gene's specific developmental and biological role. Over the years a huge number of mutant fly strains have accumulated in numerous different laboratories. These mutants are the primary resource for functional analysis of these open reading frames (ORFs) and genes.

Outside of genome-based efforts, much of the data created in characterizing a specific gene is not relevant for this particular gene and is therefore often lost, even though it may become interesting for someone else later on. Nonsystematic analyses can often be redundant as well. For instance, the gene *neb* (= *Klp38B* = *Mothra*) at chromosomal location 38B4 was cloned and genetically characterized by several laboratories (Alphey *et al.* 1997; Molina *et al.* 1997; Ohkura *et al.* 1997; Ruden *et al.* 1997). This sort of laborious effort can be rendered obsolete by genome-scale mapping projects. As many existing mutant strains were generated outside of coordinated genome efforts, and have been mapped to varying degrees of precision, we started to systematically collect and exhaustively map available mutants in the proximal 2L region. Here we report the genetic map of the cytological interval 38, the alignment of the genetic and physical maps, and the experimental identification of transcription units in the region.

Because much of the focus on Drosophila research is on identifying developmental processes, which are well conserved between Drosophila and mammals (Merriam *et al.* 1991; Rubin *et al.* 2000), we also analyzed the developmental expression pattern of the various transcription units within region 38. These expression data provide experimental support for computer-predicted transcripts and give valuable information to re-

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TABLE 1

Description of deficiency chromosomes

Deficiency	Genetic background	Source[a]	Reference
Df(2L)Acon-21	*Df(2L)Acon-21 sp cn/CyO*	A. Carpenter	
Df(2L)be408		S. Hijal & B. Suter	
Df(2L)bur-K1	*w; Df(2L)bur-K1 cn/CyO*	A. Carpenter	
Df(2L)DS5	*Df(2L)DS5 b pr cn/CyO*	A. Carpenter	Moore *et al.* (1983)
Df(2L)DS6	*Df(2L)DS6, b[1] pr[1] cn[1]/CyO*	BL-2386	Moore *et al.* (1983)
Df(2L)DS8	*Df(2L)DS8 b pr cn/CyO*	A. Carpenter	Moore *et al.* (1983)
Df(2L)DS9	*Df(2L)DS9 b pr cn/CyO*	A. Carpenter	Moore *et al.* (1983)
Df(2L)E55	*Df(2L)E55, rdo[1] hk[1] Lar[E55] pr[1]/CyO*	BL-3076	Wright *et al.* (1976)
Df(2L)Fs(2)Ket-RX32	*Df(2L)Fs(2)Ket-RX32 lt bw/Bc Gla*	J. Szabad	Erdelyi *et al.* (1997)
Df(2L)pr-A16	*Df(2L)pr-A16, cn[1] bw[1]/CyO bw*	BL-567	Ganetzky (1977)
Df(2L)pr-A20	*Df(2L)pr-A20, cn[1] bw[1]/CyO*	BL-2375	Ganetzky (1977)
Df(2L)pr-M1	*Df(2L)pr-M1 dp[ov1] bw[1]/CyO*	A. Carpenter; now BL-5084	
Df(2L)pr1	*w[m4h]; Df(2L)pr1, Cy Roi/Sco*	H. D. Lipshitz	Wustmann *et al.* (1989)
Df(2L)pr2b	*w[m4h]; Df(2L)pr2b/Cy Roi*	H. D. Lipshitz	Wustmann *et al.* (1989)
Df(2L)pr7	*w[m4h]; Df(2L)pr7, Sco/In Gla*	H. D. Lipshitz	Wustmann *et al.* (1989)
Df(2L)pr11	*w[m4h]; Df(2L)pr11, Cy Roi/Sco*	H. D. Lipshitz	Wustmann *et al.* (1989)
Df(2L)pr37	*w[m4h]; Df(2L)pr37, Cy Roi*	H. D. Lipshitz	Wustmann *et al.* (1989)
Df(2L)pr40	*w[m4h]; Df(2L)pr40/Cy Roi*	H. D. Lipshitz	Wustmann *et al.* (1989)
Df(2L)pr49	*Df(2L)pr49 bw/CyO*	H. D. Lipshitz	Brittnacher and Ganetzky (1983)
Df(2L)pr1122	*Df(2L)pr1122, cn/CyO bw*	S. R. Halsell & H. D. Lipshitz	
Df(2L)pr1123	*Df(2L)pr1123, cn/CyO*	S. R. Halsell & H. D. Lipshitz	
Df(2L)pr8311	*Df(2L)pr8311, cn/CyO*	S. R. Halsell & H. D. Lipshitz	
Df(2L)pr9133	*Df(2L)pr9133, cn/CyO*	S. R. Halsell & H. D. Lipshitz	
Df(2L)pr9201	*Df(2L)pr9201, cn/CyO*	S. R. Halsell & H. D. Lipshitz	
Df(2L)pr9202	*Df(2L)pr9202, cn/CyO*	S. R. Halsell & H. D. Lipshitz	
Df(2L)pr9204	*Df(2L)pr9204, cn/CyO*	S. R. Halsell & H. D. Lipshitz	
Df(2L)pr11163	*Df(2L)pr11163, cn/CyO*	S. R. Halsell & H. D. Lipshitz	
Df(2L)pr11164	*Df(2L)pr11164, cn/CyO*	S. R. Halsell & H. D. Lipshitz	
Df(2L)Sd14	*Df(2L)Sd14/CyO*	H. D. Lipshitz	Brittnacher and Ganetzky (1983)
Df(2L)Sd37	*Df(2L)Sd37/SM5*	BL-3779	Brittnacher and Ganetzky (1983); Ganetzky (1977)
Df(2L)Sd57	*Df(2L)Sd57/CyO*		Brittnacher and Ganetzky (1983)
Df(2L)Sd77	*Df(2L)Sd77/CyO*	BL-3178	Brittnacher and Ganetzky (1983)
Df(2L)TW1	*w; Df(2L)TW1/SM6a*	D. Huen	Wright *et al.* (1976)
Df(2L)TW2	*Df(2L)TW2, Tft[1] l(2)74i[1]/CyO*	UM-41631	Wright *et al.* (1976)
Df(2L)TW9	*Df(2L)TW9, Tft[1] cn[1]/CyO*	UM-41640	Wright *et al.* (1976)
Df(2L)TW50	*Df(2L)TW50, cn[1]/CyO, Dp(2;2)M(2)m[+]*	BL-3189	Wright *et al.* (1976)
Df(2L)TW65	*Df(2L)TW65/CyO*	BL-1602	Wright *et al.* (1976)

(*continued*)

TABLE 1

(Continued)

Deficiency	Genetic background	Source[a]	Reference
Df(2L)TW84	*Df(2L)TW84, Tft[1] l(2)74i[1] Lar[TW84]/CyO*	BL-3346	Wright *et al.* (1976)
Df(2L)TW150	*Df(2L)TW150, cn[1] bw[1]/CyO*	UM-41750	Wright *et al.* (1976)
Df(2L)TW158	*Df(2L)TW158, cn[1] bw[1]/CyO*	UM-41760	Wright *et al.* (1976)
Df(2L)TW161	*Df(2L)TW161, cn[1] bw[1]/CyO, bw*	BL-167	Wright *et al.* (1976)
Df(2L)VA6	*Df(2L)VA6, cn bw/CyO*	M-#1619	Brittnacher and Ganetzky (1983)
Df(2L)VA12	*Df(2L)VA12,cn[1] bw[1]/CyO*	BL-3171	Hirsh and Davidson (1981)
Df(2L)VA17	*Df(2L)VA17, noc[Sco] pr[1]/CyO*	UM-42684	Hirsh and Davidson (1981)
Df(2L)VA19	*Df(2L)VA19, noc[Sco] rdo[1] pr[1]/CyO*	UM-42684A	Brittnacher and Ganetzky (1983)

[a] BL indicates a stock from the Bloomington Stock Center; UM, one from the Umeå Stock Center; M, one from the Mid-America Stock Center.

searchers who expect a specific expression pattern based on a mutant phenotype.

The *Drosophila melanogaster* genome consists of four chromosome pairs that can be visualized under the light microscope by looking at the polytene chromosomes of larval salivary glands. As early as the 1930s these polytene chromosomes were mapped according to the banding pattern seen by histological staining (Bridges 1935). This cytological map made Drosophila the first organism to have a physical map, albeit of a low resolution, ~100 kb (Rubin 1996). The cytological map divides the chromosomes into numbered regions, which in turn are subdivided into lettered intervals.

Polytene region 38 is situated on the left arm of the second chromosome. It is divided into six lettered intervals that are subdivided into a total of 45 numbered intervals: A, 1–8; B, 1–6; C, 1–10; D, 1–5; E, 1–10; and F, 1–6. Region 38 contains ~1 Mb of genomic sequence. It was chosen for analysis in part as a result of its location adjacent to previously characterized regions, in particular the *Adh* region from 34C to 36A (Ashburner *et al.* 1999) and the 37 region around *Ddc* (37B/C; Stathakis *et al.* 1995). Collections of lethal, maternal effect lethal, and female sterile mutations were available for the mapping project (Schüpbach and Wieschaus 1989, 1991; Erdelyi *et al.* 1997; Kozlova *et al.* 1998; Spradling *et al.* 1999). Because these mutants were isolated in different laboratories, most of them had not previously been systematically mapped with respect to one another.

MATERIALS AND METHODS

Genetic analysis: Genetic mapping of point mutations was achieved through complementation analysis with deficiency stocks that break within the 38 interval. With the exception of *purple* and *valois*, the mutants were mapped by their recessive lethal or recessive sterile phenotypes. Point mutations mapping to the same deficiency interval were subsequently tested for complementation. The majority of the tested alleles were created by either EMS or *P*-element mutagenesis. Deficiency chromosomes and point mutations were isolated in a number of different laboratories and are listed in Table 1 and Table 2.

Sequence analysis: Our molecular work was based on P1 and bacterial artificial chromosome (BAC) genomic sequences produced by the Berkeley *Drosophila* Genome Project (BDGP; Table 3). Data were obtained from http://www.fruitfly.org/sequence/drosophila-regions.html.

The program GENSCAN (Burge and Karlin 1997) was chosen to analyze the 38 sequence (http://genes.mit.edu/GENSCAN.html). In this study the vertebrate option with default parameters (ISOCHORE 1) was used, except where indicated. The GENSCAN predictions were the basis for all transcript analysis within region 38 and probes were designed for predicted genes on the basis of probability scores and prediction of exon structure.

Peptide homologies and EST searches: As GENSCAN results are based on gene prediction algorithms only, additional sequence annotations were carried out to identify sequence similarities with those in the public domain. Each predicted

TABLE 2

Description of point mutations

Allele	Mutagen	Genetic background	Source[a]	Reference
l(2)37Fc[1]	EMS	*l(2)37Fc[1] rdo[1] hk[1] pr[1]/CyO*	BL-3564	
l(2)37Fe[1]	EMS	*l(2)37Fe[1] rdo[1] hk[1] pr[1]/CyO*	BL-3451	
scw[l1]	EMS	*scw[l1] rdo[1] hk[1] pr[1]/CyO*	BL-4351	Arora and Nüsslein-Volhard (1992)
l(2)k08115	P{lacW}	*y w; P{lacW}/CyO*	BDGP; now BL-10771	Spradling *et al.* (1999)
l(2)k15716	P{lacW}	*y w; P{lacW}/CyO*	BDGP	Spradling *et al.* (1999)
fs(2)ltoPP43	EMS	*fs(2)ltoPP43 cn bw/CyO, l(2)DTS513*	T. Schüpbach	Schüpbach and Wieschaus (1991)
l(2)k08103	P{lacW}	*y w; P{lacW}/CyO*	BDGP	Spradling *et al.* (1999)
vls[1]	EMS	*vls[1] cn[1] bw[1]/CyO*	BL-982	Schüpbach and Wieschaus (1989)
vls[3]	EMS	*vls[PG] cn[1] bw[1]/CyO*	C. Nüsslein-Volhard #M292; now UM-M292	Schüpbach and Wieschaus (1989)
l(2)38Aa[1]	EMS	*l(2)37Ea[1] l(2)38Aa[1] pr[*]/Dp(2;Y)G-M15/CyO*	P. Gay & T. Wright: now BL-5317	Lindsley and Zimm (1992)
l(2)03552	P{PZ}	*P{PZ}, cn[1]/CyO ; ry[506]*	BDGP	Ruden *et al.* (1997); Spradling *et al.* (1999)
l(2)k03903	P{lacW}	*y w; P{lacW}/CyO*	BDGP	Alphey *et al.* (1997); Spradling *et al.* (1999)
l(2)05217	P{PZ}	*P{PZ}, cn[1]/CyO ; ry[506]*	BDGP	Ruden *et al.* (1997); Spradling *et al.* (1999)
l(2)k05702	P{lacW}	*y w; P{lacW}/CyO*	BDGP	Alphey *et al.* (1997); Spradling *et al.* (1999)
l(2)k09314	P{lacW}	*y w; P{lacW}/CyO*	BDGP	Spradling *et al.* (1999)
l(2)38Ab[1]		*l(2)38Ab[1]/CyO*	P. Gay & D. Contamine; now BL-5318	Lindsley and Zimm (1992)
spir[3]	EMS	*spir[QF70] cn bw/CyO*	T. Schüpbach	Schüpbach and Wieschaus (1991)
spir[PJ56]	EMS	*spir[PJ56] cn bw/CyO*	T. Schüpbach	Manseau and Schüpbach (1989)
l(2)38Db[52]	EMS	*y[1] w[*]; l(2)38Db[52] dp[ov1] bw[1]/CyO, y[+]*	T. Kozlova; now BL-5323	Kozlova *et al.* (1998)
l(2)38Ea[36]	EMS	*l(2)38Ea[36] dp[ov1] bw[1]/CyO*	T. Kozlova; now BL-5322	Kozlova *et al.* (1998)
l(2)38Ea[41]	EMS	*y[1] w[*]; l(2)38Ea[41] dp[ov1] bw[1]/CyO, y[+]*	T. Kozlova	Kozlova *et al.* (1998)
l(2)38Ea[46]	EMS	*y[1] w[*]; l(2)38Ea[46] dp[ov1] bw[1]/CyO, y[+]*	T. Kozlova	Kozlova *et al.* (1998)
l(2)38Ea[47]	EMS	*y[1] w[*]; l(2)38Ea[47] dp[ov1] bw[1]/CyO, y[+]*	T. Kozlova	Kozlova *et al.* (1998)
l(2)38Ea[66]	EMS	*y[1] w[*]; l(2)38Ea[66] dp[ov1] bw[1]/CyO, y[+]*	T. Kozlova; now BL-5324	Kozlova *et al.* (1998)
l(2)02306	P{PZ}	*P{ry[+t7.2]=PZ} Hr38[02306] cn[1]/CyO; ry[506]*	BDGP; now BL-11181	Kozlova *et al.* (1998); Spradling *et al.* (1999)
l(2)38Eb[27]	EMS	*l(2)38Eb[27] dp[ov1] bw[1]/CyO*	T. Kozlova	Kozlova *et al.* (1998)
l(2)38Eb[51]	EMS	*y[1] w[*]; l(2)38Eb[51] dp[ov1] bw[1]/CyO, y[+]*	T. Kozlova; now BL-5325	Kozlova *et al.* (1998)
l(2)38Eb[54]	EMS	*y[1] w[*]; l(2)38Eb[54] dp[ov1] bw[1]/CyO, y[+]*	T. Kozlova; now BL-5326	Kozlova *et al.* (1998)
l(2)38Eb[63]	EMS	*y[1] w[*]; l(2)38Eb[63] dp[ov1] bw[1]/CyO, y[+]*	T. Kozlova	Kozlova *et al.* (1998)
Fs(2)Ket[RX3]	X-ray	*Fs(2)Ket[RX3] lt[1] /In(2LR)Gla, wg[Gla-1]*	J. Szabad; now BL-5314	Erdelyi *et al.* (1997)
Fs(2)Ket[31]	EMS	*Fs(2)Ket[31] dp[ov1] bw[1]/CyO*	T. Kozlova	Kozlova *et al.* (1998)
Fs(2)Ket[49]	EMS	*y[1] w[*]; Fs(2)Ket[49] dp[ov1] bw[1]/CyO, y[+]*	T. Kozlova	Kozlova *et al.* (1998)
fs(2)lto2[1]	EMS	*fs(2)lto2[1] cn[1] bw[1]/CyO, l(2)DTS513[1]*	T. Schüpbach; now BL-4994	Schüpbach and Wieschaus (1991)
l(2)k07135	P{lacW}	*y w; P{lacW}/CyO*	BDGP; now BL-10659	Spradling *et al.* (1999)

(*continued*)

TABLE 2

(Continued)

Allele	Mutagen	Genetic background	Source[a]	Reference
ms(2)04138	P{PZ}	*P{PZ}, cn[1]/CyO ; ry[506]*	BL-P1762; now BL-11762	Castrillon *et al.* (1993)
dia[38]	EMS	*dia[38] dp[ov1] bw[1]/CyO*	T. Kozlova	Kozlova *et al.* (1998)
dia[40]	EMS	*y[1] w[*]; dia[40] dp[ov1] bw[1]/CyO, y[+]*	T. Kozlova	Kozlova *et al.* (1998)
dia[50]	EMS	*y[1] w[*]; dia[50] dp[ov1] bw[1]/CyO, y[+]*	T. Kozlova	Kozlova *et al.* (1998)
dia[55]	EMS	*y[1] w[*]; dia[55] dp[ov1] bw[1]/CyO, y[+]*	T. Kozlova	Kozlova *et al.* (1998)
l(2)38EFa[2]	EMS	*l(2)38EFa[2] dp[ov1] bw[1]/CyO*	T. Kozlova; now BL-5319	Kozlova *et al.* (1998)
Bl[1]	spontaneous	*Bl[1] L[2]/SM5*	BL-238	Meyer (1952)
cad[3]	EMS	*b[1] pr[1] cad[3]/In(2LR)Gla, wg[Gla-1]*	C. Nüsslein-Volhard #B235; now BL-5316	Macdonald and Struhl (1986)
l(2)38EFb[8]	EMS	*l(2)38EFb[8] dp[ov1] bw[1]/CyO*	T. Kozlova; now BL-5320	Kozlova *et al.* (1998)
l(2)01528	P{PZ}	*P{PZ}, cn[1]/CyO ; ry[506]*	BDGP; now BL-12306	Spradling *et al.* (1999)
l(2)k13715	P{lacW}	*y w; P{lacW}/CyO*	BDGP	Spradling *et al.* (1999)
l(2)04530	P{PZ}	*P{PZ}, cn[1]/CyO ; ry[506]*	BL-P1380; now BL-11380	Spradling *et al.* (1999)
l(2)38EFd[15]	EMS	*l(2)38EFd[15] dp[ov1] bw[1]/CyO*	T. Kozlova; now BL-5321	Kozlova *et al.* (1998)

[a] BL indicates a stock from the Bloomington Stock Center; UM, one from the Umeå Stock Center; and BDGP, one from the Berkeley *Drosophila* Genome Project.

peptide resulting from a GENSCAN prediction was run through the National Center for Biotechnology Information BLAST server (http://www.ncbi.nlm.gov/BLAST) using the BLASTP program with default parameters to search for homology with other gene products. Matches with a smallest sum probability of $<1 \times 10^{-4}$ were taken as having significant homology and were noted. Expressed sequence tagged (EST) databases were searched with the entire genomic sequence of the region. In each case, the appropriate section of genomic DNA was used to search the dbEST from the BDGP web server (http://www.fruitfly.org/blast/). ESTs matching in >90% of their length were noted.

Generation of sequence tag sites: The *P* elements with flanking genomic DNA were recovered from the stocks by inverse PCR (J. Rehm; http://www.fruitfly.org/about/methods/inverse.pcr.html). The amplified sequences were cloned into vectors before being isolated for sequencing. Sequencing was done with an Applied Biosystems (Foster City, CA) ABI 373 DNA Sequencer using 250–500 ng of the sample DNA and 3.2 pmol of T7 primer.

Northern and Southern blots: Northern blots were prepared and hybridized as previously described (Suter *et al.* 1989). Probes were made from PCR-amplified recombinant P1 bacteriophages containing the genomic region of interest. PCR primers were designed in accordance to recommendations by Applied Biosystems with an oligonucleotide length of 19–23 bases containing 12 G or C nucleotides and 7–11 A or T nucleotides, with an A or a T nucleotide at the 3′ end.

To produce short probes for predicted ORFs, the primers were designed to amplify sequences within a predicted internal exon and/or to the exon with the highest probability score. They were, on average, 100–200 bp apart and used to amplify directly from the appropriate P1 clone. These probes were primarily used for the detection of transcripts.

For the 10-kb genomic fragments, primers were designed so that each set of primers overlapped by ~100 bp. The fragments were amplified using the Expand Long Template PCR System (Boehringer/Roche Diagnostics) and the appropriate P1 clone as a template. The PCR reaction was performed with 1 ng DNA template, 2.5 μl buffer 3, 3.5 μl dNTP (2.5 mm), 2.5 μl each primer (300 mm), 0.5 μl Expand Taq (3.5 units/μl), H_2O to a final reaction volume of 25 μl. PCR cycles were 10 sec at 92° and 8 min at 68° (×30) and the reaction was done using a hot start. These probes served primarily for the detection of aberration breakpoints. Labeling of amplified fragments was done by incorporation of [α-^{32}P]dCTP through random priming.

RESULTS AND DISCUSSION

The genetic map of 38: *Procedure and definition of distal and proximal ends of the genetic map:* Cytological division 38 is completely eliminated by the deficiency *Df(2L)TW65.* We selected mutants that failed to complement this deficiency for detailed mapping, even though *Df(2L)TW65* extends into division 39. The distal end of *Df(2L)TW65* defines the distal end of our genetic map of 38 (Figure 1). A total of 41 deficiency stocks, providing a total of 54 breakpoints within or at the border of the interval, were used to genetically map all available lethal and sterile mutations to the smallest possible deficiency intervals. All mutations mapping to the same deficiency interval were tested against each other for cross-complementation. The most distal complementation group in-

TABLE 3

Genomic clones covering the 38 region

BAC or P1	Accession no.
BACR08D17	AC006402
DS00863	AC004364
DS01096	AC006215
DS02109	AC002443
DS04178	AC004735
DS04217	AC004759
DS05187	AC002503
DS05709	AC002474
DS05972	AC002445
DS08416	AC002442

cluded in this genetic map is *scw* (Figure 1), which cytologically maps to 38A1-2 (Arora *et al.* 1994). On the proximal end of the genetic map we included complementation groups that mapped to *Df(2L)DS9* but are excluded from *Df(2L)bur-K1*. On the basis of these criteria, *l(2)38EFd* is the most proximal complementation group. This gene was mapped to 38F5-6 by *in situ* hybridization (Spradling *et al.* 1999).

Not counting *P*-element insertion lines already known to be allelic to other lines, a total of 45 independent *P*-element lines (supplemental material 1 at http://www.genetics.org/supplemental) and 48 EMS, X-ray, and spontaneous alleles suspected to represent 27 potentially different complementation groups were considered (supplemental material 2 at http://www.genetics.org/supplemental). Fourteen of the *P*-element alleles targeted genes within the 38 interval that are essential for viability or fertility. These alleles define 7 complementation groups. The EMS, X-ray, and spontaneous mutations were found to define a total of 16 complementation groups that map to the 38 region. In addition, the frequently used marker gene *pr* also maps to 38. Because of overlap between these classes of complementation groups, the completed analysis revealed 21 distinct complementation groups (Figure 1).

In addition to these 21 complementation groups, *trans*-heterozygous combinations of deficiencies reveal seven more regions containing genetic functions essential for viability or fertility. The results of these crosses are shown in the appendix. Failure of complementation between *Df(2L)pr8311*, *Df(2L)pr49*, *Df(2L)pr1122*, *Df(2L)TW1*, and *Df(2L)TW161*, on one side, and *Df(2L)Sd37* and *Df(2L)TW50*, on the other side, appears to define a lethal and sterile region in 38A/B. This noncomplementation does not seem to be caused by a second-site mutation on the *Df(2L)TW50* or *Df(2L)Sd37* chromosome in the proximal 38 region, because even deficiencies that eliminate a large region from *barren* into cytological region 39/40 [*Df(2L)pr-M1, Df(2L)pr11163*] complement *Df(2L)TW50* and *Df(2L)Sd37*, whereas a comparatively small deficiency from *barren* to 38D/E (*Df(2L)pr8311*) does not. The fact that five deficiencies from different sources fail to complement at least part of the lethal and sterile region in 38A/B also argues against a second-site mutation in the 37D–38A region that causes the noncomplementation. The different phenotypes of *trans*-heterozygotes can best be explained by postulating the presence of four different subregions in the lethal and sterile region in 38A/B. From distal to proximal these are a lethal, a female sterile, a male sterile, and another lethal region (Figure 1).

Df(2L)Fs(2)Ket-RX32 has its distal breakpoint between *spir* and *l(2)38Db*, and *Df(2L)40* has its proximal break between these two complementation groups. These two deficiencies are lethal when *trans*-heterozygous (see the appendix). With the caveats discussed above, this result indicates the presence of at least one more essential gene in the 38C region.

The phenotype of *trans*-heterozygous combinations of deficiencies with breakpoints between *l(2)38Db* and *l(2)38Ea* define another two genetic functions in the 38D/E region (see the appendix). *Df(2L)DS5* and *Df(2L)DS9* have their distal breakpoints in this region and they were crossed to each of the following deficiencies that have their proximal breakpoints in this interval: *Df(2L)pr9201*, *Df(2L)pr-A16*, *Df(2L)pr37*, *Df(2L)pr2b*, and *Df(2L)pr8311*. The phenotypes of these various *trans*-heterozygous deficiency combinations can be best explained by making the following assumption: between the *l(2)38Db* and the *l(2)38Ea* complementation groups, from distal to proximal, there are a male sterile region and a (semi)-lethal region with escapers being female sterile. This raises the total of genetically identified functions and

Figure 1.—Genetic map of cytological region 38. The borders of 38 are indicated with two big arrowheads on top of the map. The Δ in the "mostly used allele" row indicates that the complementation group is defined by overlapping deficiencies. The cytology of *P*-element alleles is according to Spradling *et al.* (1999) and for the deficiencies according to FlyBase. Bold column borders separate complementation groups that map to different deficiency intervals. Faint column borders indicate that neighboring complementation groups could not be mapped relative to one another because they map to the same deficiency interval. Such complementation groups may therefore trade places in the future. The results of the complementation analyses are shown as follows: "mutant" indicates lethality, sterility, or a visible mutant phenotype (for *pr* only) and "wt" means wild type. In general, no or only very few escapers were found. However, for some alleles [*e.g.*, *l(2)03552*] escapers were often seen. A question mark means that the result was ambiguous and an asterisk means that the complementation test was done with an alternative allele and not with the mostly used one. "2nd" site means that the observed lethality is caused by a second-site mutation. It is not clear whether *l(2)38Aa* is a lethal allele of *pr* or a different gene.

gene name			l(2)37Fc	l(2)37Fe	scw	l(2)38Ac	lethal and sterile region in 38A/B				barr	vls	l(2)38Aa	pr	neb	l(2)38Ab	spir
							lethal in 38A/B	fs in 38A/B	ms in 38A/B	lethal in 38A/B							
mostly used allele			l(2)37Fc[1]	l(2)37Fe[1]	scw[l1]	l(2)k08115	Δ	Δ	Δ	Δ	l(2)k08103	vls[3]	l(2)38Aa[1]		l(2)03552	l(2)38Ab[1]	spir[3]
cytology of P-elements						38A5-6					38B1-2				38B4-6		
		further into region 37															
Df(2L)Acon-21		no															
Df(2L)be408					wt	wt				wt	mutant	mutant	wt	wt		wt	
Df(2L)bur-K1	39A2 ; 39B2-3	no															
Df(2L)DS5	38C7-10 ; 39D3-E1	no									wt				wt	wt	wt
Df(2L)DS6	38E2 ; 39E7	no									wt	wt	wt		wt	wt	wt
Df(2L)DS8	38E10-F3 ; 39C2-4	no															
Df(2L)DS9	38C7-D2 ; 39A1-3	no									wt					wt	wt
Df(2L)E55	37D2 ; 38A1	yes	mutant	wt/sl?		wt							2nd site				
Df(2L)Fs(2)Ket-RX32	38D1-3 ; 38E4-6	no													wt	wt	wt
Df(2L)pr-A16	37B2-10 ; 38D2-5	yes										mutant		mutant			mutant
Df(2L)pr-A20	38A1 ; 38B6-C1	no	wt	mutant	mutant	mutant					mutant	mutant	mutant	mutant	mutant	mutant	wt
Df(2L)pr-M1	38B3-6 ; 40A3				wt	wt				wt?	mutant	mutant		mutant	mutant		
Df(2L)pr1		no			wt						wt	wt	mutant		mutant	mutant	wt
Df(2L)pr2b	38B5 ; 38D2-E1	no			wt	wt	wt	wt	wt	wt	mutant	mutant	mutant	mutant	mutant	mutant	mutant
Df(2L)pr7		no									wt	wt	mutant	mutant	wt	wt	wt
Df(2L)pr11		no	wt	mutant		mutant							mutant		mutant	mutant	mutant
Df(2L)pr37	38B2-C1; 38E2-4	no			wt	wt	wt	wt	wt	wt	mutant	mutant?	mutant	mutant	mutant	mutant	mutant
Df(2L)pr40	38A2-5 ; 38C6-D1	no	wt	wt	wt	mutant					mutant	mutant	mutant	mutant	mutant	mutant	mutant
Df(2L)pr49	38B3-6 ; 38C6-10	no			wt	wt	wt	wt	wt	mutant	mutant	mutant	mutant	mutant	mutant	mutant	mutant?
Df(2L)pr1122		no			wt	wt	wt		mutant?	mutant	mutant			mutant	mutant		
Df(2L)pr1123		no				wt*					wt	wt	wt	mutant	wt?	wt	wt
Df(2L)pr8311		no			wt	wt	wt	wt?	mutant	mutant	mutant	mutant	mutant	mutant	mutant	mutant	mutant
Df(2L)pr9133		no				wt					wt	wt	wt	mutant	wt	wt	
Df(2L)pr9201		yes				mutant					mutant	mutant		mutant	mutant	mutant	mutant
Df(2L)pr9202		yes				mutant*					mutant	mutant		mutant	mutant	mutant	wt
Df(2L)pr9204		no	wt	wt	wt	mutant					mutant	mutant	mutant	mutant		mutant	
Df(2L)pr11163		no			wt	wt			wt	wt?	mutant	mutant		mutant	mutant		mutant
Df(2L)pr11164		no				wt					wt	wt	mutant	mutant	wt		
Df(2L)Sd14	37D2 ; 38C1-2	yes				mutant*								mutant	mutant?	mutant	wt
Df(2L)Sd37	37D2-5 ; 38A6-B2	yes?	mutant	mutant	mutant	mutant				mutant	wt	wt			wt		
Df(2L)Sd57	37C6-7 ; 38 C1-2	yes												mutant	mutant	mutant	wt
Df(2L)Sd77	37C2-7 ; 38C1-2	yes				mutant								mutant	mutant	mutant	wt
Df(2L)TW1	38A7-B1; 39C2-3	no			mutant	wt	wt	mutant	mutant	mutant	mutant		mutant				
Df(2L)TW2	37D2-E1; 38E6-9	yes				mutant*								mutant			
Df(2L)TW9	37E2-F1 ; 38B5-C1	yes		mutant		mutant					mutant	mutant		mutant	mutant	mutant	wt
Df(2L)TW50	36E4-F1 ; 38A6-7	yes		mutant	mutant	mutant	mutant	mutant	mutant	wt	wt	wt	2nd site	wt		wt	wt
Df(2L)TW65	38A1 ; 39F1	no	wt	wt	mutant	mutant					mutant			mutant	mutant		mutant
Df(2L)TW84	38A1; 39D3-E1	no	wt	mutant		mutant*								mutant			
Df(2L)TW150	37F5-38A1 ; 38B5-C1		wt	mutant		mutant					mutant	mutant*		mutant	mutant	mutant	wt
Df(2L)TW158	37B2-8 ; 37E2	yes	wt								wt		2nd site			wt	
Df(2L)TW161	38A6 ; 40A4-B1	no	wt	wt	wt	wt	mutant				mutant	mutant	mutant	mutant			
Df(2L)VA6	37D2 ; 38C6-E3	yes												mutant			
Df(2L)VA12	37C2-5 ; 38B5-C1	yes		mutant							mutant			mutant	mutant	mutant	wt
Df(2L)VA17	37C1 ; 37F5	yes	mutant	wt?	wt	wt											
Df(2L)VA19	37C2-7 ; 38A6-B1	yes		mutant		mutant					mutant			mutant	mutant	mutant	wt
synonyms					l(2)37Ff						barren	valois	l(2)E124P	purple	nebbish	l(2)A113	spire
					screw							val			Mothra		p150-Spir
															KLP 38B		
alleles			l(2)37Fc[1]	l(2)37Fe[1]	scw[l1]	l(2)k08115					l(2)k08103	vls[1]	l(2)38Aa[1]		l(2)k03903	l(2)38Ab[1]	spir[3]
						l(2)k15716						vls[3]			l(2)03552		spir[PJ56]
						fs(2)ltoPP43									l(2)05217		
															l(2)k05702		
															l(2)09314		

gene name		lethal reg. in 38C	l(2)38Db	lethal & sterile region in 38D/E		l(2)38Ea	Hr38	l(2)38Eb	Fs(2)Ket	dia	l(2)38EFa	Bl	cad	l(2)38EFb	l(2)38EFg	l(2)38EFd	
		lethal in 38C		ms in 38D/E	lethal in 38D/E												
mostly used allele		Δ	l(2)38Db[52]	Δ	Δ	l(2)38Ea[36]	l(2)02306	l(2)38Eb[54]	Fs(2)Ket[RX3]	l(2)k07135	l(2)38EFa[2]	Bl[1]	cad[3]	l(2)38EFb[8]	l(2)k13715	l(2)04530	
cytology of P-elements							38E1-2			38E5-6			38E2-E9		38F1-2	38F5-6	
																	into region 39
Df(2L)Acon-21										wt	wt	wt	wt	mutant	mutant	mutant	yes
Df(2L)be408																	no?
Df(2L)bur-K1	39A2 ; 39B2-3	wt													wt	wt	yes
Df(2L)DS5	38C7-10 ; 39D3-E1		wt	mutant	mutant	mutant	mutant		mutant	mutant	mutant		mutant	mutant	mutant	mutant	yes
Df(2L)DS6	38E2 ; 39E7					wt	mutant	mutant	mutant			mutant?	mutant		mutant	mutant	yes
Df(2L)DS8	38E10-F3 ; 39C2-4						wt		wt	wt	wt	wt	wt	wt	mutant	mutant	yes
Df(2L)DS9	38C7-D2 ; 39A1-3		wt	mutant	mutant	mutant*	mutant	mutant	mutant	mutant	mutant	mutant?	mutant?	mutant	mutant	mutant	no
Df(2L)E55	37D2 ; 38A1								wt*								no
Df(2L)Fs(2)Ket-RX32	38D1-3 ; 38E4-6	mutant	mutant			mutant	mutant	mutant*	mutant	mutant	wt	wt	wt	wt	wt	wt	no?
Df(2L)pr-A16	37B2-10 ; 38D2-5		mutant	mutant	wt	wt			wt	wt		wt	wt			wt?	no
Df(2L)pr-A20	38A1 ; 38B6-C1		wt						wt*			wt	wt			wt	no
Df(2L)pr-M1	38B3-6 ; 40A3																yes
Df(2L)pr1							wt		wt	wt*		wt					no
Df(2L)pr2b	38B5 ; 38D2-E1		mutant		mutant	wt	wt		wt	wt*		wt	wt		wt	wt	no
Df(2L)pr7							wt		wt	wt?							no
Df(2L)pr11			mutant		mutant	mutant	mutant	wt	wt	wt?	wt		wt		wt		no
Df(2L)pr37	38B2-C1; 38E2-4		mutant	mutant	mutant	wt	wt		wt	wt		wt	wt				no
Df(2L)pr40	38A2-5; 38C6-D1	mutant	wt				wt		wt				wt		wt	wt	no
Df(2L)pr49	38B3-6; 38C6-10	wt	wt				wt		wt			wt	wt		wt	wt	no
Df(2L)pr1122									mutant	mutant	mutant			mutant	mutant	mutant	yes?
Df(2L)pr1123									wt						wt?	wt?	no
Df(2L)pr8311			mutant		mutant	wt	wt		wt				wt		wt	wt	no
Df(2L)pr9133									wt				wt				no
Df(2L)pr9201			mutant	wt	wt		wt		wt	wt			wt			wt	no
Df(2L)pr9202		wt	wt						wt				wt				no
Df(2L)pr9204					mutant	mutant			mutant	mutant					mutant	mutant	yes
Df(2L)pr11163						mutant	mutant	mutant	mutant	mutant			mutant		mutant	mutant	yes
Df(2L)pr11164									wt								no
Df(2L)Sd14	37D2; 38C1-2	wt	wt			wt	wt		wt			wt	wt				no
Df(2L)Sd37	37D2-5; 38A6-B2																no
Df(2L)Sd57	37C6-7 ; 38 C1-2												wt				no
Df(2L)Sd77	37C2-7; 38C1-2		wt						wt				wt			wt?	no
Df(2L)TW1	38A7-B1; 39C2-3																yes
Df(2L)TW2	37D2-E1; 38E6-9					mutant	mutant		mutant	mutant	mutant	mutant	mutant	mutant	mutant	wt	no
Df(2L)TW9	37E2-F1 ; 38B5-C1		wt						wt				wt			wt	no
Df(2L)TW50	36E4-F1 ; 38A6-7		wt						wt	wt*		wt				wt	no
Df(2L)TW65	38A1 ; 39F1						mutant	mutant	mutant	mutant*		mutant	mutant		mutant	mutant	yes
Df(2L)TW84	38A1; 39D3-E1									mutant							yes
Df(2L)TW150	37F5-38A1 ; 38B5-C1		wt						wt				wt				no
Df(2L)TW158	37B2-8 ; 37E2																no
Df(2L)TW161	38A6 ; 40A4-B1								mutant*								yes
Df(2L)VA6	37D2 ; 38C6-E3						mutant			mutant	mutant	mutant	mutant	wt	wt	wt	no
Df(2L)VA12	37C2-5 ; 38B5-C1		wt									wt	wt				no
Df(2L)VA17	37C1; 37F5																no
Df(2L)VA19	37C2-7 ; 38A6-B1		wt						wt*				wt			wt	no
synonyms							Dhr38		ketel	diaphanous		Bristle	caudal		l(2)01528		
									fs(2)ltoΔ2	ms(2)04138							
									fs(2)ltoHD43								
alleles			l(2)38Db[52]			l(2)38Ea[36]	l(2)02306	l(2)38Eb[27]	Fs(2)Ket[RX3]	l(2)k07135	l(2)38EFa[2]	Bl[1]	cad[3]	l(2)38EFb[8]	l(2)01528	l(2)38EFd[15]	
						l(2)38Ea[41]		l(2)38Eb[51]	Fs(2)Ket[31]	ms(2)04138					l(2)k13715	l(2)04530	
						l(2)38Ea[46]		l(2)38Eb[54]	Fs(2)Ket[49]	dia[38]							
						l(2)38Ea[47]		l(2)38Eb[63]	fs(2)ltoΔ2[1]	dia[40]							
						l(2)38Ea[66]				dia[50]							
										dia[55]							

FIGURE 1.—*Continued.*

complementation groups to 28. A small number of the results shown in the APPENDIX are inconsistent with the proposed genetic map. These inconsistencies presumably result from additional hits on the chromosome. This is likely because many of these strains have been kept balanced in the laboratory for many years and, without selection, they accumulate additional lethal mutations and dominant modifiers.

The stock *l(2)38Aa[1]* and some of the deficiency stocks used for the analysis, *Df(2L)be408* and in particular *Df(2L)Fs(2)Ket-RX32*, had additional lethal mutations elsewhere on the chromosome (see the APPENDIX and supplemental material 2 at http://www.genetics.org/supplemental/), thus complicating the complementation analysis. The second-site hit for *l(2)38Aa[1]* is in *l(2)37Ea*. Noncomplementation due to this second-site hit with deficiencies in the 37 region is indicated on Figure 1 as such. According to FlyBase (SPRADLING *et al.* 1999), *l(2)k13715* has several *P*-element hits, one in 38F1-2 [allelic with *l(2)01528*], one in 87C6-7, and another lethal hit in 39A [allelic with *l(2)05287*]. We do not have any indication that our *l(2)k13715* chromosome carries more than the lethal mutation in 38F [allelic with *l(2)01528*] because it complements *l(2)05287* and *Df(2L)burK1*, a small deficiency that removes *l(2)05287*.

Phenotypes of complementation groups in 38: A total of 23 of the 28 genetically identified functions shown in Figure 1 have recessive lethal phenotypes. Two are male sterile [the 38A/B region between the proximal breakpoint of *Df(2L)TW50* and the distal breakpoint of *Df(2L)pr8311* and the 38D/E region between the proximal breakpoint of *Df(2L)pr-A16* and the distal breakpoints of *Df(2L)DS5* and *Df(2L)DS9*] and three are female sterile [*spire*, *vls*, and the female sterile defined by the distal breakpoints of *Df(2L)pr8311* and *Df(2L)TW1* in the lethal and sterile region in 38A/B]. Several of the lethal complementation groups have additional phenotypes: a recessive female sterile allele [*fs(2)ltoPP43*] is allelic to *l(2)38Ac*, *Ketel* has dominant and recessive female sterile alleles, and the essential gene *diaphanous* has a male sterile allele [*ms(2)04318*] and a maternal effect lethal allele (CASTRILLON *et al.* 1993; AFSHAR *et al.* 2000). Hypomorphic mutations of the recessive lethal *pr* have a recessive visible phenotype. Various alleles of *Bristle* are either recessive lethal or female sterile and show different dominant and recessive visible phenotypes. *spire* and *Bristle* were mapped by their female sterility and *vls* by its grandchildless phenotype.

The physical and transcript map of 38: Most of the work was done with the sequence provided by the BDGP (http://www.fruitfly.org), which initially subdivided the region in different contigs that were available from the BDGP website (http://www.fruitfly.org/sequence/drosophila-regions.html; Table 3). Once the complete genome sequence became available (ADAMS *et al.* 2000), this map was updated, the sequences of nine P1s and one BAC were fused, and transcription units renamed. We chose to set the limits for the physical map on the basis of contigs sequenced by BDGP. The entire region is ~760 kb and covers the cytological regions 38B–38E (Figure 2). We have subdivided the physical map of 38 into the alphabetical subregions. The border between the subregions is placed according to the *in situ* mapping data for the P1 clones and BACs used to establish the sequence.

Identification of transcription units: All mapping of transcription units was based on predictions by the gene-finding program GENSCAN (BURGE and KARLIN 1997). Additional information, such as homology to other polypeptides and EST hits, was determined separately by sequence database searches. Polypeptide homologies were established using the BLASTP program of BLAST (ALTSCHUL *et al.* 1990) and EST hits were identified using the BDGP BLAST server (http://www.fruitfly.org/

FIGURE 2.—Molecular map of the cytological region 38B–38E. The blue line represents the genomic DNA with a black mark every 10 kb. Triangles above the blue line indicate insertion sites for *P* elements for which the lethality or sterility was genetically mapped (see also Figure 1). The extension of the sequenced clones is shown with green arrows. The cytology of the P1s was used to define the cytology of the region. Hence, 38B is defined as starting with DS00863 and ending immediately before the start of DS01096 (position 1 → 78,614); 38C is defined by the beginning of DS01096 and it ends where DS08416 starts (position 78,615 → 406,914); 38D is defined by the beginning of DS08416 and ends immediately before DS04217 ends (position 406,915 → 616,781); and 38E is defined by DS04217 (position 616,782 → 759,580). A total of 759,580 bp (black line) were analyzed with the GENSCAN gene prediction program, and a total of 135 transcription units were predicted. According to the position of their 5′ ends, they were named *38B.1–38B.23*, *38C.1–38C.56*, *38D.1–38D.32*, and *38E.1–38E.24* (in this figure "38" is omitted from their name). These cytological designations may not always precisely reflect the cytological position of the transcription unit. *: *38B.23*, *38D.32*, and *38E.24* are nested genes identified by using an appropriate genomic fragment. Transcripts for these three genes identified by Northern blots may therefore originate from a neighboring gene. *38B.23* and *38E.24* were identified with the ISOCHORE2 of GENSCAN. A total of 63 transcription units are encoded on the positive strand (arrows above the black line) and 72 on the negative strand (arrows under the black line). The transcription units that correspond to known genes bear their names written in black, and the ones that correspond to known genes for which mutant fly stocks are available also bear their names in red below. The genomic region to which deficiency breakpoints were mapped through restriction fragment length polymorphism is indicated with dashed bidirectional brown arrows. The extent of the deleted genomic region of the corresponding deficiency is shown with a brown line. An arrowhead at the end of a brown line means that the deficiency continues beyond the analyzed region. A number of complementation groups shown in Figure 1 could not be mapped to a single transcription unit. The physical interval to which they were mapped by various methods is shown with red dashed bidirectional arrows.

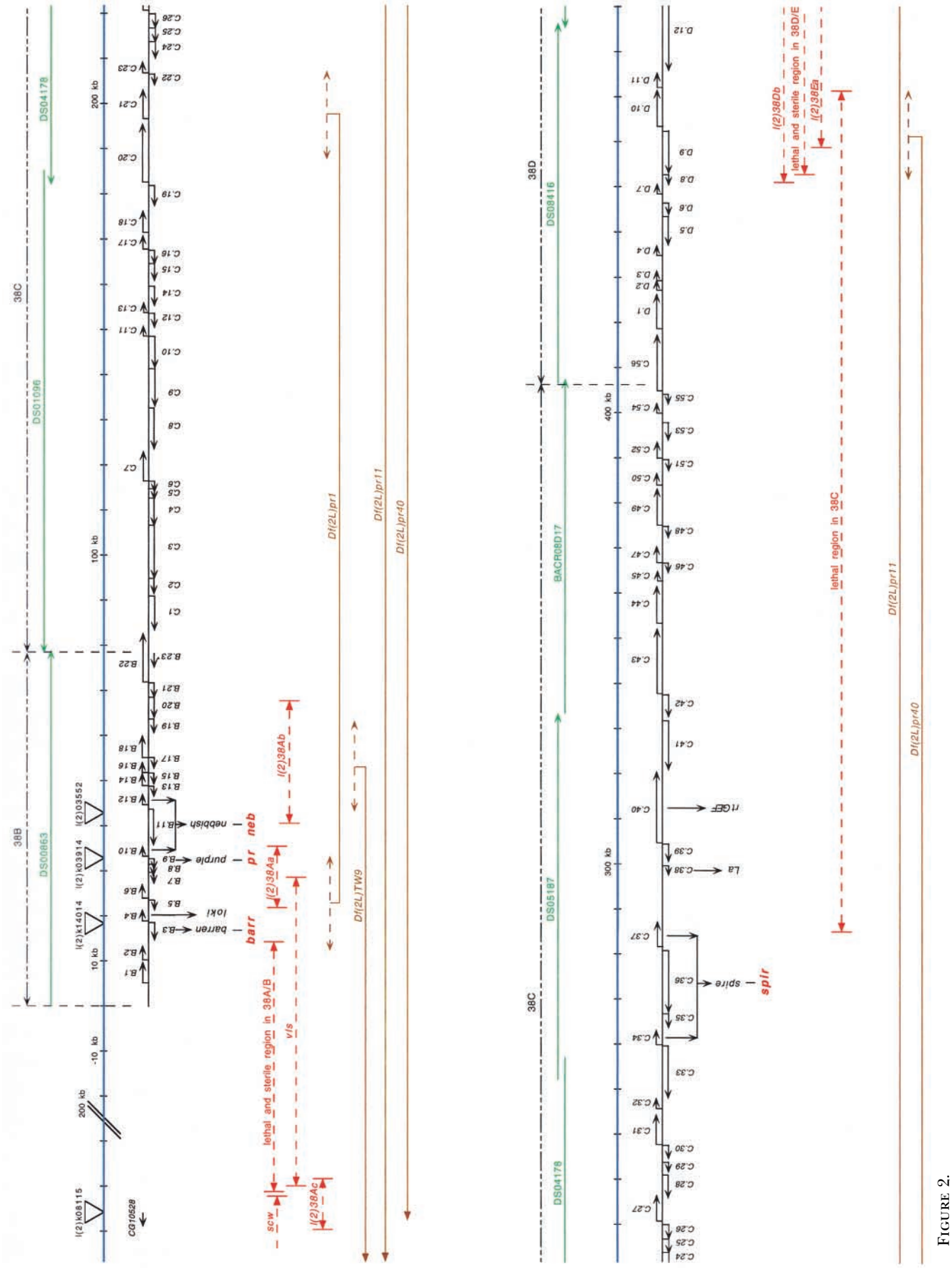
38C
38B
38D
DS04178
DS01096
DS00863
DS09416
BACR08D17
DS05187
200 kb
100 kb
10 kb
-10 kb
300 kb
400 kb
l(2)k08115
l(2)k14014
l(2)k03914
l(2)03552
CG10528
barren
ioki
purple
nebbish
barr
pr
neb
scw
lethal and sterile region in 38A/B
v/s
l(2)38Ab
l(2)38Aa
l(2)38Ac
Df(2L)pr1
Df(2L)TW9
Df(2L)pr11
Df(2L)pr40
rtGEF
La
spire
spir
l(2)38Db
lethal and sterile region in 38D/E
l(2)38Ea
lethal region in 38C

FIGURE 2.

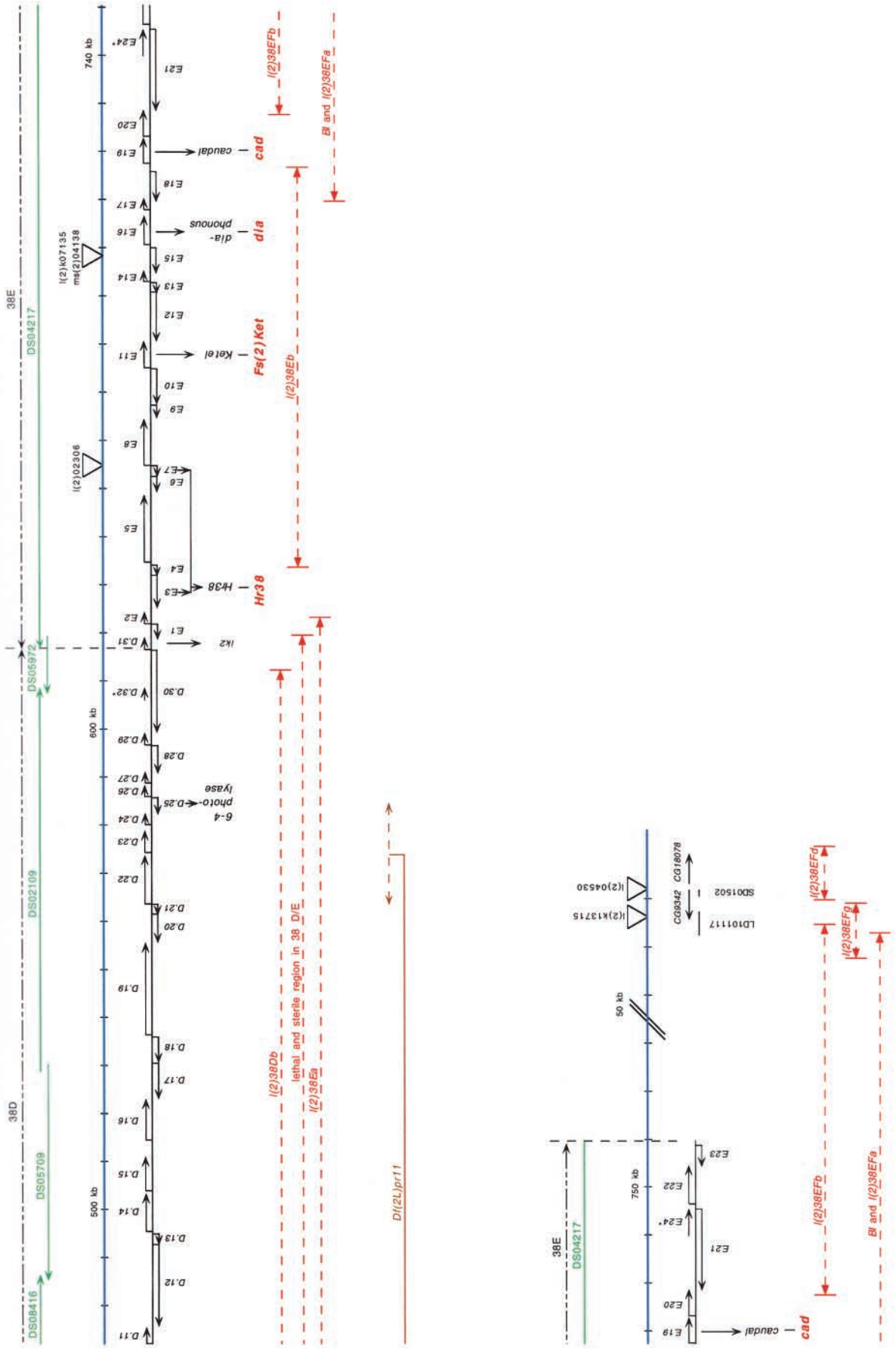

FIGURE 2.—*Continued.*

blast/) against the Drosophila EST data set. The probability scores provided in the following text for each prediction refer to the exon to which the probe was designed; most of the time this is also the exon with the highest probability score for that particular prediction. Figure 2 shows the position and direction of the predicted transcription units. Table 4 and supplemental material 3 (at http://www.genetics.org/supplemental/) list their names, positions, homologies, probability scores, and other characteristics.

GENSCAN predictions and expression of predicted genes: GENSCAN predicted a total of 135 genes to lie within the 760 kb of sequence analyzed. Of these, 17 correspond to genes that have previously been characterized and another 22 are at least partially homologous to mobile genetic elements such as transposons and retroviruses (Table 5). To test these gene predictions and to determine the expression patterns of predicted genes, probes were designed for 121 known and predicted genes, and developmental Northern blots containing mRNA from six different stages and tissues were probed. The chosen stages reflect most of the fly life cycle plus isolated ovaries. In total, these experiments allowed us to determine the expression pattern for an additional 64 of the 96 potential new transcription units (in addition to the previously published ones and the mobile elements). GENSCAN predictions, the autoradiographs of Northern blots, and a summary table of their developmental expression profile can be seen on http://www.mcgill.ca/Biology/labs/MDGP/transcripts.html.

About half of the predicted genes, 68, had similarity to known proteins. Similarity was determined using BLASTP and matches with a smallest sum probability of $<1 \times 10^{-4}$ were noted. Of the 46 predicted polypeptides with a homology other than to mobile elements, 45 or 98% were confirmed experimentally by detection of a signal on a Northern blot.

Thirty-six, or about one-half of the 67 predicted peptides with no significant homology, revealed detectable transcripts on Northern blots. EST searches carried out for the remaining 31 predictions validated an additional 3. In cases where probes were designed to an exon with a probability score of <0.5, 57% failed to give a signal on a blot. Most primers were designed to amplify one exon of each predicted ORF in order to generate a probe for use on developmental Northern blots. Lack of detection of transcripts by specific probes accordingly may be caused either by lack of mRNA expression or accumulation or by alternative processing that eliminates this particular exon in a specific stage and tissue.

Large number of male-specific transcripts: The poly(A)$^+$ mRNA used on each blot was isolated from 0- to 4-hr embryos, 8- to 20-hr embryos, the three larval instars, mature males, mature females, and ovaries. In general, the majority of transcripts detected were not ubiquitous to all stages and differential expression was detected in >80% of cases. No obvious clustering of transcripts with similar expression profiles was noted.

Of the 64 new predictions that we confirmed by Northern blots, 24 had at least one transcript that is expressed only in mature males. Nine of the probes did not detect any other transcript. The absence of transcripts in the other stages we analyzed may suggest that the gene has a male-specific role. However, the large number of such transcripts argues against this interpretation because only two genes with male sterile phenotypes are known to map to 38. One of these is an allele of *diaphanous* and the other is *ms(2)38C* (Brittnacher and Ganetzky 1983), which cytologically maps to 38C5-C10. While it is possible that phenotypes for the other genes may be found with appropriate mutant screens, it seems just as likely that adult males express many genes that are not essential for viability and normal development and are shut down in females and most other developmental stages.

Only one of the 10 predictions encoding male-specific transcripts, 38C.2, has homology to a known gene; 38C.2 is homologous to *antigen 5-related*, a gene encoded on the X chromosome of *D. melanogaster*, which in turn has partial homology to a sperm-coating protein from rat epididymis. However, a direct search with 38C.2 did not pick up the sperm-coating protein. There is one more prediction with homology to a male-specific protein, 38D.26, which is homologous to a human sperm acrosomal protein. Surprisingly, however, the 38D.26 transcript is expressed in all stages of development and much more strongly in females, ovaries, and 0- to 4-hr embryos than in males.

Genome organization in the 38 region: A heterochromatic region of close to 200 kb extending from proximal 38B to the 38C region [between *l(2)38Ab* and *spir*] contains a large number of mobile genetic elements. This region is highly repetitive in nature and contains no complementation group. On the basis of 81 confirmed transcription units that do not correspond to mobile elements (Table 4 and supplemental material 3 at http://www.genetics.org/supplemental), the overall gene density in the 38 region is approximately one gene in 9.2 kb. However, in some parts of the region the gene density can be much higher than that, reaching one gene per 3.2 kb in the central part of 38B.

Alignment of genetic and physical maps: The alignment of the genetic and the physical map was achieved primarily by using two sources of sequence tag sites (STS). The cloning of a number of genes from the 38 region was reported in the past few years. These include *barr* (Bhat *et al.* 1996), *pr* (Kim *et al.* 1996), *neb* (Alphey *et al.* 1997; Molina *et al.* 1997; Ohkura *et al.* 1997; Ruden *et al.* 1997), *spir* (Wellington *et al.* 1999), *Hr38* (Kozlova *et al.* 1998), *Fs(2)Ket* (Erdelyi *et al.* 1997), *dia* (Castrillon and Wasserman 1994), *cad* (Mlodzik *et al.* 1985), and others. Additional STS were obtained by sequencing the genomic regions flanking the *P*-element insertion sites. After eliminating known allelic *P*-element

TABLE 4

Correspondence of predicted genes used in this study with previously described genes

Gene prediction	Previously described gene	Gene prediction	Previously described gene	Gene prediction	Previously described gene	Gene prediction	Previously described gene	Gene prediction	Previously described gene
38B.1		*38C.5*	TE	*38C.32*		*38D.3*		*38D.30*	*CG17466, CG17465*
38B.2	*CG13968*	*38C.6*	TE	*38C.33*	TE	*38D.4*		*38D.31*	*ik2*
38B.3	*barr*	*38C.7*	TE	*38C.34*	*spir* (5′)	*38D.5*	*CG15476*	*38D.32*[b]	
38B.4	*lok*	*38C.8*		*38C.35*		*38D.6*		*38E.1*	*CG1962*
38B.5	*CG10728*	*38C.9*	TE	*38C.36*		*38D.7*		*38E.2*	*CG2617*
38B.6	*CG13969*	*38C.10*	TE	*38C.37*	*spir* (3′)	*38D.8*		*38E.3*	*Hr38* (3′)
38B.7	*CG10730*	*38C.11*	TE	*38C.38*	*La*	*38D.9*		*38E.4*	
38B.8		*38C.12*	TE	*38C.39*		*38D.10*		*38E.5*	
38B.9	*pr*	*38C.13*	TE	*38C.40*	*rtGEF*	*38D.11*		*38E.6*	
38B.10	*neb* (5′)	*38C.14*	TE	*38C.41*	*CG11012, CG16798*	*38D.12*		*38E.7*	*Hr38* (5′)
38B.11	*CG10746*	*38C.15*		*38C.42*	*CG10947*	*38D.13*		*38E.8*	
38B.12	*neb* (3′)	*38C.16*	TE	*38C.43*		*38D.14*		*38E.9*	*CG9316*
38B.13	*CG10747*	*38C.17*	TE	*38C.44*	TE	*38D.15*		*38E.10*	*CG9318, CG9317*
38B.14	*CG10721*	*38C.18*		*38C.45*		*38D.16*	*CG17485*, TE	*38E.11*	*Fs(2)Ket*
38B.15	*CG10756*	*38C.19*		*38C.46*	*CG10949*	*38D.17*		*38E.12*	TE
38B.16	*CG10722*	*38C.20*		*38C.47*	*CG10954*	*38D.18*		*38E.13*	*CG9319*
38B.17	*CG10757*	*38C.21*		*38C.48*	*CG15130*	*38D.19*	*CG15475*	*38E.14*	*CG9320*
38B.18	*CG10723*	*38C.22*	*CG17571*[a]	*38C.49*	*CG18597, CG11019*	*38D.20*		*38E.15*	
38B.19	*CG13970, CG13971*, TE	*38C.23*		*38C.50*		*38D.21*		*38E.16*	*dia*
38B.20	*CG13971*, TE	*38C.24*	*CG17570*[a]	*38C.51*		*38D.22*		*38E.17*	
38B.21	TE	*38C.25*		*38C.52*	*CG11017*	*38D.23*	*CG17472*	*38E.18*	
38B.22	TE	*38C.26*		*38C.53*	*BcDNA.GH02384*	*38D.24*		*38E.19*	*cad*
38B.23[b]		*38C.27*	TE	*38C.54*	*CG2508*	*38D.25*	*phr6-4, CG17470*	*38E.20*	*CG9324*
38C.1		*38C.28*	TE	*38C.55*	*CG2493*	*38D.26*	*CG2608*	*38E.21*	*CG9326*
38C.2	*CG10651*	*38C.29*		*38C.56*		*38D.27*	*CG2611*	*38E.22*	*CG9328*
38C.3		*38C.30*		*38D.1*		*38D.28*	*CG2478*	*38E.23*	*CG9329*
38C.4	TE	*38C.31*	*CG12617*	*38D.2*		*38D.29*	*CG2614*	*38E.24*[b]	

Note that there are often differences between our gene predictions and the previously described genes. TE identifies predicted transposable elements (see also Table 5).

[a] CG17571 and CG17570 were mapped to 39DE by Adams *et al.* (2000).

[b] 38B.23, 38D.32, and 38E.24 are nested genes predicted by using an appropriate genomic fragment. For 38B.23 and 38E.24 ISOCHORE2 of GENSCAN was used.

TABLE 5

Repetitive genes and their corresponding mobile elements

Prediction	Homologous gene	Repetitive element	Accession no.	BLASTp score (hits)	BLASTp expect[a]
38B.19	Reverse transcriptase	*D. yakuba* retrotransposon Helena	gi|3282366 (AF012049)	92.4	8e -18,
38B.20	Reverse transcriptase	*D. melanogaster* BS element	pir‖S55544	471	e- 131
38B.21	Reverse transcriptase	*D. yakuba* retrotransposon Helena	gi|3282366 (AF012049)	153	2e -36
38B.22	Gag-specific protein	*D. melanogaster* mobile element jockey	gi|158625 (M38643)	100	2e -20
38C.4	Hypothetical protein	*D. melanogaster* transposon pogoR11	pir‖S20478	943	0
38C.5	Hypothetical protein	*D. melanogaster* transposon pogoR11	pir‖S20478	250	4e -66
38C.6	Hypothetical protein	*D. melanogaster* transposon pogoR11	pir‖S20478	250	4e -66
38C.7	Polyprotein	*D. melanogaster* retrotransposon copia	pir‖OFFFCP	1967	0
38C.9	Prgag-pol	*D. melanogaster* gypsy transposable element	gi|2801517 (AF033821)	1992	0
38C.10	Prgag-pol	*D. melanogaster* gypsy transposable element	gi|2801517 (AF033821)	1992	0
38C.11	Prgag-pol	*D. melanogaster* gypsy transposable element	gi|2801517 (AF033821)	1992	0
38C.12	Transposase HFL1	*D. melanogaster* hobo element	pir‖A39652	255	1e -67
38C.13	Transposase HFL1	*D. melanogaster* hobo element	pir‖A39652	80.8	3e -15
38C.14	Reverse transcriptase	*D. melanogaster* F-element	pir‖A32713	91.3	1e -17
38C.16	Reverse transcriptase	*D. melanogaster* F-element	pir‖A32713	91.3	1e -17
38C.17	Reverse transcriptase	*D. melanogaster* F-element	pir‖A32713	56.6	2e -07
38C.27	Polyprotein	*D. melanogaster* retrotransposon copia	pir‖OFFFCP	2044	0
38C.28	orf	*D. melanogaster* retrotransposon mdg1	emb|CAA65152.1| (X95908)	561	e -158
38C.33	Hypothetical protein 4	*D. melanogaster* retrotransposon mdg1	pir‖S70430	1379	0
38C.44	Polyprotein	*D. melanogaster* blastopia	pir‖S38635	2593	0
38D.16	Hypothetical protein 4	*D. melanogaster* retrotransposon mdg1	pir‖S70430	1714	0
38E.12	Reverse transcriptase	*D. melanogaster* ZAM retroelement	emb|CAA04050| (AJ000387)	1245	0

[a] Expectation value: number of sequences in the database that are expected to give the same or higher score of alignment by chance (Altschul *et al.* 1990).

l(2)k09314 **3'end** (between *purple* and *nebbish*)**:**

GCCGCCCTTCACTAGAAAAAAAATAACAAACGACGCCGCGCAATAATTCGAAA

CTCTTCGGC

l(2)k13715 **3'end** (in 38F)

TTGCACTATCACCATTTCTATGATTCATTTGTTATTTTGATAATAAATTGCAATTC

ACGTGGACTAAAGGGTTCAATCATGATATGATTGTTCTTTGTGATTCATTGTG

FIGURE 3.—The stocks *l(2)k09314* and *l(2)k13715* have a *P*-element insertion in 38. DNA adjacent to the 3′ end of these *P* elements was sequenced and the generated STS are shown. The sequences were submitted to GenBank [accession nos.: AZ575479 for *l(2)k13715* and AZ575480 for *l(2)k09314*].

insertions, 14 *P* elements that cytologically mapped to the 38 interval were found to have their lethality in the 38 region. Of these 10 are alleles of either *barren*, *nebbish*, *Hr38*, or *diaphanous*, all of which are cloned and thus their positions already known on the physical map. Supplemental material 1 (http://www.genetics.org/supplemental) summarizes the information about these *P* elements. The BDGP has generated STS from the 5′ ends of the *P* elements disrupting the complementation groups *l(2)38Ac* and *l(2)38EFd*. The remaining *P*-element lines are stocks containing two insertions and no STS were made from these lines. The first line, *l(2)k09314*, genetically maps within the 38B interval but proved difficult to map more finely as many crosses result in semilethal phenotypes. The second line, *l(2)k13715*, is allelic to *l(2)01528* and together they define the new complementation group *l(2)38EFg*. We generated 3′-end STS for the *P*-element insertions in *l(2)k09314* and *l(2)k13715* (Figure 3). Each STS was tested for alignment to the 38 sequence. One of the STS generated from *l(2)k09314* aligned between *purple* and *nebbish* (GenBank accession no. AZ575480). It localizes between the predicted *nebbish* promoter and the transcription start site (Figure 2). One of the STS from *l(2)k13715* matched to the 38F region and is at the proximal end of the map in Figure 2 (GenBank accession no. AZ575479).

Physical mapping of selected deficiency breakpoints: We mapped selected deficiency breakpoints to further align the genetic and physical maps. To reduce the number of polymorphisms in the analyzed heterozygous stocks, all but two deficiency chromosomes were first crossed to an isogenic balancer chromosome (*CyO bw*). The exceptions are *Df(2L)pr1* and *Df(2L)pr11*, which were induced on a *CyO* balancer chromosome. These deficiencies were kept over a *Sco* chromosome and were always blotted side by side where they could serve as controls for each other.

Genomic fragments of 10 kb were amplified and used to probe filters containing restriction-digested genomic DNA from balanced deficiency flies. A total of 317.5 kb of sequence was covered in these experiments. Five deficiency breakpoints were identified in this way, and their locations are shown in Figure 2. One of the breakpoints, distal *Df(2L)pr1*, breaks genetically between *vls* and *pr* in 38B. Two deficiency breakpoints, proximal *Df(2L)TW9* and proximal *Df(2L)pr1*, fall between *l(2)38Ab* and *spire*. Identification of the physical location of these last two breakpoints places *l(2)38Ab* distal to position 70 kb and *spire* proximal to position 185 kb. The recent cloning of *spire* confirmed that it maps to the region between 260 kb and 290 kb (Figure 2 and WELLINGTON *et al.* 1999). Although the breakpoints of these two deficiencies are genetically in the same region, physically they are separated by ~130 kb. Two other breakpoints were uncovered proximal to *spire*: proximal *Df(2L)pr40* and proximal *Df(2L)pr11*. Identification of *Df(2L)pr40* places the lethal region in 38C distal to position 475 kb, and *l(2)38Db*, the lethal and sterile region in 38D/E, and *l(2)38Ea* proximal to position 450 kb (Figure 2). The molecular mapping of proximal *Df(2L)pr11* contradicts the genetic mapping. Genetically, the deficiency removes *Hr38*, but its molecular mapping puts the breakpoint distal to *Hr38*. It is therefore possible that this deficiency chromosome has an additional mutation in the *Hr38* gene.

Conclusion: The expression profiling of the predicted transcripts of the 38 region provides experimental evidence for 81 of 113 predicted single copy genes. The developmental profile gives further useful information for researchers with interest in developmental biology. The high resolution genetic map of the 38 region presented here identifies the genetic breakpoints of 41 deficiency chromosomes. The analysis of the various types of genetic aberrations in the region revealed a total of 28 functions on this map. By creating new links between the genetic and the physical map we were able to further improve the genetic map's resolution. The detailed map now provides the *D. melanogaster* research community with the necessary information to more efficiently use the genetic resources available in region 38.

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APPENDIX

Mutant regions defined by complementation tests with deficiencies

Cross	Viability	Df/Df + Df/CyO	Female fertility	Male fertility	Comments
		38A/B[a]			
Df(2L)TW50 × *Df(2L)be408*	Lethal	0 + 205			Lethality is caused by a second-site mutation somewhere else
Df(2L)Sd37 × *Df(2L)be408*	Viable	52 + 90	Fertile		
Df(2L)TW50 × *Df(2L)pr2b*	Viable	56 + 115			
Df(2L)TW50 × *Df(2L)pr2b*	Viable	74 + 95	Fertile		
Df(2L)TW50 × *Df(2L)pr2b*				Fertile	
Df(2L)Sd37 × *Df(2L)pr2b*	Viable	38 + 64	Fertile	Fertile	
Df(2L)TW50 × *Df(2L)pr37*	Viable	68 + 151			
Df(2L)TW50 × *Df(2L)pr37*	Viable	65 + 66	Fertile		
Df(2L)TW50 × *Df(2L)pr37*				Fertile	
Df(2L)Sd37 × *Df(2L)pr37*	Viable	57 + 99	Fertile	Fertile	
Df(2L)TW50 × *Df(2L)pr11163*	Semilethal	31 + 187			
Df(2L)TW50 × *Df(2L)pr11163*	Semilethal	32 + 126	Subfertile	Fertile	
Df(2L)Sd37 × *Df(2L)pr11163*	Viable	59 + 159	Subfertile	Fertile	
Df(2L)TW50 × *Df(2L)pr-M1*	Semilethal	16 + 116	Fertile		
Df(2L)TW50 × *Df(2L)pr-M1*				Fertile	
Df(2L)pr-M1 × *Df(2L)Sd37*	Viable	38 + 89	Fertile	Fertile	
Df(2L)TW50 × *Df(2L)pr49*	Viable	47 + 135			
Df(2L)TW50 × *Df(2L)pr49*	Viable	36 + 99	Subfertile		
Df(2L)TW50 × *Df(2L)pr49*				Fertile	
Df(2L)Sd37 × *Df(2L)pr49*	Lethal	0 + 133			
Df(2L)TW50 × *Df(2L)pr8311*	Viable	45 + 139			
Df(2L)TW50 × *Df(2L)pr8311*	Viable	49 + 146	Subfertile		
Df(2L)TW50 × *Df(2L)pr8311*				Sterile	
Df(2L)pr8311 × *Df(2L)Sd37*	Lethal	0 + 197			
Df(2L)TW50 × *Df(2L)pr1122*	Viable?	10 + 56	Not possible		
Df(2L)TW50 × *Df(2L)pr1122*				Sterile	
Df(2L)Sd37 × *Df(2L)pr1122*	Lethal	0 + 41			
Df(2L)TW50 × *Df(2L)TW1*	Semilethal	26 + 123	Sterile	Sterile	
Df(2L)Sd37 × *Df(2L)TW1*	Lethal	0 + 27			
Df(2L)TW50 × *Df(2L)TW161*	Semilethal	1 + 138			
Df(2L)Sd37 × *Df(2L)TW161*	Lethal	0 + 29			

(*continued*)

APPENDIX

(Continued)

Cross	Viability	Df/Df + Df/CyO	Female fertility	Male fertility	Comments
		38C[b]			
Df(2L)pr49 × *Df(2L)Fs(2)Ket-RX32*	Viable	44 + 146	Fertile	Fertile	
Df(2L)Fs(2)Ket-RX32 × *Df(2L)pr49*	Viable	73 + 107	Fertile	Fertile	
Df(2L)Fs(2)Ket-RX32 × *Df(2L)pr40*	Lethal	0 + 221			
Df(2L)Fs(2)Ket-RX32 × *Df(2L)pr40*	Lethal	0 + 77			
Df(2L)Fs(2)Ket-RX32 × *Df(2L)pr40*	Lethal	0 + 47			
		38D/E[c]			
Df(2L)DS5 × *Df(2L)pr9201*	Viable	73 + 181			
Df(2L)DS5 × *Df(2L)pr9201*	Viable	48 + 115	Fertile	Fertile	
Df(2L)DS9 × *Df(2L)pr9201*	Viable	40 + 81	Fertile	Fertile	
Df(2L)DS5 × *Df(2L)pr-A16*	Viable	38 + 106			
Df(2L)DS5 × *Df(2L)pr-A16*	Viable	51 + 153	Fertile	Sterile	
Df(2L)DS9 × *Df(2L)pr-A16*	Viable	26 + 61	Fertile	Sterile	
Df(2L)DS5 × *Df(2L)pr37*	Semilethal	17 + 99			
Df(2L)DS5 × *Df(2L)pr37*	Viable	14 + 48	Sterile	Sterile	
Df(2L)DS5 × *Df(2L)pr37*	Semilethal	11 + 137	Sterile		
Df(2L)DS9 × *Df(2L)pr37*	Semilethal	6 + 58	Sterile		
Df(2L)DS5 × *Df(2L)pr2b*	Semilethal	31 + 162	Sterile		
Df(2L)DS9 × *Df(2L)pr2b*	Semilethal	16 + 109	Sterile		Only two males survived
Df(2L)DS5 × *Df(2L)pr8311*	Semilethal	8 + 159			Females and males very weak,
Df(2L)DS9 × *Df(2L)pr8311*	Semilethal	21 + 77			but survivors produced progeny
Df(2L)DS5 × *Df(2L)pr11*	Lethal	0 + 32			*l(2)38Ea* and *Hr38* are in the overlap
Df(2L)DS9 × *Df(2L)pr11*	Semilethal	2 + 56			*l(2)38Ea* and *Hr38* are in the overlap

Three regions in 38 were found to encode essential genes by overlapping deficiencies.

[a] The lethal and sterile region in 38A/B can be subdivided into four subregions: two lethal regions, one male sterile, and one female sterile region. It is spanned by two distal deficiencies [*Df(2L)TW50* and *Df(2L)Sd37*] and five to six proximal deficiencies [*Df(2L)pr49*, *Df(2L)pr8311*, *Df(2L)pr1122*, *Df(2L)TW1*, *Df(2L)TW161*, and possibly *Df(2L)pr11163*]. Another four proximal deficiencies do not remove this region: *Df(2L)be408*, *Df(2L)pr2b*, *Df(2L)pr37*, and *Df(2L)pr-M1*. The extent of *Df(2L)be408* is known; it removes *barren*, *38B.4*, and *38B.5* (S. Hijal, personal communication). *Df(2L)TW1* and *Df(2L)TW50* partially complement each others' lethality but escapers are sterile. Surprisingly, *Df(2L)TW1* and *Df(2L)TW50* both do not complement *scw*.

[b] The lethal region in 38C is defined by the overlap of the proximal deficiency *Df(2L)Fs(2)Ket-RX32* with the distal deficiency *Df(2L)pr40*. The distal deficiency *Df(2L)pr49* does not span this lethal region.

[c] The lethal and sterile region in 38D/E can be subdivided into two subregions: one male sterile and one lethal region. Furthermore, escapers of the latter are female sterile. The region is uncovered by two proximal deficiencies [*Df(2L)DS5* and *Df(2L)DS9*] and four distal deficiencies [*Df(2L)pr-A16*, *Df(2L)pr37*, *Df(2L)pr2b*, and *Df(2L)pr8311*], while the distal deficiency *Df(2L)pr9201* does not extend into the region.