Mapping Second Chromosome Mutations to Defined Genomic Regions in Drosophila melanogaster

Lily Kahsai and Kevin R. Cook
Bloomington Drosophila Stock Center, Department of Biology, Indiana University, Bloomington, Indiana 47405
ORCID IDs: 0000-0003-3429-445X (L.K.); 0000-0001-9260-364X (K.R.C.)

ABSTRACT Hundreds of Drosophila melanogaster stocks are currently maintained at the Bloomington Drosophila Stock Center with mutations that have not been associated with sequence-defined genes. They have been preserved because they have interesting loss-of-function phenotypes. The experimental value of these mutations would be increased by tying them to specific genomic intervals so that geneticists can more easily associate them with annotated genes. Here, we report the mapping of 85 second chromosome complementation groups in the Bloomington collection to specific, small clusters of contiguous genes or individual genes in the sequenced genome. This information should prove valuable to Drosophila geneticists interested in processes associated with particular phenotypes and those searching for mutations affecting specific sequence-defined genes.

The phenotypes associated with mutations often provide insights into the functions of genes. Indeed, much of genetics research involves explaining how mutations give rise to phenotypes. Newer methods for inducing mutations such as transposon excision, homologous recombination, and CRISPR-based disruption are particularly good for deleting coding sequences. Such knock-out mutations are undeniably important in understanding the cellular roles of genes, but other kinds of mutations—such as those that reduce gene expression or protein activity or affect only certain protein isoforms or domains—can be informative in ways that knockout mutations are not (Venken and Bellen 2014). While point mutations can now be engineered, they can reveal novel protein structure-function relations and elicit unexpected phenotypes.

Knowing the importance of mutations, the Bloomington Drosophila Stock Center devotes considerable effort to maintaining stocks carrying mutations. Many of these mutations have been characterized phenotypically, but they have not yet been associated with sequence-defined genes. These stocks are potentially valuable, but they are requested infrequently. Geneticists interested in particular processes might be more likely to study mutations with relevant phenotypes if they knew they would be relatively easy to associate with sequence-defined genes. The usefulness and popularity of these stocks would be improved tremendously by anchoring these mutations to the genome sequence map so that their relationships to annotated genes could be recognized more readily.

Our ability to map mutations to specific genomic intervals in Drosophila improved enormously when simple techniques became available for generating chromosomal deletions with breakpoints known at single-nucleotide resolution. Three large-scale projects, including one conducted at the Bloomington Drosophila Stock Center, generated deletions with molecularly defined breakpoints (Parks et al. 2004; Ryder et al. 2007; Cook et al. 2012). Altogether, these deletions provide >98% genomic coverage and subdivide the genome into intervals of a median of nine genes (Cook et al. 2012). Using these deletions, mutations can now be mapped to very small chromosomal regions, or even single genes, with simple complementation tests. Mutations can often be mapped even more closely with follow-up complementation tests involving chromosomal duplications (Cook et al. 2010; Venken et al. 2010), or mutations affecting single genes.
## Table 1 Mapping complementation groups to specific genomic intervals

| Complementation group | Genomic interval from deletion mapping | Complementing mutations | Noncomplementing mutations | Candidate genes | Comments |
|------------------------|----------------------------------------|-------------------------|-----------------------------|-----------------|----------|
| Abb                    | 2R:23666959;23713811                   | GsT<sup>1</sup>, Sam-S<sup>T</sup> | db<sup>Eps</sup>, l(2)g<sup>H</sup> |                               |          |
| Bhe                    |                                        |                         |                             |                 |          |
| C                        |                                        |                         |                             |                 |          |
| Cass                    |                                        |                         |                             |                 |          |
| Dv-24E                  |                                        |                         |                             |                 |          |
| Eay                    | 2R:18173570;18230554                   |                         |                             |                 |          |
| Fiz                    | 2R:8976399;9031045                     | Np<sup>M10240</sup>, Np<sup>M10279</sup> |                              | CG8213<sup>M10468</sup> |          |
| Frd                    | 2R:23001651;23068684                   |                         |                             |                 |          |
| Fs(2)Abc                | 2R:15375176;15386324                   |                         |                             |                 |          |
| Fs(2)ito3               | 2L:19464056;19517610                   |                         |                             |                 |          |
| Fs(2)itoQe45            | 2L:3656901;3713827                     |                         |                             |                 |          |
| Fs(2)itoRM7             | 2R:23385467;23395914                   |                         |                             |                 |          |
| Hum                    | 2R:7395885;7447410                     |                         |                             |                 |          |
| l(2)21Ba                | 2L:67365;159063                        | Sam-S<sup>R23</sup>, Sam-S<sup>T</sup>, GsT<sup>1</sup> |                             |                 |          |
| l(2)23Ab                | 2L:2677694;2753125                     |                         |                             |                 |          |
| l(2)24Dc                | 2L:4162968;4197800                     |                         |                             |                 |          |
| l(2)24Dd                | 2L:4031318;4162968                     | ed<sup>1</sup>          |                             |                 |          |
| l(2)24De                | 2L:4162968;4197800                     |                         |                             |                 |          |

Our complementation tests with deletions (Table S4 in File S1) and these mutations indicate bhe<sup>T</sup> is a multigene, terminal deletion (Df(2L)bhe) with a breakpoint between db<sup>e</sup> and Sam-S. Polytene analysis showed a breakpoint at 21AS-B1. This is consistent with J. Kennison’s observation of at least one bhe allele failing to complement l(2)gl (cited in Lindsley and Zimm 1992). The mutant embryonic phenotype likely results from the disruption of several genes.

Our data are consistent with unpublished identifications of c as S<sub>m</sub>-Mick by Rodriguez (2004) and E. Spana and E. Green (personal communication).

These results show cass is the same gene as Aac11.

Our identification of fl<sup>T</sup> as CG8213 is consistent with the independent, unpublished results of Anne Uv (cited in Geberemedhin 2011).

Our mapping is based on the recessive lethality of Frd<sup>T</sup>. Frd<sup>T</sup> mutants carry an intragenic deletion in PPO3 (Sugumaran and Chase 2004).

Heitzler et al. (1993) mapped hum left of so, reducing candidates to nine.

Caggese et al. (1988) showed l(2)21Ba is not the same gene as GsT. Larson et al. (1996) mapped l(2)21Ba right of Sam-S and left of Gs1, reducing candidates to five.

Littleton and Bellen (1994) mapped l(2)23Ab left of Pgtk, reducing candidates to seven.

Szidonya and Reuter (1988) showed l(2)24Dc is not the same gene as ed.

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### Table 1, continued

| Complementation group | Genomic interval from deletion mapping | Complementing mutations | Noncomplementing mutations | Candidate genes | Comments |
|------------------------|----------------------------------------|-------------------------|---------------------------|-----------------|---------|
| \( \ell 243Ef \) | 2R:7665795;7708707 | tor\(^1\), U2A\(^1\) | | | Heitzler et al. (1993) showed \( \ell 243Ef \) is not the same gene as U2A or tor and maps right of U2A. Nagengast and Salz (2001) showed a U2A transgene did not rescue \( \ell 2 \) 43Ef mutations. This reduces candidates to four. |
| \( \ell 243Eg \) | 2R:7665795;7708707 | U2A\(^1\) | | | Heitzler et al. (1993) showed \( \ell 243Eg \) maps right of U2A and is not the same gene as tor, reducing candidates to four. |
| \( \ell 246Ca \) | 2R:9875312;9922003..9927457 | tea\(^{1755}\) | Etf-QO\(^{056-40}\) | | These results show \( \ell 2 \) 46Ca is the same gene as Etf-QO. |
| \( \ell 246Cb \) | 2R:9875312;9922003..9927457 | | Etf-QO\(^{056-40}\), tea\(^{1755}\) | | O’Brien et al. (1994) showed \( \ell 246Cb \) is not the same gene as FMRFa, reducing candidates to seven. |
| \( \ell 246Cd \) | 2R:9958120;10025288 | eve\(^1\), eve\(^5\), Pal1\(^{10-1}\), elf3\(^{13906}\) | | | These results show \( \ell 2 \) 46Db is the same gene as TER94, even though \( \ell 2 \) 46Db\(^{26}\) shows a complex complementation pattern with other TER94 alleles. |
| \( \ell 246De \) | 2R:9959818;10025288 | eve\(^1\), eve\(^5\), Pal1\(^{10-1}\), elf3\(^{13906}\) | TER94\(^{03775}, \, \text{TER94}^{26-8}, \, \text{TER94}^{22-30}\) | | |
| \( \ell 246Dc \) | 2R:10025288;10030539 | | | | |
| \( \ell 246Dd \) | 2R:10030539;10078293 | | | | |
| \( \ell 246Ds \) | 2R:10058120;10025288 | | | | |
| \( \ell 246Df \) | 2R:10030539;10078293 | | | | |
| \( \ell 249Fa \) | 2R:12894105..12941-055 | eve\(^5\), elf3\(^{13906}\) | | | |
| \( \ell 249Fb \) | 2R:13197974..131984-92;13219347..13219-349 | | | | |
| \( \ell 251Ea \) | 2R:15218008;15262942 | | scb\(^2\) | | Lasko and Pardue (1988) showed \( \ell 2 \) 49Fa is not the same gene as Orc3, reducing candidates to nine. |
| \( \ell 257Ba \) | 2R:21000163;2105679 | | | | |
| \( \ell 257Bd \) | 2R:21056798;21088247 | | | | |
| \( \ell 257Cb \) | 2R:21056798;21088247 | | | | |
| \( \ell 257Cc \) | 2R:21143577;21177310 | | | | |
| \( \ell 257Cd \) | 2R:21143577;21177310 | | | | |
| \( \ell 257Ce \) | 2R:21180990;21215223 | | | | |
| \( \ell 257Db \) | 2R:21301798;21341647 | | | | |

(continued)
| Complementation group | Genomic interval from deletion mapping | Comments |
|------------------------|---------------------------------------|----------|
| [(2)2]57Eb             | 2R:21497209;21607081                  | J. O'Donnell et al. (1989), Price et al. (1989), and Schejter and Shilo (1989) showed [(2)2]57Eb is not the same gene as Egr. Lane and Kalderon (1993) showed [(2)2]57Eb to be another candidate to 15. The results showed that [(2)2]Egfr was not the same gene as Egr, reducing candidates to 15. Our data place mat[(2)2]Egfr in the same general region as Clegg et al. (1993), but they placed it right of da and right of RpS27A. |
| [(2)2]57Ec             | 2L:9272496;9368459                    | J. O'Donnell et al. (1989), Price et al. (1989), and Schejter and Shilo (1989) showed [(2)2]57Ec is not the same gene as Egr, reducing candidates to 15. The results showed that [(2)2]Egfr was not the same gene as Egr, reducing candidates to 15. Our data place mat[(2)2]Egfr in the same general region as Clegg et al. (1993), but they placed it right of da and right of RpS27A. |
| DA2                   | 2L:9522946;9560489                    | Lane and Kalderon (1993) showed DA2 is not the same gene as Cks30A, reducing candidates to 20. Lane and Kalderon (1993) showed DA2 to be another candidate to 19. The results showed that DA2 is not the same gene as Cks30A, reducing candidates to 19. |
| DB2                   | 2L:9897536;9908459                    | Lane and Kalderon (1993) showed DB2 is not the same gene as Cks30A, reducing candidates to 20. Lane and Kalderon (1993) showed DB2 to be another candidate to 19. The results showed that DB2 is not the same gene as Cks30A, reducing candidates to 19. |
| DB4                   | 2L:9205076;9388129                    | Lane and Kalderon (1993) showed DB4 is not the same gene as Cks30A, reducing candidates to 20. Lane and Kalderon (1993) showed DB4 to be another candidate to 19. The results showed that DB4 is not the same gene as Cks30A, reducing candidates to 19. |
| FE3                   | hoipk07104                            | These results show FE3 is the same gene as hoip. It was mapped with cytologically defined deletions (Table S4 in File S1), but not molecularly defined deletions, so no genomic interval is given. |
| N7-6                  | 2L:9205076;9388129                    | Lane and Kalderon (1993) showed N7-6 is not the same gene as Cks30A, reducing candidates to 19. Lane and Kalderon (1993) showed N7-6 to be another candidate to 19. The results showed that N7-6 is not the same gene as Cks30A, reducing candidates to 19. |
| PC4-A                 | 2R:18051197;18118348                  | Mohr and Gelbart (2002) mapped [PC4-D, to Ubc10, CG5033 or Dhit, reducing candidates to two. |
| PC4-D                 | 2R:18051197;18118348                  | Mohr and Gelbart (2002) mapped [PC4-D, to Ubc10, CG5033 or Dhit, reducing candidates to two. |
| PC4-M                 | 2R:18051197;18118348                  | Mohr and Gelbart (2002) mapped [PC4-D, to Ubc10, CG5033 or Dhit, reducing candidates to two. |
| PC4-P                 | 2R:18051197;18118348                  | Mohr and Gelbart (2002) mapped [PC4-D, to Ubc10, CG5033 or Dhit, reducing candidates to two. |
| PC4-Q                 | 2R:18051197;18118348                  | Mohr and Gelbart (2002) mapped [PC4-D, to Ubc10, CG5033 or Dhit, reducing candidates to two. |
| syn-E                 | 2L:10349604;10381214                  | Our data place mat[(2)2]Egfr in the same general region as Clegg et al. (1993), but they placed it right of da and right of RpS27A. |
| moa                   | 2R:2272936;22764935                   | Littleton and Bellen (1994) mapped moa right of Drp1, reducing candidates to five. Our results are compatible with moa being associated with a small deletion as proposed by Littleton and Bellen. These results show sat is the same gene as Orc1. |
| ms                    | 2L:9205076;9388129                    | Mohr and Gelbart (2002) mapped [PC4-D, to Ubc10, CG5033 or Dhit, reducing candidates to two. |
| mrd                   | 2L:9205076;9388129                    | Mohr and Gelbart (2002) mapped [PC4-D, to Ubc10, CG5033 or Dhit, reducing candidates to two. |
| p{f+1}30B             | 2L:9205076;9388129                    | These crosses show a lethal mutation (hereafter p{f+1}30Ba1) is caused by the p{f+1}30B insertion or is closely linked to it. |
| pd                    | 2R:2381400;2384351                    | These results show sat is the same gene as Orc1. |
| gui                   | 2R:2381400;2384351                    | These results show sat is the same gene as Orc1. |
| fht                   | 2R:2381400;2384351                    | These results show sat is the same gene as Orc1. |
| Orc1                  | 2R:2381400;2384351                    | These results show sat is the same gene as Orc1. |
| Orc1                  | 2R:2381400;2384351                    | These results show sat is the same gene as Orc1. |
| Orc1                  | 2R:2381400;2384351                    | These results show sat is the same gene as Orc1. |

*Excludes genes with complementing mutations from the set of contiguous genes defined by deletion breakpoints (Table S3 in File S1). Ranges reflect deletion breakpoint uncertainty. Candidate genes are listed in Table S4 in File S1.*
Table 2 Complementation groups mapped to single genes

| Complementation group | Summary |
|-----------------------|---------|
| cassowary (cass)      | cass mutations were isolated as recessive lethal mutations that result in lack of adhesion between wing surfaces in homozygous mitotic clones (Prout et al. 1997). cass is allelic to Aac11, which encodes an inhibitor of apoptosis homologous to human Apoptosis Inhibitor 5 (API5) (Morris et al. 2006). |
| filzig (fö)           | fö mutations were isolated as recessive lethal mutations affecting the patterning of the embryonic cuticle (Nüsslein-Volhard et al. 1984). We found fö to be allelic to CG8213, which encodes a serine protease (Ross et al. 2003). Subsequently, we learned fÖ was also identified as CG8213 by Anne Uv (unpublished results cited in GebereMedhin 2011). |
| fs(2)abc              | fs(2)abc (abnormal chromatin) mutations were isolated as recessive paternal-effect lethals causing abnormal embryonic nuclear divisions and defective chorions (Schüpbach and Wieschaus 1989; Vessey et al. 1991). fs(2)abc is allelic to SRPK, which encodes a Serine–Arginine Protein Kinase necessary for dorsoventral egg patterning, karyosome formation, and meiotic divisions (Barbosa et al. 2007; Loh et al. 2012). |
| l(2)46Ca              | The recessive lethal l(2)46Ca is allelic to Electron transfer flavoprotein-ubiquinone oxidoreductase (Etf-QO), which encodes a component of the electron-transport chain that generates ATP from the breakdown of fatty acids (Wathamough and Fierman 2010). |
| l(2)46Db              | The recessive lethal l(2)46Db is allelic to TER94, which encodes a chaperone that targets ubiquitin-tagged proteins to the proteasome (Meyer et al. 2012). |
| l(2)FE3               | The recessive lethal l(2)FE3 is allelic to hoi-polloi (hoip), which encodes a small nuclear ribonucleoprotein component of spliceosomes (Mount and Salz 2000). |
| satin (sat)           | Schüpbach and Wieschaus (1991) showed homozygous sat£c£a females lay eggs with thin eggshells. sat is allelic to Origin recognition complex 1 (Orc1), which is needed for chion gene amplification (Park and Asano 2008). |
| l(2)51Ea              | The recessive lethal l(2)51Ea is allelic to scab (scb), which encodes an α integrin involved in cell adhesion (Stark et al. 1997). |

We report here the localization of 77 complementation groups in the Bloomington Drosophila Stock Center collection to defined genomic intervals and the mapping of eight complementation groups to individual genes. This work ties these mutations to single genes or small groups of closely linked genes, and increases the value of an underutilized set of stocks.

MATERIALS AND METHODS

The data in this report came from fly crosses made on standard medium, reared under routine conditions, and evaluated by customary standards (details provided upon request). Genomic coordinates are given in terms of the Release 6 assembly, and gene annotations are those shown in the June 20, 2017 FlyBase release (FB2017_3). Supplemental Material, Table S1 in File S1 provides a list of stocks used and our sources.

Data availability

The accompanying tables contain complete mapping data. Stocks may be obtained from the Bloomington Drosophila Stock Center or Drosophila Genomics and Genetics Resources at the Kyoto Institute of Technology as indicated in Table S1 in File S1.

RESULTS AND DISCUSSION

We identified a large set of second chromosome mutations in the Bloomington Drosophila Stock Center collection that had not been associated with annotated genes and using mapping information archived in FlyBase (http://flybase.org/), or recorded in publications to estimate the chromosomal positions of the mutations (Table S2 in File S1). We then made complementation crosses between stocks carrying the mutations and molecularly defined chromosomal deletions to place the mutations in defined genomic intervals that refine previous mapping (Table S3 in File S1). Subsequent crosses tested the mutations for allelism with mutations in sequence-defined genes. Table 1 summarizes our results. The number of candidate genes in each interval was initially determined by the overlap of deletions with transcribed gene regions. (We recognize this criterion is potentially misleading as it is possible for a deletion to remove gene regulatory regions and disrupt gene function even if transcribed gene regions are not deleted. Nevertheless, it is a reasonable and commonly employed practice for deletion studies.) From this total, we subtracted the number of genes with complementing mutations. (This criterion could also be misleading, because partial loss-of-function alleles can show intragenic complementation. Nevertheless, it is also a reasonable simplification for a preliminary mapping study.) We have provided a list of candidate sequence-defined genes for each complementation group in Table S4 in File S1. Table S5 in File S1 provides a full list of the informative mapping crosses. In every cross, we had experimental evidence indicating that both stocks were valid as follows. Every mutation being mapped failed to complement at least one deletion. Most stocks used to map the mutations were validated with independent control crosses to stocks carrying relevant, previously characterized, loss-of-function mutations or chromosomal deletions (Table S6 in File S1). For a dozen deletion stocks, noncomplementation of the deletion with one of the mutations we were mapping was taken as evidence the stock was intact, and no independent control cross was undertaken.

We were able to map 77 complementation groups to the smallest chromosomal intervals possible using existing molecularly defined deletions (Table S3 in File S1). With follow-up complementation tests using existing point mutations and transposon insertions in annotated genes, we were able to map eight complementation groups to single annotated genes, but we did not exhaust all possible tests of this sort. In the final tally, we were able to map 84 of the 85 complementation groups to 26 genes or fewer. (We found the remaining complementation group, blle, to be a multigene deletion.)
Table 2 summarizes information on the mutations mapped to single annotated genes, and shows the diversity of interesting genes affected. This work has identified the first nontransposon alleles of two genes (Aac11 and CG8213), and has added potentially important EMS- or irradiation-induced alleles to the other genes. While we have not attempted to assess the allelic strength of most of the mutations, we know the female-sterile mutation sat[SC46] mapped to Orc1 must be a partial loss-of-function allele because knockout alleles are recessive lethal (Park and Asano 2008). Mutations affecting a particular motif in the Orc1 protein have been shown to cause the same defective eggshell phenotypes as sat[SC46] (Park and Asano 2012), which suggests it too is domain specific. This result illustrates the importance of point mutations maintained in stock for their loss-of-function phenotypes: they can reveal aspects of gene function that would not be apparent from the phenotypes of gene knockouts.

In conclusion, we have refined the mapping of a large number of second chromosome mutations that have been preserved at the Bloomington Drosophila Stock Center for their mutant phenotypes. This information will provide Drosophila workers opportunities to make connections between these mutations and genes they might be studying in defined chromosomal regions.

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