



The genes of the *elbow-no-ocelli* genetic complex in *D. melanogaster*.

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The *elbow-no-ocelli* region of chromosome 2L is made up of several genes that show a high degree of interaction. These are from distal to proximal: *el<sup>B</sup>*, *pu*, *crle*, *el<sup>A</sup>*, and *noc<sup>A</sup>* (Davis *et al.*, 1990, 1997). The chromosomal region 35B was sequenced in 1999 (Ashburner *et al.*, 1999) and it became possible to map the genetic complementation groups, *el<sup>B</sup>* and *noc<sup>A</sup>* to actual genes (Ashburner *et al.*, 1999; Adams *et al.*, 2000; Reese *et al.*, 2000). Unfortunately it has proved difficult to assign genes to the other complementation groups in this complex, even though there are sufficient gene predictions available between *el<sup>B</sup>* and *noc<sup>A</sup>*, leading to some speculation as to the existence of some of these genes (Dorfman *et al.*, 2002). This has been due to lack of cDNA sequences and a lack of refinement in some of the genetic mapping of the complementation groups (Davis *et al.*, 1990, 1997). In this communication I wish to summarise the available evidence and argue in favour of the existence of the genes for *pu*, *crle*, and *el<sup>A</sup>*.

*The pu gene.* This gene is predicted to lie between the two *elbow* genes (Davis *et al.*, 1990; 1997) and is possibly associated with the gene prediction CG15284. Deletions that remove DNA proximal to *Df(2L)el6*, e.g., the synthetic deletion *el<sup>6L-A379R</sup>*, are mutant for *pu*, whereas the deletion *Df(2L)el16* (goes proximal) is not mutant for *pu*, indicating that the *pu* gene is between these two breaks (they are approximately 3 kb apart). The gene prediction CG15284 lies between these two breaks suggesting that this is the *pu* gene (Davis, 2001a). Unfortunately, due to a possible miss-mapping of the deletion *Df(2L)el<sup>14</sup>*, the genetic data is contradictory as this deletion would delete CG15284 and *Df(2L)el<sup>14</sup>* is not mutant for *pu*. As the genomic sequence is now available (Adams *et al.*, 2000) I think that *Df(2L)el<sup>14</sup>* has been miss-mapped and breaks very close to *Df(2L)el<sup>16</sup>* and neither would delete CG15284 (Davis, 2001a).

The gene CG15284 (*pu*) has a low degree of homology to the 3' end of the human mucin genes and the growth factor for human Norrie's disease (Figure 1). The domain of homology is a cystine-knot cytokine domain. I appreciate that the overall homologies are low, but all the conserved residues required for the cystine-knot are 100% conserved (Meitinger *et al.*, 1993). The cystine-knot is required to fold in a specific way and to cross-link units. Meitinger states that the overall homologies for this class of proteins is 10-30%; the essential requirement is for the cystine-knot domain cys residues (the *pu* homology is greater than 10%). The putative pupal protein has reasonable homology to the cystine-knot domain and all the required cysteines are conserved. In addition the pupal protein would have a good signal peptide indicating an excreted protein. If this match is more than coincidence then *pu* could be a *Drosophila* version of the cystine-knot and thus be a homolog of a member of this growth factor family. The gene CG15284 has recently been identified in *D. pseudoobscura* (Davis, 2003a) and also has this structure conserved in a second predicted *Drosophila* gene CG13419 (Davis, 2001b). The recent protein interaction data (Giot *et al.*, 2003) indicate with a high confidence level that CG15284 interacts with the protein product of CG15623.

*The crle gene.* The existence of this gene relies on the phenotype arising from overlapping deletions. When this 'locus' is deleted, or affected by aberrations, the surviving flies have a crippled leg phenotype, e.g. *Df(2L)el<sup>16</sup>/Df(2L)b83d29a* which only homozygously deletes CG3474, is *pupal<sup>+</sup>*

*elbow*<sup>+</sup> *crle*<sup>-</sup> (Davis *et al.*, 1997). There are as yet no point mutations for '*crle*'. The predicted gene *CG3474* lies precisely where the *crle* locus would be located as it would be deleted in *Df(2L)el*<sup>14</sup> and *Df(2L)el*<sup>16</sup> that are mutant for *crle* and not in the *crle*<sup>+</sup> deletion *Df(2L)TE35BC-8*. The locus *CG3474* encodes a putative pupal cuticle protein. This protein is also conserved in *D. yakuba* and *D. simulans* (Schmid and Aquadro, 2001), and is predicted with high confidence to interact with *CG31332*, and several other proteins (Giot *et al.*, 2003), including the *dec-2* gene that is involved in the formation of insect chorion.

**A**

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pu      MHVQELLFVAAILVPQCLRALRYSQGTGDEN

pu      CETLKSEIHLIKEEFDELGRMQRTCNADVIVNKCEGLCNSQVQPSVITPTGFLKECYCC
muc6    CS-----VREQQEEITF--KGCMA NVTVTRCEGACISAAS-FNIITQQVDARCS CC
muc2    CSTVPVTTEVS YA-----GCTKT VLMNHCSGSCGTFV-MYSAKAQALDHSCSCC
muc5    CAVYHRSLIIQQQ-----GCSSS-----CRGNCGDSSSMYSLEGNTVEHRCQCC
muc5B   CQCRINTTILWHQ-----GCETE VNITFCEGSCP GA-SKYSAEAQAMQH QCTCC
        *                               *           ^     *                       *  **

pu      RESFLKEKVITLTHCYDPDGTRLTSPEMGSMDIRLREPTECKC--FKCGDF----TR
Muc6    RPLHSYEQQLEL-PC--PDPSTPGRRVLVLT LQVFSH---CVCSSVACGD
Muc2    KEEKTSQREVVL-SC--PNGGSLTHTYTH-IES-----CQCQDTVCGLPTGTSRR
Muc5    QELRTSLRNVT L-HC--TDGSSRAFSYTE-VE-----ECGMGRRC PAPATPSTR
Muc5B   QERRVHEETVPL-HC--PN GSAILHTYTH-VD-----ECGCTPF-CVPAPM
                *                           *       *         *
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**B**

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NDP      MRKHVLAASFMSLSLLVIMGDTDSKTDSSFIMDS DPRR
pu        MHVQELLFVAAILVPQCLRALRYSQGTGDEN-----
CG13419   MLRHLLRHENN KVFLILLYCVLVSI LKLCTAQP DSSVAATDNDITHLGDD

          1                   a               2   3
NDP      CMR-----HHYVDSISHP LYKCSSKMVLLARCEGHC-SQASRSEPLVSFSTVLKQP
pu        CETLKSEIHLIKEEFDELGRMQRTCNA D-VIVNKCEGLCNSQVQ P-----SVITPTGF-
CG13419   CQ-----VTPVIHV LQYPGCVPKPI PSFACVGRCASYIQVSGSKI WQ-----

              b *4                  c                      5 6 d
NDP      FRSSCHCRPQT SKLKALRL-RC--SGGMRLTATYRYIL-----SCHCEE CN S
pu        LK-ECYCCRESFLKEKVITLTHCYDPDGTRLTSPEMGSMDIRLREPTECKCFKCGDFTR
CG13419   MERSCMC COESGEREAAVSL-FC PKVKP GERK-FKKVLT KAPLE----CMCRPCTSIEESG
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Figure 1. Comparison of the *CG15284* gene with: A, human mucins. This is the 3'-terminal of the mucin proteins. An \* indicates the conserved cys residues, ^ is an important conserved Glycine. B, human Norie's disease protein (NDP) and *Drosophila* predicted gene *CG13419*. The conserved cys residues are numbered and form cys-cys bonds as follows: 1:4, 2:5, 3:6. The cys marked with \* is essential to cross link monomer units. The cys residues marked a to d also form part of the tertiary structure of this class of cystine-knot proteins.

*The  $el^A$  gene.* This existence of the  $el^A$  gene relies upon the observation that the strong  $el$  mutation  $el^1$  is associated with a 25 kb deletion situated between the  $el^B$  and  $noc^A$  genes (Davis *et al.*, 1990, 1997). In addition there are numerous deletions that do not affect the  $el^B$  gene that are strong  $el$  in phenotype and are thus deleted for  $el^A$  (Davis *et al.*, 1990, 1997). In the  $el^1$  deletion lies the predicted gene *CG15283* (Ashburner *et al.*, 1999; Adams *et al.*, 2000; Reese *et al.*, 2000), a gene that

has also been found conserved in *D. pseudoobscura* (Davis, 2003b). This predicted gene is of unknown function but has a recently defined structural domain that is found in several eukaryotic and prokaryotic genes (Figure 2) (Davis, 2002). The recent protein interaction study has clearly identified *CG15283* as a real gene and has predicted with high confidence that it interacts with the putative gene *CG14168* (Giot *et al.*, 2003). Interestingly, *CG14168* is predicted to interact with *CG7224* with the same confidence level. The latter gene is known from cDNA data and has the same structural domain as seen in *CG15283* (Figure 2).

CG15283	EPLKPWPNNTPYTGEIGGQAGPEPTRYGDWERKGRKSTF
CG7224	EPLKPWPNTNPYTGEIGGPAGPEPTRYGDWERKGRVSDF
C6orf57	EPLEKFPDDVNPVTKEKGGPRGPEPTRYGDWERKGRCIDF
	*** * ** * * * * *

Figure 2. Alignment of the structural domains of *Drosophila* proteins *CG15283*, *CG7224* and human protein *C6orf57*. An asterisk indicates identity in all three proteins.

In conclusion a host of genetic, sequence, gene prediction, cross-specific conservation, and protein interaction data support the existence of three genes between the well-defined genes *el<sup>B</sup>* and *noc<sup>A</sup>*. I would define these genes as follows: *CG15284* is *pu*, *CG3474* is *crle*, and *CG15283* is *el<sup>A</sup>*.

References: Adams, M.D., *et al.*, 2000, *Science* 287: 2185-2195; Ashburner, M., *et al.*, 1999, *Genetics* 153: 179-219; Davis, T., J. Treneer, and M. Ashburner 1990, *Genetics* 126: 105-119; Davis, T., M. Ashburner, G. Johnson, D. Gubb, and J. Roote 1997, *Hereditas* 126: 67-75; Davis, T., 2001a, <http://flybase.bio.indiana.edu/bin/fbidq.html?FBfr0138570>; Davis, T., 2001b, <http://flybase.bio.indiana.edu/bin/fbidq.html?FBfr0139870>; Davis, T., 2002, <http://flybase.bio.indiana.edu/bin/fbidq.html?FBfr0145621>; Davis, T., 2003a, <http://flybase.bio.indiana.edu/bin/fbidq.html?FBfr0157279>; Davis, T., 2003b, <http://flybase.bio.indiana.edu/bin/fbidq.html?FBfr0157278>; Dorfman, R., L. Glazer, U. Weihe, M.F. Wernet, and B.Z. Shilo 2002, *Development* 129: 3585-3596; Giot, L., *et al.*, 2003, *Science* 302: 1727-1736; Meitinger, T., A. Meindl, P. Bork, B. Rost, C. Sander, M. Haasemann, and J. Murken 1993, *Nature Genet.* 5: 376-380; Reese, M.G., D. Kulp, H. Tammana, and D. Haussler 2000, *Genome Res.* 10: 529-538; Schmid, K.J., and C.F. Aquadro 2001, *Genetics* 159: 589-598.



Generation and authentication of *Df(3L)H99 FRT80B* recombinant lines.

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## Introduction

The *Drosophila* proteins Rpr, Hid and Grim are important regulators of cell death (Bangs, Franc, and White, 2000). They share an N-terminal RHG motif with the mammalian pro-apoptotic proteins Smac/Diablo and Omi/HtrA2 (Verhagen and Vaux, 2002). These proteins bind to inhibitor of apoptosis proteins (IAPs) using the RHG sequence, and relieve IAP inhibition of caspases.

A useful mutation for examining the null phenotypes of *rpr*, *grim* and *hid* is the deletion *Df(3L)H99*. This deletion removes all three genes plus several additional predicted genes

(<http://flybase.bio.indiana.edu>). *Df(3L)H99* homozygotes are late embryonic lethal and lack almost all normal embryonic programmed cell death (PCD) (White *et al.*, 1994).

To study the function of genes of this interval in adult flies, we generated *Df(3L)H99 FRT80B* recombinant lines. The proximity of the *H99* deletion to the centromere makes generation of the lines slightly challenging. We describe here the generation of six independent *Df(3L)H99 FRT80B* lines and their authentication. These lines can be obtained through the *Drosophila* stock center at Bloomington.

### Selection of Recombinants

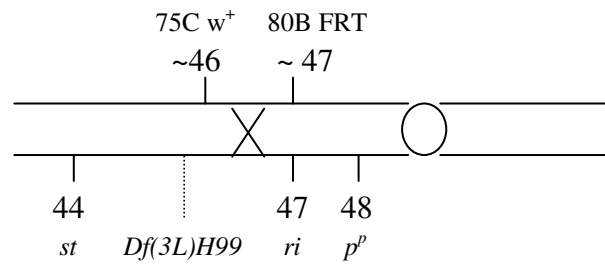
The genetic distance between the *Df(3L)H99* deletion and the *FRT80B* insertion is predicted to be no more than 2 cM, based on the linked markers of the *Df(3L)H99* line and the physical map position of *FRT80B*. Our initial attempts to select recombinants using the neomycin resistance gene of *FRT80B* did not succeed. This was probably due to a combination of the low frequency of the desired recombination and the weakness of the *Df(3L)H99* stock. We therefore selected recombinants based on their genetic markers, using the crossing scheme shown in Figure 1. We expected and obtained 0.25 % of the F2 progeny with the desired markers  $w^-$  and  $ri^+$ . These indicated recombination between the  $w^+$  of the 75C  $w^+ FRT80B$  parental chromosome and the  $ri^-$  marker of the *Df(3L)H99 ri* chromosome. We obtained 11  $w^-$  and  $ri^+$  males. Seven produced progeny when crossed to balancer females. In all seven of the stocks obtained, we verified the loss of *rpr*, *grim* and *hid* and the presence of the *Df(3L)H99* mutant phenotype and a functional FRT (see below). Of the seven original stocks, six have been stable for several years.

### Verification of Recombinant Lines

Acridine orange (AO) staining was used to determine the patterns of programmed cell death in stage 12-14 putative recombinant *Df(3L)H99 FRT80B/TM3 GFP* and *Df(3L)H99 FRT80B* mutant embryos (Figure 2A, B). The *Df(3L)H99* deletion eliminates nearly all developmental cell death, resulting in almost total loss of AO signal in stage 12-14 homozygous embryos (White *et al.*, 1994). Heterozygotes of the putative *Df(3L)H99 FRT80B* lines at these stages showed the expected normal pattern of AO signal (Figure 2A). Homozygotes showed the almost total absence of AO positive cells (Figure 2B) that is characteristic of *Df(3L)H99* embryos (White *et al.*, 1994). All six lines gave this result. The putative *Df(3L)H99 FRT80B* embryos also showed the head involution defective (*hid*) phenotype.

To further verify the absence of *rpr*, *grim*, and *hid* in the putative *Df(3L)H99 FRT80B* lines, we performed PCR analysis of genomic DNA from control *TM3 GFP* and putative *Df(3L)H99 FRT80B* embryos. DNA isolated from *TM3 GFP* embryos gave the expected size products using primers to all three genes and a positive control gene, *Drosophila TRAF1*. In contrast, all *Df(3L)H99 FRT80B* embryos gave a *TRAF1* product, but no *rpr*, *grim*, or *hid* (Figure 2C).

To determine whether the FRT sequences in the putative *Df(3L)H99 FRT80B* lines were functional for FLP mediated mitotic recombination, we crossed virgins of each *Df(3L)H99 FRT80B/TM3 GFP* line to  $w\ eyFLP; +; P\{w^+\}75C\ P\{FRT\}80B$  males and examined the pigmentation patterns in the eyes of adult progeny. Mitotic patches and twin spots were observed in virtually all female non-balancer progeny (Figure 2D, E). Most flies gave many small mitotic patches (Figure 2D) and a minority gave large “tiger stripes” (Figure 2E). In many of the mosaic eyes, numerous dark-red “twin” spots without associated white mitotic patches were observed in the central regions of the eye. In these eyes, the white patches were clearly observed closer to the eye perimeter. We speculate that



**P** ♀♀  $w; +; P\{w^+\}75C P\{FRT\}80B$  x ♂♂  $\frac{w; +; Df(3L)H99 ri p^p}{Y \quad TM3 Sb^1 ri}$



$w; +; \frac{P\{w^+\}75C P\{FRT\}80B}{Df(3L)H99 ri p^p}$

**F<sub>1</sub>** ♀♀  $w; +; \frac{P\{w^+\}75C P\{FRT\}80B}{Df(3L)H99 ri p^p}$  x ♂♂  $\frac{w; +; TM3 Sb^1 ri}{Y \quad TM6B Tb^1}$



$w; +; \frac{Df(3L)H99 P\{FRT\}80B}{TM3 Sb^1 ri}$

**F<sub>2</sub>** ♀♀  $w; +; \frac{Sb}{TM3 GFP Ser}$  x ♂  $\frac{w; +; Df(3L)H99 P\{FRT\}80B}{TM3 Sb^1 ri}$



$w; +; \frac{Df(3L)H99 P\{FRT\}80B}{TM3 GFP Ser}$

Figure 1. Crossing scheme for the generation of  $Df(3L)H99$  FRT80B recombinant lines.

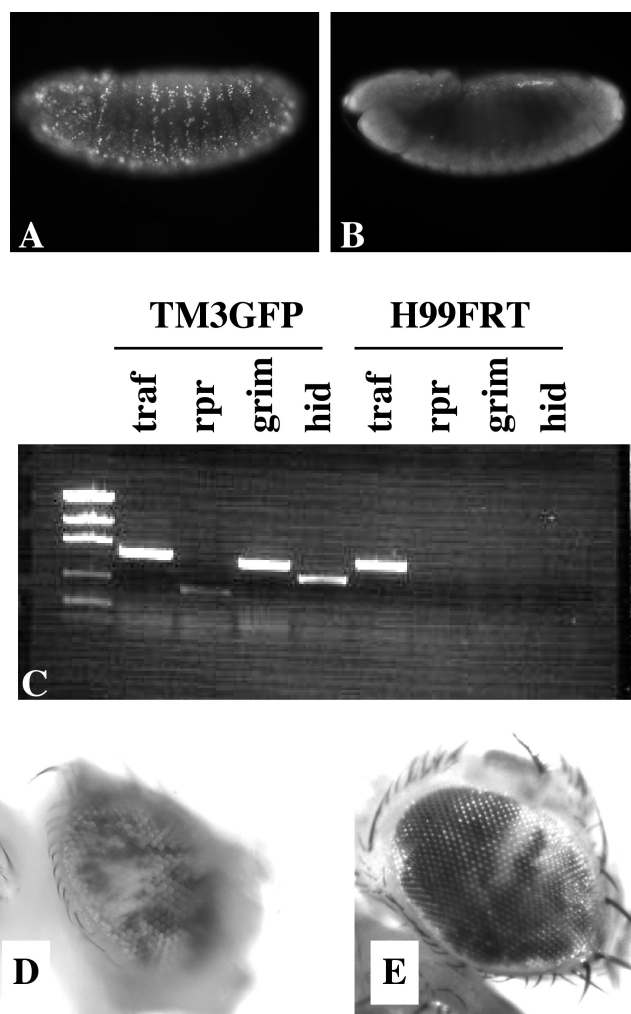


Figure 2. Verification of *Df(3L)H99 FRT80B* mutant lines. (A) *Df(3L)H99 FRT80B* Line #3 / *TM3 GFP* embryo stained with acridine orange, showing the normal patterns of acridine orange-positive dying cells for this stage. (B) *Df(3L)H99 FRT80B* Line #3 embryo of the same age, showing the H99 mutant phenotype. Note the virtual absence of acridine orange staining and the head involution defect (C) PCR amplification of genomic DNA of control (*TM3 GFP*) and putative *Df(3L)H99 FRT80B* Line# 11 (H99 FRT) homozygous mutant embryos, using primers to *Drosophila* TRAF1 (*traf*) as a positive control and *rpr*, *grim*, and *hid*. (D, E) *w<sup>eyFLP/w</sup>; +; Df(3L)H99 FRT80B/P{w<sup>+</sup>}75C FRT80B* flies showing mitotic patches and twin spots in the adult eye. Representative mosaic eyes from *Df(3L)H99 FRT80B* lines #3 (D) and #11 (E). Results for individual lines are shown; similar results were obtained with all six *Df(3L)H99 FRT80B* lines.

cells lacking the H99 interval may be at a growth disadvantage in a non-cell-autonomous fashion. That is, they may respond differently to cytokines or growth factors supplied by the retina or surrounding tissues. Future use of the lines in studies of eye development might provide an explanation.

We conclude that the putative *Df(3L)H99 FRT80B* lines reported here are null for *rpr*, *grim*, and *hid* and contain functional *FRT80* sites that should make them useful for studies of post-embryonic tissues. The results indicate that the interval defined by *hid* on the left and *rpr* on the right

is deleted in all lines. The lack of AO staining in homozygous mutant embryos further suggests that either the entire *Df(3L)H99* deletion was maintained, or that the interval delimited by *hid* and *rpr* is all that is required for normal embryonic PCD.

## Materials and Methods

### Fly stocks

*Df(3L)H99*, *kni<sup>ri-1</sup> p<sup>p</sup>/TM3*, *Sb<sup>1</sup>, w\**; *Sb<sup>1</sup>/TM3*, *P{w<sup>+mC</sup>=ActGFP}JMR2*, *Ser<sup>1</sup>*, and *y<sup>1</sup> w<sup>1118</sup>*; *P{w<sup>+mC</sup>=piM}75C* *P{ry<sup>+7.2</sup>=neoFRT}80B* flies were obtained from the Bloomington *Drosophila* Stock Center at Indiana University. From these, standard genetic methods were used to make *Df(3L)H99*, *kni<sup>ri-1</sup> p<sup>p</sup> / TM3*, *P{w<sup>+mC</sup>=ActGFP}JMR2*, *Ser<sup>1</sup> (Df(3L)H99 ri/ TM3 GFP)* and *Df(3L)H99*, *kni<sup>ri-1</sup> p<sup>p</sup> P{ry<sup>+7.2</sup>=neoFRT}80B/ TM3*, *P{w<sup>+mC</sup>=ActGFP}JMR2*, *Ser (Df(3L)H99 FRT80B/TM3 GFP)* flies. *Df(3L)H99/ TM3* flies survived poorly on our standard corn meal food formula, and were therefore maintained on wheat flour-based food supplemented with 0.25 mg/ml  $\beta$ -carotene, at 20-25°C and 65% relative humidity.

### Acridine orange staining of *Drosophila* embryos

Embryos were collected for three hours on molasses plates with yeast paste, rinsed free of yeast paste with dH<sub>2</sub>O, and replated. After an overnight incubation at 18°C, embryos were rinsed again with dH<sub>2</sub>O and placed in snap-cap tubes containing equal volumes of heptane and 5  $\mu$ g/ml acridine orange in 0.1M phosphate buffer (made by mixing 72 ml of 0.1M Na<sub>2</sub>HPO<sub>4</sub> with 28 ml of 0.1M NaH<sub>2</sub>PO<sub>4</sub>). After vigorous shaking for 3-5 min by hand, both phases were removed, fresh heptane was added and embryos were placed on slides. The heptane was allowed to evaporate and the samples immediately covered with a drop of halocarbon oil. Following the addition of a cover slip, slides were viewed under UV illumination (100W Zeiss Attoarc HBO lamp) using a Zeiss Axioplan II microscope fitted with a rhodamine filter set (Chroma)

### Embryo PCR

Embryos were collected for two hours, rinsed and replated as described above. The viable heterozygous *Df(3L)H99 FRT80B/TM3 GFP* embryos were allowed to hatch into larvae by incubating the plates for 24 h at 25°C. Remaining embryos were either *TM3 GFP* with 2 copies of GFP, or *Df(3L)H99 FRT80B* with no GFP. Embryos were sorted based on the presence or absence of GFP fluorescence, using a Zeiss Stemi SV11 stereomicroscope fitted with an Attoarc 100W HBO lamp and GFP filter set (Chroma). Single embryo extracts were made by incubation of each embryo in 10  $\mu$ l of lysis buffer (10mM Tris-HCL, pH8.2, 1mM EDTA, 25 mM NaCl, 100 $\mu$ g/ml Proteinase K) for 20 min at 37°C, followed by a 2 min incubation at 95°C. Extracts of 4 individual embryos of the same genotype were pooled, and 3  $\mu$ l of extract was used in each 10  $\mu$ l PCR reaction with 1pmol/ $\mu$ l of each primer, 250  $\mu$ M dNTPs and 1 $\mu$ l ThermoZyme polymerase (1U/ $\mu$ l Invitrogen ,Cat# E120-01). Cycles: 1x: 95°C for 5min, 30x: 95°C for 30sec, 53°C for 30 sec, 72°C for 40 sec, 1x: 72°C for 7 min. Primers:

*rpr* (forward) 5'-AGTCACAGTGGAGATTCCT-3',  
*rpr* (reverse) 5'-CATTATCACAATCGCTTG-3',  
*grim* (forward) 5'-ATGAGGACGACGTTACC-3',  
*grim* (reverse) 5'-TTCTTGTTGCTGCGGTTG-3',  
*hid* (forward) 5'-CGCATTGATCTCATGG-3',  
*hid* (reverse) 5'-GGGGATAAAGCAAGGAT-3',

*traf* (forward) 5'-GCTCCTTGGTCTTTTGCATTCA-3',  
*traf* (reverse) 5'-CCCCGGGGAATCGGACCAGCATCGCATCTCTG-3'.

Acknowledgements: We thank Maurice L. Edwards for excellent assistance with photography.

References: Bangs, P.M., N. Franc, and K. White 2000, Cell Death and Differentiation 7: 1027; Verhagen, A.M., and D.L. Vaux 2002, Apoptosis 7: 163; <http://flybase.bio.indiana.edu/bin/fbgrmap?spp=fly&chr=3L&self=1&range=17552449..18720058&mimetype=text%2Fhtml&cl=cyto def&cl=gene>; White, K., M.E. Grether, J.M. Abrams, L. Young, K. Farrell, and H. Steller 1994, Science 264: 677.



Use of cross-species genome comparison to deduce possible importance of the various *D. melanogaster fruitless* transcripts: A putative vital function for the type C transcription factor?

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The *Drosophila fruitless (fru)* gene product has been postulated to be a neural sex-determination factor that directs the development of at least two male-specific characteristics, namely courtship behaviour and the formation of the muscle of Lawrence (Ito *et al.*, 1996; Ryner *et al.*, 1996; Vilella *et al.*, 1997; Usui-Aoki *et al.*, 2000). The *fru* gene encodes a member of the BTB/POZ Zn finger family of transcription factors and is extensively sex-specifically spliced (Ryner *et al.*, 1996). There are three sex-specific transcripts in each sex with the female splice forms being larger than the male forms (Goodwin *et al.*, 2000; Usui-Aoki *et al.*, 2000). The male splice forms encode peptides with an additional sequence at their 5' ends of unknown function. At the 3' ends of these transcripts are found the Type A, B, and E Zn finger domains (Usui-Aoki *et al.*, 2000; Baker *et al.*, 2001; Davis and Ito, 2001). It is believed that these transcription factors specify all aspects of male sexual behaviour by regulating a specific set of sex-specific genes (Baker *et al.*, 2001), and it has recently been shown that the male Type B protein is sufficient to sex-specifically up-regulate the *y* gene in female CNS, whereas the male Type A protein is not (Drapeau *et al.*, 2002). The sex-specific transcripts are altered by the insertion of P-elements upstream of the BTB domain-encoding sequence and are initiated from promoter 1 (Anand *et al.*, 2001). The P-element mutants result in a variety of sexual behaviour defects affecting male sexual courtship: indeed the *satori* mutation results in males that will only court other males (Ito *et al.*, 1996). In contrast, deletion of the *fru* gene results in lethality at the late larval/early pupal stage of development (Ito *et al.*, 1996; Ryner *et al.*, 1996; Anand *et al.*, 2001).

In addition to the sex-specific transcripts there are a series of sex-non-specific transcripts that may be initiated from promoters 2-6 (Goodwin *et al.*, 2000; Anand *et al.*, 2001; Davis and Ito, 2001). Of these, one is initiated from promoter 2 and spliced to the type A C-terminal, and a second is initiated from promoter 3 and is spliced to the Type E C-terminal (Baker *et al.*, 2001): both these would be affected by the *fru* P-element mutations (Goodwin *et al.*, 2000). Transcripts initiated from promoters 3 or 4 have been shown to be required for viability (Anand *et al.*, 2001). Finally there are several sex-non-specific transcripts that do not appear to be significantly altered in expression in the various P-element *fru* mutations (Goodwin *et al.*, 2000). It is not known what peptides these latter



Type A

	C	C	H	H	C	C	H	H	
D.mel	LPTLYRCVSCNKIVSNRWHHANIHQPQSHECPVCGQKFTRRDNMKAHCKIKHADIKDR-FFSHYVHM								
D.het	.	.	.	.	.	.	.	.	-.
D.sil	.	.	.	.	.	.	.	.	-.
An.gam	F....S...H.T.....V..PELR.-.YN.I..								
Ap.mel	NLGTEC.KL.G.S.A.IKK.MKS.F.DKYQ.QI.MISL..S..L.R.I.L.G-.REGSLI.PFMRL								
	*		*	*	*	*	*	*	*

[illegible]

	C	C	H	H
D.mel	F	D	L	Q
D.het	.	.	.	.
D.sil	.	.	.	.
An.gam	. . . K .	A E E . Y . Y . M Y . R . C G D I P .	. . . . . D . . A . . . T .	D A S . V R R A
Ap.mel	Y S . . . . . R S . L . . . . Y . . . .	D S M P E . . . . . A A . G . . . . .	. . . . . D . . A . N .	V A K . S E A R G . Y
	*	* *		*

C C H H C C H H  
D.mel GGCNLHRCKLCGKVVTTHIRNHYHVHFPGRFECPLCRATYTRSDNLRTHCKFKHPM  
D.het .....  
An.gam .....  
Ap.mel PYTYTSK.....RI.SNLK...LT.N..NYV....GCCR..L...KG.I.Q...E  
\*       \*\*   \*   \*\*                   \*                               \*

The *fru* gene has recently been cloned and sequenced in the Diptera *Anopheles gambiae* (malaria mosquito: ENSANGG00000017352) and the Hymenoptera *Apis mellifera* (honeybee: contig 1134). The *An. gambiae* genomic structure is conserved with that of *Drosophila* with the same intron/exon boundaries. The amino acid sequence of the BTB domain (exons III and IV) is very well

conserved, whereas the amino acid sequences of the less well-conserved exons (V, VII, VIII, IX, X and XI) have diverged considerably, but are recognisable (for exon definition see Davis and Ito, 2001). The *Ap. mellifera* genomic structure differs from that in the Diptera in that exons III, IV and V (encoding the BTB domain) are fused into a single exon, and the amino acid sequence corresponding to exon V is much shorter. However, those amino acids that mark the intron/exon boundaries in *Drosophila* are conserved and the putative nuclear localisation sequence appears in the correct place at the end of the exon. Interestingly exons III and IV are also fused in the Damsel fly *Ischnura asiatica* [Order Odonata] (Davis *et al.*, 2000b). The C-terminal peptides are very difficult to distinguish due to low homology (Figure 1), but have been putatively assigned, as they are in the correct order in the genomic sequence, though no homologies to Zn finger type B (exon XI) were identified. In addition, the start of the putative 3' terminal exons are frame-shifted by the same amount of bases as is found in *D. melanogaster* for both mosquito and honey bee, as is the last of the common exons (VIII) from which these are spliced, thus maintaining the correct reading frame.

The low homologies for some of these terminal peptides are illustrated in Figure 1. It can clearly be seen that the Type A Zn finger is barely recognisable in *Ap. mellifera*, and actually clusters with the Type E peptides in a phylogeny of the Zn fingers constructed using ClustalW (Figure 2). This peptide has been deduced solely based upon a slightly higher homology to the Type A peptide of *D. melanogaster*, and due to its position in the genomic sequence (equivalent to exon IX). The type B Zn finger has not been found in *Ap. mellifera* despite extensive searching, so has either not been sequenced as yet or is absent from the genome. The other Type B Zn fingers cluster strongly in the

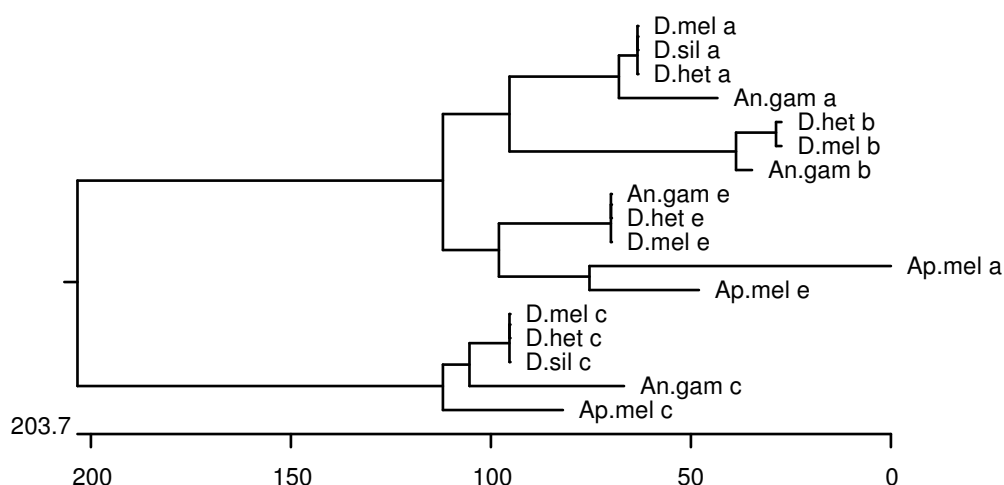


Figure 2. Phylogeny of the Zn finger domains using ClustalW.  
The species names are as in Figure 1.

phylogeny. The type E Zn finger is also poorly conserved in *Ap. mellifera* (Figure 1) and most of the homology is limited to the amino acids adjacent to the conserved Cys and His residues of the Zn finger, although it does cluster with the Type E peptides in the phylogeny (Figure 2). The most highly conserved of the Zn fingers is that encoded by the Type C peptide (Figure 1) and the homology is not confined to the amino acid required for the Zn finger. Interestingly, the Type C peptide has only one Zn finger, suggesting that it functions as a part of a hetero-dimeric protein. As a Zn finger in this sequence has only just been recognised it is possible that the significance of this peptide for the function of the *fru* gene has been overlooked, as of the three references that mention this transcript (Davis *et al.*, 2000a, 2000b, Usui-Aoki *et al.*, 2001) none have examined possible

functions for this transcript. As parts of the sexual behaviour gene cascade are not well conserved among the various insect phyla (Müller-Holtkamp *et al.*, 1995; Sievert *et al.*, 1997; Meise *et al.*, 1998; Saccone *et al.*, 1998), it is possible the sex-specific-behaviours controlled by the transcription factors from the *fru* gene that encode the types A, B and E Zn fingers are also poorly conserved. Conversely, it would seem necessary that any function required for fly viability would be more highly conserved. Thus it is proposed that the type C Zn finger may play an essential role.

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A summary of the new polytene chromosome bands seen by EM.

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The Bridges' drawn maps for *D. melanogaster* polytene chromosomes (C.B. Bridges, 1938; P.N. Bridges, 1941a,b, 1942; Bridges and Bridges, 1939; reprinted in Lindsley and Zimm, 1992) were made using the best possible squash preparations for light microscopy (Bridges, 1942). The band number in the above maps is about 3800 when the so called double bands are counted as one band each. The size of bands ranges from very faint and thin to dark and thick. Some bands have the look of large, complex structures.

Thin sections made from chromosome squashes and studied with the electron microscope (EM) should show finer detail than light microscopy. When we started to publish our micrograph maps, our goal was to identify as many Bridges' bands as possible (see Sorsa, 1988). Even though we regard about 320-350 Bridges' bands as missing in thin sections (for a summary, see Saura, 2003), electron micrographs have shown quite a few new bands (Table 1). A new band means a band that does not seem to have a counterpart on the Bridges' maps. New bands are named here with the number of the preceding band provided with a superscript prime as proposed by Semeshin *et al.* (1985a). In general new bands are very faint and thin structures.

Some new bands visible in certain micrographs only are, however, distinct (as are 5D1-2', 22F1-2', 17E4-5' and 37A1-2') and might represent structural variation between strains and/or

individuals. Some new bands were detected regularly in all or in most micrographs, but many bands only in a few or in one micrograph. New bands could be best seen in formaldehyde-fixed (FAR) material, which is post-stained with lead citrate unlike the acetic-methanol (AM) fixed material.

Table 1. A list of new bands that were detected from electron micrographs. Chromocentral parts (20B-F, 40B-F, 41A-E, 80B-F) are mostly omitted from detailed mapping. The most uncertain new bands are included in parentheses.

Chromosome arm and region	Name of the new band. Numbers denoting chromosome divisions are in <b>bold</b>	Reference (see also Saura <i>et al.</i> , 1997)
X1-2	<b>1A3'</b> , B1-2', B9-10', B14', C2-3', C4-5', (E1-2'), F3', F3'', F4'; <b>2A1-2'</b> , A1-2'', A3-4', (B1-2', C9', D3-4', D6')	Sorsa and Saura (1980a)
X3-5	<b>3A1-2'</b> , A1-2'', A4', (A6'), B2', B3-4', (C11-12', C11-12''), D4', D5-6'; E8', F1-2', F1-2'', (F3'); <b>4(A3')</b> , B1-2', B5', (C1-2', C5-6', C7-8', D1'); <b>5(A5')</b> , D1-2', (E4-5'), F3', (F4')	Sorsa and Saura (1980b)
X6-10	<b>6C1-2'</b> , (C6'), C8', D3', (E6'); <b>7(A7', A8', B1-2')</b> , B8', (B8''), C1-2', C4', D11', (E1-2'); <b>8A5'</b> , (C3', D1-2', D3'), D7', (E10-11'); <b>9(A2', A2'')</b> ; <b>10(A1-2', E6', F10', F11')</b>	Sorsa <i>et al.</i> (1983)
X11-20A	<b>12(B8')</b> ; <b>14E4'</b> ; <b>17D3-4'</b> , E4-5'; <b>18B5'</b> , D3-4', D3-4'', D10-11', D13', F1-2'; <b>19A3-4'</b> , E3-4', (E3-4''), E7-8', F1-2', F4', (F4''), F5'	Saura <i>et al.</i> (1993)
2L21-22	<b>21(A4', B1', B1'')</b> , B2', B3', B6', B7', B8'), C1-2', C3', C4', C8', E1-2', E3' (or E4'); <b>22(A1-2', A1-2'', A7', A8', D1', D5', D6')</b> , E1-2', F1-2', F4'	Saura and Sorsa (1979a)
2L23-26	<b>23B8'</b> , C1-2', C3', C4', D3', E1-2', E6'; <b>25A8'</b> , B3', B3'', B4-5', C1-2', C3', C5-6', F1-2', F5'; <b>26B4'</b> , B7', D8', E1-2', E3', F1-2', F5', F6'	Saura (1980)
2L27-29	<b>27B3'</b> , (E1-2'); <b>29A4'</b> , C3', F6-7'	Saura and Sorsa (1979d)
2L30-31	<b>30B4'</b> , B12'; <b>31(C4')</b> , D8-9', D10', (E2')	Saura and Sorsa (1979b)
2L32-36(-40)	<b>34D4'</b> ; <b>36(B4', F3', F3'', F3''')</b>	Saura (1983)
2L37-40A	<b>37A1-2', C5', (E1-2', E5')</b> ; <b>39E7'</b>	Saura and Sorsa (1979c)
2R41F-50	<b>41F1-2'</b> ; <b>43A3'</b> , B1-2', C6', E12', E13', F3'; <b>44C3'</b> , C4-5', C6', C6''	Saura (1986)
2R51-60	<b>52(C9')</b> ; <b>53F3'</b> , F11-12' (or F13'); <b>55(E6-7')</b> ; <b>56F8-9'</b> ; <b>57A1-2'</b> , B20', F8'; <b>58E10'</b> ; <b>59B4-5'</b> , B8', C5', E4', F1-2', F3', (F4'); <b>60B13'</b> , C6', C7-8', D8-9', (D15')	Saura <i>et al.</i> (1991)
3L61-63	<b>61A6'</b> , A6'', (B1'), B2', B2'', B3', B3'', (C2'), C3', C4', (C4'', C6', C6''), C7', C8', C8'', (D1') E1', F1', F2', F2'', F4', F7', F8'; <b>62(A3', A4')</b> , A5', (A12', B4-5'), B6', B6'', B7-8', (B7-8''), B9', B10-11', B12', C3', C4', D1-2', (D3'), D3'', D5-6', D5-6'', D7', E3', (E7''), E7''; <b>63(A1-2')</b> , A4', A4'', (A5'), C2', (C4'), D1', D2', (E2-3')	Sorsa <i>et al.</i> (1984)
3L64-70	<b>64A5'</b> , C8', D2'; <b>65B4'</b> , B5', D3', F11'; <b>66A8-9'</b> , A10', A12', A13', A22', B2', B11', B12', D1-2', D1-2'', D8', D14', D15', E3', E4', F6'; <b>67A1-2'</b> , A8', B7', B10', C1', C3', C8', D8', E4', E5', E7'; <b>68A3'</b> , A7', A8', B1-2', C8', C15', D4-5', E4'; <b>69B3'</b> , D6', F3', F7'; <b>70D1'</b> , D3', E3', E6'	Saura <i>et al.</i> (1989)
3L71-73	<b>71A4'</b> , B1', (B2'), B8', B8'', C1-2', C1-2'', C1-2''', D1-2', (D3', E3', E4'), E5', (F1-2'), F5'; <b>72A1-2'</b> , (D5'), D9'; <b>73A3'</b> , A8', D4', (E5', E6', F2'), F4'	Sorsa <i>et al.</i> (1987)
3L74-80A	<b>75C6'</b> , D8'; <b>77A3'</b> , B5', B9'; <b>78B1'</b> ; <b>79F1'</b> , 79F1''	Saura <i>et al.</i> (1988)
3R81-90	<b>82A1-2'</b> , A3', A4', C5', D7', E5'; <b>83B6-7'</b> (given that adjacent bands are correctly named), C1-2'; <b>84D9-10'</b> ; <b>85B2'</b> , B9', C1-2', F16'; <b>87A8'</b> , B6', C9', D14'; <b>88E3'</b> , F5' (if neighboring bands are correctly identified); <b>90B1-2'</b>	Saura <i>et al.</i> (1994)
3R91-100	<b>94(D10-11')</b> ; <b>96(A5')</b> , A6', B9', C7'; <b>98E3'</b> , F4'; <b>99A5-6'</b> , D1-2', D7', E4', E5', E5'', F5'; <b>100C7'</b>	Saura <i>et al.</i> (1996)
4:101-102	<b>102A5'</b> , A6', B2', B6', D5', E2', E5'	Saura <i>et al.</i> (2002)

Should we follow the EM mapping rules of Semeshin *et al.* (1985a), many of the 280 -370 new bands of Table 1 would probably be omitted, since the amount of evidence needed to demonstrate a band might be considered to be too meager. For example, most of the new bands in the tip of 2L were located in the difficult regions; counting of bands in these regions can not be done as reliably as in clearer regions. Many new bands are, however, certainly real structures. Schalet and Lefevre (1976; see also Semeshin *et al.*, 2001) suggested that there are new bands in 19E and F, and Hochman drew the

band 102E5' on his map (in King, 1975). In addition, Semeshin and coworkers (e.g., Semeshin and Szidonya, 1985; Semeshin *et al.*, 1985a,b) have confirmed that some submicroscopic bands like 1C5', 21E4', 25A8', 25B3', 62E3', 67A8', 68C15', 79F1' exist.

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Genetics and molecular analysis of a *Drosophila willistoni* spontaneous mutation similar to *eyeless*.

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## Abstract

A *Drosophila willistoni* mutation similar to the *eyeless* (*ey*) phenotype of *D. melanogaster* is described here. This allele segregates like an autosomic recessive gene. The structural integrity of the *D. willistoni ey* gene was investigated by Southern blot analysis using as a probe the *D. melanogaster eyeless* cDNA. The results show no structural detectable alteration at the *ey* locus. This could be the result in one or more of the following: *i*) it can be related to a point mutation; *ii*) it can be caused by a mutation at the vicinity of the *ey* gene region assayed by the *ey* cDNA probe, and *iii*) the mutation occurred in another gene whose phenotype is similar to *eyeless*.

## Introduction

In 1915 Hoge described a *Drosophila melanogaster* mutation characterized for a complete or a partial absence of eyes, which was called *eyeless* (*ey*) (reviewed in Halder *et al.*, 1995). Afterwards, the *ey* gene was characterized as being a member of the *Pax-6* family (Quiring, 1994), which codifies a transcription factor that plays a fundamental role in the eye morphogenesis and contains two highly conserved domains: a paired-domain and a homeodomain (Ton *et al.*, 1991; Walther and Gruss, 1991). The contribution of Halder *et al.* (1995) was conclusive to propose the *ey* gene as a master control gene to the eye development in *Drosophila*, since its ectopic expression can transform many other tissues to develop in an eye, including legs, wings, and antennae.

The *Drosophila ey* gene is homologous to the *Small eye*(*Sey*) in the mouse (Hill *et al.*, 1991) and to the *Aniridia*(*AN*) gene in humans (Glaser *et al.*, 1994; Jordan *et al.*, 1992). Mutants of these genes, either in *Drosophila* (Quiring *et al.*, 1994) or in mammals (Gruss and Walther, 1992) result in the reduction or the complete loss of the eye structures. Similar sequences to the *ey* had also been identified in amphibians, fish, amphioxus, sea squirts, sea urchins, squid, nematodes, ribbon worms, and planarians (reviewed by Gehring and Ikeo, 1999). These findings suggest that *Pax-6* might be the universal master control gene for the eye development. This hypothesis is reinforced by the demonstration that the mouse *Pax-6* gene expression can induce an ectopic compound eye formation (Halder *et al.*, 1995; Gehring, 2002).

Spontaneous mutation of the *ey* gene has been associated to transposable elements (TEs). Quiring *et al.* (1994) characterized two *Drosophila ey* mutants and detected that both mutations were caused by insertions of TEs into the first intron of *ey*.

Transposable elements (TEs) are DNA sequences with the ability to move themselves from site to site within of the genome promoting genetic variability as they are mutagenic, causing insertions, deletions, and others chromosomal abnormalities. The TEs are found in all organisms with a variable number of copies, making up approximately 15% of the *D. melanogaster* genome; 50% of the maize genome (Kidwell and Lisch, 2000) and more than 45% of the human genome (International Human Genome Sequencing Consortium, 2001). TEs can be functional or defective. An active system of TEs can increase the normal mutation rates (Robertson, 1978; Engels *et al.*, 1989; Kidwell and Lisch, 2002). According to Ashburner (1992), up to 80% of all mutations in *D. melanogaster* are induced by TEs. The genetic detection of hypermutable strains in *Drosophila* can also be associated to the existence of active TEs in the genomes (Loreto *et al.*, 1998). Therefore, a large number of spontaneous mutations that arise in *Drosophila* can be associated with the TEs mobilization. The aim of this report is to show data of the genetics and molecular biology of a spontaneous mutation of *D. willistoni*, named hereafter “*eyeless*” (*ey*) because its similarity to the *eyeless* mutation of *D. melanogaster*, in search for the causal association to the TE mobilization.

## Materials and Methods

*Drosophila stocks:* Table 1 shows the *Drosophila* stocks used in this study. Mutant strains were established after isolating spontaneous mutations from the progeny of isofemales lines collected in the wild, as described in Goñi *et al.* (2002).

Table 1. Stocks of *Drosophila willistoni* used in this study.

Strain*	Origin, location and date of collection	Phenotype
TB44.02	Isofemale, Rocha, Uruguay, V.2002	Spontaneous eye mutation: phenotype similar to <i>eyeless</i>
SG2.01	Isofemale, Montevideo, Uruguay, IV.2001	wild type
WIP4	Ilha das Cobras, Bahia, Brazil, 1970	wild type
EM1.00	Female, Montevideo, Uruguay, IV.2000	Spontaneous mutations: <i>yellow</i> ( <i>y</i> ) and <i>white</i> ( <i>w</i> )
EY10.00	Isofemale, Montevideo, Uruguay, IV.2000	Spontaneous eye color mutation: phenotype similar to <i>white coffee</i> ( <i>w<sup>cf</sup></i> )
Manaus	Manaus, Amazonas, Brazil	Spontaneous mutations: <i>yellow</i> ( <i>y</i> ), and segregate <i>roughoid</i> ( <i>ru</i> )
ES13.99	Isofemale, Montevideo, Uruguay, IV. 1999	Spontaneous mutations: <i>Clipped</i> ( <i>Cl</i> ), segregate <i>apterous</i> ( <i>ap</i> )

\* WIP4 (see Dos Santos-Colares *et al.*, 2003), and EM1.00 (see Goñi *et al.*, 2002)

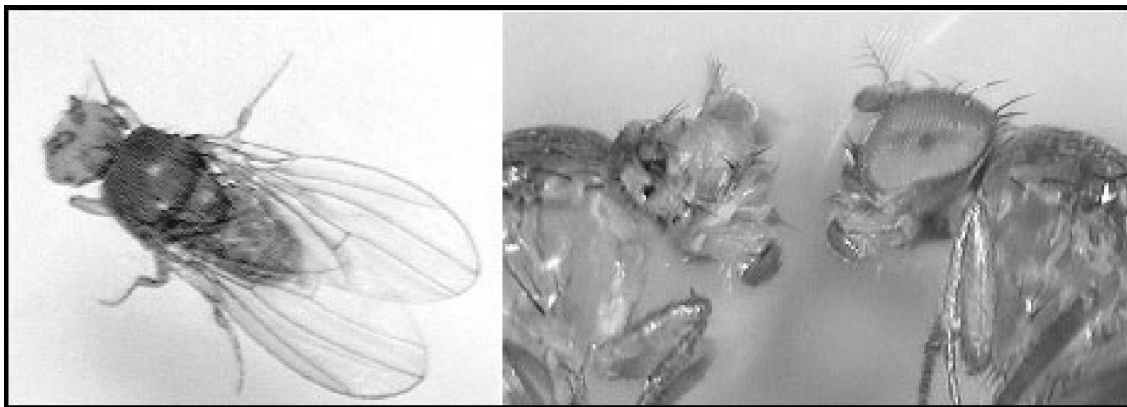


Figure 1. Flies of *eyeless* TB.44.02 mutant strain (left and center) and of wild type (right).

*Genetic analysis:* For the linkage test, reciprocal crosses between individuals of *ey* TB44.02 and *Cl* ES13.99 were performed. *Cl* is the dominant character, distinguished by variable incisions on the inner margin of the wing. As described by Ferry *et al.* (1923), *Cl* is homozygous lethal and located on the chromosome 2. It is being kept in laboratory by routine selection. The progeny of each reciprocal cross were examined, and the F<sub>1</sub> male offsprings expressing *Clipped* phenotype were selected and mass mated to *ey* virgin flies. The resulting F<sub>2</sub> offsprings were examined and classified into four phenotypic classes. For mating experiments virgin flies were collected every three hours and mated to young males. Flies were anethesized with triethylamine vapors. Progeny was scored until the 21st day to avoid overlapping generations. Stocks and matings were cultured at 25° ± 1°C with standard corn-yeast-agar media.

*Southern Blot analysis:* Genomic DNA of each strain was prepared from 50 adult flies according to the method described by Loxdale *et al.* (1998). DNA samples (approximately 15µg) were digested with the restriction endonuclease *EcoRI*, following the recommendations of the manufacturers. The DNA fragments were separated by electrophoresis on 0,8% agarose gel and

transferred to a nylon membrane (Hybond-N+ - Amersham). The probe used was a plasmid, which contains the *ey* cDNA of *D. melanogaster*, kindly sent by Dr. W. J. Gehring.

## Results and Discussion

**Mutant eye phenotype:** *D. willistoni* mutant flies showed eye size variably reduced, from flies having extreme phenotypes with no eyes to those which developed only few ommatidia in one or both the eye regions (Figure 1). In all cases, the arista of the antennae in mutant flies is remarkably short and thin in comparison to the wild type. This feature is not reported in the *eyeless* mutants of *D. melanogaster* though duplications of antennae or antennal segments with or without duplication of arista are found in some flies with the *ey*<sup>2</sup> allele (Lindsley and Zimm, 1992). Flies of the *ey* TB44.02 mutation have good viability and are phenotypic stable at 18°C temperature.

**Genetic analysis:** Examination of the F<sub>1</sub> progeny from the reciprocal crosses between *ey* TB44.02 and *Cl* ES13.99 mutant flies revealed that the *eyeless* is an autosomic recessive gene. In both cases, an excess of *Cl*<sup>+</sup> individuals was observed in a proportion of 3:1 (data not shown). The results of the linkage analysis (Table 2) showed that the *ey* locus segregates independently from the chromosome 2, this being likely located on the chromosome 3. The absence of flies of the “*Cl ey*” class among the F<sub>2</sub> progeny could be attributed to zygote inviability.

**Southern blot analysis:** Southern blot analysis was used to detect and characterize TE-insertion(s) into the *ey* locus of the *D. willistoni* TB44.02 mutant flies. Several strains having normal

Table 2. Results of linkage test of the *ey* TB.44.02 gene in *D. willistoni*

F <sub>1</sub> <i>Cl</i> /males*	Progeny (F <sub>2</sub> )				Total
	+	<i>Cl</i>	<i>ey</i>	<i>Cl ey</i>	
A	220	86	225	0	531
B	183	68	189	0	440
Total	403	154	414	0	971

\*The female parent in “A” is *Cl/Cl*<sup>+</sup>, and in “B” is *ey/ey*.

eye morphology were used as control. However, no alteration of the restriction pattern was detected in the genomic region of the *ey* locus of the mutant studied compared with the control strains (Figure 2). In all cases, the restriction fragments sizes observed were of 4.2Kb and

0.62Kb. These results allow some interpretations, not being possible to distinguish, at present, the following:

- 1- The mutation could be caused by a point mutation, nucleotide substitution or a small deletion or insertion, which cannot be detected under the molecular approach used here.
- 2- The mutation could have been caused by an alteration of the *ey* gene expression induced by a genomic mutation near the *ey* locus.
- 3- The mutation could have occurred in another gene involved in the eye development that leads to a similar phenotype. Halder *et al.* (1995) estimates that more than 2000 genes are involved in eye morphogenesis, being directly or indirectly controlled by the *ey* gene. Of these, at least four loci, when mutated, result into a similar eye phenotype to *ey*: *eye gone* (*eyg*), *sine oculis* (*so*), *eyes absent* (*eya*), and *dachshund* (*dac*) (Desplan, 1997). The *ey* mutant of *D. willistoni* studied here could be a new eye mutant locus.

Finally, in the course of this study the genome of other *D. willistoni* spontaneous mutations (*w*, *w<sup>cf</sup>* and *y*) were analyzed with the use of *D. melanogaster* genes as a probe. These genes must have acquired a significant degree of genetic difference, since no DNA hybridization was observed (data not shown) in view of the time of divergence between these species, estimated as about 50



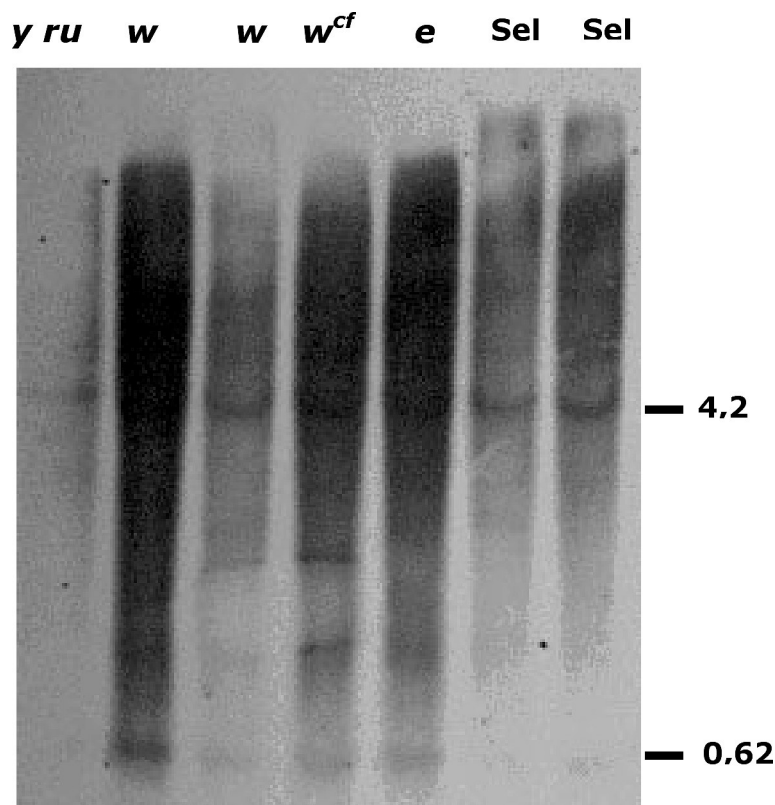


Figure 2. Southern blot of *D. willistoni* genomic DNA. The samples were digested with *EcoRI* and hybridized with the plasmid pHSE, which contains the *ey* cDNA of *D. melanogaster*. The strains are: *y ru* Manaus; *w y* EM1.00; *w* EM1.00; *w<sup>cf</sup>* Ey10.00; *ey* TB44.02; and Sel 1 and Sel 2 wild strains. All the strains showed the same bands corresponding to the restriction fragments 4.2 Kb and 0.62 Kb.

million years (Clark, 1995). On the other hand, the *ey* gene is a highly conserved gene between species in accord of the strong genomic hybridization signal observed in this study.

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