

Genomic organisation and characterisation of the behaviour gene *fruitless (fru)* in the Hawaiian species *Drosophila heteroneura* and *Drosophila silvestris* and the conservation of the *fru* BTB domain throughout evolution.

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The *fru* gene from two Hawaiian picture-wing species has been cloned and sequenced at the genomic and cDNA levels. The gene from *D. heteroneura* has at least eight exons covering a region of 18-kb, which encodes four transcripts, *fruA*, *fruB*, *fruC* and *fruF*. The *fruF* transcript has a small deletion within exon, encodes a smaller protein and appears to be an allelic variation. The first seven exons of the *D. silvestris fru* gene, which encode the *fruA* and *fruC* transcripts, have also been cloned. The genomic structure of these transcripts is identical in the three species except that the exons I and II of *D. melanogaster* that encode a male-specific peptide were not found. Two transcripts from *D. melanogaster*, *fruD* and *fruE*, were also not found in the Hawaiian *Drosophila*, though an exon with 100% homology to the terminal sequence of *fruE* was found in *D. heteroneura*. This latter was not found as a cDNA and thus may not be expressed.

The FRU peptides are highly conserved with the *D. melanogaster* proteins, particularly the BTB protein-protein-binding domain that is encoded by exons I and II in the Hawaiian *Drosophila*, and is 100% conserved. The peptide encoded by exon III has several sequence differences but these are confined mostly to regions of repetitive sequence and exons IV to VI are well conserved. The only major peptide difference is encoded by exon VII that encodes the FRU type A alternative 3' end. This peptide is semi-conserved for the 5' end and 100% conserved for the Zinc finger domains; the rest of the peptide is virtually unconserved. The exon VII sequence was also isolated from another Hawaiian species, the modified-mouthparts species *D. mimica*, and the sequence is well conserved with the other Hawaiian *Drosophila*, though there are several differences. The FRU type B and C alternative 3' ends (exons IX and V respectively) are well conserved. No sequences homologous to the sex-differentially spliced upstream exons of *D. melanogaster* have been found in *D. heteroneura*, suggesting that the *D. melanogaster* male-specific peptide is absent in the Hawaiian *Drosophila*.

The expression pattern of the *fru* gene is complex in *D. melanogaster*. There is a series of transcripts common to both adult sexes of about 3.8 to 4.0-kb. In addition there are sex-specific transcripts ranging from 5.5 to 9.0-kb with the female transcripts about 1.1 to 1.6-kb larger than the male transcripts. In *D. heteroneura*, however, the *fru* gene is expressed in one to seven days old adult

males and has transcripts of 4.0, 4.5 and 5.1-kb, which presumably correspond to the type A, B and C transcripts. It is assumed that these transcripts correspond to the sex-common transcripts of *D. melanogaster*, though they are not seen in females. Thus it appears that, at least in adults, the expression of the *fru* gene is very different to that seen in *D. melanogaster*.

The *fru* gene is involved in male courtship behaviour; mutations in *fru* exons I and II alter the male courtship resulting in flies attempting to court other males. In the Hawaiian flies males participate in lek behaviour in which the males fight to protect a specific area from other males. This is presumably a prelude to mating as the females are known to visit the lek areas. The mutant behaviour in *D. melanogaster* superficially resembles this lek behaviour and the lack of the *D. melanogaster* exons I and II in the Hawaiian flies suggests that the ancestors to *D. heteroneura* lost these exons, with the resulting behaviour defect evolving into the lek behaviour.

The origin of the Hawaiian *Drosophila* is of major interest in the evolution of the Drosophilidae. They are believed to have originated in East Asia, particularly Japan, as a single introduction and are thought to be akin to the *robusta* species group of the *Drosophila* subgenus. As sexual selection, and in particular sexual behaviour, is believed to play a major role in evolution I decided to clone and sequence part of the *fru* gene in a diverse group of Drosophilids so as to address the origin of the Hawaiian *Drosophila*. I have chosen the BTB-protein-protein binding domain as it is a region that should show a high degree of sequence conservation due to it presumably being essential for the function of the FRU protein. The DNA sequence encoding the BTB domain of the *fru* gene has been cloned from 21 species of Diptera. The protein-coding sequence is highly conserved and the amino acid sequence is identical except for two changes in the Tephritidae. The intron sequences are completely unconserved except between very closely related species such as the Hawaiian *Drosophila*. The phylogeny produced using the BTB exon sequences agrees very well with other molecular studies and suggests that the most closely related mainland *Drosophila* species is *D. moriwakii* of the *robusta* species group, thus providing evidence for an East Asian origin. The genus *Scaptomyza* is believed to have evolved in Hawaii early in the Hawaiian *Drosophila* radiation and then spread to the mainland. The BTB phylogeny shows that the *Scaptomyza* are closely related to the Hawaiian *Drosophila* and also to the *robusta* group species *D. moriwakii*, so supporting the proposed Hawaiian origin for the *Scaptomyza*. The genus *Zaprionus* is placed in the subgenus *Drosophila* closely related to *D. immigrans* along with the genera *Samoaia* and *Liodrosophila*.

In conclusion, I have cloned the *fru* gene from two Hawaiian *Drosophila* species and shown that the genomic structure differs from *D. melanogaster* in that the sex-specific splicing domain appears to be absent. I have also shown that a phylogeny derived from a sexual behaviour gene will give similar results as using other genes.