

Supplementary

Characterization of *Gfat1* (*zeppelin*) and *Gfat2*, Essential Paralogous Genes Which Encode the Enzymes That Catalyze the Rate-Limiting Step in the Hexosamine Biosynthetic Pathway in *Drosophila melanogaster*

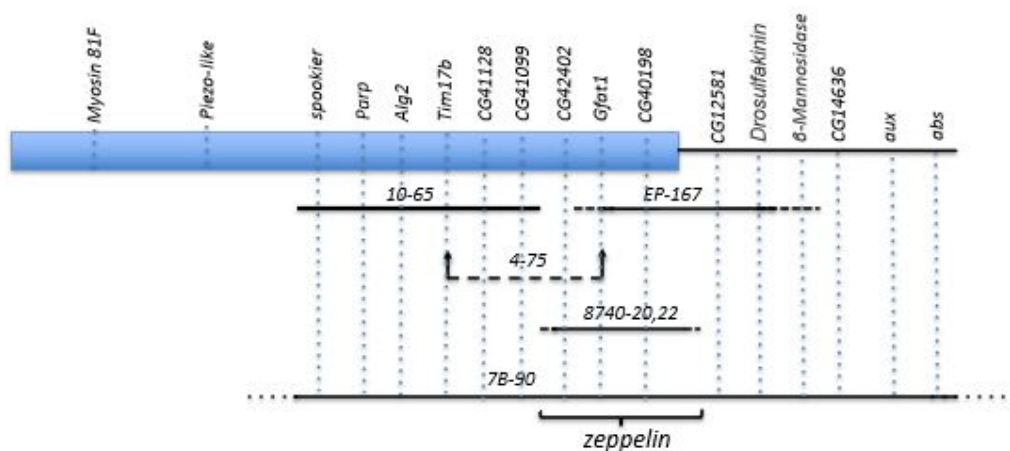


Figure S1. Map of proximal 3R correlating the molecular location of the *Gfat1* gene with the genetic position of *zep*. The extents of the various rearrangements used are shown below. *In(3R)4-75* is a multi-break inversion, whereas the other rearrangements are deletions in proximal 3R [35, 35, 41].



Figure S2. *Splayed*-like phenotype displayed by *hs-GAL4/2664-1-5M-CH2* fly raised at 29°C. Note the melanin deposit on the leg as indicated by the red arrow.

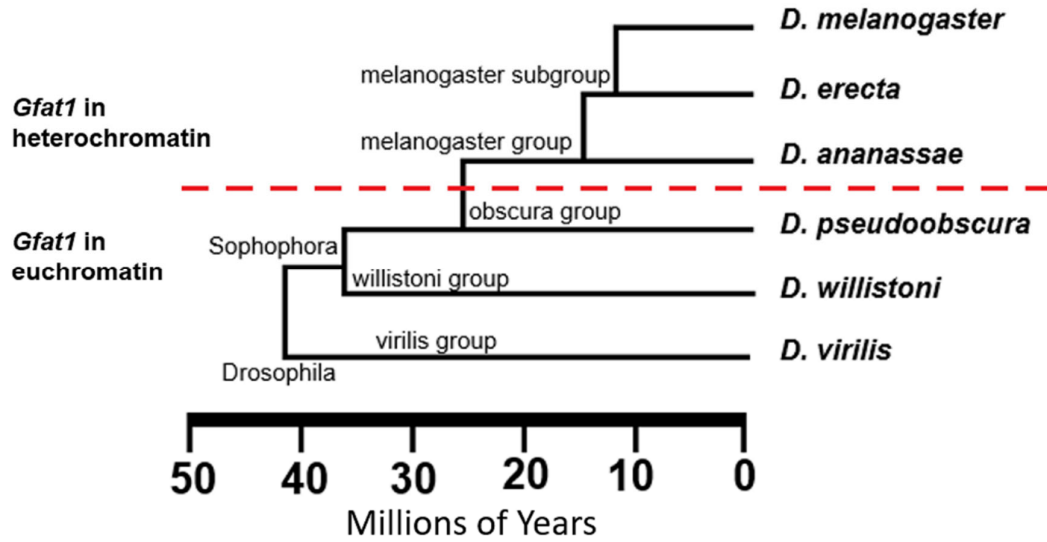


Figure S3. Schematic representation of *Drosophila* evolution over the last 50 million years. The dashed red line separates the species group in which the *Gfat1* gene is located in heterochromatin (*melanogaster* group) from that in which the gene is located in euchromatin (*obscura* group).

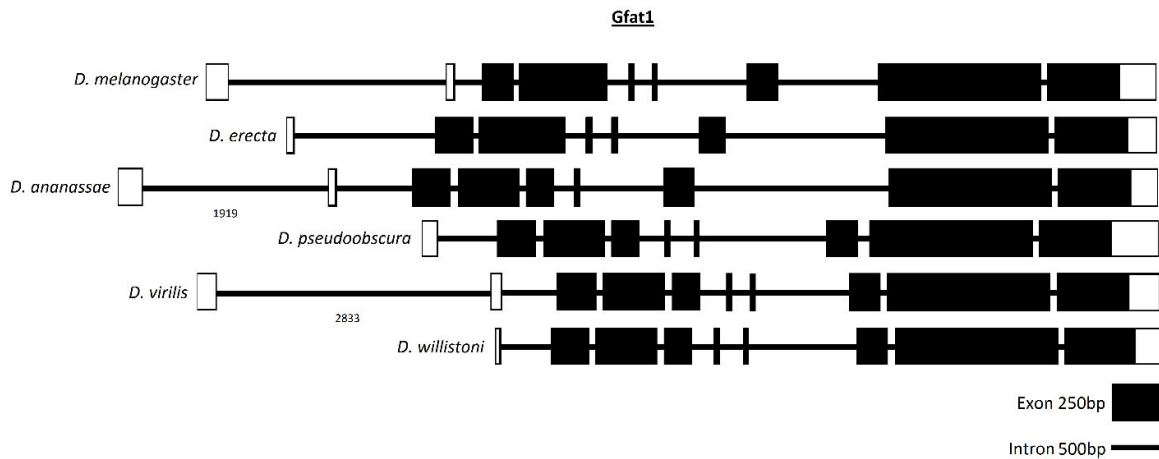


Figure S4. Gene architecture of the longest versions of *Gfat1* in six species of *Drosophila*. Translated exons are represented by black boxes. 5' and 3' untranslated regions (UTRs) are represented by white boxes. Introns are represented by black lines. Data from FB2017_05 release.



Figure S5. Gene architecture of *Gfat2* in six species of *Drosophila*. Translated exons are represented by black boxes. 5' and 3' UTRs are represented by white boxes. Introns are represented by black lines. Data from FB2017_05 release.

Table S1. RNAi strains from *Drosophila* stock centres used in the present study.

| Stock Number and Genotype | Origin | Gene | Designation |
|---|--------|--------------|-------------|
| v24539: <i>w¹¹¹⁸</i> ; <i>P{GD7732}v24539</i> | VDRC | <i>Gfat1</i> | v24539 |
| 42892: <i>y sc* v sev²¹</i> ; <i>P{TRiP.HMS02585}attP40</i> | BDSC | <i>Gfat1</i> | B42892 |
| v105129: <i>P{KK101769}VIE-260B</i> | VDRC | <i>Gfat2</i> | V105129 |
| 34740: <i>y sc* v sev²¹</i> ; <i>P{TRiP.HMS01220}attP2/TM3, Sb</i> | BDSC | <i>Gfat2</i> | B34740 |
| 50909: <i>y v</i> ; <i>P{TRiP.HMJ03131}attP40</i> | BDSC | <i>Ogt</i> | B50909 |

Table S2. Tests for viability effects of *Gfat1* RNAi transgenes when driven ubiquitously. Chromosome 2 insertions crossed with *Act5C-GAL4/CyO*; Chromosome 3 insertions crossed with *Tub-GAL4/TM3, Sb*.

| Transgene and Location | <i>CyO</i> or <i>TM3, Sb</i> Progeny | RNAi with Driver |
|------------------------|---|------------------|
| Chromosome 2 | | |
| 2664-1-2M-CH2 | 100 | 0 |
| 2664-1-5M-CH2 | 55 | 0 |
| 2664-1-9M-CH2 | 42 | 0 |
| Chromosome 3 | | |
| 2664-1-6M-CH3 | 58 | 0 |
| 2664-1-7M-CH3 | 47 | 0 |
| 2664-1-8M-CH3 | 40 | 0 |

Table S3. Primers used for the sequencing analysis of zep alleles. Primers span the entire coding sequence of the *Gfat1* gene (including exon/intron splice junctions). They are depicted 5' to 3' from left to right. *Gfat1* sequencing primers.

| GFP-F caagagtgccatgccgaag | GFP-R gacagggccatgcgaattg |
|--------------------------------------|----------------------------------|
| Grp84-01 acgcttcctgctgatggac | Grp84-02 tccagtaactggattgagt |
| Gfat1-1F tcttggttctgtcgtgctacc | Gfat1-1B aaccgagtttggctgctgat |
| Gfat1-2F tcgcttcgcatttcacaag | Gfat1-13 ccaacgtgggtcaggacgggttc |
| Gfat1-4F actacaaggttcaacattctg | Gfat1-4B gggaaatgaattgggaggtgt |
| Gfat1-5F ctgatggccttacgttactgt | Gfat1-5B atgtgtccaagcaatgtgatg |
| Gfat1-15 ctgcgcgcaatcaacatacgatt | Gfat1-6B caagtgtaatggtaggtgc |
| Gfat1-10F gtaagcacagcaatggtt | Gfat1-14F gccacttgctagaaggtgga |
| Gfat1-24R cggcaatatgataagacagtagtagc | |

Table S4. qPCR primer sequences. Gene Amplified Primer and Probe Sequences.

| <i>RpL32</i> | Forward primer 5'- GTCGGATCGATATGCTAAGCTG -3' Reverse primer 5'- CAGATACTGTCCCTTGAAGCG -3' Probe 5'-/56-FAM/ TCGATATGC /ZEN/ TAAGCTGTCGGCACAAA /3IABkFq/-3' |
|---------------------|--|
| <i>Gfat1</i> | Forward primer 5'- ACCTATACCAACACAAGTCGC -3' Reverse primer 5'- CTTCAATTCACCGGCCATG -3' Probe 5'- 56FAM/TGCCTAGAA/ZEN/GGTGCATTGAAAGTCAAAGAG /3IABkFQ/-3' |
| <i>Gfat2</i> | Forward primer 5'- TTGCCAAGGAGTTGTACGAG -3' Reverse primer 5'- TCCGTGTTTCAGTTCTCCAG -3' Probe 5'-/56-FAM/ AGTTGAAGC /ZEN/ CCCTGCCCATAATGA /3IABkFQ/-3' |

Table S5. Microsynteny of two genes flanking *RpL15* or its orthologue in six *Drosophila* species. Data from Schulze et al., 2005 and the FB2017_05 release; note that *RpL15* is located in heterochromatin in *D. virilis*, but in euchromatin in *D. pseudoobscura* (Schulze et al., 2005); sequence identity of orthologous *RpL15* proteins versus *D.melanogaster*. *RpL15* range from 96% to 100%.

| Species and Designation of <i>RpL15</i> gene | rad21 or Ortholog 5' to <i>RpL15</i> | 5' to 3' Orientation Relative to <i>RpL15</i> | Dbp80 or Ortholog 3' 5' to <i>RpL15</i> | Orientation relative to <i>RpL15</i> |
|---|---|--|--|---|
| melanogaster CG17420 | yes | same | yes | same |
| erecta GG163267 | GG13128 | opposite | yes | same |
| ananassae GF20530 | yes | opposite | GF27128 | opposite |
| pseudoobscura GA22222 | GA31871 | same | yes | opposite |
| willistoni GK12236 | GK10847 | opposite | GK10846 | opposite |
| virilis GJ22514 | GJ22512 | opposite | GJ22515 | same |