# Gene model for the ortholog of *Glys* in *Drosophila ananassae*

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## Abstract

Gene model for the ortholog of *Glycogen synthase* (*Glys*) in the May 2011 (Agencourt dana_caf1/DanaCAF1) Genome Assembly (GenBank Accession: GCA_000005115.1) of *Drosophila ananassae*. This ortholog was characterized as part of a developing dataset to study the evolution of the Insulin/insulin-like growth factor signaling pathway (IIS) across the genus *Drosophila* using the Genomics Education Partnership gene annotation protocol for Course-based Undergraduate Research Experiences.

## Introduction

*This article reports a predicted gene model generated by undergraduate work using a structured gene model annotation protocol defined by the Genomics Education Partnership (GEP; thegep.org) for Course-based Undergraduate Research Experience (CURE). The following information in quotes may be repeated in other articles submitted by participants using the same GEP CURE protocol for annotating Drosophila species orthologs of Drosophila melanogaster genes in the insulin signaling pathway.*

"In this GEP CURE protocol students use web-based tools to manually annotate genes in non-model *Drosophila* species based on orthology to genes in the well-annotated model organism fruitfly *Drosophila melanogaster*. The GEP uses web-based tools to allow undergraduates to participate in course-based research by generating manual annotations of genes in non-model species (Rele et al., 2023). Computational-based gene predictions in any organism are often improved by careful manual annotation and curation, allowing for more accurate analyses of gene and genome evolution (Mudge and Harrow 2016; Tello-Ruiz et al., 2019). These models of orthologous genes across species, such as the one presented here, then provide a reliable basis for further evolutionary genomic analyses when made available to the scientific community." (Myers et al., 2024).

"The particular gene ortholog described here was characterized as part of a developing dataset to study the evolution of the Insulin/insulin-like growth factor signaling pathway (IIS) across the genus *Drosophila*. The Insulin/insulin-like growth factor signaling pathway (IIS) is a highly conserved signaling pathway in animals and is central to mediating organismal responses to nutrients (Hietakangas and Cohen 2009; Grewal 2009)." (Myers et al., 2024).

"*Glycogen synthase* (*Glys*; aka. *GS*) is a gene within the Insulin-signaling pathway in

*Drosophila* and encodes a glycosyltransferase that catalyzes linkage of glucose monomers into glycogen. Glys activity is regulated allosterically by glucose 6-phosphate and phosphorylation/dephosphorylation allowing for control of cellular glycogen levels (Plyte et al., 1992; Roach et al., 2012). Null *Glys* mutants exhibit growth defects and reduced larval viability in *Drosophila* (Yamada et al., 2019)." (Backlund et al., 2025).

"*D. ananassae* (NCBI:txid7217) is part of the *melanogaster* species group within the subgenus *Sophophora* of the genus *Drosophila* (Sturtevant 1939; Bock and Wheeler 1972). It was first described by Doeschall (1858). *D. ananassae* is circumtropical (Markow and O'Grady 2005; https://www.taxodros.uzh.ch, accessed 1 Feb 2023), and often associated with human settlement (Singh 2010). It has been extensively studied as a model for its cytogenetic and genetic characteristics, and in experimental evolution (Kikkawa 1938; Singh and Yadav 2015)." (Lawson et al., 2024).

We propose a gene model for the *D. ananassae* ortholog of the *D. melanogaster Glycogen synthase* (*Glys*) gene. The genomic region of the ortholog corresponds to the uncharacterized protein LOC26515236 (RefSeq accession XP_032308811.1) in the May 2011 (Agencourt dana_caf1/DanaCAF1) Genome Assembly of *D. ananassae* (GenBank Accession: GCA_000005115.1; Drosophila 12 Genomes Consortium, 2007). This model is based on RNA-Seq data from *D. ananassae* (SRP006203, SRP007906; PRJNA257286, PRJNA388952; Brown et al., 2014; Rogers et al., 2014; Yang et al., 2018) and *Glys* in *D. melanogaster* using FlyBase release FB2024_02 (GCA_000001215.4; Gramates et al., 2022; Jenkins et al., 2022; Larkin et al., 2021).

***Synteny***

The reference gene, *Glys*, occurs on chromosome 3R in *D. melanogaster* and is flanked upstream by *Guanylyl cyclase at 88E* (*Gyc88E*) and *Myofilin* (*Mf*) and downstream by *CG3987* and *CG3984*. The *tblastn* search of *D. melanogaster* Glys-PA (query) against the *D. ananassae* (GenBank Accession: GCA_000005115.1) Genome Assembly (database) placed the putative ortholog of *Glys* within scaffold_13340 (CH902617.1) at locus LOC26515236 (XP_032308811.1) — with an E-value of 0.0 and a percent identity of 86.86%. Furthermore, the putative ortholog is flanked upstream by LOC6500618 (XP_044570880.1) and LOC6499946 (XP_001953578.2), which correspond to *Gyc88E* and *Mf* in *D. melanogaster* (E-value: 0.0 and 0.0; identity: 85.35% and 94.05%, respectively, as determined by *blastp*; Figure 1A, Altschul et al., 1990). The putative ortholog of *Glys* is flanked downstream by LOC6500617 (XP_032308829.1) and LOC6500614 (XP_032308765.1), which correspond to *CG3987* and *CG3984* in *D. melanogaster* (E-value: 4e-144 and 6e-100; identity: 55.19% and 60.32%, respectively, as determined by *blastp*). The putative ortholog assignment for *Glys* in *D. ananassae* is supported by the following evidence: The genes surrounding the *Glys* ortholog are orthologous to the genes at the same locus in *D. melanogaster* and local synteny is completely conserved, supported by results generated from *blastp*; we conclude that LOC26515236 is the correct ortholog of *Glys* in *D. ananasssae* (Figure 1A).

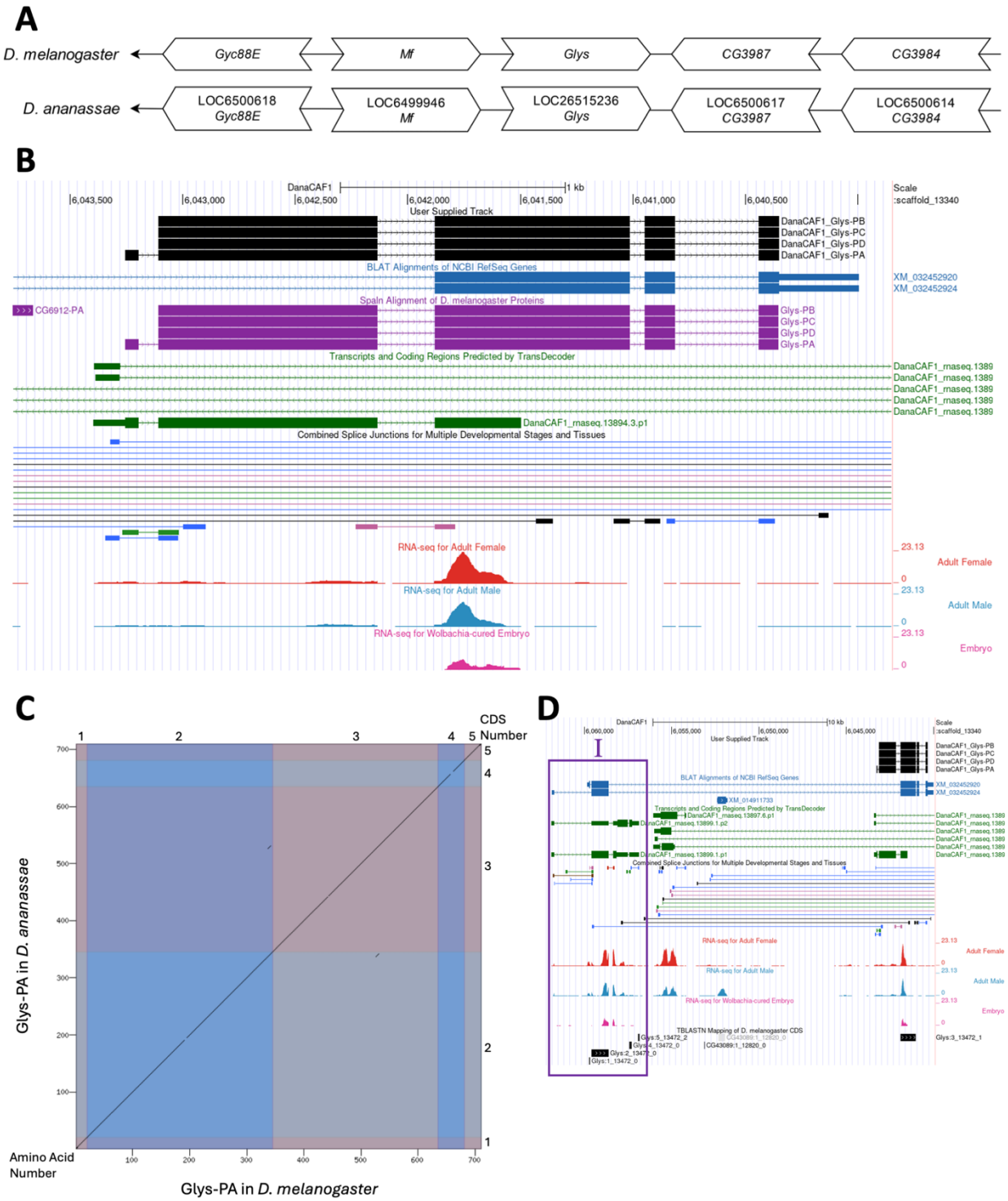


**Figure 1: Glys gene model comparison between *Drosophila ananassae* and *Drosophila melanogaster* orthologs**

**(A) Synteny comparison of the genomic neighborhoods for *Glys* in *Drosophila melanogaster* and *D. ananassae*.** Thin underlying arrows indicate the DNA strand within which the reference gene–*Glys*–is located in *D. melanogaster* (top) and *D. ananassae* (bottom). Thin arrows pointing to the left indicate that *Glys* is on the negative (-) strand in *D. ananassae* and *D. melanogaster*. The

wide gene arrows pointing in the same direction as *Glys* are on the same strand relative to the thin underlying arrows, while wide gene arrows pointing in the opposite direction of *Glys* are on the opposite strand relative to the thin underlying arrows. White gene arrows in *D. ananassae* indicate orthology to the corresponding gene in *D. melanogaster*. Gene symbols given in the *D. ananassae* gene arrows indicate the orthologous gene in *D. melanogaster*, while the locus identifiers are specific to D. *ananassae*. **(B) Gene Model in GEP UCSC Track Data Hub (Raney et al., 2014).** The coding-regions of *Glys* in *D. ananassae* are displayed in the User Supplied Track (black); coding CDSs are depicted by thick rectangles and introns by thin lines with arrows indicating the direction of transcription. Subsequent evidence tracks include *BLAT* Alignments of NCBI RefSeq Genes (dark blue, alignment of Ref-Seq genes for *D. ananassae*), *Spaln* of *D. melanogaster* Proteins (purple, alignment of Ref-Seq proteins from *D. melanogaster*), Transcripts and Coding Regions Predicted by *TransDecoder* (dark green), RNA-Seq from Adult Females, Adult Males, and Wolbachia-cured Embryos (red, light blue and pink, respectively; alignment of Illumina RNA-Seq reads from *D. ananassae*), and Splice Junctions Predicted by *regtools* using *D. ananassae* RNA-Seq (SRP006203, SRP007906; PRJNA257286, PRJNA388952). The splice junctions pertaining to the *Glys* ortholog, JUNC00120677 (black), JUNC00120692 and JUNC00120675 (blue), JUNC00120691 (green), and JUNC00120683 (pink) have read-depths of 7, 20, 28, 57 and 381, respectively. **(C) Dot Plot of Glys-PA in *D. melanogaster* (x-axis) vs. the orthologous peptide in *D. ananassae* (y-axis).** Amino acid number is indicated along the left and bottom; CDS number is indicated along the top and right, and CDSs are also highlighted with alternating colors. Line breaks in the dot plot indicate mismatching amino acids at the specified location between species. **(D) Upstream region of *Glys* ortholog gene model displayed in *D. ananassae* UCSC Genome Browser.** The coding-regions of *Glys* in *D. ananassae* are displayed in the User Supplied Track (black); coding CDSs are depicted by thick rectangles and introns by thin lines with arrows indicating the direction of transcription. Subsequent evidence tracks include *BLAT* Alignments of NCBI RefSeq Genes (dark blue, alignment of Ref-Seq genes for *D. ananassae*), Transcripts and Coding Regions Predicted by *TransDecoder* (dark green), RNA-Seq from Adult Females, Adult Males, and Wolbachia-cured Embryos (red, light blue and pink, respectively; alignment of Illumina RNA-Seq reads from *D. ananassae*), Splice Junctions Predicted by *regtools* using *D. ananassae* RNA-Seq (SRP006203, SRP007906; PRJNA257286, PRJNA388952) and *tblastn* Mapping of *D. melanogaster* CDS (black and light grey). Splice junctions shown have a read-depth of 10-49, 50-99, 100-499, 500-999, >1000 supporting reads in blue, green, pink, brown, and red, respectively. The *BLAT* Alignments of NCBI RefSeq gene prediction that includes the putative ortholog in *D. ananassae* appears to exhibit shifted CDSs in its upstream neighborhood, highlighted in purple box (I).

### *Protein Model*

*Glys* in *D. ananassae* has five CDSs within the genome sequence. The first unique protein sequence (Glys-PA) is translated from one mRNA isoform (*Glys-RA*; Figure 1B). The next unique protein sequence (Glys-PC, Glys-PB and Glys-PD) is translated from three mRNA isoforms that differ in their UTRs (*Glys-RC, Glys-RB* and *Glys-RD*; Figure 1B). Relative to the ortholog in *D. melanogaster*, the CDS number and protein isoform count are conserved, although the *BLAT* Alignments of NCBI RefSeq gene prediction for *D. ananassae* to the *D. ananassae* May 2011 (Agencourt dana_caf1/DanaCAF1) assembly incorrectly displays the first two CDSs of *Glys-RA* and the first CDS of *Glys-RC* of the putative ortholog located farther upstream (Figure 1B; Figure 1D). The sequence of Glys-PA in *D. ananassae* has 97.32% identity (E-value: 0.0) with the protein-coding isoform Glys-PA in *D. melanogaster*, as determined by *blastp* (Figure

1C). Coordinates of this curated gene models (Glys-PA, Glys-PB, Glys-PC, Glys-PD) are stored by NCBI at GenBank/BankIt (accession **BK064519, BK064520, BK064521, BK064522**, respectively). This gene model can also be seen within the target genome at this TrackHub.

***Special characteristics of the protein model:*** The BLAT Alignments of NCBI RefSeq Genes predicted the first two CDSs of *Glys-RA* and the first CDS of *Glys-RC* upstream of the putative ortholog, highlighted by the purple box denoted I in Figure 1D. This finding might imply a tandem gene duplication of *Glys*. However, a more current RefSeq assembly, *D. ananassae* Sep. 2021 (University of Maryland, ASM1763931v2/DanaRefSeq2), does not support the presence of the potential gene duplication and exhibits a single conserved ortholog to *Glys* in *D. melanogaster*. It is possible there was a tandem duplication of *Glys* in the strain sequenced for the assembly used in this gene annotation report (GCA_000005115.1) or that there was a misassembly inducing an apparent duplication. Given the failure to replicate the apparent duplication in the second assembly, however, leads us to the conservative conclusion that there is most likely no duplication event of *Glys* in the *D. ananassae* lineage and that there instead is likely as misassembly in this region in the focal assembly.

**Methods**

"Detailed methods including algorithms, database versions, and citations for the complete annotation process can be found in Rele et al. (2023). Briefly, students use the GEP instance of the UCSC Genome Browser v.435 (https://gander.wustl.edu; Kent WJ et al., 2002; Navarro Gonzalez et al., 2021) to examine the genomic neighborhood of their reference IIS gene in the *D. melanogaster* genome assembly (Aug. 2014; BDGP Release 6 + ISO1 MT/dm6). Students then retrieve the protein sequence for the *D. melanogaster* reference gene for a given isoform and run it using *tblastn* against their target *Drosophila* species genome assembly on the NCBI BLAST server (https://blast.ncbi.nlm.nih.gov/Blast.cgi; Altschul et al., 1990) to identify potential orthologs. To validate the potential ortholog, students compare the local genomic neighborhood of their potential ortholog with the genomic neighborhood of their reference gene in *D. melanogaster*. This local synteny analysis includes at minimum the two upstream and downstream genes relative to their putative ortholog. They also explore other sets of genomic evidence using multiple alignment tracks in the Genome Browser, including BLAT alignments of RefSeq Genes, Spaln alignment of *D. melanogaster* proteins, multiple gene prediction tracks (e.g., GeMoMa, Geneid, Augustus), and modENCODE RNA-Seq from the target species. Detailed explanation of how these lines of genomic evidenced are leveraged by students in gene model development are described in Rele et al. (2023). Genomic structure information (e.g., CDSs, intron-exon number and boundaries, number of isoforms) for the *D. melanogaster* reference gene is retrieved through the Gene Record Finder (https://gander.wustl.edu/~wilson/dmelgenerecord/index.html; Rele et al*.,* 2023). Approximate splice sites within the target gene are determined using *tblastn* using the CDSs from the *D. melanogaste*r reference gene. Coordinates of CDSs are then refined by examining aligned modENCODE RNA-Seq data, and by applying paradigms of molecular biology such as identifying canonical splice site sequences and ensuring the maintenance of an open reading frame across hypothesized splice sites. Students then confirm the biological validity of their target gene model using the Gene Model Checker (https://gander.wustl.edu/~wilson/dmelgenerecord/index.html; Rele et al., 2023), which compares the structure and translated sequence from their hypothesized target gene model

against the *D. melanogaster* reference gene model. At least two independent models for a gene are generated by students under mentorship of their faculty course instructors. Those models are then reconciled by a third independent researcher mentored by the project leaders to produce the final model. Note: comparison of 5' and 3' UTR sequence information is not included in this GEP CURE protocol." (Gruys et al., 2025)

**Supplemental Files**

1. Zip file containing a FASTA, PEP, GFF files for the gene model
2. Figure 1 in high resolution

**Metadata:** Bioinformatics, Genomics, *Drosophila*, Genotype Data, New Finding

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**References**

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J Mol Biol 215(3): 403-10. doi: 10.1016/S0022-2836(05)80360-2. PMID: 2231712.

Backlund AE, Cohen L, White J, Grillo L, Ianniello T, Swedrowski A, Yang MA, Jemc J, Rele CP, Reed LK. 2025 Gene model for the ortholog of *Glys* in *Drosophila busckii*. MicroPubl Biol. Apr 14;2025:10.17912/micropub.biology.001095. doi: 10.17912/micropub.biology.001095. PMID: 40303838

Bock IR, Wheeler MR. 1972. The Drosophila melanogaster species-group. The University of Texas Publication. 7213: 1-102. FBrf0024428

Brown JB, et al., 2014. Diversity and dynamics of the Drosophila transcriptome. Nature. 2014 Aug 28;512(7515):393-9. doi: 10.1038/nature12962. PMID: 24670639

Doleschall CL. 1858. Derde bijdrage tot de kennis der dipterologische fauna van Nederlandsch Indië. Natuurkundig tijdschrift voor Nederlandsch Indië. 17: 73. FBrf0000091
Drosophila 12 Genomes Consortium. 2007. Evolution of genes and genomes on the Drosophila phylogeny. Nature 450: 203-218. doi: 10.1038/nature06341. PMID: 17994087.

Drosophila 12 Genomes Consortium, et al., 2007. Evolution of genes and genomes on the Drosophila phylogeny. Nature. 2007 Nov 8;450(7167):203-18. doi: 10.1038/nature06341. PMID: 17994087.

Gramates LS, Agapite J, Attrill H, Calvi BR, Crosby MA, Dos Santos G, et al., the FlyBase Consortium. 2022. FlyBase: a guided tour of highlighted features. Genetics 220(4). doi: 10.1093/genetics/iyac035. PMID: 35266522

Grewal SS. 2009. Insulin/TOR signaling in growth and homeostasis: a view from the fly world. Int J Biochem Cell Biol 41(5): 1006-10. doi: 10.1016/j.biocel.2008.10.010. Epub 2008 Oct 18. PMID: 18992839.

Gruys ML, Sharp MA, Lill Z, Xiong C, Hark AT, Youngblom JJ, Rele CP, Reed LK. Gene model for the ortholog of *Glys* in *Drosophila simulans*. MicroPubl Biol. 2025 Jan 7;2025:10.17912/micropub.biology.001168. doi: 10.17912/micropub.biology.001168. PMID: 39845267; PMCID: PMC11751727.

Hietakangas V, Cohen SM. 2009. Regulation of tissue growth through nutrient sensing. Annu Rev Genet 43: 389-410. doi: 10.1146/annurev-genet-102108-134815. PMID: 19694515

Jenkins VK, Larkin A, Thurmond J, FlyBase Consortium. 2022. Using FlyBase: A Database of Drosophila Genes and Genetics. Methods Mol Biol 2540: 1-34. doi: 10.1007/978-1-0716-2541-5_1. PMID: 35980571.

Kent WJ, Sugnet CW, Furey TS, Roskin KM, Pringle TH, Zahler AM, Haussler D. 2002. The human genome browser at UCSC. Genome Res 12(6): 996-1006. doi: 10.1101/gr.229102. PMID: 12045153

Kikkawa, H. 1938. Studies on the genetics and cytology of *Drosophila ananassae* . *Genetica* **20**, 458–516. doi: 10.1007/BF01531779

Larkin A, Marygold SJ, Antonazzo G, Attrill H, Dos Santos G, Garapati PV, et al., FlyBase Consortium. 2021. FlyBase: updates to the Drosophila melanogaster knowledge base. Nucleic Acids Res 49(D1): D899-D907. doi: 10.1093/nar/gkaa1026. PMID: 33219682

Lawson ME, McAbee M, Lucas RA, Tanner S, Wittke-Thompson J, Pelletier TA, Ozsoy Z, Sterne-Marr R, Rele CP, Reed LK. 2024 Gene model for the ortholog of *Ilp5* in *Drosophila ananassae*. MicroPubl Biol. Dec 8;2024:10.17912/micropub.biology.000782. doi: 10.17912/micropub.biology.000782. PMID: 39717145; PMCID: PMC11664428.

Markow TA and O'Grady P. 2005. Drosophila: A guide to species identification and use. London: Academic Press. ISBN: 978-0-12-473052-6

Mudge JM, Harrow J. 2016. The state of play in higher eukaryote gene annotation. Nat Rev Genet 17(12): 758-772. doi: 10.1038/nrg.2016.119. Epub 2016 Oct 24. PMID: 27773922

Myers A, Hoffman A, Natysin M, Arsham AM, Stamm J, Thompson JS, Rele CP, Reed LK. 2024. Gene model for the ortholog *Myc* in *Drosophila ananassae*. MicroPubl Biol. Nov 30;2024:10.17912/micropub.biology.000856. doi: 10.17912/micropub.biology.000856. PMID: 39677519; PMCID: PMC11645546.

Navarro Gonzalez J, Zweig AS, Speir ML, Schmelter D, Rosenbloom KR, Raney BJ, et al., Kent WJ. 2021. The UCSC Genome Browser database: 2021 update. Nucleic Acids Res 49(D1): D1046-D1057. doi: 10.1093/nar/gkaa1070. PMID: 33221922

Plyte SE, Hughes K, Nikolakaki E, Pulverer BJ, and Woodgett JR, 1992. Glycogen synthase kinase-3: functions in oncogenesis and development. *Biochimica Et Biophysica Acta Bba - Rev Cancer*, vol. 1114, no. 2–3, pp. 147–162, doi: 10.1016/0304-419X(92)90012-N. PMID: 1333807

Raney BJ, Dreszer TR, Barber GP, Clawson H, Fujita PA, Wang T, et al., Kent WJ. 2014. Track data hubs enable visualization of user-defined genome-wide annotations on the UCSC Genome Browser. Bioinformatics 30(7): 1003-5. oi: 10.1093/bioinformatics/btt637. Epub 2013 Nov 13. PMID: 24227676

Rele CP, Sandlin KM, Leung W, Reed LK. 2023. Manual annotation of Drosophila genes: a Genomics Education Partnership protocol. F1000Research 11: 1579. doi: 10.12688/f1000research.126839.2

Roach PJ, Depaoli-Roach AA, Hurley TD, Tagliabracci VS. 2012. Glycogen and its metabolism: some new developments and old themes. Biochem J. Feb 1;441(3):763-87. doi: 10.1042/BJ20111416. PMID: 22248338

Rogers RL, Shao L, Sanjak JS, Andolfatto P, Thornton KR. 2014. Revised annotations, sex-biased expression, and lineage-specific genes in the Drosophila melanogaster group. G3 (Bethesda). Oct 1;4(12):2345-51. doi: 10.1534/g3.114.013532. PMID: 25273863

Singh BN. 2010. Drosophila ananassae: a good model species for genetical, behavioural and evolutionary studies. Indian J Exp Biol. Apr;48(4):333-45. PMID: 20726331.

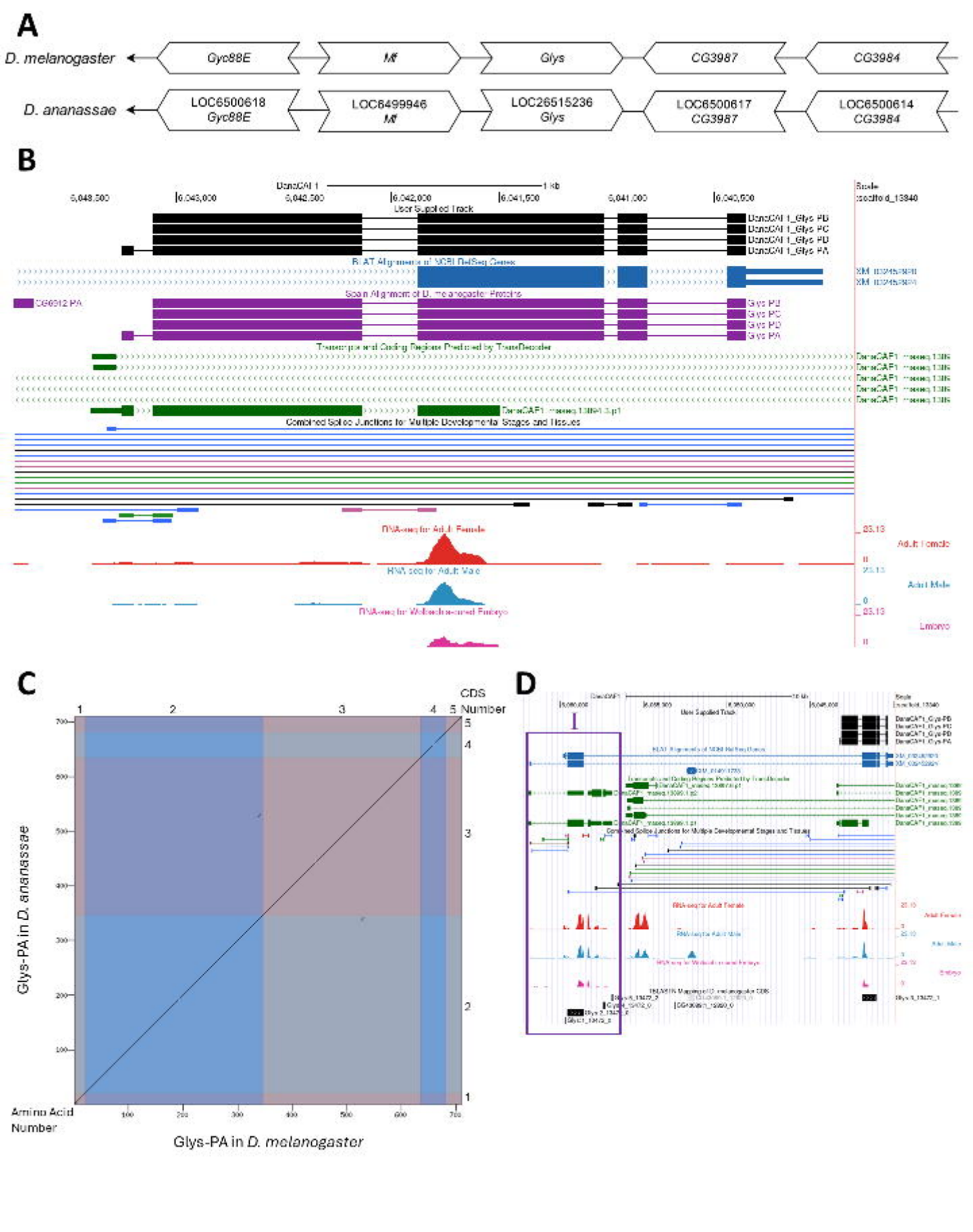
Singh BN, Yadav JP. 2015. Status of research on Drosophila ananassae at global level. J Genet. Dec;94(4):785-92. doi: 10.1007/s12041-015-0577-y. PMID: 26690536.

Sturtevant AH. 1939 On the Subdivision of the Genus Drosophila. Proc Natl Acad Sci U S A. Mar;25(3):137-41. doi: 10.1073/pnas.25.3.137. PMCID: PMC1077728; PMID: 16577879

Tello-Ruiz MK, Marco CF, Hsu FM, Khangura RS, Qiao P, Sapkota S, et al., Micklos DA. 2019. Double triage to identify poorly annotated genes in maize: The missing link in community curation. PLoS One 14(10): e0224086. doi: 10.1371/journal.pone.0314495. PMID: 31658277

Yamada T, Habara O, Yoshii Y, Matsushita R, Kubo H, Nojima Y, Nishimura T. 2019. The role of glycogen in development and adult fitness in *Drosophila*. Development. Apr 15;146(8):dev176149. doi: 10.1242/dev.176149. PMID: 30918052.

Yang H, Jaime M, Polihronakis M, Kanegawa K, Markow T, Kaneshiro K, Oliver B. 2018. Re-annotation of eight *Drosophila* genomes. Life Sci Alliance. Dec 24;1(6):e201800156. doi: 10.26508/lsa.201800156. PMID: 30599046


A
D. melanogaster
Gyc88E
Mf
Glys
CG3987
CG3984
D. ananassae
LOC6500618
Gyc88E
LOC6499946
Mf
LOC26515236
Glys
LOC6500617
CG3987
LOC6500614
CG3984
B
User Supplied Track
BLAT Alignments of NCBI RefSeq Genes
Scan Alignment of D. melanogaster Proteins
Transcripts and Coding Regions Predicted by TransDecoder
Combined Splice Junctions for Multiple Developmental Stages and Tissues
RNA-seq for Adult Female
RNA-seq for Adult Male
RNA-seq for Wolbachia-cured Embryo
C
CDS Number
Glys-PA in D. ananassae
Amino Acid Number
Glys-PA in D. melanogaster
D