Figures and figure supplements

Natural variation in sugar tolerance associates with changes in signaling and mitochondrial ribosome biogenesis

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Figure 1. Differential macronutrient spaces of Drosophila simulans and sechellia with respect to sugar tolerance. (A) Larvae of D. simulans and D. sechellia showed differential pupariation time (h after egg-laying) and survival on high dietary sugar. Larval development was monitored on a 5 x 5 grid of varying yeast and sucrose levels. Pupariation index takes into account both survival and pupariation time. n = 5 replicates of 30 larvae/replicate for each genotype and diet. (B) Tolerance of high dietary carbohydrate was restored in the D. sechellia x D. simulans F1 hybrids. n = 5 replicates of 30 larvae/replicate for each diet.

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Figure 1—figure supplement 1. Feeding behavior did not differ significantly between the species. The rate of mouth hook extensions was quantified in the presence and absence of 20% sucrose. n = 4 replicates of 10 larvae/rePLICATE for each genotype and diet. Error bars display standard error of the mean. ANOVA showed no significant difference between the groups.

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Figure 2. Introgression of D. simulans sugar tolerance into D. sechellia genome through repeated backcrosses on selective diet. (A) Construction of the sugar selected and control backcross (B.C.) lines through phenotype-based introgression. Dietary sugar content of 20% provided a strong selection, since no survivors of the D. sechellia parental line were observed in these conditions. (B) Sugar tolerance of selected lines was similar to that in the parental D. simulans line, while the sugar tolerance of the control line resembled to that of D. sechellia. Error bars display standard error of the mean. n = 5 replicates of 30 larvae/replicate for each genotype. Dunnett’s test (|d| = 2.70, α = 0.05) showed that D. sechellia and the control backcross line had significantly reduced sugar tolerance compared to D. simulans while sugar tolerance of the two HSD-selected backcross lines did not differ from that of D. simulans. (C) The sugar intolerant control line showed impaired clearance of hemolymph glucose, similar to D. sechellia. Hemolymph glucose was measured from larvae on LSD, after 2 hr on HSD, and after 2 hr of transferring of HSD-fed larvae back to LSD. Error bars display standard error of the mean. n = 5 replicates of 10 larvae/replicate for each genotype and diet. Dunnett’s test (|d| = 2.62, α = 0.05) showed that after feeding for 2 hr on HSD, D. sechellia and the control backcross line had significantly elevated hemolymph glucose compared to that of D. simulans while that of the selected line did not differ from the D. simulans level. **p < 0.01, ***p < 0.001.

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Figure 3. Global gene expression changes associated with sugar tolerance. (A) Schematic representation of RNAseq sample preparation. Parental lines and backcrossed hybrid lines were fed on LSD or transferred acutely (8 hr) on HSD, followed by RNA extraction and RNA sequencing. (B) Sample clustering reveals tight association between global gene expression profiles and sugar tolerance. Sample clustering was based on Pearson correlation and it was performed using R/Bioconductor package pvclust. Correlation was used as distance matrix. (C) Summary of selected functional groups significantly enriched among genes displaying differential expression in sugar tolerant vs. intolerant lines.

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Figure 4. Significant overlap between gene expression profiles of sugar intolerant lines and Mlx mutants. (A) Comparison of genes differentially expressed in sugar tolerant vs. intolerant lines with Mlx target genes. Gene expression profiles associated with sugar intolerance show highly significant overlap with Mlx target genes in both tolerant and intolerant lines.

Figure 4 continued on next page.
overlap with profiles of $mlx^1$ mutants. (B) Heat maps of the overlapping gene sets show similarities in gene sugar responsiveness in sugar intolerant lines and $mlx^1$ mutants. Sugar tolerance/intolerance phenotypes of the analyzed lines are indicated in color. (C) Known sugar tolerance genes sugarbabe ($sug$) and astray ($aay$) show weaker sugar induction in sugar intolerant lines, resembling $mlx^1$ mutants.

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**Figure 5.** *D. simulans* SNP signature was introgressed into a mostly *D. sechellia* SNP signature background. (A) Color shows the frequency distribution of *D. simulans*-specific SNPs displayed along the chromosomes. (B) Frequency distribution of *D. simulans*-specific SNPs displayed on chromosome arm 2R for the sugar selected (top) and not-selected control (bottom) backcross lines. Black lines above the heat maps indicate the three sugar tolerance-associated introgressed regions. (C) Limited overlap between introgressed genes and genes that are either up- or downregulated in sugar tolerant lines.

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Figure 6. Low expression of mitochondrial ribosome genes contributes to sugar intolerance. (A) Pupariation kinetics of control and mRpl43 RNAi larvae (Ubi-GAL4>), n = 7 replicates of 30 larvae/replicate for each genotype and diet. Error bars display standard deviation. (B) Pupariation kinetics of control and CG4882 RNAi larvae (Ubi-GAL4>), n = 8 replicates of 30 larvae/replicate for each genotype and diet. Error bars display standard deviation. (C) Pupariation kinetics of control and bonsai RNAi larvae (Fb-GAL4>), n = 3 replicates of 30 larvae/replicate for each genotype and diet. Error bars display standard deviation. (D–F) Relative expression of mRpl43, CG2882, and bonsai genes in sugar tolerant (hybrid and D. simulans) and intolerant (ctrl and D. sechellia) lines on low- and high-sugar diets identified by RNAseq. dAEL: days after egg laying.

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Figure 6—figure supplement 1. Macronutrient space of *Drosophila melanogaster* shows high sugar tolerance. Larval development was monitored on a 5 × 5 grid of varying yeast and sucrose levels. Pupind: pupariation index. n = 5 replicates of 30 larvae/replicate for each diet. DOI: https://doi.org/10.7554/eLife.40841.015
Figure 7. Several genes involved in signaling influence sugar tolerance. (A) Pupariation kinetics of control and SERCA RNAi larvae (Ubi-GAL4>), n = 7 replicates of 30 larvae/replicate for each genotype and diet. Error bars display standard deviation. (B) Pupariation kinetics of control and PPP1R15 RNAi larvae (Ubi-GAL4>), n = 13 replicates of 30 larvae/replicate for each genotype and diet. Error bars display standard deviation. (C) Pupariation kinetics of control and PI3K59F RNAi larvae (Tub-GAL4>), n = 5 replicates of 30 larvae/replicate for each genotype and diet. Error bars display standard deviation. (D–F) Relative expression of SERCA, PPP1R15, and PI3K59F genes in sugar tolerant (hybrid and D. simulans) and intolerant (ctrl and D. sechellia) lines on low- and high-sugar diets identified by RNAseq. dAEL: days after egg laying.

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Figure 7—figure supplement 1. Genes with a modest impact on sugar tolerance. (A) Total eclosion % of control and GlcT-1 RNAi larvae (tub-GAL4>), n = 4 replicates of 30 larvae/replicate for each genotype and diet. (B) Total eclosion % of control and Dpit47 RNAi larvae (Ubi-GAL4>), n = 4 replicates of 30 larvae/replicate for each genotype and diet. (C) Total eclosion % of control and Taldo RNAi larvae (Ubi-GAL4>), n = 19 replicates of 30 larvae/replicate for each genotype and diet. Error bars display standard deviation.

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Figure 8. Genomic variation of SERCA promoter leads to differential promoter activity. (A) SNP density maps comparing D. sechellia to D. simulans on regions surrounding the SERCA (Ca-P60A) gene. mRNA transcript models for each gene region are shown above SNP density heat maps with green and red representing coding regions on the (+) and (-) strand, respectively, and grey indicating non-coding regions. Direction of transcription is also indicated with a grey arrowhead. Heatmaps represent the density of SNP differences between D. sechellia and D. simulans in overlapping windows of 100 nt slid in 25 nt increments along the region. The promoter fragment cloned into the in vivo reporter is indicated as violet dashed line. (B) Relative mRNA (qPCR) expression of lacZ reporter gene downstream of 1.2 kB fragments of D. simulans and D. sechellia SERCA promoters reveals lower activity of D. sechellia-derived promoter. n = 8 replicates of 8 larvae/replicate for each genotype and diet. Error bars display standard deviation. **p < 0.01 (student’s t-test).

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Figure 8—figure supplement 1. Genomic variation in the mitochondrial ribosome encoding gene regions. SNP density maps comparing D. sechellia to D. simulans on regions surrounding the CG4882, mRpL43, and bonsai genes. mRNA transcript models for each gene region are shown above SNP density heat maps with green and red representing coding regions on the (+) and (-) strand, respectively, and grey indicating non-coding regions. Direction of transcription is also indicated with a grey arrowhead. Heatmaps represent the density of SNP differences between D. sechellia and D. simulans in overlapping windows of 100 nt slid in 25 nt increments along the region.

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Figure 9. Trade-off between sugar tolerance and growth on low-energy diet. (A) Compared to D. simulans, D. sechellia larvae had lower tolerance of sugar, but showed an advantage in pupariation on low nutrient diets. Surface shows [(D. sechellia pupind) - (D. simulans pupind)]. (B) On a low nutrient (2.5% yeast) diet, D. sechellia and the sugar intolerant control lines had shorter egg to pupa time and greater larval survival than did D. simulans and the sugar-selected lines. Error bars display standard error of the mean. n = 5–9 replicates of 30 larvae/replicate for each genotype and diet. (C) Pupariation kinetics of PPP1R15 RNAi (cg-GAL4>) on 2% yeast diet, n = 28 replicates of 30 larvae/replicate for each genotype and diet. Error bars display standard deviation. dAEL: days after egg laying.

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Figure 9—figure supplement 1. Morinda toxin tolerance is not associated with sugar tolerance. (A) Pupariation indices on HSD of lines after three generations of introgression and selection on HSD, LSD, and LSD + Morinda toxins. Mean of 5 replicate (30 larvae/replicate for each genotype and diet) vials were compared by Student’s t-test. ** p < 0.01. (B) Two sugar-selected introgression lines show full tolerance to Morinda toxins, in contrast to parental D. simulans line. n = 5 replicates of 30 larvae/replicate for each genotype and diet.

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