Supplemental Figures

Microbiota-dependent elevation of Alcohol Dehydrogenase in *Drosophila* is associated with changes in alcohol-induced hyperactivity and alcohol preference

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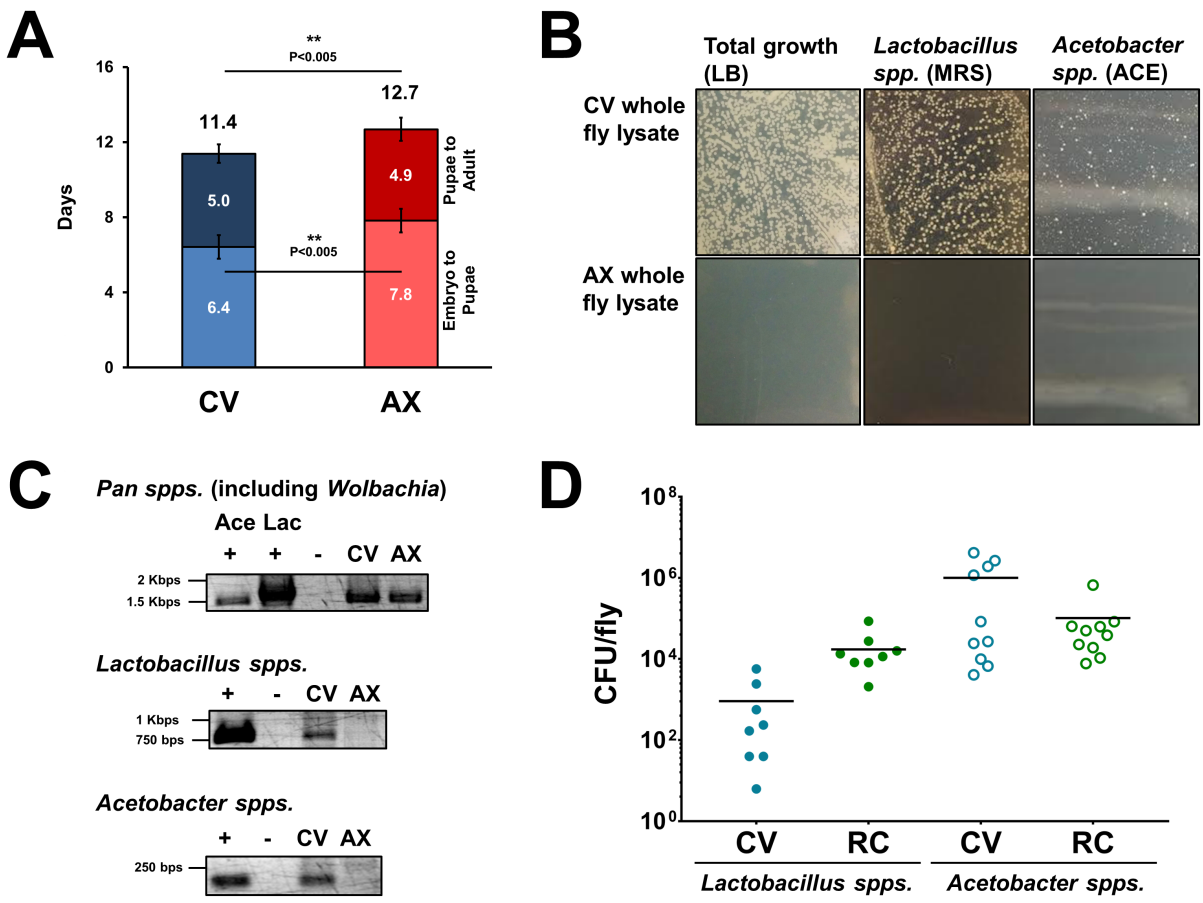
SFIGURE 1

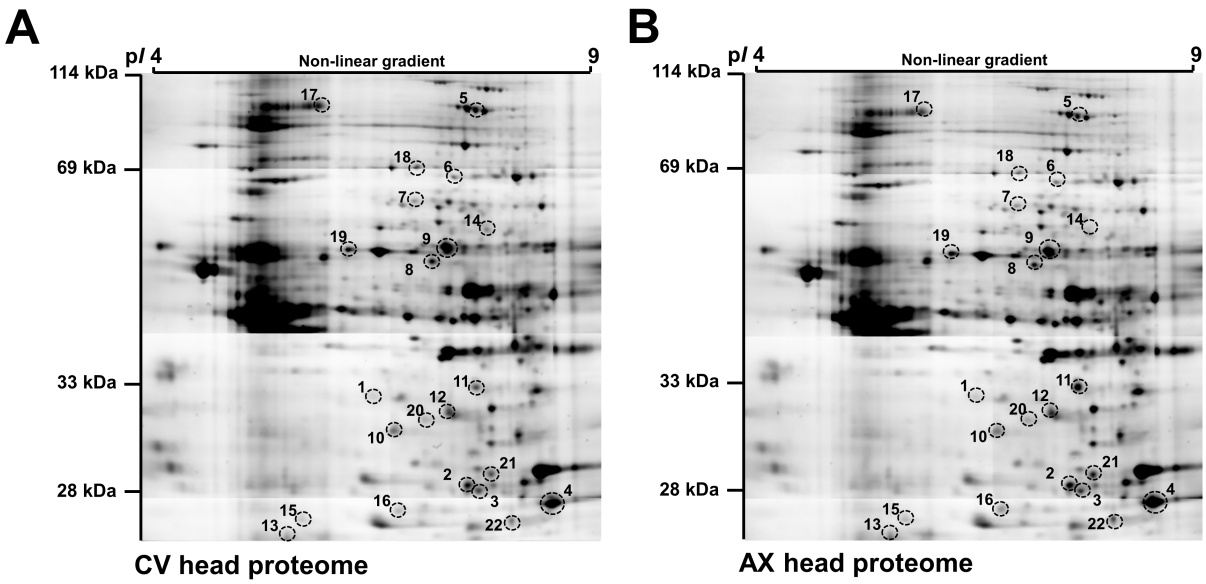


Supplemental Figure 1. Characterization of the microbiota in CV, AX, and RC males.

(A) Developmental time at 22ºC from embryo to adult was monitored for CV and AX cultures demonstrating that AX cultures take significantly longer to eclose than their CV siblings. Because AX cultures take significantly longer to pupariate than their CV siblings due to an extended larval stage, developmental time was used as a quality control check for the cultures. n = 69 bottles (CV) and 59 bottles (AX). Error bars indicate standard error of the mean and statistical significance was assigned by a Mann-Whitney test. **(B)** Homogenates from ten surface-sterilized whole CV or AX flies were cultured on three different types of media as indicated. No growth was observed from AX homogenates. **(C)** PCR using universal 16S rRNA (*Pan spps.*), *Lactobacillus*, or *Acetobacter* specific 16S rRNA primers on CV and AX male homogenates containing two flies each. + indicates a pure culture positive control and the – indicates the no template negative control. An amplicon was observed for both AX and CV using

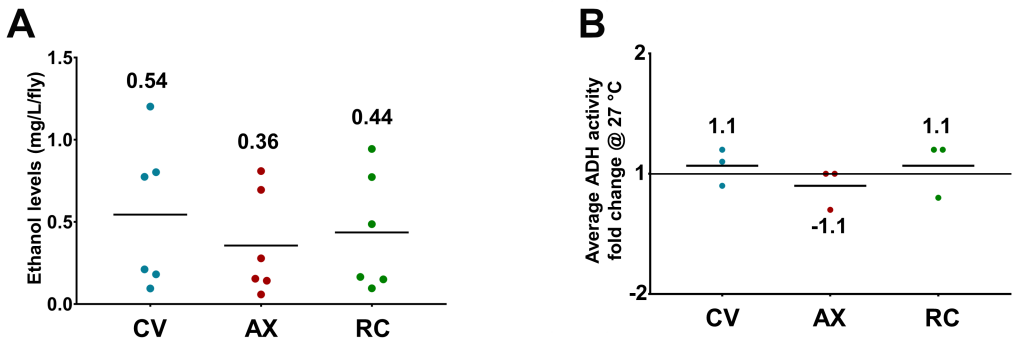
the universal 16S rRNA primers due to the presence of *Wolbachia* as determined by Sanger sequencing of the amplicons (not shown). Primers specific to *Lactobacillus* and *Acetobacter* yield amplicons in CV, but not AX flies. **(D)** The total number of bacterial colony-forming units (CFU) from whole fly CV and RC homogenates. Homogenates contained ten surfaced-sterilized 5-6 day old male flies. Error bars indicate SEM. A single dot represents one biological replicate containing ten male flies, and 10 biological replicates were performed for each condition. Only 8 dots are shown for *Lactobacillus spps.* in both CV and RC because in two cases for each there was no bacterial growth.

SFIGURE 2



Supplemental Figure 2. The *Drosophila* head proteome responds to the microbiota. Representative separate gray scale images of the CV **(A)** and AX **(B)** head proteomes from one 2D-DIGE experiment. The spot numbers correspond to the protein numbers in Fig. 1B.

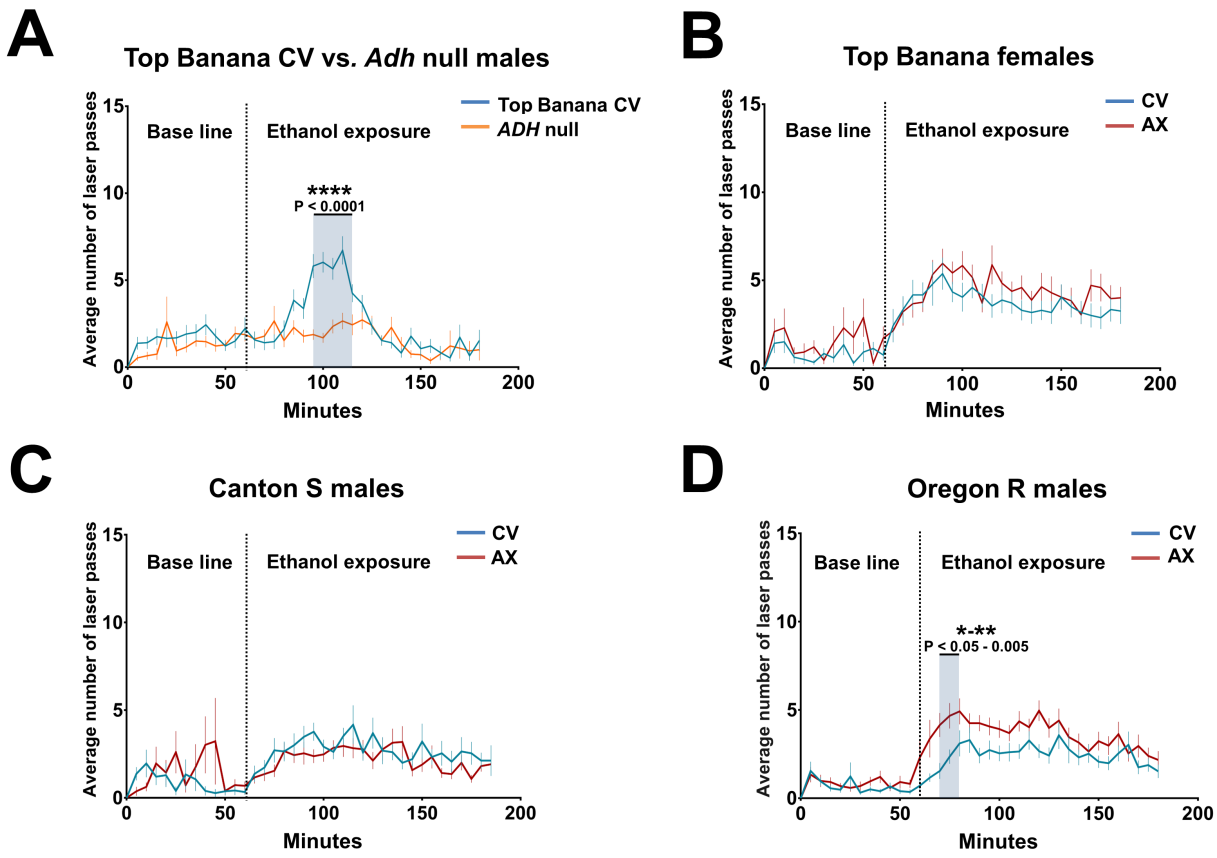
SFIGURE 3



Supplemental Figure 3. Systematic ethanol metabolism is not altered in AX male flies.

(A) Ethanol level in CV, AX, and RC whole males after exposure to 10% ethanol vapor for 30 min. Each dot represents a biological replicate containing twenty whole flies. Solid black bars indicate the mean. There is no significant difference between the means (solid black bars). **(B)** ADH enzymatic activity in CV, AX, and RC whole males after exposure to 10% ethanol vapor for thirty minutes. Each dot represents a biological replicate containing ten whole flies. Pre-exposure ADH enzymatic activity was subtracted from post-exposure ADH enzymatic activity and a fold change was calculated relative to pre-exposed male heads. There is no significant difference between the means (solid black bars).

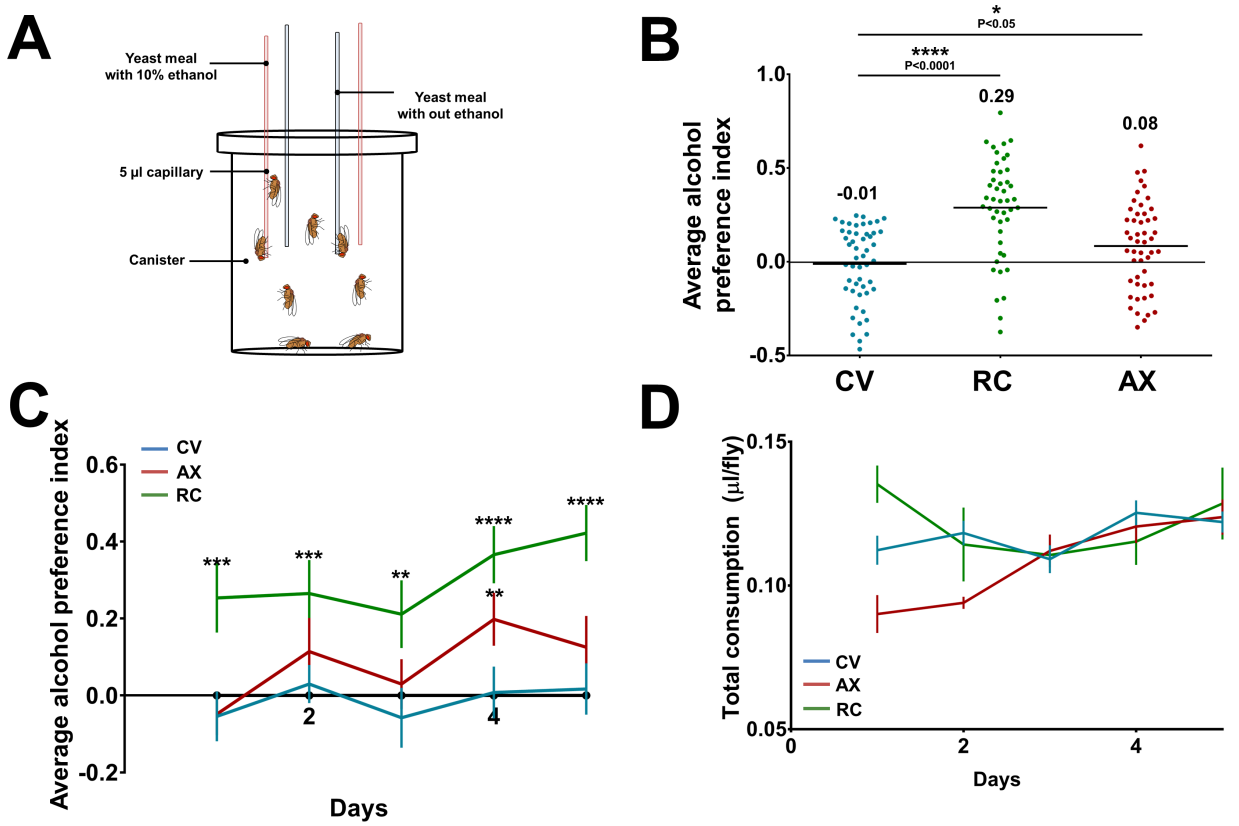
SFIGURE 4



Supplemental Figure 4. Alcohol induced hyperactivity is influenced by ADH, sex, and host genetic background.

(A-D) For all experiments, the flow ratio was H_2O:EtOH (10:1). All error bars indicate standard error of the mean and statistical significance (blue shaded regions) was assigned by a two-way ANOVA (Sidak's multiple comparisons post hoc test). **(A)** *ADH* null males did not exhibit AIH. n = 32 flies in 4 trials/condition. **(B)** Top Banana females did not experience a microbe-dependent increase in AIH. n = 32 flies in 4 trials/condition. Analysis of AIH in Canton S **(C)** and Oregon R **(D)** CV and AX males revealed genetic background differences in host response. Only Oregon R males exhibited a microbe-dependent increase in AIH. n = 48 flies in 6 trials/condition.

SFIGURE 5



Supplemental Figure 5. Manipulation of the microbiota alters alcohol consumption in the CAFÉ assay.

(A) A schematic of the two-choice capillary feeder (CAFÉ) assay to test alcohol consumption preference. Each chamber housed up to eight flies. **(B)** Average alcohol preference index for CV, AX, and RC males. Each dot represents the PI from one chamber for one day of the five-day assay. Solid black bars indicate the mean and statistical significance was assigned by a one-way ANOVA (Dunnett's multiple comparisons post hoc test). **(C)** Average alcohol preference index for CV, AX, and RC males for each day of the five-day assay. The n tested for CV, AX, and RC was 71, 77, and 63 flies (10, 10, and 9 chambers), respectively. Bars indicate standard error of the mean and statistical significance was assigned by a two-way ANOVA (Dunnett's multiple comparisons post hoc test). **(D)** Average total daily food consumption (ethanol and non-ethanol food) for CV, AX, and RC males. Error bars indicate standard error of the mean and there was no statistical difference between conditions.