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The impact of long-term different dietary regimes on microbiota communities in Drosophila melanogaster

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INTRODUCTION & AIM	 Beta Diversity Highlights Sex comparison: Diet — especially carrot — shapes microbiota
 Drosophila gut microbiota (GM) is critical for host physiology, development, ehavior, and overall fitness GM aids nutrient catabolism, immune defense, stress tolerance, longevity, eproduction, and development (1) 	 No significant differences in microbial composition Diet comparison (A): Diet has a strong impact on microbiota composition Carrot diet (C) stands out as most distinct
 Laboratory-reared Drosophila offers a simplified model (~8 species) compared to rild flies (~30 species), mainly Proteobacteria & Firmicutes (2) Core genera (Lactobacillus, Acetobacter) shape gut community, but less common nicrobes also play key functional roles 	 Sex × Diet (B): Combined sex and diet grouping shows stronger overall differences Clustering mainly driven by diet, with some sex-based separation Only male & female flies on carrot diet do not cluster together
 Lab-maintained GM is largely transient, relying on continuous microbial influx from iet Past studies have mostly explored how diet composition affects fly fitness and raits (3), but less is known about how diet shapes microbiota itself 	A) Distances to Carrot B) Axis 2 (28.17%) Female-Apple 1.75 1.50 1.25 1.00 Distances to Carrot B) Female-Apple Female-Standard Female-Standard Female-Standard Female-Apple Male-Apple
Goal : Examine how long-term diets shape adult <i>Drosophila</i> microbiota and	0.75 0.50 Male-Banana Male-Carrot

its metabolic potential

Why It Matters

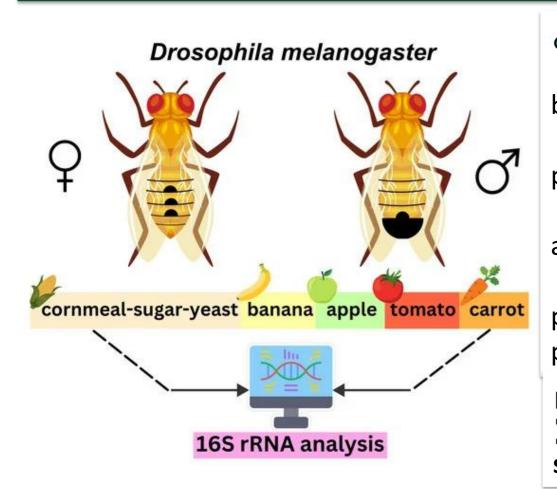
Drosophila is a key model organism for studying human disease mechanisms and host-microbe interactions

Understanding diet-microbiota links helps reveal mechanisms on the **nutrition**behavior axis

Shows how microbiota complements **host metabolic needs**

Provides insights into broader principles of symbiosis and microbial community function

METHODS



What we did:

• Tested five diets \rightarrow cornmeal, apple, banana, carrot, tomato

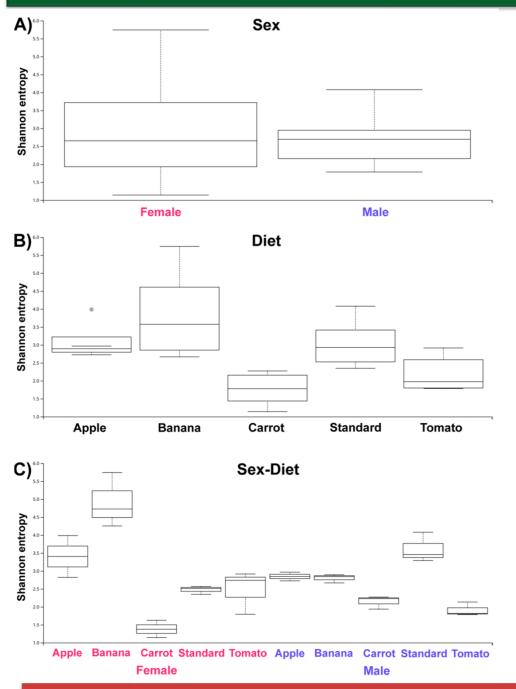
• Used **16S rRNA sequencing** \rightarrow to profile gut microbial communities

 Applied functional prediction → to assess microbial metabolic capacity

• Employed machine learning \rightarrow to predict fly diet and sex from microbiota profiles

Origin: Natural population **Rearing:** >450 generations under standard laboratory conditions

RESULTS & DISCUSSION



Alpha Diversity Highlights Sex comparison (A):

- Similar diversity in males and females
- More variation among female samples Diet comparison (B):
- High-sugar diets (banana, standard, apple) \rightarrow higher microbial diversity
- Carrot & tomato diets \rightarrow lower diversity

Axis 3 (16.28%) Taxonomic Composition Highlights \checkmark Dominant phyla: Proteobacteria & Firmicutes; Rare families: Actinobacteriota, Bacteroidota

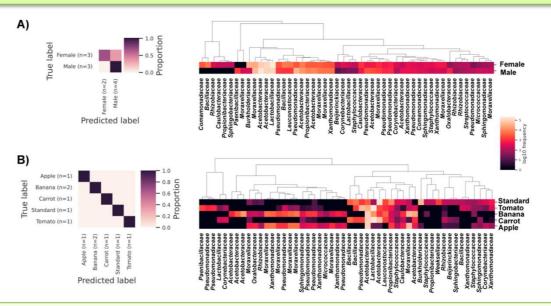
- Top families: Acetobacteraceae,
- Lactobacillaceae, Moraxellaceae
- Key group differences:
- A (apple) diet:

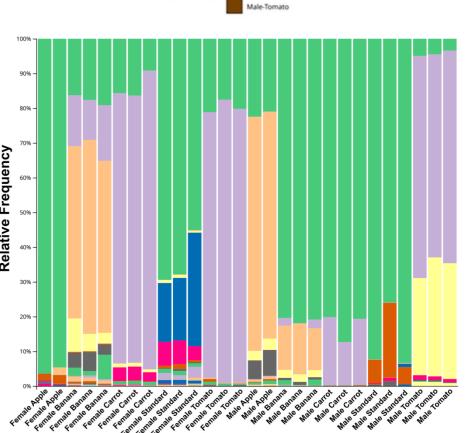
– Females → mostly Acetobacteraceae

- Males \rightarrow mostly *Moraxellaceae*, then Acetobacteraceae

- B (banana) diet:
 - Females → high *Moraxellaceae*
 - Males → dominant Acetobacteraceae
- S (standard) diet:
 - Both sexes \rightarrow Acetobacteraceae dominant
 - Females → Burkholderiaceae next - Males \rightarrow *Propionibacteriaceae* next
- C (carrot) diet:
 - Females \rightarrow Lactobacillaceae dominant
 - Males \rightarrow Acetobacteraceae dominant
- T (tomato) diet:
 - Females → more Lactobacillaceae
 - Males → more *Pseudomonadaceae*

Diet shapes which bacterial families dominate, with notable sex-based shifts in community structure across diets.





Axis 1 (33.70%)

teobacteria;c__Gammaproteobacteria;o__Burkh Bacteria;p_Firmicutes;c_Bacilli;o_Bacillales;f_Bacillaceae Bacteria:p Actinobacteriota:c Actinobacteria:o Propionibacteriales:f Propionibacteriacea Proteobacteria; Gammaproteobacteria; Xanthomonadales; d_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Caulobacterales;f_Caulobacteracea d Bacteria:p Firmicutes:c Bacilli:o Paenibacillales:f Paenibacillacea Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Sph Bacteria:p Firmicutes:c Bacilli:o Staphylococcales:f Staphylococcaceae oteobacteria:o Burkholderiales:f Oxalobacterace Bacteria;p Bacteroidota;c Bacteroidia;o Bacteroidales;f Rikenellaceae Bacteria;p_Firmicutes;c_Bacilli;o_Exiguobacterales;f_Exiguobacteraceae eria;p_Proteobacteria;c_Gammaproteobacteria;o_Legionellales;f_Legior Bacteria:p Proteobacteria:c Alphaproteobacteria:o Rhizobiales:f Beije

Predictive Features (ML) **Analysis**)

Predictive strength:

- Highest performance: predicting diet from GM composition
- Moderate performance: predicting sex from GM composition
- Key insights:
- Distinctive features span both

Banana diet shows the highest overall

diversity Sex × Diet (C):

Clear differences across groups

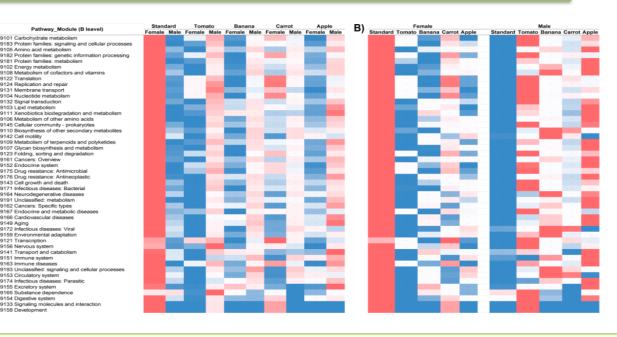
Sugar-rich diets boost diversity, especially in females

Diet has a stronger impact on gut microbiota diversity than sex, with sugar-rich diets driving higher microbial richness, especially in female flies.

CONCLUSION

 \checkmark Sugar-rich diets increase microbial richness, but not overall community performance \checkmark Core phyla consistent across diets, with varying key family abundances < Low-abundance bacterial families differ between diets, adding distinct community signatures < Functional predictions point to fermentation as a key metabolic process influenced by diet V Diet plays a critical role in shaping microbiota composition and host phenotype interactions

A Machine learning reveals that both major and minor bacterial players contribute to group-specific microbiota patterns, with diet being the strongest differentiator.



abundant and less abundant bacterial families

• Non-core microbes critically shape fly gut ecosystems

Metabolic Potential **Prediction**

• Female S-diet microbiota → high predicted metabolic activity Male S-diet microbiota → low activity

• Opposite trend in A, B, T diets (males higher than females) • C-diet microbiota \rightarrow most distinct, highest sex-based differences

Diet and sex jointly shape the **functional capacity** of fly microbiota, with unique patterns emerging especially under carrot (C) and standard (S) diets.

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