Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Microbiology

Review

Structure, function, and quantitative biology of the *Drosophila* gut microbiome

William B Ludington[1,2], Haolong Zhu[1,2], Kevin Aumiller[1,2], Angela Xu[1,2] and Josh Derrick[1,2]

Drosophila melanogaster is one of the most powerful animal models for developmental genetics, and it has a relatively simple microbiome. Flies can easily be reared germ-free, then reassociated with defined microbial strains to study colonization and bacterial effects on the fly, making a highly tractable model for microbiome studies. This review provides a biological background on the *Drosophila* microbiome for quantitative researchers interested in using the system. We discuss the current understanding of which microbes compose the fly gut microbiome, how they colonize, how they affect the fly, and how the overall microbiome structure influences its function. We also discuss existing large data sets and quantitative theory of the *Drosophila* microbiome.

Addresses
[1] Department of Biosphere Sciences and Engineering, Carnegie Institution for Science, Baltimore, MD 21218, USA
[2] Department of Biology, Johns Hopkins University, Baltimore, MD 21218, USA

Corresponding author: Ludington, William B (will.ludington@gmail.com)

Current Opinion in Microbiology 2025, **87**:102653

This review comes from a themed issue on **Microbial Systems and Synthetic Biology**

Edited by **Alvaro Sanchez** and **Seppe Kuehn**

For complete overview of the section, please refer to the article collection, "Microbial Systems and Synthetic Biology (2024)"

Available online 18 August 2025

https://doi.org/10.1016/j.mib.2025.102653

Introduction

How is a microbiome constructed? What are its parts and what functions do they serve? How does the overall microbiome work in terms of its inputs, outputs, and dynamics? How do the individual parts of the microbiome affect its host? And is the whole simply the sum of its parts? These simple questions are difficult to address in model systems such as mice with 100s of bacterial species. Low complexity, small animal model systems can address some of these limitations [1].

The microbiome of *Drosophila melanogaster* has been studied as an experimental variable for nearly a century [2], and it has clear impacts on the fly's physiology, including effects on lifespan and fecundity. However, there are relatively few quantitative studies exploring the relationship between individual members of the fly microbiome, how they colonize, and how they affect the fly. As with other host organisms, major questions about the relationship between the microbiome and its host concern: (i) whether changes in the microbiome are a cause or consequence of host physiological changes, (ii) whether causative effects of the microbiome are direct or indirect, and (iii) whether microbiome–host interactions are specifically co-evolved or more generic.

A major challenge in the microbiome field has been disentangling the complexities of microbial communities, which include the species diversity, the interactions with nutrients, the interactions with the host physiological systems such as immunity, and the various feedbacks in each of these relationships. Flies are already one of the most powerful models for animal developmental genetics, with the ability to turn individual genes on or off in specific cell types at defined time points in the animal's lifespan [3–8]. Nutrition is also well studied in flies, and defined diets are in wide use with known consequences for fly physiology, allowing dietary factors to be disentangled from microbial ones [9–17]. Moreover, *D. melanogaster* from wild and laboratory environments all over the world are associated with a consistent community of roughly 5 to 20 species of bacteria from only two phyla [18], and it is straightforward to make germ-free flies and reassociate them with defined microbial communities [19]. These experimental advantages make flies an excellent choice for microbiome experiments.

In this review, we address the structure of the commensal *Drosophila* microbiome in terms of the species composition and the physical locations of these species. We address the function of the *Drosophila* microbiome in terms of how the microbiome species convert nutrients, how the species affect one another, and how all these factors affect the host. Where possible, we focus the

review on quantitative studies that measure with precision how one factor changes as a function of another that is varied, for instance, the dose–response of colonization as a function of inoculum size [20]. We indicate data sets that can be utilized for theoretical work, and we emphasize current open areas for theory.

Structure of the *Drosophila* microbiome

The *D. melanogaster* commensal gut microbiome has been thoroughly inventoried both in the laboratory and the wild [21–24]. It is primarily composed of yeasts, acetic acid bacteria, lactic acid bacteria, and Enterobacteria [21] (Table 1). While the majority of studies have profiled only the taxonomic abundances of the microbes, some studies have spatially localized bacterial taxa within the gut.

Yeasts in the fly gut

Live yeasts are a major source of food and a stimulant for reproduction in adult flies; the fermentation products from live yeasts serve as attractants, with ethanol being the major metabolic end product [23,25–29]. Certain *Saccharomyces cerevisiae* strains from wild flies are the most attractive to flies, and these are best transmitted between flies [29]. The wild yeasts, *Hanseniaspora uvarum*, *S. cerevisiae*, *Pichia kudriavzevii* occur in the larval gut, aid development, and can survive pupariation [29–31]. However, yeast survive only transiently in the gut, suggesting that the gut environment is not a primary niche for yeasts [32]. Genetic screens for the factors required for yeast survival in the gut identified only sporulation genes, indicating that yeasts live in the gut as spores [33]. Hence, yeast have a strong and persistent relationship with *Drosophila*, but they appear not to be major inhabitants of the gut.

Bacteria in the fly gut

The total bacterial load in the fly gut has been quantified by both quantitative polymerase chain reaction (qPCR) and colony-forming unit (CFU) counts, with a wide range of 10^2 to 10^6 microbial cells per fly across laboratory and wild flies [34]. The relative abundances of different bacterial taxa in the fly gut also vary widely across studies in wild flies, with the sample substrate, for example, apple versus peach, and environmental factors such as temperature potentially driving the major differences [35,36]. For example, lactic acid bacteria vary between ~0 to 75% across different studies and sample sites, while acetic acid bacteria vary between ~25 to 100% in the same samples [22,35–37]. Lactic acid bacteria and acetic acid bacteria are readily culturable, making them favored for laboratory experiments. In laboratory stocks of flies collected from different populations of wild flies from all over the world (e.g. [21,24,38–40]), roughly the same species of bacteria occur, particularly *Lactiplantibacillus plantarum*, *Levilactibacillus brevis*, and a handful of *Acetobacter* species, including *A. pasteurianus* and *A. tropicalis*. These species occur at roughly equal abundances and are stable in the fly, whereas some strains outcompete others on the food [41], suggesting that these are specific to the fly rather than the food, e.g. see [21,38,42]. In comparison with wild flies, the overall bacterial diversity is lower but composed of roughly the same microbial groups [22,24]. In addition to lactic acid bacteria and acetic acid bacteria, the Orbaceae are Enterobacteria that are often found as commensals in insects, and sequencing studies have identified them in *Drosophila* strains from wild and laboratory environments [22,43–46]. The Orbaceae were recently isolated in culture [44], and future studies will examine them under laboratory conditions.

Bacteriophage in the fly gut

Bacteriophage are important members of microbial communities. Currently, a single metagenomic study of bacteriophage in wild flies exists, which identified 167 phage genomes that were inferred to infect lactic acid bacteria, acetic acid bacteria, and Enterobacteria [45], in rough agreement with the known bacterial relative abundances in wild flies. The study of phage dynamics in the *Drosophila* gut microbiome is an open area for investigation.

Table 1

Common *D. melanogaster* microbes.

Taxon	Location	Function	References
Yeasts: *Hanseniaspora*, *Saccharomyces*, *Pichia*, *Candida* others	Food	Nutrition, stimulate reproduction, necessary for development	[23,29,32,34,64,101]
Acetic acid bacteria: *Acetobacter*, *Acidobacteria*, *Gluconacetobacter*, *Gluconobacter*, others	Food, gut	Protein and fat production, sugar content reduction, vitamin production, acetate production, some pathogens	[22,24,38,61,102,103]
Lactic acid bacteria: *Lactiplantibacillus*, *Levilactibacillus*, *Fructilactibacillus*, *Lactococcus*, *Leuconostoc*, *Enterococcus*, others	Food, gut, cuticle	Lactate production, acidification, pathogen inhibition, growth factors	[17,22,38,42,46]
Gammaproteobacteria: *Serratia*, *Morganella*, *Orbus*, *Gilliamela*, others	Food, gut	Pathogens, commensals	[22,43–45,104,105]

Spatial localization of bacteria in the fly gut

There are several studies on the spatial localization of microbiota in the fly gut. Bacteria have been observed predominantly in the posterior midgut [47], but experiments to differentiate transiently passing cells from the fly food versus bacteria that stably associate with the gut found that most fly gut bacteria are transient [48,49]. However, specific strains of *L. plantarum*, *A. thailandicus*, and *A. indonesiensis* stably colonize a distinct physical niche in the *Drosophila* foregut, including the proventriculus, crop, and crop duct [20,49,50]. *L. plantarum* cells form a static adhesion with host tissue [50] using a specific glycan-binding adhesin that is expressed on the bacterial cell wall [51]. Colonization of the foregut niche by *L. plantarum* facilitates secondary colonization by *A. indonesiensis* and *A. pasteurianus*, which also colonize the foregut niche [49,50]. The same bacteria that live in the fly's gut live on the external cuticle, and these microbes appear to be deposited via grooming and serve to limit pathogenic yeasts [46], including entomopathogenic species [52].

Drosophila viruses comprise another component of the microbiome that has been extensively reviewed, for example, Ref. [53], and is not covered here.

Function of the *Drosophila* microbiome

Microbes draw nutrients from host food, expel byproducts that affect other microbial species as well as the host, and use the host as both habitat and a vector to other food sources and other hosts. There are three distinct ways in which the microbiome contributes to the dietary composition: (1) consumption of compounds present in the food, (2) generation of secondary metabolites, and (3) serving as a direct nutrition source for the fly. The wild *Drosophila* diet largely consists of decaying fruit matter and microorganisms that produce ethanol, short-chain fatty acids, including lactate and acetate, lipids, and amino acids. Yeasts, *Lactobacillus*, and *Acetobacter* strains reduce carbohydrate content of laboratory fly food [10,54,55]. Bacterial colonization of *Drosophila* also appears to be protective against the negative physiological effects of ethanol, a common yeast fermentation product [56]. The microbiome produces a variety of secondary metabolites or metabolite precursors that are essential for the fly: B vitamins [57,58], amino acids [57], specifically the modified N-acetylamino acids [59], and nucleic acids [59]. Other non-essential metabolites affect physiology. For instance, acetate modifies host insulin signaling to promote weight gain through lipogenesis [17,60,61] and increases histone acetylation around antimicrobial peptide genes, increasing their expression, which inhibits bacteria [62]. In low-nutrient environments, bacteria also serve as a source of calories, lipids, and proteins [63,64]. The fly also benefits the bacteria. Specifically, *Drosophila* larvae actively secrete a substrate containing N-acetylglucosamine that facilitates *Lactobacillus* growth on the fly food and *in vitro* [65].

The role of the microbiome in lipid uptake

The role of the microbiome in host lipid metabolism requires further study [66]. The effects of the *Drosophila* microbiome on lipid uptake have been studied indirectly with interesting results. Treatment of wild Hawaiian flies with antifungals increased their total lipid content [67]. Antibacterial treatment slightly decreased total lipids and cuticular hydrocarbons in females, and significantly decreased testes lipid content in males [67]. Microbial colonization appears to decrease total lipid content when compared to axenic flies, although this effect is linked to the reduction of carbohydrate composition of the food [54,55]. Combining chemically defined diets and host genetics in gnotobiotic flies would be powerful for deciphering the mechanisms.

Influence on immunity

In controlled studies of antiviral immunity, it was found that Gram-negative bacteria, including *Escherichia coli* and *A. pomorum*, induce resistance to *Drosophila* C virus through bacterial priming of the antiviral ERK pathway via NF-kB stimulation of Pvf2 ligand production [68]. Particular commensal gut species can also influence the host survival of bacterial pathogens, such as *Vibrio cholera*, which is more virulent when given to flies with *Acetobacter* than to flies with lactobacilli due to the Gram-negative *Acetobacter* activating the *V. cholera* type VI secretion system, which injects toxins into fly epithelial cells in the gut [69]. In addition to anti-pathogen immunity, the onset of intestinal cancer and cellular structure of the gut are also influenced by microbiome composition [70–72]. There are numerous additional knowledge gaps with regard to the influence of the microbiome on distinct developmental processes of which the immune system is but one component.

Influence on lifespan

The microbiome composition is both correlated with and causative of aging. Elderly *Drosophila* reared germ-free express reduced stress response and innate immunity genes compared to equally aged flies with commensal bacteria [73]. Bacteria can provision nutrients and change the nutritional composition of the fly food through metabolic processes. For instance bacterial genes for consumption of cysteine and methionine were causally linked to extended fly lifespan [74,75]. Increased microbial load is a consistent factor associated with aging — higher numbers of gut bacteria are correlated with shorter host lifespan [76], and the proportion of lactobacilli decreases while Acetobacters increase in older flies [43]. Age-related shifts in microbiome compositions are associated with age-related intestinal dysfunction [71,77,78], evidenced by loss of stem cell compartmentalization in the posterior midgut [43,70,71], loss of intestinal barrier

integrity [43,79], systemic inflammation [77], and neurodegeneration in *Drosophila* Alzheimer's models [80]. While *L. plantarum* can inhibit pathogens, *L. brevis* and *Gluconobacter* switch from commensal to pathogenic [38,81]. An open question is why and how these bacterial strains switch from a commensal state to a pathogenic one. Detailed studies assessing the role of each combination of individual species show that microbial interactions have large effects on fly lifespan [41,76,82] through unknown mechanisms. A key finding from these experiments is that lactobacilli regulate the interactions between other taxa in the microbiome that influence host lifespan [82]. However, the effects of the microbiome on lifespan are diet dependent. On low-nutrient food, bacteria can increase fly lifespan [40], whereas on high-nutrient food, bacteria decrease fly lifespan [14,63]. Strain-dependent effects further complicate the issue. For instance, while *L. plantarum* often extends lifespan [41,83], one strain shortens lifespan by disrupting intestinal homeostasis through impaired epithelial renewal [84].

Theory for the *Drosophila* microbiome

What determines the microbiome composition of an individual animal? Why do two individual animals of the same species have differences in their microbiome composition? Does the order in which species arrive influence the final composition? To what extent do deterministic versus stochastic processes play a role?

Colonization theory

To explain whether an individual exposure to a defined number of bacterial cells will result in stable colonization of the host, a stochastic framework based on the dose–response was introduced to account for the observation that two identical germ-free flies inoculated with the same dose of bacteria can end up with different outcomes: either colonized or remaining germ free [20]. This model calculates the odds of colonization from a binomial distribution where each bacterial cell inoculated into the fly has an equal probability of establishing stable colonization [20]. Obtaining reliable data requires precise measurement of the input dose, the resulting colonization outcome (colonized or not), and a large number of experimental replicates in individual germ-free flies, which can be accomplished by high-throughput methods, for example, [85].

Integration of these findings with pulse-chase experiments, where flies are first colonized by a strain labeled with one fluorescent protein and then subsequently fed the same strain labeled with a different color, allows one to calculate the rate of population turnover and use microscopy to spatially localize the site of turnover [50]. These measurements in *L. plantarum* and *A. indonesiensis* allowed refinement of the theory to account for spatial turnover of the population, which differentiates two important modes of bacterial colonization [50] (Figure 1). The first mode we call population-level colonization, where colonization is maintained by the bacteria growing faster than they are lost due to death inside the host and flushing by peristaltic flow (Figure 1). This mode has ecological features like Allee effects, where a population cannot be maintained below a certain threshold size. Population-level colonization is stochastic and can be lost due to perturbations [20]. Bacterial growth rates and population dynamics can be tracked by measuring the probabilistic loss of plasmid from labeled strains with neutral markers [20].

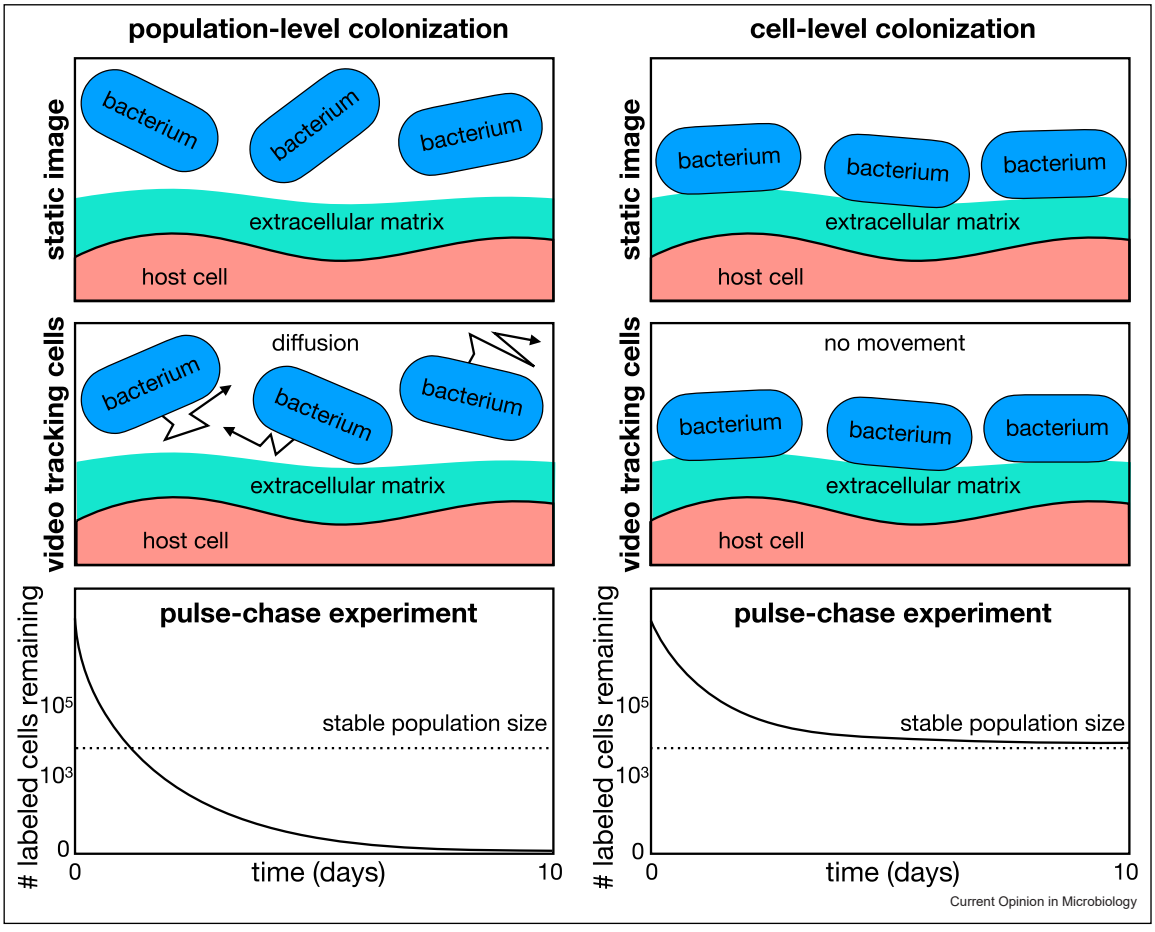
The second mode we call cell-level colonization, where colonization is stable at the single cell level due to adhesive contacts that cause individual bacterial cells to bind the host and therefore resist peristaltic flow (Figure 1). These adhesive contacts make colonization deterministic, if they are specific for a particular gut region where the bacteria have the correct physiological conditions for growth. Both types of colonization occur in *Drosophila*. Among the colonizers, *L. plantarum*, *A. thailandicus*, and *A. indonesiensis* strains from wild-caught flies can stably colonize at the single cell level in the foregut, while most bacteria are population-level colonizers [20,49,50].

Interactions between colonizers result in priority effects where the order of introduction modifies the probability of colonization [86]. The more different the two species, the more positive their interactions [86]. As the diversity of a community increases, the number of possible interactions increases as $2^n - 1$, giving need for theory of complex interaction networks.

Higher-order interaction theory

The theory of higher-order interactions has been extended and applied to microbial communities, including the fly gut microbiome. Interactions between bacterial species that affect the function of the community can be viewed as a microbial equivalent of genetic epistasis, where the interacting species are equivalent to genes and the function can be either a host trait, like lifespan [41,82], or a community function, like usage of a complex carbon source [87,88]. This framework applies to higher-order interactions, including context dependence. A challenge with applying traditional epistasis frameworks to higher-dimensional interactions is that while there are many interactions that are not calculated by standard frameworks, there are far too many interactions to calculate with exhaustive approaches [89,90]. A new mathematical framework called 'epistatic filtration' addresses this challenge by analyzing the complete interaction landscape by an unbiased method that reveals the important interactions without exhaustively testing each one. In particular, this approach identifies master regulators of complex microbial communities — species that regulate the interactions of the rest of the community in their effects on the host [82].

Figure 1



Distinction between population-level bacterial colonization and cell-level bacterial colonization. A bacterial population can stably colonize with continuous turnover of cells. Bacterial cells can be gained by consumption in the diet and cell division. They can be lost by cell death and washout by peristaltic flow. Cell-level colonization occurs when individual bacterial cells stably colonize host tissue. These two modes of colonization can be distinguished experimentally by video tracking via Bellymount [92] or pulse-chase experiments [50].

Stronger interactions occurred between bacterial strains isolated from a single wild *D. melanogaster* than between the same bacterial species isolated from laboratory flies [82]. This approach identifies important members of microbial communities for follow-up mechanistic work. For instance, the strong colonizer, *L. plantarum*, was found to be a master regulator. Thus, the fly can control its microbiome and its own physiology by proxy through *L. plantarum* [83]. A convenient, web-based interface is provided so that users can load data and receive the analysis [82].

Theory for the evolution of host–microbe associations

Deterministic interactions are typically driven by molecular mechanisms, which are encoded by genes that favor fitness. The evolutionary problem of how animal-microbe associations might evolve through initially nonspecific interactions has been approached from a disease ecology perspective on transmission dynamics [91]. Overlapping habitat preferences between microbes and host — perhaps around a shared, transient food source such as fallen fruit — and transmission dynamics in a patchy landscape are sufficient to drive co-occurrence between microbes and hosts and could serve as a starting point for the evolution of host–microbe specificity [91]. An open area for future theory could examine how these associations develop from nonspecific initial associations into complex communities. The relatively simple fly microbiome, with a stable community of as few as five bacterial species, allows these questions of complexity to be examined within a tractable framework both in terms of experiments and theory.

Quantitative data sets for microbiome theory in *Drosophila*

Given the naturally low microbiome diversity and experimental tractability of the *Drosophila* system, experimentalists have generated data sets that can be useful for

Table 2

Data sets.

Reference	Traits measured	Bacterial species	Relevance for future research
Obadia *et al*, 2017[20]	Colonization dose–response curves	Three *L. plantarum* strains as focal species; 17 total strains from genera including *Acetobacter*, *Enterococcus*, *Lactococcus*, and *Leuconostoc*.	Theory of colonization including priority effects
Dodge *et al,* 2023 [50]	Colonization dose–response and priority effects	Focal species are *L. plantarum* and *A. indonesiensis*. Limited data in supplement for *A. pasteurianus*.	Theory of colonization including priority effects
Gould *et al*, 2018 [41]	Colonization, development, fecundity, lifespan	*L. plantarum*, *L. brevis*, *A. pasteurianus*, *A. tropicalis*, and *A. orientalis*.	Theory of higher-order interactions
Jones *et al,* 2022[86]	Colonization	*L. plantarum*, *L. brevis*, *A. pasteurianus*, *A. tropicalis*, and *A. orientalis*.	Theory of microbial interactions in colonization
Eble *et al,* 2023[82]	Lifespan	*L. plantarum*, *L. brevis*, *A. pasteurianus*, *A. tropicalis*, *A. orientalis*, *A. cerevisiae*, and *A. malorum*	Theory of higher-order interactions

theorists. Different aspects of colonization can be measured using (1) frequent transfer to sterile food to push out unstable colonizers [48], (2) pulse-chase using labeled bacteria to measure population turnover [50], and (3) single-cell microscopy in live fly guts to measure the adhesion of individual bacterial cells [92]. (4) High-throughput colonization quantification enables the study of stochastic processes [20,85,93]. (5) Fecal time series can infer population dynamics within the gut [94]. Advances in fecal sampling have increased the throughput and precision [95] to allow a large number of flies to be sampled with a minimally invasive technique. There are limitations to each technique. For instance, the fluorescent proteins labeling strains can change the fitness of the strain they label; single-cell microscopy is low throughput; and high-throughput experiments miss the spatial localization of bacteria in the gut. However, the techniques cover a wide range of precision and effort, letting experimenters select the technique most suited to their application.

Quantification of colonization across a wide range of bacterial strains with sequenced genomes was used to detect bacterial genes associated with colonization in a whole metagenome-wide association study (MGWAS) [74,96,97]. In the MGWAS framework, a microbial gene is correlated with a trait of interest using a large panel of microbial strains for which the complete genome sequences are available. *Drosophila* also provides a rapid system for genome-wide association studies where fly genes are associated with traits conferred by bacteria [98]. These experimental frameworks allow researchers to screen for genes of interest, and the approach can be applied to study the genetic basis of many different microbial interactions.

Dose–response curves relate the odds of colonization for a single inoculation of an individual fly to the number of bacterial cells in that inoculation. These curves clearly differentiate strong colonizers from weak ones, with weak colonizing stochastically and strong colonizing deterministically. The Obadia *et al* data set contains dose–response curves constructed from thousands of individual flies that either began the experiment germ-free or colonized with a conventional microbiome [20]. The Dodge *et al* data set contains dose–response curves for flies that began the experiment either (1) germ free, (2) colonized by the same strain as the inoculum but carrying a different label, or (3) colonized by a different species [50]. Priority effects, where an early colonizing strain influences the colonization of a later arrival, were observed both where the first strain inhibited the second and where the first strain facilitated the second. Thus, these data sets provide a resource for theory on ecological priority effects.

To study higher-order interactions, combinatorial data sets are needed, where all $2^n - 1$ possible combinations of bacterial strains are constructed and measured for a focal trait. Combinatorial approaches, where each possible combination, for example, of one, two, three, four, and five species was inoculated into germ-free flies, were used to generate datasets to model both the effects of microbes on one another for colonization studies [41,86] as well as to model the effects of differing microbial compositions on the host physiology [41,76,82]. This combinatorial experimental design allows the interactions between the different bacteria to be calculated. The data include measurements of CFU counts for each bacterial strain in the combinations as well as measurements of fly development time, fecundity, and lifespan, which can be combined to calculate fitness (Table 2). The combinatorial data sets for colonization can be reanalyzed to further develop theories of priority effects, and the data sets for fly physiology can be reanalyzed to further develop the theory of higher-order interactions. Notably, the experiments to generate these data sets were performed on acidic fly food, where the bacteria cannot grow, which means the measured phenotypes are indicative of interactions inside the fly rather than on the food.

These microbial data sets help elucidate bacterial communities that are important for fly physiology. It should

be noted that a major advantage of *Drosophila* as a model system is the availability of genetics tools to determine the cell physiological mechanisms of host phenotypes. The recent discovery of the host cell types where colonization occurs and development of new genetic tools to control gene expression in these cell types opens up new opportunities to study the cellular and molecular mechanisms of the host–microbe interactions that were discovered through these quantitative data sets [99].

Conclusions

From an ecological and evolutionary perspective, it is important to consider how host–microbe interactions evolved. In what context is there a mutual fitness advantage in host–microbe specificity? Which interactions can persist without a co-evolved mechanism? While some host–microbe traits are nonspecific due to ecological filtering [100], others are highly specific [20,49,50]. Quantitative studies in flies and bacteria provide precise traits, which can be mapped to genes in the respective genomes of bacteria and flies.

An open and understudied area for investigation is what are the evolved molecular traits that confer specificity to host–microbe interactions. A related and understudied area, for which the fly model system is well suited, is the quantitative study of host-to-host transmission and how it has shaped the molecular repertoire of colonization factors in the bacteria and their fly hosts. Flies provide an excellent host model for these studies because of their extremely tractable genetics, high-throughput microbiome techniques, and staggering host genetic resources available.

By taking quantitative approaches integrated with theory, the field has gained critical insights into the process of gut colonization. These types of critical insights provide a model for understanding more complex host-microbiome systems such as those of humans.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

WBL reports financial support was provided by the National Institutes of Health, by the National Science Foundation, and by the Research Corporation for Science Advancement. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

WBL acknowledges SCIALOG grant SA-MND-2023-031, NIH grants R01DK128454 and R21AI173779, NSF grants IOS-2144342 and IOS-2032985.

References and recommended reading

Papers of particular interest, published within the period of review since 2020, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Vega NM, Ludington WB: **From a parts list to assembly instructions and an operating manual: how small host models can re-write microbiome theory**. *Curr Opin Microbiol* 2021, **64**:146-151.

2. Steinfeld HM: **Length of life of Drosophila melanogaster under aseptic conditions**. *Diss Thesis* 1927.

3. Ryder E, Ashburner M, Bautista-Llacer R, Drummond J, Webster J, Johnson G, Morley T, Chan YS, Blows F, Coulson D, *et al.*: **The DrosDel deletion collection: a drosophila genomewide chromosomal deficiency resource**. *Genetics* 2007, **177**:615-629.

4. Brand AH, Perrimon N: **Targeted gene expression as a means of altering cell fates and generating dominant phenotypes**. *Development* 1993, **118**:401-415.

5. Kanca O, Zirin J, Garcia-Marques J, Knight SM, Yang-Zhou D, Amador G, Chung H, Zuo Z, Ma L, He Y, *et al.*: **An efficient CRISPR-based strategy to insert small and large fragments of DNA using short homology arms**. *Elife* 2019, **8**:142.

6. Perkins LA, Holderbaum L, Tao R, Hu Y, Sopko R, McCall K, Yang-Zhou D, Flockhart I, Binari R, Shim HS, *et al.*: **The transgenic RNAi project at Harvard medical school: resources and validation**. *Genetics* 2015, **201**:843-852.

7. Pfeiffer BD, Ngo T-TB, Hibbard KL, Murphy C, Jenett A, Truman JW, Rubin GM: **Refinement of tools for targeted gene expression in Drosophila**. *Genetics* 2010, **186**:735-755.

8. Venken KJT, Schulze KL, Haelterman NA, Pan H, He Y, Evans-Holm M, Carlson JW, Levis RW, Spradling AC, Hoskins RA, *et al.*: **MiMIC: a highly versatile transposon insertion resource for engineering Drosophila melanogaster genes**. *Nat Methods* 2011, **8**:737-743.

9. Lee KP, Simpson SJ, Clissold FJ, Brooks R, Ballard JWO, Taylor PW, Soran N, Raubenheimer D: **Lifespan and reproduction in Drosophila: new insights from nutritional geometry**. *Proc Natl Acad Sci* 2008, **105**:2498-2503.

10. • Lesperance DNA, Broderick NA: **Meta-analysis of diets used in drosophila microbiome research and introduction of the drosophila dietary composition calculator (DDCC)**. *G3 Genes|Genomes|Genetics* 2020 **10**:2207-2211.
This study provides a calculator to quantify dietary differences in key factors like sugar, fat, and protein.

11. Piper MDW, Blanc E, Leitão-Gonçalves R, Yang M, He X, Linford NJ, Hoddinott MP, Hopfen C, Soultoukis GA, Niemeyer C, *et al.*: **A holidic medium for Drosophila melanogaster**. *Nat Methods* 2013, **11**:100-105.

12. Barnes AI, Wigby S, Boone JM, Partridge L, Chapman T: **Feeding, fecundity and lifespan in female Drosophila melanogaster**. *Proc Biol Sci/R Soc* 2008, **275**:1675-1683.

13. Obadia B, Keebaugh ES, Yamada R, Ludington WB, Ja WW: **Diet influences host–microbiota associations in Drosophila**. *Proc Natl Acad Sci USA* 2018, **115**:1-2.

14. Bruce KD, Hoxha S, Carvalho GB, Yamada R, Wang H-D, Karayan P, He S, Brummel T, Kapahi P, Ja WW: **High carbohydrate-low protein consumption maximizes Drosophila lifespan**. *Exp Gerontol* 2013, **48**:1129-1135.

15. Deshpande SA, Carvalho GB, Amador A, Phillips AM, Hoxha S, Lizotte KJ, Ja WW: **Quantifying Drosophila food intake:**

comparative analysis of current methodology. *Nat Methods* 2014, **11**:535-540.

16. Keebaugh ES, Yamada R, Ja WW: **The nutritional environment influences the impact of microbes on Drosophila melanogaster life span**. *MBio* 2019, **10**:670-676.

17. Storelli G, Defaye A, Erkosar B, Hols P, Royet J, Leulier F: ***Lactobacillus plantarum* promotes drosophila systemic growth by modulating hormonal signals through TOR-dependent nutrient sensing**. *Cell Metab* 2011, **14**:403-414.

18. Ludington WB, Ja WW: **Drosophila as a model for the gut microbiome**. *PLoS Pathog* 2020, **16**:1-6.

19. Koyle ML, Veloz M, Judd AM, Wong AC-N, Newell PD, Douglas AE, Chaston JM: **Rearing the fruit fly Drosophila melanogaster under axenic and gnotobiotic conditions**. *J Vis Exp* 2016 **113**:e54219.

20. Obadia B, Güvener ZT, Zhang V, Ceja-Navarro JA, Brodie EL, Ja WW, Ludington WB: **Probabilistic invasion underlies natural gut microbiome stability**. *Curr Biol* 2017, **27**:1999-2006.

21. Broderick NA, Lemaitre B: **Gut-associated microbes of Drosophila melanogaster**. *Gut Microbes* 2012, **3**:307-321.

22. Chandler JA, Lang JM, Bhatnagar S, Eisen JA, Kopp A: **Bacterial communities of diverse Drosophila species: ecological context of a host-microbe model system**. *PLoS Genet* 2011, **7**:e1002272.

23. Chandler JA, Eisen JA, Kopp A: **Yeast communities of diverse Drosophila species: comparison of two symbiont groups in the same hosts**. *Appl Environ Microbiol* 2012, **78**:7327-7336.

24. Staubach F, Baines JF, Künzel S, Bik EM, Petrov DA: **Host species and environmental effects on bacterial communities associated with drosophila in the laboratory and in the natural environment**. *PLoS One* 2013, **8**:e70749.

25. Becher PG, Flick G, Rozpedowska E, Schmidt A, Hagman A, Lebreton S, Larsson MC, Hansson BS, Piškur J, Witzgall P, *et al.*: **Yeast, not fruit volatiles mediate Drosophila melanogaster attraction, oviposition and development**. *Funct Ecol* 2012, **26**:822-828.

26. Qiao H, Keesey IW, Hansson BS, Knaden M: **Gut microbiota affects development and olfactory behavior in *Drosophila melanogaster***. *J Exp Biol* 2019, **222**:jeb192500.

27. Schiabor KM, Quan AS, Eisen MB: **Saccharomyces cerevisiae mitochondria are required for optimal attractiveness to Drosophila melanogaster**. *PLoS One* 2014, **9**:1-18.

28. Fischer C, Trautman EP, Crawford JM, Stabb EV, Handelsman J, Broderick NA: **Metabolite exchange between microbiome members produces compounds that influence Drosophila behavior**. *Elife* 2017, **6**:e18855.

29. •• Guilhot R, Lagmairi A, Olazcuaga L, Fellous S: **Microbiota acquisition and transmission in Drosophila flies**. *iScience* 2023, **26**:107656, https://doi.org/10.1016/j.isci.2023.107656.
This study quantifies wil bacterial and wild yeast colonization and transmission in wild flies with ecologically realistic experiments.

30. •• Mure A, Sugiura Y, Maeda R, Honda K, Sakurai N, Takahashi Y, Watada M, Katoh T, Gotoh A, Gotoh Y, *et al.*: **Identification of core yeast species and microbe-microbe interactions impacting larval growth of drosophila in the wild**. *Elife* 2023,1-37.
The authors identify wild bacteria and yeasts transmitted by adult flies to fruits along with laid eggs. They quantitatively identify interactions between bacteria, yeast, and flies that promote larval growth, determining the key nutrients provisioned.

31. Quan AS, Eisen MB: **The ecology of the Drosophila-yeast mutualism in wineries**. *PLoS One* 2018, **13**:e0196440-25.

32. Hoang D, Kopp A, Chandler JA: **Interactions between Drosophila and its natural yeast symbionts-Is Saccharomyces cerevisiae a good model for studying the fly-yeast relationship?** *PeerJ* 2015, **3**:e1116.

33. Coluccio AE, Rodriguez RK, Kernan MJ, Neiman AM: **The yeast spore wall enables spores to survive passage through the digestive tract of Drosophila**. *PLoS One* 2008, **3**:e2873.

34. Elya C, Zhang V, Ludington WB, Eisen MB: **Stable host gene expression in the gut of adult Drosophila melanogaster with different bacterial mono-associations**. *PLoS One* 2016, **11**:1-23.

35. •• Gale JT, Kreutz R, Morgan SJG, Davis EK, Hough C, Cancino WAC, Burnside B, Barney R, Hunsaker R, Hoyt AT, *et al.*: **Environment and diet shape the geography-specific Drosophila melanogaster microbiota composition**. *bioRxiv* 2024.
This study provides an in-depth reanalysis of wild fly microbiome collections and performs new experiments to test key variables driving differences, finding that food substrate, length of fermentation, and temperature drive the major differences, with lactic acid bacteria more prevalent on specific fruits and compost.

36. • Henry LP, Ayroles JF: **Drosophila melanogaster microbiome is shaped by strict filtering and neutrality along a latitudinal cline**. *Mol Ecol* 2022, **31**:5861-5871.
This study performs extensive wild fly microbiome sampling on D. melanogaster and D. simulans on apple and grape substrates, suggesting that neutral processes determine 93% of the microbiome composition in their survey.

37. Fink C, Staubach F, Kuenzel S, Baines JF, Roeder T: **Noninvasive analysis of microbiome dynamics in the fruit fly Drosophila melanogaster**. *Appl Environ Microbiol* 2013, **79**:6984-6988.

38. Ryu J-H, Kim S-H, Lee H-Y, Bai JY, Nam Y-D, Bae J-W, Lee DG, Shin SC, Ha E-M, Lee W-J: **Innate immune homeostasis by the homeobox gene caudal and commensal-gut mutualism in Drosophila**. *Science* 2008, **319**:777-782.

39. • Walters AW, Hughes RC, Call TB, Walker CJ, Wilcox H, Petersen SC, Rudman SM, Newell PD, Douglas AE, Schmidt PS, *et al.*: **The microbiota influences the Drosophila melanogaster life history strategy**. *Mol Ecol* 2020, **29**:639-653.
This study combines environmental sampling with laboratory experiments and finds that Maine and Florida flies are associated with different bacterial compositions that differentially influence fly survival and reproduction.

40. Brummel T, Ching A, Seroude L, Simon AF, Benzer S: **Drosophila lifespan enhancement by exogenous bacteria**. *Proc Natl Acad Sci* 2004, **101**:12974-12979.

41. Gould AL, Zhang V, Lamberti L, Jones EW, Obadia B, Korasidis N, Gavryushkin A, Carlson JM, Beerenwinkel N, Ludington WB: **Microbiome interactions shape host fitness**. *Proc Natl Acad Sci USA* 2018, **115**:E11951-E11960.

42. Wong AC-N, Chaston JM, Douglas AE: **The inconstant gut microbiota of Drosophila species revealed by 16S rRNA gene analysis**. *ISME J* 2013, **7**:1922-1932.

43. Clark RI, Salazar A, Yamada R, Fitz-Gibbon S, Morselli M, Alcaraz J, Rana A, Rera M, Pellegrini M, Ja WW, *et al.*: **Distinct shifts in microbiota composition during drosophila aging impair intestinal function and drive mortality**. *Cell Rep* 2015, **12**:1-12.

44. •• Phillips LE, Sotelo KL, Moran NA: **Characterization of gut symbionts from wild- caught Drosophila and other Diptera: description of Utexia brackfieldae gen. nov., sp. nov., Orbus sturtevantii sp. nov., Orbus wheelerorum sp. nov, and Orbus mooreae sp. nov**. *Int J Syst Evol Microbiol* 2024, **74**:006516.
This study isolates in culture and characterizes Orbaceae bacteria that colonize Drosophila melanogaster. These bacteria are related to bee symbionts but have previously only been observed in flies by sequencing.

45. • Comeault AA, Orta AH, Fidler D, Nunn T, Ellison AR, Anspach TA, Matute DR: **Phylogenetic and functional diversity among Drosophila-associated metagenome-assembled genomes**. *mSystems* 2025 **25**.
This study identifies bacterial genomes stably associated with many lines of wild-caught Drosophila from all over the world.

46. •• Hong S, Sun Y, Sun D, Wang C: **Microbiome assembly on Drosophila body surfaces benefits the flies to combat fungal infections**. *iScience* 2022, **25**:104408.

The authors demonstrate that *Drosophila* gut bacteria, deposited on the cuticle through natural fly behavior, inhibit fungal pathogenesis by preventing spore germination.

47. Broderick NA, Buchon N, Lemaitre B: **Microbiota-induced changes in Drosophila melanogaster host gene expression and gut morphology**. *MBio* 2014, **5**:e01117-14.

48. Blum JE, Fischer CN, Miles J, Handelsman J: **Frequent replenishment sustains the beneficial microbiome of Drosophila melanogaster**. *MBio* 2013, **4**:e00860-13.

49. Pais IS, Valente RS, Sporniak M, Teixeira L: **Drosophila melanogaster establishes a species-specific mutualistic interaction with stable gut-colonizing bacteria**. *PLoS Biol* 2018, **16**:e2005710.

50. •• Dodge R, Jones EW, Zhu H, Obadia B, Martinez DJ, Wang C, Aranda-Díaz A, Aumiller K, Liu Z, Voltolini M, *et al*.: **A symbiotic physical niche in Drosophila melanogaster regulates stable association of a multi-species gut microbiota**. *Nat Commun* 2023, **14**:1-13.

The authors identify the *Drosophila* foregut as a site of stable colonization by wild species of fly gut bacteria. They quantitatively determine positive and negative priority effects between different bacteria that affect the colonization of the foregut.

51. • Gutiérrez-García K, Aumiller K, Dodge R, Obadia B, Deng A, Agrawal S, Yuan X, Wolff R, Zhu H, Hsia R, *et al*.: **A conserved bacterial genetic basis for commensal-host specificity**. *Science* 2024, **386**:1117-1122.

This study identifies the bacterial gene that directs L. plantarum bacteria to colonize a specific subregion of the Drosophila gut and finds that this gene is conserved across many host-associated bacteria.

52. Elya C, Lok TC, Spencer QE, McCausland H, Martinez CC, Eisen M: **Robust manipulation of the behavior of Drosophila melanogaster by a fungal pathogen in the laboratory**. *Elife* 2018, **7**:1-34.

53. Webster CL, Waldron FM, Robertson S, Crowson D, Ferrari G, Quintana JF, Brouqui JM, Bayne EH, Longdon B, Buck AH, *et al*.: **The discovery, distribution, and evolution of viruses associated with Drosophila melanogaster**. *PLoS Biol* 2015, **13**:1-33.

54. McMullen JG, Peters-Schulze G, Cai J, Patterson AD, Douglas AE: **How gut microbiome interactions affect nutritional traits of *Drosophila melanogaster***. *J Exp Biol* 2020, **223**:jeb227843.

55. Newell PD, Douglas AE: **Interspecies interactions determine the impact of the gut microbiota on nutrient allocation in Drosophila melanogaster**. *Appl Environ Microbiol* 2013, **80**:788-796.

56. Chandler JA, Innocent LV, Martinez DJ, Huang IL, Yang JL, Eisen MB, Ludington WB: **Microbiome-by-ethanol interactions impact Drosophila melanogaster fitness, physiology, and behavior**. *iScience* 2022, **25**:104000.

57. • Consuegra J, Grenier T, Akherraz H, Rahioui I, Gervais H, da Silva P, Leulier F: **Metabolic cooperation among commensal bacteria supports Drosophila juvenile growth under nutritional stress**. *iScience* 2020, **23**:101232.

This study discovers specific metabolites that are crossfed and support the Drosophila microbiome, which benefits fly fitness.

58. Sannino DR, Dobson AJ, Edwards K, Angert ER, Buchon N: **The Drosophila melanogaster gut microbiota provisions thiamine to its host**. *MBio* 2018, **9**:e00155-18.

59. da Silva Soares NF, Quagliariello A, Yigitturk S, Martino ME: **Gut microbes predominantly act as living beneficial partners rather than raw nutrients**. *Sci Rep* 2023, **13**:1-12.

60. Shin SC, Kim S-H, You H, Kim B, Kim AC, Lee K-A, Yoon J-H, Ryu J-H, Lee W-J: **Drosophila microbiome modulates host developmental and metabolic homeostasis via insulin signaling**. *Science* 2011, **334**:670-674.

61. Kang D, Douglas AE: **Functional traits of the gut microbiome correlated with host lipid content in a natural population of Drosophila melanogaster**. *Biol Lett* 2020, **16**:20190803.

62. Jugder BE, Batista JH, Gibson JA, Cunningham PM, Asara JM, Watnick PI: **Vibrio cholerae high cell density quorum sensing activates the host intestinal innate immune response**. *Cell Rep* 2022, **40**:111368.

63. Keebaugh ES, Yamada R, Obadia B, Ludington WB, Ja WW: **Microbial quantity impacts drosophila nutrition, development, and lifespan**. *iScience* 2018, **4**:247-259.

64. Yamada R, Deshpande SA, Bruce KD, Mak EM, Ja WW: **Microbes promote amino acid harvest to rescue undernutrition in Drosophila**. *Cell Rep* 2015, **10**:865-872.

65. Storelli G, Strigini M, Grenier T, Bozonnet L, Schwarzer M, Daniel C, Matos R, Leulier F: **Drosophila perpetuates nutritional mutualism by promoting the fitness of its intestinal symbiont *Lactobacillus plantarum***. *Cell Metab* 2018, **27**:1-16.

66. Brown EM, Clardy J, Xavier RJ: **Gut microbiome lipid metabolism and its impact on host physiology**. *Cell Host Microbe* 2023, **31**:173-186.

67. Medeiros MJ, Seo L, Macias A, Price DK, Yew JY: **Bacterial and fungal components of the gut microbiome have distinct roles in Hawaiian Drosophila reproduction**. *ISME Commun* 2024, **4**:ycae134.

68. Sansone CL, Cohen J, Yasunaga A, Xu J, Osborn G, Subramanian H, Gold B, Buchon N, Cherry S: **Microbiota-dependent priming of antiviral intestinal immunity in Drosophila**. *CHOM* 2015, **18**:571-581.

69. Fast D, Petkau K, Ferguson M, Shin M, Galenza A, Kostiuk B, Pukatzki S, Foley E: **Vibrio cholerae-symbiont interactions inhibit intestinal repair in Drosophila**. *Cell Rep* 2020, **30**:1088-1100.

70. Guo L, Karpac J, Tran SL, Jasper H: **PGRP-SC2 promotes gut immune homeostasis to limit commensal dysbiosis and extend lifespan**. *Cell* 2014, **156**:109-122.

71. Li H, Qi Y, Jasper H: **Preventing age-related decline of gut compartmentalization limits microbiota dysbiosis and extends lifespan**. *CHOM* 2016, **19**:240-253.

72. Ferguson M, Petkau K, Shin M, Galenza A, Fast D, Foley E: **Differential effects of commensal bacteria on progenitor cell adhesion, division symmetry and tumorigenesis in the Drosophila intestine**. *Development* 2021, **148**:dev186106.

73. • Shukla AK, Johnson K, Giniger E: **Common features of aging fail to occur in Drosophila raised without a bacterial microbiome**. *iScience* 2021, **24**:102703.

This study reports differences in the age-related decline of flies reared with and without bacteria, highlighting a lower expression of antimicrobial peptides that accompanies the longer lifespan in germfree flies.

74. Matthews MK, Wilcox H, Hughes R, Veloz M, Hammer A, Banks B, Walters A, Schneider KJ, Sexton CE, Chaston JM: **Genetic influences of the microbiota on the life span of Drosophila melanogaster**. *Appl Environ Microbiol* 2020, **86**:e00305-20.

75. Judd AM, Matthews MK, Hughes R, Veloz M, Sexton CE, Chaston JM: **Bacterial methionine metabolism genes influence Drosophila melanogaster starvation resistance**. *Appl Environ Microbiol* 2018, **84**:e00662-18.

76. Lee H-Y, Lee S-H, Lee J-H, Lee W-J, Min K-J: **The role of commensal microbes in the lifespan of Drosophila melanogaster**. *Aging* 2019, **11**:1-14.

77. •• Zane F, Bouzid H, Marmol SS, Brazane M, Besse S, Molina JL, Cansell C, Aprahamian F, Durand S, Ayache J, *et al*.: **Smurfness-based two phase model of ageing helps deconvolve the ageing transcriptional signature**. *Aging Cell* 2023, **22**:e13946.

This study identifies two distinct modes of fly aging based on precise quantification of gut permeability. The authors use transcriptomic analysis to characterize the two phases, providing solid support for their model.

78. •• Roget T, Macmurray C, Jolivet P, Méléard S, Rera M: **A scenario for an evolutionary selection of ageing**. *Elife* 2023, **13**:1-27.

The authors introduce a quantitative model for a two-phase aging progression and show how this model can lead to evolutionary selection on lifespan as a trait.

79. Rera M, Clark RI, Walker DW: **Intestinal barrier dysfunction links metabolic and inflammatory markers of aging to death in Drosophila**. *Proc Natl Acad Sci* 2012, **109**:21528-21533.

80. Wu S-C, Cao Z-S, Chang K-M, Juang J-L: **Intestinal microbial dysbiosis aggravates the progression of Alzheimer's disease in Drosophila**. *Nat Commun* 2017 **8**:1-8.

81. Lee K-A, Kim S-H, Kim E-K, Ha E-M, You H, Kim B, Kim M-J, Kwon Y, Ryu J-H, Lee W-J: **Bacterial-derived uracil as a modulator of mucosal immunity and gut-microbe homeostasis in Drosophila**. *Cell* 2013, **153**:797-811.

82. •• Eble H, Joswig M, Lamberti L, Ludington WB: **Master regulators of biological systems in higher dimensions**. *PNAS* 2023, **120**:1-12.

The authors introduce a new mathematical framework to quantify higher-order interactions and show how it can be used to identify species that regulate the interactions of other species within the gut microbiome. This study provides large data sets for modeling work on how microbiome composition affects aging.

83. Yang JL, Zhu H, Sadh P, Aumiller K, Guvener ZT, Ludington WB: **Commensal acidification of specific gut regions produces a protective priority effect against enteropathogenic bacterial infection**. *Appl Environ Microbiol* 2025.

84. Fast D, Duggal A, Foley E: **Monoassociation with *Lactobacillus plantarum* disrupts intestinal homeostasis in adult Drosophila melanogaster**. *MBio* 2018, **9**:e01114-18.

85. •• Jaime MDLA, Salem GH, Martinez DJ, Karott S, Flores A, Palmer CD, Mahadevaraju S, Krynitsky J, Garmendia-Cedillos M, Anderson S, *et al.*: **Whole Animal Feeding FLat (WAFFL): a complete and comprehensive validation of a novel, high-throughput fly experimentation system**. *G3 Genes|Genomes|Genet* 2023, **13**:1-12.

The authors present a high-throughout technique to precisely feed and quantify fly feeding and locomotion behavior

86. •• Jones EW, Carlson JM, Sivak DA, Ludington WB: **Stochastic microbiome assembly depends on context**. *PNAS* 2022, **119**:1-8.

This study introduces a probablistic framework to model colonization of the gut based on species interactions. This study provides large data sets for modeling work on how microbiome interactions affect colonization.

87. Sanchez A: **Defining higher-order interactions in synthetic ecology: lessons from physics and quantitative genetics**. *Cell Syst* 2019, **9**:519-520.

88. Sanchez A, Bajic D, Diaz-Colunga J, Skwara A, Vila JCC, Kuehn S: **The community-function landscape of microbial consortia**. *Cell Syst* 2023, **14**:122-134.

89. Beerenwinkel N, Pachter L, Sturmfels B: **Epistasis and shapes of fitness landscapes**. *Stat Sin* 2007, **17**:1317-1342.

90. Beerenwinkel N, Pachter L, Sturmfels B, Elena SF, Lenski RE: **Analysis of epistatic interactions and fitness landscapes using a new geometric approach**. *BMC Evol Biol* 2007, **7**:1-12 60.

91. • Sieber M, Traulsen A, Schulenburg H, Douglas AE: **On the evolutionary origins of host-microbe associations**. *Proc Natl Acad Sci USA* 2021, **118**:e2016487118.

This study uses a computational framework to show how prevalent host-microbiome associations might occur based on ecological co-occurences without specific, co-evolved, molecular mechanisms.

92. Koyama LAJ, Aranda-Díaz A, Su Y-H, Balachandra S, Martin JL, Ludington WB, Huang KC, O'Brien LE: **Bellymount enables longitudinal, intravital imaging of abdominal organs and the gut microbiota in adult Drosophila**. *PLoS Biol* 2020, **18**:e3000567-19.

93. • Dodge R, Ludington WB: **Fast colony forming unit counting in 96-well plate format applied to the drosophila microbiome**. *J Vis Exp* 2023, **191**:e64298.

This study presents a ~100-fold faster technique to measure colony-forming units. The authors also provide instructions for constructing the inexpensive apparatus.

94. Inamine H, Ellner SP, Newell PD, Luo Y, Buchon N, Douglas AE: **Spatiotemporally heterogeneous population dynamics of gut bacteria inferred from fecal time series data**. *MBio* 2018, **9**:e01453-17.

95. • Wu Q, Yu G, Park SJ, Gao Y, Ja WW, Yang M: **Excreta quantification (EX-Q) for longitudinal measurements of food intake in drosophila**. *iScience* 2020, **23**:100776.

This study provides an inexpensive, robust, and highly sensitive assay for quantifying the intake of food in flies.

96. Chaston JM, Newell PD, Douglas AE: **Metagenome-wide association of microbial determinants of host phenotype in Drosophila melanogaster**. *MBio* 2014, **5**.

97. White KM, Matthews MK, Hughes R, Sommer AJ, Griffitts JS, Newell PD, Chaston JM, Metagenome-Wide Association A: **Study and arrayed mutant library confirm acetobacter lipopolysaccharide genes are necessary for association with Drosophila melanogaster**. *G3 Genes|Genomes|Genetics* 2018 **8**:1119-1127.

98. Dobson AJ, Chaston JM, Newell PD, Donahue L, Hermann SL, Sannino DR, Westmiller S, Wong AC-N, Clark AG, Lazzaro BP, *et al.*: **Host genetic determinants of microbiota-dependent nutrition revealed by genome-wide analysis of Drosophila melanogaster**. *Nat Commun* 2015, **6**:1-7.

99. •• Zhu H, Ludington WB, Spradling AC: **Cellular and molecular organization of the Drosophila foregut**. *Proc Natl Acad Sci* 2024, **121**:e2318760121.

This study identifies the cell types of the foregut tissue, which serves as a commensal niche for stable colonizing bacteria. The authors also identify Gal4 driver lines for manipulating gene expression in each of the cell types.

100. Adair KL, Bost A, Bueno E, Kaunisto S, Kortet R, Peters-Schulze G, Martinson VG, Douglas AE: **Host determinants of among-species variation in microbiome composition in drosophilid flies**. *ISME J* 2020, **14**:217-229.

101. Sang JH, King RC: **Nutritional requirements of axenically cultured Drosophila melanogaster adults**. *J Exp Biol* 1961, **38**:793-809.

102. Douglas AE: **Simple animal models for microbiome research**. *Nat Rev Microbiol* 2019.

103. Henry LP, Fernandez M, Wolf S, Abhyankar V, Ayroles JF: **Wolbachia impacts microbiome diversity and fitness-associated traits for Drosophila melanogaster in a seasonally fluctuating environment**. *Ecol Evol* 2024, **14**:1-15.

104. Jensen EC, Amburn DSK, Schlegel AH, Nickerson KW: **A collection of Serratia marcescens differing in their insect pathogenicity towards Manduca sexta larvae**. *bioRxiv* 2020, https://doi.org/10.4324/9780203041000-16

105. • Wang Z, Li S, Zhang S, Zhang T, Wu Y, Liu A, Wang K, Ji X, Cao H, Zhang Y, *et al.*: **Hosts manipulate lifestyle switch and pathogenicity heterogeneity of opportunistic pathogens in the single-cell resolution**. *Elife* 2024, **13**:1-25.

This study shows that larval flies drive Serratia marcescens from pathogen to commensal through antimicrobial peptides that change the metabolomic profile of the bacteria to a more nutritive one for the fly.