Lactobacillus plantarum favors the early emergence of fit and fertile adult Drosophila upon chronic undernutrition

Mélisandre A. Téfit and François Leulier*

ABSTRACT

Animals are naturally surrounded by a variety of microorganisms with which they constantly interact. Among these microbes, some live in close association with a host and form its microbiota. These communities are being extensively studied, owing to their contributions to shaping various aspects of animal physiology. One of these commensal species, Lactobacillus plantarum, and in particular the L.p.WJL strain, has been shown to promote the growth of Drosophila larvae upon nutrient scarcity, allowing earlier metamorphosis and adult emergence compared with axenic individuals. As for many insects, conditions surrounding the post-embryonic development dictate key adult life history traits in Drosophila, and adjusting developmental timing according to the environment is essential for adult fitness. Thus, we wondered whether the growth acceleration induced by L.p.WJL in a context of poor nutrition could adversely impact the fitness of Drosophila adults. Here, we show that the L.p.WJL-mediated acceleration of growth is not deleterious; adults emerging after an accelerated development are as fit as their axenic siblings. Additionally, the presence of L.p.WJL even leads to a lifespan extension in nutritionally challenged males. These results demonstrate that L.p.WJL is a beneficial partner for Drosophila melanogaster through its entire life cycle. Thus, commensal bacteria allow the earlier emergence and longer survival of fit and fertile individuals and might represent one of the factors contributing to the ecological success of Drosophila.

KEY WORDS: Microbiota, Symbiosis, Fertility, Fitness, Lifespan

INTRODUCTION

In nature, animals are constantly surrounded by a variety of microorganisms, whose presence has contributed to shaping life as we know it (McFall-Ngaí, 2015). The interactions existing between microbes and animals cover a broad spectrum, with outcomes ranging from obligate symbiosis to lethal infection (Casadevall and Pirofski, 2000; Hentschel et al., 2000). Among these microbial species, some live in close association with an animal host with which they establish commensalistic or mutualistic relationships. The community they form is referred to as the microbiota, which has a crucial impact on several key life history traits at the adult stage or across different life stages, and thus the length of the larval period, early and late life fecundity, adult longevity and stress resistance. Such trade-offs can involve traits either from the same life history trade-offs and the rather striking effect of L.p.WJL on larval development, we wondered about the potential repercussions of various physiological traits. Indeed, in several mammalian, nematode or arthropod models, the microbiota has been shown to shape development, immunity, metabolism and even behavior (Kostic et al., 2013; Lee and Hase, 2014). In this fast expanding research field, Drosophila has been a fruitful model. Thanks to its ease of manipulation and genetic tractability, as well as the low complexity of its microbiota, the fruit fly represents a powerful tool to delve into the mechanistic underpinnings of host–microbiota interactions (Lee and Brey, 2012; Ma et al., 2015). Studies have revealed that the presence and composition of microbiota impact various traits throughout the Drosophila life cycle such as larval growth, developmental timing, stress resistance, immune response, metabolism, lifespan and behavior (Brummel et al., 2004; Ryu et al., 2008; Sharon et al., 2010; Shin et al., 2011; Guo et al., 2014; Petkau et al., 2014; Venu et al., 2014; Wong et al., 2014; Clark et al., 2015). As the microbiota is closely associated with its animal partner and, in the case of Drosophila, is an integral part of its nutritive substrate, it is not surprising to see its influence on so many biological functions. Moreover, as for many insects, the larval stage is highly plastic in the fly life cycle. Indeed, biotic and abiotic factors surrounding the development of an organism participate in shaping this process (Gilbert, 2001; McFall-Ngaí, 2002), and in turn have a crucial impact on several key life history traits at the adult stage, such as reproductive capacity, stress resistance or lifespan (Tu and Tatar, 2003; Andersen et al., 2010; Sisodia and Singh, 2012; Burns et al., 2012).

Previously, we showed that, upon mono-association, some strains of the commensal bacterial species Lactobacillus plantarum (a member of the dominant phyla of Drosophila microbiota) are able to sustain the systemic growth of Drosophila larvae, to the same extent as a more complex microbiota (Storelli et al., 2011; Erkosal et al., 2015). Upon yeast deprivation during the larval stages, mono-association of germ-free animals with the strain Lactobacillus plantarumWJL (L.p.WJL) isolated from the intestine of lab-raised Drosophila melanogaster (Ryu et al., 2008) increases larval growth and reduces developmental timing, thus allowing earlier entry into metamorphosis of mono-associated individuals (Storelli et al., 2011).

Several studies using Drosophila lines generated in a laboratory evolution experiment of postponed senescence selection (Rose, 1984) have described a series of trade-offs between key life history traits. This occurs when the optimization of a trait correlates with a negative impact on another parameter; for example, increased reproductive capacity usually comes at the cost of a shortened lifespan. Such trade-offs can involve traits either from the same life stage or across different life stages, and thus the length of the larval period, early and late life fecundity, adult longevity and stress resistance were shown to trade-off with one another (reviewed in Zera and Harshman, 2001). Given the numerous examples of life history trade-offs and the rather striking effect of L.p.WJL on larval development, we wondered about the potential repercussions of
this accelerated growth on adult fitness. We speculated that L.p.WJL-mediated acceleration of growth in an otherwise nutritionally challenging environment might be deleterious at later stages such that it would lead to the emergence of unfit adults. To address this question, we assessed several fitness parameters in young adult flies and observed that, overall, L.p.WJL association was not detrimental for adult fitness. Furthermore, for adult males it proved to be an advantageous partner; L.p.WJL-associated males not only emerged several days before their germ-free siblings but also survived longer in nutritionally challenging conditions. L.p.WJL is thus a true beneficial partner for Drosophila across its entire life cycle, and even more so in a poor nutritional environment. This study therefore supports the notion that bacterial members of the fly microbiota might represent one of the factors contributing to the ecological success of Drosophila melanogaster.

MATERIALS AND METHODS
Fly stocks and husbandry
yw fly stocks were reared on a standard yeast/cornmeal diet containing (for 1): 50 g inactivated yeast (Bio Springer, Springaline BA95/0-PW), 80 g cornmeal (Westhove, Farigel maize H1), 10 g agar (VWR, ref. 20768.361), 5.2 g methylparaben sodium salt (Merck, ref. 106756) and 4 ml 99% propionic acid (Carlo Erba Reagents, ref. 409553). All experimental flies were kept in incubators at 25°C, with a 12 h:12 h light:dark cycle. The low-yeast diets were made by decreasing the quantity of yeast to 30, 12, 8 or 6 g l⁻¹ and the quantity of agar to 7.2 g l⁻¹. Unless stated otherwise, only mated flies were used in this study.

Generation of axenic Drosophila stocks and bacterial mono-association
To generate axenic flies, eggs were collected overnight and treated in sterile conditions with successive 2 min baths of bleach and 70% ethanol. Bleached embryos were then rinsed in sterile water for another 2 min and placed on sterile standard food supplemented with an antibiotic cocktail (50 μg ampicillin, 50 μg kanamycin, 50 μg tetracycline and 15 μg erythromycin per liter of fly food). Emerging adults were tested for axenicity by crushing and plating of 50 μL.p.WJL Fifty germ-free embryos were associated with an antibiotic cocktail (50 μg tetracycline and 15 μg ampicillin, 50 μg g l⁻¹ yeast) or kept axenic, as described above. Larvae were then left to develop under low nutrient conditions (low-yeast diet, 8 g l⁻¹ yeast) and the number of pupae appearing each day was recorded until the last larvae of the population reached pupariation.

Fecundity and fertility assessment
At emergence, groups of five females and five males were distributed in vials and transferred every 24 h to a new tube. The number of eggs laid was recorded every day for 10 days and the subsequent number of emerging adults was used to calculate the fertility ratio (number of emerging progeny divided by the number of eggs laid). In experiments where bacterial association was done only at the adult stage, the fecundity/fertility assays were started at day 7 or 10 after adult emergence and continued for 3–7 days.

Number of ovarioles
Mated females, 4–5 days old, were used to assess the number of ovarioles after development on either standard (50 g l⁻¹ yeast) or low-yeast (8 g l⁻¹ yeast) diet. Newly emerged adult flies were kept on standard food until the time of dissection. Ovaries were dissected in cold PBS and directly fixed in 4% formaldehyde for 20 min. They were then stained with DAPI (1:1000) for 15 min and transferred to 80% glycerol for preservation. After fixation and staining, ovarioles were teased apart under a dissecting microscope and mounted on slides for counting.

Adult wet mass and resistance to full starvation
Either virgin (0–7 h old) or mature and mated adults (7 or 10 days old) were collected and pooled in groups of five to be weighed on a Sartorius analytical balance CPA324S (Sartorius Weighing Technology GmbH, Goettingen, Germany). Flies of the same age were also used for full starvation assays, in tubes providing only water supply to the flies. Specifically, the starvation tubes contain a cotton ball soaked in a water reservoir to prevent them from drying. The cotton is covered with a piece of Whatman paper on which the flies are placed. Survival of the flies was recorded twice a day until all individuals were dead.

Lifespan
After larval development on either standard (50 g l⁻¹ yeast) or low-yeast (8 g l⁻¹ yeast) diet, newly emerged adults were kept all together for 3–4 days before males and females were separated for the subsequent experiments. Groups of 10 mated flies were transferred to fresh vials containing either standard or low-yeast diet. Flies were transferred to fresh fly food tubes twice a week and survival was recorded daily until all individuals were dead. Depending on the condition and on the experiment, 5–10 replicates were performed.

Statistical analyses
For comparison of GF and L.p.WJL-associated conditions, Mann–Whitney test (for mass, fecundity, fertility) and logrank test (for survival curve comparison) were performed using GraphPad Prism software version 6.0f for Macintosh (GraphPad Software, La Jolla, CA, USA; www.graphpad.com). The results of the Brown–Forsythe test for comparison of standard deviations were also obtained with this software. Whiskers of the boxplots represent the minimal to maximal values. For all experiments, the P-values are reported on the corresponding figure panels only when <0.05.

RESULTS
L.p.WJL does not directly impact Drosophila adult fitness
To determine whether L.p.WJL had an impact on fly physiology at the adult stage, we first assessed the direct effect of L.p.WJL on adult Drosophila, by associating newly emerged flies with the bacteria. After larval development on a normal diet in axenic conditions (germ-free, GF; devoid of microbiota), the young emerging adults...
were either associated with $L.p.WJL$ or kept axenic (Fig. 1A). The flies were left to mature for several days on diets with decreasing amounts of yeast and were then tested for fecundity, fertility and resistance to full starvation. After 8 days in various nutritive conditions, there was a clear effect of diet composition on the number of eggs laid per female and on the number of adult progeny emerging from these eggs; with decreasing amounts of yeast in the diet, the flies laid fewer eggs (Fig. 1B) and the fertility ratio (number of emerging progeny/number of eggs laid) showed a statistically significant increase in variability (Fig. 1C; Table S1). The ability of females to endure complete starvation was also impacted by the amount of yeast in the diet. Indeed, 7 day old females survived longer when they had been kept on a low-yeast diet after emergence (Fig. 1D, left panel). In contrast, the diet composition did not matter for their male counterparts, which died at the same rate regardless of the diet they were kept on after emergence (Fig. 1D, right panel). The association with $L.p.WJL$, however, did not impact any of these adult fitness traits. In addition, we tested the same parameters in flies that were raised on a normal diet in the presence of $L.p.WJL$ during larval life. In such rich nutritional conditions, the developmental time was similar for the axenic and the $L.p.WJL$-associated flies, and here again there was a clear impact of diet composition on fecundity, but no bacterial contribution was revealed for either fecundity or resistance to full starvation (Fig. S1). We next assayed the lifespan of these flies raised with or without $L.p.WJL$ on a normal diet, and kept as adults on either the same rich diet or a low-yeast food (Fig. 1E,F). Here, we saw a significant increase in the lifespan of axenic females kept in nutritionally rich conditions throughout their life cycle (Fig. 1G, left panel). For their male counterparts, however, as well as for female and male flies that went from a larval development on a normal diet to adult life on a low-yeast diet, there was no significant impact of $L.p.WJL$ presence (Fig. 1G, right panel, and 1H). Taken together, these results show that apart from the previously described sexually dimorphic lifespan shift on a normal diet (i.e. increased lifespan in GF females; Petkau et al., 2014; Clark et al., 2015), association of Drosophila with $L.p.WJL$ does not seem to have a direct impact on adult fitness when flies develop on a normal diet.

$L.p.WJL$-mediated larval growth acceleration is not deleterious for adult fitness

While searching for a direct effect of $L.p.WJL$ on the adult stage, we did not detect any significant impact of the commensal bacteria on the tested fitness parameters. There was, however, a quite striking larval effect, as nutritionally challenged individuals developed faster and pupariated several days earlier when they were associated with $L.p.WJL$ compared with the axenic ones (Storelli et al., 2011; Erkosar et al., 2015). While faster larval growth and precocious

Fig. 1. Lactobacillus plantarumWJL ($L.p.WJL$) does not directly impact Drosophila adult fitness. (A) Immediately after emergence, axenic (germ-free, GF; devoid of microbiota) adults developed on a normal diet were associated with $L.p.WJL$ (Lp) or sterile PBS. (B–D) When mature, they were tested for fecundity (B) or fertility (C) at 8 days of age, and for resistance to complete starvation (D) at 10 days after emergence. (E–H) Axenic eggs were inoculated with $L.p.WJL$ or sterile PBS and developed on a normal diet (50 g l$^{-1}$). The lifespan of the adults was then assessed on either the same normal diet (E,G) or a diet with a reduced amount of yeast (8 g l$^{-1}$; F,H).
emergence of the adult represent an obvious ecological advantage, doing so under nutritionally challenging conditions may in turn be deleterious for adult fitness and reproductive success. Indeed, adjusting developmental timing to environmental cues is key to Drosophila adult fitness (Nylin and Gotthard, 1998), yet upon L. p. WJL association animals develop faster even though the nutritional conditions are poor. To investigate whether the growth acceleration mediated by L. p. WJL upon nutrient scarcity would adversely impact subsequent adult fitness, we tested flies raised on a low-yeast diet with or without the bacteria, as depicted in Fig. 2A. As previously described, when raised on a low-yeast diet, larvae associated with L. p. WJL pupariate several days before their axenic siblings (Storelli et al., 2011; Erkosar et al., 2015; Fig. 2B). We then assessed the potential repercussions of the L. p. WJL association on the reproductive capacity of flies that underwent larval development in such nutritionally challenging conditions. Similar to what we observed when the flies were grown in nutrient-rich conditions and challenged only as adults, fecundity (Fig. 2F) and fertility (Fig. 2I) were both greatly impacted by adult diet composition (Fig. 2C–E). The higher the yeast content in the diet, the more eggs were laid per female per day (Fig. 2F–H). In addition, the number of adult progeny emerging from these eggs was impaired on the low-yeast diet. Indeed, as we observed for the low-yeast diet in Fig. 1C (6 g l⁻¹ of yeast), on the 8 g l⁻¹ yeast diet the fertility ratio was very variable (Fig. 2I–K; Table S1). For these two parameters, again, there was no impact of the association with L. p. WJL. Furthermore, these comparable fecundity results were supported by the fact that the number of ovarioles (the functional units of Drosophila ovaries) of females raised on a low-yeast diet was similar, regardless of their microbiota status (Fig. S2A). We are confident that our experimental setup can efficiently manipulate the ovariole number because, as expected, we observed a decreased count after development on a low-yeast diet compared with a nutritionally rich situation (Fig. S2A; Hodin and Riddiford, 2000; Tu and Tatar, 2003). As anticipated, the similar number of ovarioles between the GF and L. p. WJL conditions translated into a similar cumulative number of eggs laid over the course of the experiment, and as expected we detected reduced cumulative egg laying when animals developed on the poor diet (Fig. S2B). Next, we assayed the mass of 0–7 h old virgin adults, along with their resistance to complete starvation, as indicators of direct consequences of larval life on their adult metabolic state (Baker and Thummel, 2007). We detected a slight tendency in males and females associated with L. p. WJL to weigh less than axenic ones (Fig. 3A,B), but there was no

**Fig. 2.** Drosophila reproductive capacity is not altered after an accelerated larval development. For larvae raised on a low-yeast diet (8 g l⁻¹; A), the presence of L. p. WJL accelerated development and shortened the time to pupariation (B; AED, after egg deposition). The D₅₀ values represent the time (days) that 50% of the population reached pupariation. After this differential development, adults were kept on a rich diet (50 g yeast l⁻¹; C), an intermediate diet (30 g yeast l⁻¹; D) or a nutritionally poor diet (8 g yeast l⁻¹; E) and assessed for fecundity and fertility from day 2 after adult emergence (AAE) to day 10. The corresponding number of eggs laid per female per day (F–H) and the fertility ratios (emerging adult progeny divided by the number of eggs laid; I–K) are shown.
impact of the growth acceleration mediated by \textit{L.p.WJL} on the flies’ ability to endure full starvation (Fig. 3C). These assays were repeated on mature adults, after 10 days of adult life on either a normal diet (Fig. 3D–F) or the same low-yeast diet (Fig. 3G–I) and, again, there was no deleterious impact of the \textit{L.p.WJL}-mediated growth promotion on these adult fitness parameters. At this age, the mass tendency was reversed, as \textit{L.p.WJL}-associated males and females were now slightly heavier than their axenic counterparts. Similarly to what we observed with newly emerged flies, this did not translate into differences in resistance to full starvation. In addition, similar results were obtained when we starved adult flies that were matured on a diet with an intermediate yeast content (Fig. S3). Notably, for some of these experiments, the statistical analyses show significant differences between the groups, but the differences they represent are tenuous and probably not of any biological relevance. Collectively, these data suggest that even though larvae associated with \textit{L.p.WJL} develop faster in an otherwise poor nutritive environment, they do so without generating fitness costs for the later stage and give rise to fit and fertile adults.

\textbf{\textit{L.p.WJL}} increases the lifespan of nutritionally challenged males

While performing the experiments, we noticed that when kept on a low-yeast diet, adult males were dying rapidly and a significant
A proportion of them were dead 10 days after emergence. We then decided to study in more detail the lifespan of flies raised in such nutritionally poor conditions. After emergence from larval development on a low-yeast diet, the adults were either kept on the same low-yeast diet (Fig. 4C) or transferred to a rich diet (Fig. 4A). We saw that, while the larval nutritional environment had a notable impact on female lifespan, with a poor larval diet translating into an increased female lifespan (Fig. S4A), association with \( \text{L.p.} \text{WJL} \) did not impact this trait (Fig. 4B). However, males maintained on a low-yeast diet throughout their entire life survived better when they were associated with \( \text{L.p.} \text{WJL} \) (Fig. 4D right panel; Fig. S4B). Notably, their median lifespan was extended by 4–16 days, depending on the experiment. This fluctuation in the actual day count across experiments is commonly seen in lifespan studies (Piper et al., 2013) but the trend persisted and was statistically significant. This result shows that in a nutritionally challenging environment, \( \text{L.p.} \text{WJL} \) association not only shortens \textit{Drosophila} developmental time but also significantly increases the lifespan of adult males.

**DISCUSSION**

The microbiota is one of the key environmental factors impacting animal development and physiology and has been increasingly studied over the last few years (Sommer and Bäckhed, 2013). Our work focuses on the association between \textit{Drosophila melanogaster} and one of its natural commensal partners, \( \text{L.p.} \text{WJL} \). The findings of this study broaden our understanding of the relationship between these two partners and show that \( \text{L.p.} \text{WJL} \) association not only extends the lifespan of adult males.

A \(+/-\) \textit{L. plantarum}

\[ \text{Axenic eggs} \]

\[ \text{Larval development} \]

\[ \text{Emergence} \]

\[ \text{Adult life} \]

\[ \text{Low yeast diet} \]

\[ \text{Normal diet} \]

B

\[ \% \text{Survival} \]

\[ \text{Time (days)} \]

\[ \text{GF} \]

\[ \text{LP} \]

C

\[ \% \text{Survival} \]

\[ \text{Time (days)} \]

\[ \text{GF} \]

\[ \text{LP} \]

D

\[ \% \text{Survival} \]

\[ \text{Time (days)} \]

\[ P=0.0002 \]

\[ \text{Fig. 4.} \ \text{L.p.} \text{WJL increases the lifespan of nutritionally challenged males.} \]

The lifespan of adult males and females was assessed after larval development under low nutrition with or without \( \text{L.p.} \text{WJL} \), when flies were kept on either a normal diet (A,B) or the same low-yeast diet as the larvae (C,D).
others have shown that various microbial effects are strain specific (Storelli et al., 2011; Chaston et al., 2014). Nevertheless, taken together, all these observations suggest that adult Drosophila fitness traits might be influenced by the presence of the Gram-negative, acetic acid-producing Acetobacter species rather than by the Gram-positive, lactic acid-producing Lactobacillus species. However, based on the potent influence on larval systemic growth of Lactobacillus strains, we suspect that adult ‘growth’-related traits such as tissue regeneration (the intestine in particular) might be impacted by Lactobacillus.

Having ruled out a direct impact of L.p. WJL on adult fitness, we wanted to investigate the potential repercussions of the bacteria-mediated larval growth acceleration on adult flies. When larvae are raised on a low-yeast diet, the presence of L.p. WJL promotes their growth and shortens their developmental time (Storelli et al., 2011; Erkosar et al., 2015; this study). However, numerous studies have demonstrated that conditions impacting larval development are known to affect several adult traits in Drosophila and a shorter larval period could negatively trade-off with adult reproductive capacity, stress resistance or longevity (Zera and Harshman, 2001). We therefore suspected that this increased growth rate upon nutritional challenge could in turn adversely impact adult fitness.

Here, we demonstrate that L.p. WJL-associated individuals are as fit as their GF siblings; they show similar reproductive capacity and resist complete starvation equally well, regardless of their developmental history. The association with L.p. WJL is thus overall profitable to the fly, as it promotes larval growth and the early emergence of the imago without impairing the fitness of this mature and reproductive stage.

Strikingly, we found that L.p. WJL extends the lifespan of males kept in poor nutritive conditions. Males that were kept on a low-yeast diet throughout their entire life cycle benefited from the bacterial presence both as larvae and as adults; they displayed a shortened developmental time as well as an increased median lifespan compared with their GF siblings. Thus, L.p. WJL-associated males not only develop faster and emerge several days before their axenic counterparts but also survive longer. In the wild, where nutrients can be scarce, longer lifespan could grant these individuals more opportunities to mate, and to produce potentially more numerous progeny. However, to confirm this hypothesis, it is imperative to show that these early-emerged and long-lived males are superior in their healthspan. In this light, it might be of interest to assay the late-life reproductive capacity of L.p. WJL-associated versus GF flies to see whether, in addition to conferring the ability to live longer, L.p. WJL also allows males to stay fit and reproductively active longer. This is an interesting future direction to follow given the growing evidence supporting a role for the microbiota in the aging process (Heintz and Mair, 2014).

Altogether, our results reveal that L.p. WJL is overall beneficial for Drosophila melanogaster; the presence of these bacteria is profitable during larval life and does not harm the adult flies. Indeed, upon nutritional challenge, L.p. WJL allows the earlier emergence of fit and fertile adults and, in certain conditions, it even increases the lifespan of males. This Lactobacillus strain thus represents an advantageous partner for the fly, and taken together our results support the idea that commensal bacteria might be one of the factors contributing to the ecological success of Drosophila.

Competing interests
The authors declare no competing or financial interests.

Author contributions
F.L. supervised the work. M.A.T. and F.L. designed the experiments. M.A.T. performed the experiments. M.A.T. and F.L. analyzed the results. M.A.T. wrote the manuscript with input from F.L.

Funding
This work was funded by a European Research Council starting grant (FP7/2007-2013 N°309704). The lab is supported by the FINOVI foundation (Fondation Innovations en Infectiologie), the Fondation Schlumberger pour l’Education et la Recherche and the European Biology Organization (EMBO) Young Investigator Program. Deposited in PMC for immediate release.

Supplementary information
Supplementary information available online at http://jeb.biologists.org/lookup/doi/10.1242/jeb.151522.supplemental

References
Andersen, L. H., Kristensen, T. N., Loeschcke, V., Toft, S. and Mayznt, D. (2010). Protein and carbohydrate composition of larval food affects tolerance to thermal stress and desiccation in adult Drosophila melanogaster. J. Insect Physiol. 56, 336-340.
Baker, K. D. and Thummel, C. S. (2007). Diabetic larvae and obese flies - emerging studies in metabolism in Drosophila. Cell Metab. 6, 257-266.
Broderick, A. N. and Lemaître, B. (2012). Out-associated microbes of Drosophila melanogaster. Gut Microbes 3, 307-321.
Brummel, T., Ching, A., Seroude, L., Simon, A. F. and Benzer, S. (2004). Drosophila lifespan enhancement by exogenous bacteria. Proc. Natl. Acad. Sci. USA 101, 12974-12979.
Burns, J. G., Svetec, N., Rowe, L., Mery, F., Dolan, M. J., Boyce, W. T. and Sokolowski, M. B. (2012). Gene – environment interplay in Drosophila melanogaster: chronic food deprivation in early life affects adult exploratory and fitness traits. Proc. Natl. Acad. Sci. USA 109 Suppl. 2, 17239-17244.
Casadevall, A. and Pirofski, L.-A. (2000). Host-pathogen interactions: basic concepts of microbial commensalism, colonization, infection and disease. Infect. Immun. 68, 6511-6518.
Chaston, J. M., Newell, P. D. and Douglas, A. E. (2014). Metagenome-wide association of microbial determinants of host phenotype in Drosophila melanogaster. mBio 5.e01631-14.
Chaston, J. M., Dobson, A. J., Newell, P. D. and Douglas, A. E. (2016). Host genetic control of the microbiota mediates the Drosophila nutritional phenotype. Appl. Environ. Microbiol. 82, 671-679.
Chippindale, A. K., Leroi, A. M., Kim, S. B. and Rose, M. R. (1993). Phenotypic plasticity and selection in Drosophila life-history evolution. I. Nutrition and the cost of reproduction. J. Evol. Biol. 6, 171-193.
Clark, R. I., Salazar, A., Yamada, R., Fitz-Gibbon, S., Morselli, M., Alcaraz, J., Rana, A., Rera, M., Pellegrini, M., Ja, W. W. et al. (2015). Distinct shifts in microbiota composition during Drosophila aging impair intestinal function and drive mortality. Cell Rep. 12, 1656-1667.
Elgart, M., Stern, S., Salton, O., Gnaiksys, Y., Heifetz, Y. and Soen, Y. (2016). Impact of gut microbiota on the fly’s germ line. Nat. Commun. 7, 11280.
Erkosar, B., Defaye, A., Bozneton, N., Puthier, D., Royet, J. and Leuiller, F. (2014). Drosophila microbiota modulates host metabolic gene expression via IMD/NF-κB signaling. PLoS ONE 9, e94729.
Erkosar, B., Storelli, G., Mitchell, M., Bozneton, L., Bozneton and Leuiller, F. (2015). Pathogen virulence impedes mutualist-mediated enhancement of host juvenile growth via inhibition of protein digestion. Cell Host Microbe 18, 445-455.
Gilbert, S. F. (2001). Ecological developmental biology: developmental biology meets the real world. Dev. Biol. 233, 1-12.
Guo, L., Karpac, J., Tran, S. L. and Jasper, H. (2014). PGRP-SC2 promotes gut immune homeostasis to limit commensal dysbiosis and extend lifespan. Cell 156, 109-122.
Hatzis, C. and Mair, W. (2014). You are what you host: microbiome modulation of the aging process. Cell 156, 408-411.
Hentschel, U., Steiner, M. and Hacker, J. (2000). Common molecular mechanisms of symbiosis and pathogenesis. Trends Microbiol. 8, 226-231.
Hedin, J. and Riddiford, L. M. (2000). Different mechanisms underlie phenotypic plasticity and interspecific variation for a reproductive character in Drosophilids (Insecta: Diptera). Evolution 54, 1638-1653.
Huang, J.-H. and Douglas, A. E. (2015). Consumption of dietary sugar by gut bacteria determines Drosophila lipid content. Biol. Lett. 11, 20150469.
Kostic, A. D., Howitt, M. R. and Garrett, W. S. (2013). Exploring host-microbiota interactions in animal models and humans. Genes Dev. 27, 701-718.
Larouche, W. and Berry, P. (2012). How microbiomes influence metazoan development: insights from history and Drosophila modeling of Gut-microbe interactions. Annu. Rev. Cell Dev. Biol. 29, 1, 130628183743001.
Lee, W.-J. and Hase, K. (2014). Gut microbiota–generated metabolites in animal health and disease. Nat. Chem. Biol. 10, 416-424.

Ma, D., Leulier, F., Storelli, G. and Mitchell, M. (2015). Studying host-microbiota mutualism in Drosophila: harnessing the power of gnotobiotic flies. Biomed. J. 38, 285-293.

Mcfall-Ngai, M. J. (2002). Unseen forces: the influence of bacteria on animal development. Dev. Biol. 242, 1-14.

Mcfall-Ngai, M. J. (2015). Giving microbes their due - animal life in a microbially dominant world. J. Exp. Biol. 218, 1968-1973.

Newell, P. D. and Douglas, A. E. (2014). Interspecies interactions determine the impact of the gut microbiota on nutrient allocation in Drosophila melanogaster. Appl. Environ. Microbiol. 80, 788-796.

Nylin, S. and Gotthard, K. (1998). Plasticity in life-history traits. Annu. Rev. Entomol. 43, 63-83.

Petkau, K., Parsons, B. D., Duggal, A. and Foley, E. (2014). A deregulated intestinal cell cycle program disrupts tissue homeostasis without affecting longevity in Drosophila. J. Biol. Chem. 289, 28719-28729.

Piper, M. D., Blanc, E., Leita̧o-Gonçalves, R., Yang, M., He, X., Linford, N. J., Hoddinott, M. P., Hopfen, C., Soultoukis, G. A., Niemeyer, C. et al. (2013). A holidic medium for Drosophila melanogaster. Nat. Methods 11, 100-105.

Rose, M. R. (1984). Laboratory evolution of postponed senescence in Drosophila melanogaster. Evolution 38, 1004-1010.

Ryu, J.-H., Kim, S.-H., Lee, H.-Y., Bai, J. Y., Nam, Y.-D., Bae, J.-W., Lee, D. G., Shin, S. C., Ha, E.-M. and Lee, W.-J. (2008). Innate immune homeostasis by the homeobox gene caudal and commensal-gut mutualism in Drosophila. Science 319, 777-782.

Sharon, G., Segal, D., Ringo, J. M., Hefetz, A., Zilber-Rosenberg, I. and Rosenberg, E. (2010). Commensal bacteria play a role in mating preference of Drosophila melanogaster. Proc. Natl. Acad. Sci. USA 107, 20051-20056.

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

Sisodia, S. and Singh, B. N. (2012). Experimental evidence for nutrition regulated stress resistance in Drosophila ananassae. PLoS ONE 7, e46131.

Sommer, F. and Blöckh, F. (2013). The gut microbiota - masters of host development and physiology. Nat. Rev. Microbiol. 11, 227-238.

Staubach, F., Baines, J. F., Künzel, S., Bik, E. M. and Petrov, D. A. (2013). Host species and environmental effects on bacterial communities associated with Drosophila in the laboratory and in the natural environment C. Quince, ed. PLoS ONE 8, e70749.

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

Tu, M.-P. and Tatar, M. (2003). Juvenile diet restriction and the aging and reproduction of adult Drosophila melanogaster. Aging Cell 2, 327-333.

Venu, I., Durisko, Z., Xu, J. and Dukas, R. (2014). Social attraction mediated by fruit flies’ microbiome. J. Exp. Biol. 217, 1346-1352.

Wong, A. C.-N., Dobson, A. J. and Douglas, A. E. (2014). Gut microbiota dictates the metabolic response of Drosophila to diet. J. Exp. Biol. 217, 1894-1901.

Zera, A. J. and Harshman, L. G. (2001). The physiology of life history trade-offs in animals. Annu. Rev. Ecol. Syst. 32, 95-126.