

1 **Title:**

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

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5

6 **Running title:** Impact of *L.p.*^{WJL} on *Drosophila* adult fitness

7 (39 characters)

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20 **Keywords:** *Drosophila* – symbiosis – fertility – fitness – lifespan

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25 **Summary statement**

26 *Lactobacillus plantarum*^{WJL} is beneficial to *Drosophila* physiology along its entire life cycle.

27 This bacteria triggers the early emergence and longer survival of fit and fertile adults.

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29 (26 words)

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36 **Abstract**

37 Animals are naturally surrounded by a variety of microorganisms with which they constantly
38 interact. Among these microbes, some live closely associated with a host and form its
39 microbiota. These communities are now extensively studied, owing to their contributions to
40 shaping various aspects of animal physiology. One of these commensal species,
41 *Lactobacillus plantarum*, and in particular the *L.p.*^{WJL} strain, has been shown to promote the
42 growth of *Drosophila* larvae upon nutrient scarcity, allowing earlier metamorphosis and adult
43 emergence compared to axenic individuals. As for many insects, conditions surrounding the
44 post-embryonic development dictate key *Drosophila* adult life history traits, and adjusting
45 developmental timing according to the environment is essential for adult fitness. The growth
46 acceleration induced by *L.p.*^{WJL} occurs in a context of poor nutrition and we wondered if this
47 could adversely impact the fitness of *Drosophila* adults. Here we show that the *L.p.*^{WJL}-
48 mediated acceleration of growth is not deleterious; adults emerging after an accelerated
49 development are as fit as their axenic siblings. Additionally, *L.p.*^{WJL}'s presence even leads to
50 a lifespan extension in nutritionally challenged males. These results demonstrate that *L.p.*^{WJL}
51 is a beneficial partner for *Drosophila melanogaster* through its entire life cycle. This
52 commensal bacteria allows the earlier emergence and longer survival of fit and fertile
53 individuals and might represent one of the factors contributing to the ecological success of
54 *Drosophila*.

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56 (220 words)

57 **Introduction**

58

59 In nature, animals are constantly surrounded by a profusion of microorganisms, whose
60 presence has contributed to shaping life as we know it (McFall-Ngai 2015). The interactions
61 existing between microbes and animals cover a broad spectrum, with outcomes ranging
62 from obligate symbiosis to lethal infection (Casadevall & Pirofski 2000; Hentschel et al.
63 2000). Among these microbial species, some live closely associated with an animal host
64 with which they establish commensalistic or mutualistic relationships. The community they
65 form is referred to as the microbiota, which over the last years has been increasingly studied
66 for its impact on various physiological traits. Indeed, in several mammalian, nematode or
67 arthropod models, the microbiota has been shown to shape development, immunity,
68 metabolism and even behavior (Kostic et al. 2013; Lee & Hase 2014). In this fast expanding
69 research field, *Drosophila* has been a fruitful model. Thanks to its ease of manipulation and
70 genetic tractability, as well as the low complexity of its microbiota, the fruit fly represents a
71 powerful tool to delve into the mechanistic underpinnings of host-microbiota interactions
72 (Lee & Brey 2012; Ma et al. 2015). Studies revealed that microbiota's presence and
73 composition impact various traits throughout *Drosophila* life cycle such as larval growth,
74 developmental timing, stress resistance, immune response, metabolism, lifespan and
75 behavior (Brummel et al. 2004; Ryu et al. 2008; Sharon et al. 2010; Shin et al. 2011; Guo et
76 al. 2014; Petkau et al. 2014; Venu et al. 2014; Wong et al. 2014; Clark et al. 2015). As the
77 microbiota is closely associated to its animal partner and, in the case of *Drosophila*, is an
78 integral part of its nutritive substrate, it is not surprising to see its influence on so many
79 biological functions. Moreover, as for many insects the larval life is a highly plastic stage in
80 the fly life cycle. Indeed, biotic and abiotic factors surrounding the development of an
81 organism participate in shaping this process (Gilbert 2001; McFall-Ngai 2002), and in turn
82 have a crucial impact on several key life history traits at the adult stage, such as
83 reproductive capacities, stress resistance or lifespan (Tu & Tatar 2003; Andersen et al.
84 2010; Sisodia & Singh 2012; Burns et al. 2012).

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

93 Several studies using *Drosophila* lines generated in a laboratory evolution experiment of
94 postponed senescence selection (Rose 1984) have described a series of trade-offs between
95 key life history traits. This occurs when the optimization of a trait correlates with a negative
96 impact on another parameter; for example increased reproductive capacities usually come at
97 the cost of shortened lifespan. Such trade-offs can involve traits from either the same life
98 stage or across different life stages, and thus the length of the larval period, early and late
99 life fecundity, adult longevity as well as stress resistance were shown to trade-off with one
100 another (reviewed in Zera & Harshman 2001). Given the numerous examples of life history
101 trade-offs and the rather striking effect of *L.p.*^{WJL} on larval development, we wondered about
102 the potential repercussions of this accelerated growth on adult fitness. We speculated that
103 *L.p.*^{WJL}-mediated acceleration of growth in an otherwise nutritionally challenging environment
104 might be deleterious at later stages such that it would lead to the emergence of unfit adults.
105 To address this question, we assessed several fitness parameters in young adult flies and
106 observed that overall, *L.p.*^{WJL}-association was not detrimental for adult fitness. Furthermore,
107 for adult males it proves to be an advantageous partner; *L.p.*^{WJL}-associated males are not
108 only emerging several days before their germ-free siblings, they also survive longer in
109 nutritionally challenging conditions. *L.p.*^{WJL} is thus a true beneficial partner for *Drosophila*
110 along its entire life cycle, and even more so in a poor nutritional environment. We therefore
111 propose that bacterial members of the fly microbiota might represent one of the factors
112 contributing to the ecological success of *Drosophila melanogaster*.

113

114 **Material & Methods**

115

116 **Fly stocks and husbandry**

117 Wolbachia-free fly stocks (*yw*) were reared on a standard yeast/cornmeal diet containing for
118 1L: 50g inactivated yeast (Bio Springer, Springaline BA95/0-PW), 80g cornmeal (Westhove,
119 Farigel maize H1), 10g agar (VWR, ref. #20768.361), 5,2g methylparaben sodium salt
120 (MERCK, ref. #106756) and 4ml 99% propionic acid (CARLO ERBA, cref. #409553). All
121 experimental flies were kept in incubators at 25°C, with a 12h/12h light/dark cycle. The low-
122 yeast diets were made by decreasing the quantity of yeast to either 30, 12, 8 or 6g/L and the
123 quantity of agar to 7,2g/L. Unless stated otherwise, only mated flies were used in this study.

124

125 **Generation of axenic *Drosophila* stocks and bacterial mono-association**

126 To generate axenic flies, eggs were collected overnight and treated in sterile conditions with
127 successive 2 minutes baths of bleach and 70% ethanol. Bleached embryos were then rinsed
128 in sterile water for another 2 minutes and placed on sterile standard food supplemented with
129 an antibiotic cocktail (50µg ampicillin, 50µg kanamycin, 50µg tetracyclin and 15µg
130 erythromycin per liter of fly food). Emerging adults were tested for axenicity by crushing and
131 plating of the fly lysate on different bacterial culture media. Germ-free flies were kept on
132 antibiotic food for a few generations and conventionally reared stocks were used to
133 regenerate axenic stocks regularly. For bacterial mono-association, 50µL of PBS containing
134 10⁸ CFU of a stationary phase culture of *Lactobacillus plantarum*^{WJL} were used to inoculate
135 the surface of the food contained in a Ø1,5cm fly tube. 50 axenic eggs from an overnight
136 collection were transferred onto the inoculated food and left to develop until adult
137 emergence. The experimental germ-free condition was obtained by inoculating the food with
138 sterile PBS. In case of association at the adult stage, fly food was inoculated as described
139 above and let to dry under a hood. Forty to fifty newly emerged adult flies (females and
140 males mixed 1:1) were then transferred into the inoculated tubes and reared for 7 days until
141 the beginning of the experiments.

142

143 **Developmental timing**

144 Fifty germ-free embryos were associated with *Lactobacillus plantarum*^{WJL} or kept axenic, as
145 described in the previous section. Larvae were then left to develop under low nutrient
146 conditions (low yeast diet, 8g/L yeast) and the number of pupae appearing each day was
147 recorded until the last larvae of the population reached pupariation.

148

149 **Fecundity and fertility assessment**

150 At emergence, groups of 5 females plus 5 males were distributed in vials and flipped every

151 24h in a new tube. The number of eggs laid was recorded every day for 10 days and the
152 subsequent number of emerging adults was used to calculate the fertility ratio (number of
153 emerging progeny/number of eggs laid). In experiments where bacterial association was
154 done only at adult stage, the fecundity/fertility assays were started at day 7 or day 10 after
155 adult emergence and carried on for 3 days to 7 days.

156

157 **Number of ovarioles**

158 4 to 5 days old mated females were used to assess the number of ovarioles after
159 development on either standard (50g/L yeast) or low-yeast (8g/L yeast) diet. Newly emerged
160 adult flies were kept on standard food until the time of dissection. Ovaries were dissected in
161 cold PBS and directly fixed in 4% formaldehyde for 20 minutes. They were then stained with
162 DAPI (1:1000) for 15 minutes and transferred to 80% glycerol for preservation. After fixation
163 and staining, ovarioles were teased apart under a dissecting microscope and mounted on
164 slides to be counted.

165

166 **Adult wet weight and resistance to full starvation**

167 Either virgin (0 to 7 hours old) or mature and mated adults (7 or 10 days old) were collected
168 and pooled by 5 to be weighted on a Sartorius analytical balance CPA324S (Sartorius
169 Weighing Technology GmbH, Goettingen, Germany). Flies of the same ages were also used
170 for full starvation assays, in tubes providing only water supply to the flies. Specifically, the
171 starvation tubes contain a cotton ball soaked into a water reservoir to prevent them from
172 drying. The cotton is covered with a piece of Whatmann paper on which the flies will be.
173 Survival of the flies was recorded twice a day until all individuals were dead.

174

175 **Lifespan**

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

182

183

184 **Statistical analyses**

185 For comparison of GF and *L.p.*^{WJL}-associated conditions Mann-Whitney test (for weight,
186 fecundity, fertility) and logrank test (for survival curves comparison) were performed using
187 GraphPad Prism software version 6.0f for Macintosh (GraphPad Software, La Jolla

188 California USA, www.graphpad.com). Whiskers of the boxplots represent the minimal to
189 maximal values. For all experiments, the p-values were reported on the corresponding figure
190 panels only when inferior to 0,05.
191

192 **Results**

193

194 ***Lactobacillus plantarum*^{WJL} does not directly impact *Drosophila* adult fitness**

195 To determine whether *L.p.*^{WJL} had an impact on the fly physiology at the adult stage, we first
196 assessed the direct effect of *L.p.*^{WJL} on adult *Drosophila*, by associating newly emerged flies
197 with the bacteria. After a larval development on a normal diet in axenic conditions (germ-free
198 (GF), devoid of microbiota), the young emerging adults were either associated with *L.p.*^{WJL} or
199 kept axenic (**Figure 1A**). The flies were left to mature for several days on diets with
200 decreasing amounts of yeast and were then tested for fecundity, fertility and resistance to
201 full starvation. After 8 days in various nutritive conditions, there was a clear effect of the diet
202 composition on the number of eggs laid per female and on the number of adult progeny
203 emerging from these eggs; with decreasing amount of yeast in the diet, the flies were laying
204 fewer eggs (**Figure 1B**) and the fertility ratio (number of emerging progeny/number of eggs
205 laid) showed an increased variability (**Figure 1C**). The ability of females to endure complete
206 starvation was also impacted by the amount of yeast in the diet. Indeed, 7 days old females
207 survived longer when they had been kept on a low-yeast diet after emergence (**Figure 1D**
208 **left panel**). In contrast, the diet composition did not matter for their male counterparts, who
209 were always dying at the same rate regardless of the diet they were kept on since
210 emergence (**Figure 1D right panel**). The association with *L.p.*^{WJL} however did not impact
211 any of these adult fitness traits. In addition, we tested the same parameters in flies that were
212 raised on a normal diet in the presence of *L.p.*^{WJL} during larval life. In such optimal nutritional
213 conditions, the developmental time is similar for the axenic and the *L.p.*^{WJL}-associated flies,
214 and here again there was a clear impact of the diet composition on fecundity, but no
215 bacterial contribution was revealed for either fecundity or resistance to full starvation (**Figure**
216 **S1**). We next assayed the lifespan of these flies raised with or without *L.p.*^{WJL} on a normal
217 diet, and kept as adult on either the same optimal diet or on low-yeast food (**Figure 1E,F**).
218 Here, we saw a significant increase in the lifespan of axenic females kept in nutritionally rich
219 conditions throughout all their life cycle (**Figure 1G left panel**). For their male counterparts
220 however, as well as for female and male flies that went from a larval development on a
221 normal diet, to adult life on a low-yeast diet, there was no significant impact of *L.p.*^{WJL}
222 presence (**Figure 1G right panel and 1H**). Taken together, these results show that apart
223 from the previously described sexually dimorphic lifespan shift on a normal diet (i.e.
224 increased lifespan in GF females; (Petkau et al. 2014; Clark et al. 2015)), association of
225 *Drosophila* with *L.p.*^{WJL} does not seem to have a direct impact on the adult fitness when flies
226 develop on a normal diet.

227 **The *Lactobacillus plantarum*^{WJL}-mediated larval growth acceleration is not deleterious**
228 **for adult fitness**

229 While searching for a direct effect of *L.p.*^{WJL} on the adult stage, we did not detect any
230 significant impact of this commensal bacteria on the tested fitness parameters. There is
231 however a quite striking larval effect, as nutritionally challenged individuals develop faster
232 and pupariate several days earlier when they are associated with *L.p.*^{WJL} compared to the
233 axenic ones (Storelli et al. 2011; Erkosar et al. 2015). While faster larval growth and
234 precocious emergence of the adult represent an obvious ecological advantage, doing so
235 under nutritionally challenging conditions may in turn be deleterious for the adult fitness and
236 reproductive success. Indeed, adjusting developmental timing to environmental cues is key
237 to *Drosophila* adult fitness (Nylin & Gotthard 1998), yet upon *L.p.*^{WJL}-association animals
238 develop faster even though the nutritional conditions are poor. To investigate whether the
239 growth acceleration mediated by *L.p.*^{WJL} upon nutrient scarcity would adversely impact
240 subsequent adult fitness, we tested flies raised on a low yeast diet with or without the
241 bacteria, as depicted in **Figure 2A**. As previously described, when raised on a low yeast diet
242 larvae associated with *L.p.*^{WJL} pupariate several days before their axenic siblings (Storelli et
243 al. 2011; Erkosar et al. 2015) and **Figure 2B**). We then assessed the potential
244 repercussions of the *L.p.*^{WJL}-association on the reproductive capacities of flies that
245 underwent larval development in such nutritionally challenging conditions. Similar to what we
246 observed when the flies were grown in optimal conditions and challenged only as adults,
247 fecundity (**Figure 2F,G,H**) and fertility (**Figure 2I,J,K**) were both greatly impacted by the
248 adult diet composition (**Figure 2C, D, E**). The higher the yeast content in the diet, the more
249 eggs were laid per female per day (**Figure 2F,G,H**). In addition, the number of adult progeny
250 emerging from these eggs was impaired on the lower yeast diet. Indeed, as we observed on
251 the low-yeast diet in **Figure 1C** (6g/L of yeast), on the 8g/L of yeast diet the fertility ratio was
252 greatly variable (**Figure 2I,J,K**). On these two parameters again, there was no impact of the
253 association with *L.p.*^{WJL}. Furthermore, these comparable fecundity results were supported by
254 the fact that the number of ovarioles (the functional units of *Drosophila* ovaries) of females
255 raised on a low yeast diet was similar, regardless of their microbiota status (**Figure S2A**).
256 We are confident that our experimental setup can efficiently manipulate the ovariole number,
257 since as expected, we observed a decreased count after a development on a low-yeast diet
258 compared to an optimal situation (**Figure S2A** and Hodin & Riddiford 2000; Tu & Tatar
259 2003). As anticipated, this similar number of ovarioles between the GF and *L.p.*^{WJL}
260 conditions translated into a similar cumulative number of eggs laid over the course of the
261 experiment, and as expected we detected reduced cumulative egg laying on the poor diet
262 (**Figure S2B**). Next, we assayed the weight of 0 to 7 hours old virgin adults, along with their
263 resistance to complete starvation, as readouts for direct consequences of larval life on their
264 adult metabolic state (Baker & Thummel 2007). We detected a slight tendency in males and
265 females associated with *L.p.*^{WJL} to weigh less than the axenic ones (**Figure 3A, B**), but there

266 was no impact of the growth acceleration mediated by *L.p.*^{WJL} on the flies' ability to endure
267 full starvation (**Figure 3C**). These assays were repeated on mature adults, after 10 days of
268 adult life on either a normal diet (**Figure 3D,E,F**) or on the same low-yeast diet (**Figure**
269 **3G,H,I**) and again, there was no deleterious impact of the *L.p.*^{WJL}-mediated growth
270 promotion on these adult fitness parameters. At this age, the weight tendency was reversed,
271 since *L.p.*^{WJL}-associated males and females were now slightly heavier than their axenic
272 counterparts. Similarly to what we observed with newly emerged flies, this did not translate
273 into differences in resistance to full starvation. In addition, similar results were obtained
274 when we tested these parameters in adult flies that were matured on a diet with an
275 intermediate yeast content (**Figure S3**). Notably, for some of these experiments, the
276 statistical analyses show significant differences between the groups, but the differences they
277 represent are tenuous and probably not of any biological relevance. Collectively, these data
278 suggest that even though larvae associated with *L.p.*^{WJL} develop faster in an otherwise poor
279 nutritive environment, they do so without generating fitness cost for the later stage and give
280 rise to fit and fertile adults.

281

282 ***Lactobacillus plantarum*^{WJL} increases the lifespan of nutritionally challenged males**

283 While performing the experiments, we noticed that when kept on a low-yeast diet, adult
284 males were dying rapidly and a significant proportion of them were already dead 10 days
285 after emergence. We then decided to study more in details the lifespan of flies raised in such
286 nutritionally poor conditions. After emergence from larval development on a low-yeast diet,
287 the adults were either kept on the same low-yeast diet (**Figure 4C**), or transferred to an
288 optimal diet (**Figure 4A**). We saw that, while the association with *L.p.*^{WJL} did not impact the
289 lifespan of adults kept on a normal diet (**Figure 4B**), males maintained on a low-yeast diet
290 throughout their entire life survived better when they were associated with *L.p.*^{WJL} (**Figure**
291 **4D right panel and Figure S4**). Notably, their median lifespan was extended by 4 to 16
292 days, depending on the experiment. This fluctuation in the actual day count across
293 experiments is commonly seen in lifespan studies (Blanc et al. 2013) but the trend persisted
294 and was statistically significant. This result shows that in a nutritionally challenging
295 environment, *L.p.*^{WJL}-association not only shortens *Drosophila* developmental time, it also
296 increases significantly the lifespan of adult males.

297 **Discussion**

298 The microbiota is one of the key environmental factors impacting animal development and
299 physiology and has been increasingly studied over the last few years (Sommer & Bäckhed
300 2013). Our work focuses on the association between *Drosophila melanogaster* and one of its
301 natural commensal partners, *Lactobacillus plantarum*^{WJL}. In this study we broaden our
302 understanding of the relationship between these two partners and show that *L.p.*^{WJL} is
303 beneficial for the fly throughout all life stages. We first tested if *L.p.*^{WJL} had a direct impact on
304 adult fitness traits, by associating newly emerging flies after a larval development in axenic
305 conditions. We show that, in our setup, the bacterial presence is completely dispensable for
306 the adult fitness; the flies lay the same amount of eggs and resist starvation equally well,
307 whether they are mono-associated or not. Notably, and contrary to *L.p.*^{WJL}, the composition
308 of the diet markedly impacts these parameters, and we observe a negative correlation
309 between starvation resistance and egg laying. When decreasing the amount of yeast in the
310 diet, we see an extension of survival upon complete starvation for female flies, together with
311 a drop in the number of eggs laid. This effect is not only microbiota-independent but also
312 sex-specific and the starvation resistance of males was not impacted by the quantity of yeast
313 in the diet. Similar observations were previously reported in a study by Chippindale and
314 colleagues, where starvation resistance is promoted by lower yeast levels in the diet, at the
315 expense of fecundity (Chippindale et al. 1993). As in the present study, this effect was
316 restricted to females, a characteristic that the authors attributed to distinct lipid requirements
317 between the sexes.

318 In a previous study, we compared the transcriptomes of germ-free versus poly-associated
319 flies that had been inoculated at adult stage with a cocktail of four bacterial species selected
320 to represent the main commensals of *Drosophila* (*Acetobacter pomorum*, *Commensalibacter*
321 *intestini*, *Lactobacillus brevis* and *Lactobacillus plantarum*). This analysis revealed a
322 differential expression of several genes pertaining to metabolic processes; out of 105
323 transcripts upregulated upon bacterial poly-association, 74 were metabolism-related
324 (Erkosar et al. 2014). With such a differential expression of metabolic genes, one could
325 expect that certain fitness parameters, like reproductive capacities or starvation resistance,
326 would be affected. The fact that our present study revealed no differences between germ-
327 free and *L.p.*^{WJL}-associated animals for these traits might be attributed to the association set-
328 up. In this study, the flies are mono-associated with one species of *Lactobacillus* while in
329 Erkosar et al. the animals were poly-associated. Moreover, the bacterial cocktail used in
330 Erkosar et al. contained a species belonging to the *Acetobacter* genus. *Lactobacillaceae*
331 and *Acetobacteraceae* bacteria are the most represented in the communities associated
332 with *Drosophila* populations, in laboratory stocks as well as in wild-caught flies (Broderick &
333 Lemaitre 2012; Staubach et al. 2013; Chaston et al. 2016). Several studies have shown the

334 impact of *Acetobacter* species, notably *A. pomorum* and *A. tropicalis*, on the metabolism of
335 adult *Drosophila*, both upon mono-association with one species or in bacterial mixture
336 (Newell & Douglas 2014; Huang & Douglas 2015; Chaston et al. 2016; Elgart et al. 2016).
337 Furthermore, these studies were also specifically addressing the differential impact of
338 *Acetobacter* species versus *Lactobacillus* species and demonstrated that, in their setup, the
339 later had little to no effect in comparison to the former (Newell & Douglas 2014; Huang &
340 Douglas 2015; Chaston et al. 2016; Elgart et al. 2016). It must be pointed out however, that
341 beyond the distinction between bacterial species, the strain considered is important. Indeed,
342 our lab and others have shown that various microbial effects are strain-specific (Storelli et al.
343 2011; Chaston et al. 2014). Nevertheless, taken together all these observations suggest that
344 adult *Drosophila* fitness traits might be influenced by the presence of *Acetobacter* species
345 rather than *Lactobacilli*.

346 After having ruled out a direct impact of *L.p.*^{WJL} on adult fitness, we wanted to investigate the
347 potential repercussions of the bacteria-mediated larval growth acceleration on the adult flies.
348 When larvae are raised on a low-yeast diet, the presence of *L.p.*^{WJL} promotes their growth
349 and shortens their developmental timing (Storelli et al. 2011; Erkosar et al. 2015 and this
350 study). However, numerous studies have demonstrated that conditions impacting larval
351 development are known to affect several adult traits in *Drosophila* and a shorter larval period
352 could negatively trade-off with adult reproductive capacities, stress resistance or longevity
353 (Zera & Harshman 2001). We therefore suspected that this increased growth rate upon
354 nutritional challenge could in turn adversely impact adult fitness. Here, we demonstrate that
355 *L.p.*^{WJL}-associated individuals are as fit as their germ-free siblings; they show similar
356 reproductive capacities and resist complete starvation equally well, regardless of their
357 developmental history. The association with *L.p.*^{WJL} is thus overall profitable to the fly, since
358 it promotes larval growth and the early emergence of the imago without impairing the fitness
359 of this mature and reproductive stage.

360 Strikingly, we find that *L.p.*^{WJL} extends the lifespan of males kept on poor nutritive conditions.
361 Males that have been kept on a low-yeast diet throughout their entire life cycle benefit from
362 the bacterial presence both as larvae and as adults; they displayed a shortened
363 developmental timing as well as an increased median lifespan compared to their germ-free
364 siblings. Thus, *L.p.*^{WJL}-associated males are not only developing faster and emerging several
365 days before their axenic counterparts, they also survive longer. In the wild, where nutrients
366 can be scarce, longer lifespan could grant these individuals with more opportunities to mate,
367 and to produce potentially more numerous progeny. However, to confirm this hypothesis, it
368 is imperative to prove that these early-emerged and long-lived males are superior in their
369 healthspan. In this light, it might be of interest to assay the late-life reproductive capacities of
370 *L.p.*^{WJL}-associated versus germ-free flies and see if, in addition to confer them the ability to

371 live longer, *L.p.*^{WJL} also allows males to stay fit and reproductively active longer. This is an
372 interesting future direction to follow given the growing evidences supporting a role of the
373 microbiota in the aging process (Heintz & Mair 2014).

374 Overall, our results reveal that *L.p.*^{WJL} is beneficial for *Drosophila melanogaster* all along the
375 fly life cycle. Indeed, upon nutritional challenge the bacterial presence allows the earlier
376 emergence of fit and fertile adults and, in certain conditions, it even increases the lifespan of
377 males. This *Lactobacillus* strain thus represents an advantageous partner for the fly, and
378 taken together our results indicate that commensal bacteria might be one of the factors
379 contributing to the ecological success of *Drosophila*.

380

381

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387 proofreading of the manuscript.

388

389 **Competing interests**

390 The authors declare no conflict of interests.

391

392 **Authors contribution**

393 FL supervised the work. MT and FL designed the experiments. MT performed the
394 experiments. MT and FL analyzed the results. MT wrote the manuscript with inputs from FL.

395

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400

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499 **Figures legends**

500 **Figure 1. *Lactobacillus plantarum*^{WJL} does not directly impact *Drosophila* adult**
501 **fitness.** Right after emergence, axenic (germ-free (GF); devoid of microbiota) adults
502 developed on a normal diet are associated with *L.p.*^{WJL} or sterile PBS (A). When mature,
503 they are tested for fecundity (B) or fertility (C) at 8 days old, and for resistance to complete
504 starvation (D) at 10 days after emergence. In panels E to H, axenic eggs were inoculated
505 with *L.p.*^{WJL} or sterile PBS and developed on a normal diet (50g/L). The lifespan of the adults
506 was then assessed, on either the same normal diet, or on a diet with a reduced amount of
507 yeast (8g/L).

508

509

510 **Figure 2. *Drosophila* reproductive capacities are not altered after an accelerated larval**
511 **development.** When larvae are raised on a low yeast diet (8g/L) (A) the presence of *L.p.*^{WJL}
512 accelerates development and shortens the time to pupariation (B, days AED: after egg
513 deposition). After this differential development adults were kept on either an optimal diet
514 (50g yeast/L, C), an intermediate diet (30g yeast/L, D) or a nutritionally poor diet (8g yeast/L,
515 E) and assessed for fecundity and fertility from day 2 after adult emergence (AAE) to day 10.
516 Panels F, G and H show the number of eggs laid per female per day and the corresponding
517 fertility ratios (emerging adult progeny divided by the number of eggs laid) are shown in
518 panels I, J and K.

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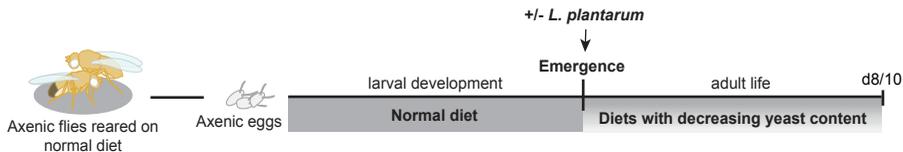
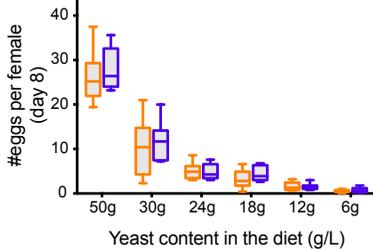
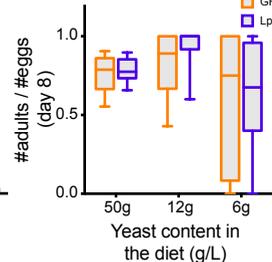
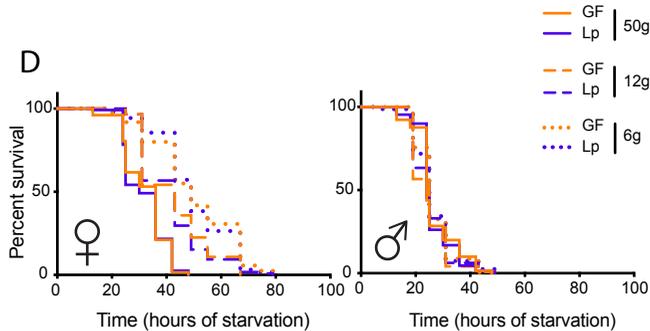
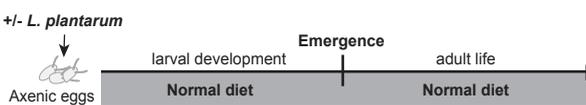
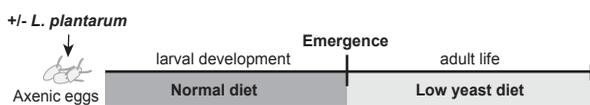
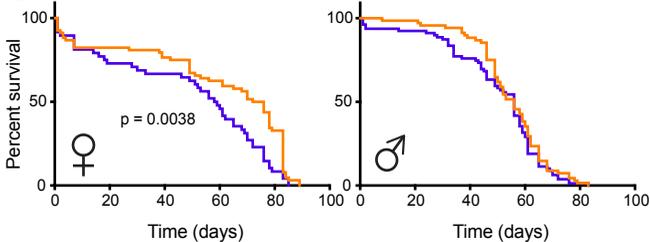
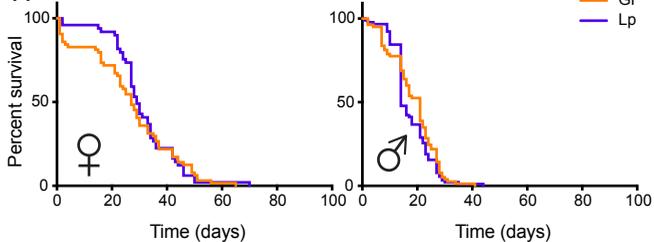
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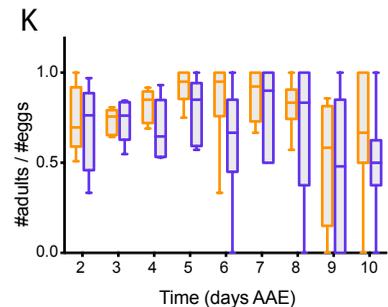
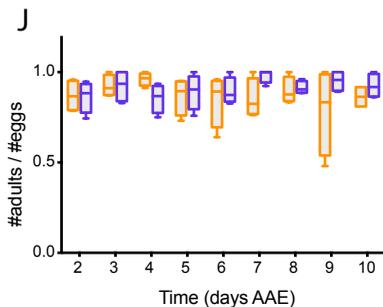
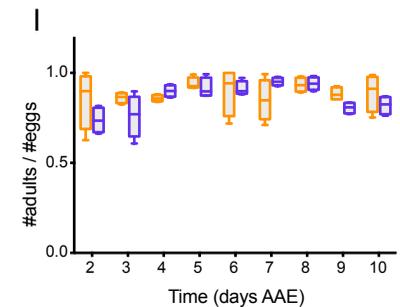
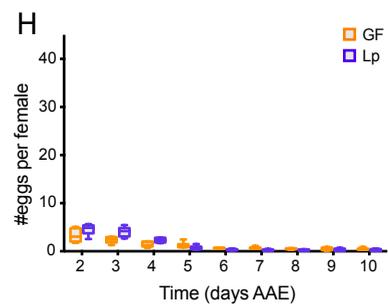
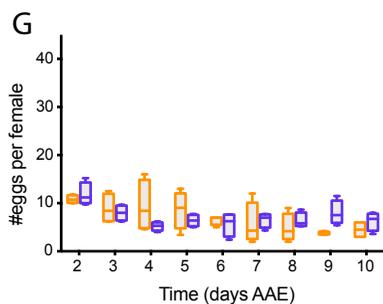
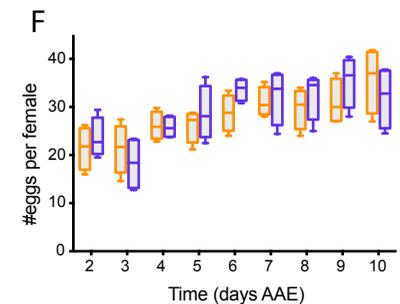
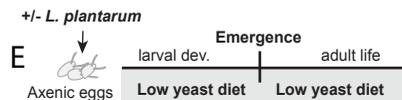
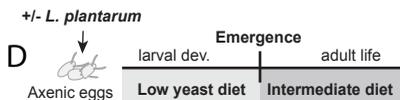
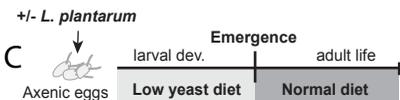
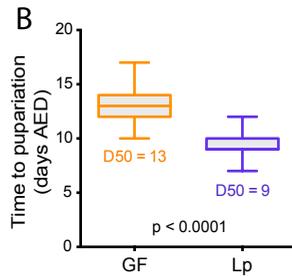
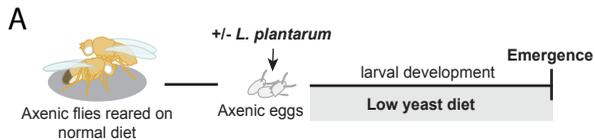
521 **Figure 3. The *Lactobacillus plantarum*^{WJL}-mediated larval growth acceleration is not**
522 **deleterious for adult fitness.** Right after emergence from larval development on a low-
523 yeast diet (A), assessment of 0 to 7 hours old flies' weight (B) and resistance to full
524 starvation (C). The same parameters were then tested on 10 days old adults kept on either a
525 normal diet (D-F) or on a low yeast diet (G-I).

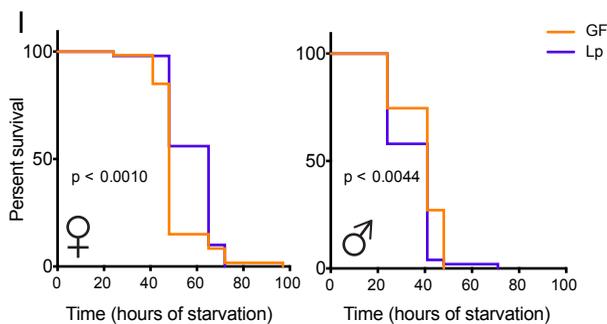
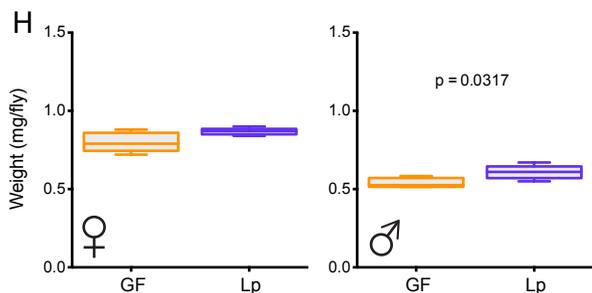
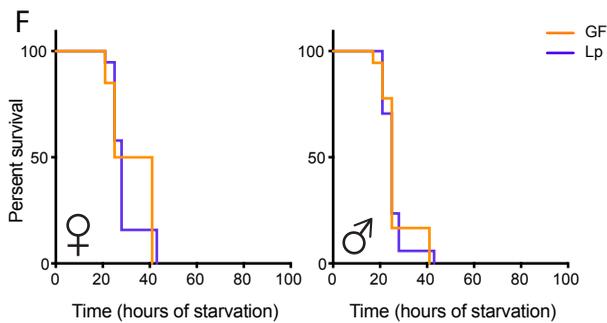
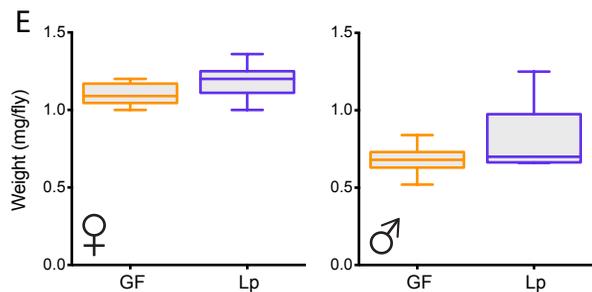
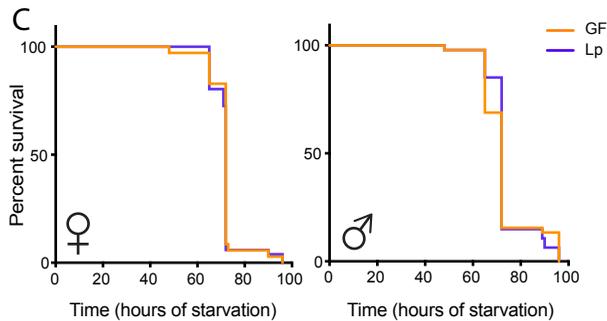
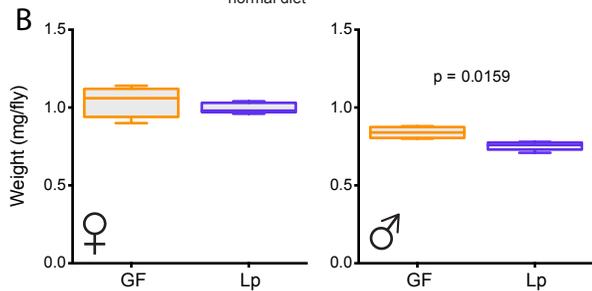
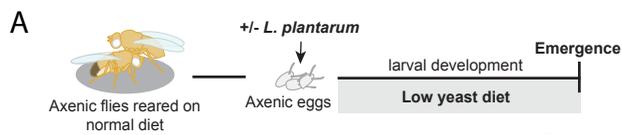
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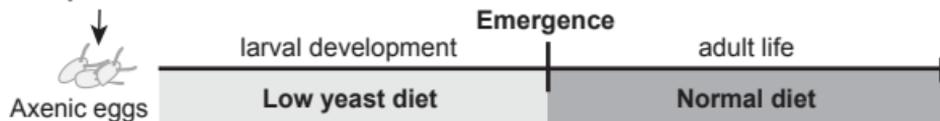
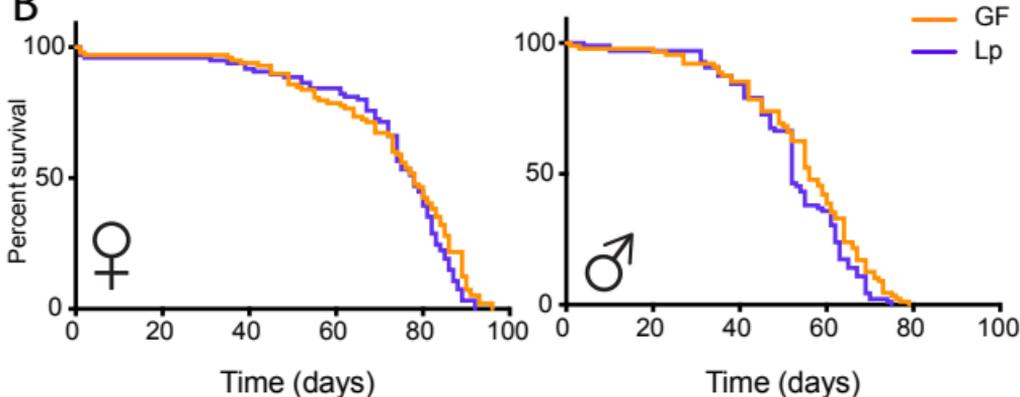
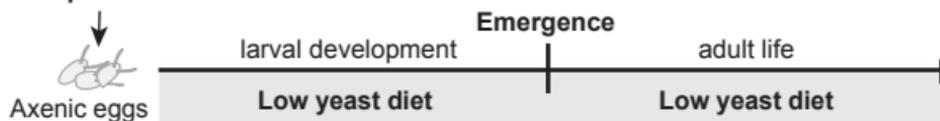
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528 **Figure 4. *Lactobacillus plantarum*^{WJL} increases the lifespan of nutritionally**
529 **challenged males.** After larval development under low nutrition with or without *L.p.*^{WJL}, the
530 lifespan of adult males and females was assessed, when they were kept on either a normal
531 diet (A, B) or the same low yeast diet as the larvae (C, D).

A**B****C****D****E****F****G****H**





A +/- *L. plantarum***B****C** +/- *L. plantarum***D**