Environmental specificity ~~and evolution~~ in *Drosophila*-bacteria symbiosis affects host developmental plasticity

Robin Guilhot[1], Antoine Rombaut[1], Anne Xuéreb[1], Kate Howell[2], Simon Fellous[1]

[1]INRA, Centre for Biology and Management of Populations (UMR CBGP), 34988 Montferrier-sur-Lez, France

[2]Faculty of Veterinary and Agricultural Sciences, University of Melbourne, Parkville, Vic 3010, Australia

Mis en forme : Gauche

Abstract

Mis en forme : Police :14 pt, Gras

~~Abstract~~

Environmentally acquired microbial symbionts could contribute to host adaptation to local ~~adaptation~~conditions like vertically transmitted symbionts do. This scenario necessitates symbionts to have different effects in different environments. ~~In~~ We investigated this idea in *Drosophila melanogaster*, a species which communities of ~~extracellular~~ bacterial symbionts vary ~~largely~~greatly among environments~~, which could be due to variable effects on phenotype. We investigated this idea with~~. We isolated four bacterial strains isolated from the feces of a *D. melanogaster* ~~lab~~laboratory strain~~,~~ and tested their effects in two ~~environments~~conditions: the ancestral environment ~~of origin~~ (i.e. the laboratory medium) and a new ~~one~~environment (i.e. fresh fruit with live yeast). All bacterial effects on larval and adult traits differed among environments, ranging from very beneficial to marginally deleterious. The joint analysis of larval development speed and adult size further ~~suggests~~shows bacteria ~~would affect~~affected developmental plasticity more than resource acquisition~~-~~. This effect was largely driven by the opposite effects of the bacteria in ~~males. The~~each environment. Our study reveals that understanding *D. melanogaster* symbiotic interactions in the wild necessitates using ecologically realistic conditions. Besides, context-dependent effects of ~~bacteria we observed~~symbionts, and ~~its~~their underlying mechanisms, ~~sheds~~shed light on how environmentally acquired symbionts may contribute to host evolution.

Mis en forme : Police :12 pt, Non Gras

Introduction

Symbiosis ~~contributes~~may contribute to host evolution through recruitment of ~~adequate~~beneficial microorganisms (Margulis & Fester 1991; Jaenike et al. 2010; Fellous et al. 2011). As the environment varies among localities, different symbionts may be most beneficial in different conditions, possibly explaining microbiota variation among populations of the same animal species (e.g. Chandler et al. 2011; McKenzie et al. 2017). Microbial symbionts may therefore ~~participate to local adaptation (Kawecki and Ebert 2004). A necessary condition to symbiont-mediated local adaptation is that~~contribute to local adaptation (Kawecki and Ebert 2004). ~~microbial effects on host fitness change with environmental conditions (Schwab et al. 2016; Callens et al. 2016). The determining of host phenotype by interactions between symbiont identity and environment (i.e. Symbiont-by-Environment interactions) would thus largely be similar to so-called Genotype-by-Environment interactions that underlie genome-based local adaptation.~~ Most studies exploring symbiont-mediated local adaptation have focused on vertically transmitted microorganisms (e.g. Moran et al. 2008). However, numerous animals form symbioses with bacteria that are in part acquired from the environment either by horizontal transmission between hosts or recruitment of free-living strains (Ebert 2013). In this context, little is known on the how microbial effects on host fitness change with environmental conditions (Schwab et al. 2016; Callens et al. 2016), a necessary condition for symbiont-mediated local adaptation (Kawecki and Ebert 2004). Here, we explore how the effects of extracellular symbiotic bacteria on ~~insect host~~ *Drosophila melanogaster* traits change when ~~hosts~~host and bacteria are studied in an environment different from ~~the~~their ancestral one ~~of origin~~.

The *Drosophila* ~~flies serve as important~~*melanogaster* fly, is a prevalent model ~~organisms~~organism for host-microbiota studies (Douglas 2018). In *Drosophila melanogaster*,

bacterial symbionts ~~participate~~contribute to a broad range of functions including resource acquisition, digestion, immunity and behavior (Broderick and Lemaitre 2012; Ankrah and Douglas 2018; Schretter et al. 2018). Several laboratory studies have established fly nutrition relies on interactions with gut bacteria (Shin et al. 2011; Storelli et al. 2011; Ridley et al. 2012; Wong et al. 2014; Huang et al. 2015; Leitão-Gonçalves et al. 2017; Téfit et al. 2017). In particular, bacterial genera frequently associated with laboratory flies, such as *Lactobacillus* and *Acetobacter*, can improve larval growth and development when laboratory food is poor in proteins (Shin et al. 2011; Storelli et al. 2011; Téfit et al. 2017). Even though some bacterial taxa are frequent in laboratory colonies, the composition of *Drosophila* bacterial gut communities largely varies among laboratories (Chandler et al. 2011; Staubach et al. 2013; Wong et al. 2013; Vacchini et al. 2017). Studies have shown that bacterial microbiota composition ~~appears to be~~is determined by ~~the~~ laboratory ~~where the Drosophila flies were reared~~conditions more than ~~by their~~*Drosophila* species (Chandler et al. 2011; Staubach et al. 2013), demonstrating these symbionts are largely acquired from ~~the~~ fly environment. Empirical studies have nonetheless shown pseudo-vertical transmission of bacteria from mothers to offspring also occurs in the laboratory (Bakula 1969; Ridley et al. 2012; Wong et al. 2015; Téfit et al. 2018). Microbiota composition differences between laboratory and field flies have led authors to argue that symbiotic phenomena as observed in the laboratory may not reflect those occurring in natural conditions (Chandler et al. 2011; Winans et al. 2017). ~~The~~Numerous variables differ between laboratory and natural environments of *D. melanogaster* flies ~~indeed differ in several aspects. The most striking~~. A substantial difference ~~may have to do with~~is the composition of the nutritive substrate upon which the adults feed, copulate, oviposit and within which larvae develop. ~~Indeed, wild~~Wild flies ~~reproduce~~live on and in fresh or decaying fruit flesh, usually colonized by yeast, whereas laboratory flies are reared on an artificial, jellified and homogeneous diet that contains long-chained carbohydrates (e.g. starch), agar,

preservatives and dead yeast cells or yeast extract. To this date, very few studies have investigated *Drosophila*-bacteria interactions in conditions comparable to those of the field. ~~It is therefore unknown whether fly~~How much *Drosophila*-bacteria interactions that occur in the laboratory are maintained in natural substrate remains largely undescribed.

~~We~~Here, we experimentally studied the symbiosis between a laboratory strain of *D. melanogaster* and four ~~of its~~ bacterial symbionts (isolated from its feces) in the ancestral laboratory ~~conditions~~medium and in a new environment (grape ~~berries~~berry) where we ~~mimicked~~reproduced natural egg and bacterial deposition from mothers. ~~The four bacteria were isolated from the feces of adult flies and chosen for their ease of cultivation and recognition on standard microbiological medium.~~ After inoculating bacteria-free eggs with these four bacterial isolates, we ~~recorded~~scored various phenotypic fly traits at the larval and adult stages. ~~Our results show drastically different effects of~~We investigated two questions. (1) We focused on the influence of environmental variation on bacterial effects analyzing each of the host's traits individually. Our aim was to unveil whether host-symbiont that occurred in the environment of origin (i.e. the laboratory) maintained in more realistic conditions. We further relate these observations to fly and microbe ecology. (2) We performed a new, simultaneous analysis of two traits in order to disentangle symbionts' effects on host developmental plasticity and resource acquisition, two non-excluding possibilities. Separating plasticity from resource acquisition is important for at least two reasons. First, long-term symbiotic associations would be more likely when symbionts ~~on the hosts in laboratory medium and natural substrate. Some differences among environments can be explained by the environment-specific mechanisms~~provide new capabilities (i.e. resources) than when they affect quantitative traits (Fellous and Salvaudon 2009) or their plasticity (Chevin et al 2010). Second, recent literature shows that the evolution of ~~bacterial benevolence. The joint analysis of~~ symbiont transmission depends on which of host's traits it affects (Brown and Akçay 2019); importantly, this

mathematical model is based on the plastic trade-off between survival and reproduction. Here, we based our analysis on another well-established trade-off in holometabolous insects, that between duration of larval development ~~time~~ and adult size ~~further suggests bacteria affect~~at emergence (Teder et al. 2014; Nunney 1996). In brief, we reasoned that bacterial effects on host developmental plasticity ~~more than~~would move host phenotypes along the trade-off axis, while resource acquisition~~.~~

would allow faster development or larger size without detrimental effects on the other trait (see Materials and Methods for details).

Mis en forme : Police :12 pt, Non Gras

Materials and Methods

Drosophila strain

~~All insects~~Insects were from the Oregon-R *Drosophila melanogaster* strain~~. This strain~~ that was founded in 1927 and has since been maintained in ~~the laboratory.~~numerous laboratories. Our sub-strain was ~~obtained~~funded ±2 years earlier from a few dozen individuals provided by colleagues~~ and~~. They had been reared on a laboratory medium comprising banana, sugar, dead yeast, agar and a preservative (Table ~~S2.1~~S1A). Before and during the experiment reported here, ~~animals~~all insects were maintained at 21 °C (stocks) or 23 °C (experiment), with 70 % humidity and a ~~14h~~14 h photoperiod.

Microbial isolates

~~We isolated a small number of~~ The starting point of this work was to isolate and cultivate symbiotic ~~bacterial strain~~ bacteria from the flies. ~~Our aim was to use~~These bacteria ~~that~~ were ~~easy~~chosen for their ease of cultivation and our ability to ~~culture and recognize~~discriminate them morphologically ~~but~~ on standard microbiological medium. Our aim was not to sample the whole community of bacteria associated with our flies stock~~. An important choice was~~ but to ~~focus on~~carry out tractable experiments using a random subset of their symbionts. Note our isolation method favored aerobic bacteria ~~that grow rapidly on standard agar plates at 25 °C, which~~ and excluded the anaerobes *Lactobacillus*~~ that are among~~spp., some of the best known symbionts of *D. melanogaster*. However, all the bacterial strains we isolated had already been identified as associated to *Drosophila* flies (Chandler et al. 2011; Staubach et al. 2013). Available literature did point to a number of taxa which interactions with *Drosophila* flies are described, and that we could have sourced from other laboratories. However, working with

Mis en forme : Police :14 pt

Mis en forme : Couleur de police : Rouge

Mis en forme : Police :14 pt

Mis en forme : Police :Italique

strains we could readily isolate from our fly colony meant we were certain to investigate fly-bacteria associations in their environment of origin.

In order to isolate bacteria present in fly feces, several groups of twenty *Drosophila melanogaster* flies were placed in sterile glass vials for 1 h. After fly removal, vials were washed with sterile PBS (Phosphate-Buffered Saline) solution, which was then plated on Lysogeny Broth (LB) agar medium (Table ~~S2.2~~S1B) and incubated at 24 °C. Four bacterial morphotypes of variable frequency were chosen based on visible and repeatable differences in size, color, general shape and transparency during repeated sub-culturing on fresh media (Figure ~~S3~~S2). A single colony of each morphotype was amplified in liquid LB medium in aerobic conditions at 24 °C for 72 h, centrifuged and washed in PBS. Several sub-samples of equal concentration were stored at -80 °C in PBS with 15 % glycerol and further used ~~in this~~for molecular identification and the main experiment (one per experimental block).

Molecular identification of each bacterium was carried out ~~with~~by Sanger sequencing. To this aim, a fresh colony of each bacterial type was picked with a sterile toothpick and dipped into sterile water, then boiled 10 min at 95 °C (Mastercycler, Eppendorf) and cooled in ice water. A sterile toothpick dipped into sterile water served as sterility control of the process. Fragments of the 16sRNA gene were amplified with bacterial primers Y2MOD (5-ACTYCTACGGRAGGCAGCAGTRGG-3') and 16SB1 (5'-TACGGYTACCTTGTTACGACTT-3') (Haynes et al. 2003; Carletto et al. 2008). PCRs were performed in a volume of 25 µl, containing each primer at 0.2 µM, 1x buffer (containing 2 mM $MgCl_2$), each dNTP at 0.2 mM, and 1 U of *DreamTaq* Taq (Thermo Scientific). PCRs cycles had an initial denaturation step at 95 °C for 15 min, followed by ten cycles at 94 °C / 40 s - 65 °C / 45 s – 72 °C / 45 s); followed by 30 cycles at 94 °C / 40 s – 55 °C / 45 s – 72 °C / 45 s; and finished with an extension step of 10 min at 72 °C. Negative PCR controls were included. PCR products were visualized under UV light in an agarose gel before sequencing. Consensus

sequences were created with CodonCode Aligner 4.2.7. Online SINA alignment service (https://www.arb-silva.de/aligner/) (Pruesse et al. 2012) and NCBI GenBank blastn service (https://blast.ncbi.nlm.nih.gov/Blast.cgi) were used to compare and assign the sequences. The four bacteria were identified as a *Staphylococcus* (likely *S. xylosus*), an *Enterococcus* (likely *E. faecalis*), an Enterobacteriaceae and an Actinobacteria (likely *Brevibacterium*). Further in this article, theses bacteria are referred to as *Staphylococcus*, *Enterococcus*, Enterobacteriaceae and Actinobacteria, respectively. All sequences were deposited in the NCBI database under the accession numbers MK461976 (*Staphylococcus*), MK461977 (*Enterococcus*), MK461978 (Enterobacteriaceae) and MK461979 (Actinobacteria).

A wild isolate of *Saccharomyces cerevisiae* yeast was used in experiments where larvae developed in fresh grape berries. The yeast was isolated from a wild Drosophilid in a vineyard in Southern France (*'Le Domaine de l'Hortus'*, Hérault, France) (see Hoang et al. (2015) for a balanced discussion on *Drosophila-Saccharomyces* interactions). The isolate was grown in YPD medium, washed, split into several samples, stored at -80 °C in sterile PBS with 15 % glycerol, that were further used in the experiment (one per block).

Experimental design

~~We followed~~Flies were associated with bacteria following a full-factorial design ~~resulting~~that resulted in twelve different treatments ~~to assay: i.~~ There were two types of fly environments ~~–~~: laboratory medium (the ancestral environment, see Table S1A for composition) and grape ~~berry~~ ~~(~~berries (the new environment, white grapes, unknown cultivar~~) , ii.~~). We had six different symbiont treatments ~~–~~: each of the four bacterial strains described above, a mix of ~~these~~the four bacteria and controls without ~~added~~ bacteria~~.~~ (Figure 1). Each treatment had 13 to 15 replicates

Mis en forme : Police :Non Italique
Mis en forme : Police :Non Italique
Mis en forme : Police :14 pt

organized in 15 blocks launched over four days. Bacterial growth was also studied in fly-free grapes but is not described here.

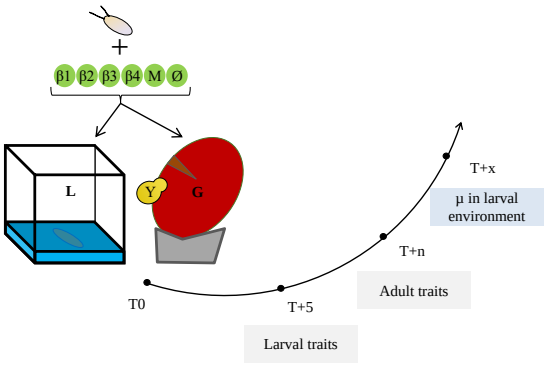


Figure 1: summary of the experimental design and the measured traits. T0: association of Drosophila eggs with bacteria (β1, β2, β3, β4), bacterial mixture (M), or nothing (Ø), in the two environments: laboratory medium (L) or grape berry (G) inoculated with live yeast (Y). T+5: larval traits scoring after five days. T+n: adult size scoring on a randomly chosen subset of adults from each replicate. T+x: analysis of the microbial content of the larval environment two days after the end of pupal formation.

Grape berries were surface-sterilized ~~with~~in a 2 % bleach solution before use. Because *D. melanogaster* females only oviposit in wounded fruit, we incised ~~5mm~~5 mm of berry skin (Figure S4) where we deposited twenty eggs free from culturable bacteria. These eggs were produced by the oviposition of flies on laboratory medium supplemented with the antibiotic streptomycin (1 mg / ml in 1 mM EDTA, Sigma-Aldrich ref. 85886). The efficacy of this method for removing culturable bacteria from egg surface was confirmed by the lack of bacterial growth after the deposition of such eggs onto LB agar plates (note however that these

conditions were not suitable for detection of anaerobic bacteria such as *Lactobacillus* ~~was not feasible in such conditions~~). Grape berries were inoculated with live yeast cells as it is a key component (Begg & Robertson 1948; Becher et al. 2012) and was necessary for fly survival in our system (Figure ~~S1~~S3). For treatments with laboratory diet we deposited 20 eggs free from culturable bacteria on incisions at the surface of 4 ml of medium placed in 2 cm * 2 cm plastic cubes. Berries and laboratory media were all placed in 75 ml plastic vials closed by a foam plug.

Bacterial cells were inoculated to laboratory medium and grape berry immediately before egg deposition. Single bacterial strain treatments received ~~2.5 x 10^3~~10^4 live bacterial cells, and the mixed treatment 2.5 x 10^3 cells of each bacterium (~~giving~~i.e. 10^4 cells in total), suspended in 10 µl of sterile PBS. The number of inoculated bacterial cells, that is < 10^4 Colony Forming Units (CFUs), was chosen based on the average number of bacteria previously reported in the guts of second-instar larvae (Bakula 1969; Storelli et al. 2011). In control treatments, sterile PBS was deposited instead of bacteria. On grape berries, 10^4 live cells of the yeast *Saccharomyces cerevisiae* were inoculated. Note fruit substrate and live yeast presence are confounded factors in our experiment because we did not intend to study the effect of live yeast onto larval growth (Becher et al 2012) but to mimic field conditions where larvae develop in presence of live yeast. Although the laboratory medium also contains yeast, ~~this preparation is inactivated~~cells are killed during industrial production (Table ~~S2.1).~~S1A).

Fly phenotyping

Mis en forme : Police :14 pt

We ~~recorded~~scored six different phenotypic traits in larvae and adults: larval size, ~~larval~~ mouthpart movement ~~speed, number of larvae~~ rate, and visible number on medium surface~~,~~ after 5 days; survival ~~until~~rate to adult emergence~~,~~; time until adult emergence and a proxy of

adult size. ~~Larval traits were measured five days after egg deposition using a stereomicroscope.~~ Larval mouthpart movement speed ~~is~~was the number of back-and-forth movements of the mouthpart that could be observed in 5 seconds. Newly formed pupae were transferred to empty sterile vials daily. We recorded male and female emergences daily.

The size of adults, and their microbial content (see below), were estimated on a subset of those that emerged from ~~the same vials.~~each vial. For each ~~experimental replicate, we~~vial, one pupa was chosen randomly ~~selected a pupa before its emergence~~ and ~~when it emerged we pooled together~~ all ~~the flies of the same sex~~adults that emerged on the same day ~~and from the same vial than the randomly selected pupae.~~as the focal pupa were collected, pooled by sex. These pools were homogenized in 200 µl of sterile PBS using a sterile pestle, ~~divided~~splat in two sub-samples and stored at -80 °C with 15% sterile glycerol. One of the two sub-samples was used to numerate live bacteria and yeast cells in newly emerged adults, the other one to estimate adult size with the spectrophotometric method described in Fellous et al. (2018). We chose this method as it allowed the simultaneous analysis of adult size and microbial content. Briefly, we used log-transformed optical density at 202 nm of fly homogenate as a proxy of adult size. This was measured several months after the experiment when samples were thawed, crushed a second time using a Tissue Lyser II (Qiagen) for 30 s at 30 Hz with Ø3 mm glass balls, centrifuged for 30 s at 2000 G. Optical density of 15 µL of supernatant was then read on a Multiskan GO spectrometer (Thermo Scientific). This metrics correlates in both males and females with wet weight and wing length (all $R^2 > 0.8$), two frequently used size proxies in *Drosophila* studies. For figures and analyses of adult size we used the Log of observed optical density divided by the number of individuals in the sample.

Analysis of ~~microbial development~~ bacterial presence and ~~evolution~~metabolism

Mis en forme : Police :14 pt

Mis en forme : Police :14 pt

Mis en forme : Police :14 pt

~~The microbial content (i.e.~~We tested the presence of inoculated bacteria and yeast~~) of newly emerged adults, as well as the microbial content of the laboratory media and of the grape berries~~ in substrates two days after the ~~removal~~appearance of the last pupa. Samples were analyzed~~.~~ by plating homogenates on LB agar medium and incubated at 24 °C. In this manuscript we only report on the presence or absence of inoculated bacteria in the larval ~~environment. We~~ substrate. Data of microorganism presence and numbers in emerging adults will ~~describe the transmission of inoculated bacteria and yeast from larvae to adults (i.e. through metamorphosis) in a separate manuscript~~be reported separately.

~~In order to better understand fly symbiosis with the~~The Enterobacteriaceae and the Actinobacteria were the main bacterial strains that affected fly phenotypes. In order to shed light on the ecologies of these two strains and therefore on their effects on hosts, we analyzed their metabolic ~~abilities profiles~~capabilities with Eco Microplates (Biolog) ~~that contain 31~~(see Text S5 for methodological details).

Bacteria and fungi morphologically different ~~carbon substrates (Text S5). In one case~~from those we ~~recorded the presence~~had inoculated were observed in samples from 17 % of the ~~Actinobacteria in a grape berry at the end of the experiment. The bacterium was isolated and its metabolic abilities were compared to that of its ancestor deposited at the beginning of the experiment. Because we suspected the grape-retrieved Actinobacteria had evolved the ability to better develop~~vials (either in ~~fruit flesh we compared its growth~~adults on in ~~grape flesh to that of the ancestor (Text S6). The two bacteria were deposited in slices of surface-sterilized berries with two initial concentrations 10^4 cells and 10^6 cells (eight replicates of each). Grape disks~~the environment). Data from these vials were ~~sampled after 24 h and 72 h and bacteria numerated on LB agar plates.~~excluded for all analyzes presented here. Both datasets are available in the open data repository Zenodo (DOI: 10.5281/zenodo.2554194).

~~**Data analysis**~~

Statistical analyses

Individual traits

To study the response of each fly ~~phenotypes~~phenotypic trait to variation of larval substrate and bacterial symbiont, we used linear mixed models (LMM) with Restricted Maximum Estimate Likelihood (REML~~) were used.~~). 'Block identity' was defined as random factor~~, while we defined as fixed~~. Fixed factors were the 'larval environment' (i.e. laboratory medium or fruit), 'bacterial treatment', 'fly sex' (for the analyses of age at emergence and adult size only), and their full-factorial interactions. A Backward, stepwise model selection was used to eliminate non-significant terms from initial, full models. Homoscedasticity and residuals normality visually complied ~~visually~~ with model assumptions. ~~Post-hoc Student's tests were used to decipher~~When the 'bacteria*environment' interaction was significant, and to investigate hypotheses based on the visual observation of the data, we used independent contrasts to test significant differences ~~among factor levels~~between bacterial treatments and controls from the same environment.

~~Bacteria and fungi different from those we inoculated were observed in 17% of the vials, which were further excluded for all analyzes presented in this article. Results of fly traits analyses were identical in the full and the curated dataset. Both datasets are available online.~~

~~The number of Actinobacteria cells (counted by colony forming units CFUs) in grape disks inoculated with the ancestral strain and the isolate retrieved from a replicate of the experiment was analyzed with a linear model. Number of cells was log(x+1) transformed to comply with model assumptions. The full-factorial model contained the factors 'bacterial strain identity~~

~~(ancestor or derived)', 'time after inoculation (24 h or 72 h)' and 'initial cell concentration (10^4 or 10^6 cells)'. Post-hoc tests were carried out by comparing with linear models cell numbers of the ancestor and derived isolate in each of the four combinations of initial density and time after inoculation. The metabolic abilities of the ancestral and derived Actinobacteria – as assayed with Eco Microplates (Text S5) in three independent observations per substrate, bacterial strain and assay duration – were further compared with Mann-Whitney tests for each individual carbon source.~~

~~Analyzes were performed with JMP (SAS, 14.1) and R (version 3.5.2).~~

~~Dataset is available in the open data repository Zenodo (DOI: 10.5281/zenodo.2554194).~~

~~Results~~

~~Larval traits~~

Joint effect of bacteria on adult age and size at emergence

The aim of this analysis was to study how bacteria affected simultaneously speed of larval development and size. Importantly, we needed to discard the general effect of the nutritive environment to single out the effects of the symbionts. Indeed, if one environment was generally more favorable than the other, main environmental effects could create a positive relationship between the two traits that would conceal how bacteria affect them. To this end, all analyses were carried out after subtracting the mean trait value of the controls (i.e. bacteria-free) in the relevant environment from the trait values of each combination of bacteria and environment. In other words, data presented in Figures 5 and S6 represent the incremental effects of the bacteria on host traits after removal of the transversal influence of the nutritive substrate.

We carried out two types of analyses. (1) In order to unveil the overall pattern (Figures 5 and S6) we worked with mean treatment effects (i.e. one single data point per treatment, two when sex was taken into account) and univariate regressions. Because of the significant interaction between sex, bacteria and environment for adult size, our initial analysis separated males from females (Figure S6). However, the linear regression of size onto developmental speed was not significantly different among sexes (Interaction Sex*Speed: $F_{1,16} = 2.93$, $p = 0.11$). Presented results hence merge observations from males and females. (2) In order to explain the factors behind the simultaneous effect of bacteria on developmental speed and adult size we carried out a multivariate analysis of variance (MANOVA) using all data points (i.e. one data point per experimental unit). MANOVA was chosen because it enables studying how factors affect

several variables jointly, in other words it considers effects onto the correlation between several variables (Zar 2009, p.319). We used a "repeated measures" personality of MANOVA and reported the tests based on the Sum response function (i.e. a M-matrix that is a single vector of 1 s; between-subject report in JMP). Model contained the factors ‘bacterial treatment’, ‘environment’ and their interaction. Homoscedasticity and residuals normality visually complied with MANOVA assumptions. The dataset used for the MANOVA analysis is available in the open data repository Zenodo (DOI: 10.5281/zenodo.3352230).

Analyzes were performed with JMP (SAS, 14.1).

Results

Effects of bacteria on individual traits reveal extensive environmental-dependence

Larval size after five days was influenced by an interaction between the environment and the bacterial treatment (Table 1, Figure ~~1A~~2A). In grapes, addition of the Actinobacteria decreased larval size relative to bacteria-free controls but had no ~~particular~~ effect in laboratory media. In laboratory media, addition of the Enterobacteriaceae ~~produced large larvae both~~ alone ~~and when mixed~~or in mixture with the other bacterial strains~~,~~ produced larger larvae than bacteria-free controls (contrast 'Enterobacteriaceae treatment' vs 'Control treatment': $F_{1.90} = 28.92$, $p < 0.0001$), which did not happen when grown on a grape substrate~~.~~ (contrast 'Enterobacteriaceae treatment' vs 'Control treatment': $F_{1.86} = 0.92$, $p = 0.3405$) (Figure 2A).

The number of larvae visible on medium surface was influenced by an interaction between the environment and the bacterial treatment (Table 1, Figure ~~1B). Presence of the Enterobacteriaceae in laboratory media led to greater numbers of visible larvae compared to all other treatments.~~2B). In laboratory media, addition of the Enterobacteriaceae alone or in mixture with the other bacterial strains led to greater numbers of visible larvae compared to bacteria-free controls (contrast 'Enterobacteriaceae treatment' vs 'Control treatment': $F_{1.131} = 20.40$, $p < 0.0001$; contrast 'Mixture treatment' vs 'Control treatment': $F_{1.131} = 6.98$, $p = 0.0092$), which did not happen when grown on a grape substrate (contrast 'Enterobacteriaceae treatment' vs 'Control treatment': $F_{1.131} = 1.63$, $p = 0.2036$; contrast 'Mixture treatment' vs 'Control treatment': $F_{1.131} = 0.93$, $p = 0.3355$) (Figure 2B).

Mouthparts movement ~~pace~~rate was influenced by an interaction between the environment and the bacterial treatment (Table 1, Figure ~~1C~~2C). Movements were generally faster in grapes than

Mis en forme : Police :Non Italique

in laboratory media. However, addition of the Actinobacteria slowed down the movements of mouthparts in grapes to a level comparable to the one of larvae reared on laboratory media. (contrast 'Actinobacteria treatment' vs 'Control treatment': $F_{1,99} = 4.54$, $p = 0.0355$) (Figure 2C).

The proportion of eggs surviving until the adult stage was only affected by the environment, with a lower survival in grapes than in laboratory media (Table 1, Figure ~~1D).~~

371 2D).

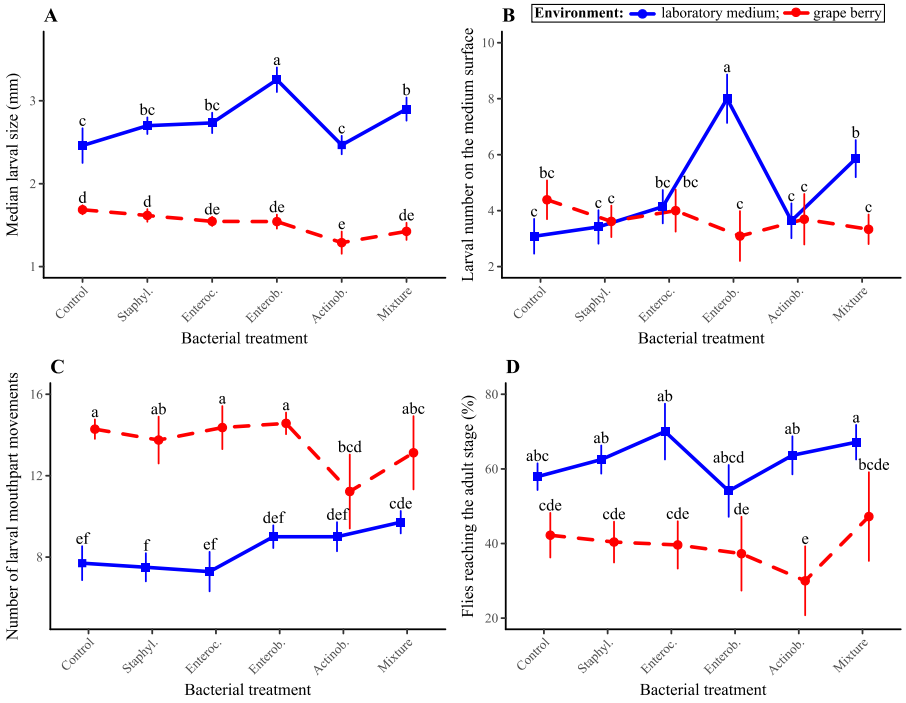
372 **Table 1: analysis of larval and adult phenotypes in response to bacterial treatment and larval environment.** Linear mixed models (REML).

~~Variable~~***Response variables***	***Median larval size***	***Number of larvae on the substrate surface***	***Larval foraging behavior***	***Developmental survival***	***Average age of emerging adults***	***Adult size proxy***
Factors						
Environment	$F_{1.18} = 137.51$ $p < 0.0001$	$F_{1.98} = 13.64$ $p = 0.0004$	$F_{1.25} = 28.43$ $p < 0.0001$	$F_{1.17} = 27.02$ $p < 0.0001$	$F_{1.18} = 77.86$ $p < 0.0001$	$F_{1.15} = 0.35$ $p = 0.5630$
Bacterial treatment	$F_{5.88} = 4.08$ $p = 0.0022$	$F_{5.131} = 2.02$ $p = 0.0806$	$F_{5.97} = 0.78$ $p = 0.5657$	$F_{5.115} = 0.78$ $p = 0.5688$	$F_{5.211} = 4.41$ $p = 0.0008$	$F_{5.183} = 0.79$ $p = 0.5609$
Environment*Bacterial treatment	$F_{5.88} = 4.64$ $p = 0.0008$	$F_{5.131} = 4.50$ $p = 0.0008$	$F_{5.97} = 2.80$ $p = 0.0211$	$F_{5.115} = 0.53$ $p = 0.7558$	$F_{5.211} = 7.85$ $p < 0.0001$	$F_{5.183} = 1.90$ $p = 0.0960$
Fly sex	-	-	-	-	$F_{1.199} = 1.68$ $p = 0.1961$	$F_{1.166} = 3.27$ $p = 0.0724$

Cellules fusionnées
Cellules fusionnées
Cellules fusionnées
Cellules fusionnées
Cellules fusionnées
Cellules fusionnées

Environment*Bacterial treatment*Fly sex	-	-	-	-	$F_{5.199} = 0.42$ $p = 0.8336$	$F_{5.166} = 2.75$ $p = 0.0204$

373 Linear mixed models, with block as random factor.


A
Median larval size (mm)
Control
Staphyl.
Enteroc.
Enterob.
Actinob.
Mixture
Bacterial treatment
B
Environment: laboratory medium; grape berry
Larval number on the medium surface
Bacterial treatment
C
Number of larval mouthpart movements
Bacterial treatment
D
Flies reaching the adult stage (%)
Bacterial treatment

374

375

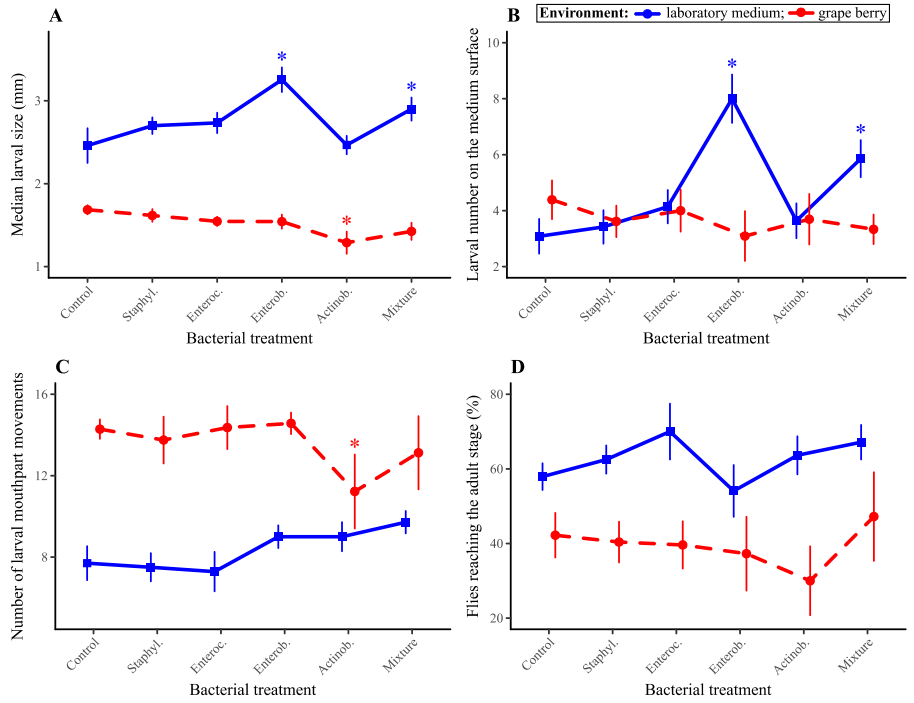


Figure ~~1~~2: larval phenotypes in response to bacterial treatment and larval environment. (A) Median larval size after 5 day; (B) Number of larvae on the medium surface after 5 days; (C) Number of larval mouthparts movements per 5 s observed after 5 days; (D) ~~Developmental survival.~~Survival from egg to adult. Symbols indicate means; error bars indicate standard errors around the mean. ~~Means not connected by the same letters are~~Stars (*) indicate treatments significantly different~~.~~ from controls in the same environment (post-hoc contrasts, $\alpha = 0.05$).

~~Metamorphosis and adult traits~~

Age at adult emergence was not different among sexes but was influenced by an interaction between the environment and the bacterial treatment (Table 1, Figure ~~2A~~3). In laboratory media, flies reared with the Enterobacteriaceae, alone or in mixture, emerged nearly two days

sooner than ~~other~~bacteria-free flies in the same environment and almost four days earlier than ~~those~~bacteria-free flies in grapes (contrast 'Enterobacteriaceae treatment' vs 'Control treatment': $F_{1.225} = 27.57$, $p < 0.0001$; contrast 'Mixture treatment' vs 'Control treatment': $F_{1.225} = 24.94$, $p < 0.0001$) (Figure ~~2A). No differences were observed among~~3). In grapes, flies reared with the bacterial ~~treatments in grapes~~mixture emerged one day later than bacteria-free flies (contrast 'Mixture treatment' vs 'Control treatment': $F_{1.222} = 6.42$, $p = 0.0120$) (Figure ~~2A~~3).

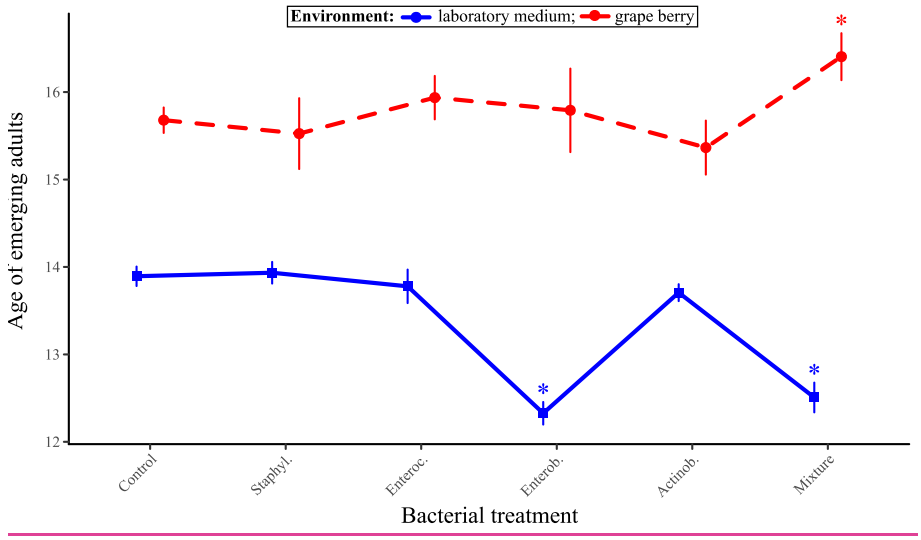
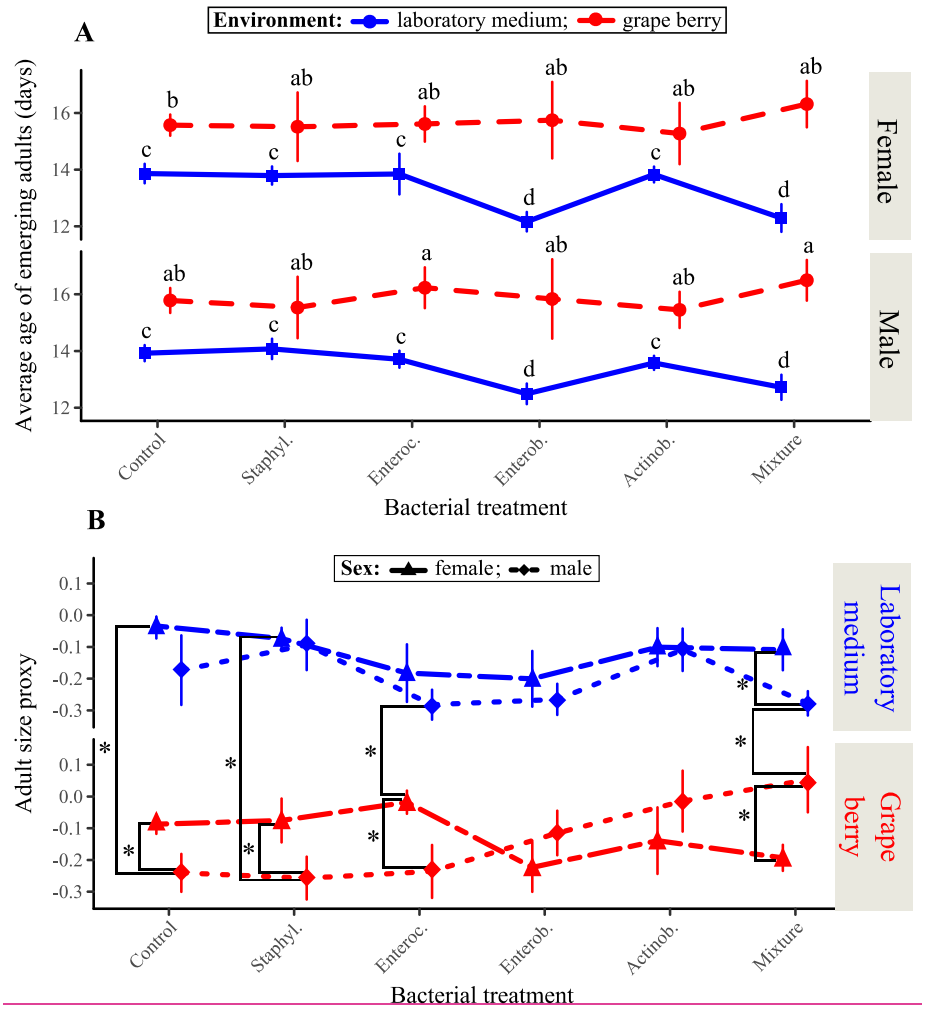


Figure 3: age of *Drosophila* adults at emergence in response to bacterial treatment and larval environment. Symbols indicate means; error bars indicate standard errors around the mean. Stars (*) indicate treatments significantly different from controls in the same environment (post-hoc contrasts, $\alpha = 0.05$).

Adult size was influenced by ~~a~~the triple interaction between sex, the environment and the bacterial treatment (Table 1, Figure ~~2B~~4). Several bacterial treatments had sex-specific effects that differed among the two environments. For example, inoculation of the mixture of ~~all~~the four bacteria produced larger males than females in grapes (contrast 'Mixture treatment' vs 'Control treatment': $F_{1.166} = 5.30$, $p = 0.0225$), but smaller males than females in laboratory media~~.~~ (contrast 'Mixture treatment' vs 'Control treatment': $F_{1.167} = 4.79$, $p = 0.0300$) (Figure 4). Similarly, inoculation of the *Staphylococcus* ~~produced~~ or *Enterococcus* led to larger males than females in ~~grapes and~~ grape (contrast '*Staphylococcus* treatment' vs 'Control treatment': $F_{1.164} = 4.97$, $p = 0.0271$; contrast '*Enterococcus* treatment' vs 'Control treatment': $F_{1.164} = 7.48$, $p = 0.0069$), but no difference in laboratory ~~media than males in grapes.~~medium (contrast '*Staphylococcus* treatment' vs 'Control treatment': $F_{1.165} = 0.11$, $p = 0.7367$; contrast '*Enterococcus* treatment' vs 'Control treatment': $F_{1.167} = 0.66$, $p = 0.4182$) (Figure 4).


A
Environment: laboratory medium; grape berry
Average age of emerging adults (days)
Female
Male
Control
Staphyl.
Enteroc.
Enterob.
Actinob.
Mixture
Bacterial treatment
B
Sex: female; male
Adult size proxy
Laboratory medium
Grape berry
Control
Staphyl.
Enteroc.
Enterob.
Actinob.
Mixture
Bacterial treatment

413

414

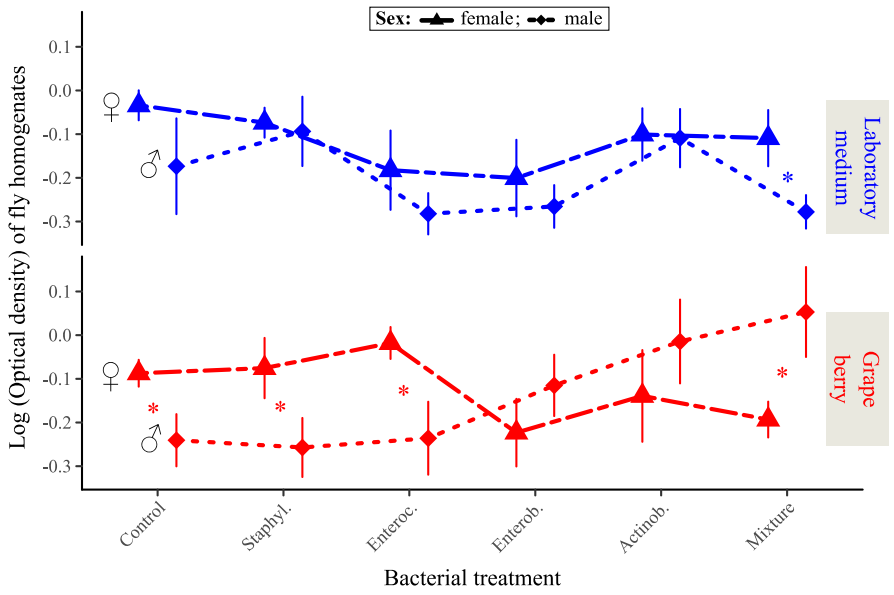


Figure ~~2:~~4: *~~Drosophila~~* adult ~~*Drosophila* phenotypes~~size proxy in response to bacterial treatment and larval environment. Symbols indicate means; error bars indicate standard errors around the mean. ~~(A) Average age of emerging adult~~ Stars (*) indicate significant differences between males and females ~~and males; (B) Adult size proxy.~~ in the same environment (post-hoc contrasts, $\alpha = 0.05$). ~~Symbols indicate means; error bars indicate standard errors around the mean.~~ ~~Means not connected by the same letters (Figure A) or * (Figure B) are significantly different.~~

Joint ~~effect~~analysis of ~~bacteria on~~adult age and size at emergence ~~and adult size~~

~~It is well established in numerous animals that, all else being equal, the speed of larval development (i.e. 1/ age at maturity) trade-offs with adult size (Teder et al. 2014). This gave us the opportunity to disentangle symbiont effects on resource acquisition (i.e. performance that~~

Mis en forme : Police :14 pt
Mis en forme : Police :14 pt
Mis en forme : Police :14 pt
Mis en forme : Police :14 pt

~~can bolster one trait with no cost to the other) from~~ **suggests bacteria affect host developmental plasticity along** ~~the trade-off. To this end, we related developmental speed and adult size using the mean trait value of each bacterial treatment (Figure 3). It was mandatory to remove the overall effects of each environment on host phenotypes, otherwise, if one environment was generally more favorable than the other it would have created a positive relationship between larval development speed and adult size that could have concealed the influence of the bacteria on the plastic relationship between these traits. We therefore divided the mean trait values of each treatment (5 bacterial treatments * 2 environments = 10 treatments) by that of the bacteria-free controls in the same environment (grape or laboratory medium). Males and females were analyzed separately owing to the three-way interaction between sex, environment and bacteria observed for adult size.~~ **a trade-off**

We expected three possible patterns when plotting average adult size in function of speed of larval development (i.e. - age at emergence): a positive relationship indicative of a similar effect of the bacteria on the two traits (i.e. bacteria modulate resource acquisition, mostly); a negative relationship indicative of bacteria affecting host position along the trade-off (i.e. bacteria modulate developmental plasticity, mostly); a lack of correlation that would have been challenging to interpret on its own as several processes could produce this result (e.g. bacterial effects on both host plasticity and resource acquisition).

The relationship between effects of bacteria on ~~duration of larval stage and~~ adult age and size at emergence was marginally significant and negative ~~for males~~ (Linear model $F_{1,8}$ = ~~6.48~~8.83, p = 0.~~0344) but not for females ($F_{1,8}$ = 3.09, p = 0.1169~~018) (Figure ~~3). Overall, in grapes bacteria produced slow-developing but large males, while they developed faster and were smaller~~ 5). A MANOVA shed light on the relative influence of the environment and the bacterial treatments on the correlated effect of the treatments on the two traits (Table 2). It

Mis en forme : Police :14 pt, Gras

Mis en forme : Police :14 pt

revealed the environment was an important factor: in laboratory ~~media~~ medium, addition of bacteria accelerated development relative to controls at the cost of producing smaller adults; in grape addition of bacteria slowed down development relative to controls but emerging adults were large (Figure ~~3B).~~5). There was no significant main effect of the bacterial treatments but a significant interaction with the environment, which confirms the bacterial treatments had different effects on host phenotype in each environment.

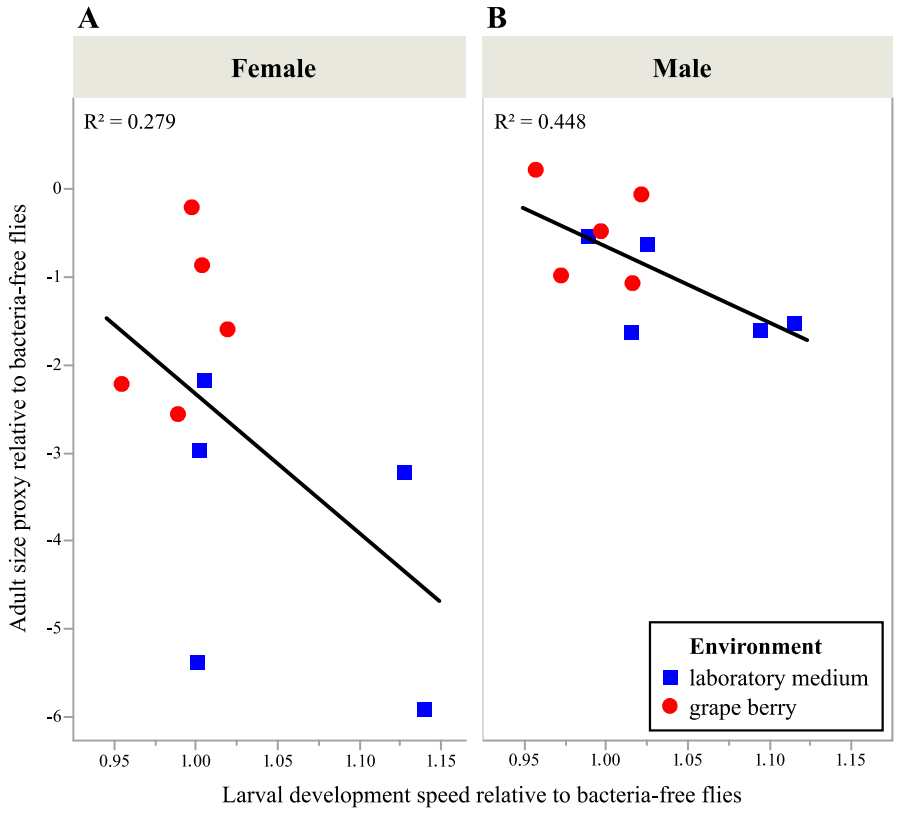


Table 2: Multivariate Analysis of Variance of the joint effect of the bacteria on 'Age at emergence' and 'Adult size'. As in Figure ~~3~~5, general effects of the environments were

Mis en forme : Police :Non Gras

removed by subtracting trait values of controls (i.e. without bacterial addition) in each environment before carrying out the analysis.

Factor	*F*	*d.f.*	*p*
Environment	14.9	1.85	0.0002
Bacterial treatment	1.65	4.85	0.17
Environment*Bacterial treatment	3.86	4.85	0.006

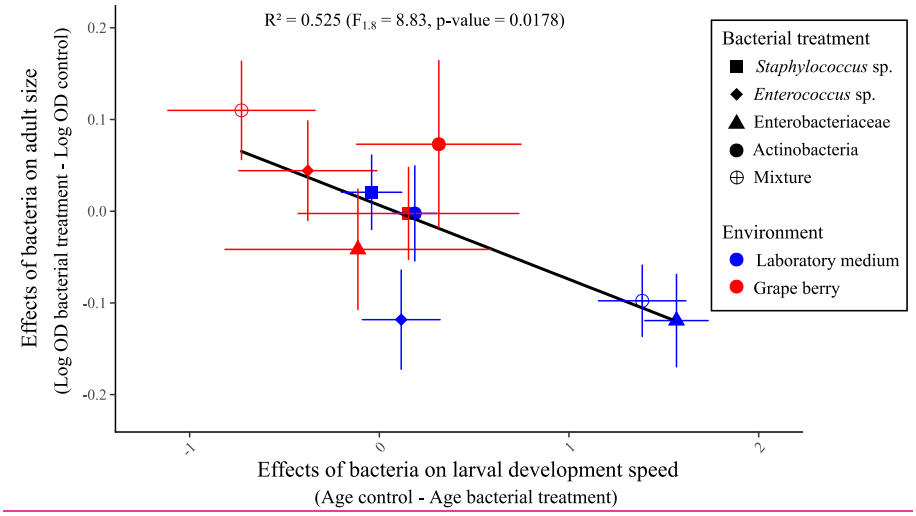
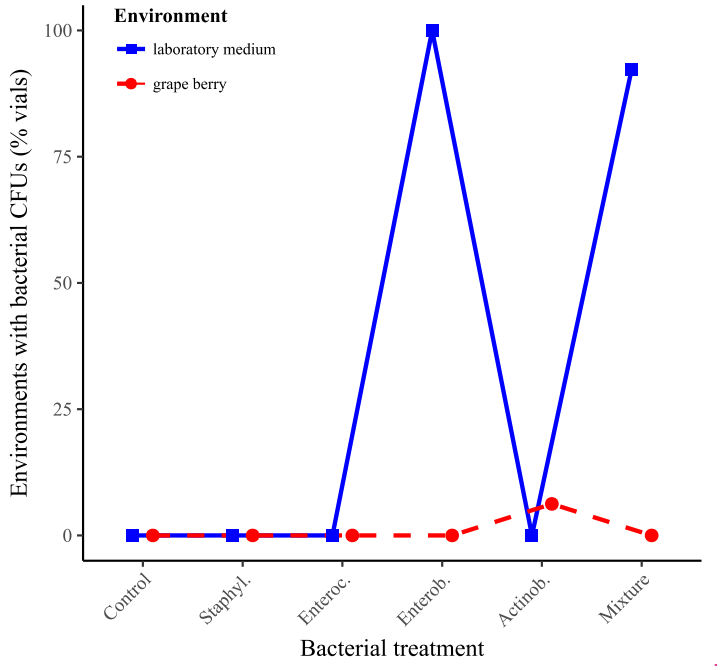


Figure 5: relationship between ~~average~~bacterial effects on age of ~~bacteria on larval developmental speed~~emerging adults and bacterial effects on adult size~~, in females (A) and females (B).~~ ~~Symbols~~. Effects of bacteria for each treatment were calculated by subtracting the mean trait value of controls in the same environment to mean trait value of the treatment.

Error bars indicate standard errors around the ~~phenotype~~ means ~~of each treatment (i.e. combination of bacterium and larval environment). All values are expressed relative to average observations of bacteria-free treatments.~~.

Bacterial ~~cells~~occurrence in the environment and ~~the flies~~their metabolism

The Enterobacteriaceae isolate was the only bacterium to be consistently retrieved from the ~~environment~~laboratory medium in which larvae had developed~~, however only in laboratory media (Figure 4).~~



Mis en forme : Police :14 pt
Mis en forme : Police :14 pt
Mis en forme : Police :14 pt

(Figure ~~**4. Proportions of environments containing bacterial cells of the strain inoculated as observed after the formation of the last pupa.** Symbols indicate percentages per treatment.~~

6). In one instance, the Actinobacteria was found in a grape berry from which no live adult fly emerged~~. This bacterium was isolated and further studied in order to investigate its possible evolution (Text S6). Comparison between the Actinobacteria ancestor and the isolate from fruit revealed a marginally non-significant interaction between bacterium identity, sampling time and initial cell density ($F_{1,56}$ = 3.25, p = 0.077) (Figure S6). Comparison between the two bacteria at all time points and initial cell densities (i.e. 4 combinations) revealed a single significant difference between the ancestor and the derived strain after 24 h with an initial density of 10^4 cells per grape disk (t = -3.33, p = 0.005). The metabolic differentiation of the two bacteria was investigated on thirty-one carbon sources. After 48 h, growth of the derived Actinobacteria was significantly greater than that of the ancestor in eleven substrates, and lower in four substrates (Figure S6A). After 120 h, growth of the derived Actinobacteria was significantly greater than that of the ancestor in three substrates, and lower in sixteen substrates (Figure S6B~~ (Figure 6). The physiological profile of the Enterobacteriaceae revealed growth of the bacterium in a broad panel of carbon sources (Figure S5A). The physiological profile of the Actinobacteria revealed substantial growth of the bacterium on the carbon sources Pyruvic Acid Methyl Ester and Tween 80 only (Figure S5B).

Mis en forme : Police :Non Gras

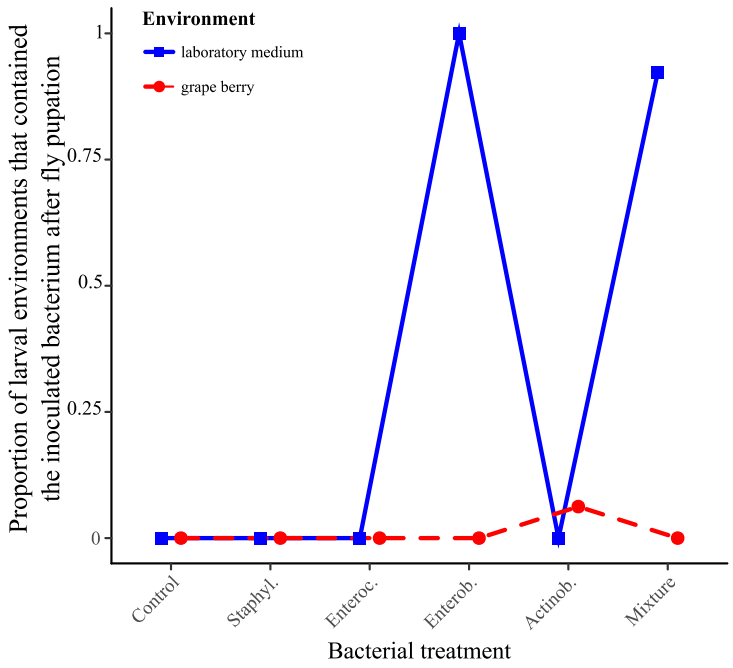


Figure 6: proportion of larval environments that contained the inoculated bacterium after fly pupation. Proportions were calculated over replicates (see after): Lab. (Laboratory medium) - Control (n = 12 replicates), Lab. - Staphyl. (n = 11), Lab. - Enteroc. (n = 7), Lab. - Enterob. (n = 10), Lab. - Actinob. (n = 10), Lab. - Mixture (n = 13), Grape - Control (n = 26), Grape - Staphyl. (n = 16), Grape - Enteroc. (n = 16), Grape - Enterob. (n = 13), Grape - Actinob. (n = 16), Grape - Mixture (n = 12).

Discussion

We studied the symbiotic interactions between a laboratory strain of *Drosophila melanogaster* and four bacterial strains isolated from ~~adult~~its feces. ~~No single effect of the bacteria~~ Our results show different effects of bacterial symbionts on host phenotype ~~observed~~ in laboratory medium ~~(i.e. the~~and in real fruit. All symbiont effects were environment-dependent, some of ~~origin) maintained in fresh fruit (i.e. the environment close to natural conditions). Some of these observations can~~which may be explained by the ecology of laboratory-associated symbionts in artificial medium. ~~Further analyses suggest combination~~The joint analysis of ~~environment~~larval developmental speed and adult size further suggests bacteria ~~affected~~influence host developmental plasticity along ~~a~~the well-known physiological trade-off between ~~larval growth speed and adult size.~~ these two traits.

Different symbiont effects in different environments

The observation that all bacterial effects on host phenotype were different in laboratory medium and grape berry prompts the question of the reason behind this discrepancy. Focusing of the Enterobacteriaceae may shed light onto the ecologies of the symbiotic bacteria we isolated, and why they differed among environments.

In laboratory medium, inoculation of the Enterobacteriaceae induced greater larval size and accelerated larval development (Figures ~~1A~~2A and ~~2A~~3). Besides, adults produced by larvae associated with the Enterobacteriaceae in laboratory medium were not significantly smaller than ~~in other treatments.~~adults produced by bacteria-free larvae (Figure 4). The bacterium hence accelerated larval growth. In its presence larvae ~~remained at the surface of the medium where they~~ could be observed in greater numbers at the surface of the medium than ~~with all other~~

Mis en forme : Police :14 pt

~~treatments~~in the absence of the bacterium (Figure ~~1B~~2B), even though there were no mortality differences among ~~them~~Enterobacteriaceae-associated and bacteria-free larvae (Figure ~~1D~~2D). The Enterobacteriaceae was also the only bacterium to be retrieved from the medium after fly pupation (Figure ~~4). These last two elements suggest the bacterium serves as food: it would grow on medium surface and be consumed by grazing larvae. This idea is further supported by the visual observation that, in absence of larvae, media inoculated with the Enterobacteriaceae harbored white microbial growth on their surface (Figure S7). Along these lines, the wide metabolic spectrum of this bacterium (Figure S5.1) is congruent with a microorganism being~~6). These elements may be explained by two mechanisms. (1) The bacterium could serve as food and be grazed on medium surface by foraging larvae. The phenomenon would be ~~a generalist that would extract resources from the medium, possibly transform nutrients (Ankrah and Douglas 2018; Sannino et al. 2018), and eventually concentrate them on medium surface. This phenomenon would be in many ways~~ similar to that described by Yamada et al. (2015) where the yeast *Issatchenkia orientalis* extracts amino acids from agar-based laboratory medium and concentrates them on medium surface where adult flies harvest them. This hypothesis is congruent with the visual observation that media inoculated with the Enterobacteriaceae harbored white microbial growth on their surface (Figure S7). Along these lines, the wide metabolic spectrum of this bacterium (Figure S5A) suggests the microorganism is a generalist that would extract resources from the medium, possibly transform nutrients (Ankrah and Douglas 2018; Sannino et al. 2018), and eventually concentrate them on medium surface. (2) Microbial growth at the surface would interfere with larval development in such way that larvae would remain at the surface. This behavior could also trigger accelerated development if excessive microbial growth revealed detrimental. The two hypotheses above are non-excluding; the joint-analysis of developmental speed and adult size sheds further light on this question (see below). Why did the effect of the Enterobacteriaceae on host phenotype differ among

environments? The physical nature of laboratory medium is very different from that of real fruit. In particular, the agar of laboratory medium permits the diffusion of simple nutrients and their absorption by bacteria and yeast. However present on surface. Besides, in fresh fruitgrape nutrients are not free to diffuse but enclosed in cells, it . Surface growth is therefore understandable that the Enterobacteriaceae did not accelerate larval growth in fresh fruit. Larvae feeding on surface growing microorganisms may therefore be more commonlikely in the laboratoryartificial medium than in the field, where larvae are rare on the surface of fruitsgrape berry, leading to different effects on larval development.

Bacteria-induced nutritional effects on *Drosophila* larvae and adults are frequently attributed to gut bacteria (In addition to physical differences between laboratory medium and fresh fruit, the nature and concentration of available nutrient are likely to differ. It is well knownShin et al. 2011; Storelli et al. 2011; Broderick and Lemaitre 2012; Leitão-Gonçalves et al. 2017). It is well established that lactic and acetic acid bacteria, two taxa that were not investigated in our experiment, can promote larval growth upon nutrient scarcity (Shin et al. 2011; Storelli et al. 2011, Téfit et al. 2017). However, it is also well established that bacteria can affect *Drosophila* phenotype through signaling (Storelli et al. 2011) as well as nutrient provisioning (Brownlie et al. 2009; Bing et al. 2018; Sannino et al. 2018). In most cases, these effects which were described from laboratory flies and in laboratory medium, are condition specific (Douglas 2018). Indeed, bacteria are often only beneficial when laboratory food has a low concentration in dead yeast (i.e. amino acids) (Shin et al. 2011; Storelli et al. 2011). Our results extend these previous observations as the *Staphylococcus*, the *Enterococcus* and the Actinobacteria we isolated and assayed here not only lost their beneficial effects when tested out of laboratory medium, but also acquired new effects. For example, in grape larval size was reduced by the Actinobacteria relative to bacteria-free controls (Figure 1A) showing symbionts can become costly when associated with host in a new environmentAlong these lines, it may seem

paradoxical the Enterobacteriaceae only accelerates larval growth in rich laboratory medium rather than in grape berry (unless the bacterium synthesized a rare nutrient). Metabolic profiling (Figure S5A) further shows the Enterobacteriaceae is a generalist bacterium able to grow on a variety of substrate. However, the Actinobacteria had a narrower metabolic spectrum (Figure S5B), suggesting it is a specialist which growth largely depends on the availability of specific nutrients. The bacterium slowed down larval growth in grape (Figure 2A) for an unknown reason - maybe because it exerted additional stress onto larvae in a relatively poor medium - but had no notable effect in laboratory medium. The environment-specific effect of the Actinobacteria compares to previous reports of *Drosophila* symbionts being beneficial in some environments only (e.g. *Lactobacillus plantarum* in rich medium), and further reveals that bacteria with little effect in an environment can become detrimental in new conditions.

Effects of bacteria on host developmental plasticity

In holometabolous insects, the duration of larval development and adult size are often negatively correlated due to a physiological trade-off: faster development reduces the duration of food intake and leads to smaller adult size (Teder et al. 2014; Nunney 1996). We propose to exploit this trade-off to separate symbionts' effects on host developmental plasticity and resource acquisition. As discussed above, symbionts of *Drosophila* flies can modify host's signaling (e.g. Shin et al. 2011; Storelli et al. ~~Whether flies' symbiotic bacteria reside durably in fly guts or are constantly excreted in the environment and re-absorbed during feeding is still debated (Ma & Leulier 2018; Pais et al. 2018). It is not possible to further this debate with our data. However, we note that only one of our four bacterial isolates, the Enterobacteriaceae, was consistently retrieved from the larval artificial medium (Figure 4). By contrast, all isolates were found in adults produced by pupae that were separated from the larval environment before~~

~~emergence (data presented in another forthcoming manuscript). These observations are congruent with the hypothesis that the *Staphylococcus*, the *Enterococcus* and the Actinobacteria we isolated are gut residents rather than grow in the medium. In the only case where we retrieved the Actinobacteria from fruit flesh, it is striking that the ability of this bacterium to maintain in fruit seemed to have evolved in the course of our experiment (Figure 4). This association between bacterium evolution and effects of host phenotype echoes the results of Martino et al. (2018) who showed *Lactobacillus* adaptation to food medium leads to greater benevolence. However, in our case adaptation of the Actinobacteria to fruit environment associated with greater cost to the host.~~

~~Host developmental plasticity~~

~~It is well established that holometabolous insects, such as fruit flies, must trade off duration of larval development (i.e. age at maturity) with adult size (Teder et al. 2014, Nunney 1996). Figure 3 displays the effects of bacterial and substrate treatments on larval and adult traits relative to treatments without added bacteria. Host trait values for each bacterial treatment were divided by values measured in controls reared on the same substrate but without addition of bacteria. The relationship between speed of larval development and adult male size was marginally significant and negative. Data therefore suggests that bacterial treatments that slowed down development led to the production of larger adult males. Because data points from fruit and artificial medium segregated in different parts of phenotypic space the results may be partly driven by the environment specific effects of bacteria on hosts.~~2011) as well as provide rare resources (e.g. Brownlie et al. 2009; Sannino et al. 2018). These two mechanisms are expected to have different effects on the trade-off between speed of development and size. Signaling (i.e. plasticity) should move hosts along the trade-off, while the provisioning of

greater resources should enable faster growth and/or larger size without sacrificing the other trait. In order to test this idea we extracted bacterial effects on host phenotype by subtracting control trait values to those of each of the bacterial treatments in each environment. The resulting plot of symbionts effects on developmental speed and adult size (Figures 5 and S6) reveals the influence of the bacteria on the host independent of the general effects of the environment (i.e. those not due to the bacteria).

Our original analysis of bacterial effects on larval development and adult size revealed a negative relationship (Figures 5 and S6). Treatments that accelerated development produced small adults and treatments that slowed down development produced large adults. The negative relationship came as a surprise as we expected nutritional symbionts to affect developmental speed and adult size either in an independent or similar fashion, which would have led to an absence or a positive relationship between these two traits, respectively. ~~Correlated,~~Instead, results suggest bacterial treatments influenced host development plastically along the trade-off between speed of development and adult size. This result contrasts with previous reports on other *Drosophila* symbionts that induce positive ~~effects of a nutritional symbiont on~~ relationships between larval and adult traits ~~were for example shown in yeast *Drosophila* mutualism~~ (Anagnostou et al. 2010; Bing et al. 2018). For example, the yeast species ~~of yeast~~ *Metschnikowia pulcherrima* ~~produce~~produces small adults that are also slow to develop (Anagnostou et al. 2010). ~~Our results suggest that bacterial symbionts, such as the ones we studied here, could alter~~Different symbionts in different contexts can therefore affect host developmental plasticity ~~in response to the ecological context. This hypothesis is congruent with the known~~or its resource acquisition.

The visual examination of Figure 5 shows bacterial effects measured in laboratory medium (blue points) group in the fast development-small size region of phenotypic space, while effects in grape (red points) occur in the small speed-large size side of the trade-off. This suggests that

the environment could determine whether bacteria accelerate development (at the cost of a smaller size) or favor size (at the cost of a slower development). A MANOVA revealed a significant effect ~~*Lactobacillus plantarum* bacteria~~ of the environment on ~~host development mediated~~the joint analysis of the two traits, hence confirming that bacterial influence on host developmental plasticity is largely determined by ~~hormonal changes (Storelli et al. 2011).~~ the environment. With only 5 bacterial treatments per environment it was not possible to test if bacteria affect host development along the trade-off within a single environment.

Whether microbial symbionts influence hosts through ~~variation of~~ effects on developmental plasticity or resource availability (i.e. general vigor ~~(~~*sensus* Fry (1993~~) or developmental plasticity (two non-excluding possibilities))~~) may change the evolutionary fate of the host-symbiont relationship. ~~Indeed~~First, symbionts that plastically alter phenotypes ~~may~~would be more dispensable that those providing functions host genomes are not capable of (Fellous and Salvaudon 2009). ~~It could further be argued the fitness effect of altering developmental plasticity may depend on environmental context more than general improvement of resource acquisition (Chevin et al 2010). As such symbiont mediated effects on host plasticity is in line with the idea that many symbionts have context-dependent effect on the fitness of their host (e.g. De Vries et al. 2004; Duncan et al. 2010; Daskin & Alford 2012; Bresson et al. 2013; Callens et al. 2016; Cass et al. 2016).~~Besides, it could be argued that the fitness effects of alternative plastic strategies may depend on the environmental context more than general improvement of resource availability (Chevin et al 2010). Therefore, symbionts that improve general performance of the host through greater resource availability may be more likely to be fixed among host individuals and populations than those that affect plasticity. By contrast, hosts may dynamically acquire and lose symbionts which effects on fitness depend on the environment, paving the way for the evolution of facultative symbiosis. Along these lines, recent modelling of host-symbiont dynamics revealed that whether symbionts affect adult

survival or reproduction (another well-known trade-off between fitness components) determines transmission mode evolution (Brown and Akçay 2019). Our experimental study only considered one trade-off between two developmental traits, possibly overlooking effects on other fitness components. Future analyses should increase in dimensionality and consider a greater number of fitness components. Similarly, a precise description of the slopes and shapes of considered trade-offs will be necessary to discriminate simultaneous effects of symbionts on plasticity and resource acquisition. We are now pursuing further investigation to determine if, and when, bacterial and yeast symbionts affect host developmental plasticity rather than ~~general performance~~resource availability in *Drosophila* flies.

~~Symbiont~~Context-dependent effects of bacteria enable symbiont-mediated ~~evolution~~adaptation

A consequence of *Drosophila* bacterial symbionts having different effects in different environments is the possibility they ~~participate~~contribute to the fine-tuning of host phenotype to local conditions (Margulis & Fester 1991; Moran 2007; Sudakaran et al. 2017). The phenomenon is ~~now~~ well established in vertically transmitted symbionts of insects that protect their hosts from parasites. For example, populations of aphids exposed to parasitoids harbor protective *Hamiltonella* symbionts at greater frequency than parasitoid-free populations (Oliver et al. 2005). Similarly, in the fly *Drosophila neotestacea*, the spread of the bacterium *Spiroplasma* allowed hosts to evolve greater resistance to parasitic nematodes (Jaenike et al. 2010). Vertically- transmitted bacterial symbionts of *Paramecium* ciliates can also improve host resistance to stressful conditions (Hori & Fujishima 2003). Whether bacteria act as parasites or mutualists ~~then~~ depends partly on the genetic ability of the host to deal with stress in absence of the symbiont (Duncan et al. 2010). However, the evolutionary role of symbionts

Mis en forme : Justifié
Mis en forme : Police :14 pt
Mis en forme : Police :14 pt

that may be acquired from the environment is less clear, in part because the mechanisms favoring the association of hosts with locally beneficial symbionts are not as straightforward as for vertical transmission (Ebert 2013). Nonetheless, several lines of evidence suggest environmentally acquired microbial symbionts may ~~participate~~contribute to local adaptation in *Drosophila*-microbe symbiosis. First, symbionts can be transmitted across metamorphosis (i.e. transstadial transmission from the larval to the adult stage) and pseudo-vertically during oviposition (i.e. from mothers to offspring) (Bakula 1969; Starmer et al. 1988; Spencer 1992; Ridley et al. 2012; Wong et al. 2015; Téfit et al. 2018). Second, host immune system participates ~~to~~in the destruction of harmful gut bacteria and the retention of beneficial ones (Lee et al. 2017; Lee et al. 2018). Third, *Drosophila* larvae ~~actively search and~~may preferentially associate with beneficial yeast species ensuring they engage in symbiosis with locally adequate nutritional symbionts (Fogleman et al. 1981; Fogleman et al. 1982). In addition to host genetic and preferential association with beneficial microbes, *Drosophila* adaptation to local conditions thanks to microorganisms further necessitates symbionts have different effects in different environments. Our results show bacteria isolated from a fly population ~~can either be beneficial, neutral or costly~~have different effects on host phenotype depending on the substrate larvae were reared in (Figures ~~1~~2, 3, 4 and ~~2). Bacterial~~5). As a consequence, extracellular bacterial symbionts may therefore ~~participate~~contribute to ~~host–~~*Drosophila* local adaptation ~~in *Drosophila* bacteria symbioses~~ through variations in symbiont community composition.

~~Host adaptation based on symbionts differs from genome-based evolution in that microbes can provide a greater amount of evolutionary novelty than mutations of nuclear genes do (Jaenike et al. 2012; Moran 2007). This arises from several factors. A single metazoan individual can associate with billions of microbial cells that each has a genome with potentially beneficial mutations. It results that populations of microbial symbionts can adapt faster to local conditions than nuclear genes. *Caenorhabditis elegans* nematodes host a diversity of bacteria, some of~~

~~which may be detrimental. A recent study demonstrated how rapid evolution in the competition between two bacterial species, one of which being pathogenic to worms, lead to host protection against the most virulent bacterium (King et al. 2016). Rapid symbiont evolution can also be beneficial to hosts in the case of nutritional symbioses as demonstrated in the relationship between *Drosophila melanogaster* and the bacterium *Lactobacillus plantarum*. It was recently shown that bacterium adaptation to nutritional substrate during 2000 generations (i.e. 313 days) in absence of hosts not only improves bacterial performance but also that of *Drosophila* larvae associated to the evolved bacterium (Martino et al. 2018). Our data suggest the pace of microbial evolution to environmental conditions may be even faster. Indeed, at the end of our experiment, we retrieved live Actinobacteria cells from one fruit. Preliminary experiments had shown this strain we had isolated from fly feces was not able to grow in grape flesh (Figure S6). We therefore hypothesized the Actinobacteria isolate had evolved a better ability to maintain in fruit flesh than the ancestor we had inoculated. Comparison between this derived strain and the ancestor indeed suggests the bacterium evolved better persistence in the environment in the time course of our experiment (Fig S6.1). However, conclusion based on this observation must not be over-stretched as our experimental setup was not initially designed to test for bacterial adaptation, we only observed this phenomenon once and the comparison between the ancestral and the derived strain is contingent on minute experimental details. On the other hand, the metabolic abilities of the derived isolate had evolved relative to the ancestor in the majority of the 31 carbon substrates they were tested on (Figure S5.2), suggesting rapid bacterial evolution did occur. The derived strain was collected in one of the replicates where larvae were smallest and with slowest mouthpart movements, where live yeast concentration was lowest, and from which no adult emerged, showing that bacterial adaptation to environmental conditions may be detrimental to insect hosts.~~

Conclusion

In this study, we found that associations between laboratory *Drosophila* flies and their microbial symbionts result in different effects on host phenotype when the symbiosis is investigated under conditions ~~close~~comparable to nature. The context-~~dependence~~dependency of bacterial effects, and the underlying mechanisms we unveiled (i.e. bacterial ecology~~,~~ and bacterial effects on host plasticity~~ and rapid bacterial evolution~~), shed light on the role of microorganisms in the evolution of their hosts. ~~Understanding~~Our results point out that in order to understand the ecology and evolution of ~~symbiosis~~symbiotic interactions in the wild ~~will necessitate working with wild strains of animals and symbionts under~~it is necessary to use ecologically realistic conditions, which is attainable in the *Drosophila* system.

Acknowledgements

We warmly thank L. Benoit, M.P. Chapuis, J. Collet, D. Duneau, R. Gallet, P. Gautier, N. Kremer, F. Leulier and N. Rode for methodological help and useful comments on an earlier version of this work. We thank Wolf Blanckenhorn, Pedro Simões and an anonymous referee for precious comments on an earlier version of the manuscript, as well as PCI (Peer Community In) Evol Biol managing board for their contribution to the advancement of science publishing.

Conflict of interest disclosure

The authors of this preprint declare that they have no financial conflict of interest with the content of this article. Simon Fellous is one of the PCI (Peer Community In) Evol Biol recommenders.

Funding

This ~~work received financial support from~~project was supported by French ~~ANR's~~National Research Agency through the 'SWING' project (ANR-16-CE02-0015~~),)~~ and by Agropolis Fondation under the reference ID 1505-002 through the 'Investissements d'avenir' programme (Labex Agro~~, CIVC, BIVB and INRA's department 'Santé des Plantes et Environnement'.~~:ANR-10-LABX-0001-01).

References

Anagnostou, C., Dorsch, M., and Rohlfs, M. (2010). Influence of dietary yeasts on *Drosophila melanogaster* life-history traits. *Entomologia Experimentalis et Applicata*, 136(1), 1-11.

Ankrah, N. Y., and Douglas, A. E. (2018). Nutrient factories: metabolic function of beneficial microorganisms associated with insects. *Environmental microbiology*. 20(6), 2002-2011.

Bakula, M. (1969). The persistence of a microbial flora during postembryogenesis of *Drosophila melanogaster*. *Journal of invertebrate pathology*, 14(3), 365-374.

Becher, P. G., Flick, G., Rozpędowska, E., Schmidt, A., Hagman, A., Lebreton, S., Larsson, M. C., Hansson, B. S., Piskur, J., Witzgall, P., and Bengtsson, M. (2012). Yeast, not fruit volatiles mediate *Drosophila melanogaster* attraction, oviposition and development. *Functional Ecology*, 26(4), 822-828.

Begg, M., and Robertson, F. W. (1948). Nutritional requirements of *Drosophila melanogaster*. *Nature*, 161(4098), 769.

Bing, X., Gerlach, J., Loeb, G., and Buchon, N. (2018). Nutrient-Dependent Impact of Microbes on *Drosophila suzukii* Development. *mBio*, 9(2), e02199-17.

Bresson, J., Varoquaux, F., Bontpart, T., Touraine, B., and Vile, D. (2013). The PGPR strain *Phyllobacterium brassicacearum* STM196 induces a reproductive delay and physiological changes that result in improved drought tolerance in *Arabidopsis*. *New Phytologist*, 200(2), 558-569.

Mis en forme : Police :12 pt, Non Gras

Broderick, N. A., and Lemaitre, B. (2012). Gut-associated microbes of *Drosophila melanogaster*. *Gut microbes*, 3(4), 307-321.

Brown, A., and Akçay, E. (2019). Evolution of transmission mode in conditional mutualisms with spatial variation in symbiont quality. *Evolution*, 73(2), 128-144.

Brownlie, J. C., Cass, B. N., Riegler, M., Witsenburg, J. J., Iturbe-Ormaetxe, I., McGraw, E. A., and O'Neill, S. L. (2009). Evidence for metabolic provisioning by a common invertebrate endosymbiont, *Wolbachia pipientis*, during periods of nutritional stress. *PLoS pathogens*, 5(4), e1000368.

Callens, M., Macke, E., Muylaert, K., Bossier, P., Lievens, B., Waud, M., and Decaestecker, E. (2016). Food availability affects the strength of mutualistic host–microbiota interactions in *Daphnia magna*. *The ISME journal*, 10(4), 911-920.

Carletto, J., Gueguen, G., Fleury, F., and Vanlerberghe-Masutti, F. (2008). Screening the bacterial endosymbiotic community of sap-feeding insects by terminal-restriction fragment length polymorphism analysis. *Entomologia experimentalis et applicata*, 129(2), 228-234.

Cass, B. N., Himler, A. G., Bondy, E. C., Bergen, J. E., Fung, S. K., Kelly, S. E., and Hunter, M. S. (2016). Conditional fitness benefits of the *Rickettsia* bacterial symbiont in an insect pest. *Oecologia*, 180(1), 169-179.

838 Chandler, J. A., Lang, J. M., Bhatnagar, S., Eisen, J. A., and Kopp, A. (2011). Bacterial
839 communities of diverse *Drosophila* species: ecological context of a host–microbe model
840 system. *PLoS genetics*, 7(9), e1002272.
841
842 Chevin, L. M., Lande, R., and Mace, G. M. (2010). Adaptation, plasticity, and extinction in a
843 changing environment: towards a predictive theory. *PLoS biology*, 8(4), e1000357.
844
845 Daskin, J. H., and Alford, R. A. (2012). Context-dependent symbioses and their potential roles
846 in wildlife diseases. *Proceedings of the Royal Society of London B: Biological*
847 *Sciences*, 279(1733), 1457-1465.
848
849 De Vries, E. J., Jacobs, G., Sabelis, M. W., Menken, S. B., and Breeuwer, J. A. (2004). Diet–
850 dependent effects of gut bacteria on their insect host: the symbiosis of *Erwinia* sp. and western
851 flower thrips. *Proceedings of the Royal Society of London B: Biological Sciences*, 271(1553),
852 2171-2178.
853
854 Douglas, A. E. (2018). The *Drosophila* model for microbiome research. *Lab Animal*, 47(6),
855 157.
856
857 Duncan, A. B., Fellous, S., Accot, R., Alart, M., Chantung Sobandi, K., Cosiaux, A., and Kaltz,
858 O. (2010). Parasite-mediated protection against osmotic stress for *Paramecium caudatum*
859 infected by *Holospora undulata* is host genotype specific. *FEMS microbiology ecology*, 74(2),
860 353-360.
861

Ebert, D. (2013). The epidemiology and evolution of symbionts with mixed-mode transmission. *Annual Review of Ecology, Evolution, and Systematics*, 44, 623-643.

Fellous, S., and Salvaudon, L. (2009). How can your parasites become your allies? *Trends in parasitology*, 25(2), 62-66.

Fellous, S., Duron, O., and Rousset, F. (2011). Adaptation due to symbionts and conflicts between heritable agents of biological information. *Nature Reviews Genetics*, 12(9), 663.

Fellous, S., Guilhot, R., Xuéreb, A., and Rombaut, A. (2018). A high-throughput spectrophotometric assay of adult size in *Drosophila* that facilitates microbial and biochemical content analysis. *Dros. Inf. Serv.*, 101, 69-74.

Fogleman, J. C., Starmer, W. T., and Heed, W. B. (1981). Larval selectivity for yeast species by *Drosophila mojavensis* in natural substrates. *Proceedings of the National Academy of Sciences*, 78(7), 4435-4439.

Fogleman, J. C., Starmer, W. T., and Heed, W. B. (1982). Comparisons of yeast florae from natural substrates and larval guts of southwestern *Drosophila*. *Oecologia*, 52(2), 187-191.

Fry, J. D. (1993). The "general vigor" problem: can antagonistic pleiotropy be detected when genetic covariances are positive?. *Evolution*, 47(1), 327-333.

Mis en forme : Motif : Transparente (Blanc)

Mis en forme : Motif : Transparente

Mis en forme : Espace Après : 0 pt

Haynes, S., Darby, A. C., Daniell, T. J., Webster, G., Van Veen, F. J. F., Godfray, H. C. J., Prosser, J. I., and Douglas, A. E. (2003). Diversity of bacteria associated with natural aphid populations. *Applied and Environmental Microbiology*, 69(12), 7216-7223.

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

Huang, J. H., and Douglas, A. E. (2015). Consumption of dietary sugar by gut bacteria determines *Drosophila* lipid content. *Biology letters*, 11(9), 20150469.

Jaenike, J., Unckless, R., Cockburn, S. N., Boelio, L. M., and Perlman, S. J. (2010). Adaptation via symbiosis: recent spread of a *Drosophila* defensive symbiont. *Science*, 329(5988), 212-215.

~~Jaenike, J. (2012). Population genetics of beneficial heritable symbionts. *Trends in ecology and evolution*, 27(4), 226-232.~~

Kawecki, T. J., and Ebert, D. (2004). Conceptual issues in local adaptation. *Ecology letters*, 7(12), 1225-1241.

~~King, K. C., Brockhurst, M. A., Vasieva, O., Paterson, S., Betts, A., Ford, S. A., Frost, C. L., Horsburgh, M. J., Haldenby, S., and Hurst, G. D. (2016). Rapid evolution of microbe-mediated protection against pathogens in a worm host. *The ISME journal*, 10(8), 1915-1924.~~

Mis en forme : Motif : Transparente

Mis en forme : Motif : Transparente (Blanc)

Mis en forme : Espace Après : 10 pt

Mis en forme : Motif : Transparente (Blanc)

Mis en forme : Espace Après : 10 pt

Lee, J. H., Lee, K. A., and Lee, W. J. (2017). Microbiota, gut physiology, and insect immunity. *Advances in Insect Physiology*, 52, 111-138.

Lee, K. A., Cho, K. C., Kim, B., Jang, I. H., Nam, K., Kwon, Y. E., Kim, M., Hyeon, D. Y., Hwang, D., Seol, J. H., and Lee, W. J. (2018). Inflammation-modulated metabolic reprogramming is required for DUOX-dependent gut immunity in *Drosophila*. *Cell host and microbe*, 23(3), 338-352.

Leitão-Gonçalves, R., Carvalho-Santos, Z., Francisco, A. P., Fioreze, G. T., Anjos, M., Baltazar, C., Elias, A. P., Itskov, P. M., Piper., M. D. W., and Ribeiro, C. (2017). Commensal bacteria and essential amino acids control food choice behavior and reproduction. *PLoS biology*, 15(4), e2000862.

Ma, D., and Leulier, F. (2018). The importance of being persistent: The first true resident gut symbiont in *Drosophila*. *PLoS biology*, 16(8), e2006945.

Margulis, L., and Fester, R., eds (1991). *Symbiosis as a source of evolutionary innovation: speciation and morphogenesis*. MIT Press.

~~Martino, M. E., Joncour, P., Leenay, R., Gervais, H., Shah, M., Hughes, S., Gillet, B., Beisel, C., and Leulier, F. (2018). Bacterial Adaptation to the Host's Diet Is a Key Evolutionary Force Shaping *Drosophila-Lactobacillus* Symbiosis. *Cell host and microbe*, 24(1), 109-119.~~

McKenzie, V. J., Song, S. J., Delsuc, F., Prest, T. L., Oliverio, A. M., Korpita, T. M., Alexiev, A., Amato, K. R., Metcalf, J. L., Kowalewski, M., Avenant, N. L., Link, A., Di Fiore, A.,

Mis en forme : Motif : Transparente (Blanc)

Mis en forme : Espace Après : 10 pt

934 Seguin-Orlando, A., Feh, C., Orlando, L., Mendelson, J. R., Sanders, J., and Knight, R. (2017).
935 The effects of captivity on the mammalian gut microbiome. *Integrative and comparative*
936 *biology*, 57(4), 690-704.
937
938 Moran, N. A. (2007). Symbiosis as an adaptive process and source of phenotypic complexity.
939 *Proceedings of the National Academy of Sciences*, 104, 8627-8633.
940
941 Moran, N. A., McCutcheon, J. P., and Nakabachi, A. (2008). Genomics and evolution of
942 heritable bacterial symbionts. *Annual review of genetics*, 42, 165-190.
943
944 Nunney, L. (1996). The response to selection for fast larval development in *Drosophila*
945 *melanogaster* and its effect on adult weight: an example of a fitness trade-off. *Evolution*, 50(3),
946 1193-1204.
947
948 Oliver, K. M., Moran, N. A., and Hunter, M. S. (2005). Variation in resistance to parasitism in
949 aphids is due to symbionts not host genotype. *Proceedings of the National Academy of Sciences*,
950 102(36), 12795-12800.
951
952 Pais, I. S., Valente, R. S., Sporniak, M., and Teixeira, L. (2018). *Drosophila melanogaster*
953 establishes a species-specific mutualistic interaction with stable gut-colonizing bacteria. *PLoS*
954 *biology*, 16(7), e2005710.
955
956 Pruesse, E., Peplies, J., and Glöckner, F. O. (2012). SINA: accurate high-throughput multiple
957 sequence alignment of ribosomal RNA genes. *Bioinformatics*, 28(14), 1823-1829.
958

959 Ridley, E. V., Wong, A. C., Westmiller, S., and Douglas, A. E. (2012). Impact of the resident
960 microbiota on the nutritional phenotype of *Drosophila melanogaster*. *PloS one*, 7(5), e36765.
961
962 Sannino, D. R., Dobson, A. J., Edwards, K., Angert, E. R., and Buchon, N. (2018). The
963 *Drosophila melanogaster* gut microbiota provisions thiamine to its host. *mBio*, 9(2), e00155-
964 18.
965
966 Schretter, C. E., Vielmetter, J., Bartos, I., Marka, Z., Marka, S., Argade, S., and Mazmanian, S.
967 K. (2018). A gut microbial factor modulates locomotor behaviour in *Drosophila*. *Nature*,
968 563(7731), 402.
969
970 Schwab, D. B., Riggs, H. E., Newton, I. L., and Moczek, A. P. (2016). Developmental and
971 ecological benefits of the maternally transmitted microbiota in a dung beetle. *The American*
972 *Naturalist*, 188(6), 679-692.
973
974 Shin, S. C., Kim, S. H., You, H., Kim, B., Kim, A. C., Lee, K. A., Yoon, J. H., Ryu, J. H., and
975 Lee, W. J. (2011). *Drosophila* microbiome modulates host developmental and metabolic
976 homeostasis via insulin signaling. *Science*, 334(6056), 670-674.
977
978 Spencer, D. M., Spencer, J. F. T., De Figueroa, L., and Heluane, H. (1992). Yeasts associated
979 with rotting citrus fruits in Tucumán, Argentina. *Mycological Research*, 96(10), 891-892.
980
981 Starmer, W. T., Peris, F., and Fontdevila, A. (1988). The transmission of yeasts by *Drosophila*
982 *buzzatii* during courtship and mating. *Animal behaviour*, 36(6), 1691-1695.
983

984 Staubach, F., Baines, J. F., Künzel, S., Bik, E. M., and Petrov, D. A. (2013). Host species and
985 environmental effects on bacterial communities associated with *Drosophila* in the laboratory
986 and in the natural environment. *PloS one*, 8(8), e70749.
987
988 Storelli, G., Defaye, A., Erkosar, B., Hols, P., Royet, J., and Leulier, F. (2011). *Lactobacillus*
989 plantarum promotes *Drosophila* systemic growth by modulating hormonal signals through
990 TOR-dependent nutrient sensing. *Cell metabolism*, 14(3), 403-414.
991
992 Sudakaran, S., Kost, C., and Kaltenpoth, M. (2017). Symbiont acquisition and replacement as
993 a source of ecological innovation. *Trends in Microbiology*, 25(5), 375-390.
994
995 Teder, T., Vellau, H., and Tammaru, T. (2014). Age and size at maturity: A quantitative review
996 of diet-induced reaction norms in insects. *Evolution*, 68(11), 3217-3228.
997
998 Téfit, M. A., and Leulier, F. (2017). *Lactobacillus plantarum* favors the early emergence of fit
999 and fertile adult *Drosophila* upon chronic undernutrition. *Journal of Experimental Biology*,
1000 220, 900-907.
1001
1002 Téfit, M. A., Gillet, B., Joncour, P., Hughes, S., and Leulier, F. (2018). Stable association of a
1003 *Drosophila*-derived microbiota with its animal partner and the nutritional environment
1004 throughout a fly population's life cycle. *Journal of insect physiology*, 106(1), 2-12.
1005
1006 Vacchini, V., Gonella, E., Crotti, E., Prosdocimi, E. M., Mazzetto, F., Chouaia, B., ... and
1007 Daffonchio, D. (2017). Bacterial diversity shift determined by different diets in the gut of the

spotted wing fly *Drosophila suzukii* is primarily reflected on acetic acid bacteria. *Environmental microbiology reports*, 9(2), 91-103.

Winans, N. J., Walter, A., Chouaia, B., Chaston, J. M., Douglas, A. E., and Newell, P. D. (2017). A genomic investigation of ecological differentiation between free-living and *Drosophila*-associated bacteria. *Molecular ecology*, 26(17), 4536-4550.

Wong, A. C., Chaston, J. M., and Douglas, A. E. (2013). The inconstant gut microbiota of *Drosophila* species revealed by 16S rRNA gene analysis. *The ISME journal*, 7(10), 1922-1932.

Wong, A. C. N., Dobson, A. J., and Douglas, A. E. (2014). Gut microbiota dictates the metabolic response of *Drosophila* to diet. *Journal of Experimental Biology*, 217(11), 1894-1901.

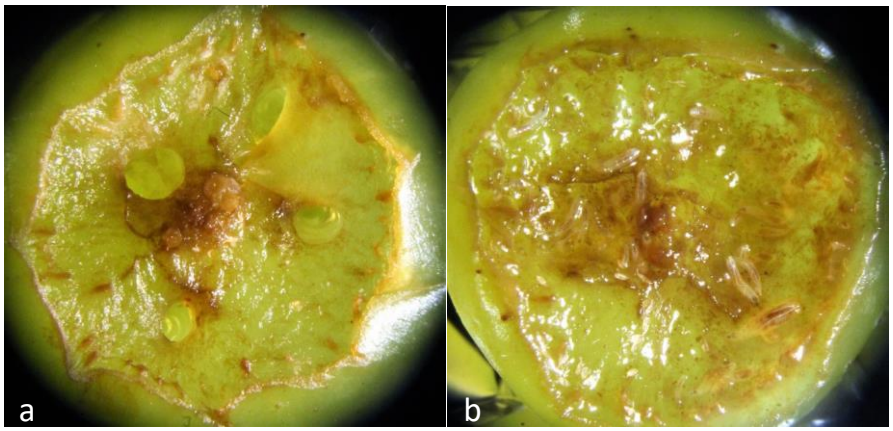
Wong, A. C. N., Luo, Y., Jing, X., Franzenburg, S., Bost, A., and Douglas, A. E. (2015). The host as the driver of the microbiota in the gut and external environment of *Drosophila melanogaster*. *Applied and environmental microbiology*, 81(18), 6232-6240.

Yamada, R., Deshpande, S. A., Bruce, K. D., Mak, E. M., and William, W. J. (2015). Microbes promote amino acid harvest to rescue undernutrition in *Drosophila*. *Cell reports*, 10(6), 865-872.

Mis en forme : Police :14 pt, Gras

Zar, J. H. (2009). *Biostatistical Analysis Fifth Edition*. Upper Saddle River, New Jersey: Pearson.

Supplementary Material 1. ~~Live yeast as a prerequisite to *D. melanogaster* larvae survival on pristine grape berry.~~



~~Figure S1: Live~~ ~~yeast is necessary for the survival of *D. melanogaster* larvae on pristine grape berry.~~ ~~Prior to the experiment, we investigated survival of *D. melanogaster* larvae on fresh grape berries. Twenty bacteria-free *D. melanogaster* eggs were deposited next to an artificial wound with or without the bacterial isolates and *Saccharomyces cerevisiae*. In absence of yeast, larvae died quickly after hatching, with or without bacteria (Figure S1a). When live yeast was added to the system, numerous larvae developed up to the 3$^{\text{third}}$ instar (Figure S1b), when we stopped monitoring.~~

Mis en forme : Justifié, Interligne : Double

Mis en forme : Police :Gras

~~Supplementary Material 2.~~ Laboratory recipes.

Table ~~S2.1: Laboratory~~S1A: laboratory medium recipe.

Component	***Amount for 1.5L***
Reverse osmosis water	1200 ml
Banana	280 g
Sugar	74 g
Dead yeast	74 g
Alcohol	30 ml
Agar	12 g
Nipagin	6 g

1049 **Table ~~S2.2: Lysogeny~~S1B:** lysogeny broth (LB) recipes.

Component	*Quantity / Volume for*			
	Liquid LB	***Agar LB***	***Anti-bacteria Agar LB***	***Anti-yeast Agar LB***
Reverse osmosis water	1000 ml	1000 ml	1000 ml	1000 ml
Proteose peptone n°3 (Conda)	10 g	10 g	10 g	10 g
Yeast extract (Merck)	5 g	5 g	5 g	5 g
NaCl (Carlo Erba)	5 g	5 g	5 g	5 g
European Bacteriological Agar (Conda)		15 g	15 g	15 g
Ampicillin (Sigma) (pure)			100 mg	
Chloramphenicol (Sigma) (100 mg/ml in ethanol)			10 mg	
Cycloheximide (Sigma) (100 mg/ml in DMSO)				1 mg

1050

Supplementary Material ~~3~~2. Bacterial strains isolated from Oregon-R *Drosophila melanogaster* and used in the experiment.

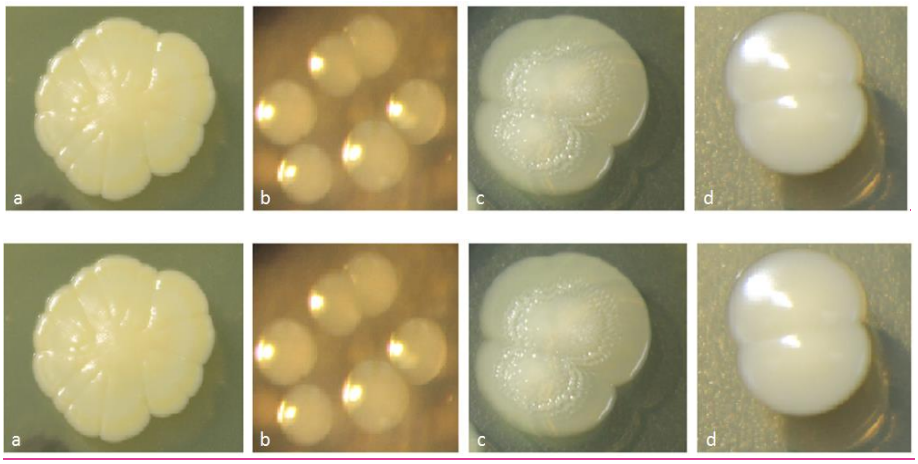


Figure ~~S3: Bacterial~~S2: bacterial strains isolated from Oregon-R *Drosophila melanogaster* and used in the experiment. (a) *Staphylococcus* sp.; (b) *Enterococcus* sp.; (c) Enterobacteriaceae.; (d) Actinobacteria.

Mis en forme : Police :12 pt

Supplementary Material 3. Live yeast as a prerequisite to *D. melanogaster* larvae survival on pristine grape berry.

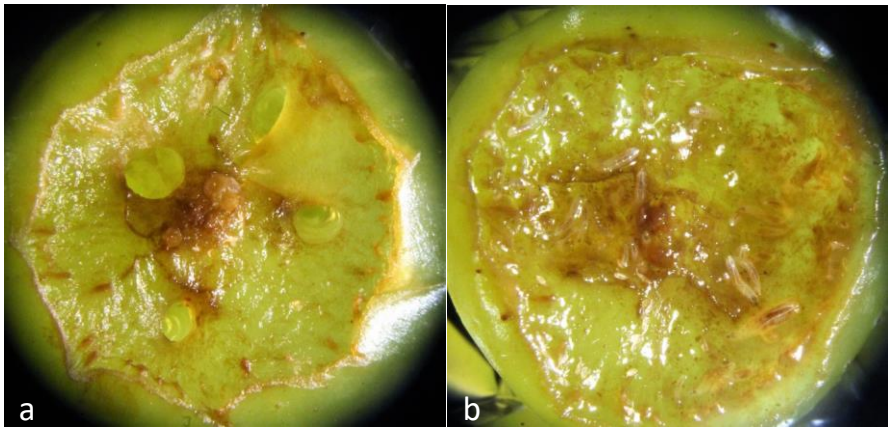
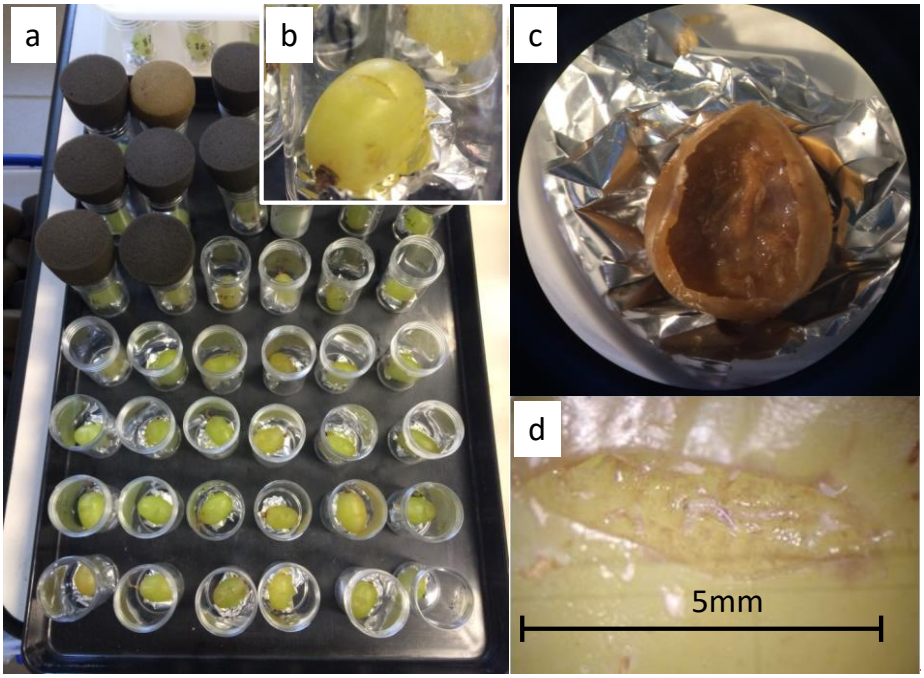


Figure S3: live yeast is necessary for the survival of *D. melanogaster* larvae on pristine grape berry. Prior to the experiment, we investigated survival of *D. melanogaster* larvae on fresh grape berries. Twenty bacteria-free *D. melanogaster* eggs were deposited next to an artificial wound with or without the bacterial isolates and *Saccharomyces cerevisiae*. In absence of yeast, larvae died quickly after hatching, with or without bacteria (Figure S1a). When live yeast was added to the system, numerous larvae developed up to the 3^{thrd} instar (Figure S1b), when we stopped monitoring.

Mis en forme : Justifié, Interligne : Double

Mis en forme : Police :Gras

Supplementary Material 4. Experimental design for the grape berry environment.



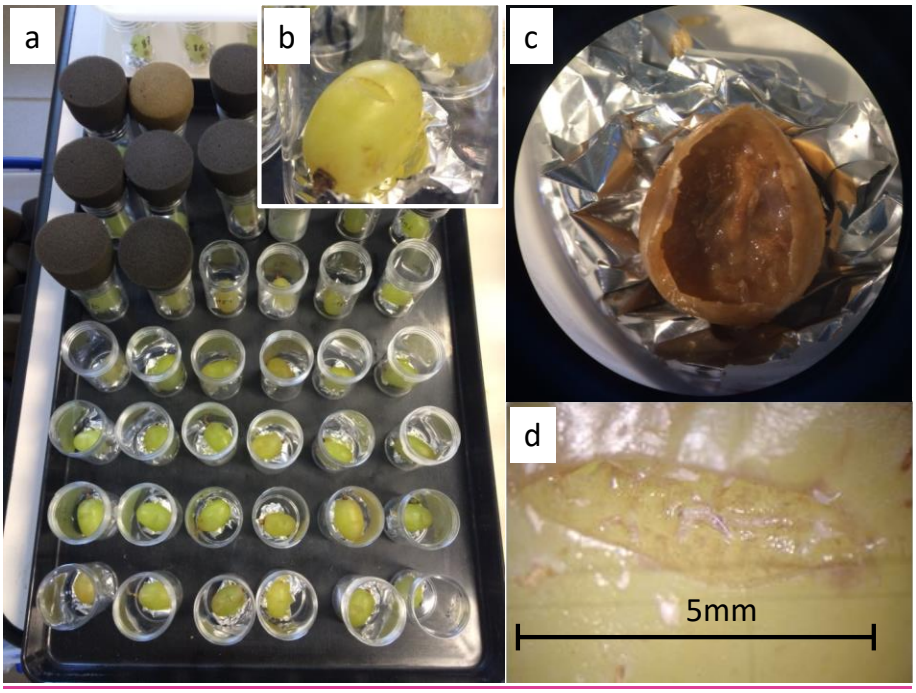


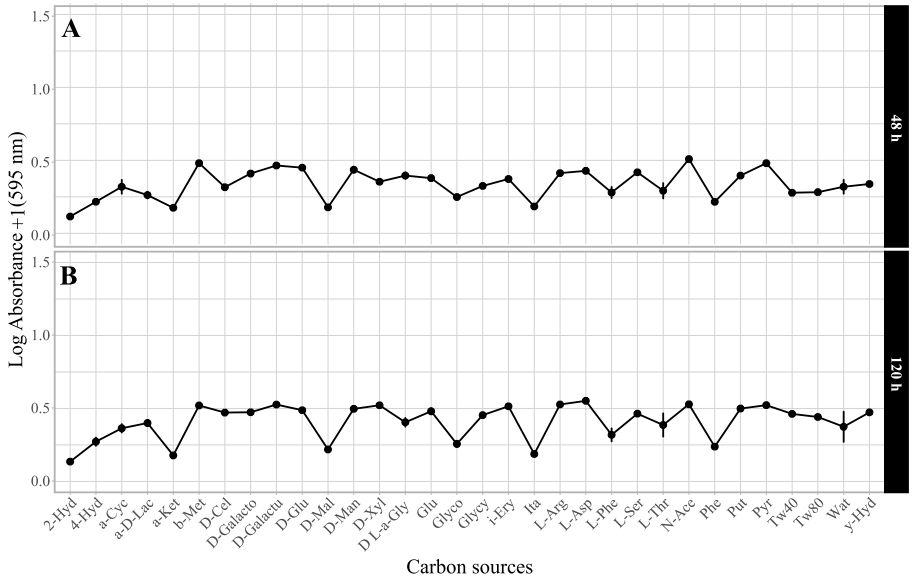
Figure S4: ~~Experimental~~experimental design for the grape berry environment. (a) Experimental block for grape berry treatments, (b) Experimental unit with grape berry, (c) Decaying grape berry with live yeast, bacteria and larvae, (d) Egg cases visible near berry incision and active larvae in fruit flesh.

Supplementary Material 5. Bacterial physiological profiles.

Mis en forme : Police :16 pt

Text S5:

Eco Microplates (Biolog) were used to have an overview of the metabolic 'fingerprint' of the Enterobacteriaceae, the Actinobacteria isolate and the Actinobacteria variant. A fixed number of fresh bacteria cells suspended in sterile PBS were inoculated in well with one of 31 different carbon sources. Each combination Bacterial isolate*Carbon source was replicated three times. The plates were incubated at 25 °C and the absorbance at 595 nm was measured with a Multiskan GO spectrometer (Thermo Scientific) after 48 h and 120 h. A tetrazolium dye included with each carbon source entrained the production of red color when bacterial respiration occurred, i.e. when the carbon source was used. Variations of red color among carbon sources allowed establishing a physiological profile of each bacterial isolate.



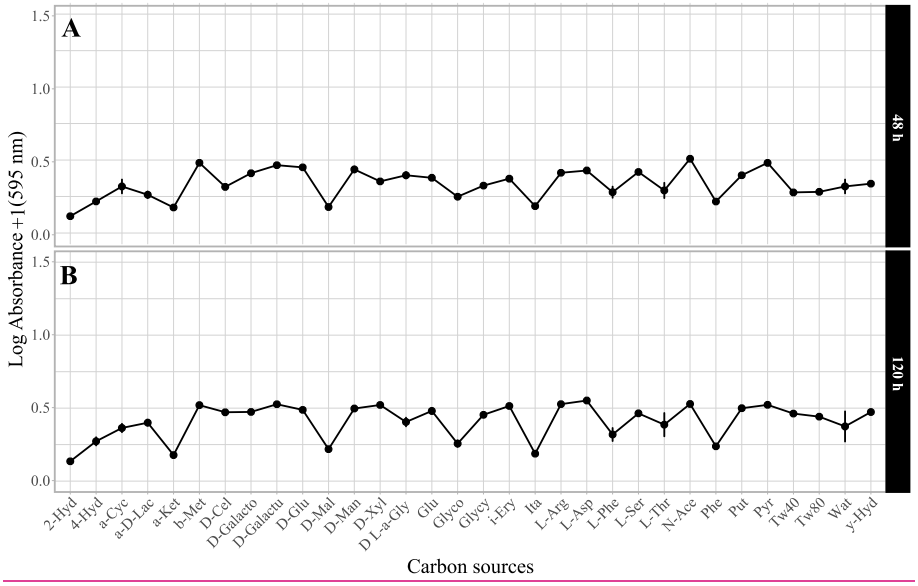


Figure S5A: physiological profile of the Enterobacteriaceae isolate after (A) 48 h- and (B) 120 h-long exposure to different carbon sources. Symbols indicate means; error bars indicate standard errors around the mean. X-axis labels correspond to abbreviations of tested carbon sources, with

Mis en forme : Justifié

2-Hyd for 2-Hydroxy Benzoic Acid; 4-Hyd for 4-Hydroxy Benzoic Acid; a-Cyc for α-Cyclodextrin; a-D-Lac for α-D-Lactose; a-Ket for α-Ketobutyric Acid; b-Met for β-Methyl-D-Glucoside; D-Cel for D-Cellobiose; D-Galacto for D-Galactonic Acid γ -Lactone; D-Galactu for D-Galacturonic Acid; D-Glu for D-Glucosaminic Acid; D-Mal for D-Malic Acid; D-Man for D-Mannitol; D-Xyl for D-Xylose; D L-a-Gly for D,L-α-Glycerol Phosphate; Glu for Glucose-1-Phosphate; Glyco for Glycogen; Glycy for Glycyl-L-Glutamic Acid; i-Ery for i-Erythritol; Ita for Itaconic Acid; L-Arg for L-Arginine; L-Asp for L-Asparagine; L-Phe for L-Phenylalanine; L-Ser for L-Serine; L-Thr for L-Threonine; N-Ace for N-Acetyl-D-Glucosamine; Phe for Phenylethylamine; Put for Putrescine; Pyr for Pyruvic Acid Methyl Ester; Tw40 for Tween 40, Tw80 for Tween 80, Wat for Water and y-Hyd for γ-Hydroxybutyric Acid.

Mis en forme : Police :10 pt

~~**Figure S5.1. Physiological profile of the Enterobacteriaceae isolate after 48 h- and 120 h-long exposure to different carbon sources.**~~

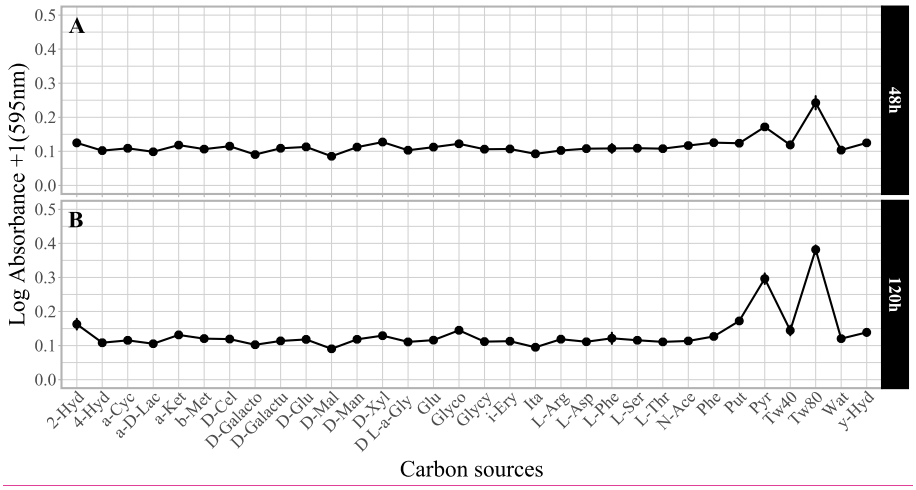
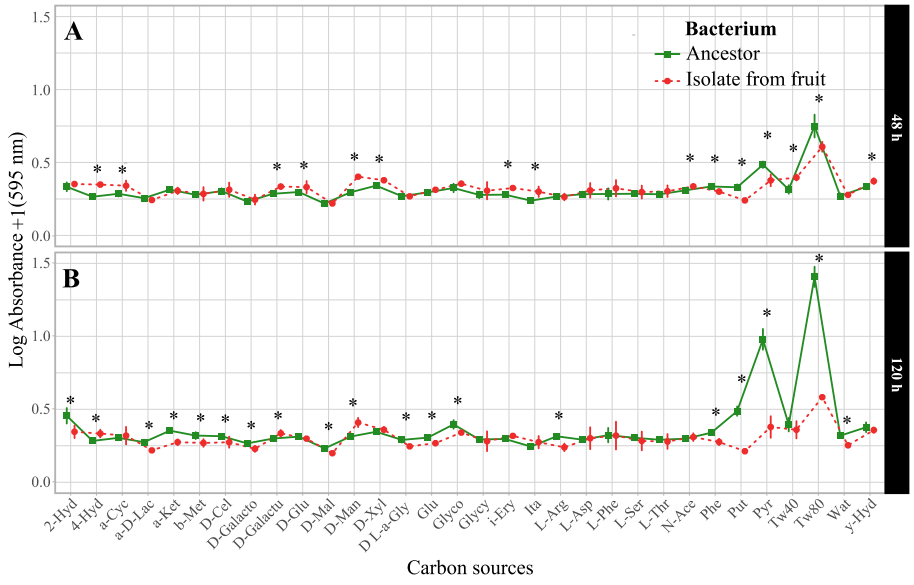


Figure S5B: physiological profile of the Actinobacteria isolate after (A) 48 h- and (B) 120 h-long exposure to different carbon sources. Symbols indicate means; error bars indicate standard errors around the mean. X-axis labels correspond to abbreviations of tested carbon sources, with 2-Hyd for 2-Hydroxy Benzoic Acid; 4-Hyd for 4-Hydroxy Benzoic Acid; a-Cyc for α-Cyclodextrin; a-D-Lac for α-D-Lactose; a-Ket for α-Ketobutyric Acid; b-Met for β-Methyl-D-Glucoside; D-Cel for D-Cellobiose; D-Galacto for D-Galactonic Acid γ -Lactone; D-Galactu for D-Galacturonic Acid; D-Glu for D-Glucosaminic Acid; D-Mal

Mis en forme : Justifié

for D-Malic Acid; D-Man for D-Mannitol; D-Xyl for D-Xylose; D L-a-Gly for D,L-α-Glycerol Phosphate; Glu for Glucose-1-Phosphate; Glyco for Glycogen; Glycy for Glycyl-L-Glutamic Acid; i-Ery for i-Erythritol; Ita for Itaconic Acid; L-Arg for L-Arginine; L-Asp for L-Asparagine; L-Phe for L-Phenylalanine; L-Ser for L-Serine; L-Thr for L-Threonine; N-Ace for N-Acetyl-D-Glucosamine; Phe for Phenylethylamine; Put for Putrescine; Pyr for Pyruvic Acid Methyl Ester; Tw40 for Tween 40, Tw80 for Tween 80, Wat for Water and y-Hyd for γ-Hydroxybutyric Acid.

Supplementary Material 6.



~~Figure S5.2. Physiological profiles of the Actinobacteria ancestor and the isolate from grape berry (vial n°419) after 48 h- and 120 h-long exposure to different carbon sources.~~

Joint analysis of bacterial effects on adult age and size for each sex.

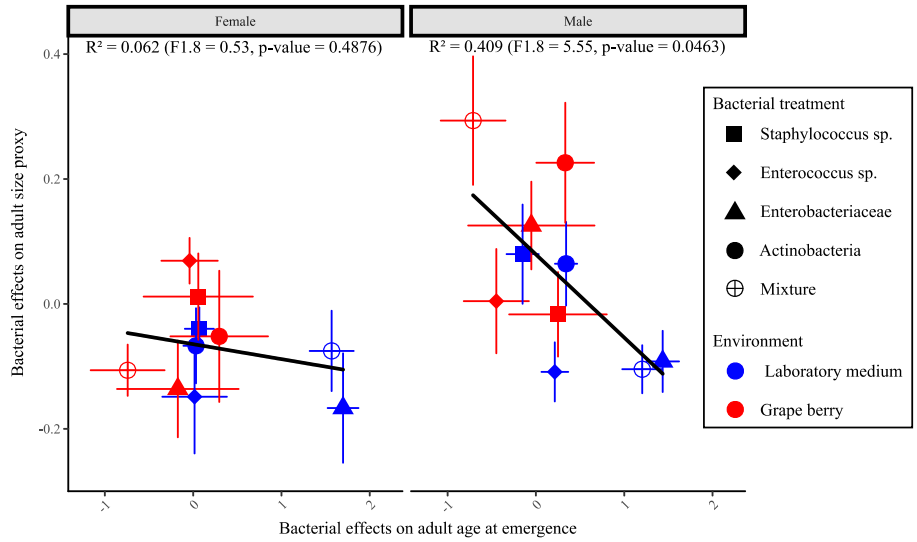


Figure S6: relationship between bacterial effects on age of emerging adults and bacterial effects on adult size, in females and males. As the linear regressions were not significantly different (Interaction Sex*Speed: $F_{1,16} = 2.93$, $p = 0.11$), data was pooled for the analysis reported in the main text. Symbols indicate the phenotype mean of each combination of bacterium and environment. Error bars mark the SE of the mean for both axes.

Symbols indicate means; error bars indicate standard errors around the mean. (*) symbols indicate significant difference between the Actinobacteria ancestor and the isolate from fruit exposed the same duration to a same carbon source. X axis labels correspond to abbreviations of tested carbon sources, with 2-Hyd for 2-Hydroxy Benzoic Acid; 4-Hyd for 4-Hydroxy Benzoic Acid; a-Cyc for α-Cyclodextrin; a-D-Lac for α-D-Lactose; a-Ket for α-Ketobutyric Acid; b-Met for β-Methyl-D-Glucoside; D-Cel for D-Cellobiose; D-Galacto for D-Galactonic Acid γ- Lactone; D-Galactu for D-Galacturonic Acid; D-Glu for D-Glucosaminic Acid; D-Mal for D-Malic Acid; D-Man for D-Mannitol; D-Xyl for D-Xylose; D L-a-Gly for D,L-α-Glycerol Phosphate; Glu for Glucose-1-Phosphate; Glyco for Glycogen; Glycy for Glycyl-L-Glutamic Acid; i-Ery for i-Erythritol; Ita for Itaconic Acid; L-Arg for L-Arginine; L-Asp for L-Asparagine; L-Phe for L-Phenylalanine; L-Ser for L-Serine; L-Thr for L-Threonine; N-Ace for N-Acetyl-D-Glucosamine; Phe for Phenylethylamine; Put for Putrescine; Pyr for Pyruvic Acid Methyl Ester; Tw40 for Tween 40, Tw80 for Tween 80, Wat for Water and y-Hyd for γ-Hydroxybutyric Acid.

Mis en forme : Police :10 pt

Supplementary Material 6. Survival on fruit without larvae of the Actinobacteria ancestor and the isolate retrieved from fruit at the end of the experiment.

Text S6:

Two concentrations (10,000 and 1,000,000 live cells) of the ancestral Actinobacteria or the Actinobacteria retrieved from the grape berry (vial n°419) suspended in sterile PBS (Phosphate-Buffered Saline) were inoculated on grape slices. The slices of surface-sterilized berries (Behar et al. 2008) were contained in petri dishes with plain agar and incubated at 24 °C. Eight grape slices were sampled and homogenized per treatment after 24 h or 72 h. Numbers of CFUs (Colony Forming Units) were measured on LB agar plates after serial dilutions.

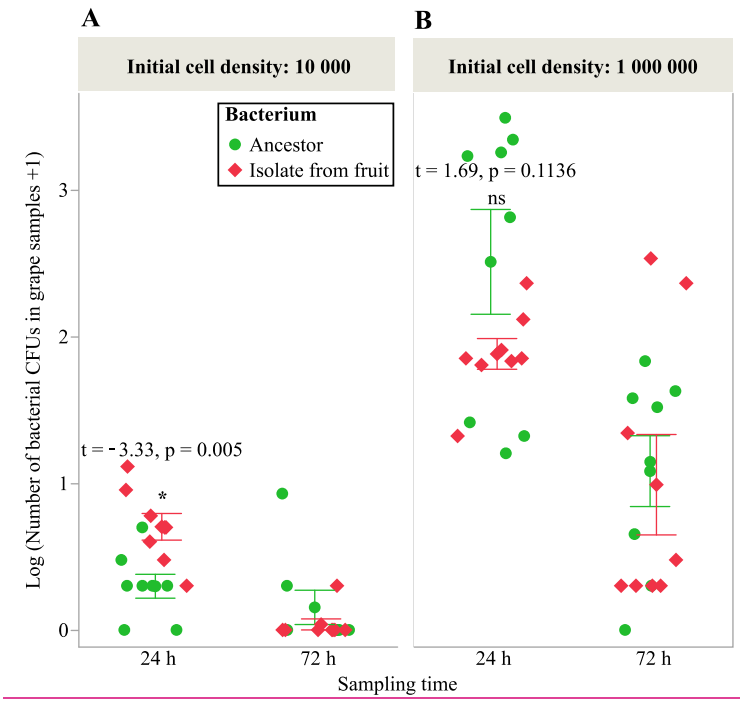
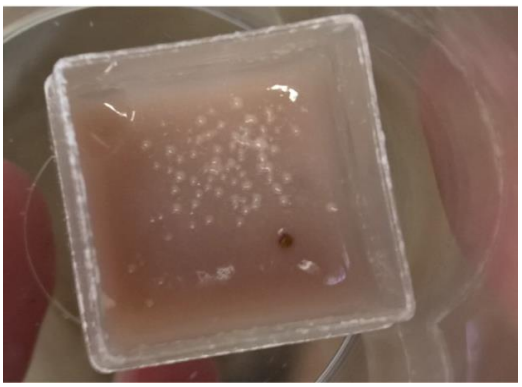


Figure S6: number of CFUs of the Actinobacteria ancestor and the Actinobacteria isolate from grape berry (vial n°419) in samples of grape slices after 24 h- and 72 h-long incubation for two initial cell concentrations. Symbols indicate individual observations; error bars indicate standard errors around the mean. (*) symbol indicates marginally significant difference between the Actinobacteria ancestor and the isolate from fruit.

Mis en forme : Gauche

Supplementary Material 7. Laboratory medium inoculated with the Enterobacteriaceae.



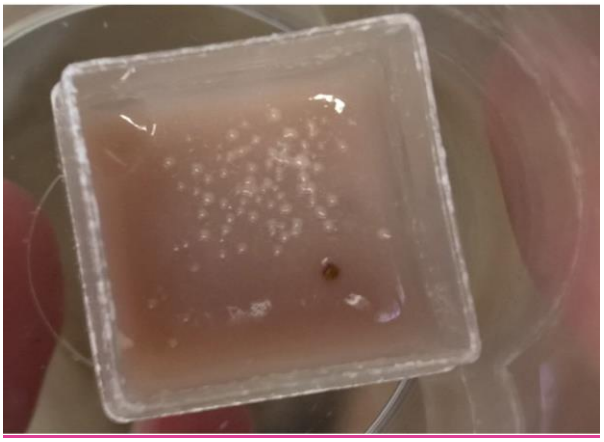


Figure S7: bacterial growth at the surface of laboratory medium five days after Enterobacteriaceae inoculation.

This picture was taken in absence of larvae, but similar growth could be observed in their presence.

Mis en forme : Police :16 pt, Gras, Anglais (États-Unis)

Mis en forme : Justifié