Microbes are potential key players in the evolution of life histories and aging in *Caenorhabditis elegans*

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Abstract

Microbes can have profound effects on host fitness and health and the appearance of late-onset diseases. Host-microbe interactions thus represent a major environmental context for healthy aging of the host and might also mediate trade-offs between life-history traits in the evolution of host senescence. Here we have used the nematode *Caenorhadbitis elegans* to examine whether host-microbe interactions might modulate the evolution of host life-history and aging. We first characterized the effects of two non-pathogenic *Escherichia coli* strains, a pathogenic *E. coli* strain and a pathogenic *Serratia marcescens* strain on the reproductive schedule and survival of an outbred *C. elegans* population, to be used in an experimental evolution study. Secondly, to investigate the dependency of these effects on host genotype, we assayed population growth rates and survival of five representative *C. elegans* inbred strains in response to these microbes. Our results show that host-microbe interactions have a substantial, host-genotype-dependent impact on reproductive effort and survival of the nematode host. Although pathogenic bacteria reduced host survival, as expected, they did not necessarily decrease host fertility or population growth rate. Given such microbe-specific genotypic differences in host life history, we predict that the evolution of reproductive schedules and senescence in this system might be critically contingent upon host-microbe interactions, a hypothesis which we will be testing using experimental evolution in future work.

Key words: *C. elegans*; microbes; host-microbe interactions; life-history evolution; aging; trade-offs.

Introduction

Microbes are thought to have major effects on the evolution and speciation of host populations due to their ubiquitous presence and ability to influence host physiology and health (Bordenstein et al. 2001; Zilber-Rosenberg and Rosenberg 2008; McFall-Ngai et al. 2013). While microbes are best known for their pathogenic or mutualistic effects, they can also modulate how hosts perceive and respond to stressful conditions. This has been observed, for example, in contexts as diverse as viral infections (Martinez et al. 2014), the autoimmune response (Langan et al. 2019), drug therapy (Pryor et al. 2019), metabolic dysfunction (Ussar et al. 2016), exposure to high temperatures (Xie et al. 2013; Howells et al. 2016), and chemical toxicity (Coryell et al. 2018). Microbes can therefore impact adaptation of host populations to conditions that are apparently unrelated to the host-microbe interaction itself (Martinez et al. 2016; Faria et al. 2016; Bates et al. 2021; Hoang et al. 2021). This ability of microbes to modulate host adaptation to various stressors might thus play a fundamental but still poorly understood role in shaping the evolution of host life history and aging.

The progressive loss of physiological function leading to a decline in fecundity and increased mortality, which defines aging, can be explained by the reduced efficacy of selection in purging mutations that have deleterious effects late in life (Fisher 1930; Haldane 1941; Medawar 1946, 1952; Williams 1957; Hamilton 1966; Rose 1991; Kirkwood and Austad 2000; Flatt and Schmidt 2009; Flatt and Partridge 2018). A major mechanism underlying the evolution of aging is antagonistic pleiotropy, i.e., the existence of alleles with antagonistic effects upon early and late life-history traits which lead to genetic trade-offs between fitness components (Medawar 1946, 1952; Williams 1957; Stearns 1989; Rose 1991; Flatt and Promislow 2009; Flatt 2020). Under this model, aging evolves because strong selection for beneficial fitness effects early in life outweighs the deleterious effects of these

alleles late in life when selection is weak (e.g., Williams 1957). A large body of work in numerous organisms, for example the nematode worm *Caenorhabditis elegans* (Anderson et al. 2011), the fruit fly *Drosophila melanogaster* (reviewed in Flatt 2020), or the fish *Poecilia reticulata* (Reznick et al. 1990), has revealed antagonistic pleiotropy underlying trade-offs by showing correlated responses to selection in major fitness components such as developmental rate, early and late-fecundity, and lifespan.

Even when populations harbor genetic variation sustaining antagonistic pleiotropic effects, they may not always experience phenotypic trade-offs nor correlated responses of life-history traits to selection, as these also depend on environmental factors (Giesel et al. 1982; Stearns 1989; Ackermann et al. 2001; Sgró and Hoffmann 2004; Gutteling et al. 2007; Swanson et al. 2016). For this reason, microbes are likely to play a relevant but underappreciated role in the evolution of aging, especially given their known effects on life-history traits (Little et al. 2002; Deckaester et al. 2003; Brummel et al. 2004; Vale and Little 2012; Leroy et al. 2012; Laughton et al. 2014; Parker et al. 2014; Diaz et al. 2015; Zurowski et al. 2020) and their evolution (Sorci and Colbert 1995; Gibson et al. 2015; Walters et al. 2020). Causal relationships between the composition of the intestinal microbiome and aging observed in humans (Claesson et al. 2011) and other organisms (Clark et al. 2015; Sonowal et al. 2017; Bárcena et al. 2019) are consistent with this notion.

Studies with the *C. elegans* model hold great promise for an improved understanding of the interplay between host-microbe interactions and the evolution of aging. For example, the worm system has been extensively used in the identification of the genetic pathways underpinning aging and longevity (Garsin et al. 2003; Kurz and Tan 2004; Antebi 2007; Evans et al. 2008; Leroy et al. 2012), many of which are shared with humans (Kurz and Tan 2004). At the same time, *C. elegans* has also been a valuable tool for studying host-microbe interactions (Tan et al. 1999; Abbalay et al. 2000; Garsin et al. 2003; Schulenburg et al. 2004;

Coolon et al. 2009; Leroy et al. 2012; Diaz et al. 2015; Dirksen et al. 2016; Schulenburg and Félix 2017) and how such interactions regulate host development, reproduction, metabolism, immunity and lifespan (MacNeil et al. 2013; Pang and Curran 2014; Chan et al. 2019). Notably, links between immunity and aging have been established in *C. elegans* (Kurz and Tan 2004), for example in the context of lifespan expansion obtained with specific bacterial metabolites (Virk et al. 2012; Han et al. 2017) or by transferring worms from their regular food source (*Escherichia coli* OP50) to other bacteria such as *Bacillus subtilis* (Aballay *et al.* 2000; Portal-Celhay et al. 2012; Donato et al. 2017). Moreover, long-lived *C. elegans* mutants have been found to be resistant to pathogenic bacteria such as *Enterococcus faecalis* and *Staphylococcus aureus* (Garsin *et al.* 2003).

The extent to which the evolution of life histories and senescence in the nematode host might be contingent upon specific host-microbe interactions remains poorly understood. To begin to address this question, we sought to examine how different pathogenic and non-pathogenic bacteria impact the reproductive schedule and survival of *C. elegans*. To this end, we first measured survival and fertility throughout the reproductive lifespan of a genetically diverse population of *C. elegans* in response to two non-pathogenic *E. coli* strains, a pathogenic *E. coli* strain and a pathogenic *Serratia marcescens* strain. Second, we sought to investigate whether microbial effects on host life history might depend on host genotype, i.e., whether host life history might be affected by interactions between microbe strain, host genotype and environment (i.e., reproductive timing). To do so, we assayed the population growth rates of five isogenic *C. elegans* strains, derived from different wild isolates, at the beginning of the reproductive period and past the reproductive peak in response to the same four bacterial strains mentioned above. Our results demonstrate that host-microbe interactions can have profound, host-genotype-dependent effects on reproductive effort and survival in *C. elegans*. Based on these results we conjecture that bacterial symbionts can modulate the

outcome of host life-history evolution in response to selection for different reproductive schedules, a prediction which we aim to test with experimental evolution in future work.

Methods

BACTERIAL STRAINS

Bacterial strains used in our experiments included two commonly employed non-pathogenic *Escherichia coli* strains, OP50 (Brenner 1974) and HT115(DE3) (Timmons et al. 2001), and two pathogenic strains, E. coli IAI1 (Picard et al. 1999; Diard et al. 2007) and *Serratia marcescens* Db11 (Flyg et al. 1980; Kurz et al. 2003). *E. coli* HT115(DE3) had been used as food during the establishment of the *C. elegans* D00 population described below. The strains *E. coli* HT115(DE3), *E. coli* OP50, and *S. marcescens* Db11 were obtained from the Caenorhabditis Genetics Center (CGC), and the *E. coli* IAI1 strain was kindly provided by Ivan Matic.

NEMATODE POPULATIONS

To assay life-history responses to the above-mentioned microbe strains we used an outbred experimental *C. elegans* population (D00) and 5 wild isolates (N2, CB4852, CB4855, CB4856, PX174). The D00 population was first described by Theologidis et al. (2014); it is a genetically diverse dioecious population with males and females, established by introgression of the *fog-2(q71)* mutant allele (Schedl and Kimble 1988) into the genetic background of a previously laboratory-adapted androdioecious population consisting of males and hermaphrodites (Teotónio et al. 2012; Chelo and Teotónio 2013). Throughout laboratory adaptation, worms were provided with *E. coli* HT115(DE3) as a food source and evolved under discrete (non-overlapping) generations imposed by a 4-day life-cycle, herein referred to as “early reproduction”. The D00 population is characterized by obligate outcrossing; its standing genetic variation results from an initial mixture of 16 isogenic strains, which were

chosen to represent a significant proportion of the known genetic diversity in *C. elegans* (Rockman and Kruglyak 2009; Teotónio et al. 2012). Here we have analyzed 5 of these 16 isolates (N2, CB4852, CB4855, CB4856, PX174).

GROWTH CONDITIONS

Bacteria were grown overnight in NGM-lite solid media at 37 ºC from LB-grown cultures. Nematode maintenance followed previously described protocols (Stiernagle 1999; Chelo 2014). On day one, L1 larvae were seeded on NGM-lite supplemented with ampicillin (100 mg/ml), carrying a confluent lawn of *E. coli* HT115(DE3). 10^3 larvae were used per plate, and development proceeded at 20ºC and 80% (RH) for 72 hours, until day four of the life-cycle. Plates were washed with M9 buffer and a KOH:sodium hypochlorite solution was added ("bleaching") to kill adults and larvae but allowing unhatched embryos to survive. Eclosion of first-stage larvae (L1) occurred overnight in 4 ml of M9 buffer with 2.5 mg/ml of tetracycline under constant shaking.

REPRODUCTIVE SCHEDULE AND SURVIVAL OF THE D00 POPULATION

Daily offspring number and survival were monitored to study the effects of different bacteria on individuals of the D00 population. Frozen (-80 ºC) stock populations were thawed and maintained for two generations prior to the assay. To set up the experiment, 10^3 L1 individuals were seeded on NGM-lite plates carrying each of the four bacteria and incubated until the beginning of day 3 (48 hours later). From each plate, 30 female larvae were placed (one larva per well) onto 24-well plates with antibiotic-free NGM-lite and matching bacteria, which had been grown from a 5 μl inoculum. Adult males from the same population and conditions, but which had been developing for one extra day, were added to the wells (two males per well). Individuals were transferred to fresh medium every 12 hours until day 6, and every 48 hours after day 6, until all individuals were found dead or considered to be missing. During the first five days, males that had died (or were missing) were replaced to ensure

mating and fertilization. After removal of adults, plates were kept in the incubator for one day and then transferred to 4ºC for a maximum of two days before counting L2-L3 larvae under the stereoscope with 10x-30x magnification. These data were used to determine total fertility (lifetime reproductive success, LRS), variation in fertility through time and the age at first reproduction (AFR). Survival was scored based on daily observations during the entire period of the experiment. Monitoring of missing or dead females occurred at the time of transfer, and individuals were considered dead in the absence of movement or response when being gently touched with a platinum wire.

GROWTH RATE OF THE D00 POPULATION AND OF INDIVIDUAL GENOTYPES

Population growth rate in response to each of the four bacterial strains was measured at two different times: at 72 hours after L1 seed (transition from day 3 to day 4), i.e., within hours of reaching sexual maturity ("early reproduction"; Anderson et al. 2011) and at 114 hours post-seed (day 5; referred to as "delayed reproduction"). Frozen populations were thawed and maintained for two generations under standard maintenance conditions, plus one generation in presence of each bacterial strain for acclimatization. In the fourth generation, L1 larvae were seeded on NGM-lite plates (10^3/plate) with a lawn of each bacterial strain and allowed to develop for 72 or 114 hours. Following our standard maintenance protocol, cultures were bleached and the number of the live L1s was estimated the following day. Each estimate was obtained by pooling individuals from three plates. The D00 population and each of the five isolates (N2, CB4852, CB4855, CB4856, PX174) were assayed in independent experimental blocks. In the assays with the isolates, each block included the N2 strain feeding on *E. coli* HT115(DE3) as a common reference, the four different bacteria and the two time points. For each bacterial strain and each time point, we used five technical replicates for D00 and N2 and four technical replicates for each of the other four isolates.

SURVIVAL OF INDIVIDUAL GENOTYPES

The effect of the four bacterial strains on survival was assayed for each of the five *C. elegans* isolates (CB4852, CB4855, CB4856, PX174). After thaw and growth for two generations under standard maintenance conditions, L1 larvae were seeded on NGM-lite media (10^3 individuals/plate) with a lawn of each of the four bacteria. 48 hours later (day 3), L4 hermaphrodites were placed on 24-well NGM-lite plates (five individuals per well), with the corresponding bacteria, as described above for the survival assay of the D00 population. Each of the four non-N2 isolates was assayed in a different experimental block, which also included included N2 as a common reference. Four plates were used per block, and every plate included all four bacterial strains. Both the N2 and one of the non-N2 isolates were used in every plate, with N2 individuals occupying one fourth of the total number of wells. This experimental design enabled the estimation of plate effects within a block. In total, 480 individuals were assayed in each block, with 120 being N2 individuals and 360 individuals from one of the other isogenic strains.

DATA ANALYSIS

Statistical analyses were performed in *R* (*R* Core Team 2019). Supplementary files with analyses and *R* code can be found at *FigShare* (see 10.6084/m9.figshare.15022566 for Supplementary Figures; and 10.6084/m9.figshare.15022599 for Supplementary Data and analysis scripts).

For fertility data, observations of 12h intervals were collapsed into daily measures until day 6 and into a single bin beyond that time. Thus, fertility reported for day 3 refers to embryos laid between 48 h and 72 h post-L1 seed, between 72 h to 96 h for day 4, between 96 h to 120 h for day 5, between 120 h and 144 h for day 6, and 144 h onwards to “day 7”. Model fitting and model comparisons were performed with generalized linear models with appropriate error distributions (see below), and analysis of deviance was used to test for

significance. Parameter estimates were retrieved and tested with *emmeans* and *pairs* function (Lenth 2018). For pairwise comparisons, we used Tukey's post-hoc tests and report adjusted *p*-values. The reproductive schedule of the D00 population was modeled with a negative binomial distribution using the *R* function *glm.nb* in the MASS package. The following model was used: *Fertility ~ Bacteria * Time*, where *Fertility* refers to the number of larvae observed per individual worm during a 24 h period, *Bacteria* represents the four bacterial strains tested, and *Time* is a categorical variable with 5 levels representing the day since the experimental set-up. Post-hoc comparisons were performed between fertility means within each day. Total fertility was modeled with a Poisson distribution using the *glm* function, as follows: *LRS ~ Bacteria, family = "poisson"(link="log"),* where *LRS* is the total number of observed larvae. A Gaussian fit was used to analyze AFR with the following code: *AFR ~ Bacteria, family ="gaussian"*, where AFR (age at first reproduction) refers to the time between L1 seed and the time at which offspring was first observed.

Cox regression (proportional hazards analysis; Cox 1972) was used to test for differences in survivorship, with *E. coli* HT115(DE3) defining the baseline risk. The following model was implemented with the functions *Surv* and *coxph* in the *survival* package in *R* (Therneau 2015): *Surv(S.time,S.event) ~ Bacteria,* with *S.time* being the time at which an individual was found dead or missing (*S.event*), assuming right-censored data. Kaplan-Meier estimation (Kaplan and Meier 1958) was used to estimate survival curves and mean lifespan.

Analysis of population growth rate was carried out using the natural logarithm (ln) of the observed rates. Whenever L1 larvae could not be detected, which would lead to growth rate estimates of zero (two samples; see Supplementary Table 2), values were replaced assuming that one L1 had been observed. To standardize the different blocks with *C. elegans* isolates, the growth rates of *C. elegans* N2 with *E. coli* HT115(DE3) were first estimated in each block and at each time point with a random-effects model using a block-specific baseline.

The following model was implemented in *R*: *log(GrowthRate) ~ Time * Bacteria * Celegans, offset=Block_offset*, where *GrowthRate* is the observed L1 growth rate in consecutive generations, *Time* is the number of hours since L1 seed, *Bacteria* represents the bacterial strains, *Celegans* represents the 5 different isolates, and *Block_offset* is the value of the block effects obtained with N2 and *E. coli* HT115(DE3).

Survival analysis of the *C. elegans* isolates was performed with Cox regression, using mixed-effect models with the *coxme* function in *R* (Thernau 2020) in order to include plate effects. The following model was used: *Surv (S.time,S.event) ~ Bacteria * Celegans + (1| Plate) + Block_offset* (see above). Mean lifespan values based on Kaplan-Meier estimation were corrected by the values obtained for each block with N2 (see Supplementary Fig. 3).

Results

DIFFERENT BACTERIA HAVE SPECIFIC EFFECTS ON THE LIFE-HISTORY SCHEDULE OF A GENETICALLY DIVERSE *C. ELEGANS* POPULATION

Reproduction and survival of the *C. elegans* D00 population were affected by the different bacteria in unique ways (Fig. 1). The presence of different bacteria had a significant effect on *C. elegans* survival (p-value < 0.0001, see Fig. 1A and Supplementary Fig. 1), with higher mortality risks observed in the presence of the pathogenic strains *E. coli* IAI1 ($p = 0.0001$ for IAI1 *vs.* HT115; $p < 0.0001$ for IAI1 *vs.* OP50) and *S. marcescens* Db11 ($p = 0.02$ for Db11 *vs.* HT115; $p = 0.005$ for Db11 *vs.* OP50). Interestingly, no consistently detrimental (i.e., pathogenic) effects were observed for fertility, even though fertility did vary with the different bacteria (Fig. 1B-C). Significant differences among bacterial strains were found for lifetime fertility ($p < 0.0001$, Fig. 1B), with the highest brood size being observed with *E. coli* HT115(DE3) (371 ± 4, mean ± standard error), followed by *E. coli* OP50 (185 ± 2), *E. coli*

IA1 (177 ± 2) and *S. marcescens* Db11 which resulted in a markedly reduced lifetime fertility (61 ± 1). These differences were also reflected in the reproductive schedule (Fig. 1C), as revealed by a significant time by bacteria interaction (likelihood ratio test, LRT = 42.5, df = 12, $p < 0.001$). Although fertility was always maximized at day 4, the relative contribution of offspring produced before and after this peak day was dependent on the bacterial strains. For instance, with *E. coli* HT115(DE3) the higher mean estimates of fertility observed throughout the entire reproductive lifespan of the host only become statistically significant after day 5 (pairwise comparisons, adjusted *p*-values < 0.05). In contrast, the initially diminished fertility of *S. marcescens* Db11 was no longer different from the majority of values observed with the three *E. coli* strains from day 4 onwards (Fig. 1C). Interestingly, comparing the start of offspring production of *S. marcescens* Db11 with the ones from all *E. coli* indicates a delay in reproduction, suggesting a possible interference with *C. elegans* development (Fig. 1D).

BACTERIA-HOST GENOTYPE INTERACTIONS AFFECT *C. ELEGANS* POPULATION DYNAMICS

The differential effects of the bacterial strains on the fertility dynamics of the host shown in Fig. 1C above suggest that the outcome of selection for early reproduction (reproduction at 72 h) versus delayed reproduction (114 h), and hence the evolution of lifespan, might depend critically on microbe-host genotype interactions. Indeed, we found that the population growth rate (i.e., a fitness proxy) of the D00 population at those two ages was dependent on the bacterial strains (Fig. 2A): this was revealed by a significant time by bacteria interaction (LRT = 2.58, df = 3, $p < 0.001$), with different slopes for *E. coli* IAI1 (CI = -0.028 to -0.014 per hour), *E. coli* HT115(DE3) (CI = -0.011 to 0.002 per hour), *E. coli* OP50 (CI = -0.009 to 0.004 per hour), and *S. marcescens* Db11 (CI = 0.006 to 0.0120 per hour). The main effects of time (LRT = 0.25, df = 1, $p = 0.03$) and bacterial strain (LRT= 1.89, df = 3, $p < 0.0001$) were also significant.

Importantly, the time-bacteria interaction effects on growth rates also varied among the five *C. elegans* genotypes, as revealed by the pervasive crossing of lines in Fig. 2B and confirmed by a significant three-way interaction term (LRT = 17.71, df = 12, $p < 0.0001$) (main effects and all two-way interactions were also significant, not shown). Individual plots by bacterial strain (Supplementary Fig. 2) indicate that these effects were comparable to those obtained for the D00 population. This can be seen, for example, with *E. coli* IAI1 which imposed the largest average decrease in growth rate with time (CI = -0.097 to -0.084 per hour), or with *S. marcescens* Db11 which caused a shallower slope (CI = -0.015 to -0.003 per hour). In this latter case, it is noteworthy that the usual reduction of growth rate with time was reversed for the CB4855 genotype in presence of *S. marcescens* Db11 (Supplementary Fig. 2D).

BACTERIA-HOST GENOTYPE INTERACTIONS MODULATE *C. ELEGANS* LIFESPAN

The different bacteria also affected the adult survival of the *C. elegans* isolates (Fig. 2C and Supplementary Fig. 3, $\chi = 629.6$, df = 3, $p < 0.001$), with a significant bacteria-host genotype interaction on lifespan ($\chi = 72.9$, df = 12, $p < 0.0001$). Interestingly, for one of these isolates we also observed a departure from the overall deleterious effect of the pathogenic bacteria on lifespan: for the PX174 genotype, lifespan in presence of *S. marcescens* (8.7 ± 0.2 days) was clearly not lower than in presence of *E. coli* HT115(DE3) (8.1 ± 0.4 days).

Discussion

Aging represents one of the most compelling examples in evolutionary biology of how trait optimization can have detrimental side effects leading to physiological dysfunction. Here, using the nematode model *C. elegans*, we have confirmed that microorganisms can shape the

host environment in which life-history traits are expressed by showing that distinct bacterial strains have specific effects on host survival, lifetime fertility and reproductive schedule (Fig. 1). Importantly, we also observed that different strains affect the nematode's reproductive dynamics and survival in a host-genotype-specific manner, suggesting that such bacteria-host interactions might affect the evolution of aging in the host.

Given the diverse type of interactions that bacteria can establish with *C. elegans* (Diard et al. 2007; Abalay et al. 2009; Coolon et al. 2009; Baeriswyl et al. 2010; Diaz et al. 2015; Dirksen et al. 2016; Stuhr and Curran 2020), the observed differences in life-history responses to specific bacterial strains (Fig. 1) were not entirely surprising. Nevertheless, the observed bacterial effects did not follow simple expectations based on our knowledge of strain pathogenicity. For instance, while the pathogenic bacteria *E. coli* IAI1 and *S. marcescens* Db11 had clear detrimental effects on host survival, they did not affect fertility; similarly, age at first reproduction was unaffected in presence of *E. coli* IAI1. Another illustration of this are *E. coli* IAI1 and *E. coli* OP50, which imposed the highest and lowest death rates (Fig. 1A), respectively, but which had similar effects on lifetime fertility (Fig. 1B), in agreement with previous findings (Baeriswyl et al. 2010). Moreover, the recent evolutionary history of the D00 population might also explain some of the observed patterns: *E. coli* HT115(DE3), the bacterium used as the food source during the previous 140 generations of laboratory adaptation (Teotónio et al. 2012; Chelo and Teotónio 2013; Theologidis et al. 2014), led to the highest lifetime fertility observed.

The apparent decoupling of the effects of pathogenic bacteria on *C. elegans* reproduction and survival (also see Diaz et al. 2015) are consistent with the existence of a degree of specificity in how bacteria interact with their nematode host's physiology, such that development, metabolism or immunity can be affected independently (Coolon et al. 2009; MacNeil et al. 2013, Maynard and Weinkove 2020). This may also explain why *C. elegans*

survival can differ considerably even between bacteria that are generally regarded as benign (Brooks et al. 2009; Baeriwisyl et al. 2010; Reinke et al. 2010; Pang and Curran 2014).

Our observation that the time-dependence of host population growth and survival differs among different host isolates in a bacterial-dependent way (Fig. 2) strongly suggests that the evolution of life-history traits might be subject to microbial modulation. In this context, bacteria can be regarded as alternative environments where the fitnesses (here given by population growth rate) of the different genotypes are ranked in an environment-specific manner.

Our results are consistent with several lines of independent evidence showing that in *C. elegans* and/or in other organisms: (i) microbes can influence the expression of life-history traits (Coolon et al. 2009; Storelli et al. 2011; Diaz et al. 2015); (ii) genotype-by-age effects are common (Leips et al. 2006; Viñuella et al. 2010); and (iii) genetic correlations between life-history traits can be subject to modulation by environmental (external) factors (Giesel et al. 1982; Stearns 1989, 1992; Gutteling et al. 2007; Swanson et al. 2016).

A demonstration of a major causal role of microbes as environmental determinants of the evolution of aging could be obtained with an experimental evolution experiment based on selection for delayed reproduction (Figure 3), an approach we are currently taking with *C. elegans*. In such an experiment, different bacterial strains might change the genetic correlations between life-history traits expressed early and late in life. Such an approach might be able to reveal to what extent the expression of genetic life-history trade-offs and the evolution of aging, subject to such trade-offs, are constrained by microbial effects.

AUTHOR CONTRIBUTIONS

M.M., T.F. and I.M.C. conceptualized and designed the project. J.S and I.M.C. collected and analyzed the data. J.S., M.M., T.F. and I.M.C. interpreted the data and wrote the manuscript.

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FIGURE LEGENDS

Figure 1. Different bacteria have specific effects on the reproductive output and survival of the genetically diverse *C. elegans* D00 host population. (**A**) shows hazard rates imposed by the benign *E. coli* HT115(DE3) and *E. coli* OP50 strains or the pathogenic *E. coli* IAI1 and *S. marcescens* Db11 bacteria (means plus standard errors SE). (**B**) shows lifetime reproductive success; and (**C**) shows the reproductive schedule. (**D**) display results for age at first reproduction, given in hours and days after L1 seed (for comparison with other panels in the figure). Shown are means plus SE. Letters above bars indicate group assignment based on post-hoc tests (adjusted p-value < 0.05, see Methods), which in (**C**) were performed within each time period.

Figure 2. Genotype-by-environment (bacteria) interactions affect *C. elegans* population growth and survival. In (**A)**, population growth rates of the genetic variable D00 population, measured at the early (72 h) and delayed reproduction period (114 h), reveal bacteria-specific effects on the temporal dynamics of reproductive output . Similarly, in **(B)**, differences between population growth at both times are bacterial dependent (color code as in (**A**)), but specific for each of the five *C. elegans* genotypes) (significant three-way interaction, *p*-value < 0.001). (**C**) shows that mean lifespan depends on the interaction between *C. elegans* genotype and bacterial type. Letters above symbols show group assignment from significant post-hoc tests (*p*-value < 0.05) obtained with data for each bacterium independently. Mean estimates and SE are shown in (**A**) and (**B**); predicted values are shown in (**C**). Note the logarithmic scale of the *y* axis in (**A**) and (**B**).

Figure 3. Experimental evolution scenarios. Using experimental evolution, different scenarios of life-history evolution may be obtained by comparison with the effects of delayed reproduction in the ancestral population. The figure depicts different hypothetical evolutionary outcomes. The white line shows hypothetical phenotypic values of *C. elegans* populations feeding on a reference bacterial strain (control); black lines show measurements taken while feeding on another strain (pathogenic or benign). In (**A**), host-microbial interactions (HM) reveal the interplay between reproductive timing and bacterial type for the genetically diverse ancestral population. These values are displayed as dashed lines in other plots for comparison. Comparing the ancestral population with derived populations maintained under control conditions (**B**) or selected for delayed fecundity (**C**) in the presence of different bacteria should allow understanding the role of *C. elegans*–bacteria interactions in the evolution of life-history and aging phenotypes (**C**), by first accounting for adaptation to the different bacteria (**B** vs. **C**).

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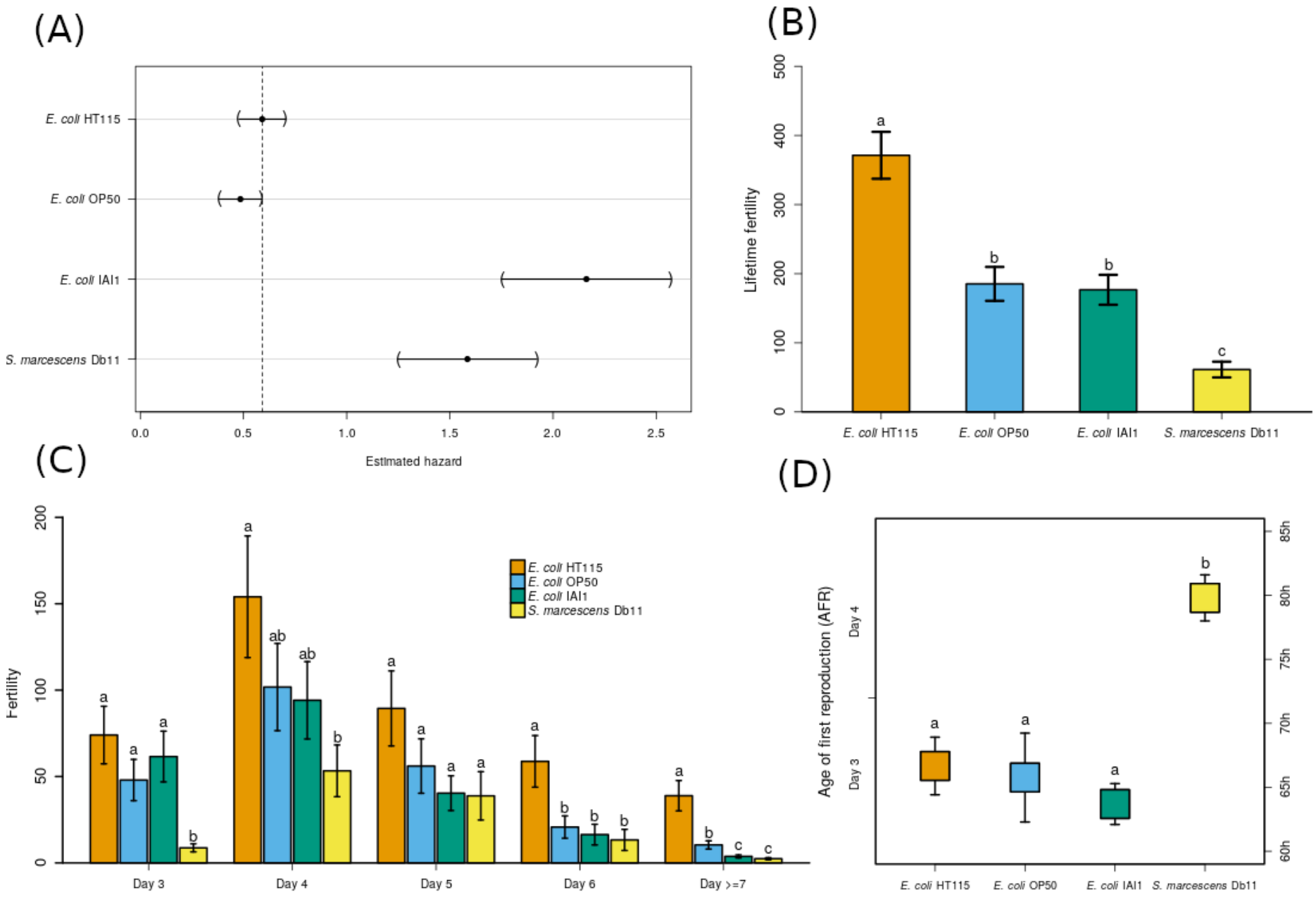
(A)
E. coli HT115
E. coli OP50
E. coli IAI1
S. marcescens Db11
0.0
0.5
1.0
1.5
2.0
2.5
Estimated hazard
(B)
Lifetime fertility
0
100
200
300
400
500
a
b
b
c
E. coli HT115
E. coli OP50
E. coli IAI1
S. marcescens Db11
(C)
Fertility
0
50
100
150
200
E. coli HT115
E. coli OP50
E. coli IAI1
S. marcescens Db11
Day 3
Day 4
Day 5
Day 6
Day >=7
(D)
Age of first reproduction (AFR)
Day 3
Day 4
60h
65h
70h
75h
80h
85h
E. coli HT115
E. coli OP50
E. coli IAI1
S. marcescens Db11

Figure 1.

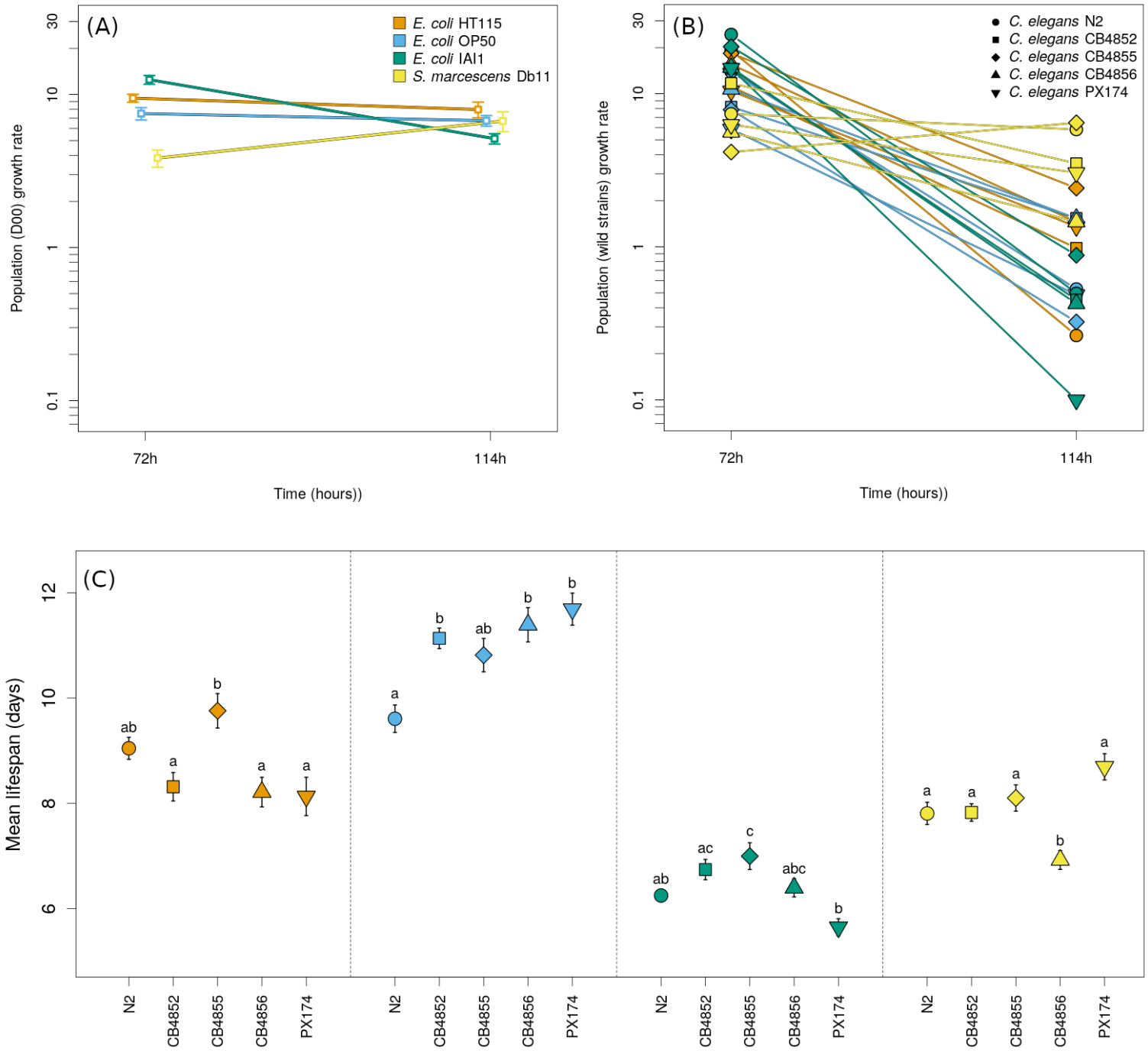
(A)
E. coli HT115
E. coli OP50
E. coli IAI1
S. marcescens Db11
Population (D00) growth rate
30
10
1
0.1
72h
114h
Time (hours))
(B)
C. elegans N2
C. elegans CB4852
C. elegans CB4855
C. elegans CB4856
C. elegans PX174
Population (wild strains) growth rate
30
10
1
0.1
72h
114h
Time (hours))
(C)
Mean lifespan (days)
12
10
8
6
ab
a
b
a
a
a
b
ab
b
b
ab
ac
c
abc
b
a
a
a
b
a
N2
CB4852
CB4855
CB4856
PX174
N2
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Figure 2.

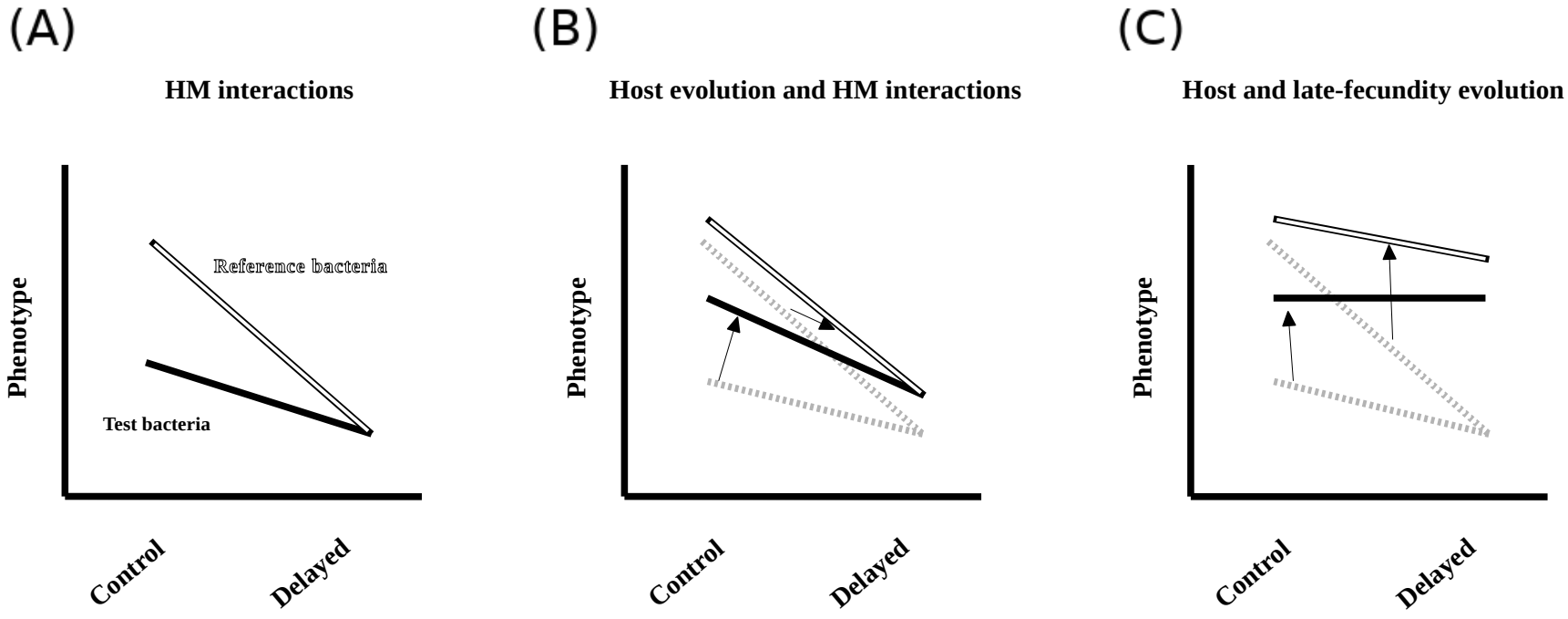
(A)
HM interactions
Phenotype
Reference bacteria
Test bacteria
Control
Delayed
(B)
Host evolution and HM interactions
Phenotype
Control
Delayed
(C)
Host and late-fecundity evolution
Phenotype
Control
Delayed

Figure 3.