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The microbiota influences the *Drosophila melanogaster* life history strategy

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Abstract

Organisms are locally adapted when members of a population have a fitness advantage in one location relative to conspecifics in other geographies. For example, across latitudinal gradients, some organisms may trade off between traits that maximize fitness components in one, but not both, of somatic maintenance or reproductive output. Latitudinal gradients in life history strategies are traditionally attributed to environmental selection on an animal's genotype, without any consideration of the possible impact of associated microorganisms ("microbiota") on life history traits. Here, we show in *Drosophila melanogaster*, a key model for studying local adaptation and life history strategy, that excluding the microbiota from definitions of local adaptation is a major shortfall. First, we reveal that an isogenic fly line reared with different bacteria varies the investment in early reproduction versus somatic maintenance. Next, we show that in wild fruit flies, the abundance of these same bacteria was correlated with the latitude and life history strategy of the flies, suggesting geographic specificity of the microbiota composition. Variation in microbiota composition of locally adapted *D. melanogaster* could be attributed to both the wild environment and host genetic selection. Finally, by eliminating or manipulating the microbiota of fly lines collected across a latitudinal gradient, we reveal that host genotype contributes to latitude-specific life history traits independent of the microbiota and that variation in the microbiota can suppress or reverse the differences between locally adapted fly lines. Together, these findings establish the microbiota composition of a model animal as an essential consideration in local adaptation.

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AUTHOR CONTRIBUTIONS

J.M.C., A.W.W. and P.S.S. designed research. A.W.W., R.C.H., T.B.C., C.J.W., H.W., S.C.P., P.D.N. and J.M.C. performed research. P.S.S. contributed reagents. J.M.C., A.W.W. and R.C.H. analyzed data. A.W.W., S.M.R., A.E.D. and J.M.C. wrote the paper.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

Keywords

acetic acid bacteria; Drosophila; lactic acid bacteria; latitude; life history; local adaptation; microbiome; microbiota

1 | INTRODUCTION

Local adaptation is where individuals in a population are better suited to live in one geography than other members of the same species that live in a different location. Decades of documenting variation in organismal genotypes and phenotypes across geographic clines have established that locally adapted phenotypes result from environmental selection on an organism's genotype (Hereford, 2009; Kawecki & Ebert, 2004; Williams, 1966). The rationale for this study comes from abundant evidence that associated microorganisms ("microbiota") substantially influence animal phenotypes and that their composition varies nonrandomly across geographic clines; thus, the microbiota should be considered in definitions of local adaptation. To date, research on interactions between microbiota and the life history strategy of the host has focused exclusively on how inter- and intraspecific variation in host life history strategies influences the microbiota (Emmett, Youngblut, Buckley, & Drinkwater, 2017; Neave et al., 2017). The reverse question—the impact of the microbiota on the life history strategy of the host—has, to our knowledge, rarely been considered (Kirschman & Milligan-Myhre, 2019; Macke, Tasiemski, Massol, Callens, & Decaestecker, 2017) and has not been investigated empirically.

Life history trade-offs have long been recognized as a widespread feature of local adaptation and have been the focus of many empirical studies (Hereford, 2009; Stearns, 1992). An animal's life history reflects its allocation of resources and time to maximize reproductive output, subject to natural selection and trade-offs along a "fast–slow" continuum (Lemaitre et al., 2015; Promislow & Harvey, 1990; Ricklefs & Wikelski, 2002). At the "fast" end, organisms develop to reproductive maturity more quickly and have high early fecundity, whereas a "slow" lifestyle favours somatic maintenance and lower initial reproduction across longer lifespan (MacArthur & Wilson, 1967; Pianka, 1970). These insights have been developed in the context of an environment-genotype centric framework, focused on geography-specific environmental selection on the organismal genotype mediated, for example, by temperature or photoperiod (Keller, Levsen, Ingvarsson, Olson, & Tiffin, 2011; Munch & Salinas, 2009). The consequent variation in genotype has been linked to various physiological and behavioural characters collectively described as the pace of life syndrome (Reale et al., 2010; Ricklefs & Wikelski, 2002).

The taxonomic identity and function of the animal microbiota can substantially influence animal life history traits (e.g., development rate, fecundity, lifespan) and their correlated physiological traits (Adair, Wilson, Bost, & Douglas, 2018; McFall-Ngai et al., 2013; Mushegian, Walser, Sullam, & Ebert, 2018; Shapira, 2017; Smith, McCoy, & Macpherson, 2007). Thus, factors that modify the microbiota composition can dramatically alter the adaptive traits of organisms in wild or laboratory settings. Relative to local adaptation, microbiota composition in some animals can vary across geographic clines such as latitude

or altitude (Suzuki, Martins, & Nachman, 2019; Suzuki & Worobey, 2014). Currently, it is not clear if geographic patterns in microbiota composition are related to local adaptation of their hosts, but this idea is suggested by previous demonstrations that some animals can mediate their phenotypes by genetic control of their microbiota (Chaston, Dobson, Newell, & Douglas, 2016; Goodrich et al., 2014), and that the microbiota can drive rapid host evolution in a wild setting (Rudman et al., 2019). Thus, while the microbiota has the capacity to augment host genetic adaptations, clear demonstrations of this phenomenon in locally adapted populations are lacking.

Drosophila melanogaster is an excellent system to address the impact of the microbiota on host life history strategy because both its life history and microbiota are well studied. Considering its life history first, trade-offs and their role in local adaptation have been demonstrated, especially in relation to latitudinal clines in allele frequencies for fitness-associated traits (Lee et al., 2011; Parkash, Rajpurohit, & Ramniwas, 2008; Schmidt, Matzkin, Ippolito, & Eanes, 2005; Sgro et al., 2010; Travers, Garcia-Gonzalez, & Simmons, 2015), candidate genes (Oakeshott, Chambers, Gibson, & Willcocks, 1981; Overgaard, Kristensen, Mitchell, & Hoffmann, 2011; Paaby, Bergland, Behrman, & Schmidt, 2014; Schmidt et al., 2008s; Umina, Weeks, Kearney, McKechnie, & Hoffmann, 2005) and genomewide patterns (Bergland, Behrman, O'Brien, Schmidt, & Petrov, 2014; Bergland, Tobler, Gonzalez, Schmidt, & Petrov, 2016; Kolaczkowski, Kern, Holloway, & Begun, 2011). In particular, *D. melanogaster* adopt different life history strategies across a latitudinal gradient in the eastern United States. Flies at high latitudes, for example Maine, occupy the “slower,” somatic maintenance-promoting end of the fast–slow continuum (long lifespans and stress survival, high fat storage), whereas flies at low latitudes, for example Florida, invest in rapid development and early reproduction (Schmidt et al., 2005; Schmidt & Paaby, 2008; Sgro & Hoffmann, 2004). Turning to the microbiota, a growing body of research has revealed that the microbiota of *D. melanogaster* is of low diversity, represented by <100 species, usually dominated by acetic acid bacteria (AAB) of the family Acetobacteraceae, including *Acetobacter* species, or lactic acid bacteria (LAB) from the order Lactobacillales, including the genera *Lactobacillus, Enterococcus* and *Leuconostoc* (Bost et al., 2018; Corby-Harris et al., 2007; Ren, Webster, Finkel, & Tower, 2007; Staubach, Baines, Kunzel, Bik, & Petrov, 2013; Wong et al., 2015; Wong, Ng, & Douglas, 2011). As in many other animals, the *D. melanogaster* microbiota varies both among individual hosts and over time within an individual animal (Rogers et al., 2014; Wong, Chaston, & Douglas, 2013), and this variation is shaped by both deterministic factors, for example host genotype, among-microbe interactions, diet composition (Chaston et al., 2016; Coyte, Schluter, & Foster, 2015; Goodrich et al., 2016, 2014; Rakoff-Nahoum, Foster, & Comstock, 2016; Smith, Snowberg, Gregory Caporaso, Knight, & Bolnick, 2015) and stochastic processes of passive dispersal and ecological drift (Adair et al., 2018; Burns et al., 2016; Jeraldo et al., 2012; Venkataraman et al., 2015). The gut microbiota of *D. melanogaster* is also readily manipulated in the laboratory: it can be eliminated by bleach treatment; the dominant taxa are fully culturable; and microbial communities of defined composition can be administered by direct inoculation to bleach-sterilized fly eggs on a sterile diet, generating gnotobiotic flies (Koyle et al., 2016). If no bacteria are reapplied, the

resultant “axenic” insects develop and reproduce with no evidence of generalized malaise (Ridley, Wong, Westmiller, & Douglas, 2012).

The basis for this study is the observation that presence and composition of the *D. melanogaster* microbiota affect key traits of *D. melanogaster* that underpin life history strategy, including development rate, lifespan and fecundity (Brummel, Ching, Seroude, Simon, & Benzer, 2004; Chaston, Newell, & Douglas, 2014; Clark et al., 2015; Deshpande et al., 2015; Newell et al., 2014; Ridley et al., 2012; Shin et al., 2011; Storelli et al., 2011; Yamada, Deshpande, Bruce, Mak, & Ja, 2015). We hypothesized that the microbiota might, therefore, influence patterns of local adaptation in *D. melanogaster*. We asked three questions: (a) How does the microbiota influence traits contributing to the life history strategy of their host? (b) Does the taxonomic composition of the microbiota in *D. melanogaster* vary with geographic location along the latitudinal cline in eastern United States? (c) What are the relative contributions of host genotype and the microbiota in shaping local adaptation of the host along this cline? Using studies of both laboratory and wild populations of *D. melanogaster*, we reveal that (a) the identity of associated microorganisms influences the position of the flies along the fast–slow axis; (b) relative abundances of key members of the microbiota in wild-caught flies correlate with life history traits and can be selected by their hosts; (c) local adaptation of the host genotype is independent of the microbiota; and (d) variation in the microbiota can amplify or suppress phenotypic differences between locally adapted fly populations. Together, these findings suggest that microbes are an essential consideration in understanding how a model animal displays its locally adapted traits.

2 | MATERIALS AND METHODS

2.1 | Fly rearing and bacterial culture conditions

Standard fly rearing conditions were at 25°C using a 12-hr light-dark cycle on a yeast–glucose (YG) diet (10% yeast, 10% glucose, 1.2% agar) containing 0.42% propionic acid and 0.04% phosphoric acid (Newell & Douglas, 2014). Fly lines are listed in Table S4. *Wolbachia* status of the flies was determined using the wsp691-R (5′-AAAAATTAAACGCTACTCCA-3′) and wsp81-F (5′-TGGTCCAATAAGTGATGAAGAAAC-3′) as described previously (Zhou, Rousset, & O'Neil, 1998) and is also reported in Table S4.

To control bacterial exposure to particular microbial partners and to test for the influence of individual microbes on life history traits, we reared flies under bacteria-free conditions or from bacteria-free eggs with an inoculated, defined microbiota. Fly eggs were collected from grape juice plates, dechorionated in 0.6% sodium hypochlorite for two 2.5 min washes, rinsed three times with sterile water and transferred to sterile YG diet (no acid preservative added) in a biosafety cabinet, as in our previous work (Koyle et al., 2016). Bacteria-free eggs were left unmanipulated, or, to rear flies with a defined microbiota, were inoculated with 50 μl bacterial culture that had been grown overnight and normalized in sterile phosphate-buffered saline to OD_{600} = 0.1. If multiple bacterial strains were used, they were first normalized to OD_{600} = 0.1, mixed in equal ratios and then inoculated to the flies in a 50 μl volume.

Bacterial strains (Table S5) were cultured on specific media: modified MRS medium (mMRS; 1.25% peptone, 0.75% yeast extract, 2% glucose, 0.5% sodium acetate, 0.2% dipotassium hydrogen phosphate, 0.2% triammonium citrate, 0.02% magnesium sulphate heptahydrate, 0.005% manganese sulphate tetrahydrate, 1.2% agar [Newell & Douglas, 2014]), potato medium (pot [Sigma P6685]), lysogeny broth (LB; 1% tryptone, 0.5% yeast extract, 0.5% sodium chloride) and brain–heart infusion (BHI, Sigma 53286). All strains were grown at 30°C except *Escherichia coli*, which was grown at 37°C. Strains grown under normoxia were shaken (liquid) or under ambient laboratory conditions (solid). Strains requiring hypoxia were grown statically (liquid) or in a sealed container flooded with CO_2 (solid).

2.2 | Bacterial abundance

Bacterial abundance was assessed in whole-body fly homogenates between 4 and 7 hr into the daily light cycle. Flies from each vial were anesthetized, and a pool of five flies was directly homogenized (no rinsing) in 125 μl homogenization buffer (10 mM Tris, pH 8, 1 mM EDTA, 0.1% Triton X-100 as in Chaston et al., 2014) with 125 μl Lysing Matrix D ceramic beads (MP Biomedicals 116540434) by shaking for 30–60 s at 4.0 m/s in a FastPrep-24 or 1,500 rpm for 2 min on a GenoGrinder 2010. The homogenate was plated onto mMRS medium twice, with dilution plating under normoxic or hypoxic conditions for enumeration of bacterial abundance, and a spot test under the reverse (hypoxia or normoxia) conditions to test for contamination by other microorganisms. After incubation, the colony morphologies were inspected visually to confirm strain identity. Where ≥200 CFU/fly of the expected bacterial strain were detected, the strain was deemed "present." Differences between *Acetobacter* strains could usually not be determined by colony morphology, so *Acetobacter* contamination of other *Acetobacter* strains cannot be ruled out.

2.3 | Development rate of the flies

Drosophila melanogaster development rate to pupariation or eclosion was determined by counting the number of pupae formed at 1, 6.5 and 10 hr into the daily light cycle. Unless otherwise noted, three separate experiments each with triplicate fly vials were performed for each treatment.

2.4 | Starvation resistance (SR)

Starvation resistance was determined in pools of ten 5- to 7-day-old flies. Between 4 and 7 hr into the daily light cycle, flies with different bacterial treatments were separated by sex under light CO_2-anaesthesia in a random order and then incubated in fly vials containing 5 ml 1% agarose under standard fly rearing conditions. Timing of fly mortality relative to the start of sorting was recorded 1, 5, 9, 13 and 17 hr after the start of the daily light cycle until all flies in a vial were dead. Unless otherwise noted, three separate experiments each with triplicate fly vials were performed for each treatment.

2.5 | Glucose content

Glucose content was measured from homogenized pools of five 5- to 7-day-old female flies as in our previous work (Chaston et al., 2014). Briefly, the pool of flies was homogenized in

homogenization buffer and analysed by the Sigma Glucose Assay kit (GAGO20-1KT) according to manufacturer instructions.

2.6 | Lifespan

Drosophila melanogaster adult lifespan was measured by recording the number and sex of dead flies and transferring surviving flies to fresh sterile diet every 2–3 days until all flies in a vial were dead. For every transfer of adult flies (P generation) to fresh diet, one spent vial per week was retained for 2–3 weeks, when the offspring (F1 generation) were homogenized to check for bacterial persistence and contamination during transfer. Where ≥200 CFU/fly of an unexpected bacterial species were detected in 2 consecutive weeks, the flies were deemed contaminated from the first date contamination was detected. In the survival analysis, flies were marked as leaving the experiment alive at that time. At least three separate experiments with triplicate fly vials were performed for each treatment.

2.7 | Fecundity

Drosophila melanogaster fecundity was defined as the number of F1 offspring per female that reached pupation and was measured in pools of 30–60 mixed sex flies aged 12–14 days post-egg deposition (approximately 2- to 4-day-old adults). First, 30–60 P generation *D. melanogaster* per vial were mono-associated with different bacterial strains. Two to four days after >90% of flies had eclosed, P generation adults were transferred to sterile YG diet between 8 and 10 hr into the daily light cycle. Eighteen hours later (between 2 and 4 hr into the daily light cycle the following day), the flies were transferred to new, sterile food. The number of F1 offspring that reached pupation in the spent vials was counted, and normalized to the number of live adult females that laid eggs in the vial. Three separate experiments with triplicate vials were performed on 3 consecutive days. If contaminating microbes were detected in emergent F1 flies, the vial was discarded.

2.8 | 16S rRNA marker gene analysis

To test for microbiota composition of wild *D. melanogaster*, the V4 region of the 16S rRNA marker gene was amplified from whole-body homogenates of wild flies collected from two locations. In the eastern United States between July and November of 2009 through 2011 (Dataset S8), wild flies were collected to empty fly vials, and *D. melanogaster* were sorted from the mixed species pools (if any) within 16 hr and stored in ethanol with <100 other flies from the sample collection. DNA was extracted from triplicate pools of 5 whole-body flies by a salting out procedure (Cenis, Perez, & Fereres, 1993). Briefly, fly bodies were homogenized in enzymatic lysis buffer with 20 mg/ml lysozyme (Amresco, 0663) and disrupted using glass beads. Cells were then lysed via incubation with 10× extraction buffer and proteinase K. After incubation with 3 M sodium acetate, DNA was extracted from the pellet using 100% isopropanol, rinsed in 70% ethanol and resuspended in sterile TE buffer. From these extracts, the V4 region of the 16S rRNA gene was amplified as described previously (Kozich, Westcott, Baxter, Highlander, & Schloss, 2013). Primer sequences are listed in Datasets S8 and S9. Sequences were normalized using the SequalPrep Normalization kit (Invitrogen) and sequenced via 2 × 250 Illumina v2 chemistry on a HiSeq 2500 at the BYU DNA Sequencing Center. Sequence reads are available at NCBI (Bioproject numbers PRJNA589702, PRJNA589709). Operational taxonomic units (OTUs)

were clustered and assigned to the sequencing data in QIIME 1.9.1 using UCLUST with open-reference OTU picking and the GreenGenes Core reference alignment at 97% similarity (Caporaso, Bittinger, et al., 2010; Caporaso, Kuczynski, et al., 2010; Edgar, 2010; McDonald et al., 2012; Wang, Garrity, Tiedje, & Cole, 2007). Taxonomy was assigned using the GreenGenes reference database (Price, Dehal, & Arkin, 2010; Werner et al., 2012). *Wolbachia* reads were filtered from the OTU table, which was rarefied to 65 reads per sample, which was still sufficient to near-saturate most samples (Figure S7). Spearman rank correlations between order-level OTU classifications and latitude were performed in R. Raw calculations and graphics are presented in Script S1. Geographic coordinates were estimated from the noted sampling locations before correlations between latitude and microbiota composition were calculated.

The microbiomes of flies from the state of Utah, USA, were analysed as above except for the following: wild flies were collected between 1991 and 1993 by Duane Jeffries and James Farmer, stored in ethanol, including at least some time at −20°C after placing in ethanol (Dataset S9). In 2019, DNA was extracted from individual male and female flies, using the salting out method above, based on which sex (sometimes both) was collected at these locations. OTUs were clustered and assigned to taxonomy using QIIME2 (Bolyen et al., 2019), including denoising with DADA2 including trimming forward reads to between bp 6 and 249 and reverse reads to between 6 and 229 based on quality scores, and rarefied to 11,025 reads per sample for subsequent analyses.

16S rRNA marker gene reads were also mined from shotgun sequencing data sets of fruit flies using standard procedures. A reference database for 16S rRNA sequences was built from the Silva 16S (90%) reference database using BWA (Li & Durbin, 2009), and reads from each data set (see accession number in Figure S3) were aligned to it using SAMTOOLS (Li, 2011; Li & Durbin, 2009). For each data set, any sequence in the Silva database that had 10 or more matches in that data set was retained for subsequent analysis. The taxonomic identity for the sequence was manually extracted from NCBI, and taxa were manually clustered as AAB, LAB, gamma-proteobacteria or other.

2.9 | Host genetic selection on the microbiota

To test for host genetic selection on microbiota composition of clinally adapted fly lines, we enumerated bacterial abundance in wild *D. melanogaster* isofemale lines that were inoculated with a defined 5-species bacterial community as in our previous work (Koyle et al., 2016). The isofemale lines were derived from collections at Rocky Ridge Orchards in Bowdoin, ME, USA, and Spice Park in Homestead, FL, USA, in 2011–2012 (Table S4). After individual capture, progeny derived from a single female were grown in Philadelphia, PA on a 21-day generation cycle on a Bloomington-style diet (1 L H_2O, 11.6 g agar, 147.5 g corn meal, 20.2 g soy flour, 34.9 g yeast, 165 ml molasses boiled and mixed together, then mixed with 1.25 L H_2O. Separately, 4.9 g methyl paraben was dissolved in 99 ml ethanol and add to the diet after it was cooled). Of several dozen derived lines, five random lines from each geography were shipped from Philadelphia, PA, to Provo, UT, where they were reared for at least two generations on the YG diet. The defined community was introduced to bleach-sterilized fly eggs as an equal-ratio mixture (normalized to OD_{600} = 0.1) of strains

DmCS_001, DmCS_002, DmCS_003, DmCS_004, DmCS_006 (Table S5). The microbial communities associated with the flies were assessed by dilution plating as described above. Visual differences in colony colour and morphology were used to distinguish *Lactobacillaceae* (large, white or yellow) and *Acetobacter* (small, tan) colonies. In a follow-up study, the same fly lines were reared with a 6-species inoculum composed of 4 *Lactobacillaceae* species isolated from wild *Drosophila* and the two previously used *Acetobacter* strains, which were isolated from laboratory *Drosophila* (strains DmW_98, DmW_103, DmW_181, DmW_196, DmCS_004, DmCS_006; Table S5). Samples were prepared, and microbiota composition was enumerated by the same methods as above.

2.10 | Life history traits in wild isofemale *D. melanogaster* lines

Using only the *Wolbachia*-free wild isofemale lines from the host genetic selection experiment (Table S4), we measured the influence of the microbiota on life history traits in flies reared under different microbial treatments: bacteria free; 5- and 6-species gnotobiotic; and in mono-association with each of LAB strains DmCS_002, DmW_098, DmW_107100, and DmW_140; and AAB strains DmCS_006, DmW_043, DmW_045, DmW_12512 (Table S5). Then, development rate and SR were measured as described above. Early fecundity was also measured as described above, but only for 1- to 4-day-old adults.

2.11 | Statistical analyses

To define the relationships between life history traits in mono-associated CantonS flies (Figure 1), Pearson (if normal by a Shapiro test) or Spearman rank (if not normal by a Shapiro test) correlations between mean phenotype values were calculated in R (R Core Team, 2018). All assays were performed on the same fly genotype using the same diet formulation, bacterial strains and general methods. One major bifurcation in the data is that the previously published TAG content, development rate, feeding rate and glucose content data were collected in Ithaca, NY, whereas the SR, lifespan and fecundity data were collected in Provo, UT. The previously published data were used for different purposes, and this is a nonredundant analysis of those data. Significant effects of bacterial treatment were defined using Cox proportional hazards model or a Kruskal–Wallis test.

To test for statistically significant differences in the CFU abundance data in Figure 2c and Figures S4 and S5, we used two approaches. First, we examined the raw CFU counts as a ratio of *Lactobacillaceae* to *Acetobacter* abundance in flies grouped by geographic cline or fly genotype using a generalized linear mixed-effects model with a binomial family (Bates, Maechler, Bolker, & Walker,2015; Hothorn, Bretz, & Westfall, 2008; R Core Team, 2018). If there was a cline-specific difference, then the difference in the microbiota of fly lines was tested using fly genotype as a fixed effect instead of geographic cline. We also compared the absolute abundances of the bacteria. A Shapiro test was used to confirm the CFU abundances were not distributed normally and differences between bacterial abundances in lines from ME and FL geographies were determined by a Kruskal-Wallis test. Differences in bacterial abundances by fly line were determined by a Dunn test with Benjamini–Hochberg correction after confirming a significant line effect by a Kruskal–Wallis test. All tests were performed in R (Dinno, 2017; Mangiafico, 2017; R Core Team, 2018).

For Figure 3 analyses, Cox proportional hazards models were used to test for differences in development rate and SR in isofemale lines from ME and FL reared under different microbiota treatments (Therneau, 2018, 2014). Differences in fecundity were determined by a Kruskal–Wallis test. Compact letter displays identifying significant differences between treatments were defined using the MULTCOMP package (Hothorn et al., 2008). The number of flies per vial in each condition is reported in Table S6.

The code used to produce each of the figures, including many raw statistical outputs, is included in Script S1.

3 | RESULTS

3.1 | The microbiota influences *Drosophila melanogaster* life history strategy

In an evaluation of previously and newly collected data sets (Datasets S1-S7; Chaston et al., 2014; Newell et al., 2014), we noticed correlated influences of AAB and LAB on *D. melanogaster* life history traits. Specifically, rearing the isogenic *D. melanogaster* CantonS line with different bacterial strains led to two distinct outcomes. First, as was shown in the previously cited analyses, rearing the flies with different bacteria led to significant variation in the phenotypes (Figure 1, blue text at top). Second, the variation conferred by the different species manifested as a trade-off between early reproduction and somatic maintenance (Figure 1, central panels). In other words, bacteria that conferred fast development rates and high early fecundity had lower lipid (TAG) levels, lifespan and starvation resistance (SR) than strains that had low early reproduction and development rates. The correlations between phenotypes were specific to the investigated traits since SR, lifespan, fecundity, and development and feeding rates were not correlated with glucose content (Figure 1), a nutritional index that is not usually considered with other life history traits (Hoffmann & Harshman, 1999). Among all tested life history traits, the correlation coefficients were consistent with established patterns of life history trade-offs in *D. melanogaster* (Hoffmann & Harshman, 1999; Kalra & Parkash, 2014) although one correlation with low replication ($n = 12$) was not significant (Figure 1). Together, this analysis reveals that variation in the identify of *D. melanogaster*-associated microorganisms can shift the phenotype of an isogenic host between a fast and slow strategy.

3.2 | The environment and host genetic selection contribute to geographic patterns in the *D. melanogaster* microbiota

To better understand the relationship between variation in the microbiota and the life history strategy adopted by an animal, we turned to the well-studied latitudinal cline in the eastern United States. Across this cline, low- and high-latitude flies, respectively, invest preferentially in fast and slow strategies (Schmidt et al., 2005; Schmidt & Paaby, 2008). We predicted that if the microbiota composition of the flies was consistent with the influence of microbes on the animal's life history strategy, *D. melanogaster* from low-latitude populations would bear more AAB than flies from high latitude populations, which would be dominated by LAB. Using 16S rRNA marker gene sequencing, we determined the relative abundance of AAB and LAB in wild male flies from six sites along the eastern United States coast in 2009, all collected within a 3-week period (Figure 2a, Dataset S8). Reads were clustered at

the order level since LAB are an order-level designation; AAB, from the family Acetobacteraceae represented 99.97% of the Rhodospirillales reads, and Rhodospirillales reads are referred to as AAB hereafter for simplicity. Consistent with our predictions, relative AAB and LAB abundances were negatively and positively correlated with latitude, respectively (Figure 2a). When we broadened our analysis across this cline to include samples collected from the same or additional sites in two subsequent years, similar, but not identical, trends were apparent. For example, the correlation coefficients for the relationship of latitude with AAB or LAB abundance had the same directionality, but the significance of the correlation between relative LAB abundance and latitude was no longer significant (Figure S1A). These findings show heterogeneity in the spatial patterns of the *D. melanogaster* microbiota and may suggest that additional factors, such as season, contribute to geographic variation in the *D. melanogaster* microbiota. Additionally, the spatial patterning in the microbiota with latitude was not restricted to the eastern United States, as the microbiota of wild-caught flies sampled from various locations in southern Utah, USA, showed that AAB and LAB read abundances were negatively and positively correlated with latitude, respectively (Figure 2b, Figure S2, Dataset S9). Therefore, while these results do not provide a comprehensive view of spatial patterning in the microbiota of *D. melanogaster*, an animal distributed across the globe, they do reveal a trade-off between the abundance of the two major taxonomic groups in the *D. melanogaster* microbiota with latitude at two locations in the United States and match a pattern previously observed in humans (Suzuki & Worobey, 2014), where the Firmicutes, which contain the LAB, are more abundant in individuals sampled at high latitudes.

We reasoned that numerous factors, including the environment or host genotype, could contribute to the geographic patterns we detected in the microbiota of wild flies. Therefore, we first tested whether latitudinal variation in the microbiota was dependent on the wild condition of the flies, by analysing the microbiota of wild-caught *D. melanogaster* that were subsequently reared in the laboratory for a short period of time (<5 generations). This was done by analysing 16S reads present in *D. melanogaster* whole-genome shotgun sequencing projects. In *D. melanogaster* collected from the eastern United States or from Australia, a second geographic area showing parallel patterns of phenotypic differentiation as is observed in the eastern United States, the negative and positive correlations of AAB and LAB reads with latitude were sometimes, but not always, detected in wild-caught flies (Figure S3, Tables S1-S3). Therefore, these patterns in microbiota are not infallibly resilient to the transfer of flies from the wild to the laboratory and depend upon unknown environmental characteristics.

We also tested if host genotype contributed to variation in the microbiota composition of geographically distinct *D. melanogaster* populations. The gold standard to test for host genetic influence on the microbiota is to eliminate inconstant access of the flies to different sets of microorganisms (Wong et al., 2013) by rearing the flies under microbiologically defined, gnotobiotic conditions (Chaston et al., 2016). We performed these analyses with flies kept in the laboratory since their collection in ME and FL, USA, several years prior, and reared each population from birth with defined, mixed communities of AAB and LAB. Unlike the previous analyses, we measured microbiota composition using culture-dependent methods because the taxonomic identity of the community was perfectly defined, fully

recoverable by culturing, and culture-dependent and culture-independent analyses of the *D. melanogaster* microbiota in the wild can yield similar outcomes (Rudman et al., 2019). The first microbial community comprised 5 bacterial species all derived from laboratory *Drosophila*. By the time these flies reached adulthood, the ratio of AAB:LAB in the ME and FL (USA) fly populations was different, even though the flies started at birth with the same ratio of the five bacteria (Figure 2c, Figure S4, Dataset S10). Because there was a low relative abundance of LAB in both fly populations, we also conducted a second experiment. The second experiment replaced the laboratory LAB with four LAB isolated from wild *D. melanogaster* and yielded a fourfold increase of LAB in ME flies relative to the first experiment, which was also statistically significant (Figure 2c, Figure S5; Dataset S11). The absolute AAB abundance did not differ significantly between ME- and FL-derived flies in either of the two experiments (Figures S4 and S5), suggesting a genetic effect primarily on LAB. Thus, host genetic selection on the microbiota composition can occur in locally adapted *D. melanogaster* adults, yielding compositional patterns consistent with the trends seen in surveys of the wild fly microbiota.

Together, the experiments using wild-caught and laboratory-reared wild flies reveal the following: (a) wild environment-dependent geographic patterns in the *D. melanogaster* microbiota composition; (b) host genetic selection on the microbiota that varied with geographic source of the flies and the bacteria; and (c) host genetic control of the microbiota was stronger for LAB than for other associated bacteria. Additionally, heterogeneity was apparent when samples were included from multiple years and sampling times, emphasizing that these patterns do not provide an absolute definition of the patterns in the microbiota for global *D. melanogaster* populations.

3.3 | Microbiota composition can influence life history differences between locally adapted *D. melanogaster*

The wild flies captured in the eastern United States (Figure 2a) harboured a microbiota that was consistent with the life history strategy naturally adopted by those flies (Schmidt et al., 2005; Schmidt & Paaby, 2008; Sgro & Hoffmann, 2004)—that is, low-latitude flies with "fast" traits naturally bore more AAB, which promote "fast" traits, whereas high-latitude flies with slow traits had higher loads of slow-trait-conferring LAB. The correlation between latitude, ratio of AAB:LAB and life history traits of the flies raised the question whether the microbiota variation was necessary for life history variation in the locally adapted fly populations. We defined this relationship by measuring life history traits in two sets of experiments: first in bacteria-free flies, exposing the phenotypic influence of host genotype alone, and then in flies bearing individual LAB or AAB species, to determine the genotype × microbiota interactions.

The development rate, SR and early fecundity in bacteria-free treatments of flies from ME and FL were significantly different, consistent with a role for host genotype in local adaptation (Figure 3a-c, Datasets S12-S14). Relative to FL flies, ME populations displayed the established trade-off between decreased development rate and increased SR, but unexpectedly had higher early fecundity. The same trends were observed when the flies were reared under gnotobiotic conditions (Figure S6, Datasets S12-S14). Thus, bacteria-free ME

flies did not display a trade-off between fecundity and development rate, suggesting that the high fecundity of ME flies could result from a condition of our laboratory experiments, such as the use of a high-nutrient diet or the relatively low abundance of LAB in gnotobiotic flies (as in Figure 2c). Overall, the results reveal that phenotypic differences between the populations are not driven exclusively by the microbiota and confirm local adaptation in the host genotype.

The second question is whether variation in microbiota composition influences the difference in phenotypes between locally adapted fly populations. When we compared the life history traits of ME and FL fly lines reared with extreme LAB:AAB ratios by using mono-association experiments, we detected that microbiota variation could reproduce, suppress or reverse the phenotypic differences found in the bacteria-free flies (Figure 3d-f, Datasets S12-S14). For example, the different fly genotypes generally showed similar phenotypic trends to the bacteria-free state when they were reared with the same microorganisms (either AAB or LAB). However, comparing traits between flies reared with different microorganisms could eliminate (fecundity in AAB-colonized FL vs. LAB-colonized ME flies) or reverse (SR and development in AAB-colonized ME vs. LAB-colonized FL flies) the difference observed between the same genotypes when bacteria free. Thus, variation in the microbiota can suppress or reinforce phenotypic differences between locally adapted populations of *D. melanogaster* and, therefore, the composition of the microbiota matters for flies to adopt a locally adapted life history strategy.

4 | DISCUSSION

Decades of work have established that organisms adapt to their environments in response to local environmental variation (Adrion, Hahn, & Cooper, 2015; Bueno et al., 2017; Koske, 1987; Reimer et al., 2017; Savage et al., 2002). Historically, life history adaptation has been examined from the perspective of an organism's genetic adaptations to environmental circumstances that vary in different geographic locations, such as temperature, photoperiod, nutrient availability and predator pressure. There is also clear evidence that the microorganisms living within, on or near a plant or animal exert substantial influence on host traits that contribute to the life history strategy, including for latitudinal clines in microbiota composition in both animals and plants (Bueno et al., 2017; Dikongue & Segurel, 2017; Koske, 1987; Reimer et al., 2017; Savage et al., 2002; Suzuki & Worobey, 2014). Three key findings in this study extend these existing findings to conclude that geographic variation in the microbiota is associated with local adaptation in a model animal. First, the life history strategy of *Drosophila melanogaster* along the “fast–slow” axis can be driven by the composition of the microbiota under experimental conditions. Second, the host genetic factors driving life history variation between locally adapted natural populations include host selection of bacterial partners with congruent effects on host life history traits. Finally, variation in the microbiota composition matters to maintain genetically controlled differences in life history traits between locally adapted fly populations.

This study revealed that the bacteria can function as a rheostat to determine the fast-slow strategy adopted by the host. The molecular basis of this effect may involve bacterial production or catabolism of key metabolites. For example, bacterial production of acetic

acid and other fermentation products, as well as branched-chain amino acids, can influence the activity of insulin-like/target of rapamycin (IIS/TOR) signalling (Shin et al., 2011; Storelli et al., 2011). IIS/TOR signalling has a central role in regulating cell and organismal growth, as well as female fecundity, and, consequently, life history strategies (McGaugh et al., 2015; Oldham, 2011). *Drosophila melanogaster* life history and survival can also be influenced by bacterial consumption of glucose (Chaston et al., 2014; Huang & Douglas, 2015) or by synthesis of methionine (Judd et al., 2018) and B vitamins (Sannino, Dobson, Edwards, Angert, & Buchon, 2018; Wong, Dobson, & Douglas, 2014), suggesting candidate bacterial functions that may mediate shifts in *D. melanogaster* life history strategy. Regardless of the mechanisms under question, the different bacterial functions may influence how the insect host detects its environment (especially diet) in the fruit or other ephemeral habitat that support larval development. Thus, the many Acetobacteraceae that are highly competitive in aerobic environments with high concentrations of sugars and other readily assimilated nutrients (Lievens et al., 2015; Wong et al., 2015) may represent a reliable cue for high-nutrient but ephemeral resources, favouring a “fast” phenotype of the host, while many Lactobacillales, which utilize complex carbon and nitrogen sources that are consumed more slowly (Duar et al., 2017), favour a “slow” phenotype of the host. Evidence from several studies suggest that the blend of fermentation products produced by microbial communities of different composition in the food and gut of *Drosophila* may represent a reliable cue for habitats of different nutritional content and persistence (Farine, Habbachi, Cortot, Roche, & Ferveur, 2017; Fischer et al., 2017; Kim, Huang, McMullen, Newell, & Douglas, 2018).

We have also obtained evidence that host genetic selection of its microbiota plays a role in shaping the fast–slow strategy adopted by *D. melanogaster*. Consistent with this finding, there is a strong overlap between *D. melanogaster* genes associated with local adaptation and host-microbe interactions. For example, of 160 previously identified genes that vary in flies across latitudinal gradients (Fabian et al., 2012), 45% have known or predicted (GWA, transcription) effects on microbiota interactions (Chaston et al., 2016; Dobson, Chaston, & Douglas, 2016; Dobson et al., 2015) relative to 31% of 13,991 total *D. melanogaster* genes ($X^2 = 7.03$, $p = .008$). A causal role of the microbiota in these correlations is indicated by the demonstration that TAG content of *D. melanogaster* is regulated in part through genetic control of microbiota composition (Chaston et al., 2016). Furthermore, the genetic determinants of *Acetobacter* abundance in *D. melanogaster* include many genes that are expressed predominantly or exclusively in neurons (Chaston et al., 2016), raising the possibility that sensory functions and behavioural traits (e.g., response to microbial volatiles, diet preference and feeding rate) can mediate differences in microbiota composition. These considerations raise the possibility that an individual fly might modify its lifespan/fecundity schedule in response to altered environmental circumstances, by seeking out and filtering a different suite of microorganisms. An important topic for future research is the significance of host genetic factors in driving microbiota composition in natural populations of *D. melanogaster*. These host effects are more diffuse than in many associations, for example legume–rhizobia and squid–Vibrio symbioses, where exquisite specificity is dictated by defined molecular interactions (Garg & Geetanjali, 2007; Gibson, Kobayashi, & Walker, 2008; Hillman & Goodrich-Blair, 2016; McFall-Ngai, 2014) because the *D. melanogaster*

association is an open system, continually exposed to microbes ingested in the food. Other deterministic factors, for example among-microbe interactions, as well as stochastic processes (see Section 1), may reinforce or suppress the effect of host factors on microbiota composition. Although the relative importance of these different factors is largely unknown, the demonstration of host genetic determinants of microbiota in various open associations in animals suggests that host determinants of microbiota composition contribute to the fitness of natural populations (Benson et al., 2010; Blekhman et al., 2015; Bonder et al., 2016; Davenport et al., 2015; Gomez et al., 2017; Goodrich et al., 2016, 2014; Human Microbiome Project Consortium, 2012; Rogers et al., 2014).

The strongest evidence that variation in the microbiota composition influences the phenotypic differences between locally adapted fly populations come from our findings that the microbiota can suppress or reverse genetically programmed differences in life history traits between fly lines. For example, a ME fly population that does not maintain high LAB loads could display a poorer SR phenotype than a FL fly population rich in LAB. Thus, the microbiota composition of *D. melanogaster* must be geography-specific to maintain the trait differences attributed to these fly lines. A major question arising from this conclusion is: How are geography-specific patterns in microbiota determined, given the established understanding that the microbiota of laboratory-reared *D. melanogaster* is inconstant and varies substantially with diet (Chandler, Lang, Bhatnagar, Eisen, & Kopp, 2011; Staubach et al., 2013; Wong et al., 2013)? While all the details to address this gap are unknown, our data confirm that both environmental and host genetic influences together contribute to these processes: wild flies reared in the laboratory for only a few generations failed to display the same patterns in microbiota composition as wild-caught flies, and wild flies reared in the laboratory with a defined starting set of microbes displayed key differences in their microbiota composition as adults. The environmental characters responsible for the variation could include temperature or diet (Chandler et al., 2011; Moghadam et al., 2018; Staubach et al., 2013), and at least some genetic factors that shape the microbiota composition of *D. melanogaster* have been described (Broderick, Buchon, & Lemaitre, 2014; Dobson et al., 2015). An additional or alternative explanation is that the characteristics described for laboratory flies may not reflect the biology of wild flies, since the interactions of *Drosophila* and their microbiota can vary depending if the partners are from the wild or the laboratory (Blum, Fischer, Miles, & Handelsman, 2013; Gould et al., 2018; Inamine et al., 2018; Obadia et al., 2017; Pais, Valente, Sporniak, & Teixeira, 2018; Winans et al., 2017). Therefore, we cannot rule out that characteristics defining interactions between laboratory *Drosophila* and their microbiota, such as inconstancy, are different for laboratory and wild flies. Regardless of the mechanism, the key importance of these findings is that models to describe local adaptation will fall short if they do not account for variation in the taxonomic and functional capacity of the microbiota.

The finding that the microbiota can mask host genetic determinants of life history traits is not without precedent. In particular, laboratory studies have revealed that the penetrance of various mutations on metabolic traits of *D. melanogaster* is altered, and frequently reduced, in flies colonized with microorganisms, relative to axenic flies (Dobson et al., 2015). However, these experiments have not previously been conducted relative to locally adapted populations. Further research is required to elucidate the underlying mechanisms and to

establish the extent to which the impact of individual microbial taxa on host life history traits in mono-associations (as used in these experiments), or controlled multispecies associations may be displayed in the taxonomically diverse microbial communities in natural fly populations.

This study extends our understanding of natural populations by combining studies of the microbiota in natural populations of *D. melanogaster* and laboratory analysis of precisely controlled host–microbe combinations. For example, axenic *D. melanogaster* is a contrived state not likely experienced by flies in the wild, but analysis of axenic flies was essential to establish the flies' genetic contributions to life history variation. Our study further emphasizes that an exclusive focus on laboratory systems can miss important interactions because it fails to reproduce the biological context underlying evolved interactions; for example, in wild flies, the difference in abundance between wild- and laboratory-fly-isolated LAB. Similarly, *Drosophila*-isolated *Acetobacter* are discordant for key functions (uric acid utilization and motility) but not taxonomy between wild versus laboratory flies (Winans et al., 2017). These issues are of general significance for the conduct of microbiome research, with parallels coming from the evidence that the microbiota of the mouse differs between laboratory inbred strains and wild (or pet-shop) mice, with associated major differences in host physiological traits, especially relating to the immune system (Beura et al., 2016; Masopust, Sivula, & Jameson, 2017; Weldon et al., 2015).

In conclusion, the impact of the microbiota on the life history strategy of *D. melanogaster* is most unlikely to be a unique trait of this insect species. Microbial influence on life history traits of animals may be widespread, representing an important, but hitherto neglected, determinant of intraspecific variation, including local adaptation. We recommend that analysis of the microbiota is included as an integral part of research on life history traits and local adaptation in animals, to determine the magnitude of microbial effects in different systems and to establish the proximate and ultimate mechanisms.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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DATA AVAILABILITY STATEMENT

All fly lines and bacterial strains will be made freely available upon request to the corresponding author. Sequence data are uploaded to the short read archive (Bioproject

numbers PRJNA589702, PRJNA589709). Raw phenotype data are available in Dryad (Walters et al., 2019).

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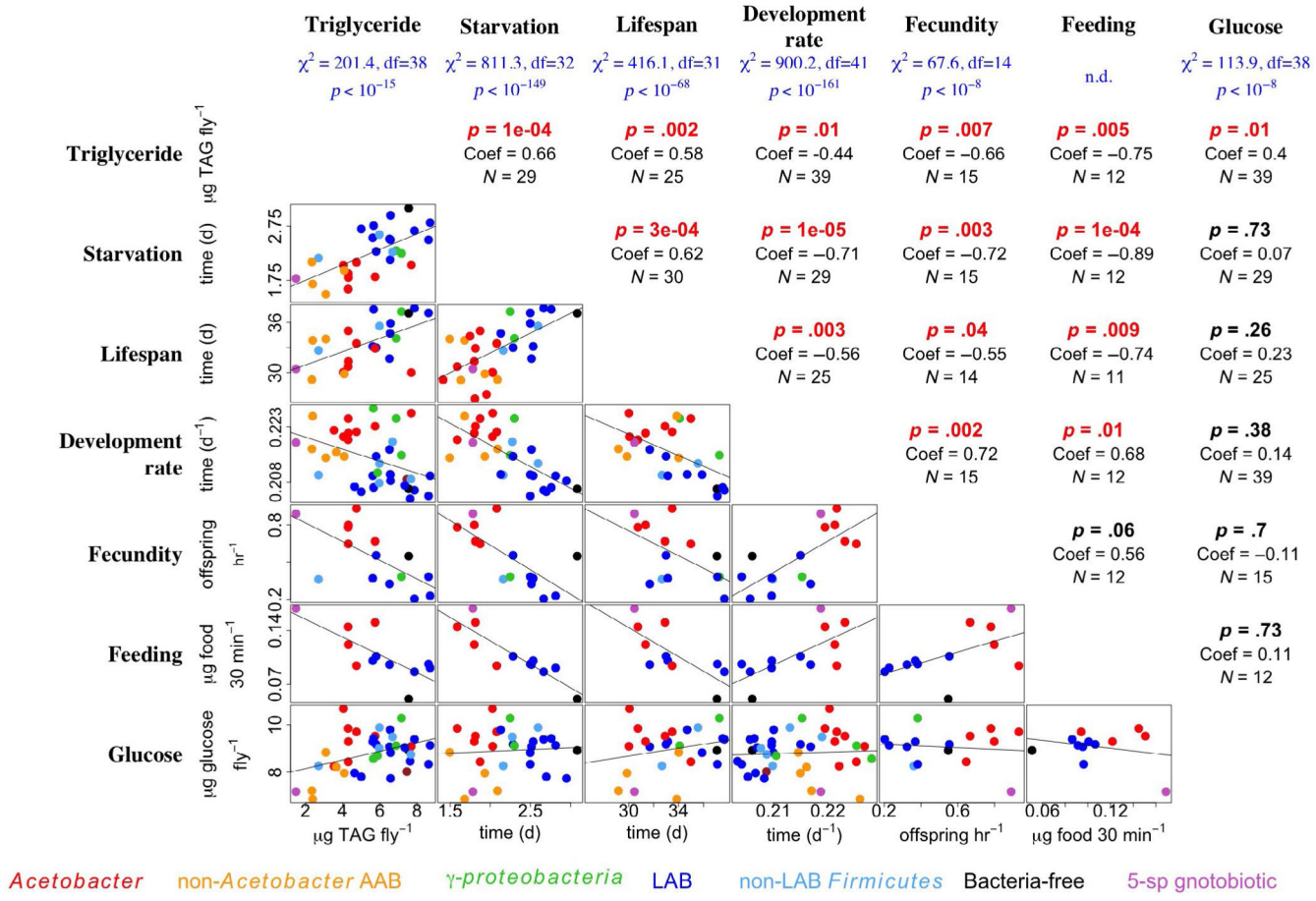

FIGURE 1.

Microbial variation influences life history patterns in a laboratory-reared isogenic fly line. Six life history traits were measured in *Drosophila melanogaster* that were mono-associated with different bacterial species and reared on a YG diet: whole-body triacylglyceride content (Triglyceride), survival under starvation conditions (Starvation), lifespan, the rate of development to pupariation (Development rate), number of pupariating offspring produced in the first 2–4 days after eclosion (Fecundity) and feeding rate (Feeding). Fly whole-body glucose content (Glucose), a trait that is not correlated with most other life history traits, was also measured. Significant influence of microbial treatment on the trait is shown at the top of the figure in blue under each trait. For triglyceride, fecundity and glucose, the chi-square statistic is from a Kruskal–Wallis test; for the other traits, it is from a Cox proportional hazards model. In the table portion, mean trait values conferred by different bacteria are plotted in the bottom half whereas the top half shows the results of correlation tests between traits in flies reared individually with the same microbe: *p*-values (*p*), correlation coefficients (Coef) and number of different mono-associations (*N*). *p*-values that were significant are shown in red. The data for triglyceride content, SR, development rate and feeding rate were published previously (Chaston et al., 2014; Judd et al., 2014; Newell et al., 2014)

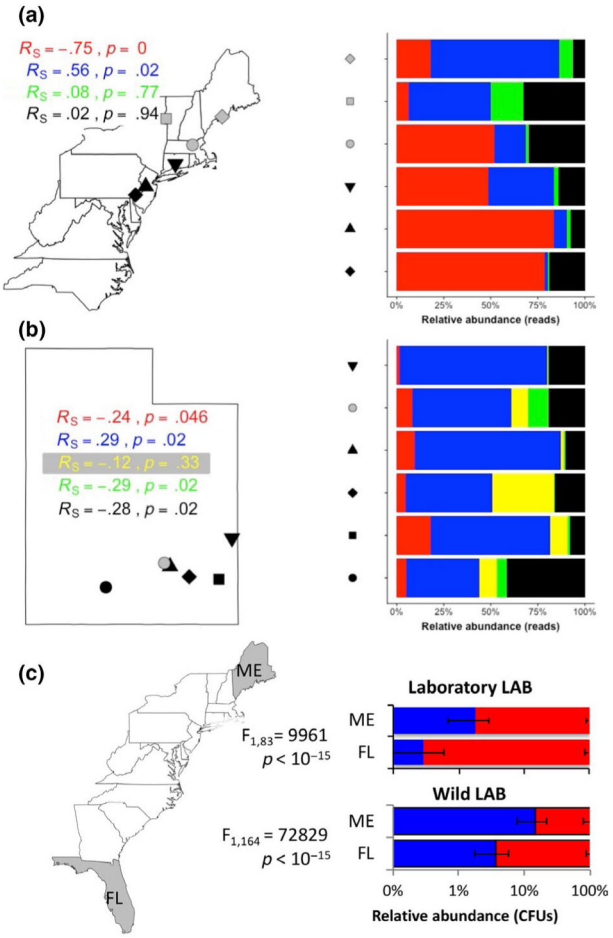

FIGURE 2.

Latitudinal variation in the microbiota of wild sampled *Drosophila melanogaster*. (a) Relative abundances of reads assigned to different bacterial orders in a 16S rRNA marker gene survey of *D. melanogaster* caught fresh in the wild in 2009. Spearman's rank correlations revealed significant positive and negative correlations between latitude and AAB (red) or LAB (blue) read abundances, respectively, but not for the Enterobacteriaceae (green) or all other bacterial reads detected (black). R_S, Spearman's rho. *p*, *p*-value. *N* = 1–3 replicate pools of 10 flies each per geographic site. (b) Relative abundances of reads assigned to different bacterial orders in a 16S rRNA marker gene survey of fresh, wild-caught flies collected in summer, 1991–1993. Spearman's rank correlations for the AAB (red), LAB (blue), Clostridiales (yellow), Enterobacteriales (green) or all other bacterial reads detected (black). *N* = 6–15 individual flies per sampled location and time. (c) Relative abundance of AAB (red) and LAB (blue) in isofemale lines derived from Maine (ME) and Florida (FL) wild populations, kept in the laboratory for several years and then reared in the laboratory under gnotobiotic conditions. Flies were reared with a 5-species microbiota, including 3 LAB isolated from laboratory *D. melanogaster* (Laboratory LAB) or with a 6-species gnotobiotic microbiota, including 4 LAB isolated from wild *D. melanogaster* (Wild LAB). The statistical difference between relative LAB and AAB abundance was determined by a generalized linear mixed (GLM) effects model using a binomial family. F, *F* statistic of

the GLM. $N = 9$ per treatment (triplicate vials in three separate experiments), except where vials were discarded for contamination

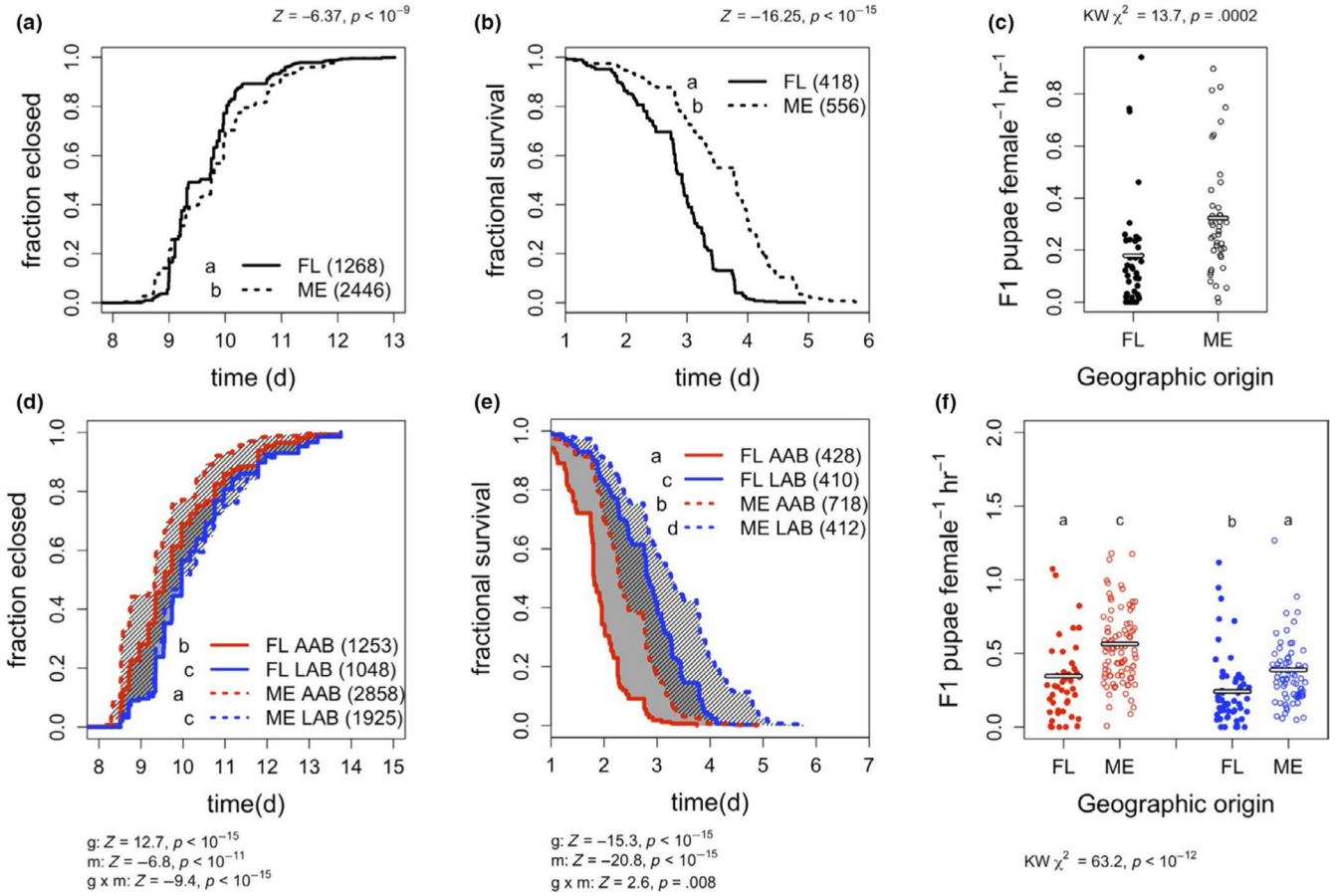

FIGURE 3.

Microbial presence and identity influence life history of wild female *Drosophila melanogaster*. Locally adapted ME (dashed lines) and FL (solid lines) flies reared with different bacterial treatments were tested for variation in time to eclosion (a, d), SR (b, e) and fecundity (c, f) when reared bacteria free (a–c); or in mono-association with AAB (red lines) or LAB (blue lines) strains (d–f). Solid or hatched shading represents the capacity for microbe-dependent variation in FL or ME fly phenotypes, respectively. Data were collected from triplicate vials in three separate experiments, except where vials were discarded for contamination or where low egg yields reduced the number of vials (exact *N* in parentheses and Table S6). Significant differences in the development or survival curves were determined by a Cox proportional hazards model (*Z*- and *p*-values are next to the genotype [g; or data in a, b], microbiota [m], or interactive [g × m] effect). Differences in fecundity were determined by a Kruskal–Wallis test, followed by a Dunn test for multiple comparisons. Different letters next to the legends represent significant differences between treatments