Population bottlenecks constrain microbiome diversity and host genetic variation impeding fitness

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Abstract

It is becoming increasingly clear that microbial symbionts influence key aspects of their host's fitness, and *vice versa*. This may fundamentally change our thinking about how microbes and hosts interact in influencing fitness and adaptation to changing environments. Here we explore how reductions in population size commonly experienced by threatened species influence microbiome diversity. Fitness consequences of such reductions are normally interpreted in terms of a loss of genetic variation and increase in inbreeding depression due to a loss of heterozygosity. However, fitness effects might also be mediated through microbiome diversity, e.g. if functionally important microbes are lost. Here we utilise *Drosophila melanogaster* lines with different histories of population bottlenecks to explore these questions. The lines were phenotyped for egg-to-adult viability and their genomes sequenced to estimate genetic variation. The bacterial 16S rRNA gene was amplified in these lines to investigate microbial diversity. We found that 1) host population bottlenecks constrained microbiome richness and diversity, 2) core microbiomes of hosts with low genetic variation were constituted from subsets of microbiomes found in flies with higher genetic variation, 3) both microbiome diversity and host genetic variation contributed to host population fitness, 4) connectivity and robustness of bacterial networks increased with higher host genetic variation, and 5) reduced microbial diversity is associated with weaker evolutionary responses in stressful environments. These findings suggest that population bottlenecks reduce hologenomic variation (in combined host and microbial genomes). Thus while the current biodiversity crisis focuses on population sizes and genetic variation of eukaryotes, an additional focal point should be the microbial diversity carried by the eukaryotes, which in turn may influence host fitness and adaptability with consequences for the persistence of populations.

Introduction

It is becoming increasingly clear that most eukaryotes live in intimate and complex relationships with microbial communities both in their external environment and on or within their body including their gut (McFall-Ngai *et al.* 2013). Numerous studies have documented how the presence and abundance of certain microbes can influence key aspects of host fitness such as lifespan, fecundity, immune responses, metabolic health, behaviour, and thermal stress tolerance traits (Sommer & Bäckhed 2013; Ericsson & Franklin 2015; Mueller & Sachs 2015; Sampson & Mazmanian 2015; Sison-Mangus *et al.* 2015; Moghadam *et al.* 2018; Douglas 2019). Conversely, the host can also control microbial composition to some extent, such as through changing nutrient availability by diet choice or host metabolism (Muegge *et al.* 2011; Jehrke *et al.* 2018), triggering immune factors (Douglas 2019), or controlling the gut mechanically such as through peristalsis (Coyte *et al.* 2015). The genetic background of the host can also interact with the microbiota (Chandler *et al.* 2011; Wong *et al.* 2014). For instance, host genes can affect the abundance of certain bacteria, allowing the microbial composition of hosts to be treated as a quantitative trait in genetic analysis (Chaston *et al.* 2016). These interactions between the host and its microbiota can in turn have a substantial impact on host fitness (Gould *et al.* 2018; Walters *et al.* 2019; West *et al.* 2019).

Because interactions between microbes and their hosts shape so many aspects of life, core biological processes including evolutionary adaptation may need to be reconsidered (Shapira 2016; Koskella & Bergelson 2020; Moeller & Sanders 2020). The 'holobiont' concept reflects the idea that eukaryote individuals do not act as autonomous units, but rather as networks consisting of the host and all its associated microbiota, and that their collective genomes – the 'hologenome' – forms an single unit of selection (Zilber-Rosenberg & Rosenberg 2008; Bordenstein & Theis 2015). Some experimental support for this idea is emerging. For instance, host selection for thermal tolerance resulted in an altered microbial composition and modulated the microbes' response to temperature (Kokou *et al.* 2018). While resident microbes respond to stressful environmental conditions, they can also subsequently aid the response of the host to such conditions (McFall-Ngai *et al.* 2013; Moghadam *et al.* 2018). More

generally, the microbial community could play a so-far underappreciated role in the broader context of population persistence which is important for research areas like conservation biology (Bahrndorff *et al.* 2016; Shapira 2016; Trevelline *et al.* 2019; West *et al.* 2019).

These conjectures raise the issue of how microbes respond to changes in a host's population size. Populations that undergo repeated or persistent reductions in size frequently suffer from inbreeding depression and genetic drift resulting in reduced genetic variation and lowered fitness (Charlesworth & Charlesworth 1987; Kristensen & Sørensen 2005), ultimately impeding evolutionary capacity and increasing the risk of extinction (Spielman *et al.* 2004; Frankham 2005; Markert *et al.* 2010; Hoffmann *et al.* 2017; Ørsted *et al.* 2019). These patterns have been investigated from the perspective of the nuclear genetic background rather than the hologenome; for instance, inbreeding depression is normally assumed to reflect the expression of deleterious alleles in their homozygotic form. However, host population size decreases may also lead to microbial perturbations, with potential feed-back effects on host fitness which could contribute to population level decline (Redford *et al.* 2012; Bahrndorff *et al.* 2016; Trevelline *et al.* 2019), although the interacting effects of microbial diversity, genetic diversity and fitness have not yet been widely tested.

Here we investigate microbial composition and abundance in 50 populations of *Drosophila melanogaster* that have experienced a variable number of population bottlenecks. These populations were subsequently phenotyped for fitness components and sequenced to obtain a genome-wide molecular measure of genetic variation (Ørsted et al. 2019). This set of lines provides a unique resource to investigate associations between host genetic variation, host fitness and the microbiome diversity (**Fig. 1**). We consider three main questions. 1) Is microbiome richness and diversity associated with host genetic variation imposed by population bottlenecks? 2) Is both microbiome diversity and genetic variation associated with host fitness, and if so, do they contribute independently? 3) How does the abundance of potential beneficial or pathogenic microbial taxa associate with host fitness?

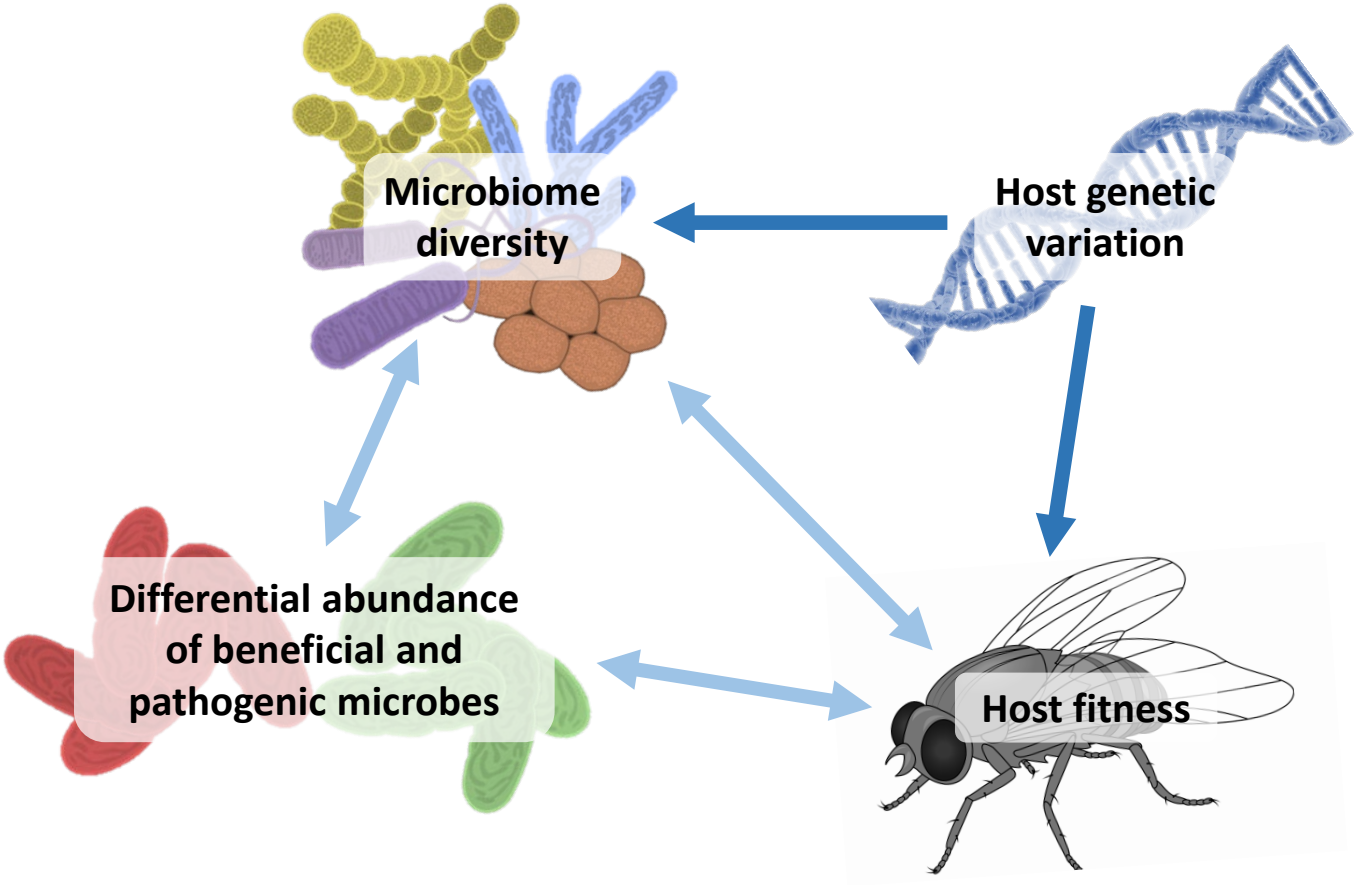

Figure 1.

Conceptual illustration of hypothetical associations between host genetic variation, microbiome diversity and host fitness in the experimental set of lines. Host genomic variation was manipulated through exposing populations to a variable number of bottlenecks and is assumed to have a causal effect on fitness and microbial diversity. The dark blue arrows represent unidirectional effects, and light blue arrows represent potential bidirectional effects. In one line of causality, low genetic variation in the host constrains the diversity (species richness and abundance) of the host-associated microbiome, which affects host fitness directly, i.e. through functional maintenance, and/or indirectly through changing the composition and relative abundance of putative beneficial and/or pathogenic microbes. In another line of causality, low host genetic variation directly affects host fitness which in turn leads to a less diverse microbiome through physiological or metabolic changes in the host "environment", or indirectly such as through a higher abundance of detrimental pathogenic microbes due to a weakened immune response.

Results

We selected 50 lines for microbiome analysis, which represent a subset of 109 lines that went through marked reductions in population size for varying number of generations and the 10 outbred control lines kept at a large population size. These original 119 lines had been sequenced using GBS to allow nucleotide diversity (π) to be estimated. In addition, the fitness component egg-to-adult viability (hereafter just viability) were estimated in each line (Ørsted *et al.* 2019). A positive association between viability and π had been established for these 119 lines (Spearman's ρ=0.45; $p<0.001$; **Supplementary Fig. S1**). For the microbiome characterization, two groups of 25 lines were selected based on measures of standardized viability and π (sum of Z-scores, see methods). One group of 25 lines had low genetic variation and low viability, and one with 25 lines had high genetic variation and high viability. The 'high' group included nine of the outbred controls as these generally had very high viability and nucleotide variability. To distinguish between effects of genetic variation within inbred lines versus inbreeding/outbreeding, we separated lines into three categories: 'low genetic variation' and 'high genetic variation' inbred lines and 'outbred' (OB) control lines (**Supplementary Fig. S2**).

Loss of host genomic variation decreases microbiome richness and diversity

Loss of host genetic variation was associated with decreased microbiome richness and diversity. The microbiomes from the outbred flies and the high genetic variation lines had a higher level of community diversity than the microbiomes of flies from the low genetic variation group (**Fig. 2**). Generally, there was an increase in microbiome diversity with increasing fly host genetic variation and viability, regardless of which measure we used to quantify the microbiome diversity. Richness indices, namely observed amplified sequence variant (ASV) richness and chao1 estimated ASV richness (**Fig. 2A-B**), showed a stronger trend than the diversity indices accounting for relative abundances (Shannon-Wiener and Simpson's; **Fig. 2C-D**). Even at the class level, the microbiomes from the low genetic variation group were less taxonomically heterogeneous than those from the high genetic variation and outbred groups (**Supplementary Fig. S3**). The majority of bacteria in the *D. melanogaster* microbiomes

127 belonged to the Alphaprotoebacteria, Bacilli, and to a lesser extent Bacteroidia and Actinobacteria. The
128 increasing level of taxonomic diversity with increasing host genetic variation was further evident at the
129 genus level, with the low genetic variation group harbouring the fewest number of genera, while the
130 outbred flies had the highest number (**Supplementary Fig. S4**). Non-metric multidimensional scaling
131 (NMDS) analysis based on the weighted and unweighted UniFrac distances (**Fig. 3**) showed that the
132 microbiomes differed depending on the fly host's level of genetic variation. Generally, there was better
133 segregation of the fly groups when only the microbiome membership (unweighted UniFrac) was
134 considered (**Fig. 3C-D)**, while the relative abundance patterns of the ASVs across the samples created
135 noise, notably among the high and outbred groups (**Fig. 3A-B)**. The host traits, nucleotide diversity and
136 viability were therefore significantly associated with the microbiome community patterns.
137
138 *Both host genetic variation and microbiome diversity contribute to host fitness*
139 In order to identify drivers of host fitness, we fitted generalized linear mixed effects models (GLMMs)
140 with egg-to-adult viability as a function of host nucleotide diversity (π) and microbiome diversity and
141 their interaction (one model for each of the four different measures of microbiome diversity; see
142 methods). The same pattern was evident regardless of the microbiome diversity metric used; both π and
143 microbiome diversity contribute to host fitness, with π contributing to a greater extent (approximately
144 2-6 larger scaled effect sizes; **Table 1**). For the richness measures, we observed significant interactions
145 between host genetic variation and microbial diversity on host fitness meaning that they do not act
146 independently. In fact, the positive interaction coefficients suggest synergism, i.e. in lines with high
147 genetic variation, the effect of increasing microbiome richness is greater than in lines with low genetic
148 variation and *vice versa* (**Supplementary Fig. S5**), while for Shannon-Wiener and Simpson's indices,
149 host genetic variation and microbial diversity contributed independently to host fitness. In all cases, the
150 full models had higher explanatory power than each individual model (π or microbiome diversity alone),
151 based on the Akaike information criterion (AIC) and χ^2 tests (**Table 1**). Using data from Ørsted *et al.*
152 (2019) from the set of lines in the present study, we also associated microbial diversity with evolutionary

153 responses in two traits (dry body mass and productivity) here defined as the slope of an ordinary linear
154 regression across 10 generations of rearing on a stressful medium. Interestingly, we found an effect of
155 microbial diversity for both traits, but no effect of genetic variation, π (**Supplementary Fig. S6**). For
156 details on assessment of these phenotypes, see Ørsted *et al.* (2019).

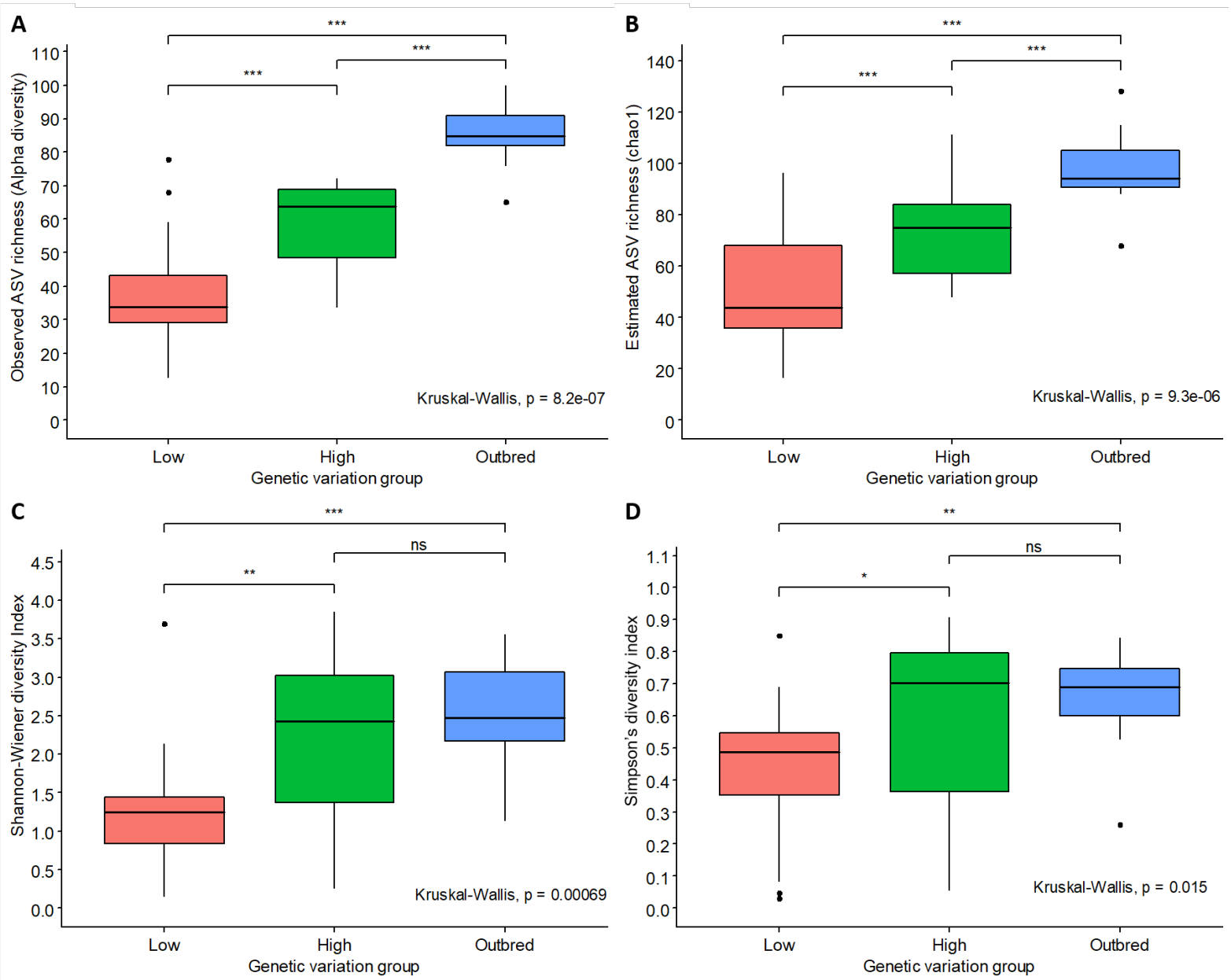

Figure 2.

Boxplots of microbiome diversity between the three genetic variation groups; low genetic variation (Low; red), high genetic variation (High; green), and outbred (blue) measured as either richness indices (**A**. observed ASV richness (Alpha diversity), and **B**. estimated ASV richness (chao1 estimation)) or measured using indices accounting for individual ASV abundances as well (**C**. Shannon-Wiener Index, and **D**. Simpson's Index). In all panels, the *p* values of a Kruskal-Wallis test show a significant effect of group, while asterisks denote the results of pairwise Wilcox's t-tests between groups; *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; and ns: $p > 0.05$.

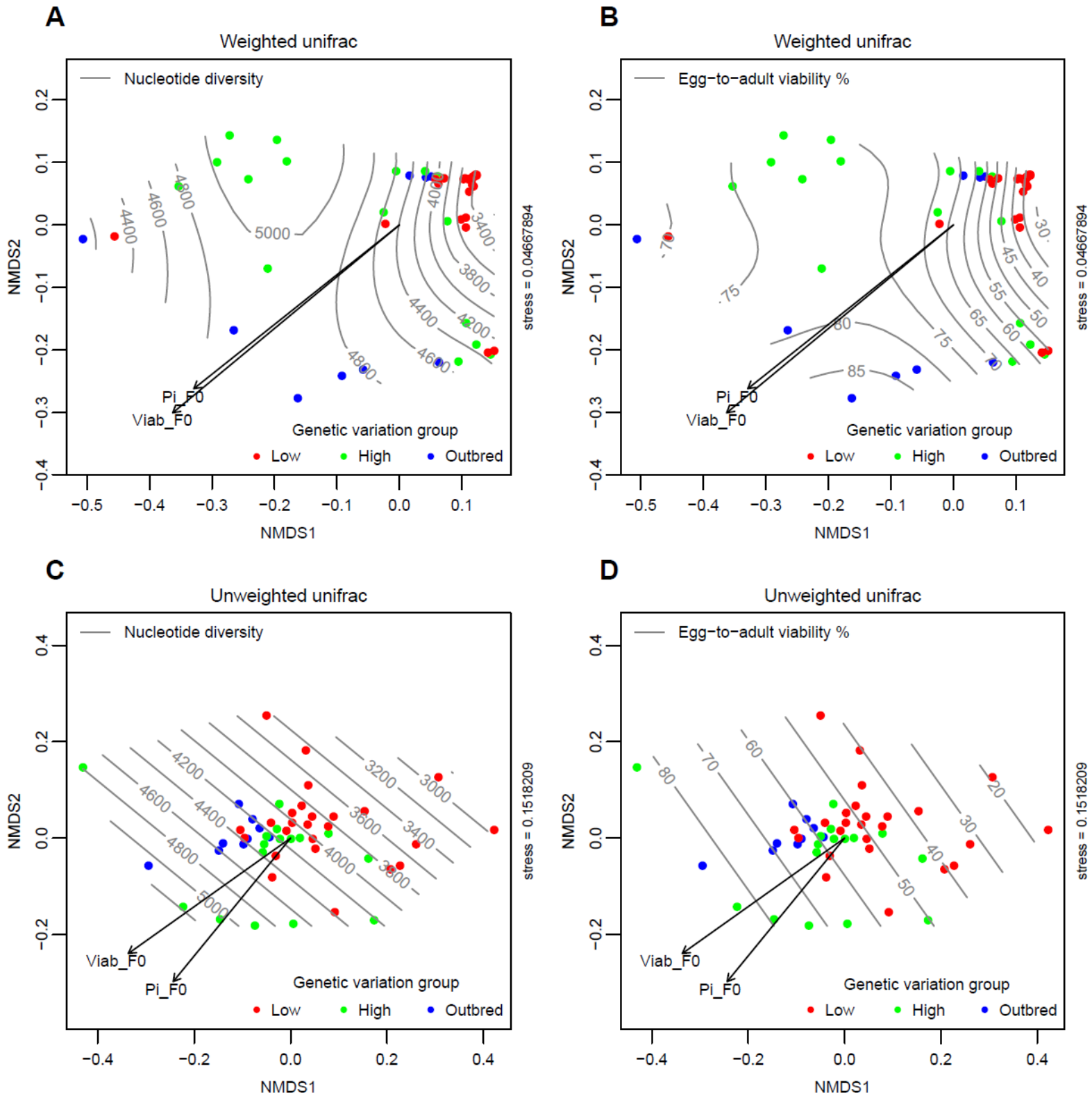

Figure 3.

Non-metric multidimensional scaling (NMDS) plots based on the UniFrac distances (weighted; **A-B**, and unweighted; **C-D**) between the 50 *D. melanogaster* lines (low genetic variation (red), high genetic variation (green), and outbred (blue). UniFrac distances account for the relative relatedness of community members, where weighted unifrac incorporates the abundance of observed organisms, while

170 unweighted unifrac only considers presence or absence. Isolines of associated covariates are shown:
171 nucleotide diversity (**A** and **C**) and egg-to-adult viability (**B** and **D**). Envfit values of significant drivers
172 ($p < 0.05$) are shown as arrows (Viab_F0 and Pi_F0 for viability and nucleotide diversity, respectively).

Table 1.

Results of general linear mixed models (GLMMs) of egg-to-adult viability as a function of nucleotide diversity (NuclDiv) and microbiome diversity and their interaction as fixed effects. The four measures of microbiome diversity are divided into alpha richness indices (Observed ASV richness (ObsAlpha) and estimated ASV richness (chao1)), and diversity indices accounting for relative abundances as well (Shannon-Wiener index (Shannon) and Simpson's index (Simpson)) in each of their own model. Both dependent and independent variables are scaled (Z-standardization) to allow direct comparison of effect sizes. Replicate vial ID were included as a random effect, as flies from the same vial are not considered independent. Conditional coefficients of determination of the GLMMs $\left(R^2_{\text{full model}}\right)$ interpreted as the variance explained by the entire model, including both fixed and random effects, is shown. Asterisks denote the significance of individual variables or interactions; *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; and ns: $p > 0.05$.

Microbiome diversity measure	Fixed effects	Estimate	Std. Error	z value	p	Sig.
Observed ASV Richness (ObsAlpha)	Intercept	0.138847	0.072575	1.913163	0.055727	
$R^2_{full\ model}$ = 0.349	NuclDiv	0.840383	0.080439	10.44742	1.51E-25	***
	ObsAlpha	0.438753	0.076743	5.717171	1.08E-08	***
	NuclDiv*ObsAlpha	0.224632	0.077655	2.892688	0.00382	**
	Random effects	**Std.Dev**				
	Vial	0.504				
	Comparison with individual models	**χ^2 df**	**χ^2**		**p**	**Sig.**
	NuclDiv; ObsAlpha; Full model	2	94.508		2.00E-16	***
	Fixed effects	**Estimate**	**Std. Error**	**z value**	**p**	**Sig.**
chao1 estimated ASV Richness (chao1)	Intercept	0.151938	0.072891	2.084454	0.037119	*
$R^2_{full\ model}$ = 0.348	NuclDiv	0.904161	0.078925	11.45594	2.20E-30	***
	chao1	0.360462	0.074083	4.865682	1.14E-06	***
	NuclDiv*chao1	0.19995	0.078566	2.544979	0.010928	*
	Random effects	**Std.Dev**				
	Vial	0.750168				
	Comparison with individual models	**χ^2 df**	**χ^2**		**p**	**Sig.**
	NuclDiv; chao1; Full model	2	110.86		2.00E-16	***
	Fixed effects	**Estimate**	**Std. Error**	**z value**	**p**	**Sig.**
Shannon-Wiener Index (Shannon)	Intercept	0.212908	0.07215	2.950924	0.003168	**
$R^2_{full\ model}$ = 0.350	NuclDiv	0.944147	0.080012	11.80009	3.90E-32	***
	Shannon	0.241357	0.076773	3.143761	0.001668	**
	NuclDiv*Shannon	0.088457	0.074131	1.193258	0.232768	
	Random effects	**Std.Dev**				
	Vial	0.789033				
	Comparison with individual models	**χ^2 df**	**χ^2**		**p**	**Sig.**
	NuclDiv; Shannon; Full model	2	123.18		2.00E-16	***
	Fixed effects	**Estimate**	**Std. Error**	**z value**	**p**	**Sig.**
Simpson's Index (Simpson)	Intercept	0.221356	0.067708	3.269264	0.001078	**
$R^2_{full\ model}$ = 0.351	NuclDiv	1.008222	0.073892	13.64459	2.17E-42	***
	Simpson	0.163998	0.07005	2.341144	0.019225	*
	NuclDiv*Simpson	0.09131	0.063324	1.441961	0.149313	
	Random effects	**Std.Dev**				
	Vial	0.804105				
	Comparison with individual models	**χ^2 df**	**χ^2**		**p**	**Sig.**
	NuclDiv; Simpson; Full model	2	151.19		2.00E-16	***

185

186 *Host genetic variation is associated with differential bacterial abundance*

187 In the outbred flies, 22 genera and 55 ASVs belonging to five bacterial classes and 11 orders were
188 significantly more abundant compared to the bottlenecked lines, i.e. the low and high genetic variation
189 groups (**Supplementary Table S1**). Among these, an additional three ASVs (ASVs 13 and 17 of
190 Nocardiaceae, and ASV 53 of *Enterococcus*) increased in relative abundance with increasing genetic
191 variation of their hosts from outbred to high to low genetic variation (**Supplementary Table S1**).
192 Generally, at both the ASV and genus levels, *Acetobacter* abundances were higher in the low genetic
193 variation flies compared to high genetic variation and outbred lines (**Fig. 4A,B,C**), with *Acetobacter*
194 ASVs 1 and 2 making up the majority (median 89.0%) of the communities in the low genetic variation
195 lines (**Fig. 4A,B**). Two *Lactobacillus* ASVs were significantly more abundant in the high genetic
196 variation flies and the outbred flies compared to flies with low genetic variation (**Fig. 4E,F**), despite a
197 genus-level analysis showing no differential enrichment of *Lactobacillus* in any of the fly groups (**Fig.**
198 **4D**). In addition, certain Corynebacteriales genera were significantly less abundant in the low
199 genetically diverse flies (**Supplementary Table S1**), while two ASVs from the genus *Corynebacterium*
200 showed an incremental increase in abundance with increasing host genetic variation (**Fig. 4G,H**).

201

202 *Core microbiomes of the low genetic variation group are subsets of the high genetic variation groups*

203 The number of ASVs that were present in at least 80% of samples was defined as the core microbiome.
204 The number of ASVs belonging to this core decreased with decreasing host genetic diversity in the fly
205 groups, with 48, 23, and 12 ASVs in outbred, high, and low genetic variation flies, respectively (**Table**
206 **2**). Nine of these ASVs persisted as part of the core microbiome across all of the fly groups, and
207 belonged to the *Acetobacter*, *Enterococcus*, *Lactobacillus*, and *Leuconostoc*. ASVs 3, 152, and 383,
208 belonging to *Wolbachia*, Bacilli, and *Leuconostoc*, respectively, formed part of the core microbiomes
209 in the low and high genetic variation groups but not in the outbred flies (**Table 2**). Meanwhile, nine
210 ASVs belonging to the *Corynebacterium*, *Empedobacter*, Nocardiaceae, *Enterococcus*, *Mesorhizobium*,
211 and *Lactobacillus*, were only in the core microbiomes of the high and outbred flies. The core

212 microbiomes of the low genetic variation lines tended to be subsets of that present in the higher genetic
213 variation lines and outbreds, with 12/12 low genetic variation group ASVs represented in the high
214 genetic variation group, and 18/23 high genetic variation group ASVs represented in the outbred group
215 (**Fig. 5, Table 2**).

Table 2.

Core microbiome ASVs in each of the three host genetic variations groups: low genetic variation (Low), high genetic variation (High), and outbred (OB). The ASVs belonging to the core microbiome for each fly group is represented by + (core) and – (not core). Here we define presence in the core microbiome if an ASV is present in at least 80% of samples of a particular group. The lowest taxonomy level is listed (o__: order, f__: family, g__:genus).

ASVs	Class	Order	Family	Genus	Species	Low	High	OB
ASV1	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	*Acetobacter*	*sp.*	+	+	+
ASV2	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	*Acetobacter*	*sp.*	+	+	+
ASV3	Alphaproteobacteria	Rickettsiales	Anaplasmataceae	*Wolbachia*	*sp.*	+	+	-
ASV4	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	*Acetobacter*	*sp.*	+	+	+
ASV5	Bacilli	Lactobacillales	Enterococcaceae	*Enterococcus*	*sp.*	+	+	+
ASV6	Alphaproteobacteria	Acetobacterales	Acetobacteraceae	*Acetobacter*	*sp.*	-	+	-
ASV7	Bacilli	Lactobacillales	Lactobacillaceae	*Lactobacillus*	*plantarum*	+	+	+
ASV8	Bacilli	Lactobacillales	Enterococcaceae	*Enterococcus*	*sp.*	-	-	+
ASV9	Bacilli	Lactobacillales	Leuconostocaceae	*Leuconostoc*	*sp.*	+	+	+
ASV10	Actinobacteria	Corynebacteriales	Corynebacteriaceae	*Corynebacterium*	*sp.*	-	+	+
ASV11	Bacteroidia	Flavobacteriales	Weeksellaceae	*Empedobacter*	*sp.*	-	+	+
ASV12	Bacilli	Lactobacillales	Lactobacillaceae	*Lactobacillus*	*sp.*	+	+	+
ASV13	Actinobacteria	Corynebacteriales	Nocardiaceae	*g__*	*sp.*	-	+	+
ASV14	Actinobacteria	Corynebacteriales	f__	*g__*	*sp.*	-	+	+
ASV15	Bacilli	Lactobacillales	Lactobacillaceae	*Lactobacillus*	*sp.*	+	+	+
ASV16	Bacilli	Lactobacillales	Lactobacillaceae	*Lactobacillus*	*plantarum*	+	+	+
ASV17	Actinobacteria	Corynebacteriales	Nocardiaceae	*g__*	*sp.*	-	+	+
ASV18	Bacilli	Lactobacillales	Enterococcaceae	*Enterococcus*	*sp.*	-	+	+
ASV19	Actinobacteria	Corynebacteriales	Corynebacteriaceae	*Corynebacterium*	*sp.*	-	-	+
ASV21	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	*Brevundimonas*	*sp.*	-	-	+
ASV22	Bacteroidia	Sphingobacteriales	Sphingobacteriaceae	*Sphingobacterium*	*mizutaii*	-	-	+
ASV23	Gammaproteobacteria	Burkholderiales	Alcaligenaceae	*Alcaligenes*	*sp.*	-	-	+
ASV24	Bacteroidia	Sphingobacteriales	Sphingobacteriaceae	*Sphingobacterium*	*mizutaii*	-	-	+
ASV25	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	*Mesorhizobium*	*sp.*	-	+	+
ASV27	Bacilli	Lactobacillales	Lactobacillaceae	*Lactobacillus*	*brevis*	-	+	+
ASV28	Gammaproteobacteria	Burkholderiales	Alcaligenaceae	*Alcaligenes*	*sp.*	-	-	+
ASV29	Actinobacteria	Corynebacteriales	Nocardiaceae	*Rhodococcus*	*sp.*	-	-	+
ASV30	Actinobacteria	Micrococcales	Microbacteriaceae	*Leucobacter*	*sp.*	-	-	+
ASV32	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	*g__*	*sp.*	-	-	+
ASV39	Bacilli	Lactobacillales	Vagococcaceae	*Vagococcus*	*sp.*	-	-	+
ASV40	Actinobacteria	Micrococcales	Microbacteriaceae	*Microbacterium*	*sp.*	-	-	+
ASV45	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	*Brevundimonas*	*sp.*	-	-	+
ASV46	Actinobacteria	Micrococcales	Brevibacteriaceae	*Brevibacterium*	*sp.*	-	-	+
ASV47	Actinobacteria	Micrococcales	Microbacteriaceae	*Leucobacter*	*sp.*	-	-	+
ASV48	Bacilli	Bacillales	Planococcaceae	*Lysinibacillus*	*sp.*	-	-	+
ASV53	Bacilli	Lactobacillales	Enterococcaceae	*Enterococcus*	*sp.*	-	+	+
ASV56	Bacilli	Lactobacillales	Lactobacillaceae	*Lactobacillus*	*brevis*	-	+	-
ASV59	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	*Mesorhizobium*	*sp.*	-	-	+
ASV72	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	*Mesorhizobium*	*sp.*	-	-	+
ASV87	Actinobacteria	Micrococcales	Microbacteriaceae	*Microbacterium*	*sp.*	-	-	+
ASV91	Actinobacteria	Corynebacteriales	Corynebacteriaceae	*Corynebacterium*	*flavescens*	-	-	+
ASV93	Bacilli	Bacillales	Planococcaceae	*Lysinibacillus*	*sp.*	-	-	+
ASV105	Bacilli	Bacillales	Planococcaceae	*Lysinibacillus*	*sp.*	-	-	+
ASV108	Bacilli	Bacillales	Planococcaceae	*Lysinibacillus*	*sp.*	-	-	+
ASV110	Bacilli	Bacillales	Planococcaceae	*Lysinibacillus*	*sp.*	-	-	+
ASV114	Bacilli	Bacillales	Planococcaceae	*Lysinibacillus*	*sp.*	-	-	+
ASV115	Bacilli	Bacillales	Planococcaceae	*Lysinibacillus*	*sp.*	-	-	+
ASV117	Bacilli	Bacillales	Planococcaceae	*Lysinibacillus*	*sp.*	-	-	+
ASV152	Bacilli	o__	f__	*g__*	*sp.*	+	+	-
ASV194	Gammaproteobacteria	Burkholderiales	Burkholderiaceae	*g__*	*sp.*	-	-	+
ASV227	Actinobacteria	Corynebacteriales	Corynebacteriaceae	*g__*	*sp.*	-	-	+
ASV383	Bacilli	Lactobacillales	Leuconostocaceae	*Leuconostoc*	*sp.*	+	+	-
ASV420	Bacilli	Lactobacillales	Enterococcaceae	*Enterococcus*	*sp.*	-	-	+

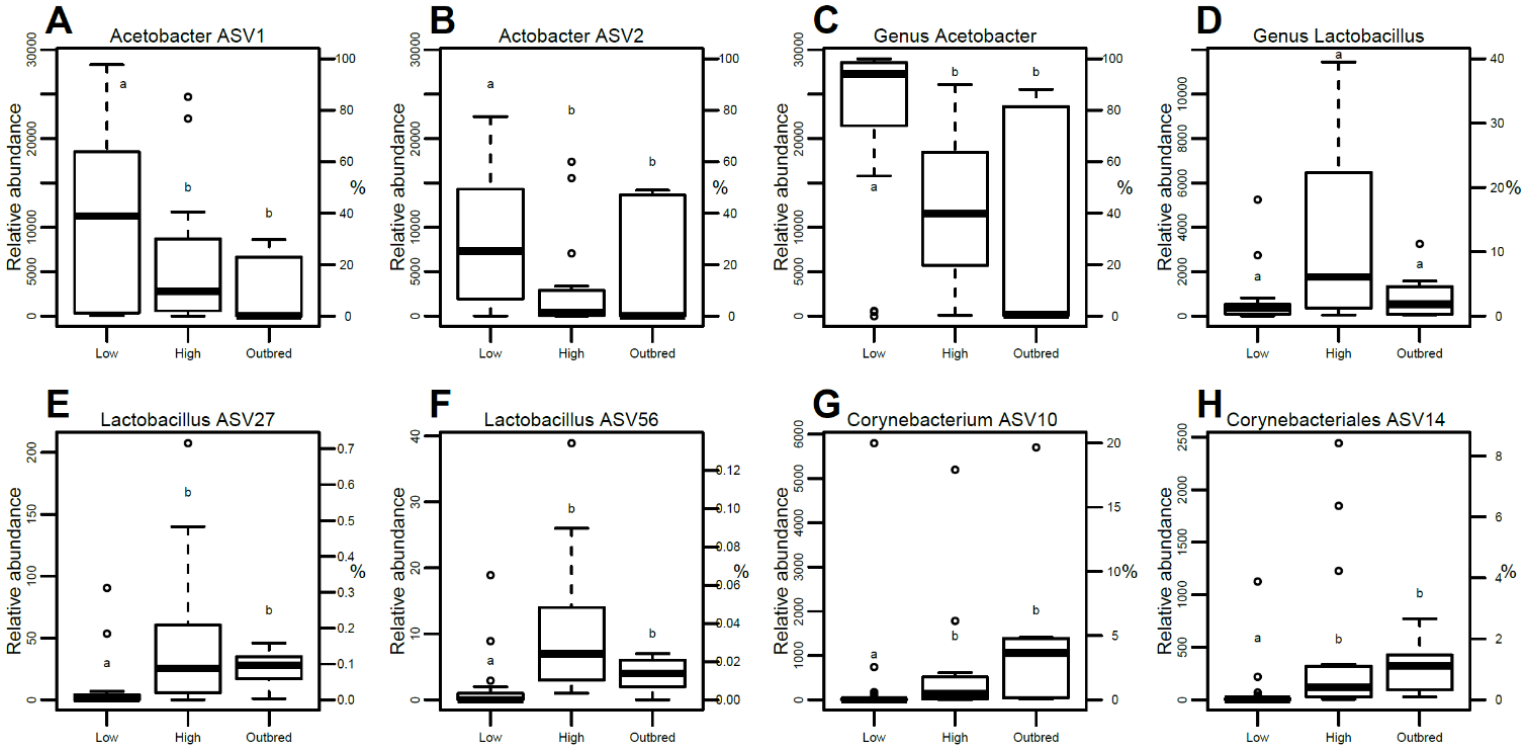

Figure 4.

Relative abundance of differentially enriched bacterial ASVs and genera **A-C.** Acetobacter ASVs and genus, **D-F.** Lactobacillus ASVs and genus, and **G-H.** Corynebacteriales ASVs in the three host genetic variations groups; low genetic variation (Low), high genetic variation (High), and outbred. The abundance axes are not scaled the same for all of the ASVs and genera because *Acetobacter* constitute the majority of the microbiome of the low genetic variation group.

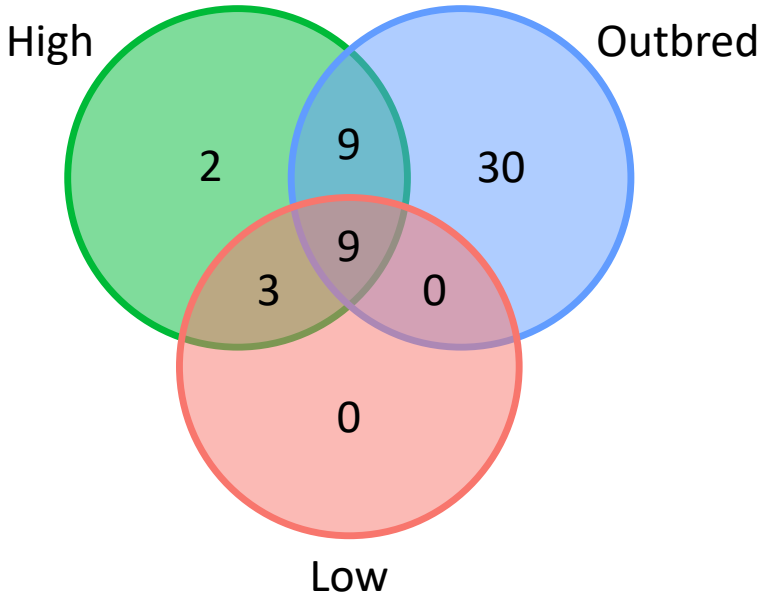

Figure 5.

Venn-diagram showing that the core microbiomes of the low genetic variation lines (Low; red) are subsets of that present in the higher genetic variation lines (High; green) and outbred lines (blue), with 12/12 low genetic variation group ASVs represented in the high genetic variation group, and 18/23 high genetic variation group ASVs represented in the outbred group. Here we define presence in the core microbiome if an ASV is present in at least 80% of samples of a particular group. The taxonomy of individual ASVs can be seen in **Table 2**.

237 *Bacterial co-abundance networks decreased in complexity with lower host genetic variation*

238 Co-abundance network analysis across the entire microbiome dataset was performed to identify
239 bacterial groups that are co-varying in relative abundance with the host fitness traits of the lines (**Fig.**
240 **6**). Fly egg-to-adult viability and nucleotide diversity co-varied with a large cluster of bacterial ASVs
241 within the same modular cluster (**Fig. 6A**). Generally, viability co-varied with a larger group of bacterial
242 ASVs than nucleotide diversity, giving 38 and 12 associations, respectively, while 11 of these ASVs
243 co-varied with both viability and nucleotide diversity. Interestingly, most of the ASVs that co-varied
244 with viability were either differentially enriched in the outbred flies or in both the outbred and high
245 genetic variation flies (**Fig. 6B**). In contrast, the two *Acetobacter* and one *Leuconostoc* ASVs that were
246 significantly enriched in the low genetic variation flies belonged to a separate modular cluster of highly
247 prevalent ASVs alongside nodes belonging to Bacilli, *Acetobacter*, and Actinobacteria
248 (**Supplementary Fig. S7A-B**). The smaller modules were often exclusively made up of related ASVs,
249 notably modules with *Lactobacillus*, *Enterococcus*, or Enterobacterales/*Providencia* species. The
250 networks of each individual fly group (**Fig. 6C,D,E**) revealed that the number of co-varying ASVs, the
251 degree of connectivity (i.e. the number of significant correlations), and the overall complexity of the
252 network decreased from the outbred flies to the low genetic variation flies, with the latter consisting of
253 few ASVs in a modular structure. Host genetic variation was positively correlated with the abundance
254 of bacteria in the outbred network, while host genetic variation did not significantly co-vary with
255 bacterial abundance in the high and low genetic variation networks (at correlation $p < 0.05$). Moreover,
256 the ASVs, belonging to the *Acetobacter* and *Leuconostoc*, that were significantly enriched in the low
257 genetic variation flies displayed no covariation patterns among the inbred flies, (Fig. 6E), in contrast to
258 ASVs that were differentially enriched in the high and/or outbred flies.

259

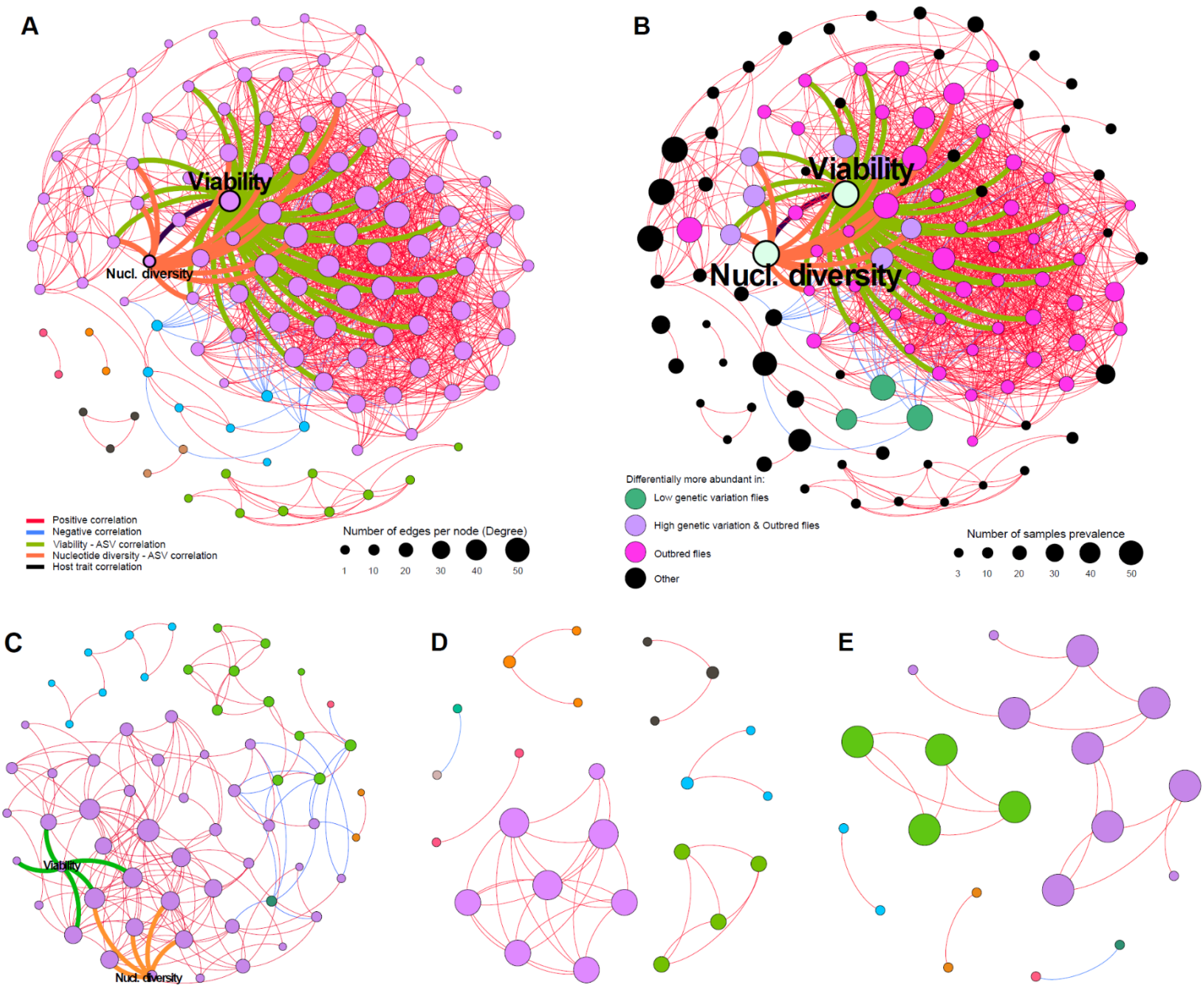

Figure 6.

Co-abundance networks of the fly microbiome. ASVs present in at least 30 reads in total and in at least three samples; correlations of > ±0.5 and fdr-corrected p-values of < 0.05 are shown. The nodes are individual ASVs and the host fitness traits, egg-to-adult viability (Viability) and nucleotide diversity (Nucl. diversity), while the edges represent positive and negative correlations, and correlations linking host fitness traits and bacterial ASVs (which were positive correlations). The network containing samples from all of the fly groups (**A**), the outbred (**C**), high genetic variation (**D**), and low genetic variation (**E**) groups are shown with node sizes displaying the degree of connectivity (relative within each figure) and colors marking the modularity structure (i.e. in which community, or cluster, the ASVs

belong to based on the leiden algorithm; **A, C, D, E**). In **B**, node sizes display the number of samples the ASVs are present within and colors mark the major groups in which the ASVs are differentially enriched. The taxonomic assignment of each node can be seen in **Supplementary Fig. S7**.

Discussion

In this study, we investigated the effects of host genetic variation on the microbiome diversity in *D. melanogaster* lines with experimentally manipulated levels of genetic variation. This was done by establishing replicate lines kept at high population sizes and lines that had been through population genetic bottlenecks. The genome-wide variation was measured in each line, and based on the combined information on levels of genetic variation and fitness we established three groups of flies denoted: 'outbred'; 'high genetic variation' and 'low genetic variation' groups in which we investigated the microbiome diversity.

Consistent with a growing body of literature (Sommer & Bäckhed 2013; Ericsson & Franklin 2015; Mueller & Sachs 2015; Sampson & Mazmanian 2015; Sison-Mangus *et al.* 2015; Moghadam *et al.* 2018; Douglas 2019), we show that the microbial community of the host is strongly linked to the fitness of the host, in our case the viability of offspring. In addition, we link reductions in host genetic variation with microbiome diversity and clearly show that microbiome diversity is lower in lines with reduced genetic variation, regardless of whether we use richness or relative abundance metrics (**Fig. 2**). Only a few previous studies have investigated the association between components of the microbiota and host genetics in invertebrates, and those that did have mainly focused on genetic variability in the host's ability to control the presence and/or abundance of specific endosymbionts (e.g., Mouton *et al.* 2007; von Burg *et al.* 2008; Chong & Moran 2016; Simhadri *et al.* 2017). Conversely, a few studies have investigated how endosymbionts can affect the genetic variation in the host through distorting sex-ratios, such as through endosymbiont induction of cytoplasmic incompatibility or parthenogenesis (Engelstädter & Hurst 2009), potentially decreasing the effective population size and increasing genetic

293 drift (Feldhaar 2011; Ferrari & Vavre 2011) or by influencing population dynamics and dispersal
294 possibly affecting standing genetic variation (Leonardo & Mondor 2006; Goodacre *et al.* 2009).

295 Advances in sequencing technologies have allowed for studies of the microbiome and this has
296 provided new opportunities for studying the importance of the microbiome in multiple disciplines
297 including evolutionary and conservation biology (Amato 2013; Antwis *et al.* 2017; Hird 2017; Cullen
298 *et al.* 2020; Moeller & Sanders 2020). This means that we can now start to experimentally validate ideas
299 centered around the importance of microbiomes for host performance including testing the hypothesis
300 that it is the combined host and microbiome genomes and environmental factors that reflect the fitness
301 of an individual and constitute the unit of selection, i.e. the holobiont concept.

302 Despite calls for an integration of microbiome research in evolutionary and conservation biology
303 (Barelli *et al.* 2015; Bahrndorff *et al.* 2016; Grueber *et al.* 2019; Hauffe & Barelli 2019; Trevelline *et*
304 *al.* 2019; West *et al.* 2019), little progress has been made on experimentally testing fundamental
305 associations between population size, host genetic variation and microbial diversity. Here we provide a
306 novel demonstration that restricting host population size has a profound impact on both the richness and
307 the diversity of host-associated microbiomes, and that effects of host genetic variation and microbial
308 richness interact synergistically in affecting host fitness. We also provide evidence that increased
309 microbial diversity in the lines is associated with a stronger evolutionary response to stressful
310 environments compared to responses seen in lines with less microbial diversity (**Supplementary Fig.**
311 **S6**). Thus, we show that populations with high host genomic variation harbor the most diverse microbial
312 community, and *vice versa* for populations with low genetic variation and that these lines are least
313 resilient to evolutionary pressure. According to the hologenome hypothesis, the holobiont constitutes a
314 single unit of selection, thus our results suggest that small and fragmented populations have a reduced
315 potential for responding to selection pressures not only due to reduced genetic variation and high rates
316 of inbreeding but also due to reduced microbiome variation, effectively reducing hologenomic variation.
317 Maintaining a diverse microbiome community may therefore be crucial for a host's responsiveness to

environmental change, adding to the importance of host genetic variation in adaptive capacity (Zilber-Rosenberg & Rosenberg 2008; Feldhaar 2011; Bordenstein & Theis 2015).

Our study sheds light on questions about the 'inheritance' of microbiomes and about hosts as 'environments' for the microbiota. While many animals, including a range of insects, have transovarial transmission of bacterial symbionts, i.e. via the egg to the embryo (McFall-Ngai *et al.* 2013; Robinson *et al.* 2019), the microbiome of captive *D. melanogaster*, especially the gut bacteria, are mainly horizontally acquired (Wong *et al.* 2016), and consist mostly of microbes expelled into the immediate environment by conspecifics or predecessors (Blum *et al.* 2013). Interestingly, our results show that the 'high genetic variation' bottlenecked populations had a markedly reduced diversity of microbes, and lower fitness compared to the outbred control group, despite there being no difference in genetic variation between the two groups. This could indicate that a reduction in host population size during a population bottleneck constrains the amount of microbial diversity available for 'sampling' for the next generation, similar to the effects of genetic drift on host genetic variation. Indeed, the greater degree of variation in the microbiome community membership, i.e. unweighted UniFrac-based ordination, among the inbred flies as compared to the outbred group strongly suggests a community shift driven by repeated population bottlenecks (**Fig. 3**). Similar to drift, this effect could be exacerbated in small populations, but unlike genetic drift, which can be very stochastic, the effects of bottlenecks yield seemingly directional and predictable effects on microbiome diversity.

Interestingly, the decreasing microbiome diversity trend with increasing host inbreeding was also found in inbred populations of other vastly different species of animal hosts, notably in inbred populations of Diannan small-ear pigs and tortoises (Yuan *et al.* 2015; Wei *et al.* 2020). This gives support to the notion that the decreased ability of inbred hosts to harbor diverse and stable microbiomes could be a general trend among many animals, although these studies did not link microbial diversity and genetic variation to fitness effects or adaptability. Previous experiments on *Drosophila* have highlighted the impact of fly and larval density on microbial communities and how this can impact fitness, producing a type of Allee effect (Wertheim *et al.* 2002). Simultaneously, a reduction in fitness

344 due to the genetic effects of population bottlenecks could in turn result in flies that cannot harbor some
345 components of the bacterial community, which could be especially critical if microbes with specific
346 metabolic functions are lost (West *et al.* 2019). We are unable to tease apart these effects in the current
347 study.

348 Despite this, we show that while host genetic variation has the strongest association with fitness, the
349 microbiome diversity also contributes, acting synergistically with host effects. We envision that
350 increased homozygosity within hosts in a population provides a less optimal habitat for maintaining
351 stable and diverse microbial communities. In this respect, a number of studies have previously reported
352 that hosts who are unhealthy and/or ill harbor different microbiomes compared to their healthier
353 counterparts (Mistry *et al.* 2017; Fei *et al.* 2019). Moreover, host genotypes have been found to
354 profoundly affect the microbiome composition, as well as their stability and beneficial effects on the
355 host (Bonder *et al.* 2016; Rayes *et al.* 2016), while other studies have shown that microbiomes, rather
356 than the host, affect host fitness, development, and even allele frequency and host evolution (Goulet
357 2015; Wang *et al.* 2018; Rudman *et al.* 2019). Given that inbred high genetic variation flies in our study
358 harbored less diverse microbiomes and had lower fitness than outbred flies despite having similar
359 nucleotide diversity, we suggest that at normal levels of genetic variation, the microbiome may be
360 relatively important in reducing fly fitness. Meanwhile, lower nucleotide diversity correlated with even
361 lower microbial diversity and host fitness, hence suggesting that at low host genetic variation due to
362 inbreeding, microbial diversity loss is primarily caused by reduced host fitness. Perhaps the less optimal
363 'habitat' for microbes in low genetic variation lines is because of reduced variation in growth conditions.
364 It has been known for a long time that productivity of *Drosophila* cultures can increase when there is
365 genetic diversity, presumably because multiple genotypes can better utilize different aspects of the
366 environment (Pérez-Tomé & Toro 1982; Hoffmann & Nielsen 1985). The genetically diverse host
367 populations could provide more variable environments for microbes, allowing a greater diversity of
368 microbes to persist. Tests of these ideas in the context of small populations with restricted genetic
369 variation require further studies that might examine the impact of antibiotics on host and microbe

370 associations with fitness. It may also be possible to transfer beneficial microbiomes to see if these can
371 "rescue" low fitness populations. Such experiments have been suggested in other contexts to improve
372 robustness and productivity of livestock and agricultural cultivars (Mueller & Sachs 2015).

373 The microbes detected in our study that varied in abundance include core *D. melanogaster* microbes
374 (Wong *et al.* 2011). Multiple ASVs belonging to Acetobacter were more abundant in lines suffering
375 from inbreeding depression and with low genetic variation, and *vice versa* for Lactobacillus and
376 Corynebacteriales (**Fig. 4**). Although the functional importance of these strains were not investigated in
377 our study, these taxa have been demonstrated to be stable colonizers of *Drosophila* (Ludington & Ja
378 2020). Indeed, many of these ASVs were also prevalent across many of our samples, thereby making
379 up the core microbiome of more than one fly group, and opportunistically increasing in abundance in
380 certain fly groups as conditions became optimal for growth.

381 The high fitness lines do have a much more complex network of co-varying, and putatively
382 interacting microbiomes (**Fig. 6**), where removal of individual species is expected to have less impact
383 whereas in low fitness lines the removal of one key species may cause more dramatic effects. This
384 suggests that high genetic variation and outbred lines harbour healthier and more robust microbial
385 'ecosystems'. This has some resemblance to stress in ecological habitats, where several studies lend
386 empirical support for this idea (Amato *et al.* 2013; Barelli *et al.* 2015; Ingala *et al.* 2019). For instance,
387 howler monkeys inhabiting degraded, more homogenous habitats had a less diverse gut microbiome
388 compared to conspecifics in non-fragmented forests, and as a result had lost the microbial metabolic
389 pathway to detoxify plant compounds in the leaves of their diet (Barelli *et al.* 2015). Diversity confers
390 resilience in macro-ecological systems (Goodman 1975; Memmott *et al.* 2004), where species-rich
391 communities are less susceptible to invasion as more niches are occupied and limiting resources used
392 more efficiently, and the same processes could be important for the robustness of microbial ecosystems
393 within hosts (Lozupone *et al.* 2012; Coyte *et al.* 2015). A high diversity of commensal microbes could
394 mean functional redundancy (Hauffe & Barelli 2019; West *et al.* 2019), thus an increased resilience

395 towards shifts in functional diversity, and better protection against pathogens (Jaenike *et al.* 2010;
396 Koskella & Bergelson 2020).

397 In conclusion, we demonstrate that restricting host population sizes is associated with a reduction in
398 diversity of host-associated microbiomes. We observe effects of both host genetic variation and
399 microbial diversity in explaining host fitness, however the patterns of causality remain unclear, i.e. we
400 suggest that it is not an all-or-nothing effect, but rather a continuum across nucleotide diversity where
401 fitness and microbiome depletion become relatively more important drivers (**Fig. 1** and **Supplementary**
402 **Fig. S5**). It is similarly difficult to establish whether fitness differences are due to beneficial or potential
403 pathogenic bacteria, partly because pathogenicity depends on many factors including the composition
404 of the whole microbial community present (Simhadri *et al.* 2017), and age of the host (Ayyaz & Jasper
405 2013). Despite this, we show clear effects of host population bottlenecks of the diversity of the
406 microbiome, similar to effects normally observed in sampling of genetic alleles during genetic drift, and
407 we show that both the reduction in microbiome diversity and genetic variation is tightly linked to host
408 fitness and evolutionary potential. Therefore, microbial diversity in environmental samples could be a
409 useful proxy for population fitness and potentially adaptability. Lastly, our results open up multiple
410 avenues for further studies, such as transplantation of microbiomes as a means of 'rescuing' populations
411 that suffer from inbreeding depression, relevant to species of conservation concern.

412 # Materials and methods

413 *Fly population bottlenecks*

414 The *D. melanogaster* flies used in the study originated from 232 wild caught females that contributed
415 equally to a mass bred population held at 1000 individuals. The flies were maintained on a 12:12 h
416 light:dark cycle at 19°C on a standard laboratory food composed of yeast (60g/L water), sucrose (40g/L
417 water), oatmeal (30g/L water), and agar (16g/L water) mixed with tap water. Following autoclaving,
418 nipagin (12mL/L water) and acetic acid (1.2mL/L water) was added to prevent fungal growth. From the
419 mass bred population, we created 40 lines of each of three different expected levels of inbreeding for a

total of 120 lines, from which we could obtain nucleotide diversity measures for 109 lines. This is described in detail in Ørsted *et al.* (2019). In summary, lines from the three levels of inbreeding experienced 2, 3, and 5 consecutive generations of bottlenecks each consisting of two males and two females (N=4) revealing expected inbreeding coefficients of 0.125, 0.219 and 0.381, respectively. Simultaneously, we established 10 control lines that were assumed outbred as they were maintained at minimum 500 individuals.

Fly lines selected for microbiome analysis

We obtained a measure of the realised genetic variation in all inbred and outbred lines using genotyping-by-sequencing (GBS) and calculating nucleotide diversity (π) from genomic SNPs (average number of SNPs ± sd = 26,877 ± 4,061; described in detail in Ørsted *et al.* (2019)). For the microbiome analysis, we aimed at comparing two groups of fly lines: one group with low genetic variation and overall low performance and one with high genetic variation and high overall performance. For a quantitative selection of these groups, we calculated a composite measure of performance as the sum of standardised values of viability and nucleotide diversity (Z-score). We selected the 25 lines lowest ranking and the 25 highest ranking lines based on this summed Z-score (50 lines total; **Fig. 1**). Because we selected lines regardless of their inbred/outbred status, nine outbred control lines were included in the 'high genetic variation' lines due to their high Z-scores sum. In all analysis, we differentiate between three groups of flies: 'low' and 'high' genetic variation lines within the inbred lines and 'outbred' (OB) lines that have not been through population bottlenecks. Following establishing the lines population sizes were flushed to ca. 200 individuals per line and in the F1 generation egg-to-adult viability were assessed by distributing 15 eggs into each of five vials per line and calculating the proportion of eggs developing successfully to the adult stage (Ørsted et al. 2019). Flies used for microbiome analysis consisted of flies emerging from the egg-to-adult viability assessment (merged across the 5 vials) that were snap frozen in liquid nitrogen at 2-3 days of age and subsequently stored at -80°C.

446 *16S rRNA gene sample preparation and sequencing*

447 The whole fly genomic DNA was extracted following the same protocol as has been described in Ørsted
448 et al. (2019). In brief, 15 randomly collected male flies from each experimental line was homogenized
449 by bead beating at 2x6 s cycles and 6500 rpm using a Precellys mechanical homogenizer (Bertin
450 Techologies, Montigny le Bretonneux, France), and the DNA was purified with the Dneasy Blood &
451 Tissue kit (QIAGEN, Hilden, Germany) with modifications specific for extracting insect tissues. The
452 V1-V3 hypervariable regions of the bacterial 16S rRNA gene was amplified and multiplexed for
453 Illumina sequencing according to Albertsen *et al.* (2015), and the library pool was sequenced on a
454 MiSeq sequencer using the MiSeq Reagent kit v3, 2x300bp paired-end configuration, and 20% PhiX
455 control spike-in.

456

457 *Microbiome analysis*

458 The demultiplexed paired-end reads were quality filtered, assembled to make consensus amplicon reads,
459 clustered into ASVs, and taxonomically assigned using the in-house workflow AmpProc version
460 5.1.0.beta2.11.1 (https://github.com/eyashiro/AmpProc), which relies on USEARCH version
461 11.0.66_i86linux64 (Edgar 2010) sequenced reads processing and FastTree version 2.1.10 (Price *et al.*
462 2009) for tree building. QIIME version 1.9.1 (Caporaso *et al.* 2010) was used to rarefy all of samples
463 to the smallest sample's size, i.e. 29,000 reads per sample (single_rarefaction.py), generate the observed
464 and estimated chao1 richness and Shannon-Wiener and Simpsons diversity values for each sample
465 (alpha_diversity.py), weighted and unweighted UniFrac matrices (beta_diversity.py), and core
466 microbiome groups (compute_core_microbiome.py). R version 3.5.0 was used for downstream analysis
467 and statistics. To assess the composition of core microbiomes, we calculated as belonging to the core
468 microbiome, the percentage to the nearest tenth if we were to accept a prevalence of an ASV in 80% of
469 the samples in each fly group.

470 Non-metric multidimensional plots based on the UniFrac matrices were generated with the R-
471 package 'vegan' (version 2.5-2 (Oksanen *et al.* 2018)). The effect of two host traits (nucleotide

472 diversity, egg-to-adult viability) (Ørsted *et al.* 2019) were calculated with the envfit function and the
473 significant envfit values ($p < 0.05$) of the fly host traits were displayed as arrows and fly host trait
474 gradients across the ordination space as isolines using the ordisurf function. Differentially enriched
475 ASVs were identified with DESeq2 (version 1.22.2 (Love *et al.* 2014)) using the Wald test, contrasting
476 across three levels (low and high genetic variation and outbred fly groups), and accepting the enriched
477 ASVs with adjusted $p < 0.1$. The same approach was used on the genus-level frequency table.
478

479 *Statistical analysis*

480 To test for differences in measures of microbiome diversity between the genetic variation groups, we
481 performed Kruskal-Wallis tests and pairwise Wilcox's t-tests between groups. To assess drivers of
482 fitness measured as egg-to-adult viability, we fitted generalised linear mixed effect models (GLMMs)
483 in the R-package 'lme4' (Bates *et al.* 2015) assuming a binomial distribution with logit link function.
484 We fitted viability as a function of nucleotide diversity and microbiome diversity (four different
485 measures as described above) and their interaction as fixed effects and replicate vials were included as
486 a random effect, as flies from the same vial are not independent. The full models were compared with
487 individual univariate models of either nucleotide diversity or microbiome diversity by AIC and χ^2
488 difference tests. We detected no over-dispersion in any of the models (residuals to df ratio > 0.31; $\chi^2 >$
489 76.02; $p > 0.99$). Conditional coefficients of determination of the GLMMs interpreted as the variance
490 explained by the entire model, including both fixed and random effects, was calculated as $R^2_{GLMM(c)} =$
491 $\frac{\sigma_f^2+\sigma_\alpha^2}{\sigma_f^2+\sigma_\alpha^2+\sigma_\varepsilon^2}$, where σ_f^2, σ_α^2, σ_ε^2 are the variances of the fixed effect components, the random effects and
492 the residual variance, respectively (delta-method, see Nakagawa *et al.* (2017)).
493

494 *Co-occurrence network analysis*

495 For co-occurrence network analysis, the ASV table of all of the fly samples and the subsetted ASV
496 tables for outbred, high genetic variation, and low genetic variation groups were used as the starting
497 frequency tables. To each of these ASV frequency tables, the fly nucleotide diversity and egg-to-adult

viability measurements were included. Given the unequal number of samples in the different fly groups, all nine samples were used for the outbred subsetted group, while the first nine samples in the list were arbitrarily used for each of the larger subsets. The ASVs present in fewer than three samples and ASVs with less than 30 reads in the respective frequency tables were removed. The 'Hmisc' R-package (Harrell 2021) was used to generate the spearman correlation matrices, and the upper triangle was used to generate the network edge list. Only edges with FDR-corrected $p < 0.05$ and correlation coefficients $> \pm 0.5$ were kept. In Gephi version 0.9.2, the network was generated with the Fruchterman Reingold algorithm and minor crossing of edges were corrected manually to improve the visualization of the clusters. From among Gephi's built-in features, the leiden algorithm was used to calculate the modularity structure, and the degree of connectivity was calculated as the number of nodes with which each node is connected by an edge.

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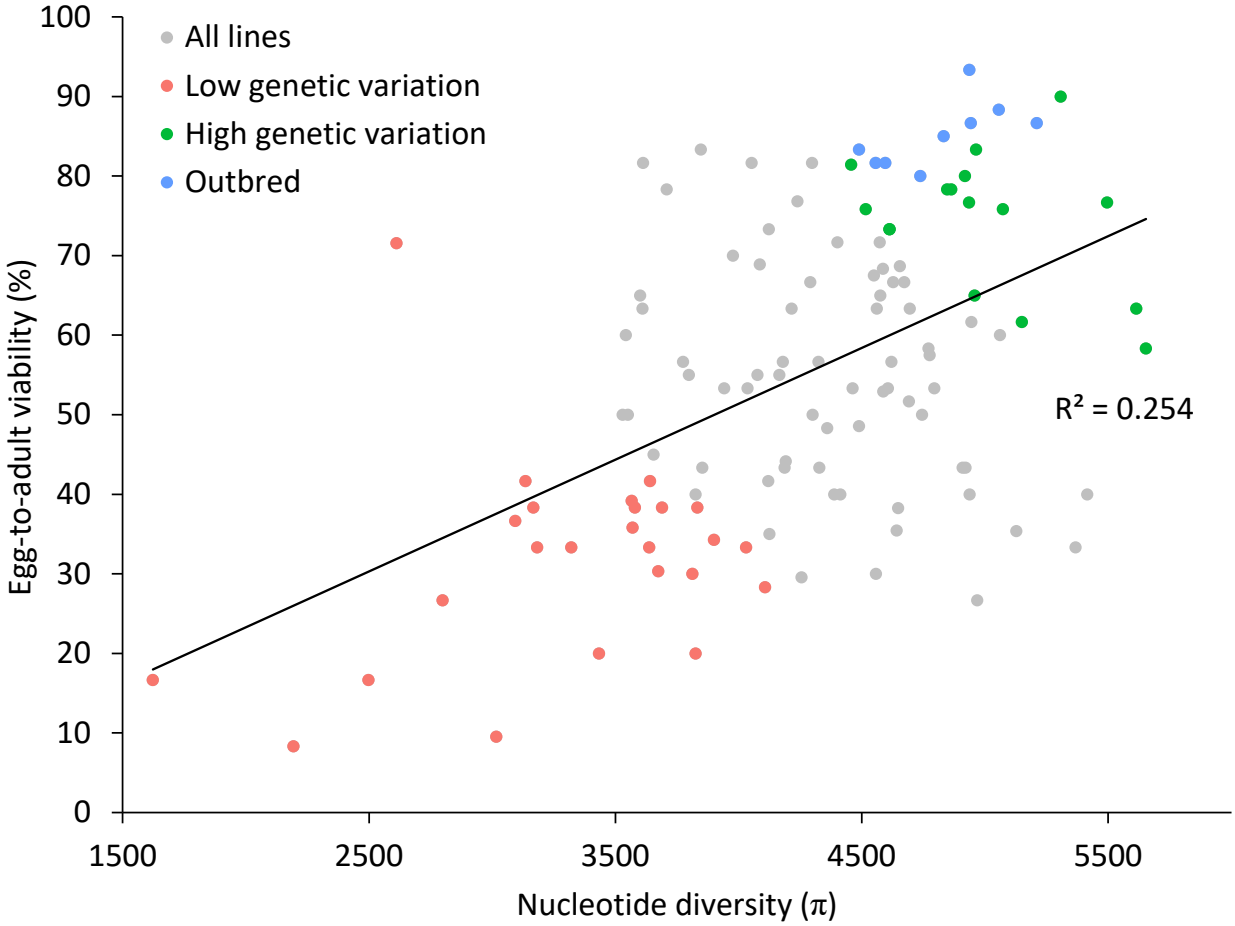
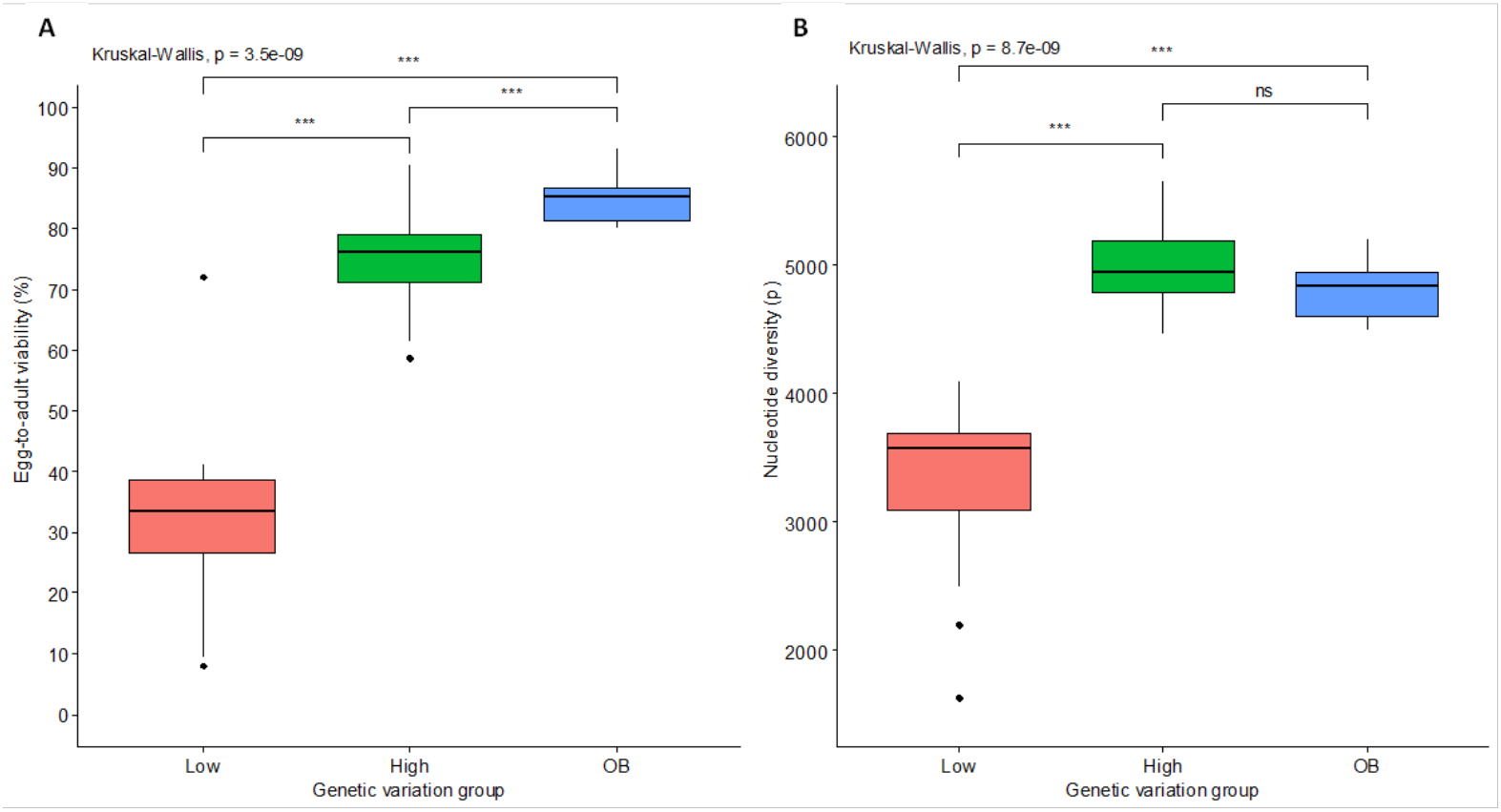
Supplementary Information



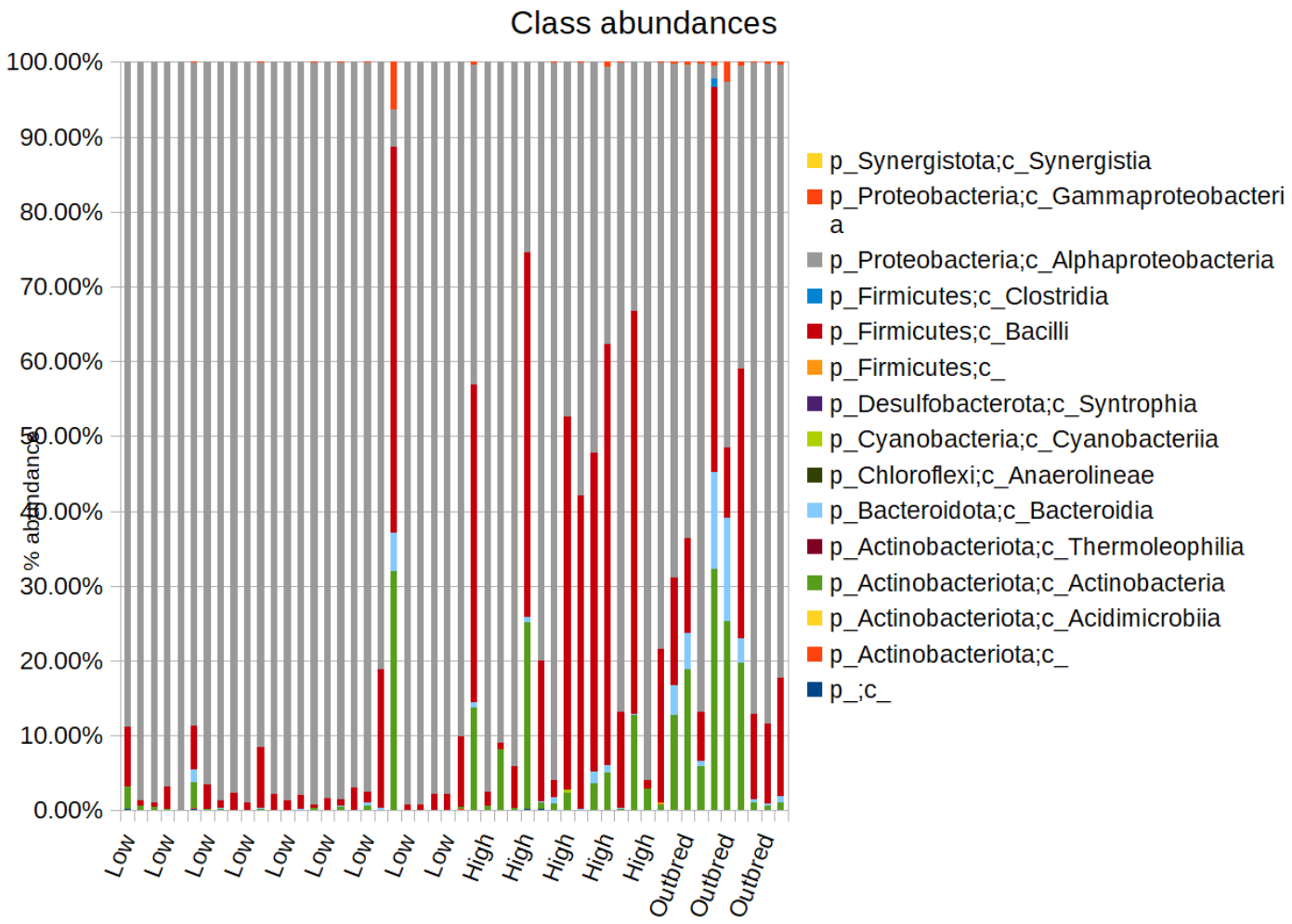
Supplementary Fig. S1.

Scatterplot of line mean egg-to-adult viability (%) and nucleotide diversity (π) as obtained through GBS of 119 lines (grey circles; from Ørsted et al. 2019). From these lines, we selected three groups of lines for analysis of the microbiome, 25 lines with low overall performance (red circles), and 25 lines with high performance (green and blue). This selection was based on a composite measure of the overall performance calculated as the sum of standardized viability and nucleotide diversity. Because we selected lines regardless of their inbred/outbred status, the 'high' group included nine of the outbred controls as they generally had very high fitness and genetic variation. To distinguish between the effects of genetic variation within inbred lines and population bottlenecks, we therefore differentiate between three genetic variation categories: 'Low genetic variation' and 'High genetic variation' lines and 'Outbred' (OB) control lines. The solid line represent the linear regression on all lines with the R^2 value shown.



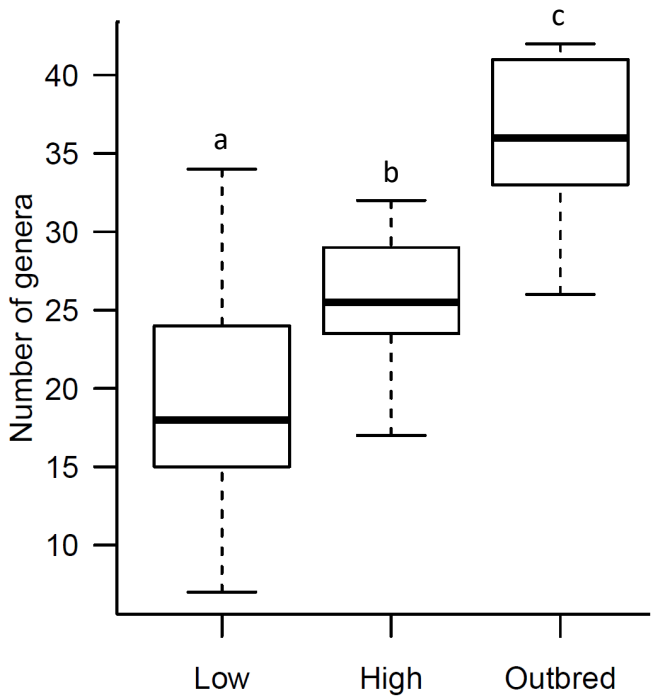
Supplementary Fig. S2.

Both the high genetic variation ('High') and the outbred ('OB') lines exhibited a significantly higher viability (**A**) and nucleotide diversity (**B**) than the low genetic variation lines ('Low'), and viability of OB lines was higher than that of the high genetic variation lines, while nucleotide diversity was indistinguishable between these two groups (two-sample Wilcox's t-tests; $p < 0.05$). The p values of a Kruskal-Wallis test show significant effects of group, while asterisks denote the results of pairwise Wilcox's t-tests between groups; *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; and ns: $p > 0.05$.



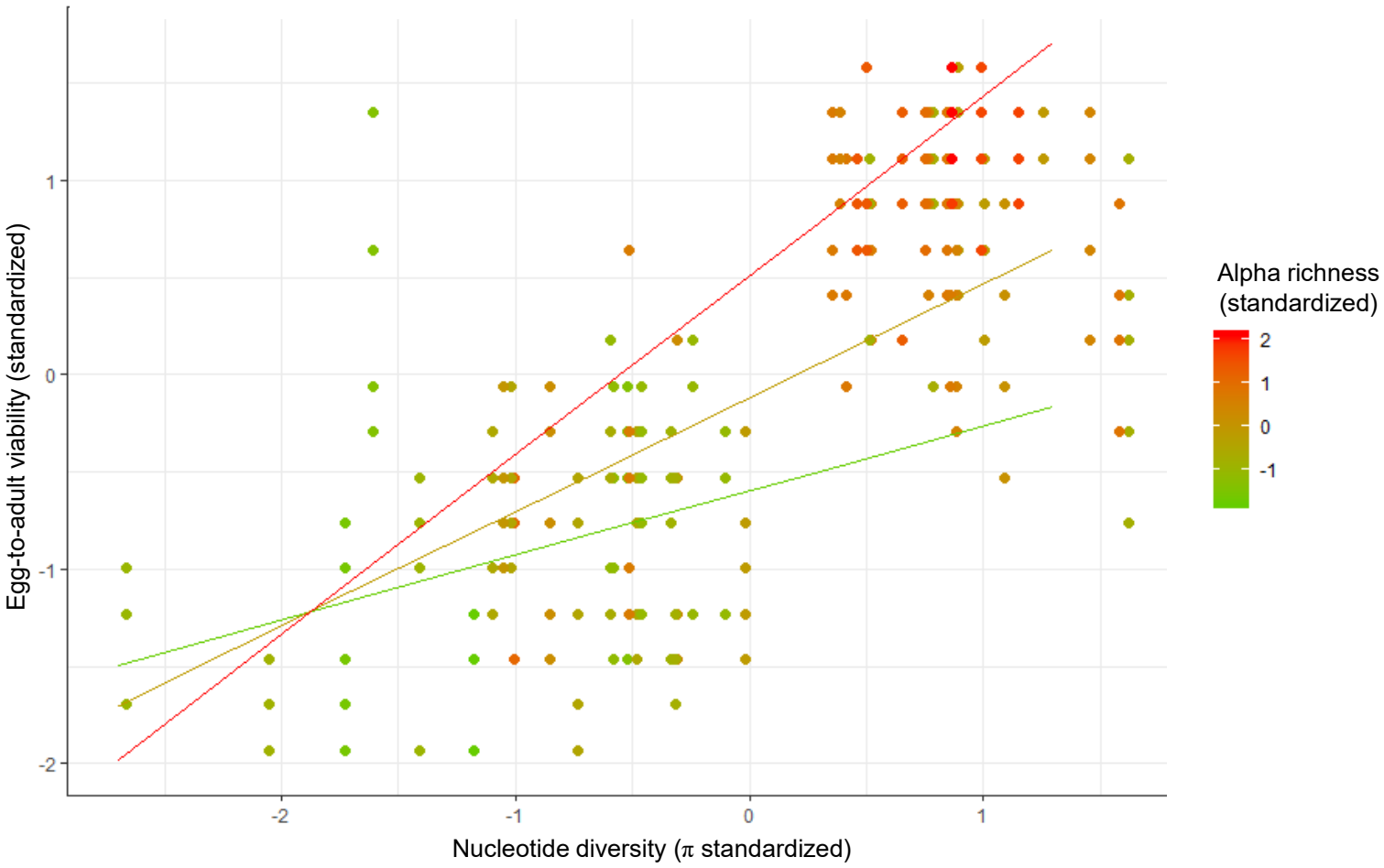
Supplementary Fig. S3.

Bar chart of class-level taxonomic composition of the fly microbiomes in the three major groups of host genetic variation (Low: low genetic variation, High: high genetic variation, and Outbred: outbred flies).



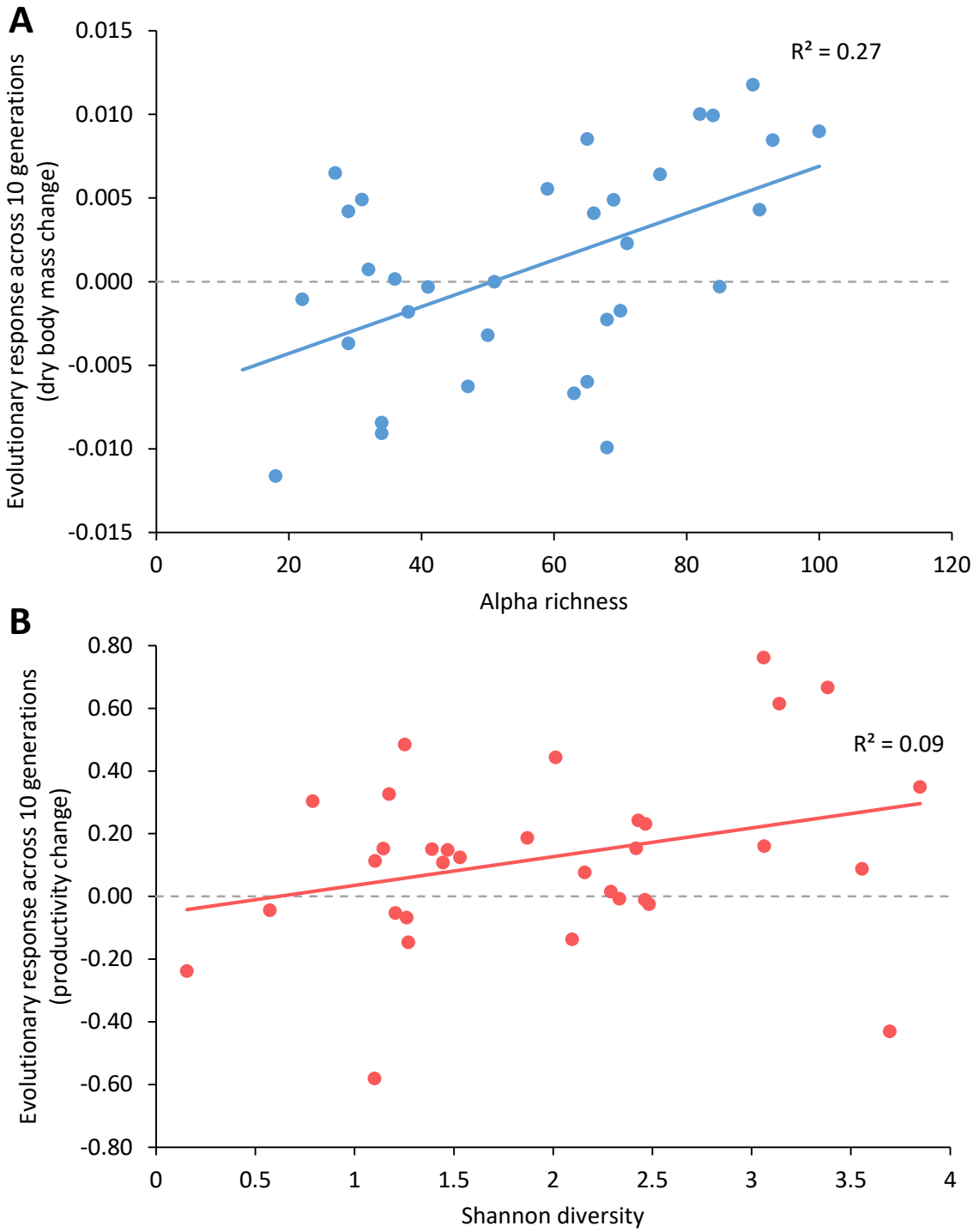
Supplementary Fig. S4.

Box plot of the number of bacterial genera harboured in each line, grouped according to fitness (Low: low genetic variation, High: high genetic variation, and Outbred: outbred flies). Letters denote significant differences in mean number of genera (Tukey's HSD test).



Supplementary Fig. S5

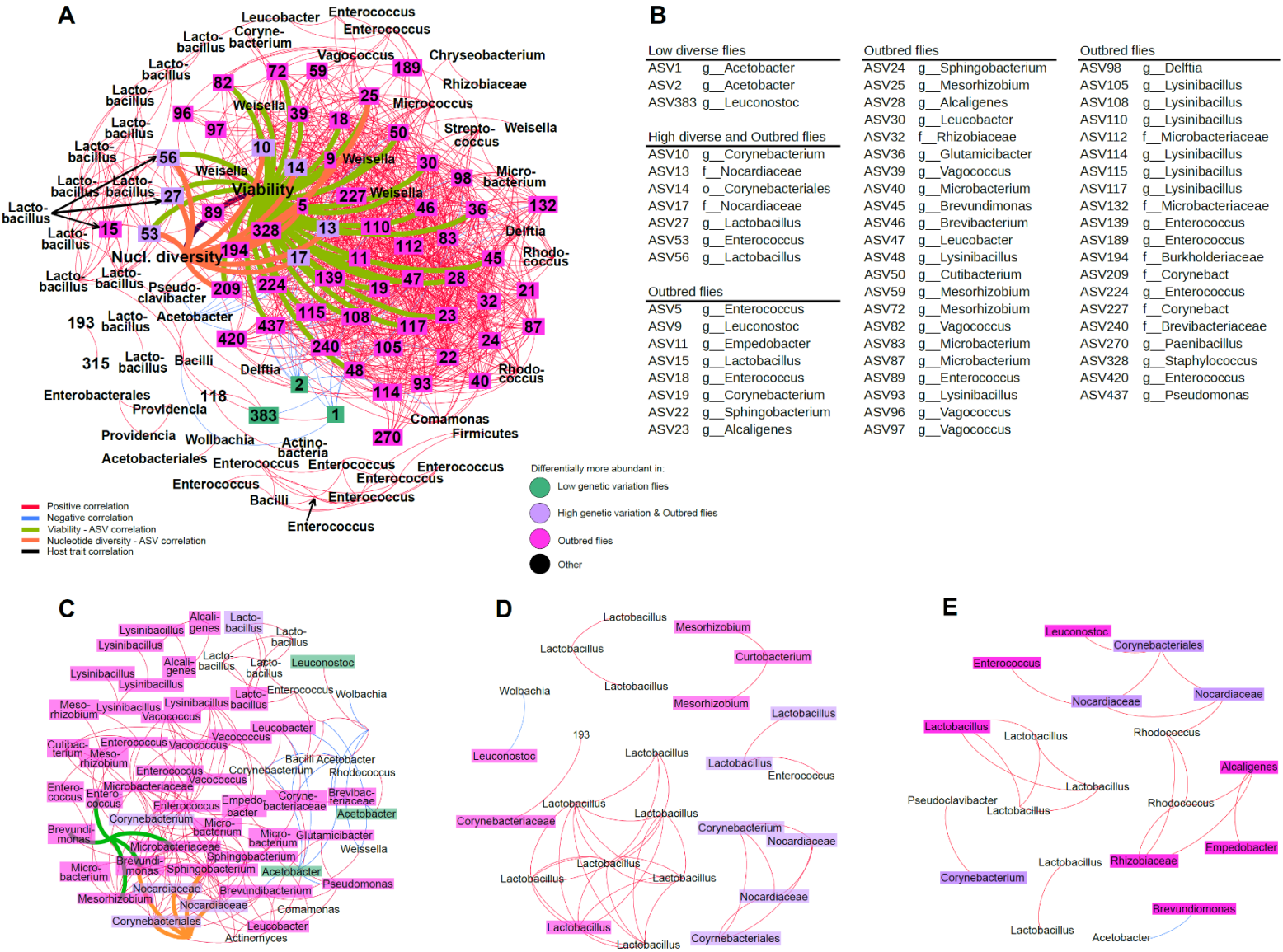
Scatterplot of egg-to-adult viability (z-standardised) from 5 vials per line as a function of nucleotide diversity (π z-standardised) for each of the 50 lines investigated for microbial diversity; the colour of points and in legend denote observed ASV richness (alpha richness, z-standardised). The three regression lines represent regressions on lines with the highest (red), median (tan), and lowest (green) alpha richness, visualizing the significant positive interaction between nucleotide diversity and microbiome richness in explaining fitness of the host population (see **Table 1** for GLMM results).



Supplementary Fig. S6

Using data from Ørsted *et al.* (2019) from the set of lines in the present study, we associate microbial diversity with evolutionary responses in two traits: **A.** dry body mass, and **B.** productivity. Here evolutionary responses are defined as the slope of an ordinary linear regression across 10 generations of rearing on a stressful medium. We correlated all four measures of microbial diversity used in the present study to these two traits (eight comparisons), but here we only included the cases where there

773 was a significant effect of microbial diversity. For both traits, we found an effect of microbial diversity
774 (alpha richness regressed on response in dry body mass: $t_{29} = 2.09$, $p = 0.045$, and Simpson's diversity
775 regressed on response in productivity: $t_{29} = 2.42$, $p = 0.022$), but no effect of genetic variation, π ($t_{29} =$
776 -1.07, $p = 0.92$, and $t_{29} = -0.14$, $p = 0.89$, for dry body mass and productivity, respectively). For details
777 on assessment of these phenotypes, see Ørsted *et al.* (2019).



Supplementary Fig. S7

Co-abundance networks of the fly microbiome (closely resembles **Fig. 6** in main text, except here we supply the ASVs for each node). ASVs present in at least 30 reads in total and in at least three samples, and correlations of > ±0.5 and fdr-corrected p-values of < 0.05 are shown. The nodes are individual ASVs and the host fitness traits, egg-to-adult viability (Viability) and nucleotide diversity (Nucl. diversity), while the edges represent positive and negative correlations, and correlations linking host fitness traits and bacterial ASVs (which were positive correlations). The network containing samples from all of the fly groups (**A**), the outbred (**C**), high genetic variation (**D**), and low genetic variation (**E**) groups are shown with the taxonomic assignment of each node. Non-highlighted nodes that could not be taxonomically assigned are left as ASV identifier numbers. The highlighted nodes

are color-coded according to the major DESeq differential abundance groups. Given the density of nodes in A and the cross-referencing of ASV identifiers in other figures, the ASVs belonging to the major DESeq groups are left as identifiers in A and the taxonomies are listed in B (o__: order, f__: family, g__: genus). The lowest taxonomic assignment down to genus level is displayed. The results of both the leiden and degree were plotted into the network graphs as node colors and node sizes, respectively. For cross-referencing the DESeq2 differentially enriched ASVs in the networks, only the most robust differential abundance patterns were displayed, notably the ASVs that were systematically enriched in the low genetically diverse flies in both the low-high and low-outbred pairwise comparisons (low), the ASVs systematically enriched in the outbred flies (outbred), and those depleted in the low diverse flies (high & outbred).

798 **Supplementary Table S1**

799 DESeq2 table is found online as a Microsoft Excel spreadsheet. The log fold change calculations are
800 done on the ASV (L7 tabs in the spreadsheet) and genus level frequency tables (L6 tabs in the
801 spreadsheet). The DESeq contrasts were done among outbred, high and low genetic variation fly groups,
802 and the pairwise contrasts are displayed in separate sheets. For the most significant pairwise
803 comparisons ($p < 0.1$) the fly group in which the ASVs or genera are enriched in is indicated.