Current Biology, Volume *27*

Supplemental Information

Probabilistic Invasion Underlies Natural Gut Microbiome Stability

Benjamin Obadia, Z.T. Güvener, Vivian Zhang, Javier A. Ceja-Navarro, Eoin L. Brodie, William W. Ja, and William B. Ludington

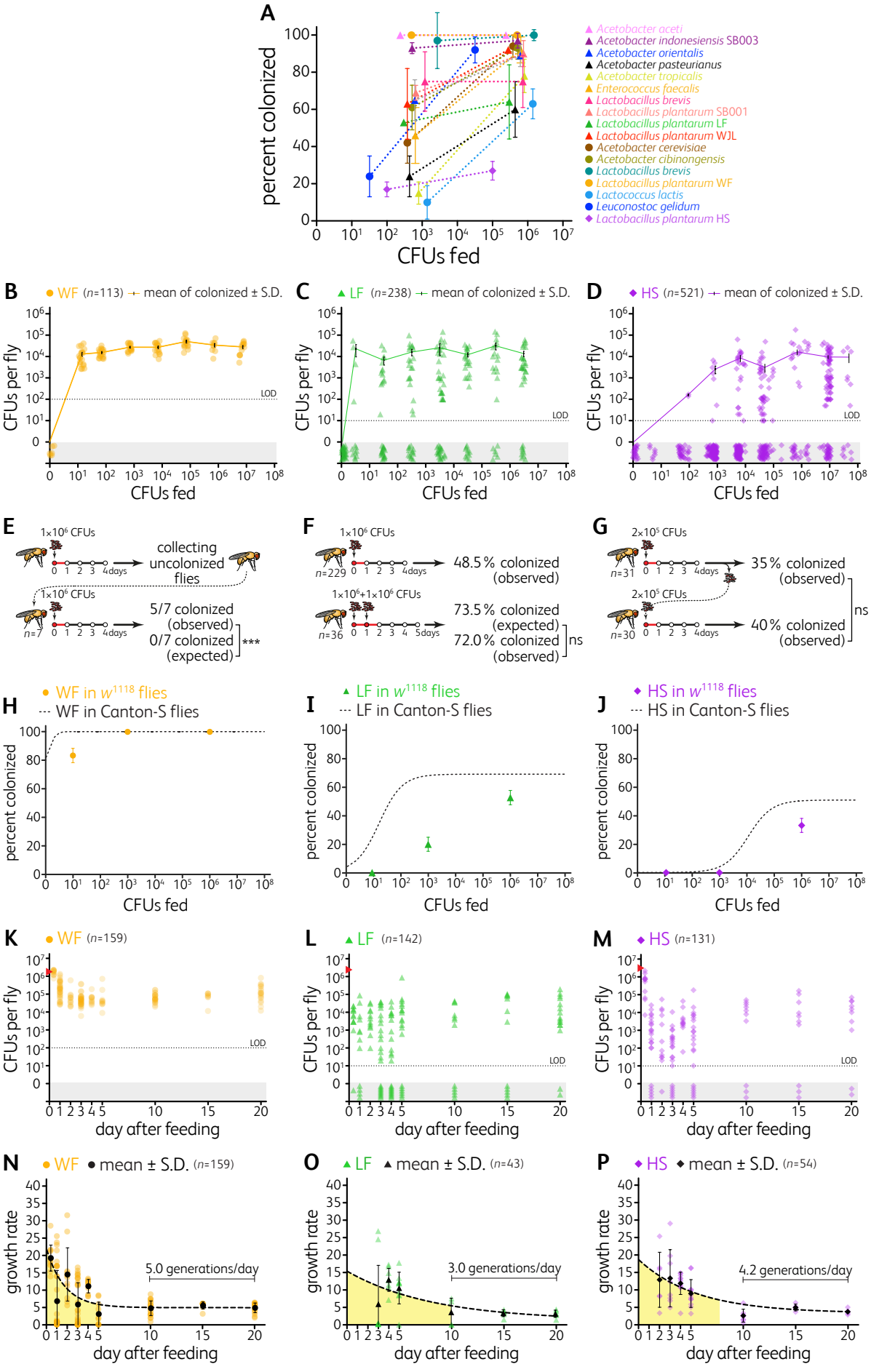
A
percent colonized
CFUs fed
Acetobacter aceti
Acetobacter indonesiensis SB003
Acetobacter orientalis
Acetobacter pasteurianus
Acetobacter tropicalis
Enterococcus faecalis
Lactobacillus brevis
Lactobacillus plantarum SB001
Lactobacillus plantarum LF
Lactobacillus plantarum WJL
Acetobacter cerevisiae
Acetobacter cibinongensis
Lactobacillus brevis
Lactobacillus plantarum WF
Lactococcus lactis
Leuconostoc gelidum
Lactobacillus plantarum HS
B
WF (n=113) mean of colonized ± S.D.
C
LF (n=238) mean of colonized ± S.D.
D
HS (n=521) mean of colonized ± S.D.
CFUs per fly
CFUs fed
LOD
E
1×10⁶ CFUs
collecting uncolonized flies
4days
1×10⁶ CFUs
n=7
5/7 colonized (observed)
0/7 colonized (expected)
***
F
1×10⁶ CFUs
n=229
48.5 % colonized (observed)
1×10⁶+1×10⁶ CFUs
n=36
5days
73.5 % colonized (expected)
72.0 % colonized (observed)
ns
G
2×10⁵ CFUs
n=31
35 % colonized (observed)
2×10⁵ CFUs
n=30
40 % colonized (observed)
ns
H
WF in w1118 flies
WF in Canton-S flies
I
LF in w1118 flies
LF in Canton-S flies
J
HS in w1118 flies
HS in Canton-S flies
percent colonized
CFUs fed
K
WF (n=159)
L
LF (n=142)
M
HS (n=131)
day after feeding
N
WF mean ± S.D. (n=159)
O
LF mean ± S.D. (n=43)
P
HS mean ± S.D. (n=54)
growth rate
5.0 generations/day
3.0 generations/day
4.2 generations/day

Figure S1. Additional Colonization Experiments Indicate Probabilistic Colonization, Related to Figure 1

(A) Natural fly commensal bacteria isolated from wild and lab fruit flies showed varied colonization efficiencies 3 days after feeding (detailed protocol in STAR METHODS). Each data point represents the mean ± S.D. of triplicates (n = 36 flies); triangles = isolated from lab flies; circles = isolated from wild flies; diamond = human isolate.

(B-D) Colonization varies between individual flies relatively independent of the inoculum dose for WF, LF, and HS. Related to Figure 1B. Dose-response 3 days after feeding germ-free Canton-S flies with (B) WF, (C) LF, or (D) HS. Bacterial loads per fly in positively colonized flies with WF, LF, and HS averaged 2.7×10^4, 1.7×10^4, and 2.0×10^4 CFUs, respectively. Data points represent bacterial load in individual flies. Mean and standard error are shown for bins of non-zero data points. Data points corresponding to 0 CFU per fly were jittered (gray area) to reveal the quantity of non-colonized flies. The limit of detection (LOD) was 100 CFUs for WF and 10 CFUs for LF and HS.

(E-G) Successive inoculations are independent events. (E) Germ-free Canton-S flies were individually inoculated with HS (1×10^6 CFUs) and the ones that shed no bacteria in their frass in a 24-h period 3 days after feeding were collected (as determined by collecting their frass and culturing it in rich medium). These flies were inoculated a second time and scored for colonization. Five of seven germ-free flies were infected, confirming that they were not colonization-resistant (***, p-value = 0, z-test). (F) Germ-free Canton-S flies were individually fed one dose or two doses one day apart of HS (1×10^6 CFUs) and scored for colonization 3 days after the last feeding. Nearly half (48.5%) of the flies fed once were colonized. If two successive colonization are independent events, the probability of colonization of the same population of flies after the second inoculation is given by $p = 0.485 + (1 - 0.485) \times 0.485 = 0.735$. Of the flies that were fed with two successive doses, 72% were colonized 3 days after feeding, indicating that successive colonizations are independent events (ns, p-value = 0.84, z-test of proportions). (G) Germ-free Canton-S flies were individually inoculated with HS (2×10^5 CFUs) and the bacteria that successfully colonized flies 3 days after feeding were recovered and directly re-inoculated to germ-free Canton-S flies. *L.plantarum* HS from the initial round colonized 35% of the flies and HS coming from colonized flies were able to colonize 40% of the flies, yielding no significant difference in colonization efficiency (ns, p-value = 0.3570, z-test).

(H-J) Influence of the fly genetic background on colonization dynamics. w^{1118} flies show a slightly lower probability of colonization than Canton-S flies 3 days after feeding with (H) WF (n = 36), (I) LF (n = 57), or (J) HS (n = 93). For comparison, dotted lines indicate the equation 1 fits of the probabilities of colonization in Canton-S flies from Figure 1B. Limit of detection was 100 CFUs in *w*1118 flies. Error bars indicate S.E.P.

(K-P) Related to Figure 1C. Time course of colonization in germ-free Canton-S flies. Individual flies were fed 1.5×10^6, 2.5×10^6, or 3.5×10^6 CFUs of WF, LF, or HS respectively. (K-M) Bacterial load over time for (K) WF, (L) LF, and (M) HS in Canton-S flies. Doses fed to flies are shown by the red triangles. Flies were transferred daily to fresh food during the time course. Data points corresponding to 0 CFU per fly have been jittered (gray area) to reveal the quantity of non-colonized flies. (N-P) Growth rate versus time after inoculation for (N) WF, (O) LF, and (P) HS in Canton-S flies. Yellow shading indicates the period of growth where the number of generations to reach carrying capacity was used to infer the relative bottleneck sizes (WF: 38 generations; LF: 62 generations; HS: 45 generations).

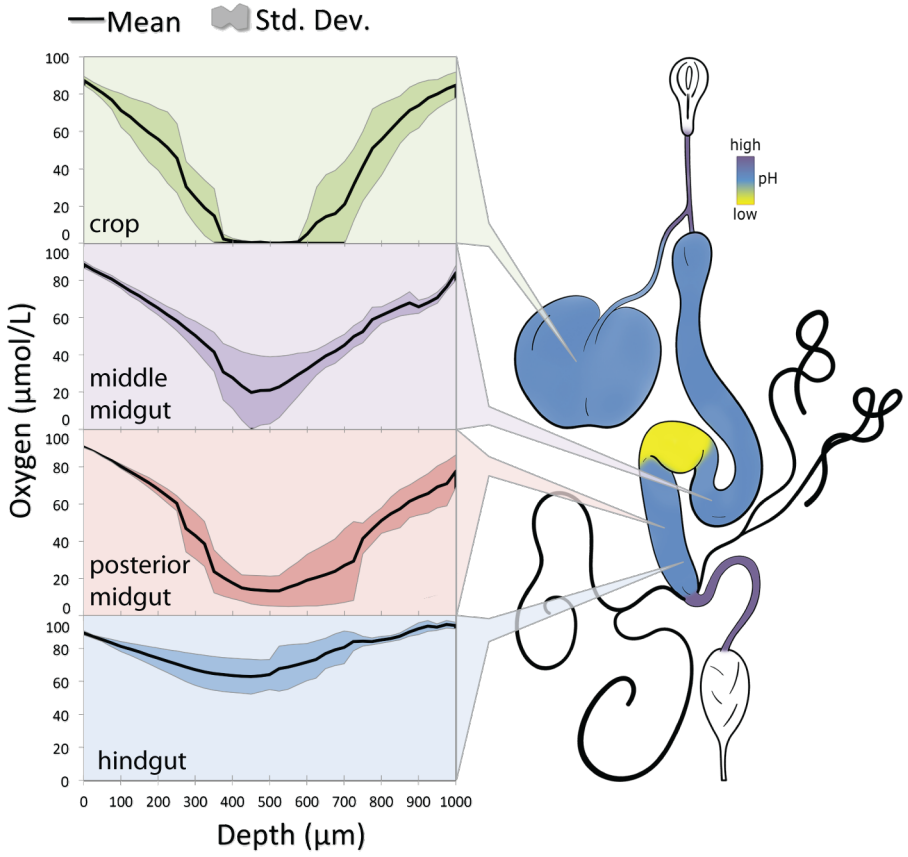

Figure S2. Oxygen Concentration Along the Fly Gastrointestinal Tract, Related to Figure 3

Dissolved oxygen concentrations were measured using a micro-O_2 probe at four locations along the fly gut. Results indicate a 200-μm anaerobic core in the roughly spherical crop as well as a low oxygen core in part of the midgut. Individual conventionally-reared flies were dissected, and their digestive tract was embedded in 40˚C agarose then immediately probed for oxygen content. At least three replicate guts were measured at each position along the gut. Shaded regions indicate S.D. The descriptive scheme of distinct pH regions compiles previous data [S1, S2]. Spatial distances for the midgut and hindgut sections are stretched due to the needle pushing the gut. For reference, the true diameter of the midgut is approximately 200 μm. Traces were manually aligned.

Phenotype	WF	LF	HS
Colonization (*in vivo*)	strong	moderate	weak
Minimum pH	2.5	2.5	2.5
Biofilm formation	moderate	strong	weak
H_2O_2 sensitivity (mM)	2.75	2.75	2.75
Motility in liquid culture	none	none	none
Anaerobic growth	strong	strong	strong

Table S1. *In vitro* physiology of *L. plantarum* strains does not explain colonization differences, Related to Figure 3

Lactobacillus plantarum WF, LF, and HS were subjected to a range of conditions. They were cultured under gradients of pH and of hydrogen peroxide (H_2O_2), showing no differences in tolerance to acidity or hydrogen peroxide. Bacteria were grown in plastic 96-well plates and then stained by Crystal violet to measure the amount of biofilm formation. Motility assays were performed both by plating in 0.5% MRS agar and by direct observation using light microscopy. Anaerobic growth was assessed in liquid media and solid media in an anaerobic chamber.

L.plantarum isolate	Average CFUs per fly	Steady state bacterial generations per day (***g***)	Number of bacteria produced per day	Average CFUs in frass	Shedding rate (***s***) (CFUs in frass per CFUs in fly)	Calculated death rate (***d***)
WF	129,100	5.02	648,082	17,858	0.138	4.88
LF	17,400	2.94	51,156	16,820	0.967	1.97
HS	16,300	4.17	67,971	7,148	0.439	3.73

Table S2. Calculation of shedding and death rates in the gut for the *Lactobacillus* strains, Related to Figure 2

Steady state growth rates were taken from previous experiments (Figure S1N-P). The shedding rate was calculated by measuring the live CFUs in frass shed for 24 h from gnotobiotic flies that were stably colonized and normalizing by the steady state gut abundance. Death rate was calculated using the equation, $\boldsymbol{g} = \boldsymbol{d} + \boldsymbol{s}$, where ***g*** is birth rate, ***d*** is death rate, and ***s*** is shedding rate (calculation in STAR METHODS). Rates are in units of bacterial generations per day. $n = 6$ replicate vials of 12 flies for each *Lactobacillus plantarum* strain.

Oligonucleotides (5'- to -3')	Identifier	Reference	Purpose (GenBank)
AGAGTTTGATCMTGGCTCAG	16S_8_F	[S3]	16S rDNA, identification of most bacterial species
GGYTACCTTGTTACGACTT	16S_1510_R	[S3]	
CTTGGTCATTTAGAGGAAGTAA	ITS1-F	[S4]	Internal Transcribed Spacer regions, identification of most yeast/fungus species
GCTGCGTTCTTCATCGATGC	ITS2	[S5]	
TGGTCCAATAAGTGATGAAGAAAC	*Wsp*_81_F	[S6]	Identification of *Wolbachia* sp. (JQ837254.1)
AAAAATTAAACGCTACTCCA	*Wsp*_691_R	[S6]	
TTTGCAAGTGAAACAGAAGG	*Wsp*B_F	[S6]	Identification of *Wolbachia* (wMel) (AE017196.1)
GCTTTGCTGGCAAAATGG	*Wsp*B_R	[S6]	
AGAGTGGCTGTGAGGCAGAT	DAV_2107_F	This paper	Identification of Drosophila A virus (NC_012958.1)
GCCATCTGACAACAGCTTGA	DAV_2353_R	This paper	
CCAGAGGGCGTTGTCGTCTCCCCCT	DCV_724_F	[S7]	Identification of Drosophila C virus (NC_001834.1)
GGGGCGATTGAACGGGTCCAGGG	DCV_1108_R	[S7]	
CGTCGAGTATTAGCGGCTTC	DXV-A_277_F	[S7]	Identification of Drosophila X virus (NC_004177)
GCCCTACGGAGTCCACATTA	DXV-A_767_R	[S7]	
CCAAACTTTGAGATAATGGCATC	SIGMAV_682_F	[S8]	Identification of Sigma virus (GQ375258.1)
GAATCCGATCATGTATGGGAAG	SIGMAV_966_R	[S8]	
ATGGCAGATTAGTGCAATGG	tLEU	[S9]	Cytochrome c oxidase (CG34069) identification of *Drosophila* spp.
GTTTAAGAGACCAGTACTTG	tLYS	[S9]	

Table S3. Oligonucleotides, Related to STAR METHODS

List of all the primers used in STAR METHODS.

Supplemental References

S1. Li, H., Qi, Y., and Jasper, H. (2016). Preventing Age-Related Decline of Gut Compartmentalization Limits Microbiota Dysbiosis and Extends Lifespan. Cell Host & Microbe *19*, 240–253.

S2. Shanbhag, S., and Tripathi, S. (2009). Epithelial ultrastructure and cellular mechanisms of acid and base transport in the Drosophila midgut. Journal of Experimental Biology *212*, 1731–1744.

S3. Weisburg, W. G., Barns, S. M., Pelletier, D. A., and Lane, D. J. (1991). 16S Ribosomal DNA Amplification for Phylogenetic Study. Journal of Bacteriology *173*, 697–703.

S4. Gardes, M., and Bruns, T. D. (1993). ITS primers with enhanced specificity for basidiomycetes--application to the identification of mycorrhizae and rusts. Mol. Ecol. *2*, 113–118.

S5. White, T. J., Bruns, T., Lee, S., and Taylor, J. W. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to ….

S6. Teixeira, L., Ferreira, Á., and Ashburner, M. (2008). The Bacterial Symbiont Wolbachia Induces Resistance to RNA Viral Infections in Drosophila melanogaster. Plos Biol *6*, e2.

S7. Goic, B., Vodovar, N., Mondotte, J. A., Monot, C., Frangeul, L., Blanc, H., Gausson, V., Vera-Otarola, J., Cristofari, G., and Saleh, M.-C. (2013). RNA-mediated interference and reverse transcription control the persistence of RNA viruses in the insect model Drosophila. Nat Immunol *14*, 396–403.

S8. Longdon, B., Obbard, D. J., and Jiggins, F. M. (2010). Sigma viruses from three species of Drosophila form a major new clade in the rhabdovirus phylogeny. Proc. Biol. Sci. *277*, 35–44.

S9. Liu, H., and Beckenbach, A. T. (1992). Evolution of the Mitochondrial Cytochrome Oxidase II Gene among 10 Orders of Insects. Mol. Phylogenet. Evol. *1*, 41–52.