Creation of experimental lines

To create the X-type-only and uninfected lines, aphids were selectively cured by allowing second and third instar nymphs to feed on aphid artificial diet spiked with an antibiotic cocktail containing 150 µg/mL of ampicillin, gentamycin, and cefotaxime for three days, modified from (1). Surviving aphids were placed on fresh fava bean (*Vicia faba*) plants until adulthood and a subset of their offspring (2nd-3rd instar) were collected to undergo DNA extraction. Nymphs were squished in 20-30 µL of lysis buffer (depending on size) with 1% proteinase K and incubated using the following protocol: 38˚ C for 35 minutes, 95˚ C for 2.5 minutes, and held at 4˚ C or on ice until screened for symbiont infection using the diagnostic PCR protocols described in (2, 3). If the antibiotic-fed mother produced offspring (F1) with the desired infection status, remaining offspring were isolated and allowed to reproduce and the screening protocol was repeated. After confirmation of symbiont infection in the F2 generation, we selected a single, parthenogenetic female to establish the experimental line, to ensure all individuals carried the desired infection status. To generate the *H. defensa*-only line, we injected hemolymph collected from superinfected aphids (XH) into the abdomens of 3rd instar aphids cured of both symbionts (UC line) using glass capillary needles with as in (2, 4). Post-microinjection, aphids were placed on fresh fava plants and underwent the same screening process as the antibiotic-exposed aphids until we confirmed infection with only *H. defensa*.

For microsatellite genotyping, aphid DNA was extracted using an E.Z.N.A. Tissue DNA Kit (Omega Bio-Tek) and stored at -20C until use. Microsatellite protocol follows (2), in which four microsatellite loci were amplified with PCR using Dye Set-30 (DS-30) fluorescent primers and a reaction cycle of: 4°C for 3 min; 45 cycles of 95°C for 30 s, 68–56°C touchdown for 13 cycles, then 55°C for 32 cycles, each cycle for 30 s, 72°C for 30 s; 72°C for final elongation, then held at 4°C. Samples were then sent to the Georgia Genomics Facility for genotyping on an Applied Biosystems 3730xl DNA Analyzer, with the ROX500 size standard. Data was analyzed using Geneious® v8.1.5.

Fitness assays

Adult aphids were placed onto fresh *V. fava* plants, allowed to reproduce for 24 hours, and offspring were collected and transferred to a fresh *V. fava* plant in cohorts of five aphids. To measure survival and fecundity, aphids were checked every three days for 30 days, at which point almost all aphids had died or ceased reproduction. The number of surviving and dead

aphids were counted at each date and any offspring produced were counted and removed (to prevent being recounted), with the total output summed as lifetime fecundity.

Exposure to a fungal pathogen

Prior to the experiment, corpses of aphids killed by *P. neoaphidis* were stored post-death but pre-sporulation at 4°C for no more than twelve weeks within a plastic screw cap container along with a desiccator bag. To rehydrate corpses and initiate sporulation, two corpses were centrally placed onto a freshly made 1.5% tap water agar plate. The plates were then sealed with Parafilm and held in the dark at 20°C for 14-16 hours to encourage sporulation. Sporulation was visually confirmed by the presence of large spore showers around the corpses, and corpses that failed to sporulate were discarded and not used in the experiment.

Quantifying symbiont titers

Fragments of the single-copy bacterial genes, *dnaK* from *H. defensa* and *gryB* from X-type, along with the polyploid *dnaK* gene from *B. aphidicola*, were amplified using RT-qPCR (2, 5-7). The X-type primers new to this study were: forward (ACG GAG GTG AGT ACC CGA AAA) and reverse (ATC AGC GTT CAT CTC TCC CA). 10 µL PCR reactions were performed on a Roche LightCycler 480 II using Roche LightCycler SYBR Green I Master chemistry and 0.5 µM of forward and reverse primers. A standard PCR cycle was used for all primers: 95˚C for 5 min; 45 cycles of 95˚C for 10 sec, 68-56˚C touchdown for 13 cycles, then 55˚C for 32 cycles, each cycle for 10 sec; 72˚C for 10 sec (7). Symbiont titers were estimated using external standard curves of each gene previously created with serial dilutions of $1x10^2$ to $1x10^9$ with duplicate standards of $1x10^7$ (8). Additionally, to correct for differing extraction efficiency between DNA samples, the aphid *Ef-1 α* gene was amplified for all samples. A correction factor was determined by dividing the highest *Ef-1 α* by the *Ef-1 α* of each sample for each time point; symbiont estimates were then multiplied by this factor.

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SI Results

Supplemental Table 1 Loci information and allele location for aphid microsatellites used to identify the genotype of 5D experimental lines. Blanks for loci indicate homozygosity.

Experimental line	Loci	Allele 1	Allele 2
5D-UC	Ap-02	228	232
	Ap-03	233	243
	Ap-05	256	264
	Aph10M	195	
5D-H	Ap-02	228	232
	Ap-03	233	243
	Ap-05	256	264
	Aph10M	195	
5D-X	Ap-02	228	232
	Ap-03	233	243
	Ap-05	256	264
	Aph10M	195	
5D-XH	Ap-02	228	232
	Ap-03	233	243
	Ap-05	256	264
	Aph10M	195	

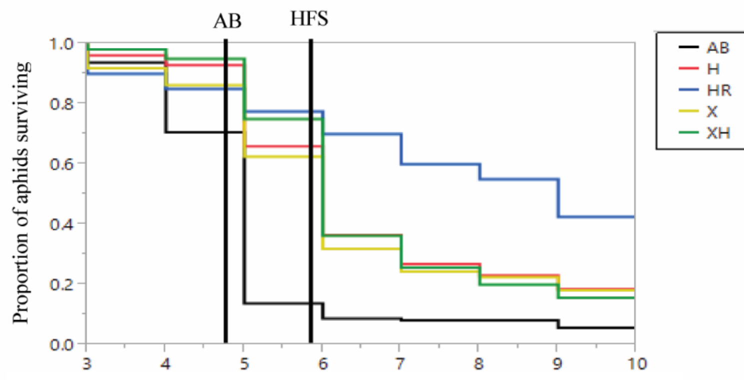
SI Table 2 X-type and *H. defensa* strain and collection location information. Loci used in strain determination reported along with accession numbers. The X-type sample with a single SNP in the *accD* locus (WI42) is also included.

Bacteria species	Location	Loci	Accession Number
X-type/5D	WI	*accD*	KY271013
		gyrB	KY271015
		hrpA	KY271016
		murE	KY271017
		recJ	KY271018
		rpoS	KY271022
		16S	KY271023
X-type/WI42	WI	*accD*	KY271014
X-type	NY	*accD*	KY274198
		gyrB	KY274199
		hrpA	KY274200
		murE	KY274201
		recJ	KY274202
		rpoS	KY274203
H. defensa	WI	*recJ*	KY271019
		recJ	KY271020
		recJ	KY271021
H. defensa	WI	*ptsI*	KY271024
		ptsI	KY271025
		ptsI	KY271026

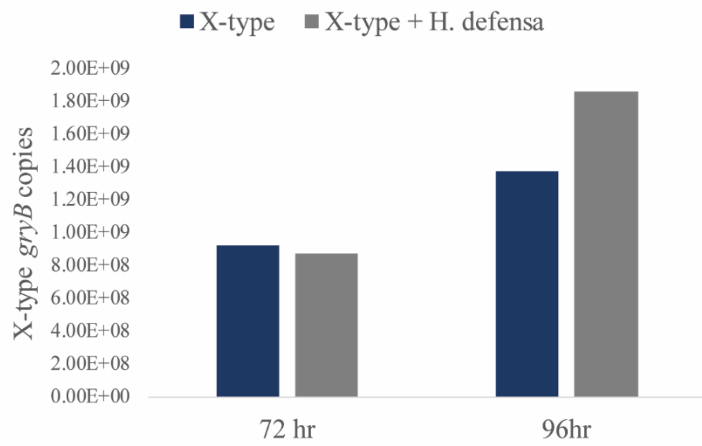
SI Table 3 Accession numbers for bacterial genes used to generate Enterobacteriaciae phylogeny (**Fig. 7**).

Species	Loci	Accession number
Arsenophonus nasoniae	*gyrB*	FN545181.1
	accD	FN545267.1
	16S	M90801.1
Blochmannia sp.	*gyrB*	CP002189.2
	accD	CP002189.2
	16S	CP002189.2
Enterobacter cloacae	*gyrB*	CP006580.1
	accD	CP006580.1
	16S	CP006580.1
Erwinia amylovora	*gyrB*	FN434113.1
	accD	FN434113.1
	16S	FN434113.1
Escherichia coli	*gyrB*	AAJT02000007.1
	accD	AAJT02000067.1
	16S	AAJT02000023.1
Ewingella americana	*gyrB*	NZ_JMPJ01000058.1
	accD	NZ_JMPJ01000067.1
	16S	NZ_JMPJ01000013.1
Hamiltonella defensa (*A. pisum*)	*gyrB*	NC_012751.1
	accD	NC_012751.1
	16S	NC_012751.1
H. defensa (*Uroleucon*)	*gyrB*	EU021842.1
	accD	EU021762.1
	16S	AF293621.1
H. defensa (*B. tabaci*)	*gyrB*	NZ_AJLH02000014.1
	accD	NZ_AJLH02000006.1
	16S	JF795506.1
Klebsiella pneumoniae	*gyrB*	AP006725.1
	accD	AP006725.1
	16S	AP006725.1
Pantoea agglomerans	*gyrB*	AXOF01000028.1
	accD	AXOF01000007.1
	16S	AXOF01000017.1
Photorhabdus luminescens	*gyrB*	BX571869.1
	accD	BX571869.1
	16S	BX571869.1
Proteus mirabilis	*gyrB*	CP012674.1
	accD	CP012674.1

	16S	CP012674.1
Proteus vulgaris	*gyrB*	CP012675.1
	accD	CP012675.1
	16S	CP012675.1
Regiella insecticola strain *5.15*	*gyrB*	AGCA01000035.1
	accD	AGCA01000305.1
	16S	AGCA01000465.1
Regiella insecticola	*gyrB*	KF575207.1
	accD	KF575191.1
	16S	AF293618.1
Salmonella enterica	*gyrB*	ABAK02000001.1
	accD	ABAK02000001.1
	16S	ABAK02000001.1
Serratia marcescens	*gyrB*	CP012685.1
	accD	CP012685.1
	16S	CP012685.1
Serratia symbiotica	*gyrB*	GL636117.1
	accD	GL636126.1
	16S	GL636115.1
Shigella flexneri	*gyrB*	AE005674.2
	accD	AE005674.2
	16S	AE005674.2
Vibrio cholerae	*gyrB*	AE003852.1
	accD	AE003852.1
	16S	AE003852.1
Wigglesworthia glossinidia	*gyrB*	CP003315.1
	accD	CP003315.1
	16S	CP003315.1
Xenorhabdus bovienii	*gyrB*	FN667741.1
	accD	FN667741.1
	16S	FN667741.1
X-type (5D)	*gyrB*	KY271015
	accD	KY271013
	16S	KY271023
Yersinia enterocolitica	*gyrB*	AM286415.1
	accD	AM286415.1
	16S	AM286415.1
Yersinia pestis	*gyrB*	ADDC01000001.1
	accD	ADDC01000001.1
	16S	ADDC01000001.1



SI Figure 1 Kaplan-Meier plot of aphid survival post exposu re to *P. neoaphidis*. Dashed lines represent estimated time of 0.5 sporulation for aphids lacking facultative symbionts (UC) and those with symbionts (HFS) (α = 0.05).



SI Fig 2 Mean X-type *gyrB* copies in 72 and 96 hour-old aphids (genotype WI12) infected with X-type (blue) and superinfected with X-type+*H. defensa* (grey).