

Supplemental Figures

Figure S1. Correlations in the relative expression levels of immune genes and bacterial density for host species, by site. All values are on a log scale. “Bt” refers to relative bacterial density, and the remainder of the labelled rows are immune genes. The results are shown for single *T. castaneum* and *T. confusum* populations isolated from the same site (column 1: Snavely site; column 2: Green River site) as well as the conglomeration of all populations (right-most column). Colors highlight the strength of the correlation (Pearson correlation; <yellow < 0.5 < blue < 0.65 < light red < 0.85 < dark red < 1). *Cec3* was not assayed for wild-derived beetles due to failure of any primer sets to achieve efficient amplification for all populations.

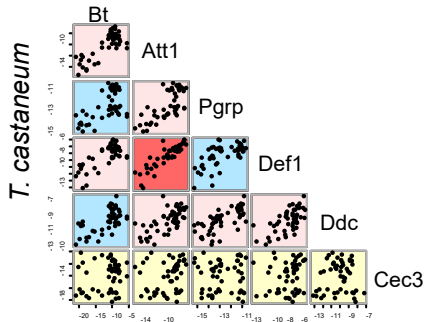
Figure S2. Survival, resistance, and immune gene expression of natural populations during *Photorhabdus luminescens* infection. Survival during acute infection was monitored for 24 hours post infection (A; N = 50-60 beetles/population). Relative bacterial density for each individual within each population at 14 hours post infection, as quantified by RT-qPCR, is calculated as the log of the linearized difference between P.lum-specific and host reference gene expression (B). The relationship between the log of bacterial density and the log of *attacin-1* (C.) and *defensin-1* (D.) gene expression 14 hours after challenge with saline or P.lum infection illustrates variation in the intercept and slope of inducible immune gene expression among populations. Lines represent linear fits for each main variable level as computed by the “lm” function in the geom_smooth algorithm of ggplot2 (R).

Figure S3. Correlations between constitutive and inducible immune parameters and phenotypic outcomes of infection with *Bacillus thuringiensis* and *Photorhabdus luminescens*. Quantification of Pearson correlation coefficients (A; bottom half = coefficient (significant ones in color), top half = relative magnitude) among phenotypes and immune parameters. Bt density at 8 hours post infection is associated with host mortality (B), but P.lum density at 14 hours shows no association with host mortality (C). Bt density at 8 hours post infection, given a low initial dose, is highly correlated to bacterial density from higher initial doses (D). Bt density at 8 hours post infection is positively correlated to the slope of *defensin-1* expression (E). HR = hazard ratio, BLUD = Bt density at death, Bt_dose and Plum_dens = bacterial density at 8 and 14 hours post infection respectively, naïve = expression in uninfected individuals, int = microbe-independent inducible gene expression intercept (saline-injected expression at 8 hours), coef = slope of expression over bacterial density. Att1 = *attacin-1*, Def1 = *defensin-1*, pg = *pgrp-sc2*, ddc = *dopa decarboxylase*. Lines represent linear fits for each main variable level as computed by the “lm” function in the geom_smooth algorithm of ggplot2 (R).

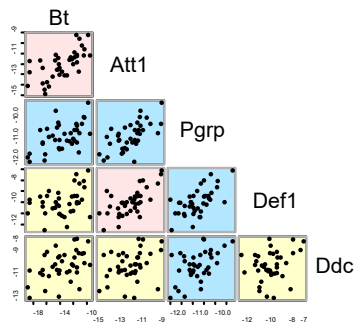
Figure S4. Patterns of constitutive and inducible *pgrp-sc2* (A), *ddc* (B), and *cecropin-3* (C) expression by population, and *attacin-1* (D) and *cecropin-3* (E) expression by RNAi treatment in *T. castaneum*. The relationship between the log of bacterial density and the log of immune gene expression 8 hours after challenge with saline or Bt infection illustrates variation in both the intercept (microbe-independent) and slope (microbe-dependent sensitivity) of inducible immune gene expression among populations. Lines represent linear fits for each main variable level as computed by the “lm” function in the geom_smooth algorithm of ggplot2 (R). Top row: color-coded by population. Bottom row: blue = injected with Male (non-target) dsRNA, yellow = injected with *imd*-dsRNA prior to infection.

Figure S5. Bacterial density and immune gene expression at the time of infection-induced mortality with Bt. Bacterial density as quantified by RT-qPCR in individuals showing moribund behavior (A) is not closely associated with the time post infection at which the individual transitions into the moribund group (B). Expression of *defensin-1* (C) and *ddc* (D) as a function of bacterial density shows variation in magnitude among populations. Lines represent linear fits for each main variable level as computed by the “lm” function in the geom_smooth algorithm of ggplot2 (R).

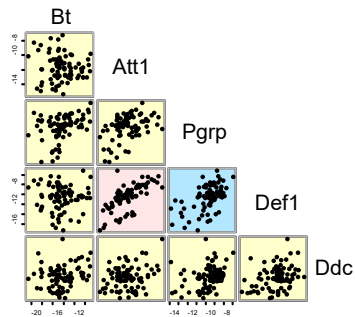
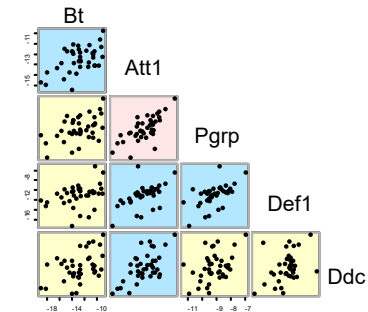
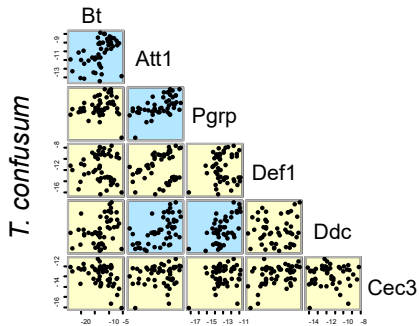
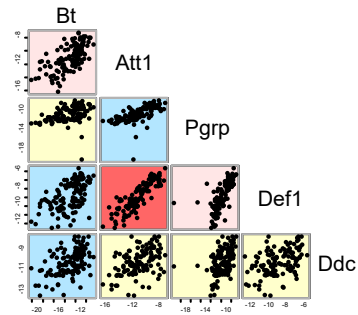
Snaveley beetles

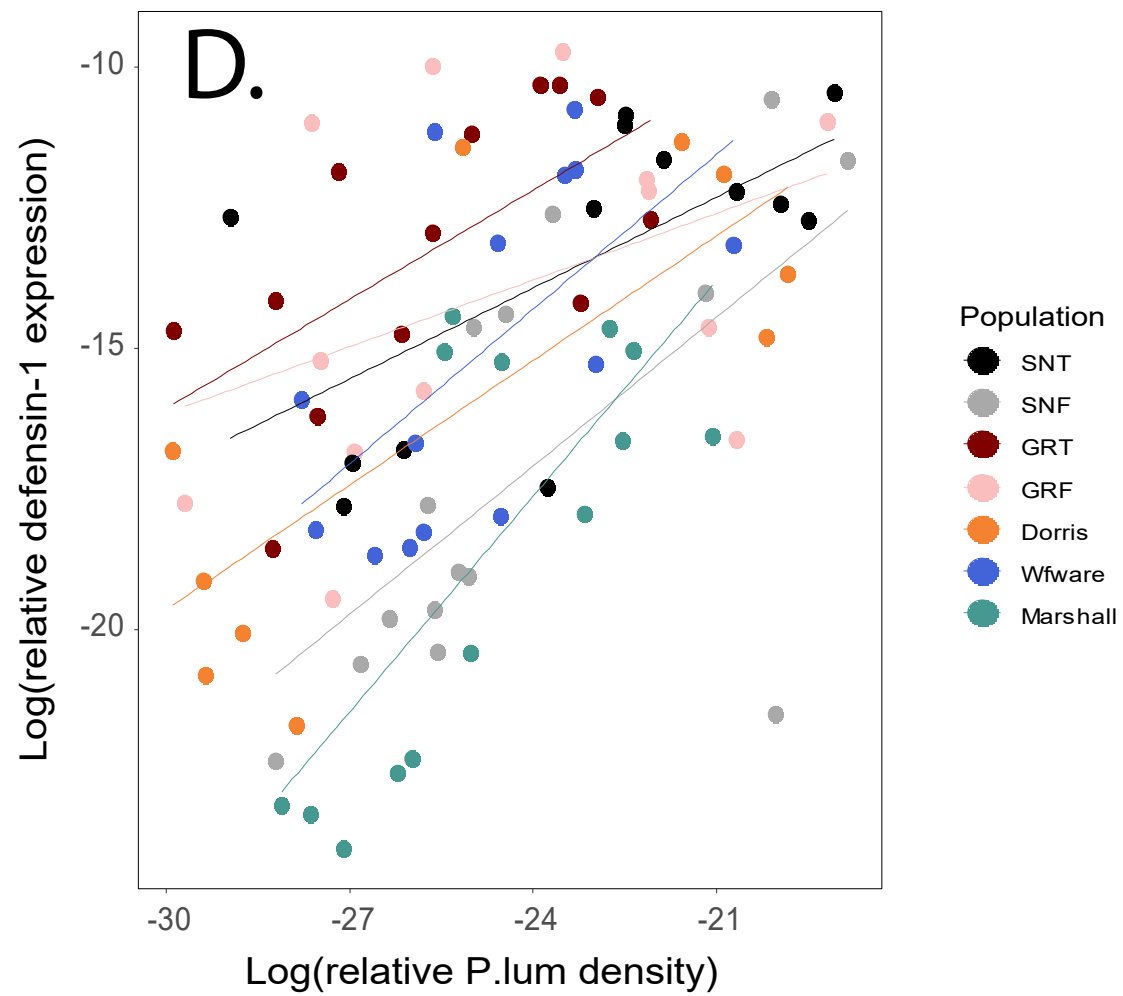
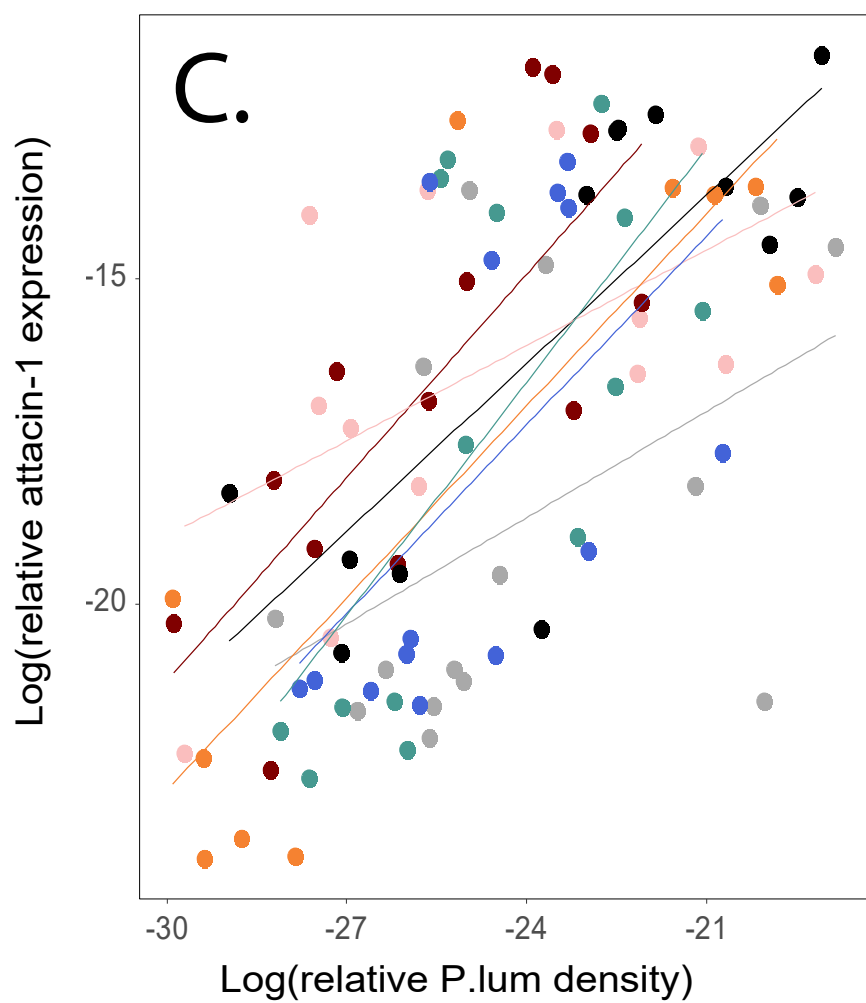
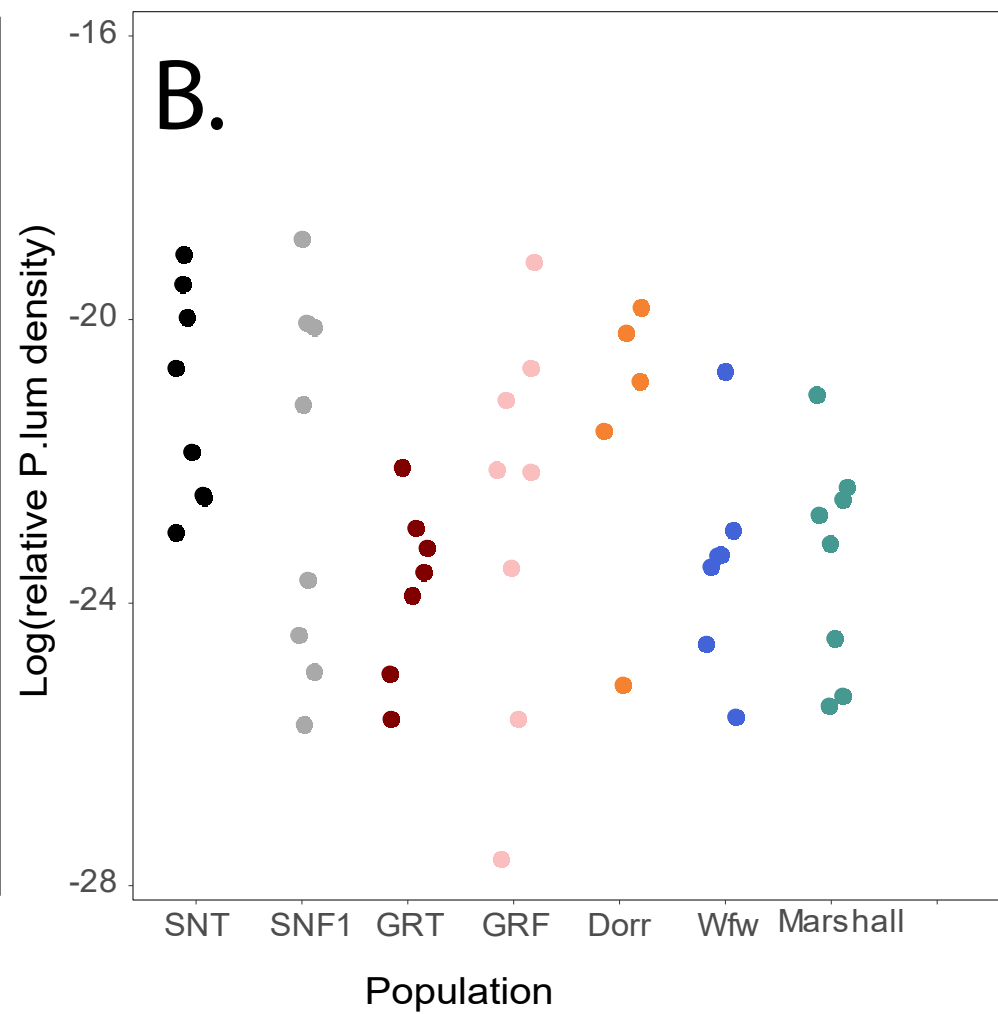
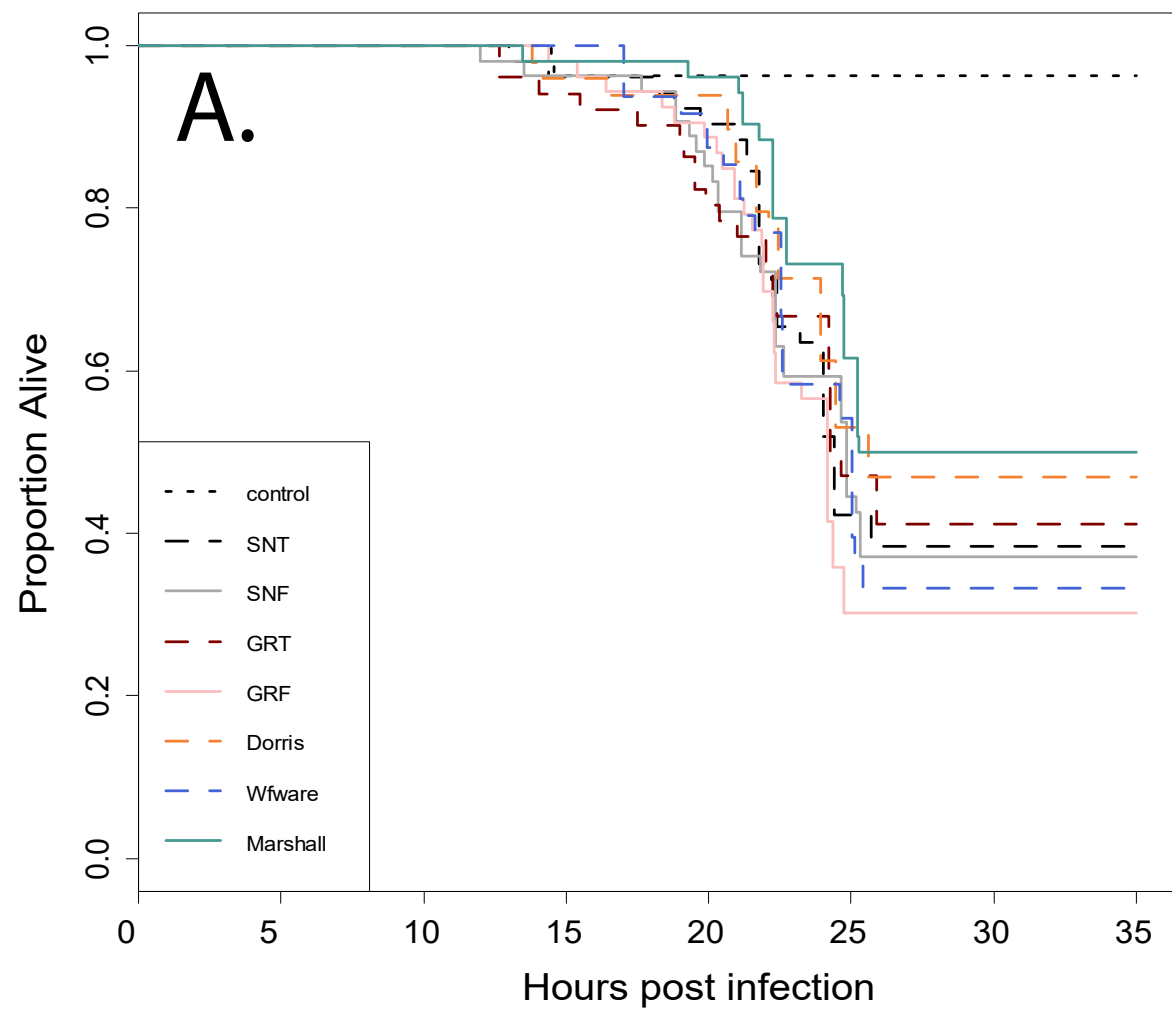


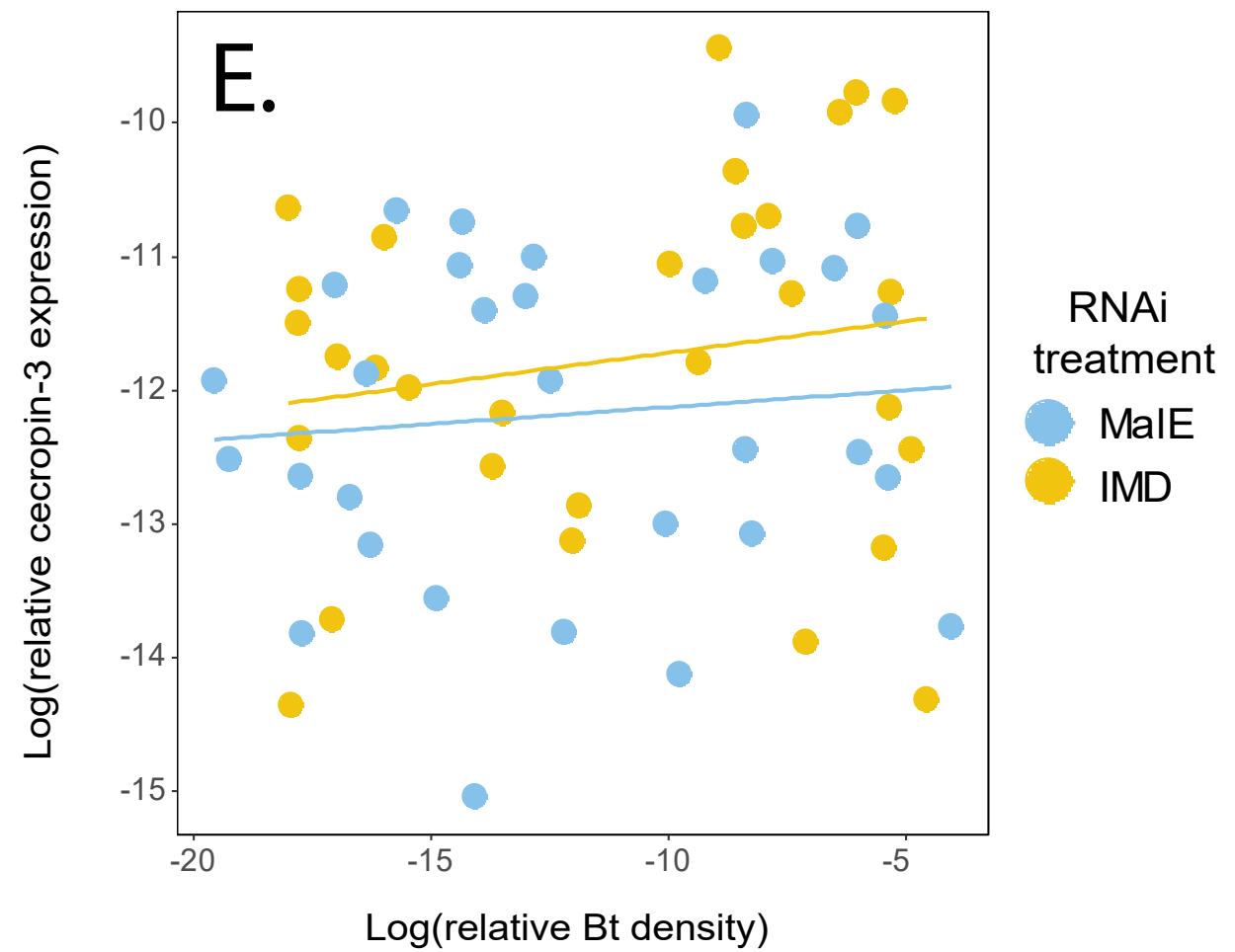
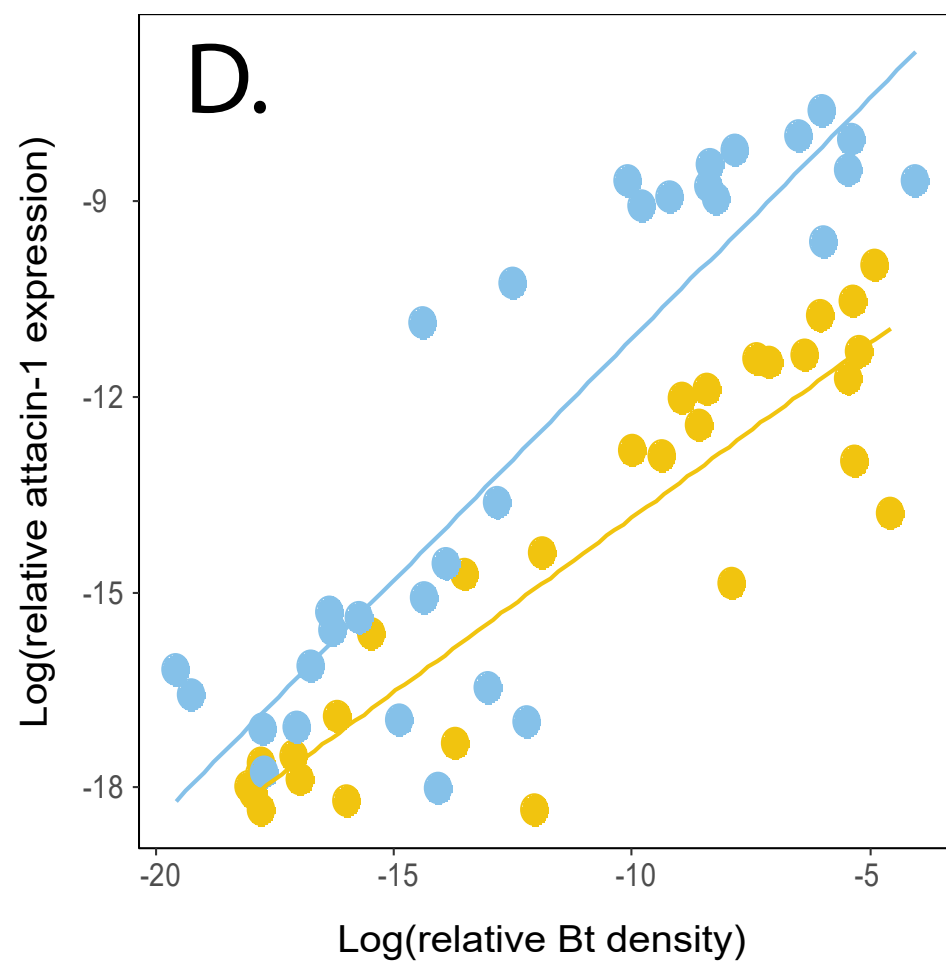
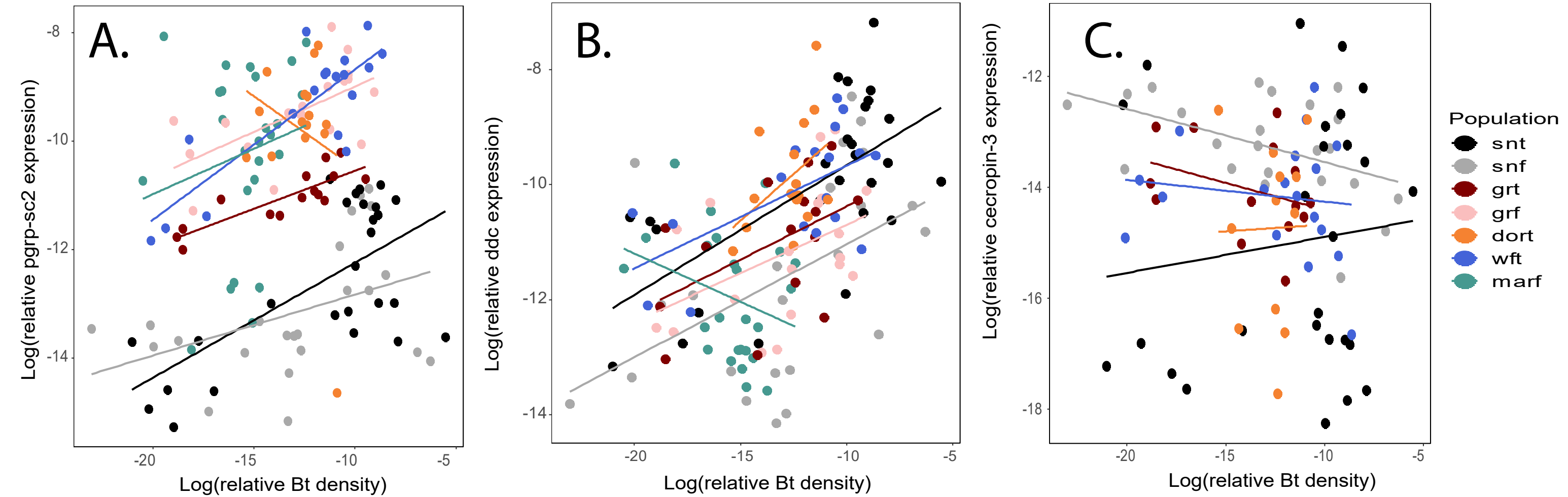
Green River beetles

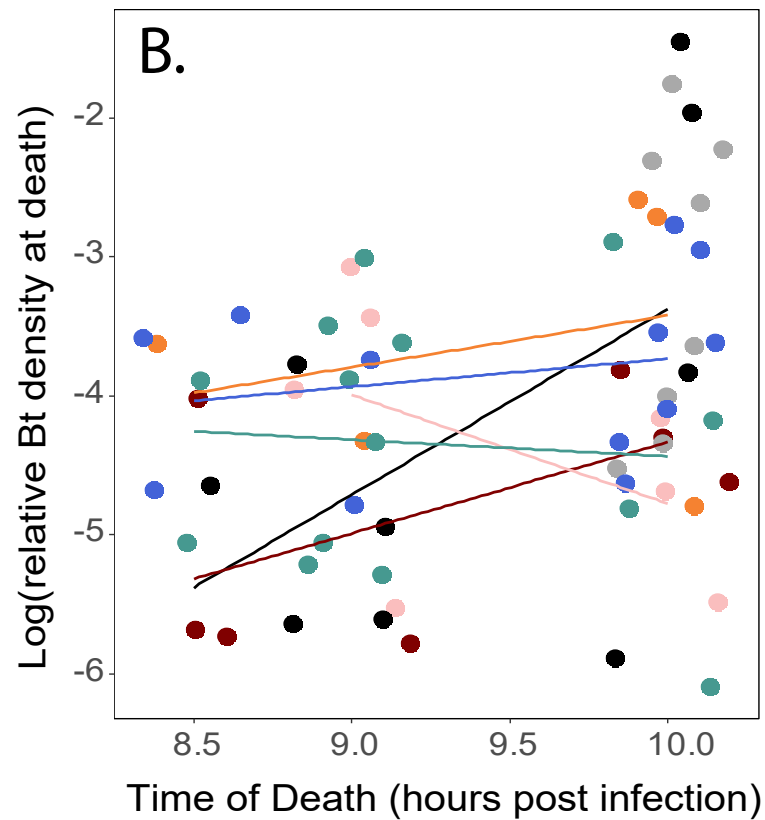
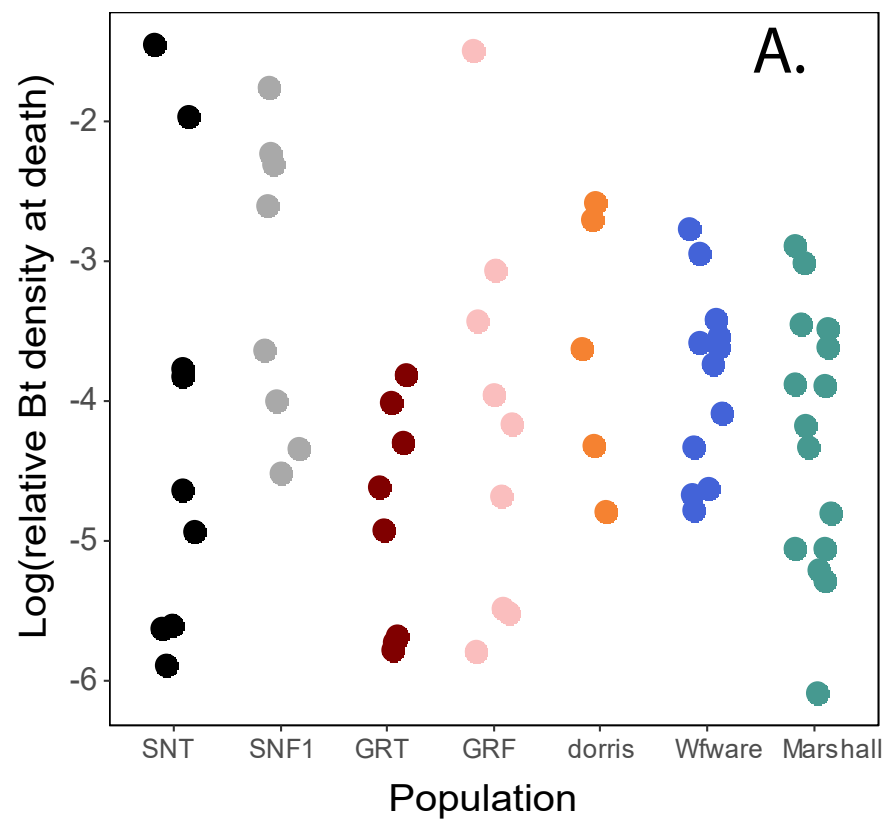


All wild beetles









Population

- SNT
- SNF1
- GRT
- GRF
- dorris
- Wfware
- Marshall

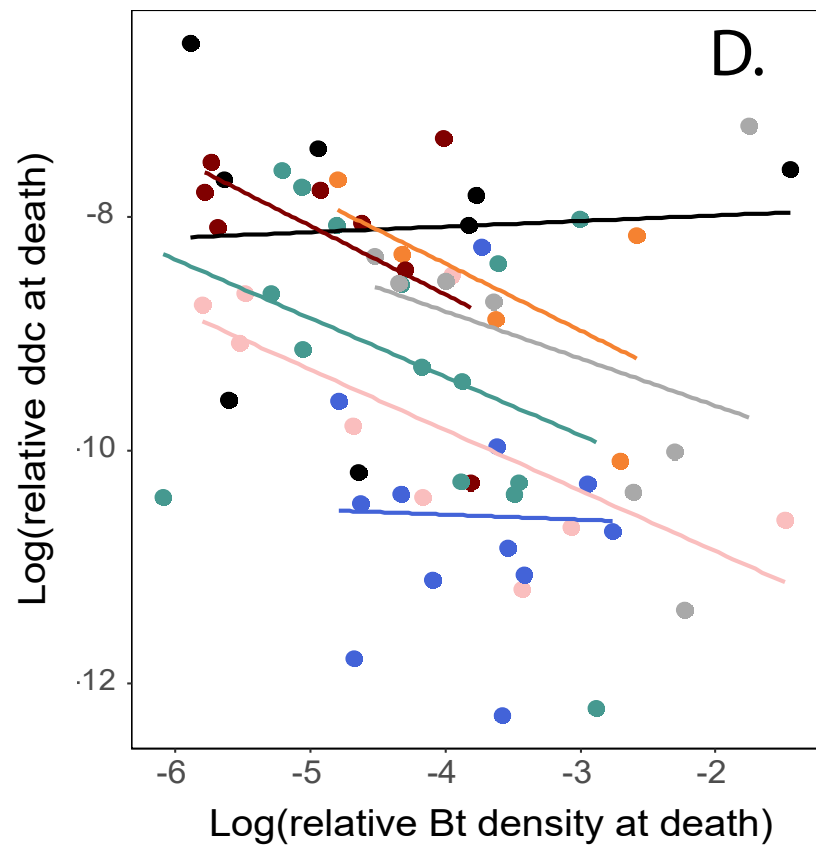
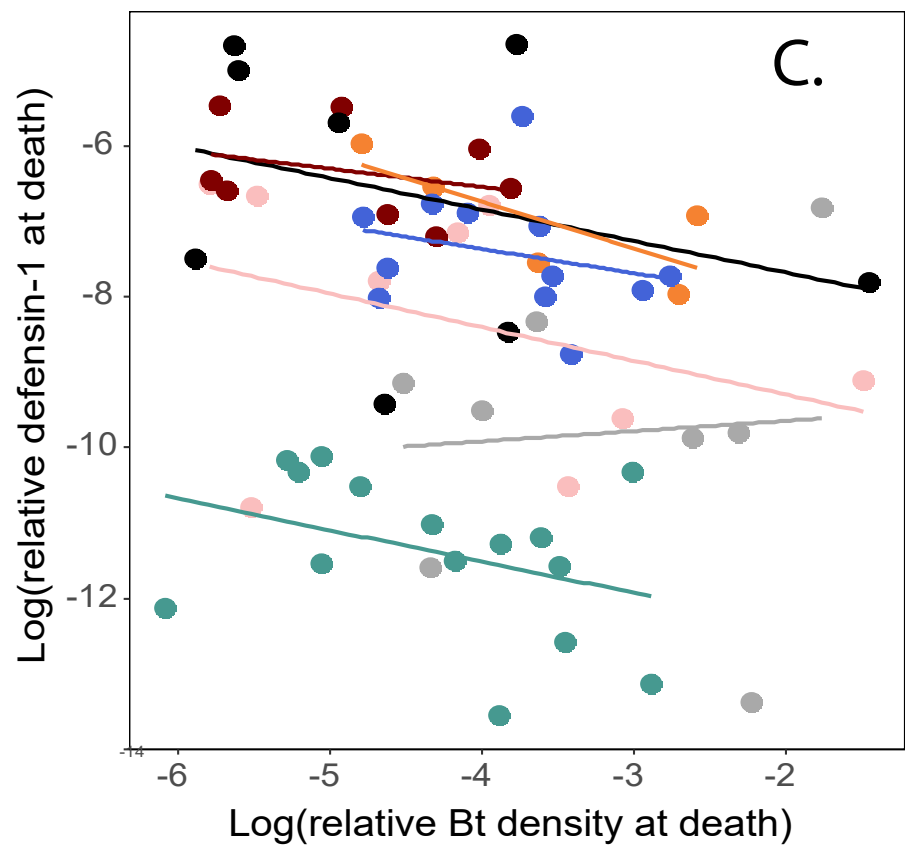


Table S1. Primer sequences used in study, in 5' to 3' order

Primer Set	Full Name/Function	Forward Oligo Sequence	Reverse Oligo Sequence	AT. (°C)
Att1	Attacin-1 (IMD AMP)	AAACARTTYCAYCCAAATGG	AGATCKGTWCCRWRITTTCTKGGT	55
Def1	Defensin-1 (Toll/IMD AMP)	TTTRYCGTTGCARTAKCCTCC	TCAARSTGAATCATGCCGCWTG	60
Cec3	Cecropin-3 (Toll AMP)	AACATGARYACCAAACITTT	CCAAYTTATMGGCTKTGGWG	55
PGRP	PGRP-SC2 (IMD recognition)	ACAGTTGGATGCKTTGAAACAGT	AACTSGTYCTGCTCCCTTG	52
Ddc	Ddc (melanization cascade)	AGAAGTCGTGATGCTKGACT	CTTGRATCACGCCGCC	55
18s	RPS18 (ribosomal protein)	CGAAGAGGTCGAGAAAATCG	CGTGGTCTTGGTGTGTTGAC	58
Bt	Bt 16s rRNA	GACTTTCTGGTCTGTAACITGACA	ACTTCAGCACTAAAGGGCGGA	55
MalE - dsRNA	Maltose binding (<i>E. coli</i>)	ATTGCTGCTGACGGGGITAT	ATGTCGGCATGATITCACCTTT	55
IMD - dsRNA	Imd	CCTCCAAGGGATGAAGTCAA	TTTCCAACAGTGGCACAATC	55
IMD - qPCR	Imd	AACTGATGCCATACCCAGCC	CAAAAGCAGATGGTCCGCTC	55

Notes: AT refers to the primer annealing temp used in a 3 step qPCR sequence. The dsRNA sequences include a 5' end T7 binding sequence, taatacgaactcactataggg

Table S2. Gene expression in naïve and saline-stabbed beetle populations, relative to SNT

Gene	Factor	Estimate	Std. Error	t value	Pval
Naïve					
<i>attacin-1</i>	Intercept	-19.69	0.50	-39.52	<2e-16
	SNF	-1.61	0.72	-2.24	0.028
	GRT	-1.02	0.70	-1.45	0.15
	GRF	-0.23	0.72	-0.32	0.75
	Dorris	-2.48	0.74	-3.37	0.0012
	Wfware	-1.78	0.70	-2.52	0.014
	Marshall	-1.99	0.70	-2.83	0.0059
<i>defensin-1</i>	Intercept	-16.60	0.50	-33.50	<2e-16
	SNF	-4.19	0.72	-5.86	9.90E-08
	GRT	0.51	0.70	0.72	0.47
	GRF	-0.50	0.72	-0.70	0.49
	Dorris	-2.40	0.73	-3.28	0.0015
	Wfware	-1.74	0.70	-2.49	0.015
	Marshall	-5.75	0.70	-8.20	3.31E-12
<i>pgrp-sc2</i>	Intercept	-17.05	0.46	-36.99	<2e-16
	SNF	-3.69	0.67	-5.54	3.75E-07
	GRT	-0.58	0.65	-0.88	0.38
	GRF	-1.12	0.67	-1.68	0.097
	Dorris	-2.43	0.68	-3.57	0.00062
	Wfware	-2.18	0.65	-3.34	0.0013
	Marshall	-3.01	0.65	-4.62	1.43E-05
Saline-stabbed, 8 hours post injection					
<i>attacin-1</i>	Intercept	-13.46	0.62	-21.55	<2e-16
	SNF	1.34	0.93	1.45	0.16
	GRT	0.71	0.99	0.72	0.48
	GRF	-0.75	0.93	-0.81	0.43
	Dorris	0.66	0.99	0.67	0.51
	Wfware	0.81	0.99	0.82	0.42
	Marshall	3.21	0.93	3.47	0.0019
<i>defensin-1</i>	Intercept	-11.09	0.62	-17.95	3.59E-16
	SNF	-1.34	0.92	-1.47	0.15
	GRT	0.91	0.98	0.93	0.36
	GRF	-1.18	0.92	-1.29	0.21
	Dorris	1.38	0.98	1.41	0.17
	Wfware	0.69	0.98	0.70	0.49
	Marshall	2.22	0.92	2.42	0.023

Notes: linear models (expression ~ population) conducted with "lm" function in R. Expression values are on a log scale.

Table S3. Population hazard ratios and gene expression means for naïve, saline-injected, and bacterial inf

Population	Bt_HR	Plum_HR	Bt_dose1	Bt_dose4	meanBLUD	Plum_dens	att1_naive	def1_naive
snt	2.53	0.98598	-9.964	-9.210	-4.192	14.651	-19.694	-16.603
snf	1	1	-13.781	-11.161	-3.175	15.514	-21.303	-20.795
grt	1.82	0.925	-13.168	-11.697	-4.859	16.473	-20.716	-16.096
grf	1.51	1.266	-13.287	-11.821	-4.177	15.777	-19.922	-17.104
dort	2.01	0.768	-12.474	-11.900	-3.608	14.921	-22.175	-19.007
wft	4.21	1.038	-11.760	-9.901	-3.842	16.244	-21.469	-18.347
marf	0.67	0.654	-15.192	-13.965	-4.283	16.218	-21.688	-22.349

Notes: Hazard ratios (HR) calculated from Cox Proportional Hazards models relative to SNF. All other values gene expression in saline-stabbed individuals after 8 hours (or 14 hours in Plum experiment (X_14int). X_co log scale. Only columns through ddc_coef are represented on corr plot

infected individuals; used in correlation plots

pg_naive	att1_int	def1_int	pg_int	ddc_int	att_coef	def_coef	pg_coef	ddc_coef
-17.053	-13.459	-11.085	-14.461	-11.687	0.377	0.376	0.111	0.330
-20.739	-12.117	-12.429	-13.861	-12.154	0.287	-0.029	0.095	0.199
-17.629	-12.747	-10.175	-11.615	-11.747	0.254	0.028	0.140	0.185
-18.171	-14.209	-12.268	-10.182	-11.624	0.250	0.235	0.167	0.169
-19.481	-12.796	-9.710	-9.706	-10.162	0.652	0.232	0.157	0.418
-19.233	-12.653	-10.400	-11.197	-11.371	0.476	0.342	0.277	0.179
-20.067	-10.249	-8.865	-10.165	-11.105	-0.127	-0.202	0.168	-0.167

μ are population means for 8 hour bacterial density given an initial dose (Bt_doseX) or at 14 hours (Plum).
 coef represents the slope of expression by Bt density for each gene. Xatdeath is the gene expression in mc

att1atdeat	def1atdeat	pgatdeath	ddcatdeath	att1_14int	def1_14int	pg_14int
-9.566	-6.696	-5.131	-7.981	-20.128	-17.846	-19.422
-11.575	-9.804	-6.548	-9.144	-22.460	-18.872	-21.674
-10.462	-6.331	-5.874	-8.161	-20.599	-16.576	-18.701
-11.198	-8.321	-7.413	-9.736	-20.310	-17.304	-18.106
-11.334	-6.982	-7.139	-8.626	-19.980	-17.184	-18.474
-12.063	-7.412	-7.802	-10.558	-20.243	-16.765	-18.575
-11.959	-11.395	-6.756	-9.229	-21.366	-21.165	-19.708

_dens). Gene_naive is average expression in unstabbed individuals. Gene_int is
 oribund individuals. Note that bacterial densities and gene expressions are on a

Table S4. Bacterial load at time of death, correlations with immune gene expression, and population

Analysis	Factor	Df	Sum Sq	Mean Sq	Fval	P val
Bacterial Load at time of death (BLUD)	Population	6	15.13	2.521	2.33	0.0442
	Time of death	1	2.26	2.262	2.09	0.1537
	Residuals	57	61.69	1.082		
Gene Expression						
<i>attacin-1</i>	Bt density	1	31.87	31.87	13.233	0.000641
	population	6	33.27	5.55	2.303	0.048221
	Bt*population	6	11.18	1.86	0.774	0.594316
	Residuals	51	122.82	2.41		
<i>defensin-1</i>	Bt density	1	16.02	16.02	8.675	0.00485
	population	6	222.23	37.04	20.06	7.03E-12
	Bt*population	6	2.49	0.42	0.225	0.96688
	Residuals	51	94.17	1.85		
<i>pgrp-sc2</i>	Bt density	1	32.42	32.42	17.86	9.84E-05
	population	6	20.11	3.35	1.846	0.109
	Bt*population	6	5.19	0.87	0.477	0.823
	Residuals	51	92.59	1.82		
<i>ddc</i>	Bt density	1	13.96	13.958	11.238	0.001516
	population	6	38.01	6.335	5.1	3.62E-04
	Bt*population	6	4.01	0.669	0.538	0.776533
	Residuals	51	63.34	1.242		

Notes: ANOVAs (expression ~ population*BLUD) conducted with "aov" function in R.

variation