

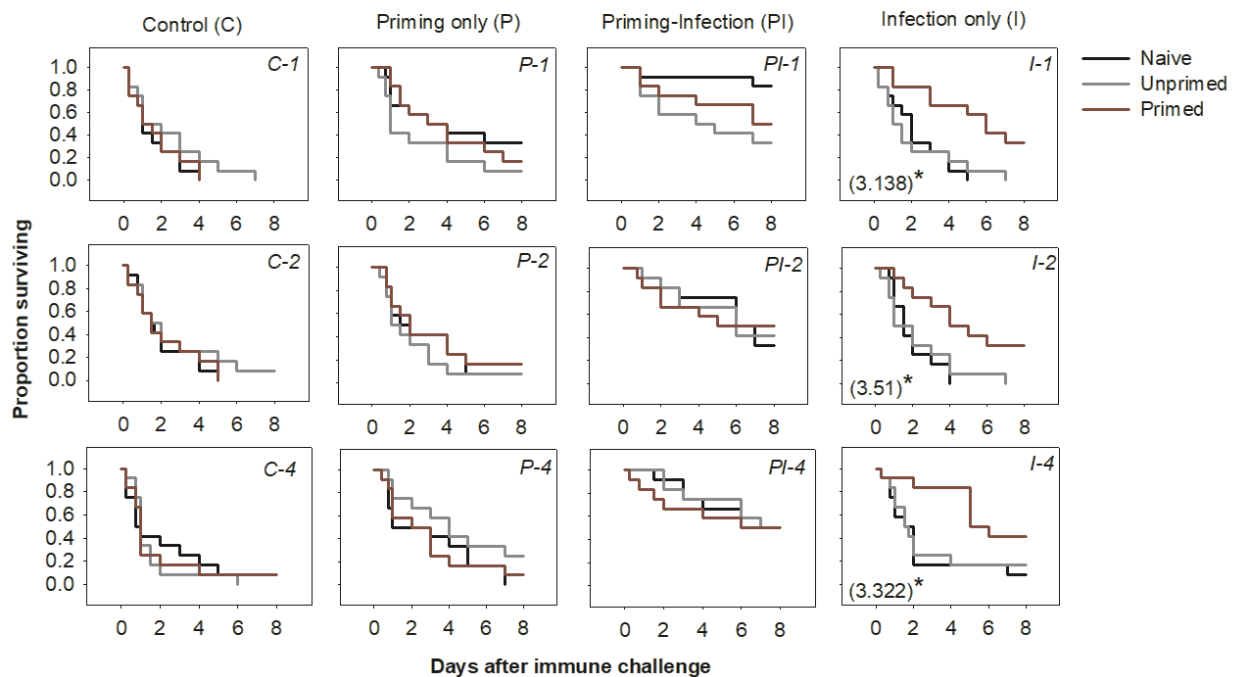
## SUPPLEMENTARY METHODS

### Priming and infection protocol

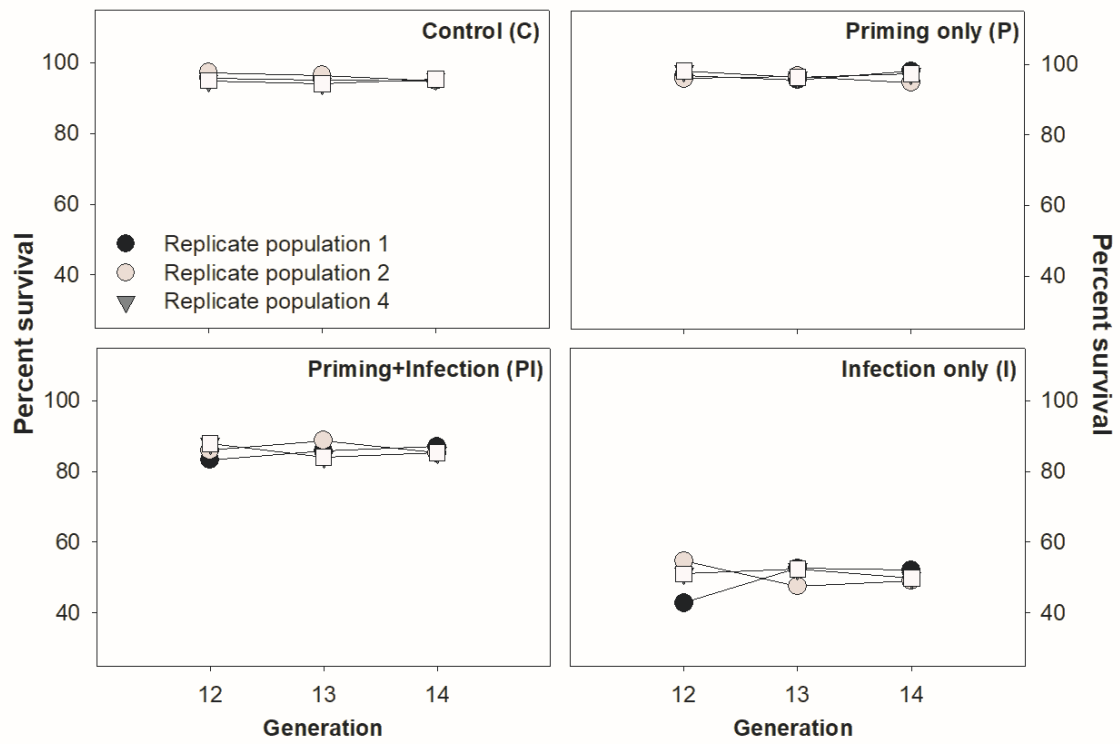
We used a strain of *Bacillus thuringiensis* (DSM 2046) (Bt) originally isolated from a Mediterranean flour moth (Roth *et al.* 2009) as a model pathogen to prime and infect adult *T. castaneum* beetles as outlined in Khan *et al.* (2017a). We used heat-killed bacteria to prime the immune system by activating the immune response without any direct cost of infection. Briefly, we first primed beetles by pricking them with a 0.1mm insect pin (Fine Science Tools, CA) dipped in heat-killed bacterial suspension prepared from freshly grown overnight Bt culture at 30°C (optical density of 0.95), or insect Ringer solution (mock priming) between their head and thorax. After six days, we infected individuals with live bacterial culture adjusted to  $\sim 10^{10}$  cells in 75  $\mu$ l insect Ringer solution (delivers approximately 12000 live cells per beetle; see Khan *et al.* 2017a) or pricked with sterile Ringer solution (mock challenge). We used the same priming and infection protocol for all subsequent assays, unless stated otherwise.

### SUPPLEMENTARY FIGURES

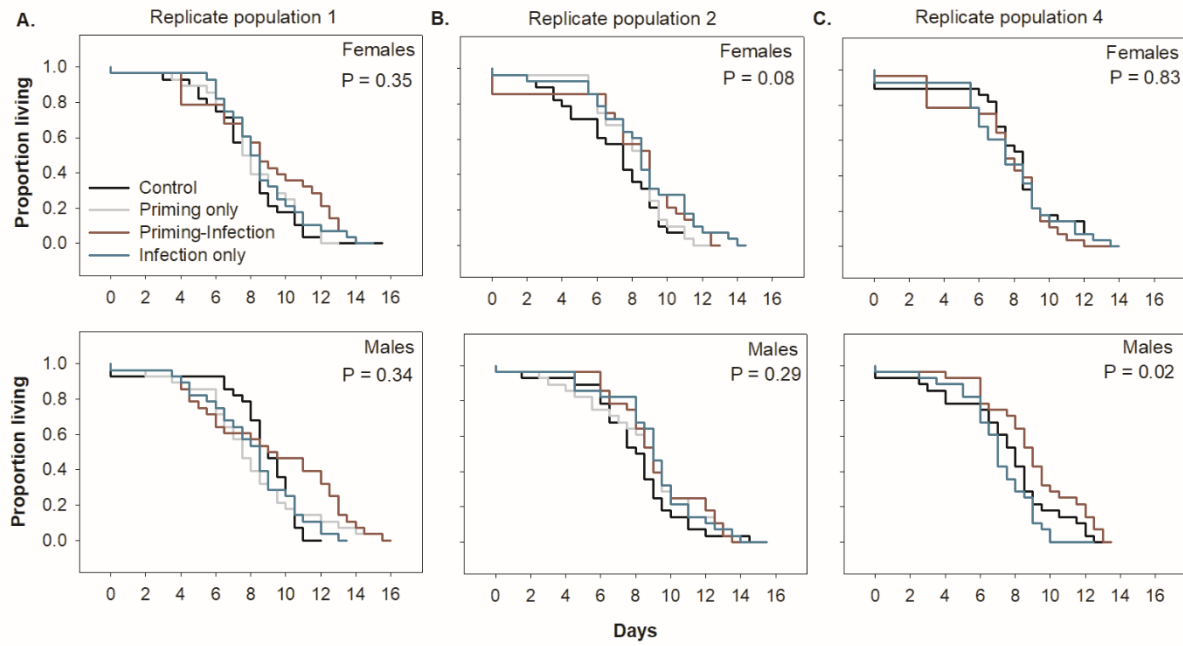
**Figure S1.** Survival curves for within-generation priming and resistance in males ( $n=12$  males/treatment/selection regime/replicate population) after 14 generations of selection. Asterisks and the numbers in parentheses for I beetles denote the hazard ratios calculated from survival curves for priming that are significantly greater than 1 ( $p<0.05$ ; a greater hazard ratio indicates higher benefit of priming)



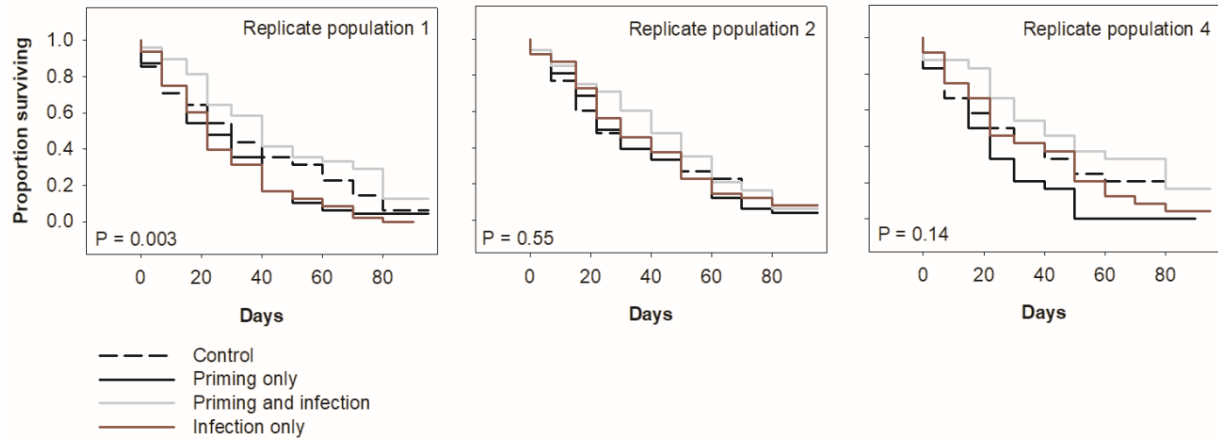
**Figure S2.** Adult survival during the first 48 h after infection with live Bt cells, during the course of experimental evolution between generation 12 and 14.



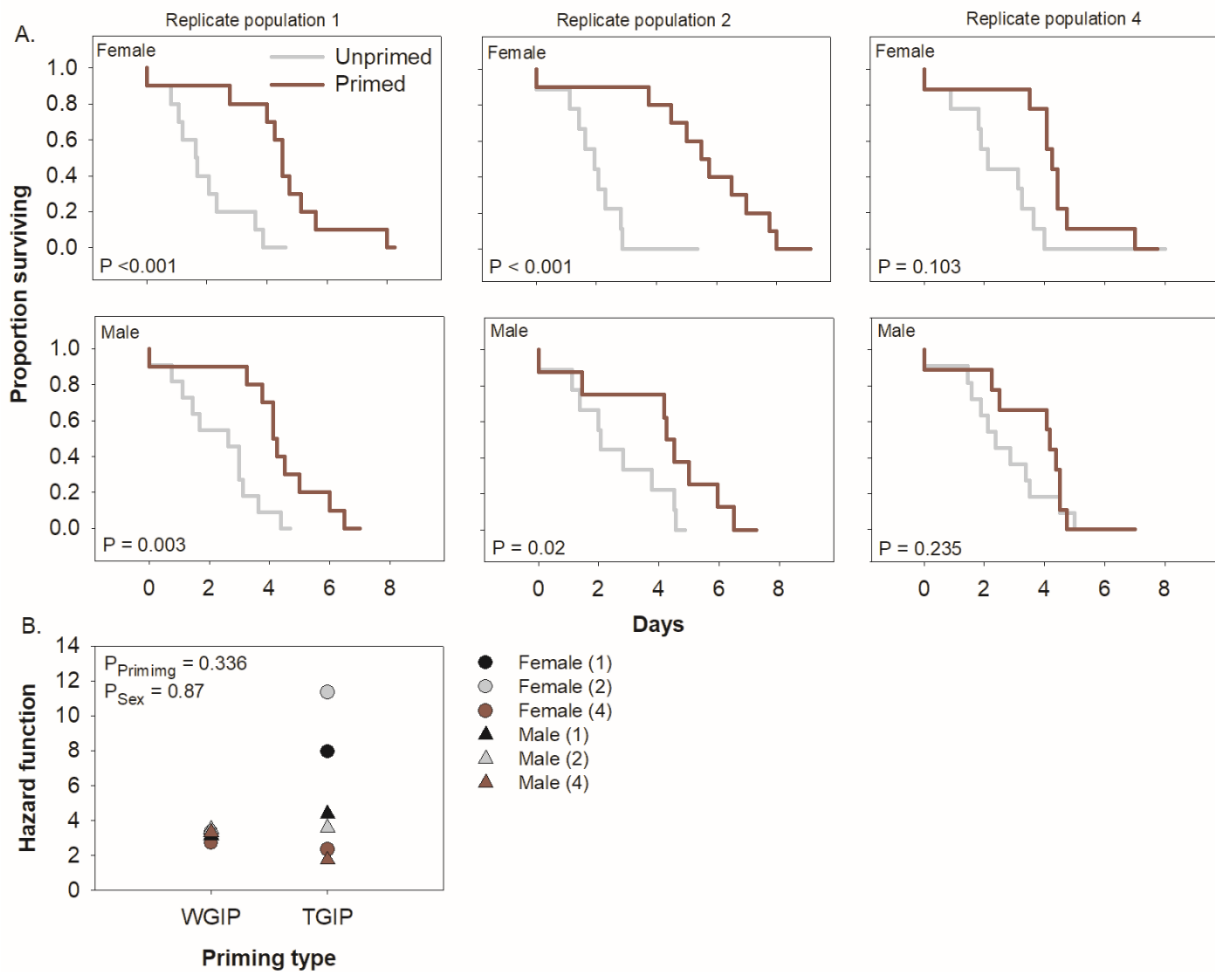
**Figure S3.** Survival curves of naïve males and females under starvation, across selection regimes. P values for the impact of selection regime are reported in each panel.



**Figure S4.** Survival curves of naïve females under normal condition up to 95 days post-emergence, across selection regimes. P values for the impact of selection regime are reported in each panel.



**Figure S5. (A)** Survival curves of the trans-generationally primed I offspring, based on the mean survival calculated from each parental pair. P values for the impact of TGIP are reported in each panel. **(B)** Survival benefits of WGIP vs. TGIP, based on the hazard function calculated from survival curves shown in panel A (offspring from unprimed parents vs. primed parents) and Fig 2 & S1 (males and females from unprimed vs. primed groups). A greater hazard ratio indicates higher benefit of priming. P values for the impact of priming type ( $P_{\text{Priming}}$ ) and sex ( $P_{\text{Sex}}$ ) are reported in each panel.



## SUPPLEMENTARY TABLES

**Table S1.** A. Number of females that were alive to reproduce after within-generation priming and infection across replicate populations, selection regimes and treatments. B. Summary of Wilcoxon Rank Sum Test on post-infection replicate size across priming treatments and selection regimes.

A.	Replicate population	Selection regime	Naïve	Unprimed	Primed
1		Control	5	7	4
		Priming only	8	9	10
		Priming-Infection	7	11	12
		Infection only	6	5	11
2		Control	7	10	10
		Priming only	6	8	10
		Priming-Infection	8	10	8
		Infection only	10	8	10
4		Control	6	8	9
		Priming only	6	9	7
		Priming-Infection	11	10	10
		Infection only	7	8	10
B.	Trait	$\chi^2$	df	P	
	Treatment	1.545	1	0.213	
	Regime	5.452	3	0.141	

**Table S2.** Summary of Cox proportional hazard analysis on survival data for within-generation priming in females.

Regime	Replicate population	df	$\chi^2$	P
Control	1	1	0.414	0.519
Infection only		1	6.502	<b>0.01</b>
Priming only		1	0.247	0.618
Priming & Infection		1	0.064	0.799
Control	2	1	0.297	0.585
Infection only		1	6.493	<b>0.01</b>
Priming only		1	1.961	0.161
Priming & Infection		1	0.005	0.942
Control	4	1	0.088	0.765
Infection only		1	4.783	<b>0.028</b>
Priming only		1	0.721	0.395
Priming & Infection		1	0.032	0.855

**Table S3.** Summary of Cox proportional hazard analysis on survival data for within-generation priming in males.

Regime	Replicate population	df	$\chi^2$	P
Control	1	1	0.743	0.388
Infection only		1	7.562	<b>0.005</b>
Priming only		1	1.388	0.238
Priming & Infection		1	0.759	0.383
Control	2	1	0.449	0.502
Infection only		1	5.766	<b>0.016</b>
Priming only		1	0.882	0.347
Priming & Infection		1	0.0002	0.988
Control	4	1	0.019	0.888
Infection only		1	4.843	<b>0.027</b>
Priming only		1	1.531	0.215
Priming & Infection		1	0.117	0.731

**Table S4.** Summary of Cox proportional hazard analysis on survival data of naïve beetles infected with Bt.

	Comparison	Replicate population	Hazard function	df	$\chi^2$	P	
Mothers	C vs. P	1	1.51	1	0.973	0.323	
		2	2.34	1	3.478	0.062	
		4	1.36	1	0.519	0.471	
	C vs. PI	1	<b>5.87</b>	1	11.265	<b>0.001</b>	
		2	<b>4.3</b>	1	7.882	<b>0.005</b>	
		4	<b>3.03</b>	1	5.318	<b>0.021</b>	
	C vs. I	1	0.95	1	0.01	0.919	
		2	1.34	1	0.504	0.473	
		4	0.958	1	0.011	0.912	
	Fathers	C vs. P	1	3.03	1	5.391	<b>0.02</b>
			2	1.41	1	0.657	0.417
			4	1.28	1	0.358	0.549
C vs. PI		1	<b>28.9</b>	1	20.407	<b>&lt;0.001</b>	
		2	<b>8.52</b>	1	13.608	<b>0.001</b>	
		4	<b>4.37</b>	1	8.24	<b>0.004</b>	
C vs. I		1	1.54	1	1.052	0.305	
		2	0.91	1	0.043	0.835	
		4	1.15	1	0.107	0.743	

**Table S5.** Summary of Wilcoxon Rank Sum test on beetle reproduction before infection as function of within-generation priming and selection regime

Block	Trait	$\chi^2$	df	P
1	Regime	6.046	3	0.109
	Treatment	1.055	2	0.589
2	Regime	2.933	3	0.402
	Treatment	1.531	2	0.464
4	Regime	7.653	3	0.0537
	Treatment	1.647	2	0.438

**Table S6.** Summary of Wilcoxon Rank Sum test for change in reproductive output with infection across replicate populations and selection regimes. C- Control; P – Priming only; PI- Priming-Infection; I- Infection only.

Regime	Replicate populations	Treatment	$\chi^2$	df	P
C	1	Naive	6.043	1	<b>0.013</b>
		Unprimed	8.337	1	<b>0.003</b>
		Primed	0.134	1	0.713
	2	Naive	6.685	1	<b>0.009</b>
		Unprimed	9.486	1	<b>0.002</b>
		Primed	5.353	1	<b>0.02</b>
	4	Naive	4.854	1	<b>0.027</b>
		Unprimed	10.54	1	<b>0.001</b>
		Primed	4.71	1	<b>0.029</b>
P	1	Naive	5.55	1	<b>0.018</b>
		Unprimed	6.247	1	<b>0.012</b>
		Primed	5.225	1	<b>0.022</b>
	2	Naive	5.089	1	<b>0.024</b>
		Unprimed	1.258	1	0.262
		Primed	4.5	1	<b>0.033</b>
	4	Naive	0.718	1	0.396
		Unprimed	7.321	1	<b>0.006</b>
		Primed	0.652	1	0.419
PI	1	Naive	1.121	1	0.289
		Unprimed	4.84	1	<b>0.028</b>
		Primed	0.067	1	0.794
	2	Naive	0.542	1	0.461
		Unprimed	0.184	1	0.667
		Primed	4.73	1	<b>0.029</b>
	4	Naive	0.095	1	0.757



		Unprimed	4.898	1	<b>0.026</b>
		Primed	1.933	1	0.164
I	1	Naive	4.486	1	<b>0.034</b>
		Unprimed	8.475	1	<b>0.003</b>
		Primed	11.91	1	<b>0.001</b>
	2	Naive	11.81	1	<b>0.001</b>
		Unprimed	8.408	1	<b>0.003</b>
		Primed	6.324	1	<b>0.011</b>
	4	Naive	4.484	1	<b>0.034</b>
		Unprimed	3.163	1	0.075
		Primed	5.66	1	<b>0.017</b>

**Table S7.** Summary of ANOVA on reproductive output after within-generation priming and bacterial infection across A. all the selection regimes; B. Control (C) vs. Priming only (P); C. Control (C) vs. Priming-Infection (PI); D. Control (C) vs. Infection only (I).

		Trait	SS	SS	F-ratio	P
A.	All regime	Replicate population (B)	90.439	2	1.128	0.324
		Regime (R)	1399.783	3	11.647	<b>3.40872e-7</b>
		Treatment (T)	317.954	2	3.968	<b>0.02</b>
		B × R	207.363	6	0.8626	0.522
		B × T	54.542	4	0.34	0.85
		R × T	639.203	6	2.6597	<b>0.016</b>
		B × R × T	504.924	12	1.05	0.403
		Error	10616.212	265		
B.	C vs. P	B	92.547	2	1.028	0.36
		R	291.311	1	6.476	<b>0.012</b>
		T	541.137	2	6.015	<b>0.003</b>
		B × R	6.848	2	0.076	0.926
		B × T	45.137	4	0.255	0.908
		R × T	276.217	2	3.07	<b>0.05</b>
		B × R × T	240.453	4	1.336	0.26
		Error	5442.71	121		
C.	C vs. PI	B	101.513	2	1.396	0.251
		R	563.698	1	15.507	<b>0.0001</b>
		T	410.017	2	5.639	<b>0.004</b>
		B × R	67.251	2	0.925	0.399
		B × T	144.875	4	0.996	0.411
		R × T	477.395	2	6.566	<b>0.001</b>
		B × R × T	96.024	4	0.66	0.62
		Error	4907.311	135		
D.	C vs. I	B	67.991	2	0.974	0.38
		R	61.381	1	1.76	0.187
		T	467.012	2	6.695	<b>0.0017</b>

B × R	25.001	2	0.351	0.699
B × T	93.715	4	0.671	0.612
R × T	311.382	2	4.464	<b>0.013</b>
B × R × T	156.131	4	1.119	0.35
Error	4289.419	123		

**Table S8.** Summary of Cox proportional hazard analysis on survival under starvation of unhandled naïve beetles across selection regimes.

Replicate population	Sex	df	$\chi^2$	P
1	Female	3	0.45	0.92
	Male	3	5.323	0.149
2	Female	3	4.156	0.245
	Male	3	1.353	0.716
4	Female	2	1.16	0.55
	Male	2	4.099	0.128

**Table S9.** Summary of Cox proportional hazard analysis on long-term survival of unhandled naïve females across selection regimes.

Replicate population	df	$\chi^2$	P
1	3	13.6393732	<b>0.003</b>
2	3	2.06145929	0.559
4	3	5.47204481	0.14

**Table S10.** Summary of (A) two-way ANOVA and (B) one-way ANOVA on early survival and developmental rate.

A. Assay	Trait	df	SS	F ratio	P
Egg-hatchability	Regime	2	1527.375	52.206	<b>3.21391e-8</b>
	Replicate	2	1328.446	45.406	<b>9.2751e-8</b>
	Regime × Replicate	4	196.92	3.3653	<b>0.031</b>
	experiment				
Pupae_3 week	Regime	2	0.0192	3.0433	0.072
	Replicate	2	0.035	5.587	<b>0.012</b>
	Regime × Replicate	4	0.006	0.494	0.739
	experiment				
Adult_4 week	Regime	2	0.045	4.886	<b>0.02</b>
	Replicate	2	0.024	2.58	0.103
	Regime × Replicate	4	0.005	0.292	0.879
	experiment				
%viability	Regime	2	1444.91	26.32	<b>4.52819e-6</b>

	Replicate	2	577.819	10.525	<b>0.001</b>	
	Regime × Replicate experiment	4	140.737	1.281	0.313	
<b>B.</b>	<b>Replicate experiment</b>	<b>df</b>	<b>SS</b>	<b>F ratio</b>	<b>P</b>	
	1	hatchability	2	416.907	20.104	<b>0.002</b>
		pupae_3	2	0.003	0.586	0.585
		%viability	2	281.635	5.642	<b>0.041</b>
	2	hatchability	2	718.315	34.241	<b>0.001</b>
		pupae_3	2	0.0104	2.88	0.132
		%viability	2	623.794	9.836	<b>0.012</b>
	3	hatchability	2	589.072	12.79	<b>0.006</b>
		pupae_3	2	0.0119	1.201	0.364
		%viability	2	680.217	13.244	<b>0.006</b>

**Table S11.** Summary of 4-way ANOVA on mean post-infection survival of offspring as a function of replicate population, selection regime, parental priming status and sex

Trait	SS	df	F-ratio	P
Replicate population (B)	1.985	2	6.688	<b>0.001</b>
Selection regime (R)	88.118	3	197.903	<b>&lt;0.001</b>
Sex (S)	0.001	1	0.006	0.933
Parental priming (P)	5.271	2	17.757	<b>&lt;0.001</b>
B × R	2.039	6	2.29	<b>0.03</b>
B × S	0.206	2	0.695	0.499
B × P	0.08	4	0.135	0.969
R × S	0.101	3	0.226	0.877
R × P	10.571	6	11.871	<b>&lt;0.001</b>
G × S	0.364	2	1.228	0.293
B × R × S	0.83	6	0.932	0.47
B × R × P	2.133	12	1.197	0.281
B × S × P	0.506	4	0.852	0.492
R × S × P	0.547	6	0.614	0.718
B × R × S × P	1.755	12	0.985	0.461
Error	586	86.97		

**Table S12.** Summary of 3-way ANOVA on mean post-infection survival of offspring as a function of selection regime, parental priming status and sex, from replicate populations that were handled together.

Replicate population	Trait	df	SS	F-ratio	P
1	Selection regime (R)	3	38.755	93.81	<b>&lt;0.001</b>
	Sex (S)	1	0.103	0.754	0.386
	Parental priming (P)	2	2.035	7.389	<b>0.001</b>
	R × S	3	0.024	0.058	0.981
	R × P	6	4.657	5.637	<b>&lt;0.001</b>

	S × P	2	0.029	0.108	0.897
	R × S × P	6	0.555	0.671	0.672
	Error	196	73.58		
2	R	3	26.168	54.598	< <b>0.001</b>
	S	1	0.004	0.025	0.873
	P	2	1.432	4.483	<b>0.012</b>
	R × S	3	0.276	0.577	0.63
	R × P	6	5.217	5.442	< <b>0.001</b>
	S × P	2	0.793	2.482	0.086
	R × S × P	6	0.567	0.591	0.736
	Error	193	30.83		
4	R	3	25.067	56.473	< <b>0.001</b>
	S	1	0.099	0.672	0.413
	P	2	1.861	6.29	<b>0.002</b>
	R × S	3	0.632	1.424	0.236
	R × P	6	2.874	3.237	<b>0.004</b>
	S × P	2	0.034	0.116	0.889
	R × S × P	6	1.161	1.308	0.255
	Error	197	61.057		

**Table S13.** Summary of Cox proportional hazard analysis on mean progeny survival from I regime

Replicate population	Sex	Hazard function	df	$\chi^2$	P
1	Female	<b>7.943</b>	1	12.491	<b>0.0004</b>
	Male	<b>4.398</b>	1	8.443	<b>0.003</b>
2	Female	<b>11.332</b>	1	14.116	<b>0.0001</b>
	Male	<b>3.589</b>	1	4.819	<b>0.02</b>
4	Female	2.337	1	2.655	0.103
	Male	1.74	1	1.408	0.235

**Table S14.** Summary of Wilcoxon Rank Sum test on progeny fitness before infection, as a function of selection regimes and priming treatments

Block	Trait	$\chi^2$	df	P
1	Regime	7.792	3	0.052
	Treatment	2.147	1	0.143
2	Regime	3.499	3	0.32
	Treatment	0.418	1	0.517
4	Regime	6.867	3	0.077
	Treatment	0.029	1	0.864

**Table S15:** Summary of Wilcoxon Rank Sum test for change in offspring's reproductive output with infection across replicate populations and selection regimes. C- Control; P – Priming only; PI- Priming-Infection; I- Infection only.

Replicate population	Treatment	Selection regime	$\chi^2$	df	P
1	Unprimed	C	3.303	1	0.0691
	primed		7.828	1	<b>0.005</b>
	Unprimed	P	0.157	1	0.691102
	primed		4.3246	1	<b>0.037563</b>
	Unprimed	PI	1.107	1	0.292
	primed		0.918	1	0.337
	Unprimed	I	10.115	1	<b>0.001</b>
	primed		0.894	1	0.344
2	Unprimed	C	3.481	1	0.062
	primed		3.951	1	<b>0.046</b>
	Unprimed	P	0.903	1	0.341
	primed		4.698	1	<b>0.03</b>
	Unprimed	PI	0.707	1	0.4
	primed		1.949	1	0.162
	Unprimed	I	5.080	1	<b>0.024</b>
	primed		9.163	1	<b>0.002</b>
4	Unprimed	C	5.08	1	<b>0.024</b>
	primed		6.33	1	<b>0.011</b>
	Unprimed	P	2.668	1	0.102
	primed		5.512	1	<b>0.018</b>
	Unprimed	PI	1.106	1	0.292
	primed		0.148	1	0.7
	Unprimed	I	9.29	1	<b>0.002</b>
	primed		8.235	1	<b>0.004</b>

**Table S16.** Summary of 3-way ANOVA on offspring fitness after infection

Traits	df	SS	F Ratio	P
Block	2	11.761	0.326	0.721
Regime	3	655.498	12.133	<b>&lt;0.001</b>
Treatment	1	59.057	3.279	0.071
Block × Regime	6	36.509	0.337	0.916
Block × Treatment	2	5.569	0.154	0.856
Regime × Treatment	3	9.839	0.182	0.908
Block × Regime × Treatment	6	58.544	0.541	0.775
Error	194	3493.667		