

## Digital supplementary material to

STUCKI, D., SUNDSTRÖM, L. & FREITAK, D. 2017: Caste-specific expression of constitutive and *Beauveria bassiana* induced immunity in the ant *Formica exsecta* (Hymenoptera: Formicidae). – Myrmecological News 25: 83-93.

**Appendix S1** contains information on the primers used for gene expression analysis and supporting information on the PCA and the post-hoc comparisons (Tabs. S1 - S6 and Figs. S1 - S4), starting below.

**Appendix S2** contains the survival data for reproductives (2013) and workers (2015), formatted for survival regression, as well as the normalized Ct-values of each gene for reproductives (2013) and workers (2015), in a separately uploaded xls file.

Tab. S1: The primer sequences used for qPCR gene expression analysis. Acronyms indicate the abbreviations used in the main text and the tables.

Gene	Acronym	Forward	Reverse	Efficiency
Arylphorin	<i>Aryl</i>	5'-ACT TAG AAC GCC TGT CCA AC-3'	5'-GAG ATT CCA TGT CCT CCA AG-3'	99.9
β-1-3-Glucan-binding protein	<i>Bl,3g</i>	5'-ATC TTA CAG GAC TGC CTC CA-3'	5'-AAC GTG AAT AAG GCA ATT CG-3'	95.7
Hymenoptaecin	<i>Hyme</i>	5'-TGG CAA AGA CGG TTT CAT CA-3'	5'-AAC CAC CAG TTA CAC CAA CC-3'	102.4
Insulin Receptor 3	<i>IR3</i>	5'-GCG AGC GTT TTC TGT AGG TA-3'	5'-CCC AGG AGC TCG ATA ACA AC-3'	105.0
LPS-binding protein	<i>LPS-bp</i>	5'-CCA CCG TGT AAC GAT AAT CA-3'	5'-TTG ACG TCA TCG GTC AAT AG-3'	102.9
Lysozyme C	<i>LysC</i>	5'-CAC CAT CCT TCT TCT TCG TT-3'	5'-AGG TGG CAT CTG TAA CAA GC-3'	91.6
Pro-Phenoloxidase	<i>PPO</i>	5'-TCC AAC TGC TGT AGG CTG TA-3'	5'-ACC TTC ACA ATC TCG GTC AT-3'	96.4
Toll-receptor	<i>Toll</i>	5'-GCT CCT TCG TGA CCT AGT CG-3'	5'-GTG AAA GCG CCA TTA TCC AT-3'	102.4
Vitellogenin	<i>Vg1</i>	5'-AGG TTG TCA TCG TCA ACG AA-3'	5'-CGT TGC TTG TAG GGG ATA GG-3'	98.3

Tab. S2: Post-hoc pairwise comparison of the survival of reproductives and workers. Lower estimates indicate higher mortality in the second listed group of each comparison. All p-values within each caste-group were FDR corrected.

Comparison	Estimate±SE	z-value	adj. p-value
<b>Reproductives</b>			
TritonX.Queens – Beauveria.Queens	-1.05 ± 0.06	18.43	< 0.0001
TritonX.Males – Beauveria.Males	-0.54 ± 0.04	13.40	< 0.0001
TritonX.Queens – TritonX.Males	-0.59 ± 0.07	9.02	< 0.0001
Beauveria.Queens – Beauveria.Males	-0.08 ± 0.05	1.73	0.0831
<b>Workers</b>			
TritonX.Nurses – Beauveria.Nurses	-1.25 ± 0.05	23.43	< 0.0001
TritonX.Foragers – Beauveria.Foragers	-1.11 ± 0.05	24.57	< 0.0001
TritonX.Nurses – TritonX.Foragers	-0.17 ± 0.06	2.80	0.0068
Beauveria.Nurses – Beauveria.Foragers	-0.03 ± 0.03	1.11	0.2670

Tab. S3: Information on a preliminary PCA without rotation. Principal components with an Eigenvalue > 1.0 were retained for analysis.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
<b>Reproductives, constitutive</b>									
Eigenvalue	3.72	1.67	1.30	1.04	0.54	0.36	0.26	0.11	0.01
Proportion of Variance	0.41	0.19	0.14	0.12	0.06	0.04	0.03	0.01	0.00
Cumulative Variance	0.41	0.60	0.74	0.86	0.92	0.96	0.99	1.00	1.00
<b>Induced</b>									
Eigenvalue	3.31	1.67	1.65	1.06	0.46	0.3	0.26	0.19	0.09
Proportion of Variance	0.37	0.19	0.18	0.12	0.05	0.03	0.03	0.02	0.01
Cumulative Variance	0.37	0.55	0.74	0.85	0.91	0.94	0.97	0.99	1.00
<b>Workers, constitutive</b>									
Eigenvalue	3.58	1.75	1.41	0.71	0.52	0.37	0.29	0.26	0.11
Proportion of Variance	0.40	0.19	0.16	0.08	0.06	0.04	0.03	0.03	0.01
Cumulative Variance	0.40	0.59	0.75	0.83	0.89	0.93	0.96	0.99	1.00
<b>Induced</b>									
Eigenvalue	3.72	1.34	0.99	0.85	0.79	0.48	0.37	0.31	0.15
Proportion of Variance	0.41	0.15	0.11	0.09	0.09	0.05	0.04	0.03	0.02
Cumulative Variance	0.41	0.56	0.67	0.77	0.85	0.91	0.95	0.98	1.00

Tab. S4: Loadings of each gene on the selected Principal Components from separate PCAs on constitutive and induced gene expression in reproductives (queens and males), and in workers (nurses and foragers). The principal components were rotated using oblique (oblimin) rotation.

	PC1	PC2	PC3	PC4	PC1	PC2	PC3	PC4
<b>Reproductives</b>	<b>Constitutive</b>				<b>Induced</b>			
<i>Aryl</i>	-0.07	-0.12	0.10	0.92	-0.20	0.02	-0.39	0.83
<i>Vg1</i>	0.94	-0.02	0.12	-0.31	0.91	-0.06	-0.15	0.05
<i>IR3</i>	-0.25	0.92	-0.19	-0.18	-0.22	0.86	0.24	-0.02
<i>PPO</i>	0.82	-0.05	-0.01	0.13	0.75	0.01	-0.40	0.10
<i>Hyme</i>	-0.44	-0.58	-0.38	-0.23	-0.24	-0.07	-0.38	-0.82
<i>LPS.bp</i>	0.30	0.58	-0.13	0.49	0.22	0.87	-0.20	0.08
<i>LysC</i>	0.26	0.59	0.46	-0.18	0.94	0.17	0.10	-0.15
<i>Toll</i>	-0.13	-0.12	0.94	0.09	0.80	-0.21	0.30	0.15
<i>β1.3g</i>	-0.90	0.03	0.26	-0.20	-0.04	0.04	0.90	-0.03
<b>Workers</b>								
<i>Aryl</i>	0.84	0.20	0.08		0.4	0.54		
<i>Vg1</i>	0.98	-0.22	-0.10		-0.15	0.84		
<i>IR3</i>	0.25	0.80	-0.29		0.56	0.25		
<i>PPO</i>	0.25	0.53	0.42		0.19	0.69		
<i>Hyme</i>	-0.73	-0.28	-0.23		-0.60	-0.04		
<i>LPS.bp</i>	0.14	0.58	0.37		-0.04	0.67		
<i>LysC</i>	-0.10	0.13	0.83		0.64	0.32		
<i>Toll</i>	0.15	-0.29	0.81		0.73	0.00		
<i>β1.3g</i>	-0.26	0.84	0.04		0.93	-0.15		

Tab. S5: Post-hoc pairwise comparisons of the differences in gene expression among treatments (TX = TritonX, BB = Beauveria) and castes/classes (Q / N = Queens / Nurses, M / F = Males / Foragers). The average difference in gene expression (inverse Ct values) is given as the parameter estimates plus/minus standard error ( $\beta \pm SE$ ). All p-values within each caste-group were FDR corrected.

	TX.Q / N - TX.M / F			BB.Q / N - BB.M / F			TX.Q / N - BB.Q / N			TX.M / F - BB.M / F		
	$\beta \pm SE$	z-value	adj.p-val	$\beta \pm SE$	z-value	adj.p-val	$\beta \pm SE$	z-value	adj.p-val	$\beta \pm SE$	z-value	adj.p-val
<b>Reproductives</b>												
<i>Aryl</i>	1.15 ± 0.39	2.96	0.0069	-1.14 ± 0.41	-2.82	0.0116	1.76 ± 0.37	4.76	< 0.0001	-0.54 ± 0.38	-1.4	0.24
<i>Vgl</i>	6.77 ± 0.45	15.12	< 0.0001	5.67 ± 0.47	12.11	< 0.0001	1.28 ± 0.43	2.99	0.0084	0.18 ± 0.45	0.4	0.69
<i>IR3</i>	-0.77 ± 0.22	-3.48	0.0026	-0.36 ± 0.23	-1.58	0.2	0.09 ± 0.22	0.42	0.68	0.50 ± 0.23	2.19	0.13
<i>PPO</i>	2.86 ± 0.32	9.04	< 0.0001	3.03 ± 0.33	9.14	< 0.0001	0.78 ± 0.30	2.61	0.0162	0.94 ± 0.31	3.04	0.0094
<i>Hyme</i>	-4.87 ± 0.94	-5.18	< 0.0001	1.26 ± 0.97	1.29	0.3	0.56 ± 0.94	0.59	0.63	6.69 ± 0.97	6.86	< 0.0001
<i>LPS.bp</i>	-0.12 ± 0.24	-0.51	0.61	0.30 ± 0.25	1.2	0.31	0.44 ± 0.23	1.91	0.14	0.86 ± 0.24	3.6	0.0057
<i>LysC</i>	1.26 ± 0.19	6.49	< 0.0001	1.94 ± 0.20	9.59	< 0.0001	-0.32 ± 0.19	-1.7	0.13	0.36 ± 0.20	1.82	0.16
<i>Toll</i>	1.24 ± 0.23	5.52	< 0.0001	2.50 ± 0.23	10.71	< 0.0001	-1.51 ± 0.23	-6.69	< 0.0001	-0.25 ± 0.23	-1.08	0.36
<i><math>\beta</math>1.3g</i>	-0.83 ± 0.21	-3.87	0.0003	0.36 ± 0.22	1.64	0.2	-1.03 ± 0.21	-4.81	< 0.0001	0.16 ± 0.22	0.74	0.52
<b>Workers</b>												
<i>Aryl</i>	0.48 ± 0.27	1.76	0.18	2.78 ± 0.28	10.02	< 0.0001	-1.55 ± 0.27	-5.68	< 0.0001	0.75 ± 0.28	2.7	0.014
<i>Vgl</i>	1.36 ± 0.40	3.41	0.0077	4.10 ± 0.41	10.12	< 0.0001	-0.20 ± 0.40	-0.49	0.62	2.54 ± 0.41	6.27	< 0.0001
<i>IR3</i>	-0.72 ± 0.25	-2.92	0.0156	1.82 ± 0.25	7.25	< 0.0001	-1.69 ± 0.25	-6.83	< 0.0001	0.86 ± 0.25	3.4	0.002
<i>PPO</i>	-0.11 ± 0.29	-0.39	0.77	2.46 ± 0.29	8.39	< 0.0001	-0.48 ± 0.29	-1.68	0.16	2.09 ± 0.29	7.13	< 0.0001
<i>Hyme</i>	-0.97 ± 0.68	-1.42	0.56	0.60 ± 0.69	0.86	0.52	0.36 ± 0.68	0.52	0.62	1.92 ± 0.69	2.77	0.0286
<i>LPS.bp</i>	-0.11 ± 0.30	-0.37	0.77	1.99 ± 0.31	6.51	< 0.0001	-0.60 ± 0.30	-1.98	0.0956	1.51 ± 0.31	4.92	< 0.0001
<i>LysC</i>	0.06 ± 0.21	0.3	0.77	1.56 ± 0.21	7.41	< 0.0001	-0.89 ± 0.21	-4.3	0.0001	0.61 ± 0.21	2.88	0.0094
<i>Toll</i>	0.66 ± 0.32	2.05	0.16	2.23 ± 0.33	6.81	< 0.0001	-2.21 ± 0.32	-6.82	< 0.0001	-0.63 ± 0.33	-1.94	0.0596
<i><math>\beta</math>1.3g</i>	0.07 ± 0.21	0.33	0.77	1.38 ± 0.21	6.57	< 0.0001	-1.70 ± 0.21	-8.24	< 0.0001	-0.39 ± 0.21	-1.88	0.0808

Tab. S6: Post-hoc pairwise comparisons of the differences in PC-scores among treatments (TX = TritonX, BB = Beauveria) and castes/classes (Q / N = Queens / Nurses, M / F = Males / Foragers). The average difference in PC scores is given as the parameter estimates plus/minus standard error ( $\beta \pm SE$ ). All p-values within each caste-group were FDR corrected.

	TX.Q / N - TX.M / F			BB.Q / N - BB.M / F			TX.Q / N - BB.Q / N			TX.M / F - BB.M / F		
	$\beta \pm SE$	z-val	adj. p	$\beta \pm SE$	z-val	adj. p	$\beta \pm SE$	z-val	adj. p	$\beta \pm SE$	z-val	adj. p
<b>Reproductives</b>												
PC1	1.62 ± 0.12	13.42	< 0.0001	2.10 ± 0.13	16.76	< 0.0001	-0.30 ± 0.12	-2.5	0.033	0.17 ± 0.13	1.38	0.17
PC2	-0.69 ± 0.32	-2.16	0.0609	-0.11 ± 0.33	-0.34	0.74	0.47 ± 0.31	1.5	0.18	1.05 ± 0.33	3.21	0.0106
PC3	-0.91 ± 0.27	-3.34	0.0022	0.29 ± 0.28	1.03	0.41	-1.71 ± 0.27	-6.26	< 0.0001	-0.51 ± 0.28	-1.79	0.13
PC4	1.22 ± 0.29	4.26	0.0001	-0.70 ± 0.30	-2.35	0.0499	0.57 ± 0.28	2.03	0.0561	-1.36 ± 0.29	-4.67	< 0.0001
<b>Workers</b>												
PC1	0.07 ± 0.17	0.41	0.68	1.41 ± 0.18	7.96	< 0.0001	-1.61 ± 0.17	-9.2	< 0.0001	-0.27 ± 0.18	-1.5	0.18
PC2	0.21 ± 0.14	1.45	0.29	2.27 ± 0.14	15.81	< 0.0001	-0.40 ± 0.14	-2.86	0.0057	1.66 ± 0.14	11.56	< 0.0001

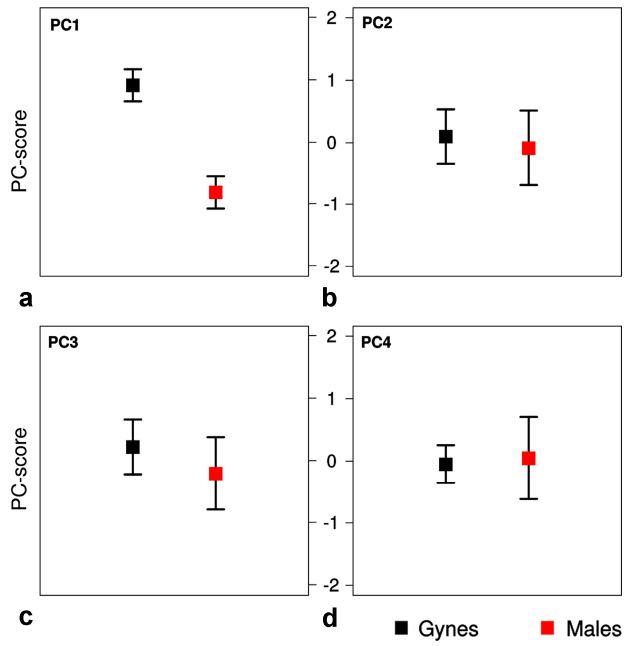


Fig. S1: Reproductive caste differences in PC scores on constitutive gene expression.

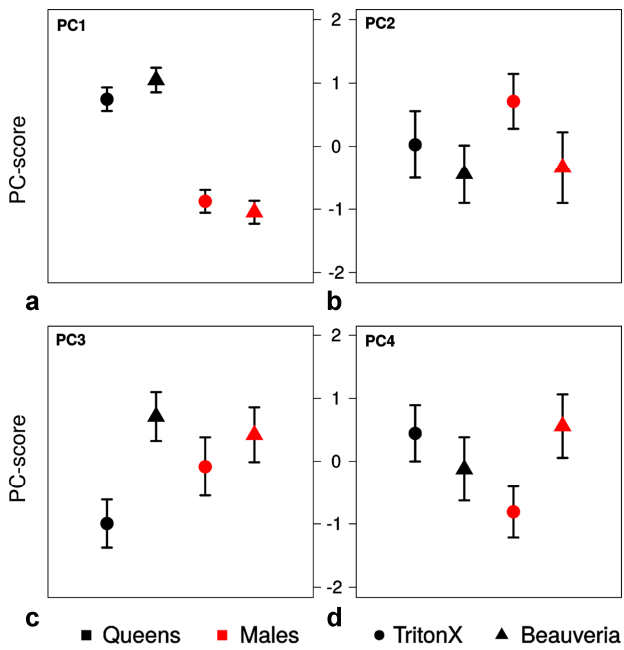


Fig. S2: Reproductive caste differences in PC scores on induced gene expression.

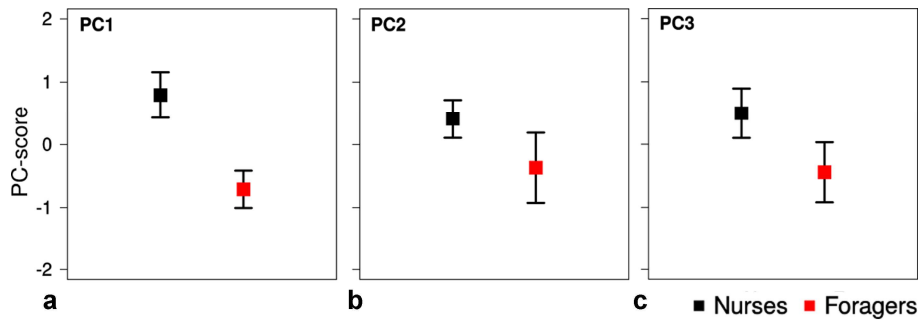


Fig. S3: Worker-class differences in PC scores on constitutive gene expression.

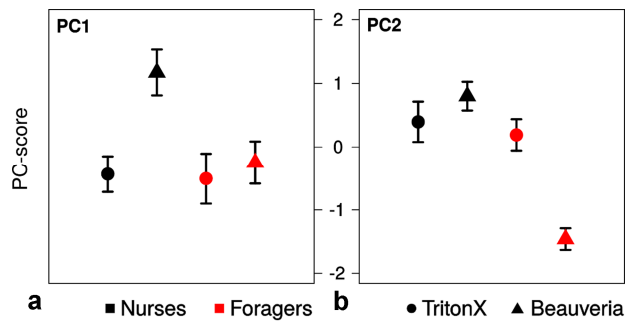


Fig. S4: Worker-class differences in PC scores on induced gene expression.