

Supporting Information

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SI Materials and Methods

In the spring of 2011, we collected queens that emerged from hibernation in northern Switzerland (B. L. Aesch and T. G. Neunforn). These queens established colonies in the laboratory. At the end of their colony cycle, these colonies produced gynes (daughter queens) and males, which we then mated; subsequently, we hibernated the gynes. On removal from hibernation, these gynes established colonies that were then used for the experiment. We intentionally used second-generation bees to avoid the known effect of maternal immunological history (1, 2). All colonies were confirmed to be free of common bumblebee parasites by repeated microscopic checks. When colonies had reached a sufficient size (~20–30 workers), we removed callow workers (i.e., workers that had emerged within the last 24 h) daily and provided them with ad libitum sugar water and pollen. Once these workers were 7 d old, they were exposed to a dose of 10,000 *Crithidia bombi* cells in 10 μ L of 50% sugar water. Treatments comprised infecting the honeybees with one of three genotypes of *C. bombi* (internal codes 08.068, 08.075, and 08.161; henceforth, shortened to nos. 68, 75, and 161) or with a sham inoculum without the parasite. After exposure, we housed them in pairs for 18 h with ad libitum sugar water, but not with pollen, during this time. Eighteen hours after exposure, we anesthetized half of the bees on ice and removed their guts. We then snap-froze the guts in liquid nitrogen and stored them at -80°C until RNA extraction. We chose this time point based on previous work showing differential expression of immune genes (3, 4). The remaining bees were given ad libitum sugar water and pollen, and they were maintained for an additional 7 d. After 7 d, we collected feces from these bees, assessed infection visually, and quantified infection load in the feces with quantitative real-time PCR (5). At this time point, the number of transmitting parasite cells in the feces correlates with infection intensities in the gut (6). Infection intensity data were analyzed with ANOVA after Yeo–Johnson transformation to improve normality and homoscedasticity of variance [CAR package (7)]. Because the sample size was unbalanced, we used type III sums of squares. We also analyzed the results of the visual checks, which were scored as presence/absence of *C. bombi* in the feces using a binomial generalized linear model with both host and parasite genotypes as crossed independent factors. We analyzed all our data with R (8).

We homogenized the guts from the bees that were killed after 18 h using 0.5-g zirconium beads at between 0°C and 10°C using an Omni Bead Ruptor 24 Homogenizer (OMNI International). We then extracted RNA using the RNeasy Plus Mini Kit (Qiagen) following the manufacturer's protocols. All RNA was assessed for quality using a 2100 Bioanalyzer (Agilent Technologies) with the RNA 6000 Nano Kit (Agilent Technologies). The RNA from three individuals per treatment per colony was pooled and then sequenced with an Illumina HiSeq 2000 sequencing system. After quality control steps to trim adapters and remove poor-quality reads, we mapped these reads to the official gene set of the *Bombus terrestris* genome project using SOAP [version 2.21 (9)]. We analyzed the resulting read counts using edgeR (10). We excluded genes where less than one read per million mapped in fewer than half of our groups, as suggested by the edgeR manual. We analyzed these data in two ways. On one hand, we considered the normalized read count of each gene as a de-

pendent variable of the respective *C. bombi* genotype and analyzed this differential transcript abundance using the glmFit function relative to the uninfected controls. Hence, the expression response is considered here as a “fingerprint” of a particular parasite genotype's infection. We also compared how each host genotype responded to each parasite genotype to explore whether host genotypes responded to the parasite genotypes differently. The results from this second analysis should be interpreted with caution, however, because each comparison is the expression of a single infected group relative to a single control group but can be viewed as a heuristic assessment of the overall patterns of infection with different parasite genotypes.

We assigned gene ontology (GO) terms by homology using Blast2GO (11). We then analyzed the over- or underrepresentation of GO categories among significantly differentially expressed transcripts using the hypergeometric test in FUNC (12), requiring at least five genes per category. We report the GO categories that are over- or underrepresented relative to a random sample of 1,000 genes within our total mapped set (Tables S1–S3). We used a cutoff of $P < 0.05$ and report the GO categories that were significant before refinement and the results after refinement by excluding categories that are significant by virtue of the importance of the daughter categories (12).

To confirm the differential expression of genes identified in the RNA-sequencing experiment, we designed quantitative real-time PCR primers of a selection of these genes using QuantPrime (3, 13) (Table S4). We then assessed the expression of 29 target genes relative to three housekeeping genes (ef1a, AK, and PLA2) in five individual bees per colony per treatment using Fluidigm 96.96 Dynamic Array IFCs on the BioMark System (Fluidigm) with EvaGreen DNA Binding Dye (Biotium) according to the Advanced Development Protocol 14 (PN 100-1208 B) developed by Fluidigm. We measured each reaction across three technical replicates and used the average cycle threshold (Ct) value of these for further analysis. We dropped three samples due to bubbles in the chip, but only one bee was dropped from any host genotype (G_H) by parasite genotype (G_P) combination.

We analyzed the expression of these genes using the difference in the Ct (dCt) of the genes of interest relative to the geometric mean of the three housekeeping genes (14). This composite average of the housekeeping genes was invariant across treatments ($F_{3,60} = 0.46$, $P = 0.71$) and colonies ($F_{3,60} = 0.62$, $P = 0.61$), and there was no interaction between these factors ($F_{9,60} = 0.82$, $P = 0.60$), suggesting that this housekeeping value is robust. We then transformed the dCt values to improve normality and homoscedasticity of variance using Yeo–Johnson power transformations. We analyzed the resulting transformed values with two separate multivariate ANOVAs with the transformed dCt value as the dependent variable. In the first analysis, we assessed the overall bumblebee response to parasite exposure by treating expression values as dependent on bumblebee genotype and exposure in a crossed model. We then explored how host and parasite genotypes interact by analyzing the expression values as explained by bumblebee genotype and *C. bombi* genotype as crossed independent factors. We also explored patterns of co-expression by producing a heat map of the correlation matrix of expression of the transformed dCt expression values of all genes.

1. Sadd BM, Schmid-Hempel P (2007) Facultative but persistent trans-generational immunity via the mother's eggs in bumblebees. *Curr Biol* 17(24):R1046–R1047.

2. Sadd BM, Schmid-Hempel P (2009) A distinct infection cost associated with trans-generational priming of antibacterial immunity in bumble-bees. *Biol Lett* 5(6):798–801.

- Brunner FS, Schmid-Hempel P, Barribeau SM (2013) Immune gene expression in *Bombus terrestris*: Signatures of infection despite strong variation among populations, colonies, and sister workers. *PLoS ONE* 8(7):e68181.
- Riddell CE, Sumner S, Adams S, Mallon EB (2011) Pathways to immunity: Temporal dynamics of the bumblebee (*Bombus terrestris*) immune response against a trypanosomal gut parasite. *Insect Mol Biol* 20(4):529–540.
- Ulrich Y, Sadd BM, Schmid-Hempel P (2011) Strain filtering and transmission of a mixed infection in a social insect. *J Evol Biol* 24(2):354–362.
- Sadd BM (2011) Food-environment mediates the outcome of specific interactions between a bumblebee and its trypanosome parasite. *Evolution* 65(10):2995–3001.
- Fox J, Weisberg S (2011) *An R Companion to Applied Regression* (Sage, Thousand Oaks, CA), 2nd Ed.
- R Development Core Team (2010) *R: A Language and Environment for Statistical Computing* (R Foundation for Statistical Computing, Vienna).
- Li R, et al. (2009) SOAP2: An improved ultrafast tool for short read alignment. *Bioinformatics* 25(15):1966–1967.
- Robinson MD, McCarthy DJ, Smyth GK (2010) edgeR: A Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* 26(1):139–140.
- Conesa A, et al. (2005) Blast2GO: A universal tool for annotation, visualization and analysis in functional genomics research. *Bioinformatics* 21(18):3674–3676.
- Prüfer K, et al. (2007) FUNC: A package for detecting significant associations between gene sets and ontological annotations. *BMC Bioinformatics* 8:41.
- Arvidsson S, Kwasniewski M, Riaño-Pachón DM, Mueller-Roeber B (2008) QuantPrime—A flexible tool for reliable high-throughput primer design for quantitative PCR. *BMC Bioinformatics* 9:465.
- Yuan JS, Reed A, Chen F, Stewart CN, Jr. (2006) Statistical analysis of real-time PCR data. *BMC Bioinformatics* 7:85.

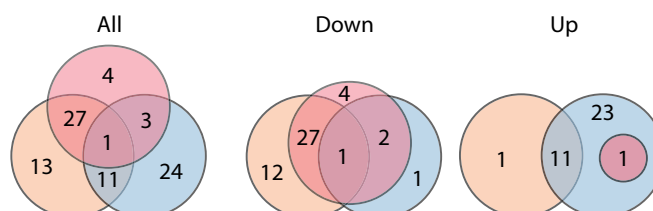


Fig. S1. Venn diagrams of the number of differentially expressed genes upon exposure to three genotypes of *C. bombi* relative to unexposed workers [nos. 68 (orange), 75 (blue), and 161 (red)] across all host colonies at a false discovery rate of 0.01.

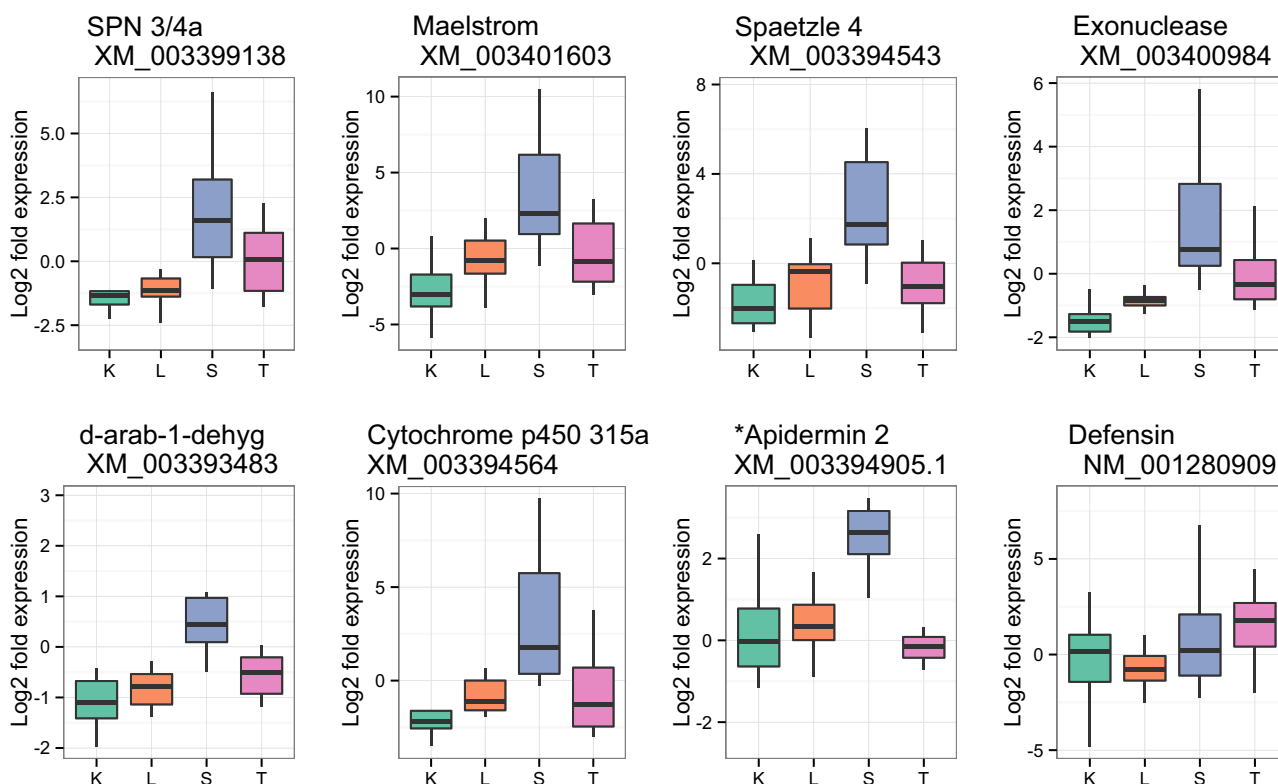


Fig. S2. Log₂ change in expression of genes upon parasite exposure across all parasite genotypes based on quantitative PCR (qPCR), where there is a significant effect of host genotype (x axis and differently colored) and parasite exposure (exposed vs. unexposed) [$G_H \times$ Exposure: $F_{3,64} = 3.17^*$, $F_{3,64} = 3.48^*$, $F_{3,64} = 3.35^*$, $F_{3,64} = 5.44^{**}$, $F_{3,64} = 6.64^{***}$, $F_{3,64} = 2.77^*$ (read from left to right and top to bottom); $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $^{\dagger}P < 0.01$] and where the interaction approached significance (apidermin 2: $F_{3,64} = 2.34^{\dagger}$). Expression of defensin varied significantly based on bee genotype only ($F_{3,64} = 3.12^*$). Values above zero can be interpreted as an up-regulation of the gene relative to unexposed individuals from the same colony. Inversely, values below zero are down-regulated upon parasite exposure. d-arab-1-dehyg, D-arabinose-1-dehydrogenase; SPN 3/4a, serpin 3/4a.

Table S1. Overrepresented (+) and underrepresented (–) GO terms for bees infected with the parasite genotype no. 68

Root node	Node name	Node ID	Genes	P	Over- or underrepresentation
BP	Negative regulation of transcription from RNA polymerase II promoter	GO:0000122	20	0.019	+*
BP	Aminoglycan metabolic process	GO:0006022	13	0.0083	+
BP	Chitin metabolic process	GO:0006030	9	0.0039	+*
BP	Amino sugar metabolic process	GO:0006040	15	0.011	+
BP	Cellular metabolic process	GO:0044237	1,524	0.015	–
BP	Cellular protein metabolic process	GO:0044267	630	0.020	–*
BP	Cellular localization	GO:0051641	262	0.050	–*
BP	Glucosamine-containing compound metabolic process	GO:1901071	12	0.0071	+
MF	Nucleotide binding	GO:0000166	634	0.023	–*
MF	Protein binding transcription factor activity	GO:0000988	28	0.035	+
MF	Transcription factor binding transcription factor activity	GO:0000989	28	0.035	+
MF	Transcription cofactor activity	GO:0003712	28	0.035	+*
MF	Chitin binding	GO:0008061	5	0.0011	+*
MF	Transcription factor binding	GO:0008134	32	0.0044	+*
MF	Peptidase activity	GO:0008233	132	0.012	+
MF	Serine-type peptidase activity	GO:0008236	29	0.037	+*
MF	Hydrolase activity, acting on carbon–nitrogen (but not peptide) bonds	GO:0016810	30	0.040	+*
MF	Serine hydrolase activity	GO:0017171	29	0.037	+
MF	Small-molecule binding	GO:0036094	645	0.021	–
MF	Anion binding	GO:0043168	492	0.024	–*
MF	Peptidase activity, acting on L-amino acid peptides	GO:0070011	108	0.026	+
MF	Carbohydrate derivative binding	GO:0097367	8	0.0030	+
MF	Nucleoside phosphate binding	GO:1901265	635	0.023	–
CC	Extracellular region	GO:0005576	80	0.031	+*
CC	Cell	GO:0005623	1,776	0.011	–
CC	Cytoplasm	GO:0005737	923	0.012	–
CC	Organelle	GO:0043226	1,196	0.031	–
CC	Intracellular organelle	GO:0043229	1,194	0.031	–*
CC	Intracellular part	GO:0044424	1,511	0.032	–
CC	Cytoplasmic part	GO:0044444	700	0.028	–*
CC	Cell part	GO:0044464	1,775	0.011	–

GO at its coarsest level breaks gene function into three basal root nodes (BP, biological process; CC, cellular component; and MF, molecular function). Root nodes provide the core classification, and the node name gives the finer scale presumed function of genes categorized to these groups.

*Categories that are significant after refinement by FUNC, which tests for overrepresentation or underrepresentation after removing daughter nodes.

Table S2. Overrepresented (+) and underrepresented (–) GO terms for bees infected with the parasite genotype no. 75

Root node	Node name	Node ID	Genes	P	Over- or underrepresentation
BP	Transition metal ion transport	GO:0000041	7	0.028	+*
BP	Meiotic spindle organization	GO:0000212	5	0.020	+*
BP	Assembly of actomyosin apparatus involved in cytokinesis	GO:0000912	8	0.032	+
BP	Cytokinesis, actomyosin contractile ring assembly	GO:0000915	8	0.032	+*
BP	Nucleobase-containing compound metabolic process	GO:0006139	708	0.036	–*
BP	Cellular aromatic compound metabolic process	GO:0006725	743	0.030	–
BP	Nitrogen compound metabolic process	GO:0006807	818	0.020	–
BP	Cellular metal ion homeostasis	GO:0006875	8	0.032	+*
BP	Response to bacterium	GO:0009617	12	0.048	+*
BP	Cellular process	GO:0009987	2,412	0.0020	–
BP	Polyol metabolic process	GO:0019751	5	0.020	+*
BP	Cytokinetic process	GO:0032506	9	0.036	+
BP	Meiotic cytokinesis	GO:0033206	5	0.020	+*
BP	Cellular nitrogen compound metabolic process	GO:0034641	752	0.029	–
BP	Hemocyte proliferation	GO:0035172	6	0.024	+*
BP	Defense response to bacterium	GO:0042742	9	0.036	+*
BP	Cellular metabolic process	GO:0044237	1,524	0.014	–
BP	Cellular biosynthetic process	GO:0044249	672	0.044	–*
BP	Cellular macromolecule metabolic process	GO:0044260	1,061	0.036	–*
BP	Heterocycle metabolic process	GO:0046483	736	0.031	–
BP	Anatomical structure development	GO:0048856	683	0.042	–*
BP	Metal ion homeostasis	GO:0055065	10	0.040	+
BP	Organic cyclic compound metabolic process	GO:1901360	754	0.028	–
MF	Carboxylesterase activity	GO:0004091	5	0.028	+*
MF	Binding	GO:0005488	1,852	0.030	–
MF	Iron ion binding	GO:0005506	21	0.0059	+*
MF	Serine-type peptidase activity	GO:0008236	29	0.011	+*
MF	Oxidoreductase activity, acting on single donors with incorporation of molecular oxygen	GO:0016701	5	0.028	+*
MF	Oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen	GO:0016705	32	0.013	+*
MF	Serine hydrolase activity	GO:0017171	29	0.011	+
MF	Organic cyclic compound binding	GO:0097159	993	0.049	–*
MF	Heterocyclic compound binding	GO:1901363	992	0.049	–*
CC	Extracellular region	GO:0005576	80	0.0001	+*
CC	Intracellular	GO:0005622	1,645	0.022	–*
CC	Cell	GO:0005623	1,776	0.0059	–
CC	Macromolecular complex	GO:0032991	750	0.033	–*
CC	Organelle part	GO:0044422	679	0.048	–*
CC	Cell part	GO:0044464	1,775	0.0060	–

GO at its coarsest level breaks gene function into three basal root nodes (BP, CC, and MF). Root nodes provide the core classification, and the node name gives the finer scale presumed function of genes categorized to these groups.

*Categories that are significant after refinement by FUNC, which tests for overrepresentation or underrepresentation after removing daughter nodes.

Table S3. Cont.

Root node	Node name	Node ID	Genes	P	Over- or underrepresentation
CC	Intracellular part	GO:0044424	1,511	0.017	–
CC	Chromosomal part	GO:0044427	70	0.025	+
CC	Cytoplasmic part	GO:0044444	700	0.021	–*

GO at its coarsest level breaks gene function into three basal root nodes (BP, CC, and MF). Root nodes provide the core classification, and the node name gives the finer scale presumed function of genes categorized to these groups.

*Categories that are significant after refinement by FUNC, which tests for overrepresentation or underrepresentation after removing daughter nodes.

†Categories that are only over- or under-represented after refinement.

