

Additional figures & tables

Figure S1 | Setup for experiment 1 to investigate the response of naïve focal *T. castaneum* cohabitated with wounded beetles at different time points. One week old virgin adult beetles were randomly assigned to one of the following treatment groups: 1. naïve focal with naïve non-focal (N^N) 2. naïve focal with wounded non-focal (N^W) 3. wounded focal with naïve non-focal (W^N) and 4. wounded focal with wounded non-focal (N^W). For each treatment 24 focal (circled) and 24 cohabitating (i.e. non-focal) beetles were used and 3 cohabitation times (6, 12 and 18 h) were independently tested. Each treatment / cohabitation time combination had four replicates resulting in a total of 2304 beetles (48 beetles x 4 treatments x 3 cohabitation times x 4 replicates). After cohabitation focal beetles were randomly assigned to evaluate either PO activity or were used for RT-qPCR to determine expression of heat shock protein, stress and immune genes. The flour on which the beetles had been for the respective time periods was used to determine hydroquinone and benzoquinone levels.



RT-qPCR: 10 animals pooled per replicate

Figure S2 | Setup for experiment 2 to investigate the response of naïve focal *T. castaneum* cohabitated with wounded conspecifics depending on their mating status and sex. One week old virgin adult beetles were randomly assigned to two mating statuses: 1. Mating and 2. No mating. For "Nating" 24 females (\mathcal{Q}) and 24 males (\mathcal{J}) were kept together for 24 h under standard conditions. For "No mating" beetles remained individualised for an additional 24 h. Afterwards beetles were separated according to sex and within their mating status and sex group assigned to one of the following treatments: 1. naïve focal beetles with naïve non-focal beetles (N^N). 2. naïve focal beetles with wounded non-focal beetles (N^N). For each treatment 10 focal (circled) and 10 cohabitating (i.e. non-focal) beetles were used and one cohabitation time (18 h) was tested. Each treatment / cohabitation time / mating status and sex combination was replicated 7 times resulting in a total of 1120 beetles (20 beetles x 2 treatments x 1 cohabitation time x 2 mating statuses x 2 sexes x 7 replicates). After cohabitation all focal beetles of each treatment were used to measure the expression of selected stress and immune genes via RT-qPCR.

Table S1. Primer-sequences with respective efficiencies (E) for RT-qPCR. ↓ in primer

Gene Symbol	Name	NCBI Accession Number	E / Fragment length bp	5' – 3' primer sequence	Primer origin
Att2	Attacin 2	TC007738	1.94 / 163	F: CAAACGACCAAAG↓GGAAACTA	This study
				R: CTTCCTCCAAGCAAAGTTGG	
Col1	Coleoptericin 1	TC005093	1.97 / 120	F: TTTGGCACTTTTTGCACTTG	This study
				R: GGGATGTCCTGTTCTACGGA	
Imd	Immune deficiency	TC010851	1.97 / 129	F: CCTCCAAGGGATGAAGTCAA	Zou et al, 2007
				R: ACTGGCAAAAG↓CAGATGGTC	
Hsp83	Heat shock protein 83	TC014606	2.00 / 107	F: CGCAGTTCATTGGCTATCCC	This study
				R: AGGAGGAAGAAGGCGAAGAC	
Hsp90	Heat shock protein 90	TC012185	1.95 / 128	F: TTGTGGTGTCACGTTTGTGC	This study
				R: TCTGCTCGTGTGTATCCGATT	
Hsp68	Heat shock protein 68	TC009706	1.85 / 140	F:CCTATTCCTGCGTCGGAGTC	This study
				R: GGCAACTTGGTTCTTGGCAG	
Thau	Thaumatin	TC000517	1.96 / 129	F: ATGGTTGCT↓ATCGAGCCGC	This study
				R: AACCCCGTTGCCATTTCTGA	
CytP450	Cytochrome P 450	TC010423	1.91 / 120	F: GTTTGTACCC CTCGGTGCCGTTCTA	This study
				R: GGTCCCGGTGGATGCCGTAAGCGAAA	
Rp49	Ribosomal protein 49	TC006106	1.95 / 132	F: TTATGGCAAACTCAAA↓CGCAAC	Konopova &
				R:GGTAGCATGTGCTTCGTTTTG	Jindra, 2007
RpL13a	Ribosomal protein L13a	TC013477	2.00 / 186	F: GGCCGCAAG↓TTCTGTCAC	This study
				R:GGTGAATGGAGCCACTTGTT	

sequences indicate where RT-qPCR primers cross exon-exon boundary.

References:

Zou Z, Evans JD, Lu Z, Zhao P, Williams M, Sumathipala N, et al. Comparative genomic analysis of the *Tribolium* immune system. Genome Biol. 2007;8:R177-R.

Konopova B, Jindra M. Juvenile hormone resistance gene Methoprene-tolerant controls entry into metamorphosis in the beetle *Tribolium castaneum*. Proc Natl Acad Sci U S A. 2007;104:10488-93.