Back to article

Fig. S1. Multiple sequence alignment between closely related species of *Tribolium castaneum* and *T. confusum* shows that there are no species similarities in the mitochondrial cytochrome oxidase I (*mtCOI*) gene

Back to article

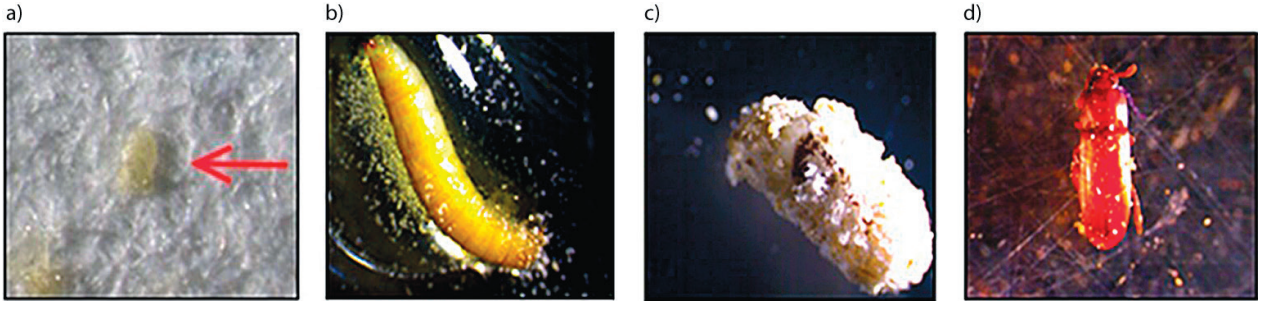

Fig. S2. Staining by iodine method of *Tribolium castaneum*: a) egg, b) larva, c) pupa, and d) adult

Back to article

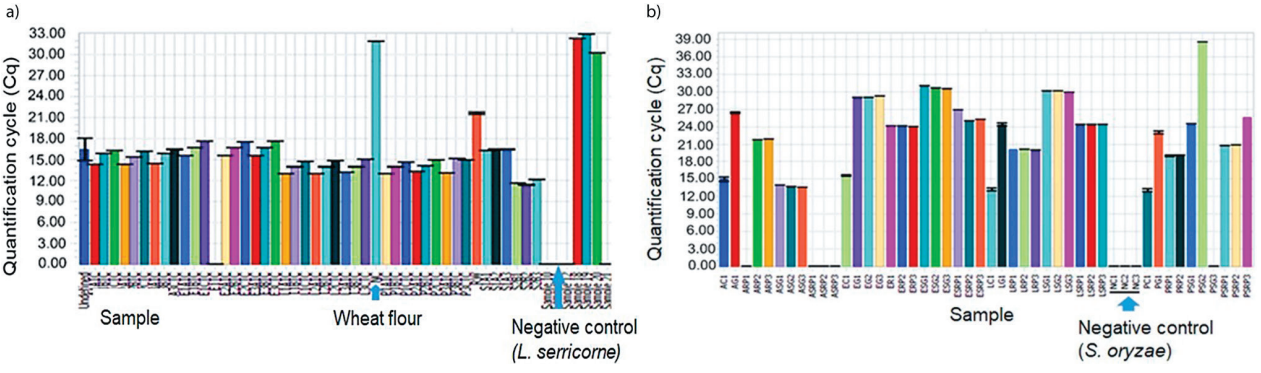

Fig. S3. Quantitative real-time polymerase chain reaction (qRT-PCR)-based amplification showing no amplification in negative control: a) *Lasioderma serricorne* and b) *Sitophilus oryzae*

Back to article

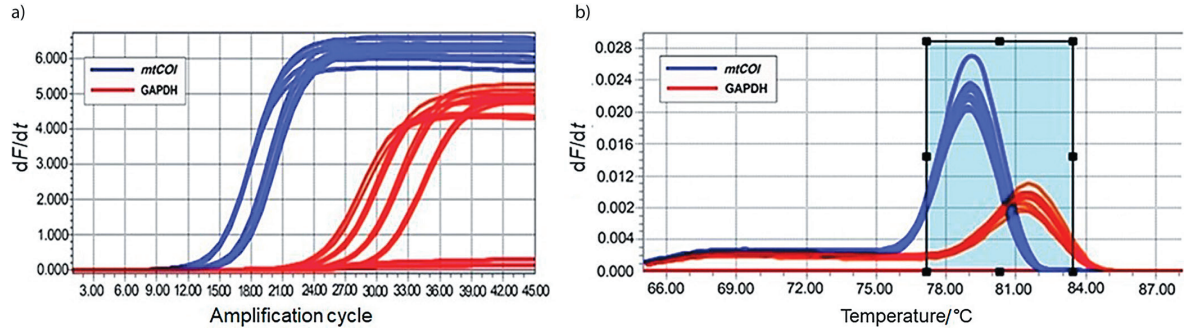

Fig. S4. Quantitative real-time polymerase chain reaction (qRT-PCR) analysis of *Tribolium castaneum* with mitochondrial cytochrome oxidase I (*mtCOI*) and GAPDH primers: a) analysis of the qRT-PCR with *mtCOI* primer, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) served as an internal control for normalization of data with all stages of *T. castaneum* (egg, larva, pupa and adult). Adults of *Lasioderma serricorne* were used as a negative control. All positive reactions amplified in the logarithmic phase before 29 cycles for GAPDH gene (dark orange) and 16 cycles for *mtCOI* gene (blue). All reactions show single melting peak and no amplification was observed for negative control, and b) melting curve analysis of *T. castaneum* DNA with *mtCOI* and GAPDH primers shows positive single peak obtained for *T. castaneum* with both primers. -d*F*/d*t*=negative derivative of fluorescence over temperature

Back to article

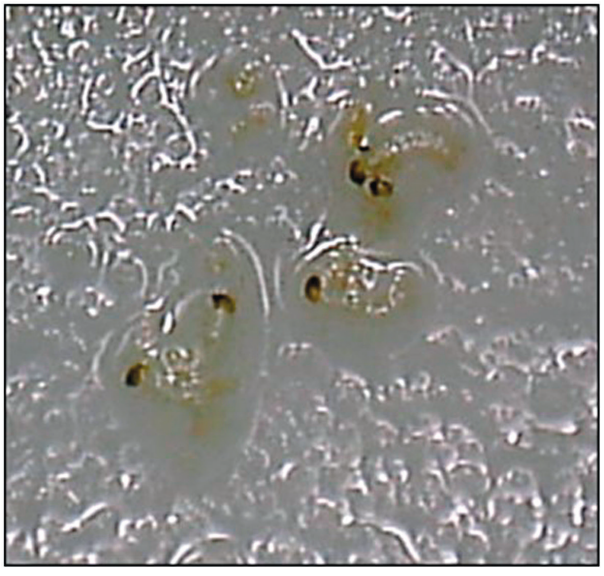

Fig. S5. *Tribolium castaneum* fragment identification in wheat flour by acid hydrolysis method

Back to article

Table S1. Data obtained from three independent experiments using regression analysis. Correlation was obtained for the cycle threshold (Ct) value with the corresponding infestation dose. The final equation derived from the analysis was used for calculating infestation in the flour in adult equivalents

Parameter for regression analysis	Obtained best-fit value for regression analysis
Slope	–2.8±0.4
y-intercept	21.9±0.68
x-intercept	7.8
1/Slope	–0.355
Confidence interval	95 %
Slope	–3.990 to –1.643
y-intercept	20.22 to 23.73
x-intercept	5.295 to 13.82
Goodness-of-fit	
R^2	0.8839
Sy,x	1.675
F value	38.06
Degree of freedom for numerator and denominator	1,5
p-value	0.0016
Deviation from horizontal axis	Significant
Data	
Number of xy pairs	7
Equation	y=–2.82·x+ 21.97

Back to article

Table S2. Frequency of DNA detection at different insect densities present in stored wheat flour

Insect equivalents as adults	*N*(*T. castaneum* beetle)/(*m*(wheat flour)/g)	Ct value
10	10/5	16.8±.02
1	1/5	22.73±0.23
0.1	1/50	26.95±0.17
0.01	Corresponds to 1/500	27.87±0.32
0.001	Corresponds to 1/5000	28.84±0.08

Each value is represented as mean±S.D. (*N*=7), Ct=cycle threshold