Protein expression parallels thermal tolerance and ecologic changes in the diversification of a diving beetle species complex

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Supplementary information

**Table S1.** Standardized volumes of the 563 spots common to all experiments for all treatments.

**Table S2.** Standardized volumes with ANOVA $P$-value < 0.05 for the Agabus brunneus population from Morocco. Information used for Figs S2,S3. RT: room temperature treatment (control). Biological replicas: r1, 2, 3.

**Table S3.** Standardized volumes with ANOVA $P$-value < 0.05 for the A. brunneus population from Iberian Peninsula. Information used for Figs S2,S3,S4. RT: room temperature treatment (control), FC: field control. Biological replicas: r1, 2, 3.

**Table S4.** Results of the comparison between treatments within each of the two populations of A. brunneus.

**Table S5.** Number of spots with significant differences between treatments for each of the comparisons.

**Table S6.** Significance of the comparisons of the expression level of the 563 common spots between treatments and populations.

**Table S7.** Principal Component Analyses (PCA) of the spot volumes with significant differences ($P$-value < 0.001) in the tested comparisons.

**Table S8.** Proteins identified in the selected spots.

**Figure S1.** Distribution of the differences between the three replicated samples of each treatment in the two populations of Agabus brunneus.

**Figure S2.** Distribution of the pairwise differences in protein spot volume between temperature treatments in each of the two populations of Agabus brunneus.
**Figure S3.** Cluster analysis of the significantly differently expressed protein spots for the two *A. brunneus* populations.

**Figure S4.** Cluster analysis of the significantly differently expressed proteins spots for Iberian population of *A. brunneus* without the field control.

**Figure S5.** Plots of the two first axes of the PCA analyses of the fold values of the 563 spots common to all experiments.

**Figure S6.** PermutMatrix representation of the cluster of the significantly different expressed spots in the comparison of the Moroccan *A. ramblae* vs. the other populations at a *P*-value < 0.001.

**Figure S7.** PermutMatrix representation of the cluster of the significantly different expressed spots in the comparison of the pooled *A. ramblae* vs. *A. brunneus* at a *P*-value < 0.0001.

**Figure S8.** Box-plots of the volumes of the three replicas by species, population and temperature treatment of the identified spots (see Tables 2,3,58 for the spot number and the identity of the proteins).
**Figure S1.** Distribution of the differences between the three replicated samples of each treatment in the two populations of *Agabus brunneus*. Vertical axis, fold change. RT, room temperature.
**Figure S2.** Distribution of the pairwise differences in protein spot volume between temperature treatments in each of the two populations of *Agabus brunneus*. Vertical axis, fold change. Note the different scales in the number of spots, due to the lower number of spots in the North Moroccan population.
Figure S3. Cluster analysis of the significantly differently expressed protein spots for the two *A. brunneus* populations. The analyses include the proteins with significantly different expression for each replicated sample, as measured with ANOVA at *P*-value < 0.01. RT, room temperature; FC, field control; r1 to r3, replicated samples. See Hidalgo-Galiana *et al.* (2014a) for details of the experimental procedure.
Figure S4. Cluster analysis of the significantly differently expressed protein spots for the iberian A. brunneus. The analyses include the proteins with significantly different expression for each replicated sample, as measured with ANOVA at $P$-value < 0.01 without the field control (FC). RT, room temperature; FC, field control; r1 to r3, replicated samples. See Hidalgo-Galiana et al. (2014a) for details of the experimental procedure.
**Figure S5.** Plots of the two first axes of the PCA analyses of the fold values of the 563 spots common to all experiments. A: all 563 spots, comparison between treatments (including room temperature, RT). B to E: only points with significantly different expression levels ($P$-value $< 0.001$) in the comparison of B: pooled *A. ramblae* populations vs. pooled *A. brunneus* populations, 4°C treatment; C: pooled *A. ramblae* populations vs. pooled *A. brunneus* populations, 27°C treatment. Squares: *A. ramblae*; circles: *A. brunneus*; filled symbols: 4°C; empty symbols: 27°C; grey symbols: RT. M: Moroccan; IP: Iberian. See Table S4 for details on the PCA results.
Figure S6. PermutMatrix representation of the cluster of the significantly different expressed spots in the comparison of the Moroccan *A. ramblae* vs. the other populations at a *P*-value < 0.001. Colours reflect the level of expression according to the scale.
Figure S7. PermutMatrix representation of the cluster of the significantly different expressed spots in the comparison of the pooled *A. ramblae* vs *A. brunneus* at a $P < 0.0001$. Colours reflect the level of expression according to the scale.
Figure S8. Box-plots of the volumes of the three replicas by species, population and temperature treatment of the identified spots (see Tables 2,3,S6 for the spot number and the identity of the proteins).