

Triple RNA-Seq characterizes aphid gene expression in response to infection with unequally virulent strains of the endosymbiont *Hamiltonella defensa*

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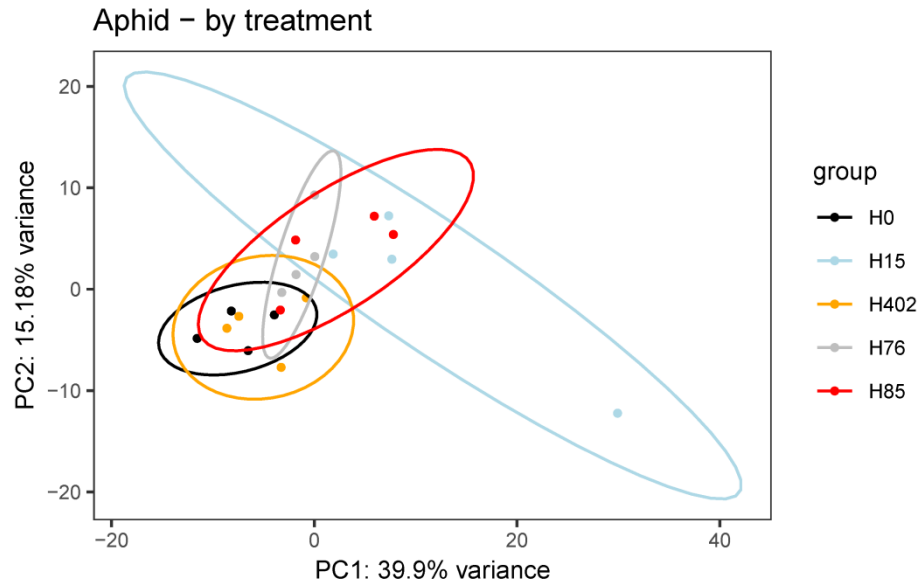
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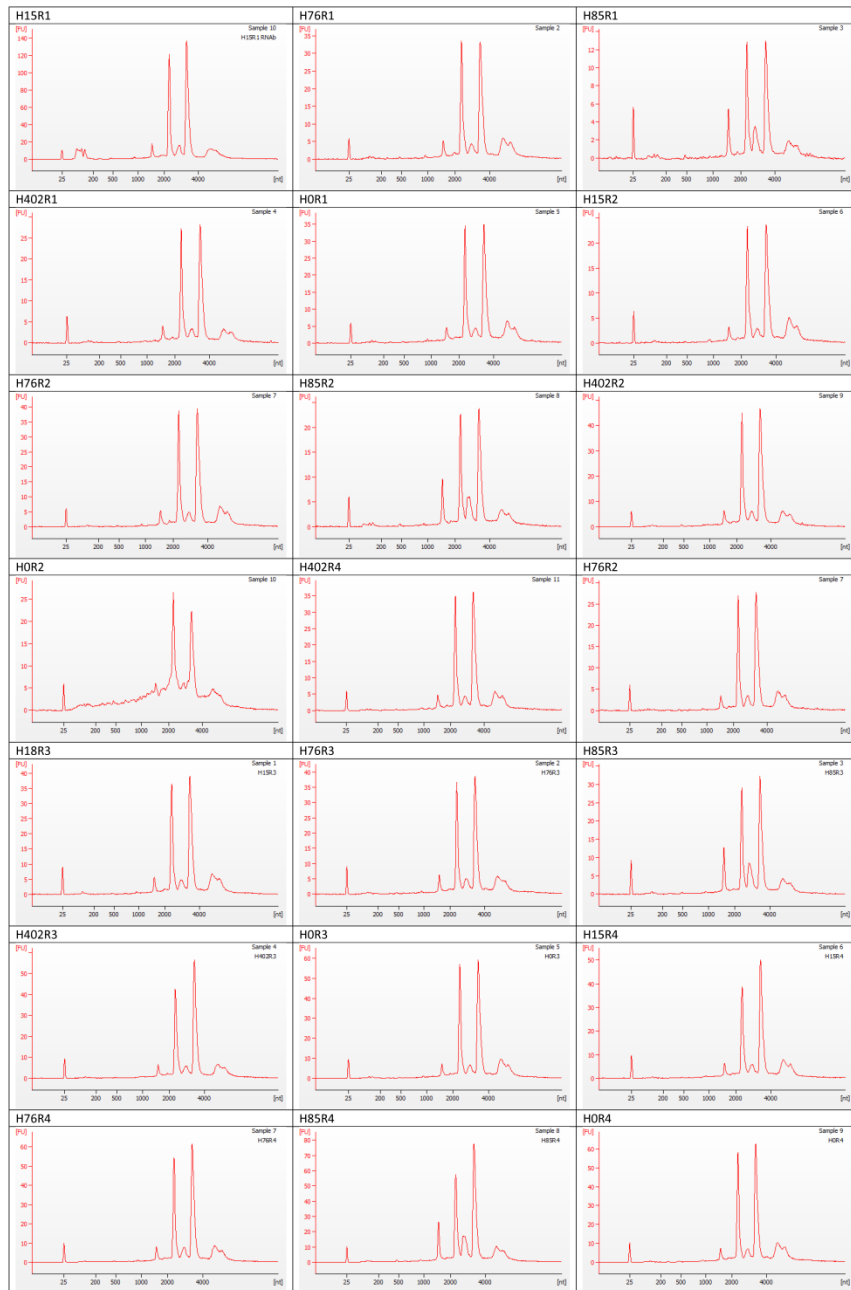
Additional File1, Supplementary Figure 1 – Library H15R1 is an outlier. Library H15R1 is located at the lower right corner of a PCA of the normalised and variance stabilisation transformed read count of all aphid genes. Aphid hosts were uninfected (H0, black) or infected (infecting strains H15 (blue), H402 (orange), H76 (grey) or H85 (red)) by *H. defensa*.

		Hamiltonella defensa													
		ApHdef-H10	ApHdef-H1	ApHdef-H11	ApHdef-H8	ApHdef-H7	ApHdef-H12	ApHdef-H9	ApHdef-H4	ApHdef-H2	ApHdef-H5	ApHdef-H3	ApHdef-H6	ApHdef-H13	
# genes		136	60	240	82	48	21	46	44	176	46	20	171	152	
Aphis fabae	ApHdef-A2	141	0.08	0.36	-0.60	-0.58	0.77	-0.82	0.58	-0.39	0.33	-0.26	0.03	0.64	-0.81
	ApHdef-A4	243	-0.46	0.39	-0.91	-0.16	0.81	-0.49	0.08	-0.62	0.74	0.24	0.01	0.92	-0.73
	ApHdef-A5	99	-0.36	-0.02	-0.59	0.10	0.57	-0.24	-0.00	-0.68	0.45	0.34	-0.33	0.61	-0.32
	ApHdef-A7	383	0.46	-0.27	0.85	0.06	-0.76	0.43	-0.02	0.65	-0.70	-0.31	0.09	-0.86	0.62
	ApHdef-A6	22	-0.01	-0.69	0.01	0.36	0.33	-0.16	0.04	-0.69	-0.13	0.32	-0.85	-0.00	0.31
	ApHdef-A10	42	-0.46	0.30	-0.38	0.14	-0.06	0.42	-0.27	-0.05	0.45	0.25	0.21	0.37	-0.18
	ApHdef-A3	22	-0.90	0.42	-0.68	0.44	0.15	0.40	-0.71	-0.23	0.85	0.66	0.30	0.65	-0.20
	ApHdef-A1	42	0.73	0.41	0.23	-0.89	-0.13	-0.39	0.82	0.52	-0.40	-0.90	0.44	-0.18	-0.48
	ApHdef-A8	97	-0.09	-0.23	0.23	0.31	-0.39	0.56	-0.23	0.06	-0.12	0.16	-0.14	-0.24	0.39
	ApHdef-A9	121	-0.05	-0.06	-0.12	-0.01	0.08	0.06	0.09	-0.18	0.07	0.03	-0.16	0.13	-0.07
	ApHdef-A11	109	0.46	0.08	0.58	-0.27	-0.55	0.20	0.19	0.70	-0.49	-0.50	0.35	-0.58	0.20

Additional File1, Supplementary Figure 2 – Correlation of aphid and *H. defensa* gene modules. Pearson correlation coefficient between eigengenes of aphid and *H. defensa* modules. Coloured: Correlation has a p-value of <0.01, red indicates positive and blue indicates negative correlation.

		Hamiltonella defensa											
		BapHdef-H6	BapHdef-H8	BapHdef-H7	BapHdef-H2	BapHdef-H1	BapHdef-H3	BapHdef-H4	BapHdef-H10	BapHdef-H5	BapHdef-H11	BapHdef-H9	
Buchnera aphidicola	# genes	60	136	240	48	21	46	44	176	171	82	152	
	BapHdef-B2	11	0.11	-0.15	-0.24	-0.61	-0.15	-0.27	-0.20	0.25	0.35	0.31	-0.32
	BapHdef-B3	22	0.41	-0.36	0.18	-0.54	-0.18	0.21	-0.56	-0.28	0.53	-0.02	-0.55
	BapHdef-B5	11	-0.05	0.06	0.15	-0.75	-0.33	-0.14	-0.40	0.04	0.14	0.04	-0.14
	BapHdef-B1	82	-0.54	0.54	0.13	0.46	0.01	-0.12	0.38	0.12	-0.71	-0.18	0.71
	BapHdef-B4	42	0.73	-0.74	-0.24	-0.28	0.20	0.24	-0.26	-0.19	0.85	0.19	-0.85

Additional File1, Supplementary Figure 3 – Correlation of *B. aphidicola* and *H. defensa* gene modules. Pearson correlation coefficient between eigengenes of *B. aphidicola* and *H. defensa* modules. Coloured: Correlation has a p-value of <0.01, red indicates positive and blue indicates negative correlation.



Additional File1, Supplementary Figure 4 – High RNA integrity after total RNA extraction. Bioanalyzer 2100 electropherograms of all libraries in batch A and B. Comparison with Schroeder et al. (2006) implied a RIN of 8 for sample HOR2 and 9- 10 for all others. Ribosomal RNA peaks of the different organisms are visible. Consider that a double 18S rRNA peak is expected for several insect species.