Figures and figure supplements

Genes associated with ant social behavior show distinct transcriptional and evolutionary patterns

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Figure 1. Behavioral and transcriptional changes associated with worker age and behavior. Numbers along the y-axis represent ages of marked worker cohorts, starting at worker eclosion as an adult (day 0). In all plots dark green represents greater values, while white represents lower values of the measure being plotted. (A) Behavioral results. Workers showed an age-dependent progression of activity, progressing from tasks such as nursing and grooming in the nest to outside tasks such as walking and foraging. (B) Heat map of expression levels over the course of worker aging (higher expression in darker green), for 23 genes most differentially expressed between nurses and foragers. The red line separates the samples classified as ‘nurses’ by K-nearest neighbor classification on the left, from ‘foragers’ on the right, suggesting a distinct transition between the two categories. (C) Correlation between patterns of expression in the 14 identified modules across worker age and behavior. The colors of the boxes are scaled with the value of correlation coefficients, ranging from white to dark green. On the right side of the heat map are the numbers of genes in each module and a dendrogram showing the inferred relationships among modules. The modules show complex patterns of expression, for example with some most highly expressed at age 0, some showing decreasing expression over time, and some increasing expression over time.

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**Figure 1—figure supplement 1.** The behaviors performed by age-marked workers changed as the workers aged, from nursing to foraging. Boxplots show the distribution of age in days for each behavior; white diamonds and the printed number show the mean age for each behavior, and the number of observations for each behavior is shown at the bottom of the graph.

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**Figure 1—figure supplement 2.** The location of age-marked workers also changed as the workers aged, from the nest area over the brood to outside the nest. Boxplots show the distribution of age with the mean and sample size for each category.

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Figure 1—figure supplement 3. The identified modules vary in expression pattern, composition of nurse-upregulated and forager-upregulated genes, and the proportion of conserved genes with identified fire ant orthologs. The total number of genes, number of nurse-upregulated genes, and forager-upregulated genes are shown, along with the proportion of identified fire ant orthologs and prominent gene ontology terms enriched for each module (see Supplementary file 4 for the full GO enrichment profiles for each module). Both the module number and associated module color are shown on the left.

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Figure 2. Connectivity, expression, and evolutionary rate for nurse-upregulated (blue), forager-upregulated (red), and non-differentially expressed genes (gray). Overall, connectivity and expression are positively correlated (F) and negatively associated with evolutionary rate (D and E), as expected. At the same time, forager-upregulated genes are much more strongly connected while nurse-upregulated genes are more loosely connected compared to non-differentially expressed genes (A); Nurse-upregulated genes have a small but significant increase in evolutionary rate (C); and both forager- and nurse-upregulated genes are more highly expressed than non-differentially expressed genes. The top panels show results for all data, while the bottom panels show results only for genes with S. invicta orthologs that had estimated evolutionary rates.

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Figure 3. Genes with identified fire ant orthologs were more highly connected and expressed, but this relationship also depended on whether the gene was nurse-upregulated (blue), forager-upregulated (red), or non-differentially expressed (NDE, gray). As shown in Figure 2, forager-regulated genes were much more highly connected, and overall, forager-upregulated genes had a higher proportion of identified fire ant orthologs (0.54) relative to nurse-upregulated and non-differentially expressed genes (0.43).

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Very similarly to Figure 3, genes with identified honey orthologs were more highly connected and expressed, but this relationship also depended on whether the gene was nurse-upregulated (blue), forager-upregulated (red), or non-differentially expressed (NDE, gray). Forager-upregulated genes had a higher proportion of identified honey bee orthologs (0.50) relative to nurse-upregulated and non-differentially expressed genes (0.38).

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