**Maternal and nourishment factors interact to influence offspring developmental trajectories in social wasps**

**Electronic Supplementary Material-3: qRT-PCR Methods, Primers, and Control Genes**

**Methods and Replication:** qRT-PCR was performed with a Bio-Rad CFX96 Touch Real-Time PCR Detection System using 2x SYBR Green Master Mix (Applied Biosystems). Absolute quantities of genes were calculated from standard curves based on genomic DNA generated from whole *P. fuscatus* thoraces. Each sample was run in triplicate with mean concentration values later used for statistical analysis. We used both internal control genes, *Ef-1α* and *RP-49,* to normalize expression levels of each candidate gene.

**Samples Selected:** Gene expression was quantified for a subset of collected pre-pupae (due to cost limitations). Sixty-one samples were subjected for gene expression analysis: 5 of 11 (2013) and all 16 (2014) foundress-reared (FR), and 12 of 76 (2013) and 25 of 38 (2014) worker-reared (WR). In 2013, WR samples were chosen non-randomly: we attempted to maximize the number of colonies represented, and chose 6 samples that had the highest and 6 samples that had the lowest lipid content. This was done in order to ensure that we included individuals across the full range of nutritional status so as not to bias our results based on nutritional differences alone. In 2014, we collected female pre-pupae from 20 colonies, so our goal was to maximize colony representation while evenly distributing the number of samples assayed among the four treatment groups. When multiple samples were assayed within a colony, the expression values were averaged and colony was the level used for statistical analysis.

**Primer design:** Primers for three genes of interest and both internal controls were developed from *Polistes fuscatus* transcriptomes (Berens et al., in prep). The primer for *hexamerin 70b* was developed from a previously published *P. metricus* transcriptome (28). All primers were designed using Primer Quest online from Integrated DNA Technologies.

**Primer sequences:**

|  |  |  |
| --- | --- | --- |
| **Gene** | **NCBI Accession Number** | **Primer Sequence** |
| *Elongation factor-1α*  (internal control) | GDFS01086685 | Forward:  5'-GTTTGACGCATATCACGAACAG-3'  Reverse:  5'-GGAGATGCTGCTATCGTTACTC-3' |
| *Ribosomal Protein-49*  (internal control) | GDFS01032632 | Forward:  5'-TCCCGATGGTAGAACACAAATAG-3'  Reverse:  5'-GGTGACGGTGCTGGATAAG-3' |
| *heat-shock protein 90α* | GDFS01066019 | Forward:  5’-TGCTCTCTCCTGTGATGTAGTA-3’  Reverse:  5’-CTTCTGCTTCGGGTGATGAA-3’ |
| *hexamerin 70b* | GBGV01000197 | Forward:  5’-TCTGGTTGGGAAACGTGATAC-3’  Reverse:  5’-CTGCCTTGTCTCTGCTGAATA-3’ |
| *inositol oxygenase* | GDFS01103214 | Forward:  5'-TTTCGACCGGAGCCTATCTA-3'  Reverse:  5'-ACACGTTCCTTGATCTCATCTG-3' |
| *rhodopsin* | GDFS01103004 | Forward:  5’-ACAAACACCGAATACACCAGTA-3’  Reverse:  5’-CCAATGGTATGTACCGGAAGG-3’ |

**Control Genes:** We assessed whether Cq values calculated for the internal control genes (*elongation factor-1* and *ribosomal protein-49*) varied across caste (foundress-reared vs worker-reared) or across the four treatment types. We used Generalized Linear Models, fit the data using the Log link function for μ, and included year in both models. We found no evidence to suggest that internal control genes varied across caste or treatment types (P > 0.50 for both analyses). Descriptive statistics are presented in the table below.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  | Mean Cq ± SE (n) | | |
|  | Treatment | Years Combined | 2013 data | 2014 data |
| *elongation factor-1* | Foundress-reared | 24.65 ± 0.32 (21) | 25.87 ± 0.96 (5) | 24.26 ± 0.24 (16) |
| No drumming, unrestricted foraging | 24.82 ± 0.19 (9) | 24.59 ± 0.0.26 (5) | 25.11 ± 0.21 (4) |
| Drumming, unrestricted foraging | 25.28 ± 0.46 (7) | 26.05 ± 0.51 (4) | 24.26 ± 0.20 (3) |
| No drumming, restricted foraging | 25.12 ± 0.79 (7) |  | 25.12 ± 0.79 (7) |
| Drumming, restricted foraging | 24.94 ± 0.51 (3) |  | 24.94 ± 0.51 (3) |
| *ribosomal protein-49* | Foundress-reared | 32.59 ± 1.28 (21) | 34.23 ± 0.16 (5) | 32.08 ± 1.67 (16) |
| No drumming, unrestricted foraging | 33.97 ± 0.40 (9) | 33.81 ± 0.74 (5) | 34.16 ± 0.10 (4) |
| Drumming, unrestricted foraging | 34.02 ± 0.35 (7) | 34.53 ± 0.42 (4) | 33.34 ± 0.31 (3) |
| No drumming, restricted foraging | 31.56 ± 1.70 (7) |  | 31.56 ± 1.70 (7) |
| Drumming, restricted foraging | 34.29 ± 0.67 (3) |  | 34.29 ± 0.67 (3) |