In development, genetics isn't everything: a collaborative case study

Instructions

- This worksheet is to be completed collaboratively by your 'article worksheet group' and submitted by September 25th (Canvas BIOL463 > Assignments > Worksheet about your assigned article). Please type your answers and ensure that all group members collaborate and understand the answers, which should represent a consensus within the group.

- Accepted formats: .doc, .docx, .rtf, .pages, .pdf

- **One rule** for this assignment: collaborate and cooperate within your group, but please do not discuss your paper or answers with other groups (yet)!

Questions

1. Please list the names of all the group members who participated:

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2. Please provide the complete reference for your assigned article (any format that includes authors, year, title and journal is fine).

W. Mao, M. A. Schuler, M. R. Berenbaum, A dietary phytochemical alters caste-associated gene expression in honey bees. Sci. Adv. **1**, e1 5000795 (2015)

3. Is your paper mostly an investigation of a factor that affects the developmental trajectory of honeybees, or of a mechanism responsible for it? Please indicate the specific factor or mechanism investigated.

The paper is mostly an investigation of a factor that affects the developmental trajectory of honeybees. The factor being investigated is p-coumaric acid. It is found in honey and beebread, but not royal jelly.

4. Before reading the article, what were your hypotheses about the main factor and/or mechanism responsible for queen vs. worker developmental trajectory?

We hypothesized that the default caste for *Apis mellifera* is the worker bee caste and that caste differentiation in *A. mellifera* is caused by a factor in royal jelly that allows queen-destined larvae to develop into adult queen bees. Before reading the article we believed that the phytochemicals being studied were found in royal jelly and that their presence

in the queen larval diet caused certain caste specific genes to be expressed at different levels than in worker bees resulting in the development of queen bees.

5. Analyze each of the data figures presented in the paper. For each one, please answer the following questions:
    1. What was the experiment that lead to the results? (What were the authors asking, what did they do, what did they measure, what were the controls).
    2. What do the data show?
    3. What can we conclude from the data?

Figure 1:

A: Before differential expression analysis, the researchers assessed the variation between the three biological replicates and between the two treatments (rearing on diets with and without 0.5 mM p-coumaric acid) using a multidimensional scaling (MDS) plot.

The researchers did an RNA-seq analysis to identify differences in gene expression in larvae reared in both control groups. RNA samples were collected from ground whole larvae after 3 days of treatment with or without p-coumaric acid. They used high throughput sequencing of 100 bp single-end reads and then mapped the reads back to the reference genome (3 replicates of 2 treatments). Read counts per gene were calculated for each replicate. They used the Bioconductor statistical software package EdgeR' to analyze and compare the RNA-seq expression profile across all probes in the biological replicates. This allowed the researchers to account for biological variability from biological replicate samples to determine whether the counts for a transcript or exon were significantly different across experimental conditions in biological replicates.

The data was visualized on an MDS plot to show the overall differences between expression profiles in the libraries. The closer the treatments are plotted on the map, the more similar they are. The farther the distance between the plot points, the greater the difference between them. The dimensions go in order of most variability. In figure 1, dimension 1 (x-axis) plotted variation among the three replicates, and dimension 2 (y-axis) plotted variation in library preparation.

B: The samples from the biological replicates of control treatments were clustered together on the MDS plot indicating that there was very little variation amongst the replicates. Samples from the biological replicates of the experimental condition, fed 0.5 mM p-coumaric acid, were not clustered together suggesting variation among replicates and among library preparations.

C: The control treatment replicates have gene expression patterns more similar to each other than to the p-coumaric acid treatment replicates. There was variation among biological replicates receiving the p-coumaric acid treatment, however, all of the replicates for the p-coumaric acid treatment were significantly separated from the clustered control replicates on the first dimension and this indicated that the sequencing data was qualified for differentially expressed gene identification. The addition of p-coumaric acid was sufficient to change the overall expression patterns across the two experimental conditions.

Figure 2:

A: The authors wanted to investigate the effect of p-coumaric acid on larval development. They used edgeR', a Bioconductor software package for examining differential expression of replicated count data, to identify differentially expressed genes (DEGs) between the control and 0.5mM p-coumaric acid treatment. The control larvae were fed a p-coumaric acid-free diet and the 0.5mM p-coumaric acid treatment larvae were fed a diet containing 0.5mM p-coumaric acid.

B: The data shows a plot of the log fold change in expression versus log counts per million. Each gene that is differentially expressed is represented as a red dot. Genes that are not differentially expressed are depicted as a black dot.

C: We can conclude that there are DEGs between the control and 0.5mM p-coumaric acid treatments. There are differences in fold change of expression for in the genes that were assessed.

Figure 3:

A: The authors wanted to characterize changes in larval development induced by p-coumaric acid by identifying cellular pathways that were significantly affected by p-coumaric acid. In order to perform a pathway analysis, Mao et al. (2015) performed an RNA-Seq experiment to study differences in gene expression between the control bees that were fed a diet lacking in p-coumaric acid (standard queen larval diet), and treated bees that were fed a diet supplemented with 0.5 mM of p-coumaric acid. Using the DAVID (Database for Annotation, Visualization and Integration Discovery) functional annotation clustering tool on the RNA-seq data, the researchers were able to identify functionally related gene groups among all the differentially expressed genes. Additionally, DAVID provided information on pathways that were affected by treatment with p-coumaric acid. GAGE (Generally Applicable Gene-set Enrichment) and Pathview were then used to characterize which larval pathways were most affected

by p-coumaric acid. Based on the pathway and gene-set analysis done by using GAGE and Pathview, researchers identified the Hippo signaling pathway as being most significantly affected by p-coumaric acid.

B: The data shows the affected genes in the Hippo signaling pathway. Upregulated genes are shown in red boxes and downregulated genes are in green boxes on the Hippo signaling pathway map. The majority of the molecular players in the Hippo signaling pathway are affected by p-coumaric acid. Five of these molecules were downregulated and 29 were upregulated in bees reared on a diet supplemented with p-coumaric acid.

C: From the data represented in Figure 3 we can conclude that p-coumaric acid is sufficient to disrupt the Hippo signaling pathway. The expression level of many of the molecules in the Hippo signaling pathway of *A. mellifera* larvae reared on a diet of 0.5 mM of p-coumaric acid were affected. The Hippo signaling pathway is involved in organ development and based on the data presented in this figure, bees fed a diet that contains p-coumaric acid as larvae may have underdeveloped organs compared to queen bees.

Figure 4:

A: The authors wanted to determine if p-coumaric acid altered larval developmental fate. In order to do this, they studied ovary development in larvae reared on the standard queen larvae diet (which contains royal actin and lacks p-coumaric acid), as well as in larvae fed an identical diet supplemented with p-coumaric acid. The control group in this experiment were the larvae reared on the standard queen diet and the second group of bees were treated with p-coumaric acid. The researchers dissected adults within 12 hours of eclosion and measured and scored the size the ovaries. Ovary development was scored on a five-point scale (where a score of 1 meant the ovaries were underdeveloped and a score of 5 meant they were fully developed).

B: The data collected from this experiment shows that larvae raised on a standard queen larvae diet supplemented with p-coumaric acid have underdeveloped ovaries. Maximum ovary development was seen in the control bees that were fed the standard diet lacking p-coumaric acid. The number of bees with underdeveloped ovaries is approximately the same for both the control and the treated group however researchers found that none of the bees fed a diet that contained p-coumaric acid attained full ovary development. The data in part A shows how ovaries were scored on a 1 (underdeveloped) to 5 (fully developed) scale. The data in part B shows the proportion of ovaries found that were scored between 1 to 5 in the control and the treated bees.

C: From the data presented in Figure 4 we can conclude that the standard queen larvae diet lacking in p-coumaric acid is necessary to achieve fully developed ovaries in adults. Ovaries of a typical virgin queen bee were only identified in the control adult group. None of the bees that were reared on a diet that contained p-coumaric acid had fully developed ovaries although they did have ovaries that ranged from underdeveloped (score of 1) to advanced development (score of 4).

6. As a group, select what you think is the most important or impactful data figure presented in your paper. Briefly explain why you think that it is the most important, and how it contributes to furthering our understanding of the regulation of development in female honeybees (and possibly, developmental regulation in general).

Figure 3 is the most impactful data figure presented in the paper. It shows a map of the Hippo signaling pathway, which is the pathway that the researchers found to be the most severely affected by p-coumaric acid. Of the two gene sets identified from the KEGG pathway database, only the Hippo signaling pathway was enriched. This figure is the only one which relates the differentially expressed gene profile associated with p-coumaric treatment with a biological mechanism for caste development in honey bees. Figure 4 shows the relationship between the treatment groups and ovary development, but it does not suggest any underlying mechanism or pathway for the difference in ovary development. Figure 3 is so important because it demonstrates all the ways the Hippo signaling pathway is affected if the diet fed to the bees is supplemented with p-coumaric acid. The Hippo signaling pathway is involved in organ development therefore this figure clearly shows that p-coumaric acid may affect organ development in bees fed p-coumaric acid versus bees fed a diet lacking p-coumaric acid. Figure 3 contributes to our understanding of the regulation of development in female honeybees because it is a visual representation of the influence of p-coumaric acid on gene expression in a signaling pathway linked with regulating organ development. In addition, the map shows which genes were differentially expressed between the control and the p-coumaric treated bees.

7. Think about the experience that you just had of working collaboratively on an assignment. As a group, how did you proceed? Was the strategy successful? How did you define "successful"? If you were to work together again, would you do anything differently?

Our group proceeded by first reading the paper individually. We then met as a group to discuss the paper in general and the questions on this worksheet. We assessed the difficulty of answering each section, particularly the analysis of each figure, and then assigned primary responsibility for each section. We drafted our response using Google

Docs so that all group members could see the current form of our response and make any changes to all parts of the document at any stage. Although each person had primary responsibility for certain sections, we would each review, make comments, changes, and additions to the entire worksheet.

We think this strategy was successful. We would define "successful" in this context as being able to collaborate efficiently. Using google docs helps because then we could all work off the same document and there was less chance of duplicating work and all members were able to make immediate changes and comments.

If we were to work together again, we would have aimed to have a second group meeting after completing all sections of the worksheet to review the entire document together as a group rather than separately.