What role does DNA methylation play in determining caste phenotypes in paper wasps?

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Introduction
Epigenetic modifications are stable modifications to DNA that do not change its sequence but can have strong effects on gene expression. Epigenetic effects, such as DNA methylation, are known to be important in mediating effects of maternal care on the growth, health and behavior of offspring [1]. Recent studies on honey bees have shown that DNA methylation modulates nutritional control of queen and worker caste differences. Genetically identical larvae can become either fertile queens or sterile workers depending on methylation levels, where reduced methylation will lead to queen phenotypes in honey bees [2]. The study of the paper wasp genus Polistes has been critical in gaining insights into the evolution of insect sociality. Polistes dominulus is a primitive social wasp and can provide insights into the role of DNA methylation in the evolution of caste differences (Figure 1) [3].

Materials and Methods
- **Polistes dominulus nests and foundress(s) collected and housed in climate controlled rearing room (Figure 4).**
- Each foundress paint marked (Figure 5) and assigned to one of four treatment groups:
  - High food + Zebularine treatment
  - High food + control treatment (honey water)
  - Low food + Zebularine treatment
  - Low food + control treatment (honey water)
- Treatments were conducted twice per day (Figure 7):
  - Zebularine groups were hand fed 1 μl of 2mM Zebularine in honey water twice per day using a pipette
  - Control groups received 1 μl of 50% honey water solution twice per day
- After 2 weeks several focal and non-focal 3rd instar larvae were analyzed for methylation levels, lipid stores and gene expression (Figure 6):
  - Total RNA was extracted from each larva
  - RNA sequencing and measuring gene expression in larvae using RNA sequencing.

Results
- We had very few larvae in the LO group that survived to pupation.
- We found no effect of food level or zebularine on larval wet weight, dry weight, lipid stores, or lipid percentage (data not shown).
- We found pupation time was shorter in zebularine groups (Figure 9).

Discussion and Future
Our data showed a shorter pupation time for wasps when treated with zebularine as compared to our control and suggested more worker-like behaviors. This data does not support the complete opposite of our original hypotheses that inhibiting DNA methylation with zebularine will result in wasps with more queen-like phenotypes. Also we found no effect of food level for any variable so food might not have been limited in our low food treatment. Possible reason for finding the opposite results include: (1) Zebularine did not actually inhibit methylation but had other effects (2) Methylation levels in workers are actually lower than in gynes or zebularine made them more worker-like. Future work will include additional behavior observations, measuring adult lipid stores and ovary size, measuring methylation levels using bisulfite sequencing and measuring gene expression in larvae using RNA sequencing.

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Literature cited
[5] Hunt, J. L., Magnus, H. J., and Forstmeier, J. M. 2010. A Behavioral Role for the Methylation of Reproductive Female Focal wasps that emerged were paint marked (Figure 8)

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Figure 2. Polistes dominulus
Figure 3. Zebularine, a DNA Methylation Inhibitor
Figure 4. Hypocritically nest
Figure 5. Focal workers
Figure 6. Polistes dominulus larvae
Figure 7. Testing larvae
Figure 8. Paint marked foundress and emerged worker
Figure 9. Pupation time by treatment group (ANOVA, HZ vs HO, LZ vs LO, p=0.034)
Figure 10. Proportion of time spent in different behaviors by individual wasps (HZ, HO, LZ)

Data trends so far suggest less time spent off nest in zebularine groups and more nest work in the low food zebularine group (figure 11).