



House Flies: Manure, Media, and Microbes

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Abstract

This study was conducted to determine if there is a difference in bacterial abundance in house flies based on sex and rearing environment (manure versus artificial media) for house flies. This is important in determining the effectiveness of the facilities where the flies are being raised. Although, previous studies have shown differences in bacterial abundance between male and female house flies, it still remains unknown whether there is a discrepancy in bacterial abundance between rearing environments in the lab. We hypothesized that there would be a greater abundance of bacteria in females than males and a greater bacterial abundance in the manure environment than the artificial media. We determined that there was no significant difference between house fly sex or the environments in which they were raised. These results are meaningful because they introduce evidence of forced interaction that could skew the bacterial counts. In the future, the results would be more telling with a larger sample size.

Purpose

The objective of this project was to determine whether there is a difference in the colony forming units (CFU) of bacteria present in male and female adult house flies reared on either manure or artificial media.

Questions and Hypotheses

Question: Based on data collected from two different rearing environments, manure vs. artificial media, is there a difference in total bacterial abundance between male and female adult house flies?
Hypotheses: If we test the abundance of bacteria between male and female flies, then there will be a greater abundance of bacteria in the females than in the males. Between those reared in manure versus those reared with artificial media, there will be a greater abundance of bacteria in the flies raised in manure than the flies that were raised on the artificial media.

Study System

House flies associate with microbe-rich environments across their life history because larvae require microbes for nutrition (Nayduch and Burrus 2017). A natural setting, such as a dairy farm, may contain multiple larval development sites such as manure, soiled bedding, and moistened spent feed, all of which support microbial growth. As a proxy for these types of habitats, laboratory rearing practices may incorporate similar substrates to sustain fly colonies. In the wild, females seek out such substrates for oviposition and also ingest the substrate and the resident microbes as a protein source to promote vitellogenesis (Thomson et al 2017). Based on this information, we predicted that even in laboratory colonies, female flies would harbor more bacteria than males due to increased contact with, and possible ingestion of, the larval developmental substrate. Further, irrespective of sex, flies reared in colonies utilizing manure would have greater bacterial abundance than those reared on plant-based substrate.

Methods and Experimental Design

- 1) Flies (n=4 males and females x 3 replicate dates) were collected from two rearing colonies (Colony A reared on wheat bran-based media and colony B reared on cow manure).
- 2) Flies were hand homogenized in 500 µl sterile phosphate buffer saline (PBS) and homogenate was 1:10 serially diluted.
- 3) Dilutions were plated in duplicate on both Tryptic Soy Agar (TSA) and Violet Red Bile Agar (VRBA).
- 4) Agar plates were incubated at 26.0 °C for 48 h and 37.0 °C for 24 h, respectively.
- 5) Colony forming units (CFUs) were enumerated by standard methods and total CFU per fly was determined.
- 6) Data analysis was performed using a two-way ANOVA, $p < 0.05$.

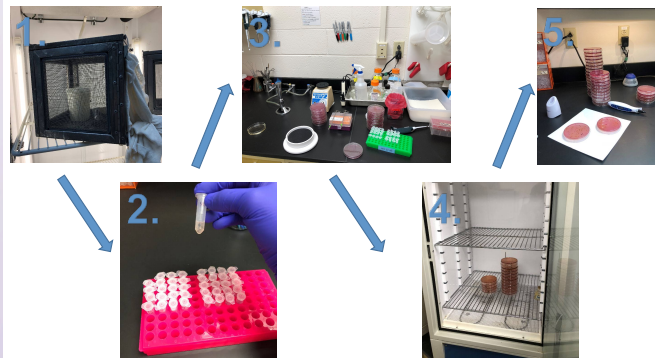


Figure 1. Schematic portrayal of methods used.

Results

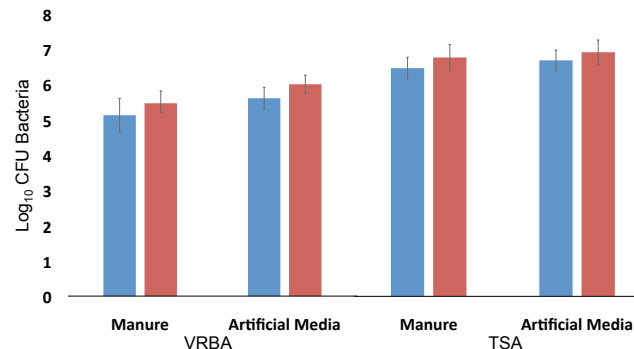


Figure 2. Bacteria cultured from male and female houseflies from VRBA (left) and TSA (right). Mean ± SEM CFU bacteria are depicted (n=12 flies per time point). There was no significant difference across means ($p > 0.05$).

- Both VRBA media and TSA media showed no significant difference in bacterial counts between sex and between rearing environment (Figure 2).
- ❖ This points to possible contamination of the artificial media rearing environment.
- ❖ The small sample size (n=4 males and females) may have contributed to our inability to reject the null hypothesis that there is no sex effect on bacterial abundance.

Discussion & Conclusions

Our results indicated that there was no significant difference in total bacterial abundance (TSA) and total coliform abundance (VRBA) between males and females and between manure and artificial media environments. If the sample size were larger, it is possible that there could have been a difference. However, based on the small sample size of this experiment, we are unable to reject the null hypothesis. In addition, the flies in their natural environment are free to disperse and are not subject to forced interaction with other flies and substrate as they are in an insectary environment. Furthermore, future studies that require "clean" colony flies should adapt additional methods to prevent microbial acquisition.

Future Directions

While there was no difference in bacterial abundance between males and females as was expected, this may have been due to their enclosed environment and forced interaction between each other. In order to amend this, a larger sample size could have resulted in a meaningful difference between genders. Using other types of artificial fly media could have produced in a more significant disparity between males and females. And, comparing the bacterial abundance of captive flies to those in a natural environment, such as a farm, could show a more distinct contrast.

References

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- Thomson, J.L., K.M. Yeater, L. Zurek, and D. Nayduch. 2017. Abundance and accumulation of *Escherichia coli* and *Salmonella typhimurium* procured by male and female house flies (Diptera: Muscidae) exposed to cattle manure. *Ann. Entomol. Soc. Amer.* 110: 37-44.

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