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Data Availability

The data associated with this publication are available at: https://github.com/chaomingxu/bee_poster_code.git

Variation of Ocelli Size Between Male and Female Bees in Species of Different Sociality

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Abstract

The project aims at exploring the impact of bees' sociality on potential variation in ocelli morphology. By measuring ocelli variance between sexes and comparing it between bees of different degrees of sociality, I will test whether sexual size dimorphism occurs in ocelli within social and solitary bee species. Another question the study investigates is whether the variation also exists within each bee species. Overall, I hope to gain a more insightful understanding of how bees' behavioral differences affect their traits.

Introduction

Bees are important pollinators. However, their numbers and diversity are in decline which poses a serious threat to plant pollination and affects food production (Tepedino et al., 2015). The Big-Bee project (<http://big-bee.net>) launched in 2021 to research bee ecology and taxonomy through the digitalization of bee specimens. In my project, bee ocelli are the primary research focus. Ocelli (Fig.1) are light-sensory organs comprised of three simple lenses located anterior dorsally on the head of insects. These simple lenses are present in most species of Hymenoptera (bees, wasps, ants), and are always present in bees. In bees, they play an important role in facilitating flight control, as they function as highly responsive light detectors that can sense and evaluate light intensity from different directions during flight. Previous studies show that ocellar size is positively correlated with the nocturnal foraging behavior of bees (Kerfoot, 1967), although our understanding of the variation of ocelli across different lineages is limited. Recent studies have also found that ocelli size varies across different sexes and castes in honeybees (Streinzer et al., 2013). Nonetheless, it is unclear whether such variation in ocelli size consistently exists within other bee species. Exploring the literature focusing on the difference between sexes or castes within a bee species, I found that bee species with a more complex sociality will have morphological, physiological, and behavioral differences between castes, whereas, in bee species of less social complexity, a caste might not even exist (Holland&Blotch, 2020). Although sex is not mentioned, sociality might still play a role in contributing to the sexual size dimorphism of ocelli, due to potential behavioral differences.

My goal for this project is to investigate differences between male and female bee ocelli size as it relates to body size and understand if any differences can be explained by variation in bee sociality. I hypothesize that male bees will have a different ocelli size than female bees within the same species and this difference will persist in social bees but not in solitary bees.

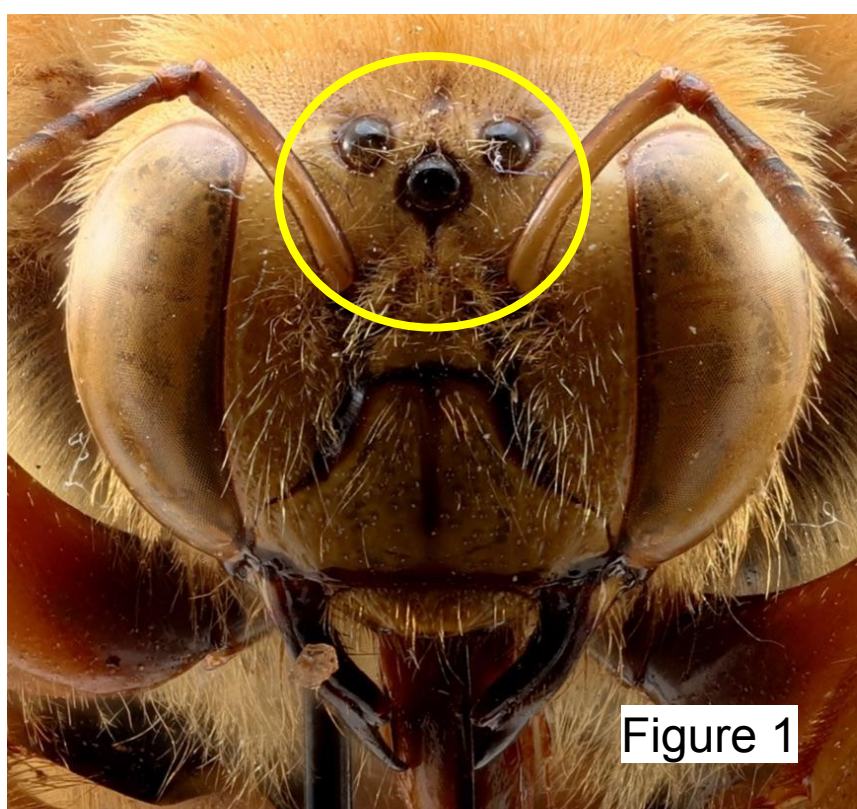


Figure 1: A photo showing the anterior view of ocelli of *Xylocopa varipuncta*. The ocelli are outlined by a yellow circle. Photograph by Zoe Wood.

Species List	Sociality	Number of Males	Number of Females
<i>Apis mellifera</i>	Social	8	10
<i>Agapostemon texanus</i>	Solitary	10	10
<i>Augochloropsis pomoniella</i>	Social	10	10
<i>Bombus melanopygus</i>	Social	10	8
<i>Bombus vosnesenskii</i>	Social	10	10
<i>Lasiglossus incompletum</i>	Solitary	9	10
<i>Xylocopa varipuncta</i>	Neither	10	10

Table 1: Bee species name, sociality, and number of males and females specimens used in this study.

Technique and Equipment for Specimen Measurement

An Olympus microscope reticle was used to measure both body size and ocelli width. The reticle required calibration using a 0.01 mm microscope stage calibration slide. Two measurements were taken from each specimen: Medium Ocellar Width (MOW; Fig.2) and Intertegular Span (ITS; Fig.3 & Fig.4). 0.8x magnification was used to measure ITS and 4x to measure MOW, thus calibration needed to be conducted separately. ITS has been shown to be an accurate estimate of the dry weights of bees (Cane, 1987).

MOW was measured transversely across the medium ocellus, and ITS was measured at the shortest distance between two tegulae. Measurements were often challenging as bee tegulae are commonly obscured by hairs. Sometimes, I used a bee brush to move any hairs away that might cover up tegulae.

MOW, plotted against ITS and the graph (Fig.5) indicates a strong linear relationship between MOW and ITS. Therefore, ocelli size increases as body size increases and larger bees would have larger ocelli. To account for their linear relationship, I used the ratio of MOW to ITS as a parameter for comparison (MOW:ITS Ratio = MOW/ITS).

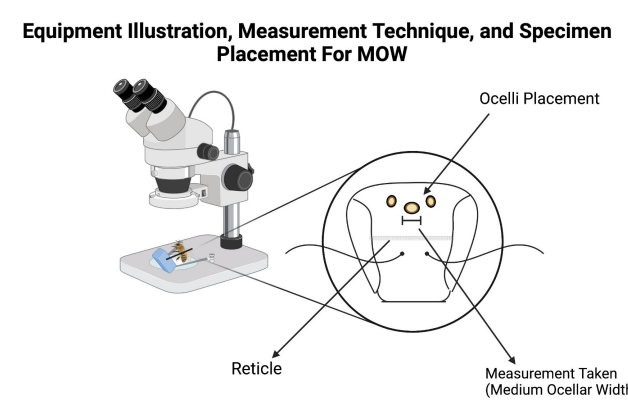


Figure 2

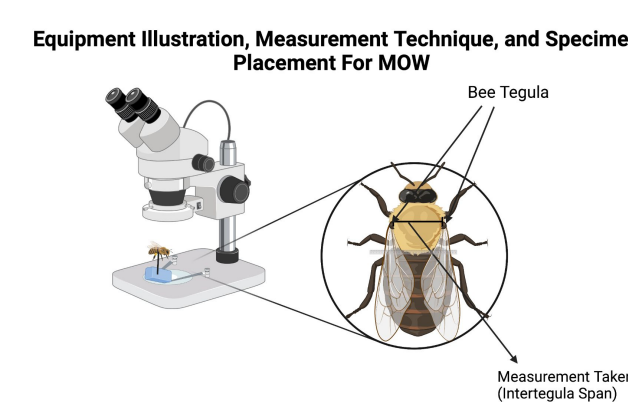


Figure 3

Figure 2: A microscopic view of Median Ocellar Width (MOW) and specimen placement on the microscope stage.
Figure 3: Microscopic view of Intertegular Span (ITS) and specimen placement.



Figure 4

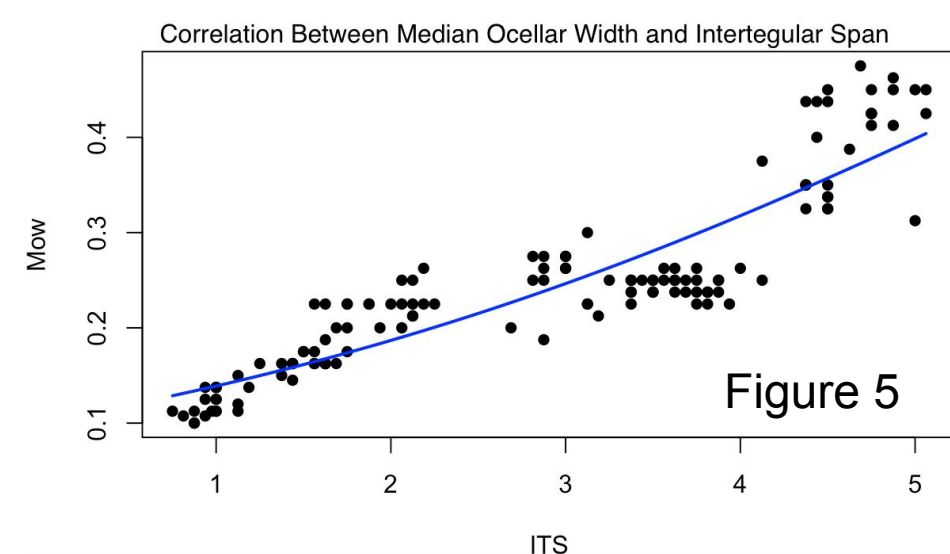


Figure 5

Figure 4: A photograph showing the Intertegular Span of *Agapostemon texanus*.
Figure 5: A graph showing the correlation between MOW and ITS.

Statistical Test

For the species level datasets, Shapiro-Wilk test was used to check for normality (p-value > 0.05) and Levene test was applied to test for equal variance (p-value > 0.05). Because of the data was normally distributed and the variance was equal, a two-sample t-test was performed on these datasets. However, the data within the All Bee Species, Social Bees and Solitary Bees datasets were not normally distributed after performing Shapiro-Wilk, thus a non-parametric Wilcoxon test was performed. For two-sample t-test and Wilcoxon test, p-values less than 0.05 were considered statistically significant. All statistical analyses was performed using base R and the car package (Fox & Weisberg, 2019). Graphs were created using ggplot2 (Wickham, 2016).

Result

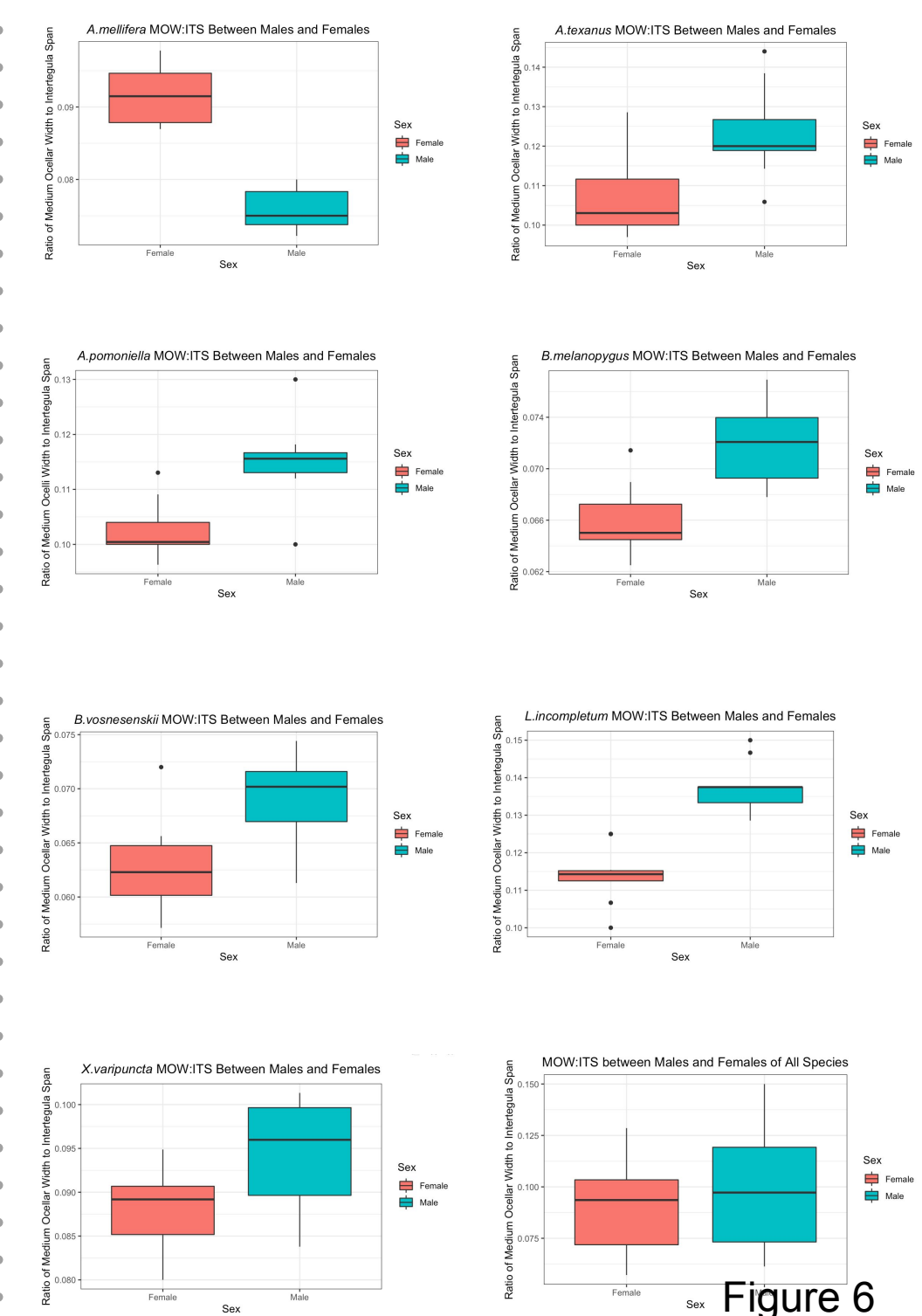


Figure 6

Figure 6: Eight boxplots plotting MOW:ITS Ratio between sex for seven bee species and for all bees. From top to bottom and left to right the species are: *A.mellifera*, *A.texanus*, *A.pomoniella*, *B.melanopygus*, *B.vosnesenskii*, *L.incompletum*, *X.varipuncta*, All Bee Species (last).

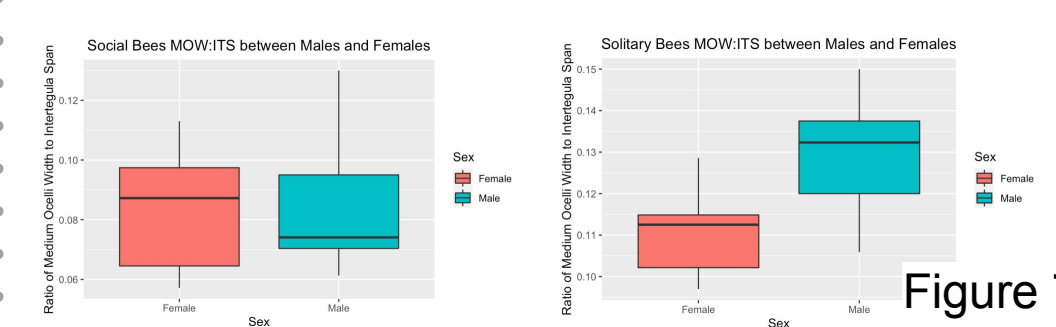


Figure 7

Figure 7: Boxplots showing MOW:ITS Ratio data distribution between social and solitary bees. From left to right: Social bees (left) and Solitary bees (right)

Two-sample T-test	Mean of Male MOW:ITS Ratio	Mean of Female MOW:ITS Ratio	t-value	p-value	95% CI
<i>Apis mellifera</i>	0.07581	0.09148	-9.2378	0.000000	-0.01228 to -0.01238
<i>Agapostemon texanus</i>	0.1251	0.1071	3.3787	0.003361	0.00648 to 0.02587
<i>Augochloropsis pomoniella</i>	0.1151	0.1071	4.4021	0.000431	0.00648 to 0.01824
<i>Bombus melanopygus</i>	0.07177	0.06606	4.0454	0.000382	0.002716 to 0.008896
<i>Bombus vosnesenskii</i>	0.06271	0.06271	3.6311	0.001911	0.002719 to 0.01019
<i>Lasiglossus incompletum</i>	0.1374	0.114	2.0718	0.000000	0.01641 to 0.03032
<i>Xylocopa varipuncta</i>	0.06448	0.08816	2.6961	0.01477	0.001388 to 0.01119

Table 2: Table shows the mean MOW:ITS Ratio of male and female bees, t-value, p-value and 95 confidence interval for all seven species

Shapiro-Wilk showed that all the species level datasets were normal, whereas the population and sociality datasets were tested to be not normal even after data transformation. The result of the two-sample t-test within species between sexes is displayed in Table 2: all the species level datasets have a p-value < 0.05. Therefore, I feel confident to conclude that there is a difference between male and female MOW:ITS Ratio within each species. However, when the datasets are combined creating an All Bee Species dataset, the p-value of the Wilcoxon test between male MOW and ITS is 0.07938, which is not significant. Therefore, I am not confident that there is a difference between male and female MOW:ITS Ratio for all bees. In the Social Bees dataset, the test shows a p-value of 0.253 (>0.05), but in the Solitary Bees dataset, the test shows a p-value of 1.56E-05 which is significant.



Discussion

The insignificant difference between male and female MOW:ITS Ratio of the All Bee Species dataset might be explained by *Apis mellifera*, which had a higher MOW:ITS ratio in females, while all other bees had a higher MOW:ITS ratio in males. For all the species-level datasets, the ratio difference was tested to be significant, thus indicating that a difference between male and female ocelli width is present in bees, but it is not necessarily greater for one sex or another across species.

The test examining the effect of sociality on ocelli size was inconclusive, with a significant difference in ocelli size between sexes in solitary bees but not in social bees. This may be due to the variation in *Apis mellifera* (as above). However, sociality in bees is also very complicated and presently not clearly defined. In our test, bees were placed into two groups but sociality may better be described as a quantitative parameter that is related to their colony sizes (Holland&Blotch, 2020).

Future Directions

This study brings up several areas for future research or inquiry on the topic that I would like to pursue.

1. It will be interesting to better understand the reason for the sex differences in bee ocelli using field and experimental methods. For example, since ocelli size is known to be correlated with nocturnality, we could conduct field research recording when male and female bees forage and see if there is a difference in their flight time.
2. To further examine the differences in ocelli size in all bees it will be more holistic to include bees from other families. Right now, my dataset only has two families of bees: Halictidae and Apidae.
3. The question of sociality ratio difference, additional queen specimens from some eusocial bees will be essential to improve the dataset because the difference in sociality primarily contributes to the morphological behavior in castes instead of sexes.

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