AGE-RELATED DIFFERENCES IN SPERM QUALITY IN MALE BUMBLE BEES (*BOMBUS IMPATIENS)*

By

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ABSTRACT

Bumble bees are among the most important and efficient pollinators of crops and wild flowering plants. Unfortunately, over the past twenty years, some bumble bee species have been facing declines. To add to the understanding of bumble bee reproduction to ultimately discover different factors associated with bumble bee species declines, such as male mating behavior and sperm quality, this research aims to develop a new method for collecting and assessing sperm quality for the common eastern bumble bee species *Bombus impatiens.* I observed male offspring at different ages and analyzed sperm motility to quantify sperm quality and concentration. Through this experiment, the impact of age on sperm quality for a stable (commercially reared) bumble bee species can be understood. Ultimately, this experiment is also designed to support the development of conservation efforts through the success of artificial queen insemination, which will help increase bumble bee populations and other pollinators through lab rearing and mating experiments, as well as provide methods for sperm collection and analysis in other insect species.

ACKNOWLEDGEMENTS

I would like to thank my faculty mentor Dr. Hollis Woodard and my graduate student mentor Blanca M. Guillén for their guidance and encouragement throughout my time in the lab. I could not have completed this Capstone without them. I would also like to thank Members of The Woodard Lab and The Baer Lab for all their help and advice.

Lastly, thank you to my family and friends who have supported me throughout my journey at UCR and always asked for updates on my Capstone project, I could not have made it this far without them as well.

Although my bumble bee research now comes to an end, I will continue to spread my love of our wonderful (and cute) pollinator species. Thank you so much to the University Honors program and the Woodard Lab for this once-in-a-lifetime opportunity to work with bumble bees.

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INTRODUCTION

Bumble bees are one of the most important pollinators worldwide (Velthuis and Van Doorn, 2006) that pollinate wild flowering plants and important common crops such as tomatoes and strawberries (Nayak et al., 2020). They are more efficient pollinators than the often abundant honey bees (Nayak et al., 2020) for some plant species due to their ability to buzz pollinate, where they attach to a flower and vibrate, or "buzz", to effectively release pollen (De Luca and Vallejo-Marín, 2013). In light of the concerning declines observed over the past two decades among many bumble bee species (Cameron et al., 2011), this research aims to develop a new method for collecting and assessing sperm quality for *Bombus impatiens*. *B. impatiens* is one of the most commonly used bumble bee species for research in North America as they are able to be commercially reared in abundant amounts (Velthuis and Van Doorn, 2006). My research intends to analyze the reproduction physiology of male bumble bees to identify adequate protocols to identify sperm quality in depth with the future goal of applying this information to different experiments on mating behavior in laboratory and wild settings.

In a mature bumble bee colony, there are three castes: fertilized queens, largely sterile female workers, and males (drones). Queens lay eggs that develop into workers, drones, and new unfertilized queens (gynes) over the course of their life cycle (Belsky et al., 2020). Workers tend to the queen's brood and forage for food to help the queen concentrate on laying eggs (Belsky et al., 2020). Drones leave the colony during late summer or early fall and mate with gynes, who will start a new colony in the spring (Belsky et al., 2020).

Male bumble bees play a significant role in queen mating behaviors and fitness through their sperm, weight, and size. The sperm of drones can affect the fitness as well as the hibernation success of inseminated *B. terrestris* queens (Baer and Schmid-Hempel 2005). Drones

also produce a gelatinous mating plug that is released post-copulation inside the queen for sperm competition (prevent sperm from other drones from inseminating the queen) (Zhao et al., 2021). *B. terrestris* species are monandrous, but there have been reports of single and multiple mating events in queens (Baer and Schmid-Hempel, 1999). It was found that hibernation success of the queens decreased when they were inseminated with sperm from two different males instead of only one (Baer and Schmid-Hempel, 2005). These experiments can be done in *B. impatiens* once sperm analysis of this experiment is completed to see if there is a difference in sperm effects on queens between the two species.

It was also found that there was a lower ratio of dead to live sperm in polyandrous insect species, such as honey bee species *Apis mellifera*, compared to monandrous species *B. terrestris* (Hunter and Birkead, 2002). This experiment also suggests that female polyandry leads to higher quality sperm (higher ratio of live to dead sperm) of drones in these colonies (Hunter and Birkhead, 2002). Because there have been reports of multiple mating events in *B. terrestris* queens (Baer and Schmid-Hempel, 1999), it suggests that monandrous species like *B. impatiens* may also be able to have a higher sperm viability if the queens began to have multiple mating events.

In *B. terrestris*, it has been found that drones born from workers had a significantly lighter weight and wing size than drones born from queens (Zhao et al., 2021). It was also found that the heavier and younger drones copulate faster with a shorter attachment period than drones that were older and lighter (Zhao et al., 2021). Although this experiment did not use worker-born males, size and weight data were collected in this experiment as this data suggests that the sperm of *B. impatiens* drones may be affected by their weight and size. There was also a positive correlation between sperm length and drone size in *B. terrestris*, but spermatheca storage of

sperm in queens did not differ with different sperm length (Baer et al., 2003). Although sperm length was not measured for this experiment, future experiments can be done with *B. impatiens* sperm and drone size to test sperm transfer from the drone to the queen's spermatheca.

Another experiment done with *B. terrestris* showed that although colony foundation was not affected by sperm, in some colonies, sperm significantly decreased the survival of the queens (Korner and Schmid-Hempel, 2003). Once queens are mated with drones or artificially inseminated with sperm, the effects of queen survival after the mating event has occurred can be studied. It was also discovered that age was unrelated to mating success of *B. terrestris* drones, but the length of the drones' fore and hind tibias affected mating success (longer tibia length correlated with higher mating success) (Amin et al., 2012). It is predicted that *B. impatiens* males at the age of highest viability will have a higher mating success although this data suggests that age may not play a role.

Artificial insemination of bumble bee queens (in *B. terrestris*, *B. lucorum*, and *B. hypnorum*) has been proven to be a successful process (Baer and Schmid-Hempel, 2000). Still, it has not been researched much since its earliest use. A potential drawback to artificial insemination may be the decrease in hibernation success of queens, but this may be due to the CO2 used for anesthesia of the queens (Baer and Schmid-Hempel, 2000) and would have to be researched further. However, through artificial insemination, there is an opportunity for large and disease resistant colonies to be developed for commercial production (Baer and Schmid-Hempel, 2000), and research as bumble bees are needed for crop pollination. Upon completion of this project, drones can be used to potentially artificially inseminate queen bumble bees in the future to predict which males have the highest reproductive success. This work is designed to support future projects that will allow for more time efficient conservation methods of declining bumble

bee populations (through artificial insemination) as well as provide a method of sperm collection and analysis for other endangered insect species.

METHODS

A condensed version of the experimental methods are illustrated in Figure 5.

AGE GROUP DISTRIBUTION

31 newly emerged (callow) drones from three commercial (Koppert®) *Bombus impatiens* colonies were used for this experiment (collected on day 0 of emergence, recognized by their silvery appearance). Drones become sexually mature starting on day six post eclosion (Belsky et al., 2020) and viability was analyzed over a drone's lifespan to see how maturity affects their sperm quality. Drones were intended to be evenly distributed into seven different age groups of 5, 7, 9, 11, 13, 15, and 17 days when their sperm would be dissected for analysis.

COLLECTION AND MAINTENANCE

Five males for each age group were attempted to be collected from each of the three colonies for each age group (n = 5, n = 35 total/colony, n = 105 total/experiment). Ultimately, due to low male production in the colonies during the time of the experiment, only 31 males were able to be collected across the 3 colonies. The callow males, after collection, were given artificial nectar made of one part cane syrup (distilled water, sucrose, sorbic acid solution (potassium sorbate and distilled water), HoneyB Healthy, and Amino Boost) and one part inverted syrup (distilled water, sucrose, sorbic acid solution, citric acid powder, HoneyB Healthy, and Amino Boost) as well as a poly floral pollen ball made of a ratio of 1:1 artificial nectar and pollen (Pollenergie France).

This sustenance was replaced every 1-4 days to prevent mold growth. The males were maintained in a laboratory insect rearing incubator (28 ± 2.5°C, 60 ± 5% RH) at standard lab conditions until the day of dissection.

DISSECTIONS

Individual males were first treated with CO2 in order to sedate them, and weight was recorded shortly afterward. Once immobilized, I separated the abdomen from the rest of the body, and the head was squeezed quickly to kill the male. Wings were collected for male size analysis as well. The marginal cell size (Figure 3) of wings was measured as it is correlated with overall body size in bumble bees (Holland et al., 2021). The abdomen was pinned down (Figure 1a) and then cut vertically down while pinning the two flaps down (Figure 1b). Two fine forceps (0.1 x 0.005 mm, Moria Ultra Fine Forceps, Fine Science Tools) were used to pinch the ejaculatory duct and pull the accessory gland out from the abdomen onto a cavity slide (Thick Cavity Slides, 3 Concavities, PK/12 United Scientific Supplies) (Figure 2a, 2b). 10 µL of Hayes medium (9.0 g/l NaCl, 0.2 g/l CaCl2, 0.2 g/l KCl, 0.1 g/l NaHCO3, pH 8.7) (Baer et al., 2009) was added to the plate onto the accessory gland to prevent drying of the genitalia. The accessory gland was separated into two even pieces vertically down the middle, and one accessory gland was moved into a 1.5 mL Eppendorf tube with 60 µL of Hayes medium for storage in a -20ºC laboratory freezer. One side of one accessory testis of the remaining accessory gland was gripped with one forceps, and the vas deferens was gripped with the other pair of forceps. The forceps holding the vas deferens was gently pulled to uncoil the accessory testis and pulled to pinch the testis to initiate sperm and seminal fluid flow onto the plate. This procedure did not work well with

thicker, dull forceps. Forceps were then slid gently across the accessory testis to aid with sperm flow. A 10 µL Hamilton glass capillary was then used to obtain 1-2 µL of sperm.

Figure 1a. Abdomen of a drone pinned down

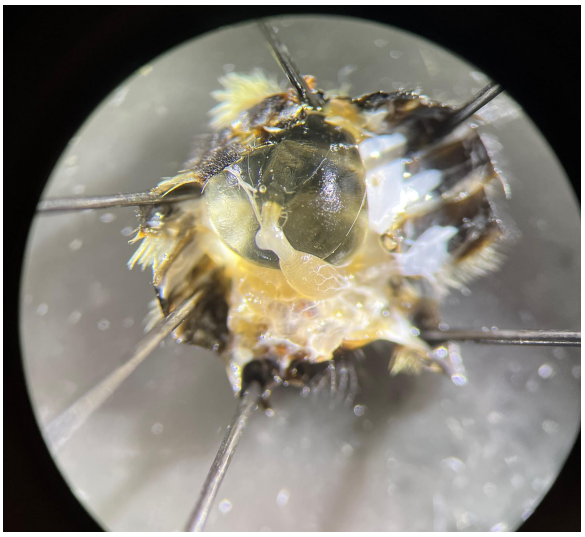

Figure 1b. Abdomen of drone opened up for dissection

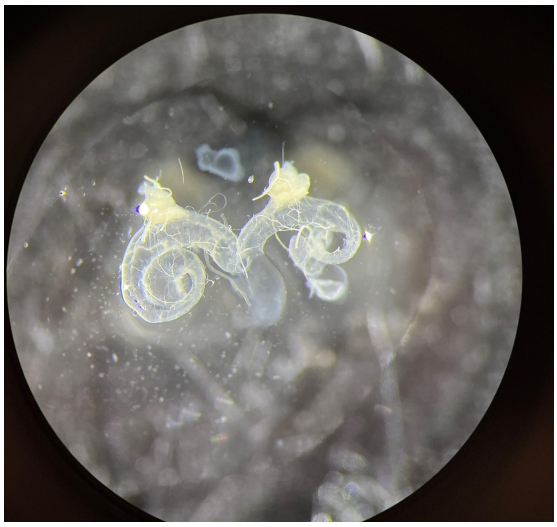

Figure 2a. Drone genitalia under microscope

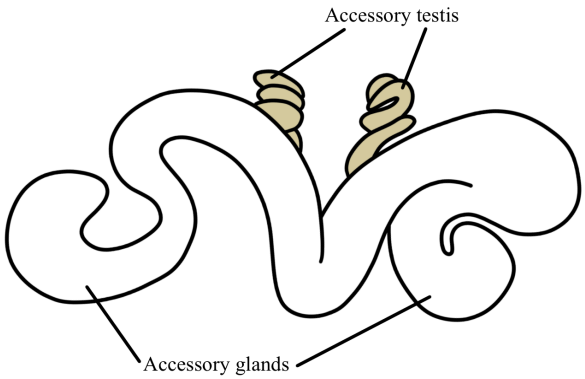

Figure 2b. Drone genitalia with labeled structures

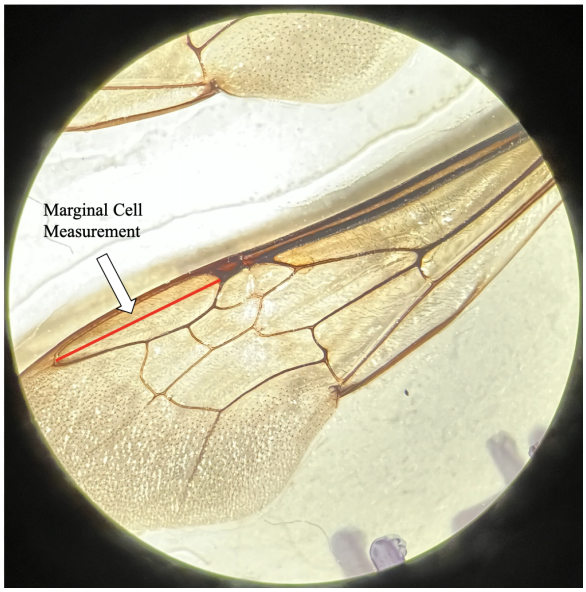

Figure 3. Labeled marginal cell of a bumble bee drone wing. Measurements are done (in mm) across the cell horizontally, as shown by the red line.

LIVE/DEAD® SPERM VIABILITY KIT (L-7011) DILUTIONS

The LIVE/DEAD® Sperm Viability Kit (L-7011) from ThermoFisher Scientific was used for sperm motility analysis. 2 µL of the SYBR 14 dye component, used to color the live sperm red, was diluted in 98 µL of Hayes saline. Each dilution was used for about 20 samples and was made as needed for preservation of the stock solution. The propidium iodide (PI) component, used to color the dead sperm, was used as a stock solution without dilution.

SPERM SAMPLE DILUTIONS AND MICROSCOPIC SLIDE PREPARATION

100 µL dilutions (including Hayes solution, SYBR-14/propidium iodide dye, and collected sperm dissected in 10 µL of Hayes solution) of the 2 µL of sperm/seminal fluid collected from the accessory testis was performed in a 1.5 µL Eppendorf tube with a pipette. The pipette was also used to gently mix the diluents for even sperm distribution. All preparation was done in the dark from this point on to prevent damage of the dyes, which included fading of the sample when

placed under the microscope. 2 µL of SYBR 14 was added into the Eppendorf tube and incubated in the dark for 10 minutes at room temperature. Then, 2 µL of PI was added to the same Eppendorf tube and incubated for another 10 minutes in the same conditions. Samples were stored inside a dark container at all times prior to sperm viability analysis.

SPERM MOTILITY/VIABILITY/CONCENTRATION ANALYSIS

A Leica DM2500 LED fluorescence microscope, homed in a dark room with a red/green filter was used for this experiment. 5 µL of sperm solution was added onto a microscope slide and covered gently with a cover glass for counting. This process was repeated 3 times total per sample. The microscopic slides were kept as horizontal as possible for cover slide intactness and placed under the objective lens. 200 total sperm were counted in one sample (600 total over 3 samples). Live sperm were dyed in green and dead sperm were colored red (Fig. 4). Sperm heads were counted as some dead tails were colored green. Sperm near the edge of the cover slide were not counted due to possible drying of the specimens and sperm colored a mix of green and red were still counted as live, as they were in the process of dying but still alive. Sperm viability was calculated based on the percentage of live sperm to total sperm.

The concentration of sperm was calculated using a hemocytometer as this is the most commonly and frequently used method to count sperm in honey bee species (Yániz et al, 2020). 10 µL of the sperm sample was loaded onto the hemocytometer and sperm in the 5 diagonal center squares were counted. Calculations were done as followed: dilution factor * count of sperm in squares * 50000 = Concentration/mL.

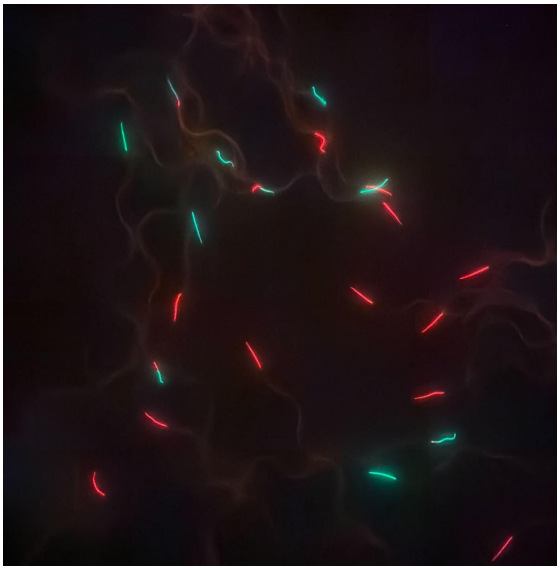

Figure 4. Stained live (green) and dead sperm (red), mounted in a glass slide under a fluorescence microscope on 20X magnification. Sperm that are stained half red and green are in the process of dying.

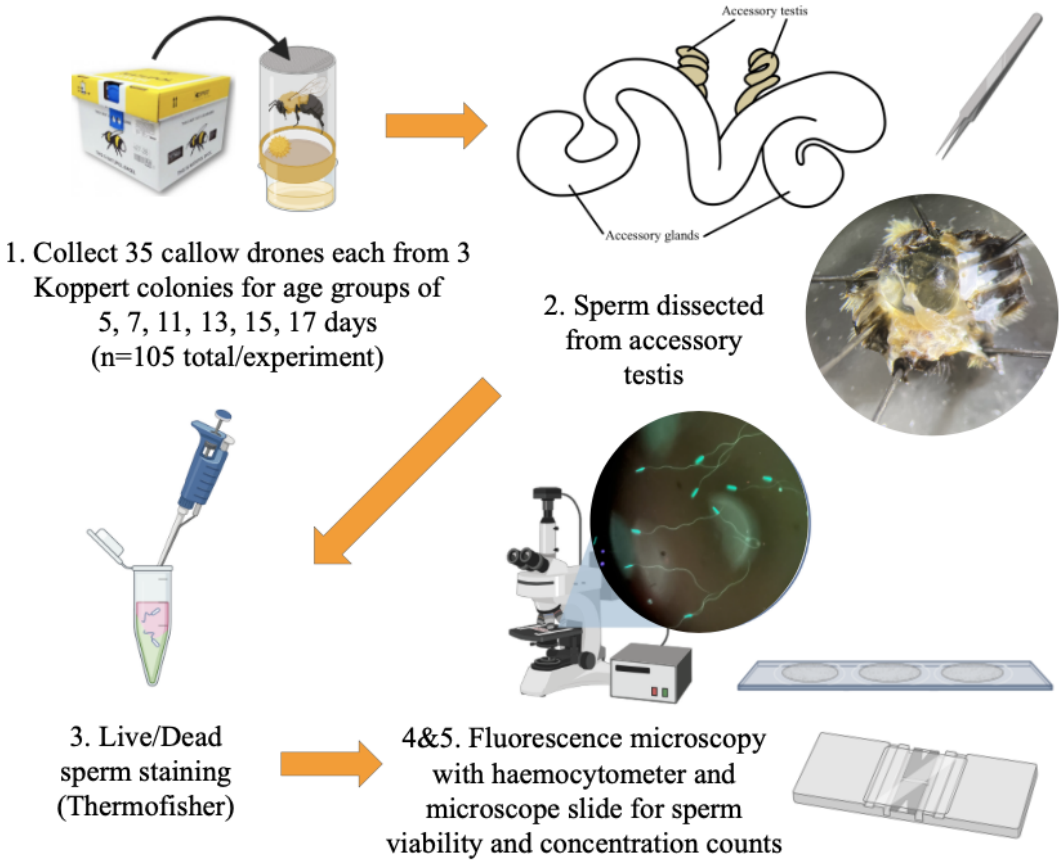

Figure 5. Illustrated methods. 20X magnification was used for live/dead imaging.

RESULTS

MALE COLLECTION

Although 105 total drones were intended to be collected for this experiment, in total only 31 were collected across 3 colonies (Figure 6). The colonies slowed down their male production during the time of the experiment and only a limited amount of drones were able to be collected. Due to low sample sizes and insufficient distribution of drone ages, no statistical analyses (ANOVA, GLM) were performed due to low data counts.

Treatment grp.	day 5	day 7	day 9	day 11	day 13	day 15	day 17
# drones							
1			M9	M13	M1		M25
2			M12	M28	M2		
3					M18		
4							
5							
BS04							Total = 35 drones

Treatment grp.	day 5	day 7	day 9	day 11	day 13	day 15	day 17
# drones							
1	M3	M5	M11	M14	M31	M15	M26
2	M4	M6		M19		M16	
3	M21	M8		M29			
4							
5							
BS05							Total = 35 drones

Treatment grp.	day 5	day 7	day 9	day 11	day 13	day 15	day 17
# drones							
1	M22	M7		M17	M20	M23	M27
2		M10		M30		M24	
3							
4							
5							
BS06							Total = 35 drones

Figure 6. Drone distribution of 31 males across 3 colonies BS04, BS05, and BS06. M = male.

SPERM VIABILITY

Sperm viability of the drones were calculated using R. Drones of age 5 (n = 4) were found to have an average sperm viability of 95.1%, and the viability continued to increase to a mean of 98.2% by day 9 (n = 3), where drones had the highest viability with the smallest standard deviation of 0.013. After day 9, average sperm viability continued to decrease to a mean of 80% by day 17 (n = 3). The standard deviation values began to decrease from 0.040 by day 9 and began to increase again until 0.13 by day 17, suggesting higher variability of sperm viability of older drones.

	Average Sperm Viability (%)	Standard Deviation of Average Sperm Viability
Day 5	95.1	0.040
Day 7	95.8	0.035
Day 9	98.2	0.013
Day 11	94.5	0.028
Day 13	93.1	0.070
Day 15	87	0.16
Day 17	80	0.13

Table 1. Values of average sperm viability with standard deviations.

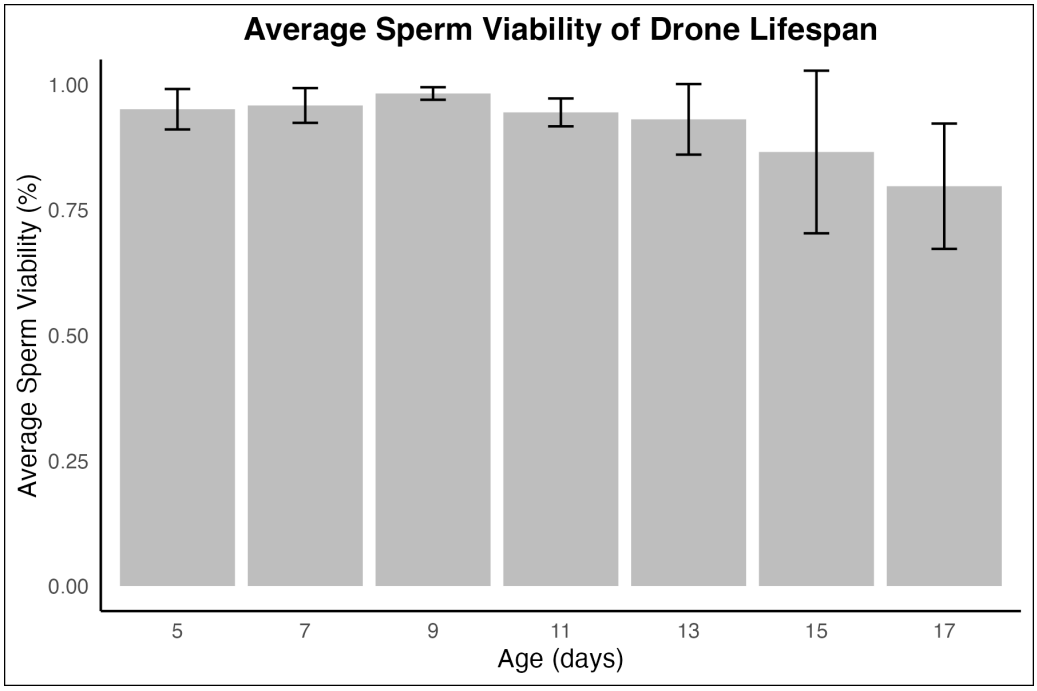

Figure 7. Sperm viability (%) of *B. impatiens* drones (n = 31) from odd ages of 5 to 17 days old with error bars. 1-2 µL of sperm concentrate was collected from only one out of the two testes for each male.

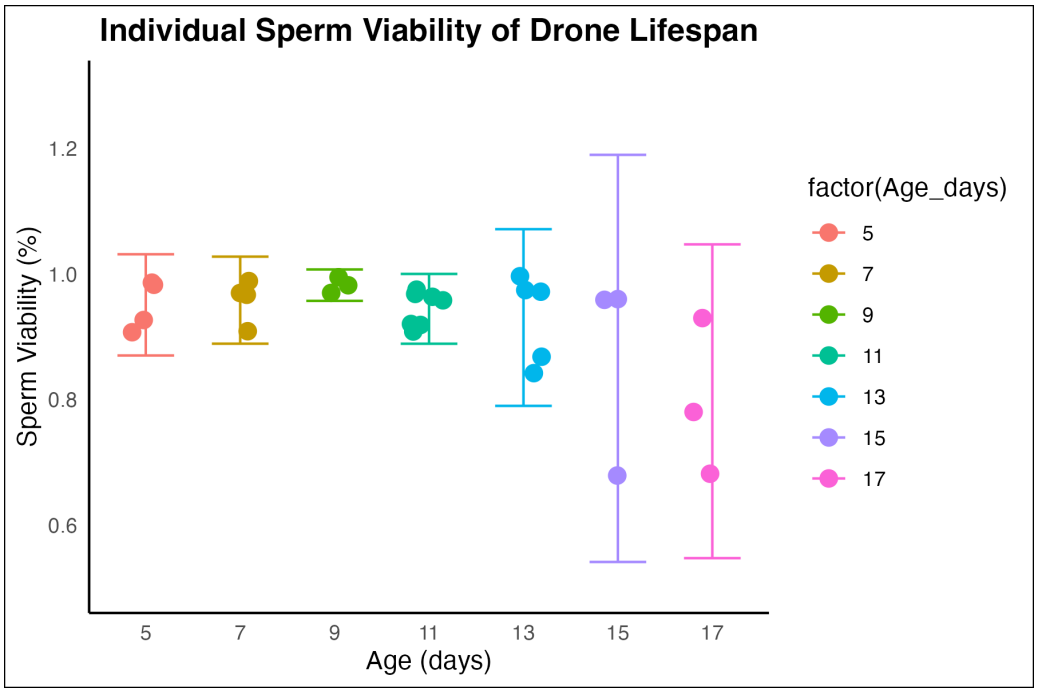

Figure 8. Sperm viability (sperm/mL) of *B. impatiens* drones (n = 31) from odd ages of 5 to 17 days old with error bars, n = 4, 5, 3, 7, 5, 4, 3 respectively). Each individual circle represents an individual drone.

CONCENTRATION

Contrary to the sperm viability data, sperm concentration did not have a bell shaped curve (Figure 9). Sperm concentration was highest at 1.3e+08 sperm per mL on day 9 (n = 4) and was lowest on day 13 (n = 5) with a concentration of 7.4e+07 sperm per mL. Standard deviation was highest at 8.4e+07 on day 9 and was lowest at 2.2e+07 on day 13.

	Average Sperm Concentration (sperm/mL)	Standard Deviation of Average Sperm Concentration
Day 5	9.9e+07	6.7e+07
Day 7	8.2e+07	5.8e+07
Day 9	1.3e+08	8.4e+07
Day 11	1.0e+08	6.1e+07
Day 13	7.4e+07	2.2e+07
Day 15	1.1e+08	6.6e+07
Day 17	1.2e+08	5.9e+07

Table 2. Values of average sperm concentrations with standard deviations.

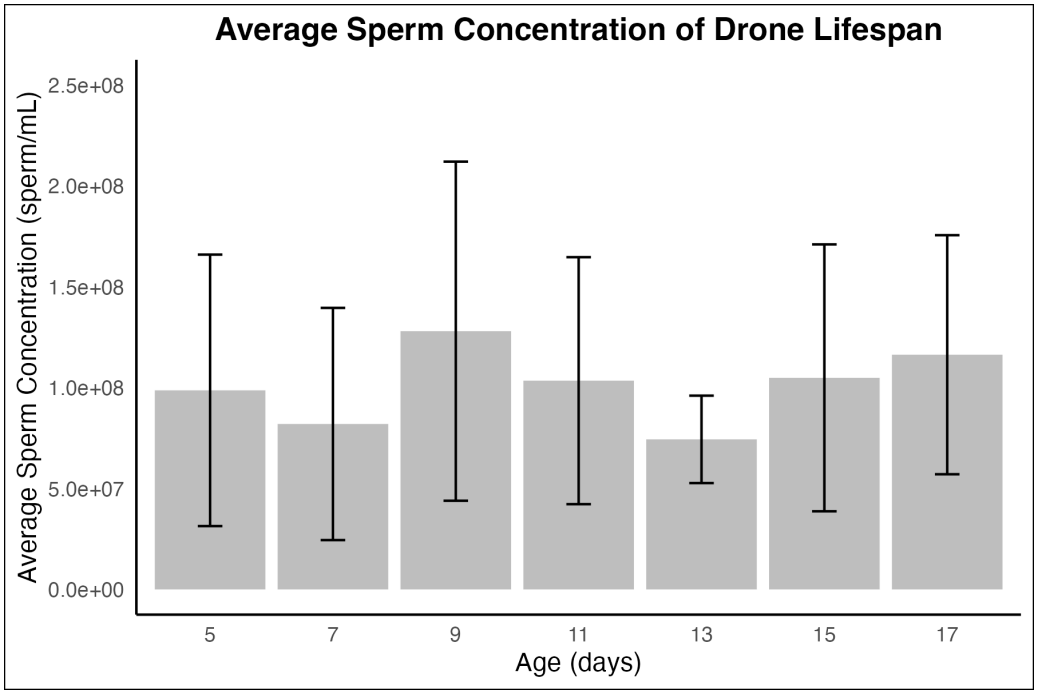

Figure 9. Average sperm concentration (sperm/mL) of *B. impatiens* drones (n = 31) from odd ages of 5 to 17 days old (n = 4, 5, 3, 7, 5, 4, 3 respectively) with error bars. 1-2 µL of sperm concentrate was collected from only one out of the two testes for each male.

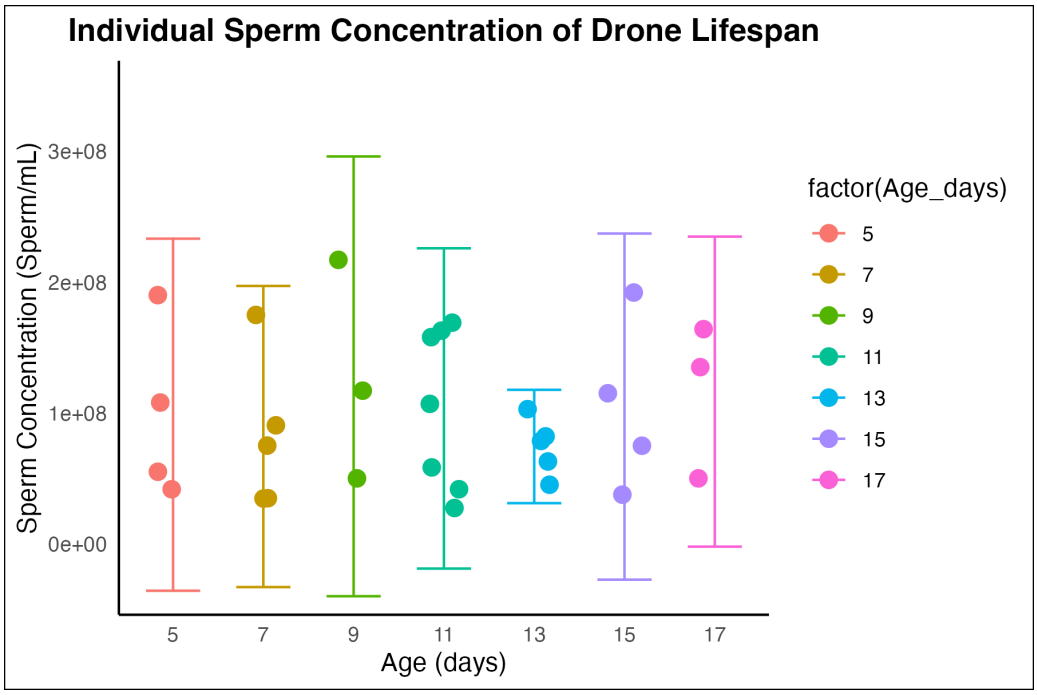

Figure 10. Sperm concentration (sperm/mL) of *B. impatiens* drone (n = 31) from odd ages of 5 to 17 days old with standard error/deviation. Each individual circle represents an individual drone.

WEIGHT OF MALES

Weight of males was compared to sperm viability and concentration using R. There was a downward trend of -0.3 g/viability (%) through a linear model applied between sperm viability and weight (Figure 11), as well as a downward trend of -7.2e+06 between sperm concentration and weight (Figure 12).

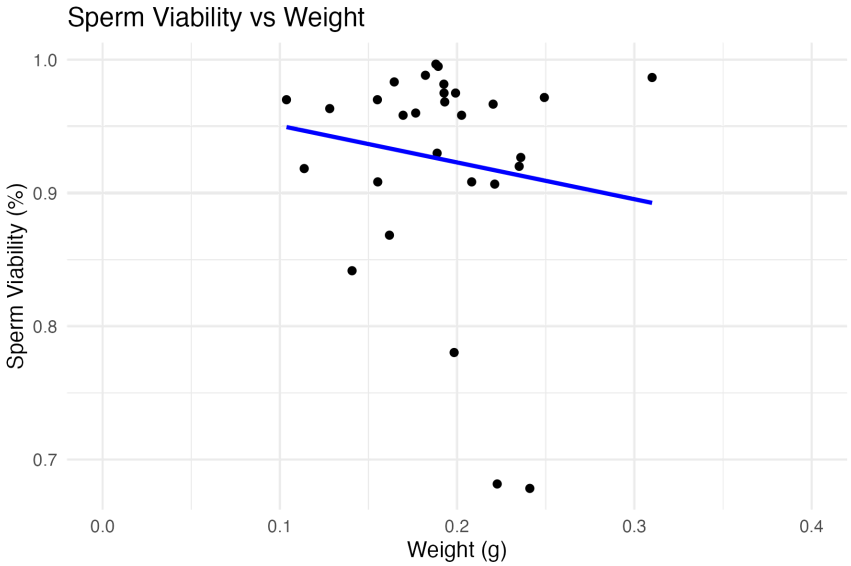

Figure 11. Sperm viability vs. weight with a linear model applied.

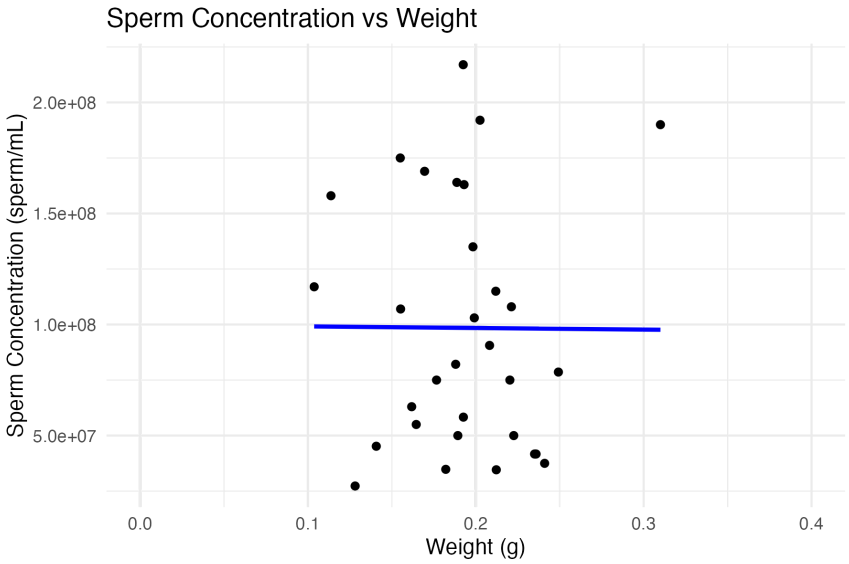

Figure 12. Sperm concentration vs. weight with a linear model applied

SIZE OF MALES

Size of males was compared to sperm viability and concentration using R. There was a downward trend of -0.05 mm/viability (%) between sperm viability and size (Figure 13), as well as a downward trend of -2.5e+06 between sperm concentration and size (Figure 14).

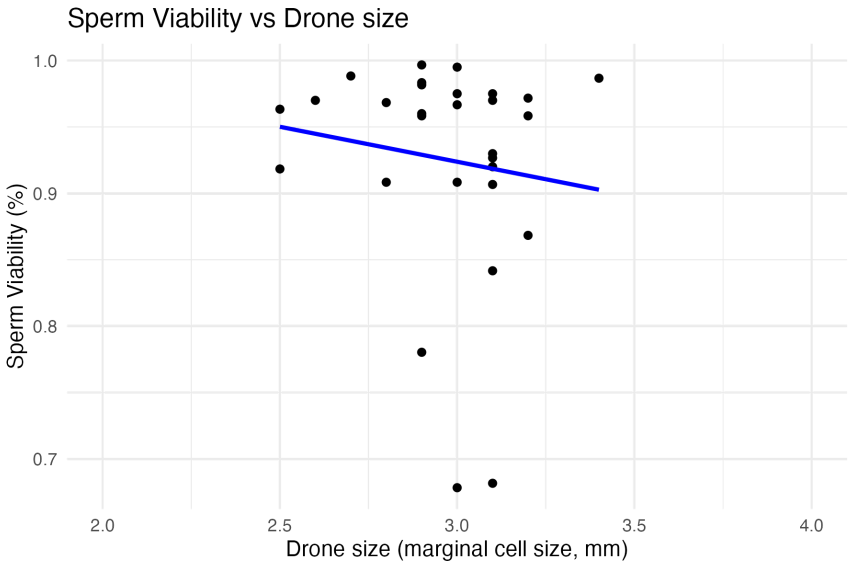

Figure 13. Sperm viability vs. drone size with a linear model applied

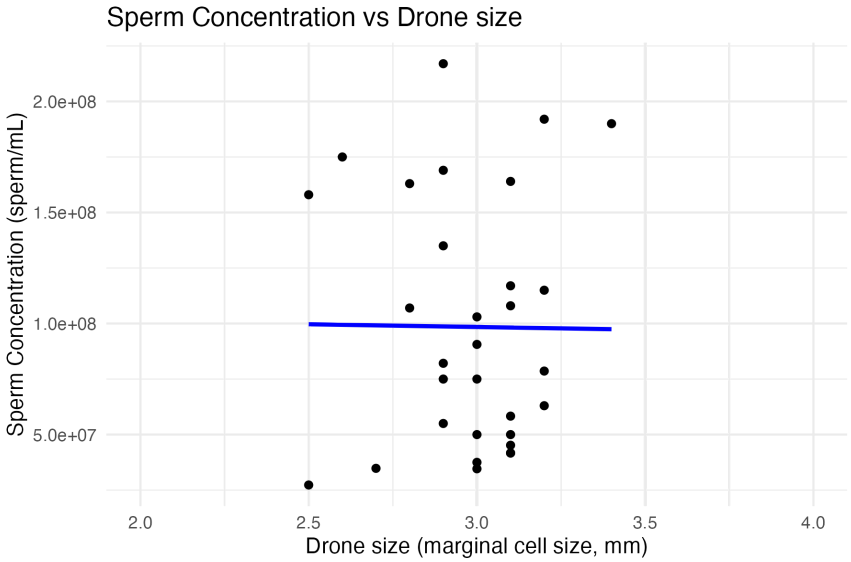

Figure 14. Sperm concentration vs. drone size with a linear model applied

DISCUSSION

SPERM VIABILITY

Sperm viability of *B. impatiens* drones was highest at day 9 after emergence in the small sample size of this experiment, but more data would be required to determine at which age sperm viability is highest.

SPERM CONCENTRATION

Sperm concentration over a *B. impatiens* drone lifespan was very fluctuant with this small sample size. It was predicted that this graph would look similar to the bell shaped curve of sperm viability because it was predicted that sperm concentration would peak at the highest age of viability to ensure mating success, but this was not the case, at least based on the minimal data collected. It is not understood why sperm concentration was lowest at day 13 but highest at ages of days 9 and 17 respectively. These data may suggest that sperm concentration in *B. impatiens* drones is not related to sperm viability at different ages, supported by a high average standard deviation of 6.0e+07.

However, the pattern I observed where sperm concentration appears highest on day 9 is consistent with data found with East Asian bumble bee species *Bombus ignitus*. In an experiment done by the The National Academy of Agricultural Science in South Korea, it was found that *B. ignitus* drones had the highest number of sperm at 443,900 ± 107,700 sperm at 9 days post eclosion (Yoon et al., 2018). There was a significant decrease in sperm ($p = 0.0001$) beginning at 35 days and up to a 12.8 fold decrease in sperm concentration by day 40 compared to day 9 (Yoon et al., 2018) as well. This bell curve shaped data from this experiment suggests that the

fluctuating data in Figure 7 is due to low sample sizes or there is actually no correlation between sperm concentration and age in *B. impatiens*.

DRONE WEIGHT & SIZE

A downward trend of the applied linear model was able to be seen in Figure 9 (sperm viability vs. weight) as well in Figure 11 (sperm viability vs. size). This suggests that smaller and lighter drones have a higher sperm viability, but additional data would have to be acquired to see if this is true. Statistics were not able to be done due to the small sample size acquired. Figure 10 (sperm concentration vs. weight) and Figure 12 (sperm concentration vs. size) depict a smaller downward trend closer to 0. This suggests that weight and size of a drone has no significant correlation with sperm concentration, but further studies would have to be done.

In a study done with solitary bee species *Osmia cornuta*, it was found that sperm concentration and weight had a significant positive correlation at 4 days old (Strobl et al., 2019). It has been shown that *O. cornuta* has overall lower sperm viability and concentration compared to eusocial bee species, but within this species larger males are able to produce more sperm and mate multiple times (Strobl et al., 2019). This suggests that *O. cornuta* males only release a portion of their sperm ejaculate during each copulation event to allow for multiple copulation events with queens (Strobl et al., 2019). This can be supported with an experiment done with *B. terrestris,* where it was also found that bumble bee drones only need 10% of their sperm to be transferred to a queen for successful colony development (Baer and Schmid-Hempel, 1999). The eusocial honey bee species *Apis mellifera* also shows a significant positive correlation between weight and sperm concentration in differently-sized drones (Schlüns et al., 2003). These data suggest it

is possible for weight to be a significant factor in sperm viability and concentration in *Bombus* species, but further research would have to be done to confirm this.

INSECTICIDE EXPOSURE, MALNUTRITION, AND HEAT

It is important to also consider wild drone bumble bee species as many species live in the wild and not commercially reared. Some other researched environmental factors, such as heat and insecticide exposure, also play a role in the changes of sperm viability and concentration.

Research done on the commercially reared European bumble bee species *Bombus terrestris* shows that insecticide exposure decreases sperm viability but not concentration (Minnamayer et al., 2021). *B. terrestris* drones were given field simulated amounts of insecticide and also had a high survival rate of over 93% (Minnamayer et al., 2021). Another similar experiment was conducted with insecticide exposure and malnutrition effects. This experiment was also done on *B. terrestris* drones and they were exposed to insecticides and malnourished of a 50% sucrose solution (Straub et al., 2022). There were no effects in survival with insecticide exposure or malnourishment of the drones (Straub et al., 2022). Sperm viability stayed the same, but concentration significantly decreased when the drones were malnourished (Straub et al., 2022). It is predicted that similar trends would be seen in *B. impatiens* drones as *B. impatiens* and *B. terrestris* are species within the same genus.

Wild bumble bees are also susceptible to climate change, and although they may not be affected by rising temperatures as their heat tolerance can reach up to 55ºC, their gametes may be affected as they have a lower tolerance (Campion et al., 2023). In one experiment, commercially reared *B.*

impatiens males were exposed to their lower temperature tolerance of 4ºC and higher temperature tolerance of 45ºC for 85 minutes (Campion et al., 2023). Males exposed to the 45ºC temperature had a significant decrease in sperm viability whereas males exposed to the 4ºC temperature showed no significant changes (Campion et al., 2023). This research could serve as a basis for future research regarding the relationship between, body heat/temperature of drones, mating, and sperm viability.

PRELIMINARY EXPERIMENT

During the development of sperm collection and analysis methods, a preliminary experiment was done with *B. impatiens* workers. Worker-produced male microcolonies with workers from 3 different colonies were set up. It was found that when 2 workers from the same colony were put together, they would begin laying eggs within 1-2 days. These microcolonies with 2 workers from the same colony also produced the most number of egg clumps before brood dissection. This is significant because it suggests that brood establishment is still possible even when workers are separated from their colony. Due to the success of this preliminary experiment, a similar experiment was conducted with wild *Bombus* species, *vosnesenskii* and *californicus*. This capstone experiment was originally to be done with 3 wild *Bombus* species (*vosnesenskii, melanopygus,* and *sonorus)*, but it was found that when 2 workers from the same species were placed together, one would die within the first 5 days. When the workers were alone, they did not produce any brood up to 27 days even after multiple rounds of CO2 treatment that allows the workers to avoid diapause, a period in which an insect has a decrease in metabolism to prepare for reproduction (Amsalem et al., 2015) (similar to hibernation in animals). Because of the

unexpected results from this experiment, commercial *B. impatiens* colonies were used to collect males instead of creating microcolonies from workers.

Although it was expected to have an abundance of males across 3 colonies for this experiment, male production boomed during methods development and began to decrease at time of collection. This was the biggest limitation in this experiment as there was also an uneven distribution of males across all ages for the 3 colonies that was unable to be controlled. Another limitation to consider is that methods had to be created and developed in the lab prior to the start of this experiment. Also, it was seen that sperm would fade when exposed to light from the fluorescence microscope for too long during counting, which may have affected the sperm counts as well. To minimize this, sperm were counted as quickly as possible once they were placed under the microscope lens, which was the best method to prevent the fading of sperm.

FUTURE DIRECTIONS

Although only 35 out of the original 105 drones were able to be collected for this experiment, new methods for sperm collection and analysis were able to be created for the lab after several months of trial and error. More drones can be dissected from more male producing bumble bee colonies to get a higher sample count as the relationships between weight, age, and viability are still unknown in male bumble bees (Zhao et al., 2021). These data could also be used as preliminary data for future experiments. This experiment is also able to set a foundation for sperm collection and analysis in other bumble bee species through the new developed methods.

Future directions include doing an experiment on the effects of starvation on sperm quality as well as an experiment on the effects of mating on sperm quality. The starvation experiment would consist of starving drones for a few hours at a certain age to see if nutrition plays a role in sperm quality as drones were given adequate amounts of nectar and pollen for this experiment. The mating experiment would be done by attempting to mate drones of a certain age with queens to see at what age drones tend to mate the most, and to see if drones mating is correlated with their sperm viability. We can also analyze if weight plays a role in mating as well, as males of heavier weight tend to mate more and have a shorter duration of copulation than lighter males in *B. terrestris* (Gosterit & Gurel, 2016). By doing these experiments, we can see if those factors play a role in sperm quality or if they are unrelated, as it is still unknown why the sperm concentration is fluctuating over a drone's lifespan in this experiment.

Future research can be done to increase conservation efforts of bee species and increase pollination efforts in the wild. This research is the platform to develop future methods of raising colonies with strong male sperm that can be reared in the lab and then placed in the wild. It has been shown that managed (honey and bumble) bees can negatively affect wild bee populations through several factors such as transmission of pathogens or resource competition (Mallinger et al., 2017). However, there are also positive effects such as increased pollination of native plant species, which suggests that managed bee populations can be used for land conservation or restoration efforts as well (Mallinger et al., 2017).

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