

Determining Species and Sex of *Chrysomya rufifacies* (Macquart) and *Cochliomyia macellaria* (Fabricius) (Diptera: Calliphoridae) Based on Pupae Weight Difference

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In both the medical and forensic fields, blow flies (Diptera: Calliphoridae) play an important role as decomposers of carrion. *Chrysomya rufifacies* (Macquart) (Diptera: Calliphoridae) and *Cochliomyia macellaria* (Fabricius) (Diptera: Calliphoridae) are species of blow flies that have been known to be some of the first insects present on carrion in the wild. They can be used to determine the Post Mortem Interval (PMI) in certain cases. A certain size difference in pupae of females and males of the two blow flies species was documented. Being able to determine the sex and species from the weight of the larvae would allow scientists to save time. Scientists would not have to wait for the blow flies to emerge as adults for this information. This experiment set to test that there was a difference in the weight of the blow fly pupae based on sex and species. The progeny of flies captured and recorded from maggots were used to weigh each species. A significant difference (P value < 0.0001) was found in the weight of *Chrysomya rufifacies* male (0.043339 grams) and females (0.061941 grams) when compared to those of *Cochliomyia macellaria* male (0.006990366 grams) and females (0.042606 grams).

Key words: Sex, Maggots, carrion, weight, pupae

Blow flies (Diptera: Calliphoridae), are often the first colonizers of carrion found in the wild and can oviposit as quickly as hours after death (Wells and Greenberg 1992, Interaction). For this reason, species like *Chrysomya rufifacies* (Macquart) (Diptera: Calliphoridae) and *Cochliomyia macellaria* (Fabricius) (Diptera: Calliphoridae) are often used in forensic science to develop a post-mortem interval (PMI). This interval can be defined as the time elapsed since a person's death. Blow flies are of medical importance due to the secondary myiasis that they can cause from infesting the flesh of animals.

Chrysomya rufifacies can cause sheep strike and can cause economic loss in the livestock they can infest (Brundage 2012). The larvae of *Chrysomya rufifacies* and *Cochliomyia macellaria* have similar food preferences and morphological features and the adults often compete for the same food source (Wells and Greenberg 1992). There are ways to determine the species based on features of the pupae, sex must be determined after the adult stage is reached (Swinger et al 2013). There was a size difference observed while rearing the two species in a student laboratory. There were no previous studies that tested the

weight relation of the two species. If the weight of pupae could determine the species and sex of the fly, it would allow scientists to more efficiently sort through specimens collected from carrion.

Materials and Methods

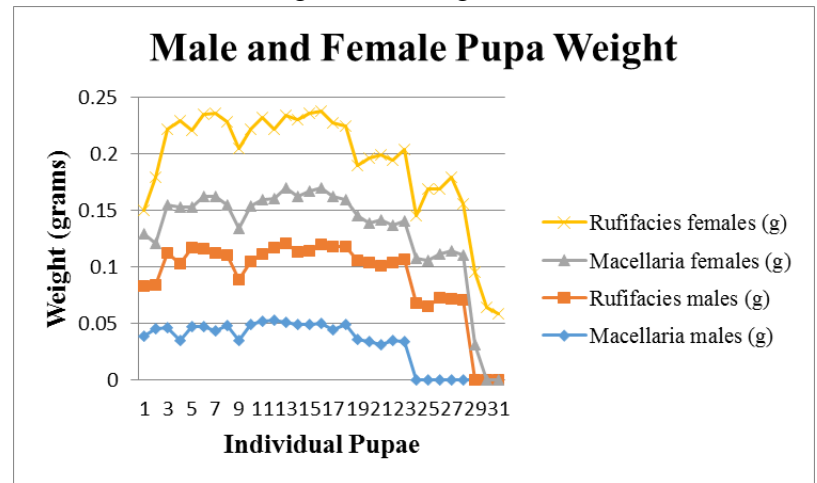
Maggots of *C. rufifacies* and *C. macellaria* found in the wild were reared and the F2 generation larvae were used to find the average weights of the two species. Once adulthood was reached and the sex was determined, correlations were recorded. *C. rufifacies* and *C. macellaria* maggots were collected from carrion in the wild on a road side in College Station, TX, USA. The maggots were then reared on food-grade cow liver (Foster Farms, Livingston, California) until they reached the adult stage. Adult flies were then placed together in a 12x12x12 cage (Bioquip, Rancho Dominguez, CA). They were given sugar and distilled water as a food source. Once the adults were three days old, cow liver was provided as a protein meal. At five days old, adult flies were provided with cow liver for them to oviposit. Once the eggs were laid, they were left on the cow liver to allow maggots to eat once they hatched. Cow liver was then placed on beds of sand in 1-pint mason jars (Anchor Hocking, Lancaster, OH) and maggots were transferred to the jars and given extra liver as needed. Once the maggots pupated, they were gathered and weighed individually. Each pupa was then placed in a separate 2 oz. cup with a lid (Newell, Hoboken, NJ). The cups were stored at about 21°C until the adults emerged, who were then sexed. The data was analyzed using a T-test in SPSS (IMB software).

Results

Overall, the average weights of the *Chrysomya rufifacies* male and female pupae were significantly higher than that of *C. macellaria* with both P values >0.0001. Figure 1 shows that *C. rufifacies*, both male and female, weight more than *C. macellaria*.

Figure 1: Male and Female difference based on weight and species

As shown in Fig. 2, the weight of the 24 *C.*



macellaria males had a lower average of 0.043339 ± 0.00145 grams, compared to the 29 *C. rufifacies* males, who had an average of 0.065225 ± 0.00156 grams. The 30 female *C. macellaria* flies had an average weight of 0.042606 ± 0.001079 grams, lower than that of the 32 *C. rufifacies* females average weight of 0.061941 ± 0.002069 grams. Standard of Deviation of *C. macellaria* males was 0.006990366, compared to that of the *C. rufifacies* males at 0.008283926. In females, *C. macellaria* had a standard of Deviation of 0.005811868, *C. rufifacies* was 0.011521625.

Figure 2: Mean and Standard Deviation of *Chrysomya rufifacies* and *Cochliomyia macellaria*

	Mean Weight (g)	Standard Deviation
Macellaria males	0.043339 ± 0.00145	0.006990366
Rufifacies males	0.065225 ± 0.00156	0.008283926
Macellaria females	0.042606 ± 0.00108	0.005811868
Rufifacies females	0.061941 ± 0.00207	0.011521625

In Fig.3, T-test P value for males shows $1.64413\text{E-}13$, meaning that *C. rufifacies* male pupae significantly weighed more than *C. macellaria* males. The T-test for females showed a p-value less than 0.0001, meaning that *C. rufifacies* females weighed significantly more than *C. macellaria* females. Males and females of the same species had similar weights concluding there was no significant difference that suggests the gender of a larva could be determined.

Figure 3: P-Values comparing the two genders. Any value below 0 indicates a significant difference

T-Test P Value	
Male Pupae	$1.6441\text{E-}13$
Female Pupae	$3.8660\text{E-}11$

Discussion

This experiment found that between male and female larvae of *C. rufifacies* and *C. macellaria*, there was a way to determine the species based on weight. The P-value for the males was found to be $1.6441\text{E-}13$ and $3.8660\text{E-}11$ for females. These values are greater than 0.05, meaning there was a significant difference between the weight for sexes of the two species. By weighing pupa found on carrion in the wild, an accurate estimate could be made on the species based on these results. Colonization of carrion by insects can happen quickly after death. Flies from the

family Calliphoridae have been shown to be some of the first arthropods to locate and feed off of this food source. This makes them play an important part in determining the post-mortem interval and help solve any questions regarding the length of time a body has been decomposing based on what life stage flies are found at. Any information that would help determine a species found could help scientists establish a PMI more efficiently due to certain species being known for colonizing carrion before others (Wells and Greenberg 1992). While the flies were raised in similar environments of 12x12x12 containers, changes like temperature, bacteria present on eggs, and food for adults and larvae can make a difference in the size of the larvae (Brundage 2012). If the developing flies do not get the nutrients they need or are negatively affected by the other factors mentioned, the overall weight of the pupae could have a lower average. There was no sterilization of the specimens prior to rearing; it is very possible this could have affected the final weight. The living conditions of the specimens raised in the laboratory could have been affected by these factors, causing them to under develop. In the future, the conditions could be tailored to the individual species, as each has an ideal habitat. A larger sample of the males and females from each species would also be helpful in calculating a more accurate average weight.

References

- Brundage AL.2012.**Fitness Effects of Colonization Time of Chrysomya Rufifacies and Cochliomyia Macellaria, and Their Response to Intra- and Inter-Specific Eggs and Egg-Associated Microbes
- Swinger SL, Hogsette JA, Butler JF.2013.** Larval Distribution and Behavior of Chrysomya rufifacies (Macquart) (Diptera: Calliphoridae) Relative to Other Species on Florida Black Bear (Carnivora: Ursidae) Decomposing Carcasses.
http://livestockvetento.tamu.edu/files/2015/07/Larval-Distribution_NeoTrop_Feb2014.pdf
- Wells JD, Greenberg B.1992.** Interaction between Chrysomya rufifacies And Cochliomyia macellaria (Diptera: Calliphoridae): the possible consequences of an invasion.
<https://www.cambridge.org/core/services/aop-cambridge-core/content/view/S0007485300051543>
- Wells JD, Greenberg B.1992.** Laboratory Interaction Between Introduced Chrysomya Rufifacies and Native Cochliomyia Macellaria (Diptera: Calliphoridae)
<https://www.cambridge.org/core/services/aop-cambridge-core/content/view/53436BBC73E6EC95338FA2C88F6113D4/S0007485300051543a.pdf/interaction-between-chrysomya-rufifacies-and-cochliomyia-macellaria-diptera-calliphoridae-the-possible-consequences-of-an-invasion.pdf>