

The Ability of Wingless Fruit Flies to Detect the Odor of Acetic Acid

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Abstract:

The study was conducted in order to investigate the fruit fly's ability to detect odor of acetic acid. It was conducted under the hypothesis that if fruit flies are able to detect the odor of acetic acid, then there are more fruit flies gathered near a banana. The experiment was done by comparing the numbers of wingless fruit flies at treatments consisting of water as control, wine, an overripe banana, and vinegar. The result of ANOVA test showed that there's an overall significant difference among the means of treatment groups, however, the results of Tukey's test showed that there's no individual significant difference between the means of each treatment group. The inconsistency in the results of a one-way ANOVA test and Tukey's test could be due to the lack of statistical power.

Introduction:

Sensing the environment is an essential technique that organisms learn in order to survive and protect themselves from the danger. One of the senses is the olfaction, or the sense of smell. Most animals, like humans, use their nose but few don't even have a nose to begin with. In the case of fruit flies, they use a pair of sensory appendages called antennae to sense the environment around them and "detect the odor" (Hines, 2014).

The fruit fly's ability to detect the odor is quite interesting considering how they always seem to be able to 'sniff' out the fruits on the kitchen counter or the garbage bins in the house. According to the study done by Juandet and Gallio, "Adult flies forage for microbes on overripe fruit, relying on their sense of smell to detect the acetic acid that accumulates as the fruit ferments" (Jouandet and Gallio, 2015). Their ability to sense the smell comes from the olfactory system, much like other vertebrates. The olfactory receptor neurons that detect the chemical, that is associated with the odor, is connected to discrete clusters of synapses within the brain called glomeruli (Jouandet and Gallio, 2015). These receptors on their olfactory neurons allow the flies to sniff out a main product generated from fruit fermentation, which is acetic acid. With the presence of certain types of bacteria, such as acetic acid bacteria, glucose and other six carbon-based monosaccharides are turned into ethanol and then to acetic acid (Duan, 2018). Juandet and Gallio also state that "flies tend to ignore or even avoid both low levels of vinegar (which suggest that the fruit is not ripe enough) and high levels of vinegar (which suggest that

the fruit might be rotten)” (Jouandet and Gallio, 2015). This is due to the fact that 1) they need to obtain the nutrients from the overripe fruits with right amount of acetic acid and 2) female fruit flies need to find a surface where it is safe enough for them to lay eggs, like a moist fermenting food (Orkins, 2019).

In this experiment, I investigated the fruit flies’ abilities to detect the scent of acetic acid. I created three treatments consisting of water as the control, white wine, a overripe banana, and vinegar. I conducted the study under the hypothesis that if fruit flies are able to detect the odor of acetic acid, then there are more fruit flies gathered near the banana. I expected to see more fruit flies near the banana because it was over ripe, which means it contains enough acetic acid for fruit flies to be attracted to it, and it had the largest surface area compared to other treatments, which means it is favourable for female fruit flies to lay their eggs on. To test this hypothesis, I conducted a one-way analysis of variance (ANOVA) comparing the mean numbers of fruit flies gathered for each treatment.

Methods:

The experiment began by collecting wingless fruit flies from a pet store because no wild fruit flies were available in my home. In order to prepare each treatment, I cut the paper towels into nine pieces and put initials of the treatments on them (e.g., w for water, v for vinegar, and wine for wine) so that identifying them would be easy. Afterwards, I soaked each piece of paper towels one-by-one in each treatment (water, wine and vinegar). Then, a transparent, 50-by-40 centimeter container was prepared, which was deep enough to keep the flies from escaping during the experiment. The cooking sheet paper was laid at the bottom of the container in order to distinguish the fruit flies, then the soaked paper towels were placed in each corner of the container. The flies were released in the middle of the container after all the treatments were placed. After waiting for 10 minutes, I noted the number of flies on each paper towel., and repeated the whole cycle a total of three times.

After the measurements were taken, I analyzed the data using a one-way ANOVA test. If the results were found to be statistically significant, a Tukey’s test would be performed.

Results:

For this experiment, there were a total of 12 measurements taken, three measurements coming from each treatment. A one-way ANOVA was performed on the entire data to analyze whether the means of the treatment groups are equal. The result of the ANOVA test showed an F value of 4.927 and a p-value of 0.0317, which is a statistically significant result. The analysis showed that the means of the treatment groups are not equal and independent. Since the result was found to be statistically significant I performed Tukey's test in order to identify which mean difference between the treatment groups is the most significant.

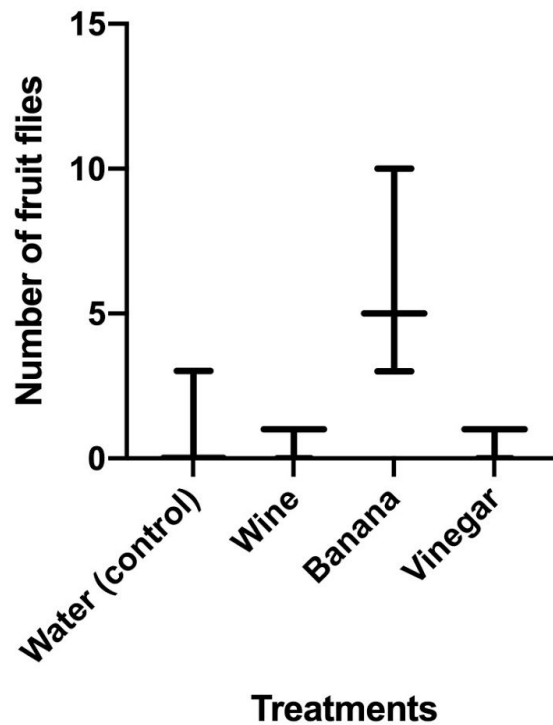


Fig 1. The mean number of wingless fruit flies that gathered on a banana was the greatest (Water: $\mu = 1.000$, Wine: $\mu = 0.6667$, Banana: $\mu = 6.000$, Vinegar: $\mu = 0.6667$). The numbers of the fruit flies were measured in a transparent container that contained each treatment (water as control, wine, banana, and vinegar) on a paper towel. Statistics performed by one-way ANOVA test with a post-hoc test, Tukey's test (result of Tukey's test shown in Fig. 2), comparing the significance of the difference in the means of the treatment groups. Results of ANOVA test: p-value = 0.0317 and F value = 4.927.

The result of Tukey's test showed that the mean differences between the treatment groups are not significant (p-values > 0.05).

Treatment Groups	95% Confidence Interval	P-Value
Water vs. Wine	[-5.004, 5.671]	0.9969
Water vs. Banana	[-10.34, 0.3373]	0.0665
Water vs. Vinegar	[-5.004, 5.671]	0.9969
Wine vs. Banana	[-10.67, 3.920×10 ⁻³]	0.0502
Wine vs. Vinegar	[-5.337, 5.337]	>0.9999
Banana vs. Vinegar	[-3.920×10 ⁻³ , 10.67]	0.0502

Fig 2. The result of Tukey's test showed no significant difference between each treatment. The Tukey's test was conducted after a one-way ANOVA test, in which showed a significant difference between the means of all the treatments. The p-value and 95% confidence interval (CI) of difference in the means of the wingless fruit flies that gathered near each treatment group. Water (control) and wine: p-value = 0.9969, 95% CI = [-5.004, 5.671]. Water (control) and banana: p-value = 0.0665, 95% CI = [-10.34, 0.3373]. Water (control) vs. vinegar: p-value = 0.9969, 95% CI = [-5.004, 5.671]. Wine vs. banana: p-value = 0.0502, 95% CI = [-10.67, 3.920×10⁻³]. Wine vs. vinegar: p-value > 0.9999, 95% CI = [-5.337, 5.337]. Banana vs. vinegar: p-value = 0.0502, 95% CI = [-3.920×10⁻³, 10.67].

Discussion:

The result of the experiment rejects the hypothesis that if fruit flies are able to detect the odor of acetic acid, then there are more fruit flies gathered near the banana. The result of the one-way ANOVA showed a statistical difference between the means of the treatment groups and the independence between them. However, Tukey's test was unable to identify which treatment group's mean is significantly different. The reason for the result of the post-hoc test not being significant is due to the lack of statistical power. Not enough data were taken for Tukey's test to determine the statistical significance of the mean difference. Though there is a possibility that the overall mean of treatment groups combined together is significant, since Tukey's test only compares the individual means of treatment group, the result came out to be non-significant.

The result of the experiment was apparent in Fig. 1, where the mean number of fruit flies on banana is the highest compared to the other groups. This experiment showed the fruit flies' tendency to choose fermented fruit over vinegar, which contains the most amount of acetic acid. The main reason could be due to the fact that female wingless fruit flies were looking for a place to lay their eggs. During reproduction, female fruit flies lay their eggs on moist and fermented food (Orkins, 2019). Since the reproductive season of fruit flies is throughout the year and their

life cycle from an egg to an adult take about 8 to 10 days (Potter), the female wingless flies that were introduced to the treatments could have been looking for a surface where they could lay their eggs on, resulting in greater amount of fruit flies gathering on the banana. During the experiment, I noticed a few fruit flies that were stuck on the surface of the banana, which could have been laying their eggs.

Though the result of the experiment supports the hypothesis, there are sources of error which could have affected the experiment result. The main error in this experiment is from the systematic error. One of the errors is from the difference in the visualization of each treatment group. Since there is a chance of fruit flies getting attracted to the colors (Marmaini and Saputra, 2016), using an actual banana while other treatments were soaked in a paper towel could have affected the result of the experiment. In order to further investigate this experiment, instead of using an actual overripe banana, banana juice seeped in a paper towel could be used in order to match the visual presentation of all the treatment groups for the future studies. Another error that may have affected the result of the experiment is the lack of data. Since this experiment consisted of only three trials, there was not enough data taken for each treatment group to conduct accurate Tukey's test. For future studies, more data should be taken in order to strengthen the statistical power and to precise results.

Conclusion:

In conclusion, the experiment demonstrated that wingless fruit flies favour the odor of acetic acid from fermented fruit than vinegar or wine. This was explained by the mean numbers of the fruit flies gathered on the banana having the highest mean value compared to the other groups, and by the result of ANOVA test shown to be statistically significant. Though the main question of the experiment was answered, there are potentials for the future studies that could look into the relationship between the colourism and fruit flies or the types of scent that will keep fruit flies away from the fermented fruits.

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Appendix:

Raw data of the numbers of fruit flies measured at each treatment:

AFTER 10 min	Water (Control)	Wine	Banana	Vinegar
Trial 1	0	0	5	1
Trial 2	3	1	10	0
Trial 3	0	1	3	1

The result of ANOVA table:

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	61.58	3	20.53	F (3, 8) = 4.927	P=0.0317
Residual (within columns)	33.33	8	4.167		
Total	94.92	11			

The result of Tukey's test:

Tukey's multiple comparisons test	Below threshold?	Summary	Adjusted P Value			
Water (control) vs. Wine	No	ns	0.9969	A-B		
Water (control) vs. Banana	No	ns	0.0665	A-C		
Water (control) vs. Vinegar	No	ns	0.9969	A-D		
Wine vs. Banana	No	ns	0.0502	B-C		
Wine vs. Vinegar	No	ns	>0.9999	B-D		
Banana vs. Vinegar	No	ns	0.0502	C-D		
Test details	Mean Diff.	SE of diff.	n1	n2	q	DF
Water (control) vs. Wine	0.3333	1.667	3	3	0.2828	8
Water (control) vs. Banana	-5.000	1.667	3	3	4.243	8
Water (control) vs. Vinegar	0.3333	1.667	3	3	0.2828	8
Wine vs. Banana	-5.333	1.667	3	3	4.525	8
Wine vs. Vinegar	0.000	1.667	3	3	0.000	8
Banana vs. Vinegar	5.333	1.667	3	3	4.525	8