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Article

# Effects of Wi-Fi EMF on *Drosophila melanogaster*

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## SUMMARY

**Wi-Fi is commonplace in developed countries, and the number of people exposed to it is increasing. The health effects of Wi-Fi electromagnetic fields (EMF) are not well-documented. This research study engineered an apparatus, a "Box Setup," to expose fruit flies, *Drosophila melanogaster,* to three concentrations of Wi-Fi EMF and a control. Wi-Fi EMF exposure produced a statistically significant decrease in the number of total progeny adults, and number of pupae. There was no significant effect on the progeny male:female ratio, nor the adult:pupa ratio. Only the highest Wi-Fi EMF concentration (100%) decreased the number of progeny adults, number of progeny females, and number of pupae; whereas even a low EMF concentration (5%) decreased the number of progeny males. This suggests that male *D. melanogaster* are more sensitive to Wi-Fi EMF than female *D. melanogaster*. Further research is needed to identify what aspects of fruit fly biology are affected by Wi-Fi EMF exposure, and why males are more sensitive than females.**

## INTRODUCTION

Electromagnetic fields (EMF) consist of electric and magnetic waves that feed off each other and produce each other, producing a forward propagation of waves (2). The EMF produced by Wi-Fi routers is non-ionizing, *i.e.* it does not carry enough energy to remove electrons from atoms (1, 3, 4, 5). Wi-Fi is used to connect computers, smartphones, and other devices to the Internet or each other. In developed countries, many households and businesses use Wi-Fi EMF at all times, which means that many humans are under constant Wi-Fi EMF exposure. Also, there is less scientific literature investigating Wi-Fi EMF's health effects than there is for cellphone EMF. Given Wi-Fi EMF's widespread and growing usage, scientists must carefully evaluate whether Wi-Fi EMF has health effects on humans.

The purpose of this research project was to determine whether Wi-Fi electromagnetic fields affect the health of *Drosophila melanogaster*. *D. melanogaster* is a model organism for scientific research, as much of its biology and physiology mirrors that of other organisms, including humans (6). The focus of this study was to investigate the effects of Wi-Fi EMF, if any, on the *D. melanogaster* as a whole organism. If statistically significant results emerged at the organismal level, targeted research might be conducted by the scientific community to determine the specific *D. melanogaster* physiology affected most by Wi-Fi EMF, which could translate to human physiology.

We hypothesized that Wi-Fi EMF Radiation would affect *D. melanogaster* as measured by changes in the number of progeny adults, the number of pupae, the number of progeny males, the number of progeny females, the progeny adult:pupa ratio, and the male:female ratio. Changes in these variables could be caused by a variety of effects, including effects on fecundity, development, or survival. The term "progeny adult" indicates an eclosed adult *D. melanogaster* that was an offspring of the male and female *D. melanogaster* pair introduced to the vial. A progeny adult was exposed to Wi-Fi EMF for its entire life cycle, and was the offspring of a *D. melanogaster* pair that had been exposed to Wi-Fi EMF for their entire life cycle. Different statistical comparisons separated progeny adults into "progeny males" and "progeny females" to investigate whether sex-selective effects occurred.

## RESULTS

At the start of the experiment, no affordable and effective apparatus suitable for a home setting could be found to produce controlled concentrations of EMF for testing with *D. melanogaster*. Therefore, a testing chamber had to be engineered that was both affordable and provided effective EMF control inside the chamber while keeping the *D. melanogaster* healthy. The Box Setup (**Figure 1**) is the testing chamber engineered for this purpose. Four Box Setups (**Figure 1A**) were constructed, one for each of the three concentrations and the control. The Box Setups each had design features (**Figure 1B**) that successfully prevented exterior EMF from entering, provided a means of ensuring that all the Wi-Fi routers were functioning, were grounded Faraday cages (7, 8), maintained adequate humidity, had consistent air circulation, and were quiet. The temperature fluctuated between 22 and 25 degrees Celsius, which did not negatively affect the *D. melanogaster* (9).

Three concentrations of Wi-Fi EMF and a control were tested (**Table 1**). The different concentrations were produced by covering the Wi-Fi router antennae with carbon fabric EMF-absorbent sleeves of varying sizes. The concentrations tested were percentages of the full Wi-Fi EMF dose from a TP-LINK® Wi-Fi router, determined from EMF measurements taken in this study. Since the TP-LINK® Wi-Fi router is

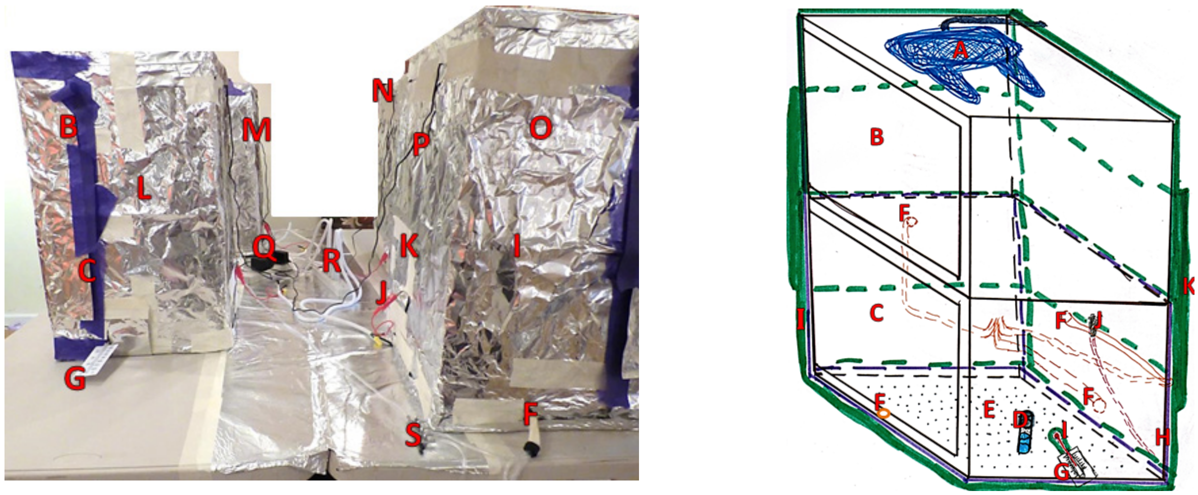


**Figure 1. Box Setup Photo and Diagram.** Not pictured are the carbon-impregnated fabric sleeves placed on the Wi-Fi router antennae to create different EMF concentrations. D. melanogaster were placed inside each Box Setup to receive EMF exposure of a specific concentration. Pictured are the (A) Wi-Fi router under the roof of the Wi-Fi Chamber, (B) Wi-Fi Chamber with a door flap for access inside, (C) Drosophila melanogaster Chamber with a door flap for access inside, (D) vial with a D. melanogaster male and female (each Box Setup had 15 vials); vial positioned in a grid square that has mean EMF measurements within the defined range for that EMF concentration, (E) Grid on floor of D. melanogaster Chamber used for EMF measurement and vial positioning, (F) Air tubes to provide air circulation in the D. melanogaster Chamber, two air tubes provided air input and two provided venting, the four tubes were gathered in a bundle to exit the shielding, (G) Thermometer with bulb covered in EMF-reflective shielding and inside D. melanogaster Chamber while the end protruded outside for easy temperature reading, (H) Carbon impregnated fabric for EMF-absorbent shielding surrounded the D. melanogaster Chamber, (I) aluminum foil EMF-reflective shielding surrounded the entire Box Setup, (J) Alligator clip for grounding system was attached to EMF-reflective shielding then plugged into the wall outlet's "Ground", (K) Piece of EMF-reflective shielding to cover the seam between the Wi-Fi Chamber and the D. melanogaster Chamber, (L) 100% EMF concentration Box Setup, (M) 5% EMF concentration Box Setup, (N) Control Box Setup, (O) 20% EMF Concentration Box Setup, (P) Power cord for Wi-Fi Router, (Q) Power strip for Wi-Fi Router's power supply, (R) Power strip for grounding system, (S) Air valve for air circulation system

intended for home use, the Wi-Fi EMF concentrations tested are likely levels that humans experience.

To analyze the data, ANOVA and t-test statistical tests were conducted and graphs were constructed. The graphs, which display the mean number of *D. melanogaster* for each Wi-Fi concentration, show a negative trend: as the Wi-Fi EMF concentration increases, the number of *D. melanogaster* decreases. This negative correlation can be observed for the comparisons involving the number of progeny adults, the number of pupae, the number of progeny males, and the number of progeny females. The trend does not appear for the male:female ratio or the adult:pupa ratio.

When collecting data, the *D. melanogaster* vials exposed to the 20% concentration were placed upright in the freezer causing the adult flies to freeze into the medium. Therefore, the data for the number of progeny males, number of progeny females, and male:female ratio could not be collected for this concentration. This disturbance compromised the accuracy of the data collected for the number of progeny adults and the number of pupae since these data could not be collected the same way as they were for the other concentrations. Though these data could not be assumed to be reliable under these conditions, the data collected for the 20% concentration did follow the negative trend observed for the other concentrations; namely, as the concentration of Wi-Fi EMF increases, the number of *D. melanogaster* decreases. Further research is needed to confirm these results for the 20% concentration.

The hypothesis was that Wi-Fi EMF Radiation would affect *D. melanogaster* as measured by changes in the number of progeny adults, the number of pupae, the number of progeny males, the number of progeny females, the progeny adult:pupa ratio, and the male:female ratio

For the number of progeny adults, high levels of Wi-Fi EMF exposure (100% concentration) reduced the number of flies, but low levels (5% concentrations) did not. ANOVA comparison ($\alpha$=0.05) of the number of progeny adults across all EMF concentrations (0%, 5%, 100%) produced a statistically significant result; the *p*-value was less than

| Wi-Fi Concentration | Range of EMF Exposure (mW/m$^2$) |
|---|---|
| 0% (Control) | 0 |
| 5% | 5 – 9 |
| 20% | 25 – 32 |
| 100% | 125 – 147 |

**Table 1.** Wi-Fi EMF concentrations tested on *D. melanogaster*.

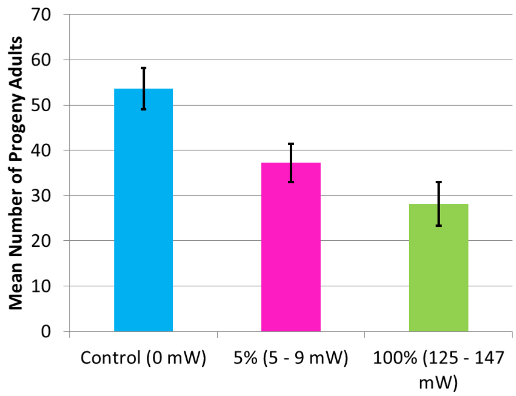


**Figure 2. Effect of EMF on Number of *D. melanogaster* Adult Progeny**. Bars represent mean number of progeny adults for each EMF concentration. Error bars represent one standard error above the mean and one standard error below the mean.

α ($p$=0.0013; **Figure 2**). Two-tailed t-test comparison (α=0.025) of the 0% and 100% concentrations produced a statistically significant result since the $p$-value was less than α ($p$=0.00076), with a Hedge's $g_s$ effect size of 1.4 and a 95% CI: [0.62, 2.3]. There was no significant difference between the 5% and 0% concentrations.

Another way that the effects of Wi-Fi were measured was by comparing male and female progeny adults. The results suggest that male flies are more sensitive to Wi-Fi EMF than female flies. Even at a low (5%) concentration, the number of progeny males was significantly fewer than the control. ANOVA comparison (α=0.05) of the number of progeny males for concentrations 0%, 5%, and 100% produced a statistically significant result ($p$=0.0036; **Figure 3**). Two-tailed t-test comparison (α=0.025) of the 0% and 5% concentrations yielded a $p$-value less than α ($p$=0.0030), with a Hedge's $g_s$ effect size of 1.2 and 95% CI: [0.41, 2.0]. Two-tailed t-test comparison (α=0.025) of the 100% and 0% concentrations produced a $p$-value less than the α ($p$=0.0069), with a Hedge's $g_s$ effect size of 1.1 and 95% CI: [0.33, 1.9].

For progeny female adults, high levels of Wi-Fi EMF exposure (100% concentration) reduced the number of flies, while low levels (5% concentration) did not. ANOVA comparison (α=0.05) of the number of progeny females for concentrations 0%, 5%, and 100% produced a statistically significant result ($p$=0.0014; **Figure 4**). Two-tail t-test comparison (α=0.025) of the 100% and 0% concentrations also yielded a $p$-value less than α($P$=0.00053), with a Hedge's $g_s$ effect size of 1.4 and 95% CI: [0.64, 2.3]. There was no significant difference between the 5% and 0% concentrations.

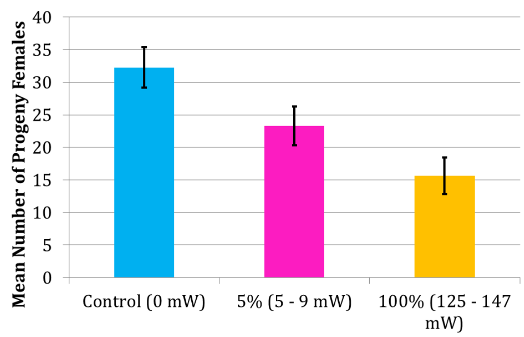


**Figure 4. Effect of EMF on Number of Progeny Female Adults**. Bars represent mean number of progeny female adults for each EMF concentration. Error bars represent one standard error above the mean and one standard error below the mean.

To investigate whether Wi-Fi EMF has developmental effects, the number of pupae was also analyzed. High levels of Wi-Fi EMF exposure (100% concentration) decreased the number of pupae, but lower levels (5% concentration) did not. ANOVA comparison (α=0.05) of the number of pupae across all EMF concentrations (0%, 5%, 100%) produced a statistically significant result ($p$ = 0.0035; **Figure 5**). Two-tailed t-test comparison (α=0.025) of the 0% and 100% concentrations yielded a $p$-value less than α ($p$=0.0019),

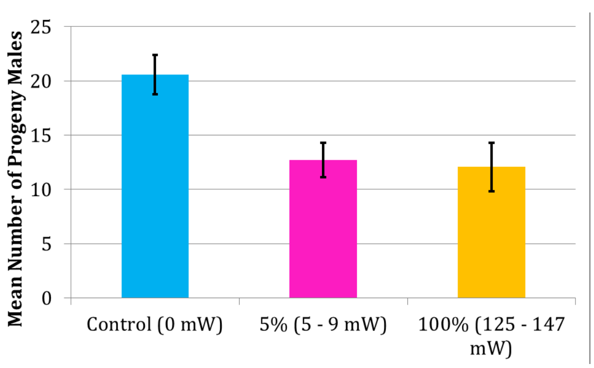


**Figure 3. Effect of EMF on Number of Progeny Male Adults**. Bars represent mean number of progeny male adults for each EMF concentration. Error bars represent one standard error above the mean and one standard error below the mean.

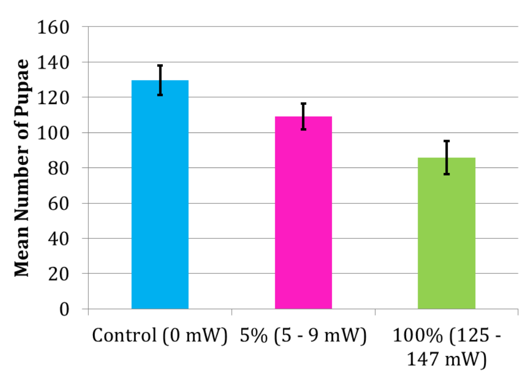


**Figure 5. Effect of EMF on Number of Pupae.** Bars represent mean number of pupae for each EMF concentration. Error bars represent one standard error above the mean and one standard error below the mean.

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with a Hedge's $g_s$ effect size of 1.3 and 95% CI: [0.50, 2.1]. There was no significant difference between the 5% and 0% concentrations.

Wi-Fi EMF's effect on the male:female ratio is unclear. A low (5%) concentration showed a marginally significant decrease, whereas the 100% concentration showed no significant difference. ANOVA comparison (α=0.05) of concentrations 0%, 5%, and 100% produced a *p*-value less than the α, and was statistically significant (*p*=0.038; **Figure 6**). Two-tailed t-test comparison (α=0.025) of the 5% and 0% concentrations (*p*=0.089) was marginally non-significant, since the *p*-value is close to the α. Two tail t-test comparison (α=0.025) of the 100% and 0% concentrations (*p*=0.23) did not produce p-values below the α, and was not statistically significant. Visual observation of the chart did not show any obvious pattern in the male:female ratio as the EMF concentration increases. Further research is necessary to determine how Wi-Fi EMF exposure affects the progeny male:female ratio.

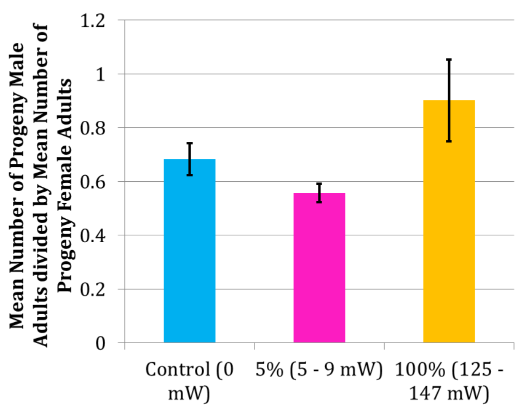


**Figure 6. Effect of EMF on Male:Female Ratio of Adult Progeny.** Bars represent mean male:female ratio for each EMF concentration. This is the data before the logarithmic transformation. Error bars represent one standard error above the mean and one standard error below the mean.

None of the statistical comparisons of the adult:pupa ratio emerged as statistically significant (**Figure 7**). This suggests that Wi-Fi EMF exposure does not affect the adult:pupa ratio of *D. melanogaster*.

## DISCUSSION

The results of this study show that Wi-Fi EMF has a statistically significant effect on the number of total progeny adults, number of progeny males, number of progeny females and number of pupae. The progeny adult male:female ratio was not significant. There was no statistically significant effect for the progeny adult:pupa ratio. This suggests that when *D. melanogaster* are exposed to Wi-Fi EMF, the number of progeny adults, number of progeny male adults,

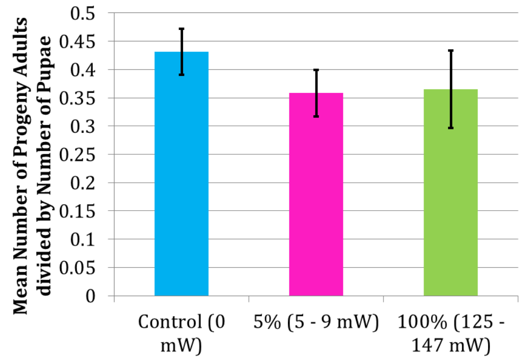


**Figure 7. Effect of EMF on Adult:Pupa Ratio.** Bars represent mean progeny adult:pupa ratio for each EMF concentration. This is the data before the logarithmic transformation. Error bars represent one standard error above the mean and one standard error below the mean.

number of progeny female adults, and number of pupae, are reduced. Results for the progeny adult male:female ratio are inconclusive. Meanwhile, the results suggest that the progeny adult:pupa ratio is unaffected. The results of this study support the hypothesis, since they suggest that Wi-Fi EMF affects *D. melanogaster*.

Since the t-tests performed were two-tailed, their statistical significance does not indicate whether the number of *D. melanogaster* increased or decreased. However, there was no reliable data collected for the 20% concentration and if this data is excluded from the charts, a clear negative trend is apparent. As the Wi-Fi EMF concentration increased, the number of flies decreased. This trend applies to the mean number of progeny adults, mean number of progeny male adults, mean number of progeny female adults, and mean number of pupae. This negative correlation does not seem to exist for the male:female ratio or the adult:pupa ratio.

Given this negative correlation, the results suggest that only a high concentration of EMF (100%) decreases the number of progeny adults, the number of progeny females, and the number of pupae, and that low (5%) EMF levels do not produce a statistically significant effect. It may be that these groups are only affected when Wi-Fi EMF surpasses a threshold concentration. In contrast, even a low EMF concentration (5%) produced a statistically significant decrease in the number of progeny males. This suggests that male *D. melanogaster* are more sensitive to EMF than female *D. melanogaster*. There was not a statistically significant difference in the progeny adult:pupa ratio for any EMF concentration, which suggests that EMF exposure does not impair eclosure of pupae. The results for the male:female ratio are inconclusive, since the ANOVA comparison produced a p-value less than the α, but the t-test comparisons to the control did not.

Further research is necessary to identify which specific aspects of *D. melanogaster* biology—such as fecundity, hormone modulation, eating behavior, or larval development—are affected to cause the decrease in the number of *D. melanogaster*. Research into specific areas of *D. melanogaster* biology, and research using more complex organisms such as mice, could shed light on any effects of Wi-Fi EMF for humans. Another relevant question is whether Wi-Fi EMF has generational effects. This could be investigated by studying multiple generations of *D. melanogaster* and focusing on generational effects. Further research is needed to determine Wi-Fi EMF's effects on the male:female ratio. Future studies could employ a two-way ANOVA to better understand Wi-Fi EMF's effect on the male:female ratio.

The Box Setup apparatus could be improved by adding a parabolic reflector to the Wi-Fi Chamber of the Box Setup, which could produce more consistent EMF measurements across the grid on the floor of the *D. melanogaster* chamber of the Box Setup. The *D. melanogaster* chamber should also be lined with a more effective EMF-absorbent material than the carbon-impregnated fabric used in this experiment to improve EMF absorbance and mitigate standing waves. The *D. melanogaster* chamber could also be modified so that EMF concentrations on the grid floor can be measured while the door flap is closed and all the shielding is in place.

A published experimental setup designed to expose small animals, such as mice, to controlled concentrations of EMF experienced many of the same physics challenges encountered in this experiment, and this setup could be utilized for future research with Wi-Fi EMF (11).

The implications of this study extend beyond Wi-Fi usage. Non-ionizing Radiofrequency EMF, which is the type of EMF used for Wi-Fi, is also produced by cellphones, smart meters, and cellphone towers (1, 3, 4, 5). These technologies may exhibit similar biological effects as Wi-Fi.

An uncontrolled variable that may have occurred is slight warming of the *D. melanogaster* vials from the EMF; however, a warmer temperature would be expected to accelerate the *D. melanogaster* life cycle (9, 10) and subsequently increase the number of progeny, not decrease the number of progeny, as occurred with Wi-Fi EMF exposure in this experiment. If the Control (0% EMF concentration) *D. melanogaster* chamber was consistently warmer than the other three *D. melanogaster* chambers, this could explain its greater number of progeny. However, this was not the case. The *D. melanogaster* chambers with the higher EMF concentrations sometimes experienced slightly higher temperatures than the others, yet they had fewer progeny. Throughout the experiment, the four *D. melanogaster* chambers were kept at approximately 24 degrees Celsius. The control *D. melanogaster* chamber and the 5% EMF concentration *D. melanogaster* chamber sometimes measured 0.5 degrees Celsius less than the *D. melanogaster* chambers for the 100% and 20% EMF concentrations. This cooler temperature was probably because the 5% and control *D. melanogaster* chambers were positioned closer to an exterior wall and this experiment was conducted in winter. Throughout the experiment, the *D. melanogaster* chambers were 22 to 25 degrees Celsius.

The *D. melanogaster* vials and foam plugs likely reflected or absorbed a small percentage of the Wi-Fi EMF. However, the nature of Wi-Fi EMF is such that it penetrates many types of materials so that it can provide Wi-Fi service throughout an entire home. The influence of the vials and foam plugs on the *D. melanogaster*'s EMF exposure is negligible for the purposes of this experiment. Furthermore, any effect the vials and foam plugs had on the Wi-Fi EMF exposure is a controlled variable, because all of the *D. melanogaster* were placed in the same type of vials with identical foam plugs.

Due to the wave nature of EMF, a change in the polarity or angle of the EMF meter, a change in the EMF shielding's position, or a three centimeter (cm) change in the position of the EMF meter caused the EMF measurement to change unpredictably. In addition, EMF measurements may have changed when the shielding was positioned over the door flap. This is a limitation of this study, but is consistent across all the Box Setups including the control.

## METHODS

### Summary of Procedure

Four Box Setup apparatuses were designed and constructed. Each apparatus consisted of two cardboard boxes on top of each other. The top box was the "Wi-Fi chamber," with a router inside, and the bottom box was the "*D. melanogaster* chamber," where the *D. melanogaster* vials were placed.

The Box Setup apparatus required intensive design and construction. First, a thermometer, air tubes, and a Wi-Fi router were installed in each Box Setup. The Box Setups were then covered with EMF shielding. EMF-reflective shielding used was Reynolds Wrap® Extra Heavy Duty aluminum foil, and EMF-absorbent shielding used was carbon-impregnated fabric. Sleeves of the carbon-impregnated fabric were placed on the Wi-Fi router antenna to attenuate the Wi-Fi EMF. Different-sized carbon fabric sleeves were used to make the different concentrations of Wi-Fi EMF Radiation. The control (0% concentration) was achieved by placing two layers of aluminum foil between the Wi-Fi chamber and the *D. melanogaster* chamber, blocking all measurable EMF. The air supply, grounding system, and electrical system were connected to each Box Setup. Grounding of the Box Setups was done based on a study in which the aluminum Faraday Cage did not successfully block EMF until it was grounded (8). A grid was marked on the floor of the *D. melanogaster* chamber and five EMF measurements were taken in each square of the grid. Microsoft Excel was used to calculate the mean of these five measurements. The squares of the grid which had mean EMF measurements which fell within the defined EMF ranges were chosen for *D. melanogaster* vial positioning.

Upon completion of a functional Box Setup for each

EMF concentration, the focus was shifted towards the *D. melanogaster*. Sixty vials were prepared with medium, distilled water, and yeast. *D. melanogaster* adults, which were 1-4 days old, were anesthetized and sexed. One male and one female *D. melanogaster* were placed in each vial. The vials were positioned in grid squares with mean EMF measurements within the defined Wi-Fi EMF ranges. There were 15 vials for each EMF concentration. After positioning the vials, the fronts of the *D. melanogaster* chambers were covered with shielding. (See **Figure 1**) The *D. melanogaster* were exposed to Wi-Fi EMF for 14 days. The *D. melanogaster* vials were then removed from the Box Setups and frozen. The data was collected and recorded for the number of progeny adults, number of progeny males, number of progeny females, number of pupae, male:female ratio, and adult:pupa ratio.

### Choice of Shielding Materials

Aluminum was chosen as an EMF reflective material because at 1.5 GHz/s and 3.0 GHz/s, 99.0% of EMF is reflected by aluminum (12). The Wi-Fi EMF used in this experiment was 2.4 GHz, which falls within this range. Very little EMF travels through aluminum at Gigahertz frequencies (12). Extra Heavy Duty aluminum foil is chiefly aluminum (98.5%), and the remaining metals are mostly iron and silicon (13).

The Skin Effect principle (14, 15) was used to confirm that Extra Heavy Duty aluminum foil (thickness of 32 μm) is suitable for EMF reflective shielding.

Skin Effect Depth equation: $\delta=\sqrt{(\rho/\pi f\mu)}$

$\delta$ is the skin effect depth in m,

$\rho$ is the resistivity of the conductor in $\Omega$ x m ,

f is the frequency of the EMF wave in Hertz ,

$\mu$ is the absolute magnetic permeability. This is the relative permeability of the conductor multiplied by $4\pi \times 10\text{-}7$ H/m.

For aluminum, the skin depth is ≈ 1 μm. So, after every 1 μm of aluminum thickness, EMF power density is decreased by 1/e. After 32 μm, the thickness of Extra Heavy Duty aluminum foil, the power density is $(1/e)^{32}$, which is approximately $10^{-14}$. Carbon is EMF absorbent in some forms (16, 17). A carbon-impregnated fabric, “Microwave Absorbing Sheet,” was obtained as an EMF absorber for this experiment (18, 19). The Less EMF website, where the carbon fabric was purchased, advertised it as an effective EMF absorbent material, providing more than 25 decibels of attenuation at 2 GHz. Carbon fabric was selected instead of other available EMF attenuating fabrics made from silver and nickel because those fabrics have antimicrobial properties. Antimicrobial properties could adversely affect the *D. melanogaster* which rely on a variety of microbes to ferment their medium.

Steps were taken to avoid build-up of heat inside the Box Setup. In theory, EMF is converted to heat as the EMF-absorbent carbon fabric absorbs it. Cardboard boxes have a thermal conductivity of 0.21 Wm-1K-1, indicating that cardboard is a poor thermal conductor, and therefore a good insulator (20). If the carbon fabric lined the inside of the cardboard box, then as it absorbed EMF, the cardboard would retain the heat. For this reason, the carbon fabric was layered on the exterior of the cardboard boxes, so that any heat produced by EMF absorption would be transferred to the environment, and not be trapped in the cardboard box. The EMF-reflective shielding, aluminum foil, was placed as the outermost layer, on top of the carbon fabric since its thermal conductivity is 210 Wm-1K-1, indicating that it is a good thermal conductor and heat is readily conducted out of it (20). So, any heat produced by the carbon fabric absorbing EMF would be easily transferred to the aluminum foil, and be transferred to the surrounding air. As expected, the temperature inside the Box Setups did not increase over time as it would if heat was trapped inside.

### Construction of "Box Setup" Testing Apparatus

Each Box Setup consisted of two 46 by 32 by 35 cm cardboard boxes stacked on top of each other. Cardboard boxes were chosen as the structure for the testing chamber because of their shape, sturdiness, and availability. The upper box, the “Wi-Fi Chamber” contained the TP-LINK® Wi-Fi router (21), the source of the EMF. This Wi-Fi router was selected because it was one of the most highly rated routers available for home use at the time of the experiment. The lower box, the “*D. melanogaster* Chamber” held the *D. melanogaster* vials.

In addition to effective control of EMF within the testing chamber, other objectives for the Box Setup were determined. Control of EMF levels inside the chamber was maintained by the twofold strategy of blocking exterior EMF from entering and absorbing EMF reflections inside the chamber. Different concentrations of EMF were created by changing the size of sleeves of absorbing EMF material placed over the Wi-Fi router antennae. To maintain the *D. melanogaster* inside the closed testing chamber, an air circulation and humidity system were installed. Relative humidity was maintained at approximately 40%, a level high enough to avoid the drying of the medium and low enough to avoid mite infestation or bacterial contamination.

So that the Control vials would also be exposed to any heat that could have been produced by the router, the Wi-Fi chamber in the Control Box Setup also contained a Wi-Fi router that was producing Wi-Fi. The control (0% concentration) was achieved by placing two layers of aluminum foil between the Wi-Fi Chamber and the *D. melanogaster* chamber.

The flat floor of the *D. melanogaster* chamber ensured that all the *D. melanogaster* vials would rest on an even surface. This was pursued because EMF exposure can change when there is a difference in position of only a few millimeters. However, after observing the nature of EMF, the authors do not think that the flat floor feature made a measurable impact on EMF exposure. Nevertheless, the flat floor was a useful feature to make the grid squares easier to draw.

A layered shielding system was used so that openings in the shielding could be made without allowing measurable amounts of exterior EMF to enter the Box Setup through them. These openings were necessary for the thermometers and air tubes. The layering system involved alternating layers of EMF-reflective and EMF-absorbent shielding.

### Rationale for Air Circulation

It was necessary that there be adequate air circulation to mitigate the carbon dioxide produced by the fermenting medium and *D. melanogaster*. An air tube and air valve system were designed and installed to provide 24/7 air circulation. The air valve system was used to distribute air equally among the four Box Setups. A Tetra Whisper® aquarium air pump designed for a 378.5 L fish tank was selected and positioned to minimize vibrations and noise as uncontrolled variables. To prevent the *D. melanogaster* medium from drying, the incoming air was humidified. The Vicks® Warm Mist humidifier was selected because it created vapor by heating water and therefore did not require an antibacterial filter. "Cool-air" humidifiers were avoided because they use antimicrobial agents to prevent bacterial contamination; exposure to antimicrobial agents would be an uncontrolled variable that could modify the fermentation of the *D. melanogaster*'s medium. Also, only distilled water was used in the humidifier to minimize potentially uncontrolled variables because of water solutes.

### Construction of Wi-Fi Chamber

The "Wi-Fi Chamber" was identical for all the Box Setups. The flaps of the cardboard box which would normally form the floor were cut off. The flaps which form the top were taped with packing tape with the short flaps facing inwards, then the long flaps. Measurements were drawn using an aluminum T-square. A utility knife was used to cut openings. A door flap was cut in the front of the box to provide access to the interior. The left edge of the box served as the hinge for the door flap. The door flap was cut from the front of the box 23 cm from the left edge, 4 cm from the top edge, and 4 cm from the bottom edge. A 5 cm sturdy ribbon was stapled to the right edge of the door flap to be used for opening it. The Wi-Fi router was attached to the ceiling of the box using zip ties. Circular openings for the two zip ties were made by punching a sharp pencil through the ceiling of the box. The openings for the first zip tie were made 21.5 cm from the front edge, 15.5 cm from the left edge and 21.5 cm from the front edge, 1 cm from the left edge. The openings for the second zip tie were made 14 cm from the front edge, 15.5 cm from the left edge and 14 cm from the front edge, 1 cm from the left edge. The plastic zip ties were threaded through the openings made. The Wi-Fi router was positioned within 8.5 cm from the front and back edge, and 7.5 cm from the left edge. The zip ties were tightened until snug, and the position of the router was double checked. If necessary, adjustments were made, and the zip ties were tightened completely. Excess zip tie length was cut off. A circular opening for the Wi-Fi router's power cord was made by punching a thick pen through the ceiling of the box 5 cm from the back edge, 13 cm from the left edge. The power cord was inserted through the opening and connected to the Wi-Fi router. The Wi-Fi router button was pressed to the "On" setting so that when power was connected, Wi-Fi EMF would begin automatically. The Wi-Fi Chamber was taped on top of the *D. melanogaster* Chamber using packing tape, making a Box Setup. The door flap was taped shut with painter's tape.

### Construction of *D. melanogaster* Chamber

The "*D. melanogaster* Chamber" was identical for all four Box Setups. Using packing tape, the floor was taped with the longer cardboard flaps facing inwards, making a flat floor for the vials to rest on. Packing tape was used to seal all edges and seams of the box. Measurements were drawn using an aluminum T-square. A utility knife was used to cut openings. A door flap was cut in the front of the box to provide access to the interior of the *D. melanogaster* Chamber. The left edge of the box served as the hinge for the door flap. The door flap was cut from the front of the box 23 cm from the left edge, 4 cm from the top edge, and 4 cm from the bottom edge. A 5 cm sturdy ribbon was stapled to the right edge of the door flap to be used for opening it. Circular openings for air tubes for the air circulation system were made by punching a sharp pencil through the walls of the box. For the front and back of the box, openings were made 2 cm from the bottom edge and 23 cm from the left edge. For the right and left sides of the box, openings were made 18 cm from the left edge and 28 cm from the bottom edge. A rectangular 2.5-cm by 1-cm opening for the thermometer was made on the right side of the box 11 cm from the left edge and 4 cm from the bottom edge. Plastic aquarium airline air tubing was then cut for the air circulation system: two 90-cm pieces and two 50-cm pieces. The 90 cm pieces were labeled "O" for output, and the 50 cm pieces were labeled "I" for input. Two rotations of 2 cm wide masking tape were wrapped around the end of each air tube piece. The 50 cm air tubes were inserted through the circular openings made in the front and back of the box, and the 90 cm air tubes were inserted through the openings in the right and left sides of the box. The piece of masking tape wrapped around the end of each tube prevented the tubes from falling out of the openings. Wood glue was used to keep the air tubes in place. Pieces of masking tape were used to secure the air tubes along the outside walls of the box, so that they all came together at the back. The air tubes were gathered in a bundle, secured with a Velcro® strip, then secured with 4 cm wide masking tape, then secured with a Velcro® strip, then secured with 4 cm wide masking tape, then secured with a Velcro® strip.

### Emitting Different Concentrations of Wi-Fi

EMF-absorbing carbon impregnated fabric shielding was placed on the Wi-Fi router antennae by rolling the fabric into hollow, cylindrical sleeves and sliding the sleeves onto each antenna. The sleeves were secured in place with masking tape on the interior of the sleeves. The carbon fabric sleeves attenuated the Wi-Fi EMF, and different dimensions of carbon fabric were used to create the different EMF concentrations. Each Wi-Fi router antennae was 17.5 cm long. The 100% concentration Wi-Fi Chamber did not receive any carbon fabric sleeves on the Wi-Fi router antennae.

For the 20% concentration Wi-Fi Chamber, six pieces of carbon fabric were cut 4.5 cm long, 7 cm wide. These carbon fabric rectangles were then rolled into hollow cylindrical sleeves with a length of 4.5 cm. On each antennae, the sleeves were positioned: first a 1 cm space with no sleeve, 4.5 cm sleeve, 1 cm no sleeve, 4.5 cm sleeve, 1 cm no sleeve, 4.5 cm sleeve, 1 cm no sleeve.

For the 5% concentration Wi-Fi Chamber, six carbon fabric pieces were cut 5.5 cm long, 7 cm wide. These carbon fabric rectangles were then rolled into hollow cylindrical sleeves with a length of 5.5 cm. On each antennae, the sleeves were positioned: first a 0.25 cm space with no sleeve, 5.5 cm sleeve, 0.25 cm no sleeve, 5.5 cm sleeve, 0.25 cm no sleeve, 5.5 cm sleeve, 0.25 cm no sleeve.

The Control (0% Wi-Fi EMF concentration) did not receive any sleeves on the router antennae because the sleeves are not capable of completely blocking EMF. The router was kept "On" so that any heat it produced would be a controlled variable among all the Box Setups. The Control (0% concentration) was achieved by placing two layers of Extra Heavy Duty aluminum foil between the Wi-Fi Chamber and the *D. melanogaster* Chamber. The aluminum foil blocked the EMF from entering the *D. melanogaster* Chamber and reflected the EMF back into the Wi-Fi Chamber. The Skin Effect calculation confirmed that two layers of Extra Heavy Duty aluminum foil would attenuate virtually all of the Wi-Fi EMF. The only difference between the Control Box Setup and the Box Setups for the other concentrations was two layers of reflective shielding blocking the Wi-Fi before it reached the vials.

The Control (0% concentration) was confirmed by changing the EMF meter to its "Sound" setting, placing the EMF meter inside the *D. melanogaster* Chamber, closing the *D. melanogaster* Chamber, and listening for EMF readings. In "Sound" mode, the intensity of the EMF level is proportional to the intensity of the meter's buzzing. Silence indicates no measurable EMF. No measurable EMF was detected by the EMF meter in the Control (0% concentration) *D. melanogaster* Chamber when the router was "On"— it did not produce any sound. Measurements were drawn using an aluminum T-square and permanent marker. A utility knife was used to cut the shielding.

### Applying Shielding to Wi-Fi Chamber

The reflective shielding was Reynolds Wrap® Extra Heavy Duty aluminum foil. This shielding was 45.7 cm wide off the roll. Three pieces of reflective shielding were cut (a) 118 cm long, 45.7 cm wide, (b) 66 cm long, 31 cm wide, and (c) 52 cm long, 45.7 cm wide.

This shielding was applied to the Wi-Fi Chamber using masking tape. The shielding was applied in a specific order: first piece (a) on left side, back side, and right side of box; second piece (c) applied on top side; edges overlapping piece (a); third (b) applied to front side; edges tuck under (c) and (a).

Measurements were drawn on shielding using an aluminum T-square and permanent marker. A utility knife was used to cut shielding.

### Applying Shielding to *D. melanogaster* Chamber

A 31- by 121.5-cm piece of EMF-absorbent shielding (carbon impregnated fabric) was wrapped around the *D. melanogaster* Chamber to cover the left side, back side, and right side and overlap slightly over the front of the Chamber.

Next, the shielding layering system for the air tube bundle was done. A 20 cm by 15 cm piece of EMF-reflective shielding was positioned on the left side of the Chamber so that it aligned with the left side's left edge and bottom edge. On top of this piece, a 33-cm by 15.5-cm piece of EMF-absorbent shielding was positioned so that it aligned with the left edge and bottom edge of the left side of the Chamber. An opening was cut in these pieces for the air tube bundle to feed through; the opening was 2.5 cm from the Chamber left side bottom edge and 9.5 cm from its left edge.

A 47-cm by 57-cm piece of EMF-absorbent shielding covered the bottom of the *D. melanogaster* Chamber. It had overlap over all four sides of the Chamber. A 92- by 45.7-cm piece of EMF-reflective shielding covered the right side and back of the Chamber; it had overlap over the bottom and left side of the Chamber. A 40- by 47.5-cm piece of EMF-reflective shielding covered the left side of the Chamber and overlapped onto its back. A 52- by 44.5-cm piece of EMF-reflective shielding covered the bottom of the *D. melanogaster* Chamber; it overlapped over all four sides of the Chamber.

For the control *D. melanogaster* Chamber only, two 52- by 44.5-cm pieces of EMF-reflective shielding covered the top of the Chamber, with overlap over all four sides of the Chamber. These blocked the EMF produced by the Wi-Fi router from reaching the *D. melanogaster* Chamber.

Where the shielding on the *D. melanogaster* Chamber met the shielding of the Wi-Fi Chamber, there was a small gap, where there was no shielding. A 45.7- by 120 cm piece of EMF-reflective shielding was used on the exterior of the Box Setup to cover this gap.

Next, the shielding layering system for the thermometer opening was conducted. A 16- by 18-cm piece of EMF-reflective shielding was centered over the place where the thermometer opening had been cut into the *D. melanogaster* Chamber cardboard box. The thermometer bulb, which was wrapped in a sleeve of EMF-reflective shielding, was inserted through the 16- by 18-cm piece of EMF-reflective shielding. Wrapping it in a sleeve did not affect the temperature readings, since aluminum foil is a good thermal conductor (20). A 14- by 17-cm piece of EMF-absorbent shielding was layered next. A

14- by 14-cm piece of EMF-absorbent shielding was the final layer for the thermometer's shielding layering system.

The last step was for shielding to be applied to the front of the *D. melanogaster* Chamber. A 57.5- by 30-cm piece of EMF-absorbent shielding covered the front side of the Chamber, and overlapped onto its left side. A 63- by 30- cm piece of EMF-reflective shielding covered the front side of the Chamber, and overlapped onto its left side. Finally, a 56- by 44-cm piece of EMF-reflective shielding covered the front side of the Chamber, and overlapped onto its left side.

All shielding was secured with masking tape, except the shielding used to cover the front of the *D. melanogaster* Chamber. That shielding was secured using Painter's tape so that it was easily removable, allowing the door flap to be opened to access the interior of the *D. melanogaster* Chamber.

### Installation of Air Circulation System

The Box Setups were placed on two adjacent tables; the air circulation apparatus (humidifier, air pump, etc.) was placed underneath the tables. Plastic garbage bags were spread on the floor and a stool was covered with a garbage bag to stay dry. The aquarium air pump was placed on the stool and the humidifier was placed next to the stool. The aquarium air pump and humidifier were plugged into a GFCI outlet, which was used for safety in case of a water spill. Two pieces of Extra Heavy Duty aluminum foil (a) 75 cm long, 45.7 cm wide, (b) 34 cm long, 45.7 cm wide, were cut and shaped into a hood to direct water vapor from the humidifier towards the intake of the aquarium air pump. The aluminum foil hood was secured to the bottom of the table and stool using masking tape.

Air valves, which each had four openings, were used. Before starting the experiment, the air valves were all opened and closed to reduce their stiffness. Pieces of plastic aquarium airline air tube (a) 1 piece: 115 cm long, (b) 4 pieces: 102 cm long, (c) 1 piece: 42 cm long—were cut for use as connectors between air valves. Piece (a) connected the air pump to the main air valve which rested on the table. Piece (b) connected the main air valve to the individual air valves for each Box Setup. Piece (c) connected to one of the openings of the Control Box Setup's air valve. This airline tube was used to measure the humidity of the air that the *D. melanogaster* were being exposed to. All four openings of the main air valve were opened. They attached to a valve for each Box Setup, which split the air flow into two tubes before leading it into the *D. melanogaster* Chamber. For the Control Box Setup, a third air valve opening connected to Piece (c). This Piece (c) opening was kept closed except when measuring humidity. The aluminum hood was adjusted until the desired relative humidity was achieved, as measured with the Humidity Measuring Procedure. The humidifier was filled with distilled water.

### Grounding System

A grounding system was installed in the Box Setups to make them grounded Faraday Cages. Four electrical cords with a plug at one end were obtained by cutting the cords off of power strips. The ground wire, which was covered in green insulation, was stripped at its end. This end was twisted around the exposed wire of an alligator clip. The joint was secured with a wire nut. The alligator clip was attached to the piece of aluminum foil which covered the small gap where the *D. melanogaster* chamber shielding met the Wi-Fi chamber shielding. The section of aluminum foil which the alligator clip clamped onto was folded to add thickness so that the alligator clip would not puncture it. Using a multimeter, the resistance between the aluminum shielding and grounding pin on the cord's plug was measured and recorded. The resistance was 0.1 Ω for all four Box Setups. The alligator clips were attached to cords, which were plugged into a power strip, which was then plugged into a wall outlet, providing a "Ground."

### EMF Measurement in Grid Squares

To measure the EMF in every square of the grid on the floor of the Box Setups, first a simple holder was made for the EMF meter. This was necessary because higher-than-normal EMF measurements were observed when the device was hand-held; this could have been caused by human body conducting stray EMF in the environment. Two erasers were taped with masking tape at one end of the bottom of a plastic ruler. The EMF meter was taped to the plastic ruler at a right angle to the ruler, directly above the two erasers. This made a holder for the EMF meter which did not seem to conduct environmental stray EMF. The backlight of the EMF meter was kept "On," to make the measurements easy to read. EMF measurements were then taken in each grid square. This was repeated for each Box Setup except the Control. The Control (0% EMF concentration) Box Setup did not have a grid.

Holding the plastic ruler, the researcher positioned the EMF meter in a grid square. The EMF meter's position was parallel to the router antennae at all times so that they shared the same polarity. The researcher waited two seconds for the EMF measurements to settle, then recorded five EMF measurements on a prepared piece of graph paper which had a sketch of the grid squares on it. The decimals were truncated to the ones place.

### Horizontal Position Effect on EMF Concentration

Due to the nature of EMF and the strong EMF-reflection properties of aluminum foil, EMF levels did not seem to have a discernable pattern based on location in the grid. For example, some of the highest EMF concentrations were measured at the edge of the floor, rather than the center. Therefore, to determine where to position the *D. melanogaster* vials, the grid system was developed. Each Box Setup had a grid drawn on its floor. Each grid square was 3 cm by 3 cm because this is approximately one third of the Wi-Fi EMF wavelength, which is 12.5 cm. This size was also chosen because it is approximately the space required for one vial of *D. melanogaster*. Since this is about one third of the wavelength, EMF levels were expected to be relatively consistent throughout an individual square. Five EMF measurements were taken in each square of the grid and the mean of those measurements was calculated. *D. melanogaster* vials were placed in grid squares with EMF levels within the concentration ranges.

### *D. melanogaster* Care

*D. melanogaster* Wild Type (+) were purchased from Ward's Science. They were kept in cellulose acetate propionate plastic vials and fed 4-24 Instant Drosophila Medium Blue, both from Carolina ® Biological Supply Company. The *D. melanogaster* were cared for following the guidelines of the Carolina ® Drosophila Manual (10).These original 16 culture vials, which were of indeterminate ages, were stored in a cool, dry location for 16 days while the Box Setups were engineered. Then, four culture vials were placed in each Box Setup for 14 days. It was assumed that each culture vial's average EMF exposure corresponded to each Box Setup's respective EMF concentration, even though the vials were not positioned in specific grid squares. During these 14 days, eggs were laid that developed into larvae and pupae, and would eventually develop into the adult *D. melanogaster* used as the parents in this experiment.

After 14 days, the Box Setups were opened and all the adult *D. melanogaster* present were removed using a vacuum cleaner, so that only eggs, larvae, and pupae remained. Then, the vials were returned to the Box Setups, and the pupae were allowed to eclose for four days, producing 1-4 days old adult *D. melanogaster* that had been exposed to the different Wi-Fi EMF concentrations for their entire lifetime. The Box Setups were opened and the vials removed so that the *D. melanogaster* adults could be anesthetized, sexed, and paired as the parent *D. melanogaster* whose progeny were studied in this investigation.

For each Box Setup, 15 vials, or 15 pairs of *D. melanogaster* were placed in the Box Setups for 14 days. Each pair of parent *D. melanogaster* laid eggs that developed into larvae, pupae, and progeny adults. These pupae and progeny adults were counted as data for the experiment. The progeny adults were 1-5 days old. Therefore, the progeny adults and pupae that were counted in this experiment were from the second generation of *D. melanogaster* to be exposed to Wi-Fi EMF for their entire life cycle. *D. melanogaster* age was calculated by assuming a life cycle of 10 days, since the temperature varied within a range of approximately 22 degrees Celsius and 25 degrees Celsius (15).

### *D. melanogaster* Vial Preparation

All materials were collected and a plastic garbage bag was spread on a table to protect the work space. Plastic cups were labeled as "Everclear," "Distilled water," "Medium," and "Dump." A large funnel was labeled as "Medium," and a small funnel as "Distilled water." The work area and utensils were sanitized by swabbing them with Everclear ® (75.5% ethanol solution) and allowed to dry. Vials were labeled with their concentration (represented by letters A-D) and numbers 1-15. An example of a vial label would be "A1."

There were a total of 60 vials. 15 mL of dry 4-24 Instant Drosophila Medium Blue was added to all the vials. Then, 15 mL of room temperature distilled water was added to all the vials. Between 5-8 grains of Dry Active Yeast were added to each vial. Using a 2-mL plastic pipette, an additional 2-mL of distilled water was added to each vial, which was then immediately covered with a foam plug to minimize evaporation.

### Anesthetization and Sexing of *D. melanogaster*

All materials were collected and a plastic garbage bag was spread on a table to protect the work space. The work area and utensils were sanitized by swabbing them with Everclear ®. Everything was allowed to dry.

The *D. melanogaster* were anesthetized using FlyNap ® (22) via the anesthetization procedure described in the Carolina ® Drosophila Manual (10). *D. melanogaster* were transferred from their culture vial to an empty vial prior to anesthetization. They were emptied onto a white paper immediately after anesthetization.

The anesthetized *D. melanogaster* were sexed to identify male/female pairs for the experimental vials. A camel hair brush was used to gently manipulate the anesthetized *D. melanogaster*. 10-15 anesthetized *D. melanogaster* were transferred onto the dissecting microscope's plate. At 10X magnification, the *D. melanogaster* were sexed based on diagrams found in the Carolina® Drosophila Manual (10). The anesthetized *D. melanogaster* were sorted into the following groups: unsorted *D. melanogaster* to the back, males to the left, females to the right, and discards to the front. Discarded *D. melanogaster* were either (a) pale indicating recent eclosure so they probably would not survive anesthetization, (b) injured, or (c) of indistinguishable sex. The discarded *D. melanogaster* were placed into an oil morgue. The camel hair brush was used to drop a male and female *D. melanogaster* in each experimental vial, then the foam plug was replaced on the vial.

### Measuring Temperature, Humidity, Watt Consumption

It was important to have a way to make sure that all the Wi-Fi routers were functioning, even when they were not visible because they were inside the Box Setups. This was necessary because if a Wi-Fi router turned off unexpectedly, the *D. melanogaster* inside would not be exposed to Wi-Fi EMF. To address this, the power adapters for all the routers were plugged into a single power strip which was connected to a Watt meter plugged into the wall outlet. The Watt readings were recorded twice a day to ensure that power consumption did not change. When all the routers were on and producing Wi-Fi, Watt usage was 5.5-5.6 W.

Approximately every 12 hours, the Humidity Measuring

Procedure was conducted (see below). While waiting the 5 minutes for the relative humidity measurement, the temperature of each Box Setup was recorded from the thermometers in each *D. melanogaster* chamber. Adjustments to the environment were made as necessary to keep the temperature within the desired range of 22-25 degrees Celsius. To increase the temperature, the furnace air vent which heats the room where the experiment was conducted was opened or the space heater temperature was increased. To decrease the temperature, the opposite was done. The height of the distilled water in the humidifier tank was measured and recorded to monitor water consumption. The humidifier was refilled with distilled water. Finally, the Watt consumption was recorded.

It was essential that adequate humidity be maintained since dry air could cause dry medium, which could starve the *D. melanogaster*. Furthermore, when medium is dry, larvae pupate directly onto it instead of on the walls of the vial, making them difficult to observe and record accurately. The relative humidity of the air pumped into the Box Setups was maintained at approximately 40%. The relative humidity inside the *D. melanogaster* Chamber could not be measured since the Box Setups were kept sealed during the experiment.

The humidity was measured approximately every 12 hours. To do this, the air valve connected to air tube Piece (c), which was connected to the Control Box Setup, was opened. The main air valve openings for Box Setups 2, 3, and 4 were closed. This way, all the air was being pumped through Piece (c). The end of Piece (c) was placed into a graduated cylinder lying on its side, and the hygrometer was placed into the graduated cylinder with its sensor facing the end of Piece (c). After 5 minutes, the relative humidity measurement was recorded. The air valves were then returned to normal.

### Statistical Analysis

Rigorous statistical tests, including control of the familywise error rate, were used to minimize the probability of the results solely occurring by chance. Statistical Analysis was conducted using Microsoft Excel's ® Analysis ToolPak ©. Data was eliminated for vials where the *D. melanogaster* did not survive anesthetization and there were no progeny adults. Statistical test data was rounded to two significant figures.

The statistical tests and comparisons conducted on the data were planned before the data was collected, to avoid bias (23). Statistical tests used were single factor ANOVA and two-sample t-test Assuming Unequal Variances. The ANOVA null hypothesis was that there was not a statistically significant difference among the groups tested. The t-test null hypothesis was that there was not a statistically significant difference between two concentrations. Null hypotheses were rejected if the *p*-value was less than the alphaα value for the ANOVA and the alphaα for one of the t-tests. Alpha represents the Type I Error.

For the t-tests, the two-tail p-value was used instead of the one-tail p-value, to investigate whether a change exists when *D. melanogaster* are exposed to Wi-Fi EMF. A change could be either an increase or a decrease. The hypothesis was that Wi-Fi EMF exposure would cause a change in the variables tested, and did not specify directionality of change, i.e. whether the change would be an increase or a decrease. For the male:female ratio and the adult:pupa ratio, statistical tests were conducted after a logarithmic transformation on the data, since ratios may not have a normal distribution (24). The male:female ratio and the adult:pupa ratio are ratios, so the Cohen's $d_s$ effect size, Hedge's $g_s$ effect size, and 95% confidence interval could not be calculated for this data.

The Bonferroni correction was used to control the familywise error rate for the t-tests by dividing the 0.05 alphaα by the number of t-tests (25). The Cohen's $d_s$ effect size was calculated using Equation 1 from an article by Lakens (26). Since Cohen's $d_s$ can be biased in samples with a count of less than 20 individuals, the Hedge's $g_s$, or corrected effect size, was calculated and used to report effect size (26). Cohen's $d_s$ effect size was only used to calculate the 95% confidence interval (CI) (27).

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