

## Filthy Flies?

### Experiments To Test Flies as Vectors of Bacterial Disease

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### ackground & Significance

Arthropods are important in disease transmission. They can serve as vectors, which are animals that transmit pathogens from one host to another. Arthropods can serve as vectors by two mechanisms: mechanical transmission or biological transmission. Mechanical transmission is a passive mechanism for the spread of infectious organisms; this would include contaminating food or tissue while walking on it. Biological transmission is an active mechanism, such as transmission during biting and feeding. For more than 75 years, flies and other insects have been known to serve as mechanical vectors of infectious disease (Hegner, 1926). Flies have been shown to harbor over 100 different species of potentially pathogenic microorganisms and are known to transmit more than 65 infectious diseases (Greenberg, 1965). For example, *Musca domestica*, the common house fly, has been shown to spread illnesses including hepatitis, polio, tuberculosis, and dysentery (Kettle, 1995).

Flies are able to transmit pathogens by three routes: body surface, vomit, or feces. One fly can carry over 33 million bacteria on the outer surface of its body (Greenberg, 1973). A significant reason for their vectoring ability is their lifestyle. Many non-biting flies, including house flies, blow flies, and flesh flies, feed on human and animal waste and lay their eggs in manure or other decaying matter. The dangerous bacteria present in these wastes stick to the deeply channeled mouthparts, footpads, and hairs of flies. The bacteria are then transferred by direct contact when the flies land on food, eyes, noses, mouths, and open wounds (Kettle, 1995). Given that an adult fly may live for four weeks (Fletcher et al., 1990) and travel over six kilometers in 24 hours (MacLeod & Donnelly, 1963), flies have ample opportunity to play a significant role in disease transmission.

This laboratory exercise is a simple way to teach students

about the potential danger that flies pose as vectors of infectious disease. It also allows for student-directed inquiry into fly behaviors that potentially reduce the risk of transmission. The exercise works with both the common house fly, *Musca domestica*, and the flesh fly, *Sarcophaga carnaria*, which is commonly found on decaying flesh. In this experiment, the flies are exposed to non-pathogenic *Escherichia coli*, and bacterial numbers are quantified.

This exercise also tests the flies' ability to clean the bacteria from their bodies, feet, and wings. The cleaning process has been shown to be important in the survival of other insects, including termites (Traniello et al., 2002). The termites groom to remove infectious bacteria. A fly continuously grooms by rubbing its feet together and sweeping its legs over its body. This grooming behavior may serve not only to rid itself of dangerous bacteria, but also to rid itself of dust and dirt that reduces its sense of taste and its ability to grip surfaces. Research conducted on fly grooming is sparse, but some data suggest that flies are able to remove macroscopic particles (Szebenyi, 1969). For example, the fly *Drosophila melanogaster* uses sweeping motions to pick up the yeast and concludes by rubbing it off its forelegs. In this exercise students can test the ability of flies to remove potentially harmful bacteria and can estimate the role of flies in the transmission of infectious bacteria.

The goal of this exercise is to investigate the role of flies as vectors of bacterial disease and fly behaviors that may reduce their vectoring ability. Students generated hypotheses to investigate the ability of two species of flies to vector diseases. In the first experiment, two species of flies were exposed to a bacterial culture to compare their abilities to serve as vectors of bacterial diseases. This initial experiment provided students with some interesting information but generated many more questions. Students then generated two more questions they wanted to address. In the second experiment, students compared the length of time that flies were exposed to bacteria to discern if the length of time is important in the ability of flies to accumulate bacteria. In the third experiment students investigated the role of fly cleaning behavior on the amount of bacteria vectored.

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# Procedure

## Maintenance & Preparation of Stocks

Bacterial cultures of *Escherichia coli* K12 were purchased from Carolina Biological Supply for about \$10 per culture. The stock culture was maintained on nutrient agar plates or slants in the refrigerator. (The *E. coli* K12 can also be purchased in liquid cultures, which should be stored in the refrigerator and used within a week for consistent results.) A small amount of the bacterium was grown in nutrient broth at room temperature 24–48 hours. The culture should be very turbid to get an inoculum of around  $1 \times 10^8$  cells/ml, which will be a volume of 0.1 ml, to spread evenly on fresh nutrient agar. After spreading the bacteria, plates should be incubated another 24–48 hours at room temperature. These plates should be completely covered with bacteria, which is called a bacterial lawn. These plates serve as the source of bacteria with which to contaminate the flies. Contamination works best if plates are used immediately after incubation and not allowed to dry out.

*S. carnaria* and *M. domestica* pupae were obtained from Carolina Biological Supply for about \$18 and were kept at room temperature in small aquaria with lids until emergence approximately one week after arrival. After emerging, flies should be provided with water on a damp paper towel or cotton ball. Flies were used for experimentation within two days of emerging. Prior to exposure to bacterial cultures, the flies were anesthetized by cooling them in the refrigerator and on ice for approximately three minutes. To catch the flies, a pair of flame-sterilized forceps can be used to grab the fly gently by the leg. Using one fly at a time for each student group worked the best.

## Experimental Procedure

### Experiment #1

After capture, the flies were transferred into the nutrient agar plates with lawns of *E. coli* K12. The lids of the plates keep the flies contained, but students had to rotate the plates to keep the flies off the lids of the plates. Flies were allowed to walk all over the plates of *E. coli* K12 for 30 seconds and then transferred to sterile nutrient agar plates for five minutes. After exposure, the plates were cooled in the refrigerator for three minutes and then the fly was removed. Flies were placed in the freezer to humanely euthanize them and then disposed of in the autoclave bags with the rest of the bacterial contamination. The nutrient agar plates were incubated at room temperature for 24 hours and subsequently examined for colony-forming units (CFU). Plates should be placed in the autoclave bag after use. The experiment was replicated ten times using different flies of each species. The data were statistically analyzed using a student's *t*-test with a probability of 0.05. We noticed that fly-exposed plates produced only what appeared to be colonies of *E. coli* K12 and that generally neither fly species caused lawns of bacteria. During the discussion of the data from this initial experiment, students developed two more hypotheses that they wanted to test: Does time of exposure matter and can flies reduce bacterial transmission with cleaning? The same basic procedure was followed for the next two experiments with minor changes.

### Experiment #2

To test the hypothesis that longer exposure times increase the amount of bacteria carried by a fly, *S. carnaria* were placed

on the culture plates of *E. coli* K12 and allowed to walk around for 5, 30, or 60 minutes. The flies were then transferred onto sterile nutrient agar plates for five minutes. After exposure, flies were removed from the plates, and the plates were incubated 24 hours at room temperature. The resulting colonies were counted. The experiment was replicated ten times using different flies. The data were statistically analyzed using a One Way Analysis of Variance with a probability of 0.05, followed by a Tukey test when differences were detected.

### Experiment #3

This experiment tested the ability of flies to clean themselves after exposure to bacteria, thereby reducing their ability to vector bacterial diseases. The flies were placed on the culture plates of *E. coli* K12 and allowed to walk around for five minutes. The flies were then transferred onto a fresh nutrient agar plate with no holding time or into a sterile Petri plate with no agar in it for a 30-minute or 60-minute holding period. After the holding time, flies were transferred to sterile nutrient agar plates for five minutes. After five minutes, the flies were removed from the plates and humanely euthanized. The experiment was replicated ten times using different flies. The plates were incubated for 24 hours at room temperature. After incubation, plate colony counts were conducted, and data were statistically analyzed using a One Way Analysis of Variance with a probability of 0.05, followed by a Tukey test when differences were detected.

## Safety Concerns

Instructors should be sure that they obtain only a Biosafety Level 1 bacterium, such as *E. coli* K12. These bacteria are not known to cause disease in healthy adults and pose minimal risk to students. Precautions that need to be taken include hand-washing and countertop disinfection, no food or drink while working on the experiment, and decontamination of wastes.

## Results

### Experiment #1

We conducted preliminary experiments using *M. domestica* and *S. carnaria* to determine if common filth flies can vector *E. coli* using simple laboratory exposure methods. The first experiment tested whether different flies could act as mechanical vectors of bacteria. *S. carnaria*, the flesh fly, spends much of its time feeding and reproducing on dead animals. *Musca domestica*, the house fly, is a filth fly. It can be found around garbage cans and dung. From this background information, the students hypothesized that both species would vector bacteria. Students found that both species of flies do serve as vectors (Figure 1). As can be seen in Figure 1, *M. domestica* is also capable of vectoring bacteria, but at a statistically significant ( $p < 0.001$ ) lower concentration than *S. carnaria*. Students can think of reasons why there may be differences. One possible difference could be size; *S. carnaria* are much larger flies than *M. domestica*. This could lead to further tests of size differences in a species based on nutrition. Our students were more interested in the length of time that flies were exposed to bacterial contamination. Their experiment had only allowed 30 seconds of exposure; they were interested in the amount of bacteria that could be vectored after extended exposure. The students devised a slight variation to the original experiment, which we have designated Experiment #2.

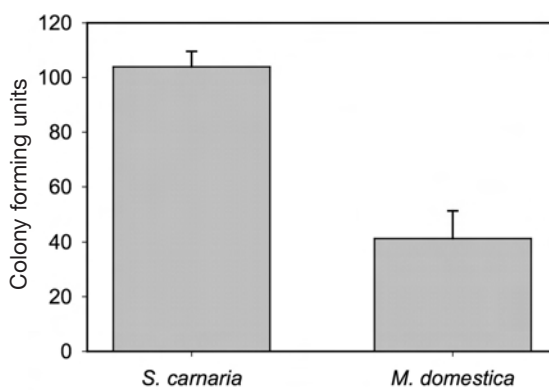
## Experiment #2

In Experiment #2, students tested whether or not different exposure time to the bacteria affects the flies' ability to vector bacteria. Students hypothesized that flies would vector more bacteria when they had been exposed to the bacteria for longer periods of time. The data in Figure 2 show typical results. Our students found that there is no significant difference between the vectoring ability of the flies after 5, 30, or 60 minutes of exposure to *E. coli* K12. Students came up with possible explanations for this phenomenon. They thought that since it does not matter how long a fly feeds, probes, or walks on a surface containing bacteria; the fly's surfaces must be completely covered with bacteria within the first five minutes of exposure. Still interested in how they might manipulate vectoring ability, students wanted to look at fly cleaning. They could not find much in the literature on the flies' ability to clean and how cleaning affects disease transmission, so students devised a third experiment.

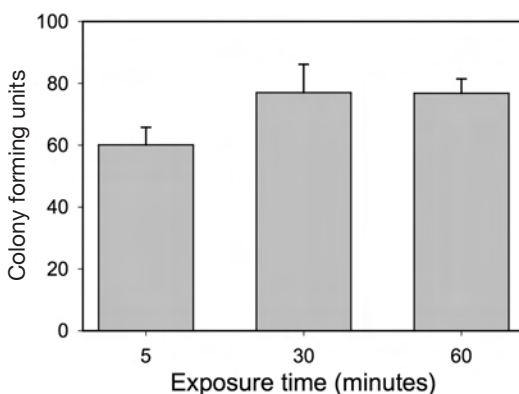
## Experiment #3

The goal of Experiment 3 was to test whether or not flies clean themselves and if their grooming practices affect the amount of bacteria they are able to vector. Students had a split opinion of whether or not flies could clean themselves enough to change disease transmission. In this experiment students exposed the flies to bacteria and then held them in a sterile Petri plate for various times before placing them on nutrient agar plates. Students were able to observe and document fly cleaning behavior during the experiment. Results showed that there is significant reduction in the amount of bacteria vectored when flies are given the opportunity to clean themselves (Figure 3). The amount of *E. coli* K12 present on the flies after each of the three holding times was significantly different ( $p < 0.001$ ). Students also noted that flies did appear to clean themselves during the first few minutes in the sterile containers. These data helped our students to realize that although flies are capable of vectoring disease-causing bacteria, flies are not as "dirty" as they may have thought.

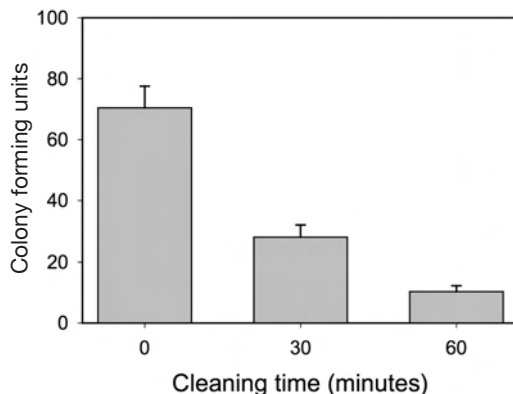
**Figure 1.** Influence of fly species on amount of *E. coli* vectored. Bars represent Mean  $\pm$  1 S.E.; N = 10. *S. carnaria* vectored significantly more *E. coli* after a five minute exposure (t-test,  $P < 0.001$ ).



**Figure 2.** Influence of exposure time on amount of *E. coli* vectored by *S. carnaria*. Bars represent Mean  $\pm$  1 S.E.; N = 10. No significant differences were observed (ANOVA,  $P > 0.05$ ).



**Figure 3.** Influence of cleaning time on amount of *E. coli* vectored by *S. carnaria*. Bars represent Mean  $\pm$  1 S.E.; N = 10. Each consecutive cleaning time significantly reduced the amount of bacteria vectored (ANOVA,  $P < 0.001$ ).



## Discussion

This exercise was straightforward and easy for students and was a great opportunity for student-directed inquiry and cooperative learning groups. There are nearly endless possibilities for students to develop questions, design experiments, and address real issues. Besides the three questions that our students addressed in this exercise, students could compare filth flies to non-filth flies, size of flies, or bacterial species to which flies are exposed. Instructors can talk about different diseases vectored by non-biting flies, which could lead students to look at transmission of yeast or mold. Students could also be asked about managing insects and how important it might be to reduce disease transmission as compared to the dangers of increasing exposure to the toxins in pesticides. Instructors can find many exciting ways to adapt this exercise to fit into their curriculum.

## Acknowledgments

This project was supported by NIH Grant #2 P20 RR16469 from the Nebraska INBRE Program of the National Center for Research Resources and the University of Nebraska at Kearney Research Services Council.

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