stocks (A and B) that have normal chromosomes. You could not mix the two stocks together and compare offspring production, because the two stocks have the same, wild type, phenotype. Yet, if you place stock A in a bottle with a compound autosome stock that is marked with a visible mutation, you could measure the proportion of A offspring and compound autosome offspring. This progeny ratio could then be compared to the results of B flies and the compound autosome. This would give the fitness of A and B stocks compared to the compound autosome stock and, therefore, the fitness of stocks A and B compared with each other. The compound-autosome technique is a one-generation test of overall fitness, including mating ability, fecundity, fertility, and viability, and has been used to measure the fitness of stocks in the presence and absence of active transposable DNA elements (Belyaeva *et al*., 1982; Woodruff *et al*., 1999) and the fitness of stocks that are resistant or sensitive to insecticides (Minkoff and Wilson, 1992).

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Drosophila smoking: using flies in a smoke-free class.

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Overview

Smoking habit is responsible for 71% of cases of lung cancers, 42% of deaths for chronic respiratory disease, and 10% of cardiovascular diseases (Öberg *et al*., 2010). According to the World

Health Organization (WHO), tobacco caused 100 million deaths in the twentieth century (WHO 2011).

Adult *Drosophila melanogaster*, like many vertebrates, when exposed to cigarette smoke or nicotine are initially stimulated, but higher concentrations are lethal to flies (Wolf and Heberlein, 2003). There is wide genetic variation for resistance to nicotine in natural populations and laboratory stocks (Passador-Gurgel *et al*., 2007). These characteristics make *Drosophila* an excellent model to class activities, allowing the students to observe directly the harmful action of smoking. We hope that this experience would collaborate to reduce the smoking habit. Also, the activity allows students to conduct experimental research using hypothesis tests and statistical analysis. It has been suggested that it is crucial for scientific literacy that the students can experience the nature of science -NOS (Miller *et al*., 2010). The activity here described offers a possibility to understand better how scientific knowledge is produced.

The experiment consists in comparing the recovery time and mortality rates for different *Drosophila* strains exposed to CO_2, cigarette smoke, and smoke provided by burning of different vegetables, such as grass, tea, or some seasonings. The flies are exposed to a saturated atmosphere with CO_2 or smoke. For the recovery time is registered the time at which the first and last fly begins to move. The flies that do not recover are registered as dead for quantification of mortality rate.

The experiment proposed can be useful in many school levels, from fundamental school to under-graduation. Of course, the amplitude and profundity should be modulated by the objectives of the course, the syllabus, age of students, and proposal of the teachers. In the fundamental school can be a concrete and immediate demonstration of the damage caused by cigarette smoke, associated with learning of healthy habits and combat to smoking. At higher levels of schooling others aspects can be added. For example, different experimental designs can be proposed, as the control of variables as exposition time, different smoke, genotype of the flies, and so forth. Also, the sample size (flies for experiment) and the number of experimental replicas can be included as themes for learning.

Constructing Some Apparatus for the Experiment

To expose the flies to cigarette smoke, the teacher could simply aspire the smoke and, using a tube, to expire the fume to the bottle in which there are the flies. However, as we want to discourage the smoking habit, this procedure is strongly not recommended. To expose flies to CO_2 and the fume, we have made the apparatus shown in Figure 1. For CO_2 we have used a small PET bottle and, in the cap, is done a hole and fixed a plastic tube using epoxy putty. Water is placed in the PET bottle and the CO_2 is produced by placing an effervescent antacid tablet in it. The CO_2 is transferred to the bottle with the flies by the plastic tube (Figure 1-A). Also, the gas from a soda can be used, putting soda in the PET bottle and shaking.

The smoke is produced using another apparatus. A wash bottle is attached to a plastic tube, and this one is linked to the cigarette into a glass vial. From the glass vial is another plastic tube leading the smoke to the bottle with the flies (Figure 1-B).

To avoid secondhand smoke it is highly recommended to use fume hoods or, alternatively, this part of the activity could be done in an open environment. The reasons of such care should be discussed with the students.

Observed Results

Figure 2 shows an example of results that can be obtained in a classroom activity. The objective was to compare the recovery time after 30 minutes of exposition to CO_2 or to cigarette smoke. Four strains of flies were used in this activity: *D. melanogaster* wild type (Dm wt), *D.*

melanogaster sepia mutant (Dm se), *D. simulans* wild type (Ds wt), and *D. simulans* yellow mutant (Ds ym).

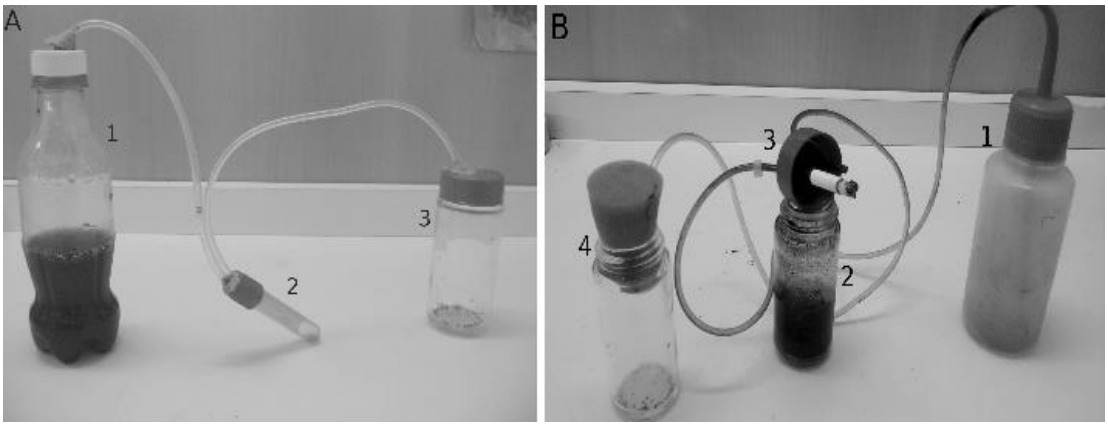

Figure 1. A) Apparatus for CO_2 exposure of flies. A PET bottle (1) with water and an effervescent antacid tablet is connected by a tube to a vial with the flies (2). We include a reservoir between the bottle that is source of CO2 and the vial with flies, as a security system, to avoid liquids reaching the flies (3). B) Apparatus for exposing the flies to smoke. A wash bottle (1) is connected by a plastic tube to a glass vial (2). In a CAP of this vial is attached a 1 ml micropipet tip to affix the cigarettes (3). From this vial one plastic tube is connected to another bottle with the flies (4).

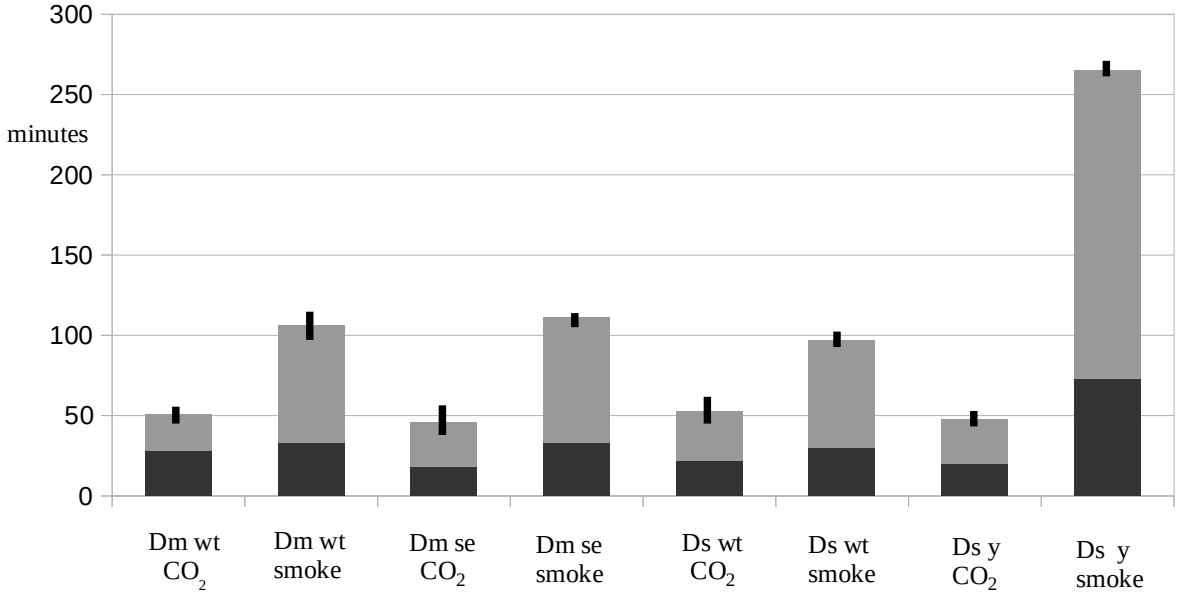

Figure 2. Time, in minutes, in that the first fly becomes active (dark gray), and that all the flies become actives (light gray) after having been exposed for 30 minutes to an atmosphere saturated with CO_2 or cigarette smoke. Dm wt = wild type strain of *D. melanogaster*; Dm se = sepia strain of *D. melanogaster*; Ds wt = wild type strain of *D. simulans*; Ds y = yellow strain of *D. simulans*. Black lines in the bars represent the standard deviation.

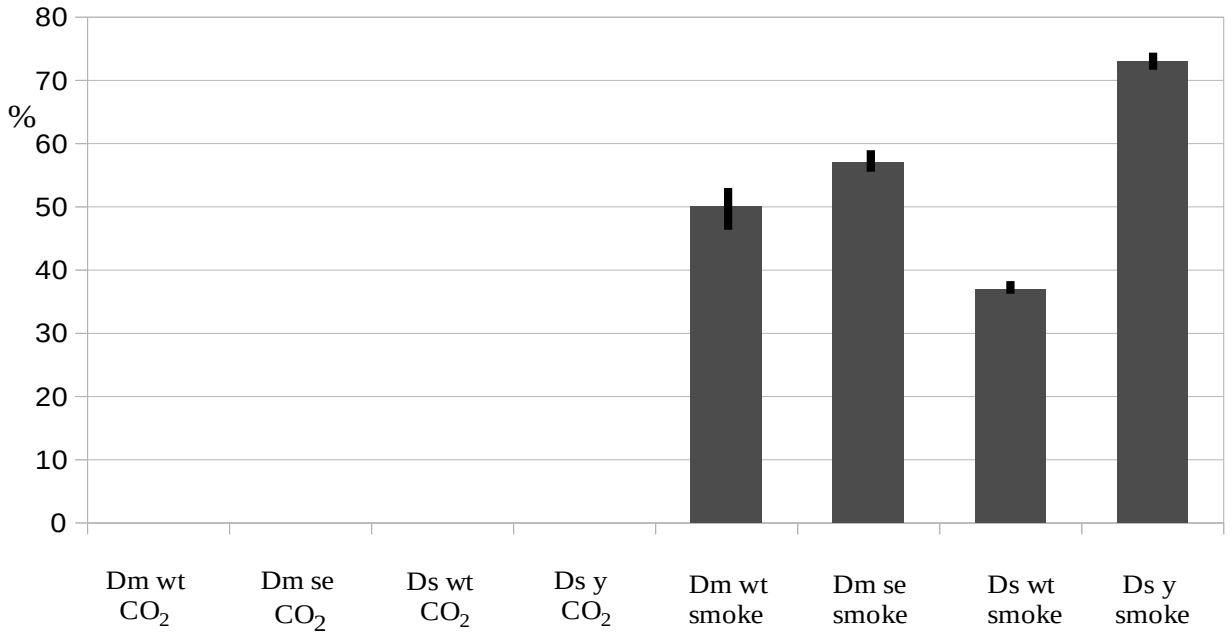

Figure 3. Mortality rate (in percentage) for flies exposed to CO_2 and to cigarette smoke. Dm wt = *D. melanogaster* wild type; Ds se = *D. melanogaster* sepia; DS wt = *D. simulans* wild type; Ds y = *D. simulans* yellow. Black lines in the bars represent the standard deviation.

The recovery time reported is the average from three independents replicas, each one using a sample of approximately 30 flies. The exposure to cigarette smoke was standardized; in each trial the flies were exposed to the burning of two centimeters of cigarette, during 30 minutes. The recovery time and mortality were registered. The recovery time was around fifty minutes for CO_2 for all strains, and for flies exposed to cigarette smoke, this time was twice. The exception was the *D. simulans* yellow strains that spent about 270 minutes to recover from the exposition to fume.

Analysis of variance (ANOVA) showed that the treatments using CO_2 and cigarette smoke differ significantly in the samples. The "t" test showed us that *D. simulans* yellow is more susceptible to cigarette smoke than other strains, and it is a good example of genetic diversity in response to environmental factors.

A more remarkable result was the high rate of mortality observed in the flies exposed to cigarette smoke as compared the absence of mortality in flies exposed to the CO_2 (Figure 3). Differences among the strains can be observed, suggesting genetic variation to the resistance or tolerance to the cigarette smoke.

Once well settled on the protocols and the procedures to expose the flies to cigarette smoke, new treatments were applied to the four *Drosophila strains*. We have done some "cigarettes" using grass (*Paspalum notatum)* or teas, as for example leaves of herb mate (*Ilex paraguariensis*). However, when the flies were exposed to smoke of these "cigarettes" the results were similar to those observed using CO_2 , showing that it is the chemical compounds present in the tobacco that are responsible for the mortality of flies and not from any fume.

Experiments using shorter exposition time, as 10 or 15 minutes, produce similar results and the class duration will be, also, shorter.

Didactic Possibilities

Different types of experiments in which *Drosophila* are exposed to CO_2, cigarette smoke, or smoke from other plants can be configured. Demonstrative activities allow the observation of the damage caused by smoking. While CO_2 and fume produced by the burning of most plants only produce anesthesia in the flies, cigarette smoke produces a high mortality. These observations can be associated to discussion and advertisements about smoke-free. Higher skills like setup of experiments, control of variables, statistical analysis, and so forth can be developed if the activities were done as a project. For example, analyses of genetic variability for nicotine susceptibility allow discussion of themes like pharmacogenetics. The literature reviews on the phenomena observed can lead to a deeper understanding in physiology, biochemistry, genetics, and other disciplines.

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54th Annual *Drosophila* Research Conference

The 54th Annual *Drosophila* Research Conference was held on 3-7 April 2013 in Washington, D.C. The 2013 Organizing Committee was Richard Mann (Columbia University, New York, NY), Hannele Ruohola-Baker (University of Washington, Seattle, WA), Kristin Scott (University of California, Berkeley, CA), and David Stern (Janelia Farm Research Campus, Ashburn, VA). The conference was sponsored by The *Drosophila* Board in association with the Genetics Society of America, 9650 Rockville Pike, Bethesda, MD 20814-3998. The Program and Abstracts Volume lists two Plenary sessions, 156 platform session talks, 807 posters, and 12 workshops.

Historical Address Speaker

Jules A. Hoffmann (IBMC, University of Strasbourg, Strasbourg, France). Innate immunity: From flies to humans.

Plenary Lectures

Marc R. Freeman (University of Massachusetts Medical School/HHMI, Worcester, MA). Molecular mechanisms of axon degeneration.

Tom Clandinin (Stanford University, CA). Genetic approaches to dissecting neural computation in the visual system.

Chris Jiggins (University of Cambridge, Cambridge, UK). The genomics of speciation and pattern evolution in (butter)flies.

Naama Barkai (Weizman Institute, Rehovot, Israel). Creating gradients by morphogen shuttling.

Leanne Jones (Salk Institute, La Jolla, CA). Maintenance of niche function and tissue homeostasis during ageing.