Epigenetic Effects of Diet on Fruit Fly Lifespan: An Investigation to Teach Epigenetics to Biology Students

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Abstract

Do our genes exclusively control us, or are other factors at play? Epigenetics can provide a means for students to use inquiry-based methods to understand a complex biological concept. Students research and design an experiment testing whether dietary supplements affect the lifespan of Drosophila melanogaster over multiple generations.

Key Words: Epigenetics, Drosophila, diet, longevity.

Traditionally, biologists understood that DNA nucleotide sequences could be passed from parent to offspring. If alterations or mutations of the DNA occurred, then these changes could be passed through multiple generations. This same idea has been taught to generations of biology students, who have learned that organisms inherit their genetic makeup solely from their parents' DNA sequences. Recent evidence shows that factors outside of the DNA sequence can be inherited (Holliday, 1990; Reik et al., 2001; Bird, 2002; Cooney, 2006, Dolinoy et al., 2006). These factors modify gene expression and heritability (Szyl et al., 2008). The change in gene expression is attributable to chemical tags composed of methyl or acetyl groups, which are responsible for modifying the way DNA is packaged (Jones & Takai, 2001; Niculescu & Zeisel, 2002). These chemical tags, known as epigenetic markers, can be used to either silence or express genes that they affect (Jaenisch & Bird, 2003; Kovalchuk, 2008). Epigenetic markers can be turned on or off at any time during the life of an organism. It is believed that environmental factors can influence whether epigenetic modifications occur (Brakefield et al., 2005; Dolinoy & Jirtle, 2008).

Translated literally, epigenetics is “above the genome.” This process changes gene expression without altering the DNA sequence and is subsequently inherited by offspring (Bird, 2002). Methylation and acetylation are two main types of epigenetic processes that contribute to gene imprinting that results in undesirable consequences for individuals and their potential offspring. The addition of methyl groups can lead to genes being silenced and therefore not expressed (Jones & Takai, 2001; Niculescu & Zeisel, 2002). Specific cancers can grow uncontrollably when the tumor suppressor gene p53 is silenced (Kim, 2005; Burbans & Weinberger, 2007). Fortunately, the effects of epigenetic imprinting can potentially be reversed in cases where environmental toxins affect the epigenome of the developing fetus (Dolinoy & Jirtle, 2008) so that methyl groups can be removed by environmental conditions (Holliday, 1990).

Acetylation can cause modification of chromatin and the corresponding histone proteins. DNA can either be tightly bound or folded, resulting in genes being silenced and not expressed. Chromatin can also be loose on the DNA sequence, which influences gene expression or activation (Kunert et al., 2003). These modifications were once believed to be uninheritable, unlike the DNA sequences that are passed to daughter cells. But recent evidence has shown that epigenetic modifications not only affect an individual, but affect their offspring for multiple generations (Dolinoy et al., 2006).

Multiple factors are hypothesized to affect the “epigenome.” One of these is change in the diet of an organism, tested by supplementation with a specific compound and determination of its effects on the organism’s epigenetic makeup. Using soy as a source of genistein, a phytoestrogen, Dolinoy et al. (2006) showed that the latter has epigenetic effects on coat color and obesity in future generations of mice. Soy is linked to lower incidences of cancer and obesity, which could lead to an increase in longevity (Barnes, 2004). In Dolinoy et al.’s (2006) study, genistein from soy increased the rate of cell methylation in mice given the supplement, and the higher methylation rates influenced coat color and fat in the offspring (Dolinoy et al., 2006). It has been suggested that dietary supplementation could have the same effect on fruit flies (Drosophila melanogaster) by increasing lifespan (Piper et al., 2005).

In another study, pregnant mice fed a diet supplemented with zinc, choline, and folate were compared with a group fed a standard lab diet. All the mice were genetically identical. The results indicated that the epigenetic markers were passed on to their children and grandchildren (Cropley et al., 2006). Evidence has shown that foods that contain these supplements alter epigenetic markers and can alter gene expression (Niculescu & Zeisel, 2002).

Effects of chemicals on the germ lines of male mice were tested in a study designed to replicate pesticide use. The environmental toxins vinclozolin...
and methoxychlor reduced F1 male sperm count and motility and increased the chances of infertility. Male offspring of the affected mice in generations F2 through F4 had an increased rate of infertility because of low sperm count and weak sperm motility. The results indicated that germ-line epigenetics can be reprogrammed to influence future generations. The reprogramming was attributed to DNA methylation patterns (Anway et al., 2005).

Understanding the role that epigenetics plays in evolution and inheritance is important for beginning biology students. Most of the relevant laboratory exercises and activities used in biology classrooms are restricted to inheritance of DNA, and little information is available regarding epigenetics. Here, we describe an inquiry-based activity that uses the fruit fly to help students understand epigenetics and its role on lifespan. Suitable at various levels of study, this investigation allows students to design and implement a long-term controlled experiment to measure the effects of diet on the lifespan of fruit flies.

The students are given a general protocol at the beginning of the investigation. They conduct research to learn the effects of various dietary supplements – some of the choices increase and others decrease lifespan – and choose the one they will investigate (two articles that the course instructor could provide for this research: Cohen, 2003; Pray, 2004). On the basis of their research, the students develop hypotheses about the effect of supplementation on the lifespan of fruit flies. Then they perform the experiment, collect and analyze data, and draw conclusions. Later they present their results to the class in the form of a peer-reviewed journal article or a poster, as if they were presenting at a conference.

Below, we present and discuss our own results from conducting the experiment with students. Note that the time needed to obtain significant results could be 16 weeks or more, and planning is of the utmost importance.

O Materials & Methods

Wild-type fruit flies (Carolina Biological Supply, catalogue no. 17-2100) were raised in 75-mL plastic vials with foam plugs. An incubator with a temperature range of 24–26°C was used to maintain the fruit flies at constant temperatures. A photoperiod of 12 hours light and 12 hours dark was maintained for the duration of the experiment. Carolina Biological (catalogue no. 17-3200) fruit fly medium was prepared according to the manufacturer’s instructions. Total food medium in the amount of 15 mL was added to each vial. Control vials contained 100% plain medium, and experimental vials contained 10% of the additive. The food supplements tested were garlic, wheat germ, and soy (tofu). Fleischmann’s brand baker’s yeast in the amount of 0.1 mg was added to the top of the food medium in each vial. For the lifespan experiment, 10 females and 10 males from stock bottles were added to each vial (three replicates were set up for each treatment). Fruit flies were allowed to mate for 96 hours, and then the parents were removed and placed into new culture vials containing the specified food additive. Newly emerged flies were allowed to grow and reproduce for three generations. Flies were transferred to new food media weekly and dead flies were recorded. Statistical analysis on survival was done by performing a Wilcoxon test using JMP software, version 4 (SAS Institute, Cary, NC), and figures were made in Microsoft Excel 2007.

To increase their learning of concepts, students can be divided into heterogeneous groups and work cooperatively. In this approach, it is important that each student has the opportunity to participate in different aspects of the activity. Students who actively participate develop a greater connection to the content and can integrate their newly acquired knowledge with material previously learned in the course.

O Results

Lifespan and a generational difference were tested for each individual supplement compared with the control. In the first generation, all the supplements produced similar results as measured in percent lifespan, with the exception of garlic, which significantly reduced percent lifespan (p = 0.0002; a 14-day reduction in lifespan; Figure 1A). In generations 2 and 3, there was no significant deviation from the unsupplemented control (Figure 1B, C). Comparing generations within treatments, the fruit flies reared on unsupplemented control media (Figure 2A) and wheat germ (Figure 2B) did not differ significantly between generations. Supplementation with garlic resulted in a significant difference in lifespan between the first and second generations (p = 0.0011; Figure 2C). Supplementation with soy resulted in a significant difference between the first and third generations (p = 0.0068) and between the second and third generations (p = 0.0115), but not between the first and the second generations (Figure 2D).

O Discussion

The hypothesis for this experiment was that the supplements added to the fruit flies’ diet would negatively affect the lifespan of future generations. The garlic supplement initially reduced the lifespan of the fruit flies within the parent generation, but this effect did not persist in the next two generations. Garlic may have caused methyl or acetyl tags to not be placed on the histone proteins, so that a heritable epigenetic marker was not created. By contrast, the soy supplement caused a significant
would be to test different diets on the same genotypes. This would deter-
mine how a specific dietary supplement would alter the lifespan of a
group of similar individuals.

Quantifying modifications of DNA methyl tags is another possible
method for determining how diet affects the epigenome. When genes
are methylated, they have the ability to become activated when normally
they would not be expressed. During development, genes are regulated at
specific times and DNA methylation patterns influenced by dietary sup-
plements could be used to detect abnormal development of the embryo
(Bird, 2002). According to Stancheva and Meehan (2000), recent studies
have demonstrated that gene expression is inactivated in the frog from
fertilization to mid-blastula stage. The DMNT1 (DNA [cytosine-5-]
methytransferase 1) gene down-regulates gene expression during this stage of
development, but if the gene is demethylated, some genes will become
prematurely activated. Studies could be conducted to determine whether
dietary supplements affect developmental genes of a variety of organisms
such as fruit flies, frogs, or mice. If developmental genes are prematurely
activated, it could be determined that DNA methylation patterns have
been altered and quantified by abnormal development and growth pat-
terns of the experimental animals. These types of experiments are not nec-
essarily feasible at the middle or high school level but could be conducted
by upper-level undergraduate students as part of an independent research
experiment supervised by a scientist trained in this area.

This project gives students an opportunity to conduct research in an area
of science they may not have known existed. Most students in introductory
biology courses have not been exposed to epigenetics and its implications,
but they can build on earlier instruction in molecular genetics to under-
stand the mechanisms of epigenetics. Learning about the effects of modified
gene expression, students begin to question whether certain traits are strictly
genetic or have an environmental influence on gene expression.

Many laboratory investigations in biology classrooms are exercises in
following a protocol provided by the instructor and obtaining results that
may or may not be understood by the student. Allowing the student to
propose a problem, research current literature, and design an experiment
gives them ownership of their learning. Hands-on use of the scientific
method contributes to a better understanding of concepts and applica-
tions introduced in lecture (National Academy of Sciences, 1996), and
the excitement of obtaining real data can open students’ minds to a possi-
bile future career in investigative research, something they may not have
considered before.

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