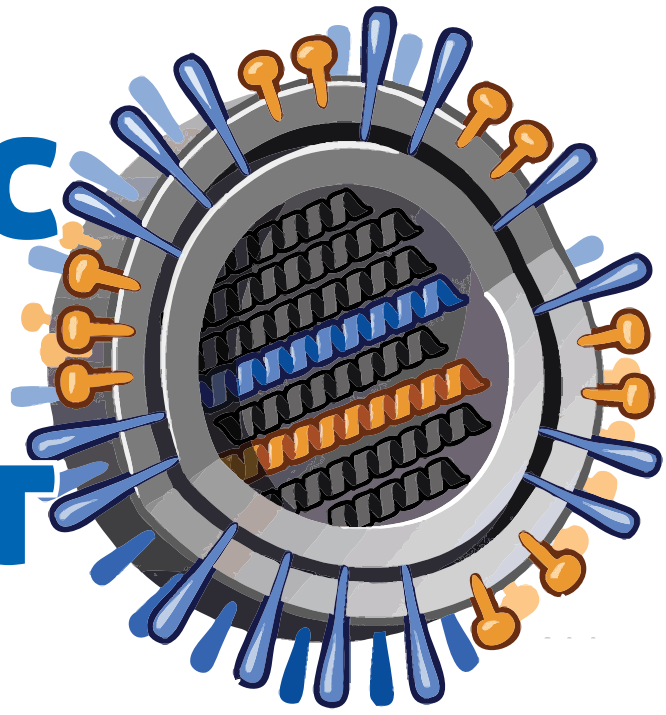
Antigenic Shift and Drift



Modeling the evolution of the influenza virus

Meena Balgopal and Cindi Bondy

It's that time of year again, when avoiding the flu is on everyone's mind. As we brace ourselves for possible flu outbreaks, the need to understand biological issues related to this virus becomes clear. Through modeling, the lesson presented in this article helps students understand how the influenza virus (or flu) evolves and how flu vaccinations are selected each year.

Modeling

Students often have difficulty understanding scientific processes they cannot see (Lawson et al. 2000), so providing them with materials they can manipulate and visualize can aid their understanding. We designed the activity presented in this article—using Styrofoam cup models of the virus, pushpin proteins, and RNA strands made of pipe cleaners or yarn—to demonstrate how medical researchers determine what type of flu virus vaccine to develop each year.

We use this lesson in an Advanced Placement Biology class. Students make models of the flu virus, learn how it can evolve quickly and unpredictably, and generate data through a role-playing activity.

The virus

Viruses are amazingly diverse biological agents known to infect the cells of a wide range of organisms. Though viruses are generally not considered living (and do not belong to a domain or kingdom), some scientists consider them to be organisms because they contain genetic material, are obligate symbionts (usually pathogenic or, in rare cases, mutualists), and have the ability to evolve (Villareal 2004).

Viruses can contain either DNA or RNA genetic material. Both enter a host cell by binding to receptor proteins; they then manipulate their host cell's machinery to produce new virions (individual virus particles). RNA viruses are sometimes retroactively transcribed into DNA before normal transcription and translation occurs.

Of the millions of viruses out there—HIV (human immunodeficiency virus), herpes virus, West Nile virus, Feline Leukemia virus, Beet Yellows virus, and so on—influenza is the most well-known in the United States because of the number of infections that occur each year. Annually, 5–20% of the U.S. population contracts the flu and up to 200,000 people may be hospitalized each year (CDC 2010).

The flu virus is a type of *Orthomyxovirus* of which there are three types: A, B, and C. The three types, or genera, of influenza virus are determined by several factors, including host range, antigenic differences, variability of surface proteins, genome organization, and morphology (Wilschut, McElhaney, and Palache 2006). Influenza C is not considered an important pathogen for humans.

Influenza A is the most medically significant type of influenza virus because it can cause a pandemic—an epidemic that spreads across more than one continent. Well-known outbreaks include the 1918 Spanish Flu, the 1957 Asian Flu, and the 1968 Hong Kong Flu. Millions of people died during each of these pandemics. More recently, the avian flu outbreak in 2004–2005 and the swine flu (i.e., H1N1) outbreak in 2009–2010 have been cause for concern (Liberatore 2008; Liberatore 2009).

How does the influenza A virus spread? It attacks the mucous membranes of the respiratory tract or eye region—causing shivers, headaches, body aches, sore throat, cold-like symptoms, and a cascade of high fevers (the body's initial defense against the virus is to heat up, which slows viral replication). Other symptoms include extreme fatigue, coughing, runny nose, aching muscles, and nausea. Coughing allows the virus to pass from one person to another through airborne droplets of sputum, a mixture of saliva and mucus.

The anatomy

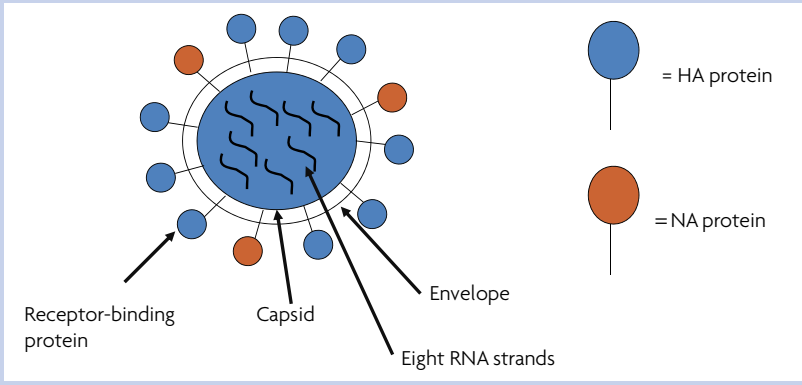
Viruses are comprised of genetic material (RNA or DNA, though influenza viruses only contain RNA segments); a protective coat called a *capsid;* and in some cases, an additional outer coat called an *envelope,* made up of proteins, sugars, and lipids stolen from the virus's host cell. Each individual virus particle is called a *virion*.

Many viruses also have spike-like proteins on the surface of their capsid or envelope. Two of these "spike" proteins are found on influenza A and B viruses: hemagglutinin (HA) and neuraminidase (NA) (Figure 1). Two viral RNA segments (of a total of eight RNA segments within each flu virion) encode for these two proteins. HA is more common; it binds the virus to the host cell's receptors, so it can enter the cell. NA is less common, but equally important; it enables "offspring" virions to exit the host cell and infect new ones.

FIGURE 1

Influenza A virus.

Influenza A virus with hemagglutinin (HA) and neuraminidase (NA) spikes around the capsid.



In medicine and in the general public, we refer to flu strains by their HA and NA combinations. Because there are 16 types of HA and 9 types of NA, there are numerous possible combinations (Doherty and Turner 2009). The 2009–2010 outbreak of "swine flu," for example, was called *H1N1* because it contained type 1 HA and type 1 NA proteins.

Each year, medical researchers try to predict the most common strain of influenza virus (i.e., the combination of the HA and NA types) to produce an effective vaccination. Vaccines are made to recognize both HA and NA proteins and are, therefore, specific to a particular virus strain. However, it is important to note that an individual can be vaccinated against the flu strain predicted to be the most prevalent for that year, and still be infected by a different—less common—strain.

Medical researchers make new flu vaccines each year because of the virus's ability to evolve quickly and unpredictably.

Evolution

Antigenic shift

The flu virus evolves using two mechanisms—antigenic shift and antigenic drift. Antigenic shift is also called *RNA reassortment*. Reassortment occurs among the RNA segments within a respiratory cell that has been infected by two strains of a virus when the two viruses exchange genetic material. Because avian, swine, and human viruses are similar, they can share RNA segments if they have infected the same cell.

Pigs, for example, can be infected with both swine and avian flu virus strains, allowing RNA reassortment to occur in those cells that are infected. This produces novel combinations of HA and NA proteins. Most avian flu viruses do not cause symptoms in birds, but pigs infected by the swine flu virus exhibit symptoms similar to humans infected with influenza. Pigs may cough or have runny noses, and humans who have contact with these animals can become infected with a new hybrid strain.

There are three types of antigenic shift:

- The first occurs when an intermediate host (often a pig) is infected with both an avian influenza A strain and a human influenza A strain. This results in a new strain of the virus that can infect humans.
- The second occurs when an avian influenza strain jumps directly from birds to humans (i.e., there is no intermediate host). *Jumping* happens when body fluids of two different species come into contact, allowing the virus to move from one organism to another.
- The third type occurs when an influenza strain that does not undergo genetic changes jumps from a bird to an intermediate host and then to a human.

Antigenic drift

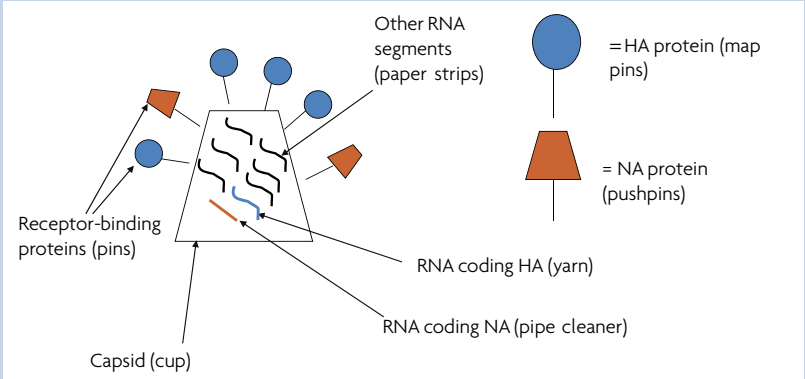
The second mechanism that allows the flu virus to evolve is a more gradual process called *antigenic drift,* which refers to mutations that occur by chance when gene sequences change on RNA segments. This type of mutation results in slight changes to the HA and NA proteins. If a person has received a vaccination that recognizes specific forms of the proteins, then antigenic drift might result in viruses that are not detected by the vaccine.

The lesson

The objective of this one-period 5E—Engage, Explore, Explain, Elaborate, and Evaluate—lesson (Bybee 1993) is to generate several combinations of HA and NA proteins, allowing students to determine which strain is most common in their classroom population and, therefore, which

FIGURE 2

Influenza A virus model.



warrants a vaccination. The activity involves modeling and can be used as a prompt for a whole-class discussion on viral biology, evolution, or public health.

To begin, students create models of the flu virus (Figure 2) using the following materials:

- Styrofoam cups labeled "pig," "duck," and "person"
- dressmaker or map pins (HA proteins)
- pushpins of various colors (NA proteins),
- 8 cm pieces of yarn (RNA encoding each type of HA protein)
- pipe cleaner pieces of various colors (RNA encoding each type of NA protein)
- strips of paper (nontranslated RNA segments)
- paper bowls labeled "pig respiratory cell"

The procedure

The following instructions guide the teacher through each step of the lesson.

Setup

Label the Styrofoam cups so that there are equal numbers of "pigs," "birds," and "humans." Label the paper bowls as "pig cell" (Figure 3) and place these on a materials table along with piles of yarn, pipe cleaners, push pins, and map pins. The colors of the yarn should correspond with those of the pushpins, and the colors of the pipe cleaners should correspond with those of the dressmaker or map pins.

Engage

Ask students whether they receive flu vaccinations each year and whether they have had the flu. Have any come down with the flu after being vaccinated, and if so, can they explain why? Have students take a pretest that combines both open-ended and recall answers (see "On the web"). We have found that the pretest helps us identify what preconceptions students have about the flu virus and viral evolution. We suggest that students take a posttest at the end of the lesson, as well—prior to the class discussion on whether antigenic shift is a mechanism of evolution. Afterward, hand out a recent newspaper article, listen to a podcast, or watch a news report to inform students about the most recent swine flu epidemic (see "On the web").

Explore

Hand each student a labeled foam cup and have them create a model influenza virus using the materials from the materials table. The labeled cups (i.e., pigs, birds, and humans) guide students as to which color string, pipe cleaners, push pins, or map pins they should use (Figure 4, p. xx). Using a teacher-made model, provide a brief introduction about viruses and their structure. Then have students use their models to describe the general properties of viruses.

Explain that viruses contain genetic material in the form of RNA or DNA (the yarn and pipe-cleaner pieces) and a capsid (the cup), and that some also have an additional coat, or envelope, which is not represented in our virus model. Remember to explain that influenza viruses only contain RNA segments. This is a good time to ask a series of questions about viruses:

- Are viruses living?
- Can viruses evolve and, if so, how?
- What are the characteristics of living organisms (e.g., made of cells, use energy, respond to stimuli, reproduce, grow, pass down genetic material)?
- Do viruses exhibit any of these characteristics?

Explain

Explain the "mixing vessel" hypothesis to students—which states that because pig respiratory cells can be infected by avian, swine, and human flu strains, when coinfection of the cell occurs, RNA from the different strains can be swapped during antigenic reassortment of RNA segments, resulting in new viral strains (Figure 3). These new viral strains can be particularly lethal to humans and are easily spread through coughing and sneezing because they infect lung tissue cells.

Then, have students form "minicommunities" of at least one pig, one duck, and one human (though there may be more than one of each organism per group). Each group picks up a paper bowl labeled "pig cell" to enact the "mixing vessel" stage. Ask the ducks and pigs in each group to remove one strand of yarn and one pipe cleaner and place them back in their group's paper bowl—the color does not

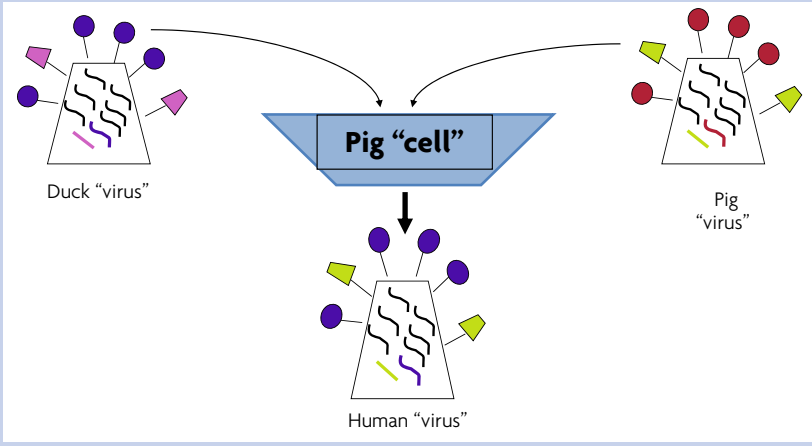
matter. Then have students randomly (perhaps with their eyes closed) take a piece of yarn and a pipe cleaner and place them back into their cups (the capsid). The colors of the yarn and pipe-cleaner pieces do not matter. This is the first step in antigenic reassortment.

The humans and pigs then repeat this step, completing the antigenic reassortment process (mixing has just occurred). Have students alter the receptor-binding proteins according to their new genetic material (i.e., use the extra pins on the materials table instead of push pins or map pins). Ask students to explain why they needed to change their push pins and map pins after reassortment occurred (the answer is because new RNA segments code for different HA and NA protein types, creating a new virus strain.)

FIGURE 3

Reassortment of genes.

Reassortment of genes in infected pig respiratory cells can result in new strains of flu virus that can be virulent in humans. The "mixing vessel" hypothesis describes how antigenic shift occurs.



Elaborate

Ask all the pigs to come to the front of the classroom. Count the number of strains the class created and record the data on the board (types of strains and how many individuals created each strain; Figure 5). Ask students if there are any strains that are more common than others and tally them.

Initiate a discussion about antigenic shift and evolution by asking students to reiterate how the mixing vessel hypothesis can explain antigenic shift. Remind students that evolution can be defined as "changes in allele frequencies in a population over at least one generation"; it does not necessarily have to result in new species. Ask whether a new viral strain should be considered a new species, and why or why not. Students will have to revisit their assumptions about the species concept they most often use (biological species concept refers to sexually reproducing, extant organisms; however, there are other species concepts). Finally, ask: "Using this definition of evolution, can antigenic shift be considered a mechanism for evolution?"

Evaluate

The Centers for Disease Control and Prevention (CDC) uses similar methods of surveying strains to predict which version of a vaccine to produce for the approaching flu season. As a class, have students determine which of their strains is most common. End the activity with a class discussion about annual vaccinations and determine whether the data set was large enough to know which vaccine to produce. In our class, we have to repeat the process so that each student has two virions, which doubles our data set of HA and NA combinations. Students might also follow up with a research project on how the CDC actually makes its yearly vaccine decision.

Conclusion

We have found this activity to be effective in helping students visualize how genetic reassortment (i.e., antigenic shift) occurs in the influenza virus. Through modeling, students learn viral anatomy and discuss evolutionary potential. This activity is a great way to encourage students to consider what they know about living organisms and determine whether viruses should be classified as such.

Some scientists (Villareal 2004) argue that because viruses have evolutionary potential, we should consider reclassifying them, using more than just their protein coat and genetic material. We ask students, "If viruses can evolve, shouldn't we consider them to be living? Why or why not?" This is one problem without a clear-cut answer.

In addition to discussions about what is living and how organisms evolve, we use this lesson as a way to integrate important public health concerns, such as how virulent virus strains spread in a population and vaccinations' role in decreasing the percentage of people who are infected. ■

Addressing the Standards.

The following National Science Education Standards (NRC 1996) are addressed in this article:

Life Science (p. 181)
- Biological evolution

Science in Personal and Social Perspectives (p. 193)
- Personal and community health

FIGURE 4

Materials for a flu virus model.

The teacher should have one bowl with 8 cm pieces of paper, yarn, and pipe cleaners and another with push pins and map pins so it is easy for students to gather their materials.

Avian influenza virus

- Sytrofoam cup
- six strips of paper representing the six RNA fragments that code for proteins
- a piece of pipe cleaner (blue or green)
- a piece of yarn (orange, red, or purple)
- six dressmaker pins that are the same color as the yarn
- two pushpins that are the same color as the pipe cleaner

Swine influenza virus

- Styrofoam cup
- six strips of paper representing the six RNA fragments that code for proteins
- a piece of pipe cleaner (yellow or green)
- a piece of yarn (orange, yellow, or green)
- six dressmaker pins that are the same color as the yarn
- two pushpins that are the same color as the pipe cleaner

Human influenza virus

- Styrofoam cup
- six strips of paper representing the six RNA fragments that code for proteins
- a piece of pipe cleaner (yellow or white)
- a piece of yarn (yellow, blue, or pink)
- six dressmaker pins that are the same color as the yarn
- two pushpins that are the same color as the pipe cleaner

Meena Balgopal (meena.balgopal@colostate.edu) *is an assistant professor of science education at Colorado State University in Fort Collins, and Cindi Bondy* (bondyc@fargo.k12.nd.us) *is a biology teacher at Fargo South High School in Fargo, North Dakota.*

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FIGURE 5

Determining the vaccination.

Determining what vaccination to make depends on calculating the most prevalent strain of the flu virus. This is accomplished by first determining the most common HA/NA protein combination. In the real world, there are 16 possible HA protein types and 9 possible NA types that can combine to form flu strains that are difficult for our immune systems to recognize. In this activity, instead of 144 possible combinations, there are 28 (7 HA proteins × 4 NA proteins).

Determine the different combinations of HA/NA proteins using abbreviations for the colors listed. Then tally up how many of each combination are present. The most prevalent combination of HA and NA proteins will likely be a strong candidate for the vaccination for the following flu season.

NSTA connections

Read more about avian and swine flu in past Health Wise columns—available in *The Science Teacher* archives at *www.nsta.org/highschool*.

On the web

Potential swine flu epidemic: *www.pandemicflu.gov/index.html*
Pre- and posttest: *www.nsta.org/highschool/connections.aspx*

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