Monitoring Changes in Species: Africanization in U.S. Honeybees

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Biography

Marianne Niedzlek-Feaver received her Ph.D. from the University of Michigan. As an evolutionary ecologist, she is interested in identifying factors that shape the mating systems of grasshoppers and katydids. She currently teaches Evolution, Invertebrate Zoology and Introductory Biology courses in the Biology Department and Biological Sciences Program. She has received various grants to improve the laboratory experience and is a member of the NCSU Academy of Outstanding Teachers.

Patricia Aune received her MS in Biology from the University of North Carolina at Chapel Hill. She is currently the Laboratory Supervisor where she organizes, coordinates and helps train the graduate students instructors who teach the science major's two semester Introductory Biology Laboratory course. She is also the co-designer and co-instructor of a hands on non-majors Biology Distance Education laboratory course.

Miriam Ferzli received her Ph.D. from NC State University in Science Education. She is a Teaching Assistant Professor in the Department of Biology at NC State University where she teaches Introductory Biology. She also teaches a graduate seminar on teaching in the life sciences and conducts educational research with a focus on scientific thinking and problem solving. She has received the Outstanding Teaching Award and is part of the Academy for Outstanding Teachers at NC State University.

Introduction

As part of our first semester of the introductory biology lab that introduces the scientific method, evolution, ecology and diversity, we were particularly interested in developing an exercise that gave students an opportunity to work with molecular data, given its increasing utility for phylogenetic studies. This exercise also enables us to introduce electrophoresis as a tool for separating DNA and proteins in general and to emphasize the use of biotechnology by ecologists and evolutionary biologists. However, our students are not exposed to cellular structure and function until the second semester of biology, so we had to develop an exercise that required little background knowledge of inheritance patterns by our students.

Information presented to students

1. Most of our students could easily understand, after a brief explanation, that both parents contribute to the phenotype and as a result, to the behavior of worker bees, while mitochondria are inherited only (with rare exception) from mothers and contain some DNA that can be analyzed via electrophoresis.

2. Students are told that, although scientists do not yet understand why, the genetic material contributing to Africanization dominates phenotypic expression, regardless of the parent providing that genetic material.

3. They are also given some information about the problem and honeybee history in North America.

Honeybees in North America were originally imported from Europe. An African subspecies was imported into South America because of its superior honey producing potential in warmer climates. Unfortunately, individuals of African bees have been introduced and have established colonies in the USA. The Africanized colonies tend to abandon commercial hives, especially when stressed, and so cannot be used as domesticated pollinators, which are normally transported in hives to areas where they are needed. This African behavior dominates in hybrid workers and so threatens the agricultural pollination industry. Students are surprised to learn how many of our domestic crops depend on bee pollination.

Data collected by students

Participants measure the wings of worker bees and correlate these measurements with measurements from known Africanized and European colonies. We actually provide an Excel® spreadsheet for students that allows students to graph their data automatically when they input measurements for their unknown sample of workers, known African and known European workers. Each group of 3-4 students work with bee samples from an unknown hive, with each student responsible for measuring 9-12 wings.

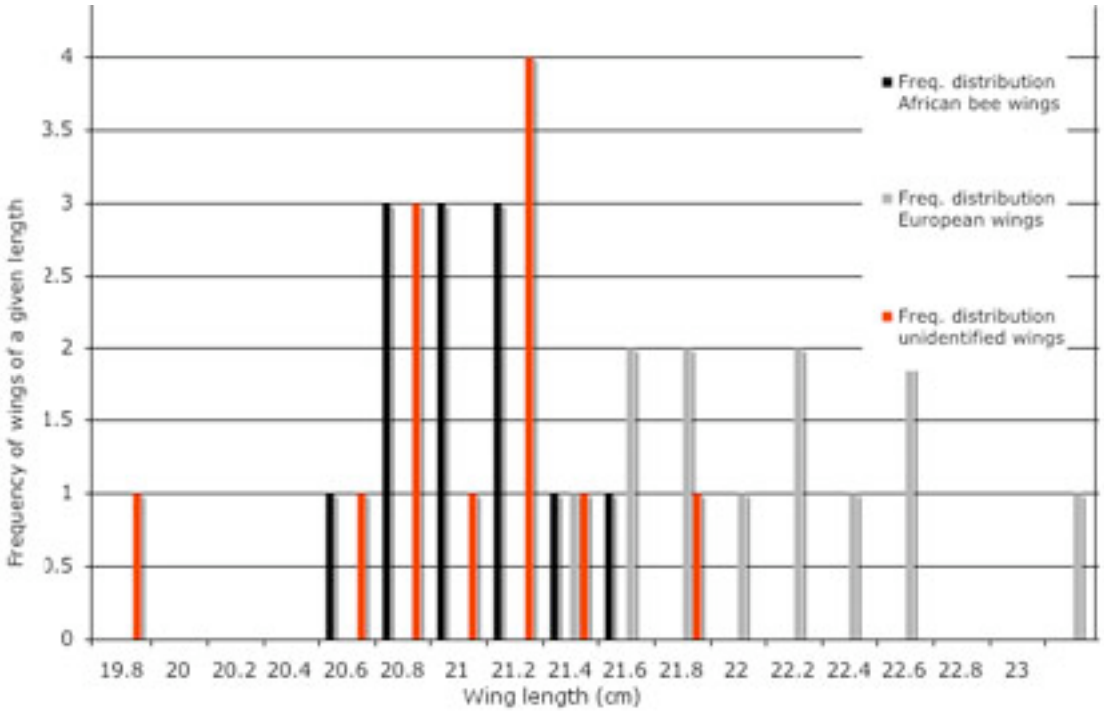


Figure 1. The graph for the unknown workers clearly shows a distribution of wing lengths from a hive that is clearly European in morphology.

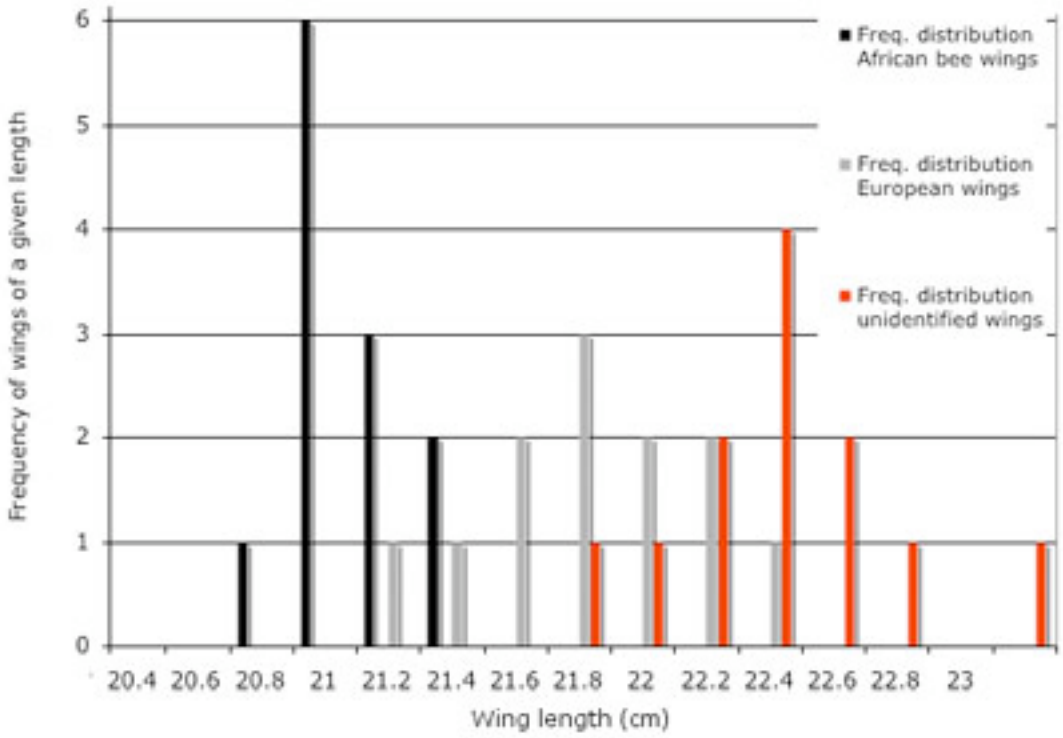


Figure 2. The graph for the unknown workers shows a distribution of wing

lengths that is difficult to characterize as European or African in morphology.

Students also participate in an simulation of mitochondrial DNA . They are told this is normally only done when phenotypic analyses of workers indicate Africanization is a possibility. The electrophoresis activity uses plasmid fragments that simulate the results obtained with restriction enzymes and mitochondrial DNA, one band is produced for hives with Africanized bees, two bands for hives with a European Queen.

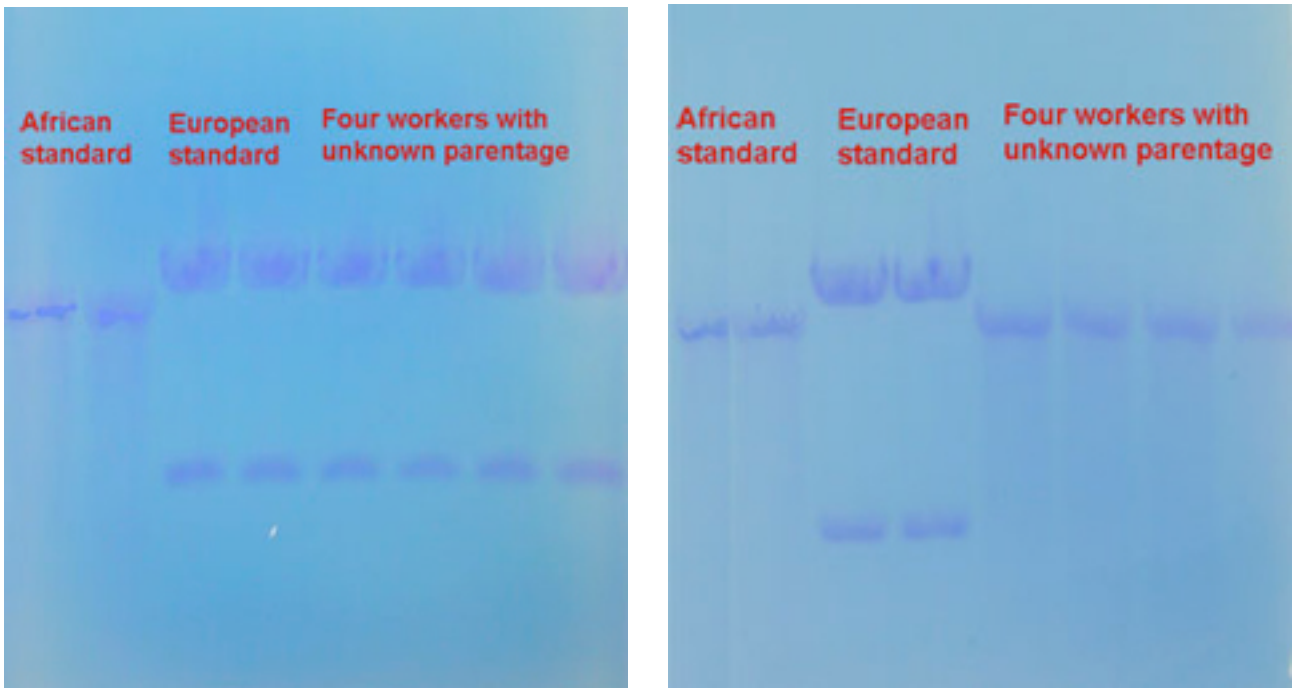


Figure 3. The gel on the left identified unknown workers as from a hive with a European queen, the graph on the right identifies unknown workers as from a hive with an African queen.

Wing samples are assembled to give different results and also results that correlate differently with the electrophoretic data to stimulate class discussion. One group of students for example, may obtain wing data that indicate Africanization, but mitochondrial data that indicate the queen is of European descent, while another obtains wing and mitochondrial data that indicate Africanization of their hive. Students then analyze the data they obtained in detail before formulating their answers to the concerned beekeepers (In reality, students justify their answer to fellow classmates and the laboratory instructor). It is an excellent exercise in showing the power and limitations of correlative data. As such, it stimulates discussion of methods and sample sizes used. It also serves as an exercise that paints a truer picture of the problems associated with potential Africanization of honeybee hives.

Resource availability

Tarpy, D. 2007. Africanized honey bees in North Carolina. North Carolina State University, A & T State University, Cooperative Extension. CD-ROM. The authors wish to thank Dr. Tarpy for his

help in developing this lab. Interested instructors should contact Dr. Tarpy for more information and CD availability. david_tarpy@ncsu.edu

The DNA Kit was developed for us and continues to be provided by Carolina Biological. Email P. Aune for more information. patty_aune@ncsu.edu,

A ZIP package will be also available on the web site, **http://www.ncsu.edu/project/interactivebiology** in 2009. This package will contain more samples of data obtained by students, and a set of photomicrographs of wings divided into six groups that instructors can use. This set is based on wings furnished by Dr. Tarpy that we were allowed to photograph**.**

Laboratory exercise

The laboratory exercise as published in the BIO 181-INTRODUCTORY BIOLOGY I, LABORATORY MANUAL follows. 2007. by Biological Sciences , Copyright © 2007 by Biological Sciences, NC State University

Honeybees are social insects that work together in a highly structured manner. Within each hive, the bees belong to one of three groups or castes: queens, drones and workers. Each hive has only one breeding female, the queen, who can lay over a thousand eggs a day and may live anywhere from two to eight years. She is larger than the workers or the drones. Drones are males. They only live about eight weeks and there are only a few hundred ever present in the hive at one time. Their sole role is to mate with a new queen if one is produced. Drones are driven out of the hive to die after the mating season. Worker bees are all sterile females. The workers maintain the hive, cleaning and feeding the larvae. As they get older they will become foragers, gathering nectar, pollen, and other hive necessities.

You will use two methods to identify Africanized hives. The first involves measuring wing lengths of a sample of worker bees and comparing those lengths to a sample of known African and European bees (Figure 1). This method FABIS (Fast African Bee Identification System) was developed by the government as a quick and inexpensive way to detect worker bees from potentially Africanized hives. Suspect hives can then be subjected to the full United States Department of Agriculture (USDA) identification method that measures 23 separate morphological characters and examines the pattern obtained with computer-assisted cluster analysis.

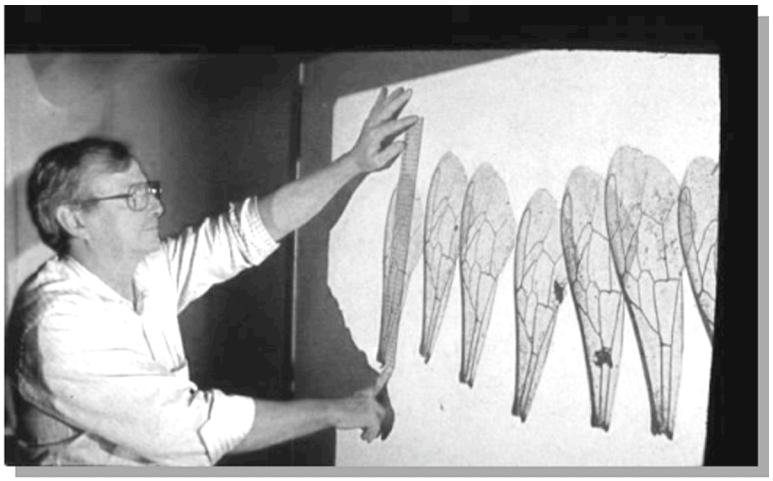


Figure 1. Measuring wings (reproduced with permission from Tarpy, 2007). Wings are magnified so that small differences in wing length are apparent.

The second method you will use involves DNA analyses. For example, mitotyping (Figure 2), a process in which a portion of mitochondrial DNA (mDNA) is examined and amplified by polymerase chain reaction (PCR). Mitochondria are small cellular organelles that are only inherited from the mother. They contain a small circular loop of their own maternal DNA. This mDNA is then subjected to an enzyme that cuts it at a specific location. If the worker bee had a European mother, the mDNA would be cut into two pieces. If the worker bee had an Africanized mother the enzyme would be unable to cut the Africanized bee mDNA loop, resulting in one piece.

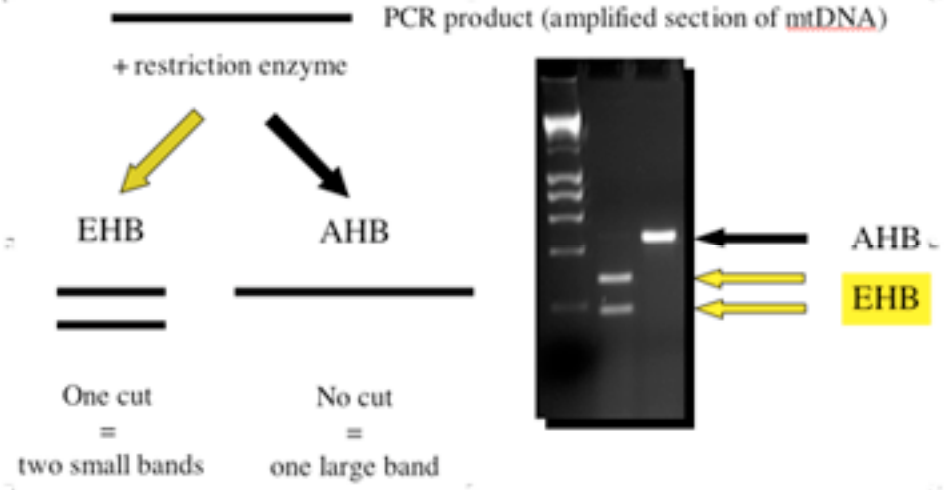


Figure 2. Mitotyping schematic: EHB = European Honeybee, AHB = Africanized Honeybee (modified with permission from Tarpy, 2007)

DNA Analyses:

These methods are much more expensive, and in the case of mitotyping, only give information about the mitochondria of the workers—material inherited from their mother, the queen of the hive. The queen may be genetically a European bee, with respect to her mitochondria, but still have mated with an Africanized drone. Their progeny, or the resulting Africanized workers, may

still display the aggressive and hive-abandonment behaviors that tend to be dominant. This is because they have still inherited 1/2 of their overall DNA from their father.

Possible Matings	Africanized mother with either father	European mother with European father	European mother with Africanized father
Type of Inherited mitochondria	Africanized mitochondria	European mitochondria	European mitochondria
Inherited Behavior	Africanized behavior	European behavior	Africanized behavior

Table 3. Possible matings and progeny

Morphological characteristics:

You will be given a file that contains pictures of wings from twelve known African worker bees, twelve known European worker bees and twelve unknown worker bees. You will compare the frequency distribution of wing size of the unknown bees to those of the known African and European bees. Remember, most African traits are dominant and so a worker that has an Africanized father or mother should favor the phenotype of the Africanized parent. Only those bees whose parents were both European bees will resemble the known European worker bees.

Procedure

1. Measure the wings of all 36 bees. Note the specific points to begin and end your measurements (see Figure 3). Each student within a student group of four should measure nine wings.

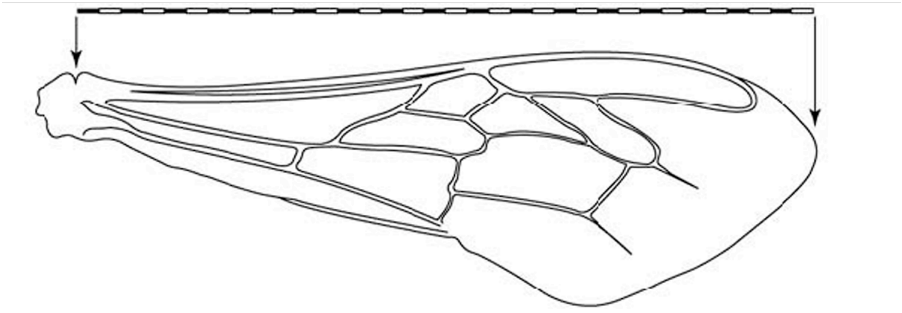


Figure 3. How to measure bee wings (reproduced with permission from Tarpy, 2007)

Table 4. Wing length of bees (cm)

African bees	**European bees**	**Unknown bees**

2. Graph the distributions using the supplied Excel® spreadsheet template. Place a copy of your graph here:

3. 3. On your graph, is there overlap between the curves of the African and European bees?

4. Does the distribution of the unknown bees fall completely within that of the African or European bees? If not, how confident can you be that the unknown bees are from an Africanized hive?

5. Africanized bees are usually smaller than European bees, but European bees in one area, under climatic stress, may be smaller than other European bees from another area. If this test is not 100% conclusive, because of possible size overlap due to stress variation in given local conditions, why use it at all?

Molecular characteristics:

We will next be running a simulation of the type of data that you would collect if you used mitotyping to identify Africanized colonies. You will be given DNA samples that have been amplified by PCR and exposed to digestion by an enzyme. We can separate DNA fragments of different sizes using **Agarose gel electrophoresis** in which the DNA is placed in a gel matrix and then subjected to an electric current. DNA is negatively charged and the DNA fragments will move toward the positive pole. The large fragments will move more slowly than the smaller fragments, allowing the fragments to separate. A marker dye in the sample moves ahead of the fragments so that you can tell when to stop applying electric current. The gel can then be removed and stained with a dye that binds to the DNA, allowing you to visualize the fragments. You should expect to get the same general pattern, two fragments with European mDNA, one fragment with Africanized mDNA. The exact spacing between different fragments will vary from student group to student group or from what you may see in the primary literature. This is due to the duration and size of gel that is run.

Procedure:

The micropipette:

1. Before beginning the experiment, you should become familiar with the use of the micropipettes (See Figure 4). Please pay careful attention as your laboratory instructor demonstrates this technique. Pipetting can be difficult, particularly when using such small portions. It is important to be able to properly measure each sample.

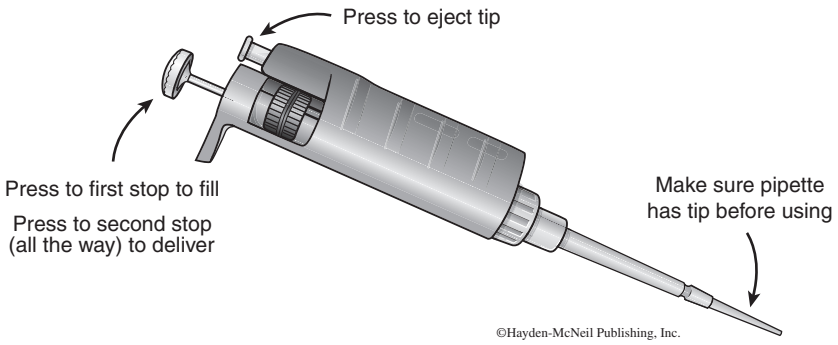


Figure 4. Parts of a micropipette

2. Use the practice gels and the practice loading solution provided to practice pipetting and loading wells on a gel. Since this is a technique that you will use in subsequent courses, take the time to learn this now. There is also a short film on pipetting that your laboratory instructor will present.
3. Your laboratory instructor will have already prepared 0.8% agarose gels for the class. The gel solution will be placed in 3 DNA electrophoretic units and covered with TAE buffer. Examine the gel. You should be able to see 8 wells at the anode (black) end of the unit. The DNA samples will migrate toward the cathode (red) end of the unit during electrophoresis.

Loading the Gel with Pre-digested DNA samples:

1. We have prepared DNA from three suspect hives. Each student group will be given bullet tubes containing DNA from two different unknown bees from one of the suspect hives and two known bees (1 Africanized and 1 European to use for the DNA comparison). Everyone within each student group will load one of these samples into the gel. You will be sharing the gel with another student group. You will be instructed as to which lane you will load and which hive you will be testing. NOTE: It is imperative that you use the assigned samples and run them in the assigned lanes (see step 5 below).
2. Pulse spin the tubes in the microcentrifuge for 2 seconds to force the liquid to the bottom of the tube. NOTE: Be sure that all tubes are in a **balanced** arrangement in the rotor of the centrifuge.
3. Place the DNA samples in a foam rack until you are ready to load the gel.
4. Load the gel in the order presented in step 5 below. Each student will load one sample into one of the three class gels. One group will load the first 4 lanes of the gel: another group, testing the same suspected hive, will load the last 4 lanes.
5. Use a separate tip for each sample and load 20 μl of each sample into the wells of the gel in the following order:
   Lane 1- Known European (20 μl)
   Lane 2- Known Africanized (20 μl)

Lane 3- Unknown #1 (20 μl)
Lane 4- Unknown #2 (20 μl)
Lane 5- Known European (20 μl)
Lane 6- Known Africanized (20 μl)
Lane 7- Unknown #1 (20 μl)
Lane 8- Unknown #2 (20 μl)

6. Secure the lid on the gel unit and connect the electrical leads to the power supply. The power supply will be set to 100V and the samples will electrophorese for about 60 minutes or until the tracking dye front is visible near the bottom of the gel. Be sure to check that you can see tiny bubbles rising from the wire electrodes when the power is running.
7. Check with your laboratory instructor when you think the gel has finished running. Turn off the power to the unit and unplug the electrical cord before removing the lid.

Staining the Separated DNA Samples:

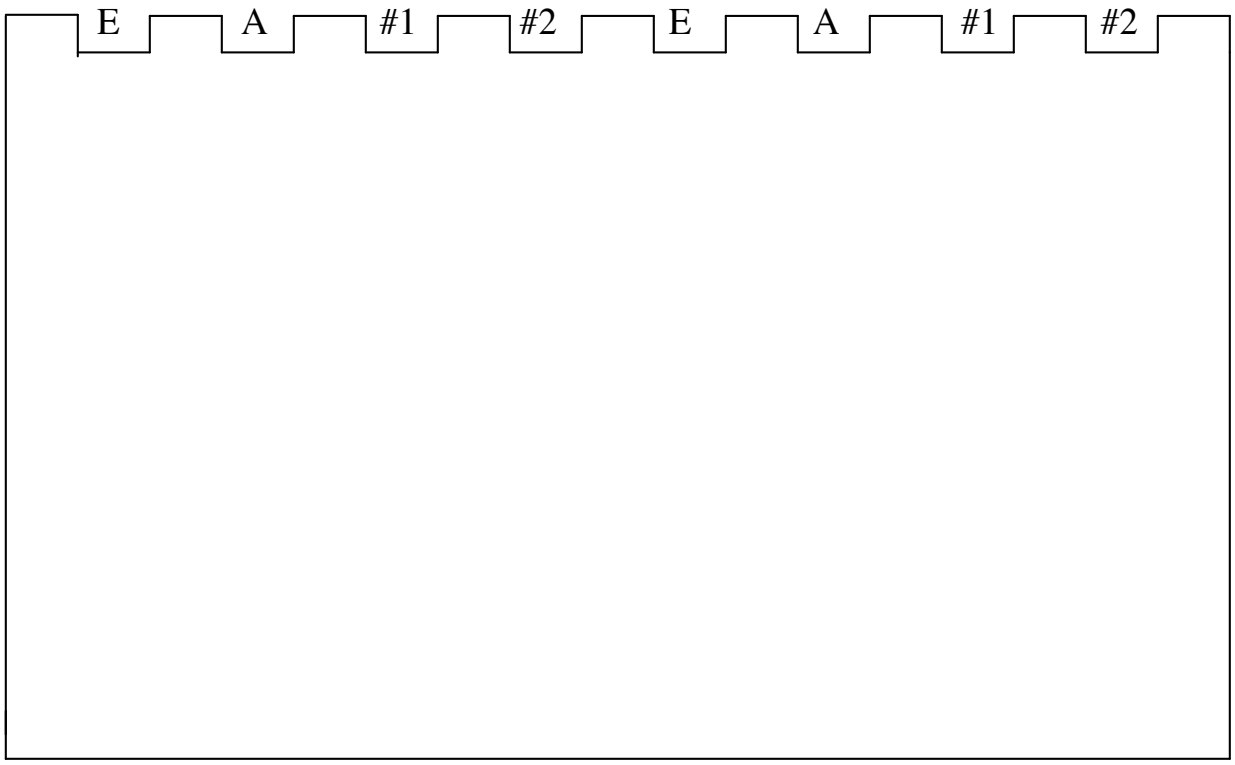
1. Slide the gel off the electrophoretic unit tray into the plastic weigh boat. Cover the gel with the Fast Blast blue stain. Leave the gel in the stain for 2-3 minutes, but no more than 3 minutes. Pour the stain back into the Fast Blast beaker. It can be re-used.
2. Transfer the gel into the plastic rinsing tray containing warm tap water. Swirl gently for 10 seconds to rinse the gel. Discard the water and refill the tray with clean warm water. Gently swirl every minute for 5 minutes. Change the water a second and third time, swirling once again every minute for 5 minutes. Discard the water.
3. Place the gel in a new weigh boat. Initially, the bands will be a little fuzzy, but will become sharper about 10-15 minutes after the final rinse. You may want to use a white light box to better visualize the bands.

References:

Tarpy, D. 2007. Africanized honey bees in North Carolina. North Carolina State University, A & T State University, Cooperative Extension. CD-ROM.

NOTE: The authors wish to thank David Tarpy, of the Entomology Department at North Carolina State University, for allowing us access to research materials dealing with Africanization of bees, such as wings from Africanized, European, and unidentified bees that we photographed. We could not have developed this lab without his generous support of our efforts.

1. Draw the resulting bands in the gel below:



1. Could you identify the unidentified bees as Africanized or European bees?
2. How do your electrophoretic results compare to the morphological data you graphed?
3. Figure 5 below is a gel resulting from a recent survey of suspected Africanized hives in North Carolina. What do the results indicate?

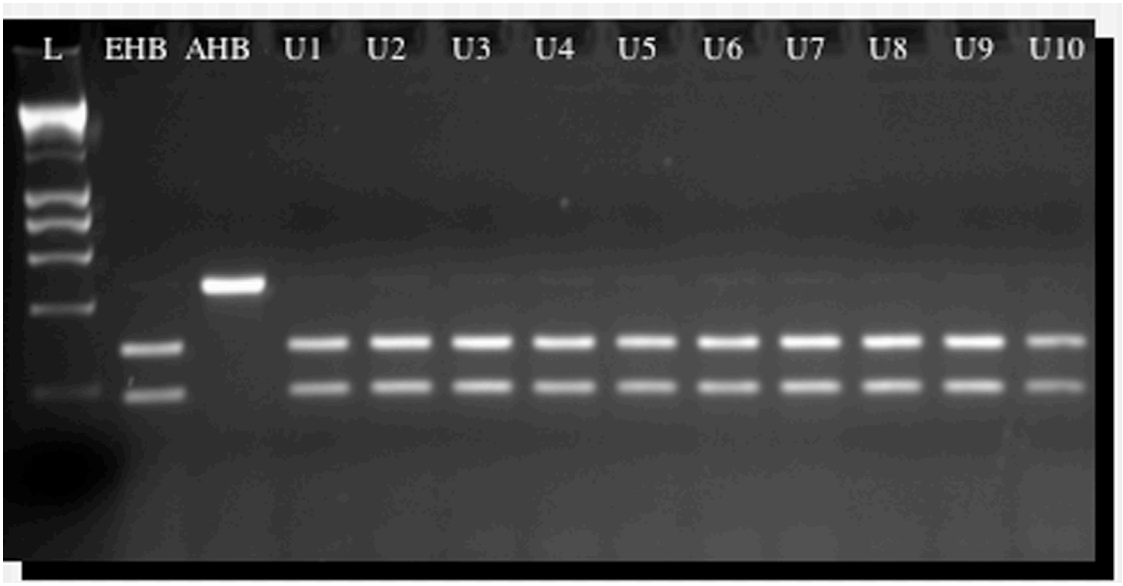


Figure 5. Electrophoretic gel from a North Carolina hive (reproduced with permission from Tarpy, 2007)

4. Why not simply conduct the molecular testing on a sample of workers and do away with the morphological examination? (Consider economics and male contribution to possible behavior.) Would knowing that Africanized behavior is dominant in hybrids make the morphological test more or less valuable as a diagnostic tool?

5. A beekeeper suspects that his hive has been Africanized due to increased defensiveness and other behavior by worker bees. He submits a large sized sample. The morphological data indicate more overlap with Africanized characteristics than with European reference populations. However, DNA analysis of mitochondria yields no evidence of Africanization. How would you advise him?