How do the patterns on butterfly wings develop?

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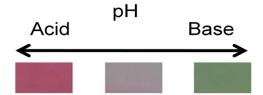
Grade levels targeted: High school

Observations:

Butterflies display diverse and beautiful patterns on their wings that can alternately make them harder for predators to see and more attractive to members of their own species. Alan Turing, a mathematician who is famed for breaking Nazi codes and as a father of computer science, suggested that these complex patterns are not set in the developing wing like a paint-by-numbers set, but emerge from the simple interaction of chemicals (morphogens) diffusing from a sources within the wing.

The wing itself can be thought of as a mosaic of cells that together form a blank canvass. Each of these cells will produce a color, but each also has a simple switch mechanism that responds to a rise in the amount of morphogen around it by producing a different color in the same manner as the thermostat on an air conditioner responds to the level of heat around its sensor to turn itself on. The level of morphogen that triggers a *reaction* in the cell is called a *threshold*, and will be found in cells either closer to or further from the source of the morphogen based on the way the chemical spreads or *diffuses* through the wing. One morphogen can produce circular patterns as cells react to the spread of the chemical trigger or *activator*, but if we add a second chemical that interferes with the *activator* and reduces its level, an *inhibitor*, a wide array of patterns can be formed. Because the cells are *reacting* to the levels of *morphogen* determined by *diffusion*, this system is known as the *reaction-diffusion system*.

In order to mimic the reaction-diffusion system of butterfly wings, we need a substrate that reacts to a threshold of a chemical in a manner that we can see. One quantity that is easily manipulated is the acidity/alkalinity or pH of a solution. If we had a substance that would react distinctly to different levels of pH, this would be similar to cells reacting to different threshold concentrations of a chemical. Luckily, an extract of red cabbage turns green/blue in an alkaline or basic solution, and more red/violet as acid is added. If we dip paper butterflies in red cabbage solution, the paper will react to a source of acid or base by changing color appropriately. The dipped paper, like the sheet of cells making up the wing, has the potential to display different colors when the local conditions change, but requires a signal to trigger it. Because acids and alkaline bases oppose each other and cancel each other out, we can use an acid as the activator and a base as the inhibitor. The paper color remains unchanged when there is no acid or base added and goes back to its original color when the two come together and cancel each other out. If we designate the green color of the base as what would occur when there is no activator present, this system has two thresholds, resulting in two colors as the pH increases.



Evolution and wing pattern: Real butterfly wing patterns evolve through natural selection. In each generation, individual butterflies are "selected" because they are more likely to survive long enough to breed if their wing pattern provides some benefit. This could be a pattern that matches the foliage on which the insect lives. Such patterns are said to be "cryptic". The pattern may also be beneficial if it makes the butterfly easy to see to potential mates, or flashy enough to startle a predator, as may be the case with large eye-spots. In fact, all of these benefits may be acting at the same time in a population, leading to wings with a variety of visual features that are shown differently when the wings are folded or closed. The butterflies that are selected to survive into the next generation, will then breed and combine the genes that code for the beneficial pattern. This may lead to even better patterns, because each individual may have had wing patterns that were beneficial in a slightly different, but complimentary way. In some cases, the result of mixing may actually look worse than what came before, if the traits mixed contrasted in some manner.

Question:

Can a Reaction-Diffusion system with two morphogens, an activator and an inhibitor, produce patterns that are similar to those seen on the wings of butterflies in the wild through selection?

Hypothesis:

Selecting the best matches for a model butterfly wing from among wing patterns derived from the differential placement of sources of activator and inhibitor will produce patterns which are similar to those seen on butterfly wing.

Materials:

- Blotting paper, scissors
- 1 head of red cabbage
- Pot
- Spoon
- Strainer
- Citric acid or lemon juice
- Baking soda
- 2 plastic cups
- 1 bowl
- 2 eyedroppers
- A six-sided die
- A camera to photograph the patterns formed on paper butterflies

Experiment 1:

- 1. Chop the red cabbage into pieces and boil in water until the liquid is red (20 min). Strain the liquid and place it in a bowl.
- 2. Cut paper into butterfly shapes.



3. Dip14 paper butterflies in red cabbage extract and place on a flat surface. They can be allowed to dry or used while still wet.



- **4.** Mix citric acid and water in one cup and baking soda and water in the other. The exact amount in each is not critical, we simply want them concentrated enough to color the red cabbage extract appropriately. **Do not mix solutions directly in the same cup!** This will result in alarming and possibly staining overflow due to foaming like that of a shaken soda can as the acid reacts with the base.
- 5. Find images of wild butterfly wings for inspiration as to the patterns you which to try to recreate. Choose a pattern to be the model that you are going to attempt to select for.

Patterns seen on wild butterfly wings

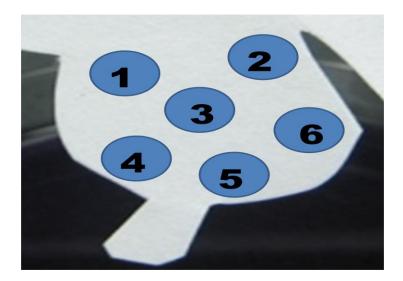
Eye Spot Curve V-shape







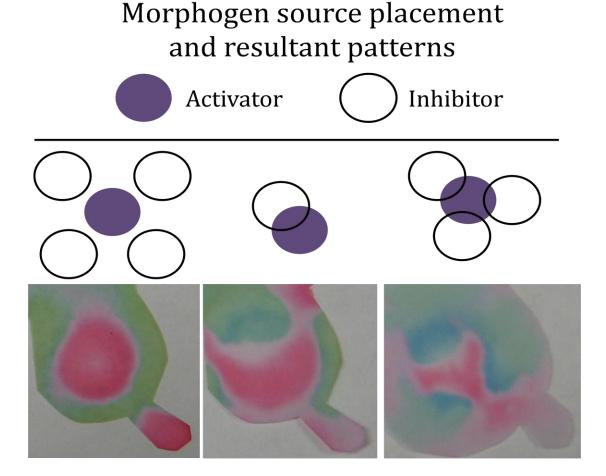
6. In order to create wing patterns, you'll need a six-sided dice. You will roll the dice and the number will tell you where to place drops of acid or base solution according to the pattern below. When you roll a number, you place 2 drops of acid or activator on that spot and record the number and type of drop. Roll the dice again and put 2 drops of base or inhibitor on the spot you rolled, even if it is the same number you have previously rolled. Yes, you may conceivably end up with one big spot if you roll the same number over and over.



By continuing this process 6 times, you will have a recipe for the wing pattern that emerges. Record the order of the numbers and types of drops, for example: 1A, 4B, 3A, 4B, 5A, 2B. This is the code for each wing pattern. You need only do one wing, but repeating the pattern on both results in an attractive butterfly.



7. Repeat the process eight times to make variations in wing pattern. Photograph all of the wings. Remember that this is a diffusion system not a painting set, so no dragging morphogen into a desired pattern.



Visually inspect the eight patterns and choose the four that appear similar to your preselected model pattern. You should now have 4 patterns and 4 corresponding codes.

8. Choose the single pattern from among these 4 that most resembles your model. You will now make up 4 new butterflies using the 4 codes you selected, but there is a catch. You will use two codes simultaneously; each wing will receive drops according to its own code and that of the single best from the original wing patterns. Instead of 2 drops, you will use 1 drop from both codes, going through each code in order, the first drops from

each one, then the second, and so on. That code which you had chosen as best will simply be repeated as you add it to itself. You are mixing the code for the best pattern into that of the others, essentially cross-breeding them. Photograph the 4 resultant patterns.

9. Now choose the best two from this round, one of which you consider to be the best overall. Make up two new butterflies, as above by mixing the best pattern code with the second best as well as with itself. Photograph the results and pick one of them to be the final winner. If all has gone well, you now have a record of the whole selection process.

Results:

Show the patterns that you have created and the placement of morphogen sources that they emerged from. Line the pictures up so that you can compare the first 8 patterns are above the next 4, then 2, then the ultimate winner. Show an image, or drawing of the pattern you chose to select for.



Discussion:

Did the reaction-diffusion system you developed result in the formation of patterns that can be compared to those on real butterflies? Describe what occurs as the acid sources diffuse to meet the base sources. Discuss the way reaction thresholds within the paper lead to color expression.

Did the patterns appear more like your chosen model after each round of selection? Was the pattern you chose as best match from among the original 8 wings the ultimate winner? Did mixing good matches produce a great match, or result in a pattern that was a poorer match?

References:

Locate sources on the various elements of your project and list them with links if they are internet sources. A true scientist would have to provide references for every step in the process that they did not develop on their own.

Contact: For help or more information about this project, contact Paul Bardunias at paulmb@ufl.edu