Research Paper On The Comparison Of The LDH Isoenzymes From Scuds From Two Different Geographic Locations

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Abstract

Two different populations of Gammarus fasciatus were studied to see if their LDH isoenzymes were similar. Samples of Gammarus fasciatus were taken from Bear's Grass Creek and Thompson Valley Creek, both in the Lower Eau Claire Watershed in Eau Claire county. This study was to determine if geographic isolation has effected the make-up of the two populations. Samples were studied using gel electrophoresis, which separated proteins, and then stained in a LDH stain. Twelve repetitions were done for each population. Stained bands were measured and analyzed. Three results showed indications of a difference between populations. The percent difference of electrophoretic migrational distance between populations was: 24.44%, 12.5%, and 2.44%.

Researcher's Background

I, Anna Cousin, am currently a junior at Augusta High School in Wisconsin. I researched on the gammarus fasciatus at the Augusta High School Science Lab between June and September of 1996. Right away, I would like to recognize and thank Mr. Paul Tweed, my Biology teacher as a mentor throughout my research and web page setup.

I first became interested in this area of research when studying genetics in Biology. I then did an independent study on DNA, proteins, and their separation by gel electrophoresis, by following pre-made labs. I finally prepared my question of individual study and carried out the research. I presented my research and the Western Wisconsin/Upper Michigan Junior Science and Humanities Symposium in September of 1996. I received sixth place overall and am an alternate to attend nationals.

I designed this web page to make my research available to others. This page is set up so that you can search my paper by section or view the complete paper as a whole.

All materials and instructions can be obtained from:
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Background

Geographic Isolation

Geographic isolation is important in allowing local gene pools to differentiate from one another. Generally, the greater the distance between local breeding populations, less gene flow occurs between them. I developed my research project by wondering if there would be any difference in a certain organism in two different locations. I decided upon studying an organism in local streams. In choosing an organism to study, I looked at availability and quantity, and that is mainly why I chose to study the scud or sideswimmer (Gammarus fasciatus).

Geographic isolation is important in allowing local gene pools to differentiate from one another. Generally, the greater the distance between local breeding populations, less gene flow occurs between them. According to the book, The Science of Evolution, by William D. Stansfield, organisms with little or no gene flow between species that live in different locations, tend to go on their own evolutionary way, adapting to different conditions in their own locations.

Migration is the only other process other than mutation by which a population can receive new kinds of genetic information. There are at least three models to represent migratory schemes. The island model shows populations exchanging genes in various quantities and directions through migrations. The river model shows a linear gene flow, and finally the distance model shows a group of organisms distributed over a large geographic area, where individuals do not have as much of an opportunity to mate. The scuds were collected from Bear's Grass Creek and Thompson Valley Creek (see appendix A). Bear's Grass and Thompson Valley are separate drainage basins in the Lower Eau Claire Watershed, which both empty into the Eau Claire River.

The organisms in ponds, lakes, streams, or rivers might be expected to show considerable differentiation because of their isolation, but quite the opposite may be true. Many fresh-water organisms are widely distributed. One way by which different fresh-water systems may be interchanged is caused by floods that periodically join rivers, lakes, etc.

Scuds (Gammarus fasciatus)
Scuds are widely distributed and common in unpolluted clear waters. They are found in vegetation or hidden under and between debris and stones near the banks of the water. The species of scuds I found in the Lower Eau Claire Watershed is the Gammarus fasciatus. According to the Fresh-Water Invertebrates of The United States, 3rd Edition, by Robert W. Pennak, very little is known about the passive transport of the amphipods from one drainage system to another.

Electrophoresis
My method of showing a similarity or difference between the organisms was separation of proteins by gel electrophoresis. Electrophoresis is the movement of charged particles in solution under the influence of an electric field. The electrophoresis setup includes: a power supply, and the electrophoretic chamber, which the electricity travels through, and also holds the gel. The agarose gel, containing samples in wells to be separated, is submerged in buffer within the electrophoretic chamber. Current from the power supply causes the proteins to migrate. Larger particles migrate slower than smaller particles, thus separating them according to size.

LDH
The extract obtained from the scuds contained hundreds of colorless proteins in addition to LDH. LDH, or Lactate Dehydrogenase, is found in various muscle tissues. LDH is an isoenzyme, which means that the LDH enzyme has different molecular forms. LDH has five distinct forms which are represented by different tissues in different amounts. Because LDH has various molecular forms, the electrophoretic mobilities of the LDH isoenzymes are different. In order to identify the LDH isoenzymes, gels are dyed in special LDH substrate

after the electrophoresis. The LDH substrate picks up the LDH bands.

Hypothesis
Using the previous information as background, I developed my hypothesis. I believe that scuds from two different geographic locations will have different LDH isoenzyme patterns. I believe this because I feel that the scuds are geographically isolated from one another.

Materials and Procedures

Field Materials
-D-frame net
-collection bottle
-tweezers
-hip waders

Procedure
1- Collect scuds from sites with an aquatic D-frame net.
2- Deposit organisms in enamel trays for field separation.
3- Store organisms in a jar with stream water -no alcohol- this will render the proteins useless.

4- Return to the lab, key out selected amphipod specimens to the species level using the keys in Fresh-Water Invertebrates of The United States, 3rd Edition.
5- Immediately after identification begin the extraction procedure.

Extraction Materials and Procedures
Extraction Materials
-mortar and pestle
-extraction buffer-(contains the detergent Nonidet P-40)
-centrifuge
-centrifuge tube
-pipets
-grease pencil
-clean microtubes

Extraction Procedure
1- Place 3 grams of scuds and 4 mL of extraction buffer into chilled mortar and pestle and grind until a homogeneous suspension is formed.
2- Pour homogenate into a centrifuge tube a centrifuge for 5 minutes.
3- Pipet the supernatant into clean microtubes, label with grease pencil, and freeze.

Preparation of the Agarose Gels
Preparation of the Agarose Gels Materials
-electrophoresis buffer

-agarose
-casting tray
-glass slide
-casting comb
-masking tape
-plastic wrap
-hot plate
-250 mL beaker
-25 mL test tube
-glass stirring rod
-safety goggles
-test tube holder
-beaker tongs
-test tube tongs
-balance

Preparation of the Agarose Gels Procedure

1- Measure and pour 15 mL of electrophoresis buffer into test tube.
2- Weigh 0.18 grams of agarose on a balance and add to test tube.
3- Stir with glass stirring rod until the agarose forms a suspension.
4- Place test tube into a boiling water bath, stirring often, until mixture turns clear. Approximately 3-5 minutes.
5- Remove test tube and allow to cool for a minute.
6- Set up casting tray by placing glass slide in casting tray, placing comb in slots, and sealing ends with tape.
7- Pour cooled agarose into gel casting tray and cover with plastic wrap.
8- Leave small amount in test tube for testing application.
9- Refrigerate gel at least 15 minutes before using. Will keep in refrigerator no longer than three days.

Detection of LDH Isoenzymes and Materials

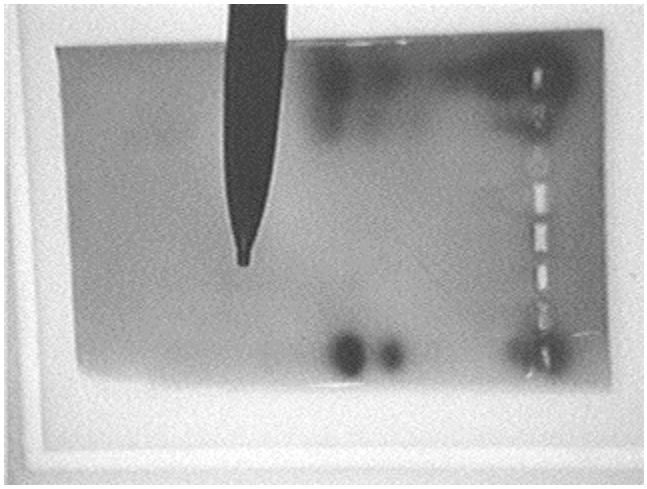
-water bath
-thermometer
-gel staining dish
-LDH substrate
-dark bottle
-graduated cylinder

Detection of LDH Isoenzymes Procedure
1- Prepare LDH Substrate Solution: 1/6th substrate is dissolved in 16 mL of distilled water. LDH is stored in a dark bottle until use.
2- Add LDH solution to a gel stain dish containing the gel. Incubate in the dark for 1 hour in a 37°C water bath.

3- After incubation, rinse in water, examine under a light box, or store covered in water in refrigerator.

Analysis and Results

When the gels were finished incubating, I examined them on a light box. Using a centimeter ruler, I carefully measured each sample and the standards from the well to the end of the band and then recorded the distance. I then outlined the gel onto a clear transparency sheet, marking the wells, banding patterns, and standard patterns, for a permanent copy of results.



Example of a gel with bands

I analyzed the data by taking the average distance of the three runs per gel from each sample site. If the average distances equaled each other, then the LDH isoenzymes were the same. Thus the organisms had similar LDH enzyme composition between the two sites. If the migration distances were not equal, then LDH isoenzyme composition was different between the two sampling locations.

With the first gel, I did not run the Calf Serum standards with it because it had not come from the company yet. Instead, dye was used, to mark the migration. The results after the electrophoretic run showed that there was a difference between sites. There was a 2.44% migration difference in the samples. Meaning that the Thompson Valley extract traveled 2.24% further than the Bear's Grass extract did. In addition to the six bands I measured for the total distance, there were also some dark streaks occurring before the last band on the gel that the dye picked up. I sketched the outlines of those dark bands onto the transparency for my results, but I did not incorporate those measurements into this data because I mainly concerned myself with the maximum distance traveled by each extract because these bands were distinct and could be compared between the two sample sites. They also could indicate a difference in the sizes of the smallest isoenzymes between sites.

The second gel showed similar results to the first gel. This time, the Calf Serum was used as standards. The standards were in the first and last wells and after the electrophoresis, they measured equally to each other. Because the standards were similar on each side, this shows that the gel is even throughout and the LDH staining is working. I could only see the banding patterns from the first extract. They were all the same length. Banding patterns from the second extract could not be seen on the gel. Several factors could have effected the absence

of the bands, which include: they ran out of the gel, the dye didn't pick up the bands, or there was not enough protein to be seen. Due to the fact that there were no bands in the second extract on this electrophoretic run, the percentage of migration difference is not applicable to this gel. The similarity between gel one and gel two were the dark streaking patterns before the band. The dark streaking patterns occurred in all six of the wells containing extract. The streaks are noted on the transparency.

Gel number three had banding patterns that were different lengths between locations. The Bear's Grass migration distances were longer than the Thompson Valley migration distances. The percent difference in migration distances was 24.44%.

The fourth gel also had banding patterns that were different lengths between locations. Bear's Grass had migration distances that were longer than the Thompson Valley migration distances. The percent difference in migration distances was 12.50%.

I ran a total of four gels with three samples per location for comparison. Therefore the organisms from each location were analyzed for LDH isoenzymes a total of 12 times. The following graph represents the distance of migration from each well for the four gels.

		Thompson Valley			
	Well #1	Well #2	Well #3	Average	
Gel	length in cm	length in cm	length in cm	length in cm	
1	4.1	4.1	4.1	4.1	
2	0	0	0	0	
3	4.5	4.5	4.5	4.5	
4	4.8	4.8	4.8	4.8	
		Bear's Grass			
	Well #1	Well #2	Well #3	Average	Percent Difference
Gel	length in cm	length in cm	length in cm	length in cm	in Migration Distance
1	4.2	4.2	4.2	4.2	2.44%
2	5.5	5.5	5.5	5.5	#DIV/0!
3	5.6	5.6	5.6	5.6	24.44%
4	5.4	5.4	5.4	5.4	12.50%

Conclusions

My hypothesis stated: I believe scuds from two different geographic locations will have different LDH isoenzyme patterns. This is because I feel that the populations of scuds are geographically isolated from one another. My research has shown that the the LDH isoenzyme patterns of scuds from two different geographic locations are different to some extent by factors of 2.44%, 12.50%, and 24.44% differences in gel migration distances. This shows a strong possibility for different molecular forms of the LDH isoenzymes. Since gel electrophoresis separates molecules by size, and because the banding patterns of the sample sites were different, the LDH isoenzymes were different. LDH is a protein and proteins are coded by DNA. If the proteins are different between two populations then the DNA must also be different between the two populations, thus indicating a genetic difference between the two.

My results support geographic isolation because generally, the greater the distance between local breeding populations, less gene flow occurs between them. Organisms with little or no gene flow between species that live in different locations, tend to go on their own evolutionary way, adapting to different conditions in their own locations. These adaptations are recorded in their DNA and expressed in proteins.

I believe that with more sampling and gel electrophoresis runs, my data and conclusions would be stronger. Time was a limiting factor in this project because the procedures required much time.

Bibilography

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