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2005 Florida Coast Deep Corals

Are You Related?

Focus
Molecular genetics of deep-sea corals

Grade Level
9-12 (Life Science)

Focus Question
How can scientists use genetic evidence to distinguish species of marine organisms?

Learning Objectives
Students will be able to define "microsatellite markers," and explain how they may be used to identify different populations and species.

Students will be able to explain two definitions of "species," and describe processes that result in speciation.

Students will be able to use microsatellite data to make inferences about populations of deep-sea corals.

Materials
- ❒ Copies of "Molecular Genetics of Deep-Sea Corals Worksheet," one copy for each student or student group
- ❒ (Optional) Copies of reference materials (see Learning Procedure, Step 1)

Audio/Visual Materials
- ❒ None

Teaching Time
One or two 45-minute class periods

Seating Arrangement
Groups of two to four students

Maximum Number of Students
30

Key Words
Molecular genetics
Microsatellite
Population
Speciation
Biodiversity
Lophelia pertusa
Deep-sea coral

Background Information
Coral reefs are one of the most species-rich ecosystems on Earth. Shallow-water coral reefs are visited by millions of tourists every year, and are familiar to many others thanks to numerous print, film, and video productions. In contrast, reefs formed by deep-water corals are virtually unknown to the general public and have received much less scientific study. Yet, deep-water reef ecosystems may have a diversity of species comparable to that of corals reefs in shallow waters, are found on continental margins around the world, and provide habitat for many fishes and invertebrates that may be of direct importance to humans. Unfortunately, deep-water reefs are increasingly being impacted by destructive fishing practices and other human activities.

Deep-water coral reefs were discovered in the Gulf of Mexico nearly 50 years ago, but very little is

known about the ecology of these communities or the basic biology of the corals that produce them. One of the most conspicuous differences between shallow- and deep-water corals is that most shallow-water species have symbiotic algae (zooxanthellae) living inside the coral tissue, and these algae play an important part in reef-building and biological productivity. Deep-water corals do not contain symbiotic algae (so these corals are termed "azooxanthellate"). Yet, there are as many species of deepwater corals (slightly more, in fact) as there are species of shallow-water corals.

The major structure-building corals in the deep sea belong to the genus *Lophelia*, which has been intensively studied on deep-water coral reefs near the coasts of Europe. Deep-sea coral structures and their associated organisms are often referred to as bioherms or lithoherms. Bioherms are mound-like ancient reefs built by marine invertebrates. Some bioherms may have a covering of live coral over mud and coral debris. Lithoherms are high-relief structures composed of consolidated limestone, which provide hard substrate for a variety of organisms. Most reports of *Lophelia* reefs in the Gulf of Mexico are the result of investigations directed toward hydrocarbon seepage and/or chemosynthetic communities. Scientists studying deep-water reefs on the Norwegian continental shelf have found that many large *Lophelia* banks occur at sites where there are relatively high levels of light hydrocarbons present in the sediments. The reason for this correlation is not known, nor is it known whether a similar correlation exists in the hydrocarbon-rich Gulf of Mexico.

While *Lophelia* corals are capable of building substantial reefs, they are also quite fragile, and there is increasing concern that these reefs and their associated resources may be in serious danger. Many investigations have reported large-scale damage due to commercial fishing trawlers, and there is also concern about damage that might result from exploration and extraction of fossil fuels. The objectives of the 2005 Florida Coast Deep Corals Expedition are to:

- Map selected deep-water, high-relief coral ecosystems on southwestern and eastern regions of the Florida shelf;
- Describe biological communities associated with these reefs;
- Identify dominant fish species associated with these coral communities; and
- Describe the geologic and hydrographic features of each site.

One of the many questions facing scientists exploring *Lophelia* coral reefs is whether *Lophelia* corals in one location are the same species as *Lophelia* corals in other locations. Historically, corals have been classified primarily on the basis of structural characteristics of their skeletons. On this basis, *Lophelia* corals are all classified as a single species. But recent research using techniques of molecular biology to study genetic characteristics has shown that *Lophelia* corals from the North Atlantic are genetically different from *Lophelia* corals collected near Brazil, suggesting that these populations are genetically isolated from one another. This finding has important implications for the conservation and protection of deep -sea coral reef resources, because if corals on different reefs are genetically distinct, then many more areas must be protected to maintain biological diversity than would be necessary if corals were genetically identical on all deep-water reefs.

In this lesson, students will investigate the concepts of gene flow, genetic isolation, and microsatellite markers, and apply these concepts to make inferences about the genetic "connectedness" in a series of hypothetical deepwater coral populations.

Learning Procedure

[Note: This activity is adapted from approaches used in classroom activities by R. P. Filson and the San Diego Zoo, available online at http://www.ucmp.berkeley.edu/fosrec/Filson.html, and http://www.sandiegozoo.org/teachers/images/curriculum_microsatellite.pdf respectively].

1. To prepare for this lesson, read the introductory essays for the 2005 Florida Coast Deep Corals Expedition at http://oceanexplorer.noaa.gov/explorations/05deepcorals/welcome.html, and "Community Genetics - All About Relationships" at http://oceanexplorer.noaa.gov/explorations/05coralbanks/background/community_genetics/community_genetics.html

   If students will not have internet access, you will also need to download the interview with Edward O. Wilson titled "Speciation and Biodiversity" from http://www.actionbioscience.org/biodiversity/wilson.html

   Worksheet activities are divided into three parts. Part I is a brief review of some basic concepts of molecular biology. If students are already familiar with this material, Part I can be skipped or used as an independent review of these concepts. Alternatively, you may want to have students review the information in "A Primer on Molecular Biology" (http://www.coris.noaa.gov/exchanges/coralgenome/sup_genome_primer.html) and discuss answers to Part I prior to completing the rest of the worksheet.

   Part II of the worksheet is a review of some concepts of speciation (how species come to exist), based on an interview with Edward O. Wilson, internationally regarded as "the dean of biodiversity." Part III is an exercise using microsatellite data as the basis for inferences about genetic "connectedness" among a series of hypothetical deepwater coral populations. Note that these data are greatly simplified for the purpose of this activity; typical microsatellite sequences for *Lophelia pertusa* are usually several hundred nucleotides long, and analysis is accomplished with computer programs. If you or your students want to see actual nucleotide sequences for *L. pertusa* or many other organisms, visit the National Center for Biotechnology Information's Nucleotides Database at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Nucleotide

2. Briefly review deep-water coral reefs, and contrast these reefs with the more familiar shallow-water reefs. Be sure students understand that both types of reef exhibit a high degree of biodiversity and are important habitat providers for many species. Much less is known about deep reefs, however, because of their location. Students should also realize that deep reefs are found on continental shelves worldwide. Briefly discuss the role of *Lophelia pertusa* in building deep-sea coral reefs, and the question of whether this is a single species with a global distribution, or whether different species occur in different areas.

3. Provide each student group with a copy of the "Molecular Genetics of Deep-Sea Corals Worksheet," as well as copies of materials discussed in Step 1 (if necessary).

4. Lead a discussion of students' answers to questions on the worksheet. The following points should be included:

   In Part I, the text should read:

   DNA is the chemical molecule that contains information needed to replicate an organism. The DNA molecule is often described as a double HELIX. If the DNA molecule were to straighten out, it would resemble a ladder. The side rails of this ladder are formed by molecules of the sugar DEOXYRIBOSE. Each sugar molecule is joined to a phosphate group and a NITROGEN base, which may be adenine, guanine, cytosine, or thymine. Each base forms one-half of a rung on the "ladder." Each base on one rail bonds with a base on the other rail to form a complete "rung" or base pair. Adenine always forms a base pair with THYMINE while cytosine always forms a base pair with GUANINE. The combination of a sugar molecule, phosphate group, and base is known as a NUCLEOTIDE. The specific sequence of bases forms a code that contains

the information needed to synthesize PROTEINS that control the activities of cells.

A segment of a DNA molecule that contains the code for a specific protein is called a GENE. In some cases, this code may have several alternative forms. A segment of DNA that codes for hair color, for example, may have a form that codes for black hair, another form that codes for red hair, another form that codes for blonde hair, etc. These alternative forms are called ALLELES, and each has a unique sequence of nucleotides.

A GENOME is defined as all of the genetic material (DNA) in the chromosomes of a particular organism. Not all of this material codes for specific proteins. In fact, only about TWO percent of the DNA in human chromosomes codes for protein synthesis. The remainder consists of NONCODING regions whose function is unknown, but may be involved in maintaining the physical structure of chromosomes and/or regulating the timing, location, and quantities of protein synthesis.

Part II

- The "classical" definition of a species is "A population or a series of populations of individuals that interbreed freely with one another." Note that in this context, "population" means a group of organisms that interbreed and share a gene pool.

- This definition does not work for species that reproduce asexually because individuals of these species do not interbreed. An alternative approach is to define a species as a group of organisms whose DNA nucleotide sequences do not differ by more than an arbitrary percentage (30% in the case of bacteria). Students should realize that two "species" identified by this definition might be quite similar or very different. In fact, a similar uncertainty results from the "classical" definition as well: There is a big difference between two populations that are biologically unable to interbreed (dogs and sparrows, for example) and two populations that are biologically capable of interbreeding but do not do so because of geographic isolation (such as fishes in two widely separated lakes).

- Factors that favor an increase in the number of species in an ecosystem include energy (abundant and diverse food sources favor more species), stability (more species accumulate in a stable ecosystem because there is more time to adapt to particular niches), and area (larger areas are able to accommodate more species).

- Speciation is the process in which related organisms change to a point at which they are different enough to be considered separate species. Sympatric speciation occurs among similar organisms in close proximity that don't interbreed because of differences in behavior, even though they theoretically could. Allopatric speciation occurs among similar organisms that theoretically could interbreed but do not because they are geographically separated.

- Population size is critical to survival. As population size decreases, there is increasing probability that lethal genes will occur. Wilson states that a population size of 500 individuals is the minimum needed to keep a species alive and healthy, while a population size of 50 individuals is only adequate for a short period of time.

- Species are disappearing from Earth's ecosystems approximately 1,000 times more rapidly than new species are appearing.

- E.O. Wilson's "hot spots" include tropical forests, freshwater systems, and coral reefs.

Part III

Repeating sequences in Table 1 are:

Location	**Locus 1**	**Locus 2**	**Locus 3**
Off Jacksonville	(AGAC)3	(AC)6	(AGC)4
Off Jupiter Inlet	(AGAC)3	(AC)6	(AGC)3
Miami Terrace	(AGAC)4	(AC)6	(AGC)3
Pulley Ridge	(AGAC)3	(AC)4	(AGC)3
Middle Grounds	(AGAC)4	(AC)4	(AGC)3

Location	**Locus 4**	**Locus 5**	**Locus 6**
Off Jacksonville	(ATA)4	(GA)3 (CG)5	(AAG)4
Off Jupiter Inlet	(ATA)4	(GA)3 (CG)5	(AAG)4
Miami Terrace	(ATA)4	(GA)4 (CG)4	(AAG)4
Pulley Ridge	(AG)3 (ATA)3	(GA)4 (CG)4	(AAG)4
Middle Grounds	(AG)3 (ATA)3	(GA)4 (CG)4	(AAG)4

Location	**Locus 7**	**Locus 8**	**Locus 9**
Off Jacksonville	(CA)4 (AC)3	(GTT)5	(GA)4 (TC)3
Off Jupiter Inlet	(CA)4 (AC)3	(GTT)5	(GA)4 (TC)3
Miami Terrace	(CA)3 (AC)4	(GTT)3	(GA)4 (TC)3
Pulley Ridge	(CA)3 (AC)4	(GTT)3	(GA)7
Middle Grounds	(CA)3 (AC)4	(GTT)3	(GA)7

Shared alleles among the ten different pair combinations are:

	Jacksonville	Jupiter Inlet	Miami Terrace	Pulley Ridge	Middle Grounds
Jacksonville					
Jupiter Inlet	8				
Miami Terrace	4	5			
Pulley Ridge	2	3	5		
Middle Grounds	1	2	6	8	

Based on microsatellite data in Table 1, corals from the the Jacksonville + Jupiter Inlet and Pulley Ridge + Middle Grounds sites are most similar with eight alleles in common. These corals are somewhat similar to corals from Miami Terrace, with 4 - 6 alleles in common. Corals from the Jacksonville and Jupiter Inlet sites are much more distantly related to corals from the Pulley Ridge and Middle Grounds sites with only 1 - 3 alleles in common. Using the 30% criteria discussed in Part II, corals from the Jacksonville and Jupiter Inlet sites would be considered a single species, corals from the Pulley Ridge and Middle Grounds sites would be considered a second species, and corals from Miami Terrace would be a third species. Three distinct populations appear to be represented by these species. This information would suggest that at least three populations would need to be protected to maintain biological diversity in the area sampled.

Students sometimes describe speciation and evolutionary processes in terms of something a species "needs to do" in order to survive. Be sure students understand that individuals of a species cannot voluntarily evolve, no matter how much they need to do so; but individuals can (and typically do) migrate to follow suitable habitats as the location of these habitats shifts in response to environmental change (this is called "habitat tracking").

The Bridge Connection

http://www.vims.edu/bridge/ – In the "Site Navigation" menu on the left, click on "Ocean Science Topics," then "Biology," then "Biodiversity" for links to topics on biodiversity and evolution.

The "Me" Connection

Have students write a short essay describing conditions that would favor the development of a new species from a population of *Homo sapiens*, and whether the process would be allopatric or sympatric speciation.

Connections to Other Subjects

English/Language Arts, Earth Science

Evaluation

Answers to worksheet questions and participation in class discussions provide opportunities for assessment.

Extensions

1. Have students visit http://oceanexplorer.noaa.gov/explorations to keep up to date with the latest discoveries by the 2005 Florida Coast Deep Corals Expedition.

2. Visit http://www.ucmp.berkeley.edu/fosrec/Filson.html, and http://www.sandiegozoo.org/teachers/images/curriculum_microsatellite.pdf for additional activities involving biogeography, molecular genetics, and evolution.

Resources

http://oceanexplorer.noaa.gov/

Roberts, S. and M. Hirshfield. Deep Sea Corals: Out of sight but no longer out of mind. http://www.oceana.org/fileadmin/oceana/uploads/reports/oceana_coral_report_final.pdf — Background on deep-sea coral reefs

http://www.ucmp.berkeley.edu/fosrec/Filson.html – "Island Biogeography and Evolution: Solving a Phylogenetic Puzzle Using Molecular Genetics" classroom activity by R. P. Filson

http://www.sandiegozoo.org/teachers/images/curriculum_microsatellite.pdf – "Buddies or Siblings? Determining Relatedness in Anegada Iguanas" classroom activity from the San Diego Zoo

http://www.coris.noaa.gov/exchanges/coralgenome/sup_genome_primer.html – "A Primer on Molecular Biology"

http://www.actionbioscience.org/biodiversity/wilson.html – "Speciation and Biodiversity," an interview with Edward O. Wilson

http://www.pbs.org/wgbh/evolution/library/glossary/ – A glossary from PBS of terms related to evolution

http://oceanexplorer.noaa.gov/gallery/livingocean/livingocean_coral.html – Ocean Explorer photograph gallery

http://oceanica.cofc.edu/activities.htm – Project Oceanica Web site, with a variety of resources on ocean exploration topics

National Science Education Standards

Content Standard A: Science as Inquiry

- Abilities necessary to do scientific inquiry
- Understandings about scientific inquiry

Content Standard C: Life Science

- The cell
- Molecular basis of heredity
- Biological evolution
- Interdependence of organisms

Content Standard E: Science and Technology

- Understandings about science and technology

Content Standard F: Science in Personal and Social Perspectives

- Population growth
- Natural resources
- Environmental quality
- Natural and human-induced hazards
- Science and technology in local, national, and global challenges

For More Information

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Molecular Genetics of Deep-Sea Corals Worksheet

Part I.

Fill in the blanks:

DNA is the chemical molecule that contains information needed to replicate an organism. The DNA molecule is often described as a double _____. If the DNA molecule were to straighten out, it would resemble a ladder. The side rails of this ladder are formed by molecules of the sugar _____. Each sugar molecule is joined to a phosphate group and a _____ base, which may be adenine, guanine, cytosine, or thymine. Each base forms one-half of a rung on the "ladder." Each base on one rail bonds with a base on the other rail to form a complete "rung" or base pair. Adenine always forms a base pair with _____, while cytosine always forms a base pair with _____. The combination of a sugar molecule, phosphate group, and base is known as a _____. The specific sequence of bases forms a code that contains the information needed to synthesize _____ that control the activities of cells.

A segment of a DNA molecule that contains the code for a specific protein is called a _____. In some cases, this code may have several alternative forms. A segment of DNA that codes for hair color, for example, may have a form that codes for black hair, another form that codes for red hair, another form that codes for blonde hair, etc. These alternative forms are called _____, and each has a unique sequence of nucleotides.

A _____ is defined as all of the genetic material (DNA) in the chromosomes of a particular organism. Not all of this material codes for specific proteins. In fact, only about _____ percent of the DNA in human chromosomes codes for protein synthesis. The remainder consists of _____ regions whose function is unknown, but may be involved in maintaining the physical structure of chromosomes and/or regulating the timing, location, and quantities of protein synthesis.

Part II

Read the interview with Edward O. Wilson titled "Speciation and Biodiversity" (http://www.actionbioscience.org/biodiversity/wilson.html), then answer the following questions: [Hint: You may want to consult the glossary of terms at http://www.pbs.org/wgbh/evolution/library/glossary/]

1. What is the "classical" definition of a species?

2. Does this definition work for species that reproduce asexually? If not, what is an alternative definition that includes these kinds of species?

3. What factors favor an increase in the number of species in an ecosystem?

4. What is speciation? What is the difference between sympatric and allopatric speciation?

5. What is the relationship between population size and survival of a species?

6. How rapidly are species disappearing from Earth's ecosystems compared to how rapidly new species are appearing?

7. What areas does E. O. Wilson identify as "hot spots" where habitats are most endangered and have the largest number of species found nowhere else?

Part III

One of the relatively new molecular tools being used by scientists studying populations of deep-sea corals involves "microsatellite markers." Microsatellites are regions (also called "loci," singular "locus") on a strand of DNA in which there is a repeating sequence of nucleotides, such as

adenine-cytosine-adenine-cytosine-adenine-cytosine-adenine-cytosine;
or A-C-A-C-A-C-A-C- for short;
or (AC)4 for shorter.

These repeating sequences occur in the non-coding regions of the DNA strand, but are inherited when the DNA replicates in the same way that genes are inherited. The length of sequences used in most analyses are two, three, or four nucleotides long, and are called di-, tri-, or tetra-nucleotides respectively. On either side of each microsatellite segment there are specific "flanking" sequences of nucleotides that are used to locate specific microsatellite segments on a DNA strand.

What makes microsatellites useful is that the number of times a particular sequence repeats often varies between individuals, populations, and species. So, there might be four nucleotide repetitions at a particular microsatellite locus in one population, while another population might have five nucleotide repetitions at this locus. Each different repetition sequence at a particular locus is designated as an allele (note that this broadens the definition of allele given in Part I). By examining the similarities between many different microsatellite loci, scientists can obtain indications of how genetically alike different organisms may be. So, if two individuals had the same alleles at ten different microsatellite loci, a scientist might infer that these individuals were more alike than two other individuals that had different alleles at these loci.

The coral *Lophelia pertusa* is one of the major deep reef building species and is found on continental shelves around the world. Historically, coral species have been identified on the basis of their skeletal structure. But recent investigations have found some corals whose skeletal structures are very different genetically, suggesting that these corals may represent different species. Microsatellites may provide a way to resolve this kind of uncertainty.

In this exercise, you will analyze nucleotide sequence information for ten microsatellite loci on DNA from specimens of the deep-sea coral *Lophelia pertusa* collected from five different locations. Then, you will use this information to make inferences about which of these specimens may represent different populations or possibly different species.

Table 1 lists the nucleotide sequences for nine microsatellite loci for each specimen. Portions of one or both flanking sequences may also be included. The first step in your analysis is to summarize the repeating sequence at each locus for each specimen using the "(AC)4" notation described above. Note that a sequence may consist of several repeating sets, such as (AGC)4(CG)7(TAG)5. You may want to spread the work around by having each member of your group summarize the sequences for a specimen from a single location.

Next, compare each specimen with the other four specimens to determine how many of the nine alleles are the same. There are ten different pair combinations possible among the five specimens. Record your results in Table 2. The first pair (specimen from off Jacksonville and specimen from off Jupiter Inlet) has been filled in as an example. These specimens have the same allele at eight of the nine microsatellite loci. Again, you can spread the work around by assigning one or two pair combinations to each group member.

Finally, use the data in Table 2 as a basis for answering the following questions:

1. Which specimens appear to be closely related?

2. How many different populations appear to be represented by these specimens?

3. How could this information affect a strategy for protecting deep-water coral reefs in the area sampled?

Table 1

Nucelotide Sequences for Nine Microsatellite Loci on DNA from *Lophelia pertusa* Collected at Five Different Locations

Location	Microsatellite Locus 1	Microsatellite Locus 2	Microsatellite Locus 3
Off Jacksonville	AGTTAGACAGACAGACAGTC	GTGAACACACACACACCATA	CGTAAGCAGCAGCAGCGTCC
Off Jupiter Inlet	AGTTAGACAGACAGACAGTC	GTGAACACACACACACCATA	CACGTAAGCAGCAGCGTCCG
Miami Terrace	TTAGACAGACAGACAGACTC	GTGAACACACACACACCATA	CACGTAAGCAGCAGCGTCCG
Pulley Ridge	AGTTAGACAGACAGACAGTC	ACGTGAACACACACCATAAC	CACGTAAGCAGCAGCGTCCG
Middle Grounds	TTAGACAGACAGACAGACTC	ACGTGAACACACACCATAAC	CACGTAAGCAGCAGCGTCCG

Location	Microsatellite Locus 4	Microsatellite Locus 5	Microsatellite Locus 6
Off Jacksonville	TGCCATAATAATAATAAGCA	GTGAGAGACGCGCGCGCGAA	TGCAAGAAGAAGAAGGTCC
Off Jupiter Inlet	TGCCATAATAATAATAAGCA	GTGAGAGACGCGCGCGCGAA	TGCAAGAAGAAGAAGGTCC
Miami Terrace	TGCCATAATAATAATAAGCA	GTGAGAGAGACGCGCGCGAA	TGCAAGAAGAAGAAGGTCC
Pulley Ridge	TCCAGAGAGATAATAATAGC	GTGAGAGAGACGCGCGCGAA	TGCAAGAAGAAGAAGGTCC
Middle Grounds	TCCAGAGAGATAATAATAGC	GTGAGAGAGACGCGCGCGAA	TGCAAGAAGAAGAAGGTCC

Location	Microsatellite Locus 7	Microsatellite Locus 8	Microsatellite Locus 9
Off Jacksonville	AACCACACACAACACACTCA	AACGTTGTTGTTGTTGTTCA	GTAGAGAGAGATCTCTCCGA
Off Jupiter Inlet	AACCACACACAACACACTCA	AACGTTGTTGTTGTTGTTCA	GTAGAGAGAGATCTCTCCGA
Miami Terrace	AACCACACAACACACACTCA	TAGAACGTTGTTGTTCACTT	GTAGAGAGAGATCTCTCCGA
Pulley Ridge	AACCACACAACACACACTCA	TAGAACGTTGTTGTTCACTT	GTAGAGAGAGAGAGAGACGA
Middle Grounds	AACCACACAACACACACTCA	TAGAACGTTGTTGTTCACTT	GTAGAGAGAGAGAGAGACGA

Table 2

	Jacksonville	Jupiter Inlet	Miami Terrace	Pulley Ridge	Middle Grounds
Jacksonville					
Jupiter Inlet	8				
Miami Terrace					
Pulley Ridge					
Middle Grounds					