Classification by Application of Modern Technologies

Outcomes:
1. Explain how scientific knowledge evolves as new evidence comes to light and as laws and theories are tested and subsequently restricted, revised, or replaced. (115-7)
2. Analyze and describe examples where scientific understanding was enhanced or revised as a result of the invention of a technology. (116-2)
3. Construct arguments to support a decision or judgement, using examples and evidence and recognizing various perspectives. (118-6)
4. Identify new questions or problems that arise from what was learned. (214-17)
5. Use organisms found in a local or regional ecosystem to demonstrate an understanding of the fundamental principles of taxonomy. (316-5)

Introduction

New and modern classification techniques have provided important diagnostic tools in the classification of organisms. The unique chemical nature of DNA can be used to identify individual organisms and/or to classify organisms. It provides a tool that can confirm relationships between individuals and also provides a means to identify a species using only the smallest fragment of biological material. All multicellular organisms have this unique fingerprint. There are many possible applications of DNA analysis. Relationships and identification using DNA evidence is widely used in courtrooms to convict perpetrators of crime as well to acquit those who are wrongly convicted.

Through a mapping of the unique chemical nature of the DNA molecule, biologists can attain valuable information to further enhance their work. As methods of DNA analysis become more refined and utilized, the science of classification is improved. The following examples illustrate some of the possibilities of these techniques and how they help science.

Fish Forensics

While on a normal patrol of the Flemish Cap a Canadian Coast Guard helicopter spots a foreign trawler. When the pilot radios in the location and name of the vessel she finds that the vessel has a quota for pollack, yet the area where the vessel is sighted is far from the grounds on which pollack would be caught. Yielding to her suspicions, the pilot has a support vessel pursue the trawler and detain it.

Imagine that you are the DFO (Department of Fisheries and Ocean) Scientist assigned to the case. You have strong reason to believe that the fish caught by the trawler is Atlantic Cod, yet the crew maintains that the filtered and salted fish are Pollack. How could you possibly prove that the fish is Atlantic Cod? Visually examining the flesh of the fish which has been filtered, iced, and/or salted would not aid in the identification due to the similar appearance pollack has to cod in texture, color, and smell. The answer lies in using DNA analysis.
The identity of the species of fish that has been caught and processed can be difficult to determine. Questions may arise as to the origin of a commercial fish product, for example, whether the catch in a boat's hold has been properly reported, or whether the label on a commercial product is accurate. Although physical identification is difficult or impossible once skin and scales are gone, the fish DNA survives processing in sufficient quantities to provide a reliable test.

![Diagram of fish species]

Figure 1: Comparison of DNA for known genetic species

In the test shown above, the identity of four salt-cured fish was questioned. DNA from each was amplified (copied), sequenced (coded), and compared to a DNA database of known cod, pollock, and hake species. The analysis produces a "family tree," which shows that each fish came from a different commercial species: walleye or Alaska pollock or Pollachius virens (Fish 4), Atlantic cod or Gadus morhua (Fish 3), Pacific cod or Gadus macrocephalus (Fish 2), and pollock/saithe or Gadus thayera (Fish 1). Similar tests can be used to differentiate many species of fish. If a sample of fish is found to contain similar DNA to a known species, then it can be concluded that the sample would be the same species.

In order to compare genetic material, the actual sequence of bases must be determined in each sample. This analysis can be done through the use of gel electrophoresis. When the actual sequence of nitrogenous bases is determined, a direct comparison can be made between, not only species, but also between the individuals. The closer the sequences are to each other, the more closely related the individuals.

The samples taken are often small, therefore, the amount of viable genetic material is often limited. To overcome this problem it is necessary to amplify or copy the DNA material. Older technologies (recombinant technologies) were capable of doing this, but it was time-consuming. The modern means of amplifying and copying DNA is known as Polymerase Chain Reaction (PCR). PCR can amplify DNA of any origin (virus, bacteria, plant, or human) hundreds of millions of times in a matter of hours, a task that would have required several days with recombinant technology. PCR is especially valuable because the reaction is highly specific, easily automated, and capable of amplifying minute amounts of sample. For these reasons, PCR has had a major impact on clinical medicine, genetic disease diagnostics, forensic science, and evolutionary biology. A diagrammatic representation of the PCR process is found in figure 2.
DNA Amplification Using Polymerase Chain Reaction

Reaction mixture contains target DNA sequence to be amplified, two primers (P1, P2) and heat-stable Taq polymerase

Reaction mixture is heated to 95°C to denature target DNA. Subsequent cooling to 55°C allows primers to hybridize to complementary sequences in target DNA.

When heated to 72°C, Taq polymerase extends complementary strands from primers.

First synthesis cycle results in two copies of target DNA sequence.

Denature DNA

Hybridize primers

Extend new DNA strands

Second synthesis cycle results in four copies of target DNA sequence.

Figure 2: PCR Reaction

The Fortune Bay "Sea Monster"

In the summer of 2001 there was a fascinating story that came from the small outpost town of St. Bernard's. Washed up on shore was an unknown creature that local residents and scientists could not visually identify. Reports were issued that describe the large creature as having fur. The description of a large marine creature with fur started many rumors of a washed up "Sea Monster". A picture of the "sea-monster" is found at the end of this section. Genetic testing using PCR, and subsequent sequencing revealed that the "sea-monster" was in fact a badly decomposed sperm whale. As for the fur, it was actually badly decomposed fatty tissue. The following is a news release that was issued.
shortly after the DNA analysis was completed.

St. John's, Newfoundland. 16 August 2001 - DNA testing has identified the "sea monster" that washed ashore at St. Bernard's, Fortune Bay, as the remnant of a sperm whale (Physeter catodon).

Based on material provided by Dr. Garry Stenson, of the Department of Fisheries and Oceans in St. John's, scientists at the Genetics, Evolution, and Molecular Systematics Laboratory in the Department of Biology at Memorial University of Newfoundland (Dr. Steve Carr, Dr. Dawn Marshall, Ms. Kim Johnstone, and Ms. Lori Lynn) performed a forensic DNA test to determine species origin. The analysis compared the DNA sequence of the creature's NADH2 gene (a specific genetic marker) with that of homologous DNA from a variety of large marine species, including sharks and whales. Comparison with this database gave an almost perfect match with a sperm whale. The few observed differences are consistent with ordinary genetic variation expected among individuals within species.

The test involves a "DNA xerowing" procedure called the polymerase chain reaction, which generates a large number of copies from a single original gene. The sequence of the gene can then be determined on an automated DNA sequencer. This type of DNA test is particularly useful in cases like the sea monster, which involve material in a poor state of preservation or of questionable origin. The identification was done as part of an ongoing collaboration between DFO and Memorial scientists to study the genetics and genomics of marine organisms.

Figure 3: The "Fortune Bay Sea Monster"

The DNA analysis technique described in the article is identical to the one used in our hypothetical case of foreigner over fishing. Careful examination of a minimal amount of genetic material enables scientists to determine organisms that otherwise would be impossible to identify.
Stock Structure in Atlantic Cod

DNA classification techniques can also be used to help make important commercial fish management decisions. Setting inshore and offshore quotas is very difficult when it is unknown if fish migrate between these two regions. This could be more easily determined if the fish could be examined to check for distinct breeding units, which can be quite significant as it pertains to setting quotas. Knowing whether the fish move between the regions would have a significant impact on quotas set by DFO.

Determining if distinct breeding exist would indicate whether fish migrate. If the fish populations were separate breeding stocks then their DNA would be different. This would mean that quotas for both could be separately established. It is widely known that present stocks of inshore cod are high, while offshore stocks are still very low. Many inshore fisherman often argue that the inshore fishery should be reopened on a larger commercial basis. DFO scientists are hesitant about this because they see a possibility of the inshore fish being used to replenish the depleted offshore stocks.

The Genetics, Evolution, and Molecular Systematics Laboratory at Memorial University has pioneered the application of DNA analysis techniques to study the natural populations of marine organisms. In particular, direct analysis of DNA sequences via the polymerase chain reaction (PCR) technology has been developed as a tool to investigate several fishery management questions. One in particular, is whether distinct breeding units exist between the inshore and offshore regions.

Conclusion

The science of classification has progressed greatly with the development of new DNA analysis technologies. These technologies have empowered biologists with an ability to gather much more information about individual organisms and their relationships to other species using small amounts of genetic material. Identification and subsequent classification of organisms creates evolutionary links between known and unknown species. For example, evolutionary links between humans are being uncovered by the Human Genome Project. Methods and technologies that help scientists classify and organize information enhances our ability to understand the world around us, i.e., it improves science.

Questions

1. How could the information gathered through genetic analysis be applied to Fisheries management practices?

2. During a routine check on an offshore trawler DFO officials discover a large supply of what they believe to be frozen cod fillets. The crew has no licence for the species but maintain that the fish is pollack for which they had a quota. Discuss how the situation could be resolved.

3. What is the significance to the Inshore fishery of the province if it can be determined that the Offshore and Inshore populations of Cod are genetically distinct?

4. There have been suggestions by some that the Beothuk Indians were amalgamated into the population of early Newfoundland. How could the techniques of Molecular (DNA) classification be used to confirm or deny this suggestion? Would the conclusions reached by this method be a valid one?

5. Area quota systems are used to effectively manage big game populations. This involves managing each area as separate "stocks". How could the science of DNA classification be used to more effectively define the boundaries that determine the areas? Why would this be (or not be) a practical means to divide the areas?