Bio 80M
Conservation in the Sea
Threats to Genetic Diversity

Outline:

1. What is genetic diversity and how do we measure it?
2. Why is genetic diversity important?
3. Threats to genetic diversity - some examples.

What is genetic diversity?

- it is possible to describe “genetic diversity” at various levels of biological organization.

1. an individual organism
- different individuals may differ in the number of different gene copies that they possess.
- in a diploid species, the maximum number of different forms of a gene that may be present at any given genetic locus is two.
- these different forms of a gene are called alleles.
- suppose we analyzed the hexokinase (\textit{Hex}) locus, an enzyme catalyzing the first reaction in glycolysis.

\[
\text{Hex}^A \text{ or } “A” \quad \text{Hex}^B \text{ or } “B”
\]

- if different alleles are present at the hexokinase gene it is said to be \textbf{polymorphic}.
- if an individual has two different alleles at a gene, it is said to be \textbf{heterozygous} - AB.
- if it has two copies of the same allele it is said to be \textbf{homozygous} – AA or BB.
- the number of heterozygous loci per individual may vary.
- an individual may be heterozygous at the hexokinase locus but homozygous at another gene locus.

2. a population of organisms
- the amount of genetic variation present in an interbreeding population of organisms usually greatly exceeds that present in individual organisms.
- this is a simple consequence of two factors.
- first, the total number of alleles present in a population at a given gene can be much greater than two (the maximum number of different forms of the gene present in a given organism.)
- second, if a gene is polymorphic in a population, not all individuals will be heterozygous at that gene.
- some will be heterozygous, some will be homozygous.
- the proportions of individuals that are heterozygous and homozygous are determined by the \textbf{frequencies of alleles} at that gene in the population.
- for example, consider a genetic locus with two alleles: A and B.
- if the species considered is diploid, then there can be three genotypes: AA, AB and BB.
- let the frequency of the A allele be $p$ and the frequency of the B allele be $q$. 
- a fundamental principle of population genetics called the Hardy-Weinberg equilibrium dictates that the frequencies of different genotypes is given by $p^2$, $2pq$, and $q^2$.

<table>
<thead>
<tr>
<th>Genotype:</th>
<th>AA</th>
<th>AB</th>
<th>BB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency:</td>
<td>$p^2$</td>
<td>$2pq$</td>
<td>$q^2$</td>
</tr>
</tbody>
</table>

- consider the following two examples.

<table>
<thead>
<tr>
<th>Allele frequencies</th>
<th>Genotype frequencies</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>0.9</td>
<td>0.1</td>
</tr>
</tbody>
</table>

- the extent of genetic variability is clearly higher when the two alleles are present at equal frequency.
- this principle holds true irrespective of allele numbers.

3. a species (a collection of species populations)
- different populations of a species may be genetically differentiated from each other to varying degrees.
- if they are, the extent of genetic diversity in the entire species exceeds that present within any one population.
- the term used to describe the genetic subdivision of a species is called **population structure**.
- as shown later in class, to adequately conserve a species - marine or terrestrial - requires knowledge of the degree of population structure of the species.

4. a group of related species.
- the final level that the term genetic diversity can be applied involves a group of related species of organisms.
- here, the term “genetic diversity” is synonymous with “species diversity”.
- preserving genetic diversity in this sense means preserving a group of related species.
- by doing so, one preserves the legacy of their evolutionary history.

**How do we measure genetic diversity?**

- true measures of genetic diversity concern parameters that are specific to **populations**, not **individuals**.
- to adequately assess levels of genetic diversity we need to obtain a random sample of individuals from natural populations and examine their genotypes at a number of independent genetic loci.
- how do we go about estimating gene diversity?

**The molecular genetics toolkit**
1. Polymorphic proteins (allozymes)

- developed originally by Oliver Smithies in the late 1950’s.
- first applied to population-level variation by Harris (1966) on humans and Lewontin and Hubby (1966) on *Drosophila*.
- one scores allozymes by obtaining tissue sample from a population - for example blood samples.
- the next step is to grind up the tissue to liberate enzymes and other proteins from within cells.
- the homogenized tissue is centrifuged and a small amount of the liquid supernatant is applied to a gel - starch or acrylamide.
- the gel is exposed to an electric field and the proteins migrate in the gel because they have a net negative charge.
- after the proteins have migrated a set distance, the positions of different enzymes are localized by incubating a slice of the gel with a solution specific to a certain enzyme (i.e., has the substrate and cofactors necessary for catalysis).
- different bands may appear in different individuals caused by mutations in the protein products that alter the net charge of the protein.

2. DNA markers

   a. mitochondrial DNA (mtDNA)

- in most vertebrates, mtDNA is a circular molecule 16,000 to 18,000 bp in size.
- it is haploid and usually maternally inherited.
- it possesses 37 genes, 22 are tRNAs, 2 are rRNAs, and 13 are polypeptides.
- the mtDNA genome lacks introns, pseudogenes and intervening sequences that are common in nuclear DNA (nDNA).
- various techniques have been developed to score polymorphisms in mtDNA.
- originally mtDNA are isolated from purified mitochondria, digested with a variety of restriction endonucleases and the resulting products run on agarose gels.
- restriction endonucleases are enzymes that cut DNA when at highly specific recognition sequences (usually 4 to 6 bp in size).
- for example, the restriction enzyme *Eco*RI cuts DNA at the sequence GATTC.
- suppose we isolated mtDNA from five individuals and cut the DNA with *Eco*RI.
- we may observe the following pattern:

- the frequencies of polymorphic sites for different enzymes can be estimated and compared among different populations of a species or among different species.
- because mtDNA is haploid, there is no heterozygosity.
- however, we can still estimate a measure of gene diversity that reflects the number of polymorphic sites present in a population and the frequencies of these sites.
- now we use the polymerase chain reaction (PCR) to amplify smaller pieces of mtDNA for sequencing or scoring of polymorphic restriction sites.

   b. nuclear DNA (nDNA)
- restriction fragment length polymorphisms (RFLPs) can also be scored in regions of nuclear DNA.
- the traditional way of scoring RFLPs is to isolate and label a clone and then hybridize it to a membrane filter containing DNA digested by a specific restriction enzyme.
- the location of the fragment is then located by autoradiography on X-ray film.
- now we use PCR to amplify pieces of DNA of various sizes for similar analyses.

c. microsatellites

- a more recently developed class of markers are called microsatellites.
- microsatellite arrays contain varying numbers of a small repeating unit that usually is 2-4 bp in size (for example, CA or GC).
- within a specific chromosomal region, the size of microsatellite arrays differ because of differences in the numbers of the repeating unit.
- for example, one microsatellite “allele” may contain 20 copies of CA.
- another allele may contain 19 copies.
- individuals may be homozygous for the 20 copy or 19 copy allele, or they may be heterozygous and have both.

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-----------CACACACACACACACA-------------- 8 “repeats”
---------CACACACACACACA------------------ 6 “repeats”
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- microsatellites are scored by using the PCR reaction to amplify the array using primers constructed from unique flanking sequences of DNA.
- some microsatellites exhibit high levels of variability - and may have high mutation rates (10^{-3} to 10^{-4} per gamete per generation).

Estimates of genetic diversity

- many thousands of species have now been surveyed for genetic diversity at allozyme loci.
- the extent of variation present in most species is large.
- heterozygosity estimates are usually in the range of 8 to 20%.
- marine invertebrates - notably molluscs and echinoderms - have the highest levels recorded.
- so an abundance of genetic diversity is there - why should we be concerned about maintaining it?

Why is genetic diversity important?

- there are two ways in which genetic diversity is important for a species.
- one deals with present conditions, the other with the future.

1. Genetic diversity is selectively important

- genetic variation may be adaptive for the species.
- for example, if the geographic range inhabited by a species is large and variable some of the genetic polymorphisms present may facilitate living in a varied set of conditions.
- an excellent example of a well-studied polymorphism that is experiencing natural selection is the leucine aminopeptidase (Lap) locus in the blue mussel, *Mytilus edulis*.

- this enzyme catalyzes the cleavage of n-terminal amino acids from di-, tri- and tetrapeptides.
- the enzyme has two distinct functions.
- one is to serve as a digestive enzyme - it is abundantly expressed in the gut lumen.
- the other function is osmoregulatory.
- marine bivalves are osmoconformers - the osmolarity of their tissues is identical to that of surrounding sea water. This osmoconformation is achieved by modifying intracellular levels of free amino acids - notably proline, glycine and alanine.
- as salinity goes up, small peptides are cleaved and the a.a. pool increases.
- as salinity falls, these a.a.’s are removed from the pool to reform small peptides.
- some amino acids are exported to the haemolymph some are ultimately excreted. This results in a net loss of nitrogen which may affect the animals energy budget.
- *Lap* functions in this capacity.

- a sharp cline exists in the frequency of the *Lap*94 allele at the entrance to Long Island Sound.
- the frequency of the 94 allele declines sharply from 0.55 in full oceanic salinity environments to 0.12 over a 50 km area.
- what is the cause of this cline?
- is there any environmental factor responsible for producing this cline?
- yes, at the entrance to Long Island Sound there is a drop in salinity from oceanic levels (33-35 ppt) to estuarine levels (25-30 ppt).

- the *Lap*-194 allele appears to be optimized for functioning in a high salinity environment.
- at the biochemical level it has been found to have a higher catalytic efficiency - about 20% greater than the other alleles (96 or 98).
- find that genotypes possessing the 94 allele have higher catalytic activity.
- in the brackish water environment of Long Island Sound, the *Lap*-194 allele is at a disadvantage
- individuals possessing this allele suffer a higher loss of nitrogen and experience higher levels of mortality than genotypes lacking the *Lap*-194 allele.
- the form of balancing selection acting to maintain the *Lap*-1 polymorphism is thus environmental heterogeneity in salinity.

2. Genetic diversity may be adaptive for future conditions

- the presence of suitable levels of genetic variation may be crucial in allowing a species to successfully adapt to changing environmental conditions at some future date.
- if this variation is lost, the capacity of the species to adapt may be compromised and it may face extinction.
- this is because the capacity of a species to adapt is dependent on its existing reservoir of genetic variation.
- the process by which new genetic variation is introduced into a species - mutation - is too slow to fuel this adaptive response.
Threats to genetic diversity

1. Genetic bottlenecks

- severe overexploitation of a marine species can cause the population to crash to low numbers.
- during the time that the population remains small, genetic diversity is easily lost.
- the population may or may not recover.
- if it does it has experienced what is called a “bottleneck”.
- an extreme example of a major genetic bottleneck involves the northern elephant seal (*Mirounga angustirostris*).
  - the northern elephant seal was almost hunted to extinction in the late 19th century - it is estimated that only 10-30 individuals survived.
  - the species has now rebounded to a population size of approximately 130,000.
  - in 1974, the Northern elephant seal was surveyed for genetic variation at 24 electrophoretic, or allozyme, loci by Bonnel and Selander in 1974.
  - surprisingly, it was found to possess no genetic variation!
  - the Northern elephant seal is an interesting case in that the population has rebounded and appears to being do fairly well without polymorphism that is commonly found in other large mammals.
- the Southern elephant seal was also extensively hunted in the last century but never experienced a decline below an estimated size of several thousand animals. It possesses levels of protein polymorphism that are comparable to other large mammals.
- the ultimate fate of the Northern elephant seal may ultimately be affected by this lost variation - it may be poised on the brink of extinction if, for example, an epidemic were to strike.

2. Inbreeding

- inbreeding occurs in a population when matings occur among related individuals more commonly than they would in a randomly mating population.
  - there are two genetic consequences of inbreeding:

  1. Increase homozygosity (i.e., prop. of homozygotes)
  2. Decrease heterozygosity (i.e., prop. of heterozygotes)

- both consequences lead to **inbreeding depression**
  - the loss of heterozygosity means that the population may lose adaptively significant variation.
  - the increase in homozygosity is bad because it will result in the manifestation of homozygotes for deleterious alleles normally “masked” in heterozygous state.
- both lead to what is called **inbreeding depression**.
- inbreeding depression causes a wide range of debilitating conditions:

  1. reduced viability
  2. sterility
  3. reduced longevity
- inbreeding depression may contribute to population extinction as the fitness of the population erodes over time.
- inbreeding depression is a serious problem faced by the aquaculture industry.
- in maintaining small numbers of individuals in hatcheries as “broodstock”, over time there will be inbreeding.
- this makes it difficult for the broodstock to be successfully propagated over time.
- it is common to “outbreed” the stock by introducing new, unrelated individuals that introduce new genes into the population.

3. Hybrization by introduced species

- a final threat to genetic diversity is caused by the inadvertent (or sometimes purposeful) introduction of species to a new part of the world.
- these are commonly referred to as invasive species – you already had a lecture on this problem earlier in the class.

- invasive species also can wreak serious genetic harm after their introduction.
- they can do this by intercrossing with closely related species that they have not been exposed to in their evolutionary past.
- this is called **hybridization**.
- the two species form what is called a **hybrid zone**.
- the hybrid zone can have serious consequences to population “health” because hybrids are usually inferior to either parental species and commonly are sterile or have greatly reduced fecundities.
- this can cause dramatic declines in population size.

- an interesting (and scary) example of this problem is occurring right in our back door involving mussels.
- a hybrid zone occurs in Monterey Bay between a native species of mussel, *Mytilus trossulus* and an introduced species, *M. galloprovincialis*.
- galloprovincialis was introduced into southern California in the early part of the last century from the Mediterranean.
- it has been rapidly moving up the coast and hybridizing with the native trossulus.
- it had reached Cape Mendocino about 5-6 years ago and stopped further expansion – we thought it may be a “barrier” to further movement north.
- no such luck, it expanded past this cape during the last El Nino and is heading on up the coast.
- the consequence for trossulus is bad

- how many other examples are there like this?
- only a few others are known.
- many, many more are probably occurring right now but we don’t know it.
- we only discovered the mussel hybrid zone because mussels are extensively studied at the genetic level.
- it is scary to consider that many other examples are likely out there waiting to be discovered.
Conserving genetic diversity

- how do we go about preserving genetic diversity?
- recently, new approaches have been developed that utilize information provided by phylogenetic trees.
- two approaches have been proposed:

1. **Preserve the evolutionary history of the group.**

   - here, high value placed on phylogenetic “distinctiveness”.
   - species (or populations) that are very different from others are given high priority for conservation.

2. **Preserve the evolutionary future of the group.**

   - here, priority is placed on trying to preserve young, rapidly diversifying groups since these are viewed as having the highest “evolutionary potential”.

   - which approach is correct?
   - unfortunately, we don’t know…