Determining Evolutionary Relationships Among Ungulates
Today's Lab:

1) Gel electrophoresis of lactate dehydrogenase isozymes
2) Morphological examination of representative ungulates
   a) Hoof morphology
   b) Dentition
   c) Headgear structures
3) Microscope skills – photos of flies
4) Transfer flies
5) Sow C-fern spores
   (sections 02 and 05 only)
Molecular Phylogeny

http://www.guardian.co.uk/science/2007/dec/19/whale.deer
http://www.youtube.com/watch?v=13GQbT2ljxs
Evolutionary Relationships

*Artiodactyla*
- Even number of toes

*Perissodactyla*
- Odd number of toes

Camel thinking: Where do I belong?
Strategies for Determining Relationships

- Morphological
  - Two Approaches
  - Molecular
  - Dentition
  - Headgear
  - Foot Structure
Strategies for Determining Relationships

- Kind/location of teeth
  - Dentition
    - Type of Cusps
      - Morphological
        - Headgear
          - Foot Structure
            - Crown Height
              - Brachydont
              - Hemimastoid
              - Lophodont
Strategies for Determining Relationships

Morphological

Dentition

Foot Structure

Horns
Permanent, non-branching

Deciduous Horns
Permanent, sheath shed annually

Antlers
Non-permanent, shed annually

Antlers
Non-permanent, shed annually
Strategies for Determining Relationships

Morphological
- Artiodactyl: even number of toes
- Perissodactyl: odd number of toes

Dentition
Headgear
Foot Structure

Perissodactyl Feet:
- Tapir
- Rhinoceros
- Horse

Perissodactyl:
- Even number of toes

Artiodactyl:
- Even number of toes

Deer
Hippopotamus

Elongated and fused metatarsals 3 and 4
Strategies for Determining Relationships

Morphological

Dentition

Headgear

Foot Structure

Two Approaches

Molecular
Molecular Approach: Lactate Dehydrogenase Isozymes

Enzyme: speeds rate of biological reactions

Molecular: Lactate Dehydrogenase
found inside cells; released into interstitial fluid as cells die

Isozymes: multiple forms (5) of LDH; same function
All have about the same molecular weight – 137kD

Each has different net charge; moves at different rate in electrical field (e.g., gel electrophoresis)
Proteins

- Proteins - products of gene expression
- Information encoded in a gene is first transcribed into messenger RNA and then translated into a protein
Proteins

- Mutations in DNA may alter gene products
- Mutations accumulate over time
- To determine how closely related two species are, compare either gene sequences or amino acid sequences for a particular protein
LDH Isozymes

**Enzymes** - usually proteins
- Composed of building blocks - amino acids
  - 20 different ones
  - Each amino acid has different physical and chemical properties - e.g., neutral, charged (positively or negatively)

**Isozymes**
- Tetramers - composed of four subunits (monomers)
- Two kinds of monomers - H and M
  - vary in amino acid composition
  - thus, each has a different charge
- Each isozyme moves at a different rate in electrical field
- We use gel electrophoresis to separate isozymes
LDH Isozymes

Each isozyme is **tetramer** (composed of 4 monomers)

Two kinds of monomers
- "H" monomer and "M" monomer
- combine in different ratios to form 5 different isozymes
  - two “pure” types of isozymes - HHHH (H\textsubscript{4}) and MMMM (M\textsubscript{4}) = homopolymers
  - three hybrid types - HHHM (H\textsubscript{3}M), HHMM (H\textsubscript{2}M\textsubscript{2}), and HMMM (HM\textsubscript{3}) = heteropolymers
- H and M monomers of species (e.g., cow) have different amino acid sequences
- H monomer - more negatively charged
- M monomer - more positively charged
- On electrophoresis, isozymes separate
LDH Isozymes of Different Species

- H and M monomers of *different* species have differences in their amino acid sequences (like when you did sequence alignment comparing COX-III)
  - Differences or mismatches in sequences may result in differences in the net charge of each isozyme
- The greater the number of mismatches or differences in H and M subunits for species, the more distantly related that species is from others from an evolutionary standpoint
- On electrophoresis, more distantly related the species will have the isozymes that move at very different rates from those of species that are more closely related
Gel Electrophoresis

• Method used to separate molecules of different sizes or charges - proteins, DNA, and RNA

• Material used is agarose
  • acts as a molecular sieve or net; molecules move through spaces in it
  • more electronegative molecules move faster toward positive electrode (anode)
  • less electronegative move more slowly
  • electropositive will move up (toward cathode), not down gel
Agarose Gel Electrophoresis

- Use serum as source of LDH from four (4) ungulates
- Sample of each serum is loaded in a separate well
- An electrical current is applied to gel
- Charged molecules in gel move based on charge and mass
- Small, electronegative molecules move toward positive electrode (anode)
- Larger, electronegative molecules move toward the anode more slowly
- Electropositive molecules move toward negative electrode (cathode)
Non-specific staining of serum proteins
How Do We Visualize Only LDH Isozymes?

Use Substrate-Specific Stain Dye-Coupled Reaction
Use a **Substrate-Specific Stain**

\[ \text{Enzyme (LDH)} \]

Substrate $\rightarrow$ Product
Substrate-Specific

\[ \text{lactate} + \text{NAD}^+ \xleftrightarrow{\text{Enzyme (LDH)}} \text{NADH} + \text{pyruvate} \]

**Substrates**

**Products**
**Lactate Dehydrogenase**

Oxidation-reduction (redox) reactions

\[
\text{lactate} + \text{NAD}^+ \leftrightarrow \text{NADH} + \text{pyruvate}
\]

*substrates* \leftrightarrow *products*

*Oxidation* = a loss of electrons (often as hydrogen atoms)

*Reduction* = a gain of electrons (often as hydrogen atoms)

*When one substance is oxidized another one is reduced*
**LDH Stain - Why Does It Turn Purple?**

**Dye-Coupled Reactions**

Oxidation-reduction (redox) reactions

\[
lactate + NAD^+ \rightarrow NADH + pyruvate
\]

\[
NADH + PMS \rightarrow NAD^+ + PMS-H
\]

\[
PMS-H + NTB (yellow) \rightarrow PMS + NBT-formazan (purple-brown)
\]

Red = reduced form
**Agarose Gel Electrophoresis**

- Work with lab partner
- Each lab pair loads 4 samples on gel:
  - horse
  - goat
  - sheep
  - cow
- Share gel with one other lab pair
- They will run the same set of samples on the same gel
- Run gel until blue dye (bromophenol blue) is about 2 cm from end of gel (~45 minutes)
- Remove tray; slide gel into bowl; stain until bands clearly visible - about 1-1.5 hours
Gel Analysis

- Count bands in each lane - should be 5 (one for each isozyme)
- Compare positions of the same band in each lane
- Which ungulates have identical band patterns?
- Which ungulate has a band that is uniquely different from the other ungulates?
LDH Agarose Gel
Plan for Lab

• Load and run gel (40-45 minutes)
• Look at demonstration materials
• Microscope skills: photograph flies (male, female, and zoom of eye phenotype)
• Transfer flies to new vial & label
• Transfer gel to stain (1-1.5 hours)
• Continue with demonstrations (worksheet)
• Photograph gel and analyze results (worksheet)
• Sections 07, 05 & 02 only:
  • Sow C-fern spores (wild type and mutant)
  • Photograph spore types (2)
**Before you leave . . .**

- Show your worksheet to your TA or instructor
- Clean up your work area
  - Used reagents and materials go in hood
    - Buffer from gel box (Buffer Waste)
    - Gels (Gel Waste)
    - Stain (Stain Waste)
  - Rinse gel box with ultra pure water (carboys); leave at your bench on paper towels to dry
  - Rinse finger bowls with ultra pure water (carboys); leave at your bench on towels to dry
  - Pipettors in plastic box
  - Used pipette tips in plastic beaker - Used tips
  - Wipe down your work area
Due Next Week:
Check RPI-LMS

- Phylogeny of Ungulates Lab Report
- Pre-lab directions-complete
- Quiz on Ungulates Lab