Introduction to Genetic Analyses in Tribal Fisheries Management

Reference:
What Does Genetics Have to Do with It?, 3rd edition, Anthony J. Gharrett
(http://seagrant.uaf.edu/bookstore/pubs/AN-18.html)
We all have learned that:

• Our “genetics” is some sort of code – a heritable set of instructions that directs how cells/tissues function

• This genetic code is contained in “DNA”

• DNA is contained in each of the cells of our body

• Kids get a mix of the “genetics” from their parents

• A population is a group of individuals that breed among each other, and thus share their “genetics”

• Individuals within a population will, therefore, be genetically, and physically, more similar to each other than to individuals from other populations
... but how does all this work?

- What is the structure of DNA, and the mechanisms for:
  - directing cell function and production of cell components?
  - copying this “code” to new cells?
  - passing on our genetics to our offspring?

- How can genetics analyses inform issues in fisheries management of interest to the tribes? Are there “markers”/“tags” within the DNA that permit us to:
  - identify offspring to parents, and assess relative productivity?
  - identify individuals to their population/stock of origin?
  - (correlate genetic markers to individual or population life history traits?)
Introductory Presentation will review:

1. Basic DNA structure

2. How this structure allows for self-replication, so that a faithful copy of the genetic code is provided to each new cell

3. How the DNA “code” is translated for the production of proteins – the molecules that form the structural elements of our cells, or are involved in catalyzing or facilitating metabolic processes
Introductory Presentation will review:

4. How the parents’ genetic code (genotype), and their physical and biochemical genetics-based traits (an organism’s phenotype) are inherited by their progeny.

5. How “markers” in this genetic code can inform questions regarding fish population structure and reproductive success, etc.

6. (Brief review of qualitative versus quantitative genetic traits)

What is DNA?

• DNA – deoxyribonucleic acid – a long linear molecule made up of a string of nucleotides

• Each nucleotide made up of:
  • Deoxyribose – a sugar
  • Phosphate – $\text{PO}_4^{-3}$
  • Purine or pyrimidine nitrogen-containing base

• DNA molecule is made of not one, but two complementary parallel strands - form a double helix
What is DNA?

Four nitrogen-containing bases, of two types:

- Adenine (A) – purine
- Guanine (G) – purine
- Thymine (T) – pyrimidine
- Cytosine (C) – pyrimidine

(note: while the base portion does have weakly basic properties, the “+” charge of the phosphate gives the nucleotide an overall acidic nature – hence DN-Acid)
Hydrogen bond

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... but why is it important that DNA be double-stranded?

- The strands are complementary – kind of like “mirror images”
- Separate the two strands, and each can be used as a template for rebuilding of the opposing strand – DNA replication
- How does DNA replication occur?

http://www.youtube.com/watch?v=zdDkiRw1PdU&feature=related
Genes and Proteins

• How does the nucleotide sequence contained in the **genome** (the full complement of DNA in an organism) direct cell function?

• Specific portions of the DNA sequence - **genes** - constitute a code for the production of **proteins**
  - protein molecules = strings of **amino acids** (n≈20)
  - proteins = structural elements, enzymes, other functions (comprise >50% dry weight of cells)
  - DNA code = each different 3-nucleotide base sequence codes for a particular amino acid

• **Transcription** - produce copy of gene DNA – messenger RNA
• **Translation** - produce protein coded by RNA message
Nonpolar, alphabetical R groups

Glycine  Aladine  Valine

Leucine  Isoleucine  Proline

Polar, uncharged R groups

Serine  Threonine  Cysteine

Lysine  Arginine  Histidine

Negatively charged R groups

Methionine  Asparagine  Glutamine

Aromatic R-groups

Phenylalanine  Tyrosine  Tryptophan

Positively charged R groups

Aspartate  Glutamate

Transcription & Translation

http://www.youtube.com/watch?v=41_Ne5mS2Is
e.g., hemoglobin – $2\alpha + 2\beta$ chains, each which binds a heme-Fe complex ($O_2$ binds to the Fe)
But, need to understand:

• How DNA is organized within the cell **nucleus**?

• How DNA replicates and provides a full copy to each of the daughter cells during cell division - for cell replacement and growth in multi-celled organisms?

• How DNA is allocated to **gametes** (egg and sperm cells) for the purpose of sexual reproduction
  • **inheritance** – how are parental genetic traits transferred to their offspring?
  • and, why doesn’t the fertilized egg (embryo) end up with twice as much DNA per cell ...?
Chromosomes

- Human genome totals approx. 3,000,000,000 bp - 6.4 pg/cell (pg=10^{-12}g); similar for salmon species
- End to end, total DNA ≈ 2 m in length
- A cell’s DNA not in a single molecule, but sub-divided among several molecules, called chromosomes (humans n=46; salmonids n=52 to 84)
- To fit within a 6μm (0.006 mm) diameter nucleus, DNA is wound-up, folded and refolded
- Chromosomes are most tightly packaged just prior to cell division; more relaxed (chromatin) during normal cell function
Chromosomes

• Eukaryotes (organisms from protozoans & algae to “higher order” animals & plants) undergo sexual reproduction, and in consequence are *diploid* – each cell contains 2 sets of *homologous* chromosomes (one set from mom, the other set from dad); humans = 2 sets of 23 chromosomes (N=46)

• **Karyotype** - image of chromosome pairs at the most condensed stage (following replication – paired chromatids; see Mitosis) just prior to cell division; arranged by size and centromere position
humans \( 2N = 46 \)

rainbow trout \( 2N = 60 \)
Diploid (2N) chromosome number in trout and salmon

<table>
<thead>
<tr>
<th>Genus / Species</th>
<th>Number</th>
<th>Genus / Species</th>
<th>Number</th>
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</thead>
<tbody>
<tr>
<td><strong>Salmo</strong></td>
<td></td>
<td><strong>Salmonidae</strong></td>
<td></td>
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<tr>
<td><em>salar</em> (Atlantic salmon)</td>
<td>58, 60</td>
<td><em>tshawytscha</em> (Chinook salmon)</td>
<td>68</td>
</tr>
<tr>
<td><em>trutta</em> (brown trout)</td>
<td>80</td>
<td><em>kisutch</em> (coho salmon)</td>
<td>60</td>
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<tr>
<td><strong>Salvelinus</strong></td>
<td></td>
<td><strong>Acipenseridae</strong></td>
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<tr>
<td><em>confluentus</em> (bull trout)</td>
<td>78</td>
<td><em>gorbuscha</em> (pink salmon)</td>
<td>52</td>
</tr>
<tr>
<td><em>malma</em> (Dolly Varden)</td>
<td>82</td>
<td><em>keta</em> (chum salmon)</td>
<td>74</td>
</tr>
<tr>
<td><em>fontinalis</em> (brook trout)</td>
<td>84</td>
<td><em>mykiss</em> (steelhead/rainbow trout)</td>
<td>58, 60</td>
</tr>
<tr>
<td><em>namaycush</em> (lake trout)</td>
<td>84</td>
<td><em>clarki</em> (cutthroat trout)</td>
<td>64, 66</td>
</tr>
<tr>
<td><em>alpinus</em> (Arctic charr)</td>
<td>78</td>
<td></td>
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</tr>
<tr>
<td><strong>Homo sapiens</strong> (us!)</td>
<td>46</td>
<td><strong>Mollusca</strong></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td><em>Anodonta oregonensis</em> (fw mussel)</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Acipenser transmontanus</em> (white sturgeon)</td>
<td>271 (including 90 microchromosomes; ≈8N)</td>
</tr>
</tbody>
</table>
Mitosis – the process by which a cell duplicates its DNA and provides a full complement of chromosomes to each daughter cell; necessary for growth and cell replacement

Steps in mitosis:
- Replication of chromosomes (paired chromatids, attached at the centromere)
- Condensation and alignment of chromosomes
- Dissolution of nuclear membrane
- Separation and random segregation of chromatids – one of each pair to opposite poles
- Division of cytoplasm into 2 new identical cells
- Reforming of nuclear membrane
Mitosis

http://www.youtube.com/watch?v=VIN7K1-9QB0
**Meiosis**

*Meiosis* - process by which a germ cell (oocyte, spermatocyte) produces mature gametes (egg or sperm), each containing only a single (*haploid*) set of chromosomes.

**Steps in meiosis:**
- Replication of chromosomes (paired chromatids)
- Condensation of chromosomes & dissolution of nucleus
- Pairing of homologous chromosomes, with crossing-over
- Meiosis I – random segregation of homologous chromosomes
- Cytoplasmic division (sperm), or formation 1\(^{st}\) polar body (egg)
- Meiosis II – random segregation of chromatids
- Cytoplasmic division (sperm), or formation 2\(^{nd}\) polar body (egg)
Meiosis

http://www.youtube.com/watch?v=D1_-mQS_FZ0
Gametogenesis

(a) Gamete formation in the male
- Spermatocyte (diploid)
- Meiosis I
- Meiosis II
- Spermatids (haploid)
- Sperm cells (haploid)

(b) Gamete formation in the female
- Oocyte (diploid)
- Meiosis I
- Meiosis II
- Polar bodies (haploid)
- Egg cell (haploid)

http://www.youtube.com/watch?v=AgEqWPyO8z0&lr=1
10 min break ...
Genetic Variation & Mutation

• DNA replication is very efficient, BUT occasional mistakes do occur, producing changes in nucleotide sequence (mutations)

• Alternative sequences - involving differences in a single or multiple nucleotides within an identifiable segment of DNA (locus) - are termed alleles

• An individual possesses 2 sets of chromosomes, thus two copies of each locus
  • **Homozygous** – 2 of the same allele
  • **Heterozygous** – 2 different alleles
Genetic Variation & Mutation

• Identification of the allelic character for a suite of loci identifies an individual’s genotype (“genetic fingerprint”)

• A population can be characterized by genotyping a representative sample of individuals for the suite of loci, and estimating the allele frequencies

• The magnitude of frequency differences can be used to estimate relatedness among populations

• And, the genotype of an individual can be used to assign it to a population of most probable origin
Genetic Variation & Mutation

• A mutation within a gene (a segment of coding DNA) may result in a change in the amino acid sequence of the protein, and the change may, or may not, alter protein character or functionality (or, render it totally non-functional)

(similar to allelic differences in DNA sequence, different functional forms of a protein (allozymes) can sometimes be observed; allozymes were the predominant genetic marker used in fisheries from the 1970s to the 1990s)

http://www.dnalc.org/resources/3d/17-sickle-cell.html
Coding (Genes) versus Non-Coding DNA

• Mutations within genes (coding DNA) that reduce, or nullify, protein functionality will be (very strongly) selected against

• Therefore, variation within genes, and even more so within proteins, is limited

(a well known exception: individuals homozygous for sickle cell anemia – a single amino acid change in β-hemoglobin – typically died in youth; BUT individuals heterozygous for the mutation show increased resistance to malaria, providing counter-balancing selection that has maintained the mutation within populations in Africa where malaria is prevalent)
Coding (Genes) versus Non-Coding DNA

• However, only a very small % of genome is actually made up of genes (segments of DNA that directly code for the amino acids), plus some adjacent segments that influence gene expression.

• Most of the genome (98% ?) DNA is non-coding; this DNA was presumed non-functional and, naively, referred to as “junk” DNA.

http://www.youtube.com/watch?v=ZvnhZIGZS4&feature=plcp&context=C35708eeUDOEgsToPDskluC-Hhgeu6UebgCCzlyyCE
Coding (Genes) versus Non-Coding DNA

• Mutations within non-coding DNA, have lesser fitness implications and tend not be selected against

• Therefore, mutations within non-coding DNA can accumulate

• Characterizing the variation in coding and non-coding loci (genetic markers) provides information about individuals and populations that can be applied to various questions in fisheries management

• How this is done will be described in the subsequent presentations
Genotypes to Phenotypic Traits

**Qualitative Traits**
- “Mendelian” traits
- trait controlled by (a mutation to) a single gene (or two genes)
- alleles show dominant or recessive effects, or incomplete or co-dominance
- traits identified in fish often associated with coloration or external physical characters (size/shape of fins, eyes, etc.)

**Quantitative Traits**
- trait controlled by multiple genes
- measures in a population show continuous distribution
- phenotypic variation ($V_P$) due to genetic $V_G$ (additive $V_A$ and dominance $V_D$) factors, and to environmental $V_E$
- heritability ($h^2$) = $V_A/V_P$
- selective breeding uses $h^2$ to shift average trait value within population
Qualitative (Mendelian) Traits

Wild type (AA, Aa)
Albino (aa)

Wild type (GG)
Palamino (G’G)
Golden (G’G’)

a. Scaled (Wild type)
SS, Ss / nn

b. Mirror
Ss / nn

c. Line
SS, Ss / Nn

d. Leather (Nude)
ss / Nn

e. _ _ / NN

Fig. 18a–d. Types of scaling in the common carp *Cyprinus carpio*. a. Scaled (SSnn and Ssnn); b. scattered (ssnn); c. linear (SSNn and SsNn); d. nude or leather (ssNn)
Quantitative Traits

- Reproductive
  - age/size at maturity
  - jack(jill) rate
  - run and spawn timing
  - spawning success
  - fecundity (eggs/kg)
  - egg size
  - incubation survival to eyed/hatch/swim-up

- Physical
  - fin spine & ray number
  - gill raker number
  - length, weight and condition factor
  - body conformation and dress-out percentage
  - skin and flesh coloration (carotenoid level)
  - flesh quality - % moisture, % lipids
Quantitative Traits

• Behavioral
  • aggressivity
  • vulnerability to fishing gear
  • cannibalism
  • feeding
  • predator avoidance

• Production
  • growth rate
  • feed-conversion rate
  • age/size at smoltification
  • physiological tolerance to temperature, low O₂, high N₂, high CO₂, pH, formalin, other chemicals)
  • disease resistance, and sensitivity/response to antibiotics and vaccines
  • enzymatic or other metabolic rates
Example: Selective breeding for a quantitative genetic trait – run timing


- Cowlitz Salmon Hatchery – coho program 1967 to 2001
- Management objective - delay coho return to avoid by-catch in coho fishery of depressed Chinook (and avoid work in cold winter weather)
- Beginning 1967, percent in hatchery broodstock:

<table>
<thead>
<tr>
<th>Run Timing</th>
<th>Early</th>
<th>Middle</th>
<th>Late</th>
</tr>
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<tbody>
<tr>
<td>(Aug to mid-Oct)</td>
<td>(mid-Oct to Nov)</td>
<td>(Dec to Feb)</td>
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</tr>
<tr>
<td>natural</td>
<td>40%</td>
<td>33%</td>
<td>27%</td>
</tr>
<tr>
<td>hatchery</td>
<td>10%</td>
<td>80%</td>
<td>10%</td>
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