

Introduction to Genetic Analyses in Tribal Fisheries Management

Reference:

**Genetic Guidelines for Fisheries Management
Kapusinski and Miller, Sea Grant MN**

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” of 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 phenotypically, 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?
 - identify individuals to particular populations/stocks?
 - (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 provided for 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, and genetic-based traits (an organism's phenotype), is 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 – 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

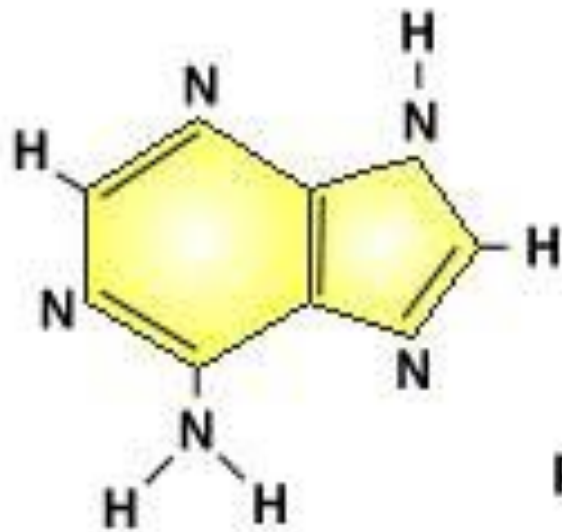
Guanine (G) - purine

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)

adenine



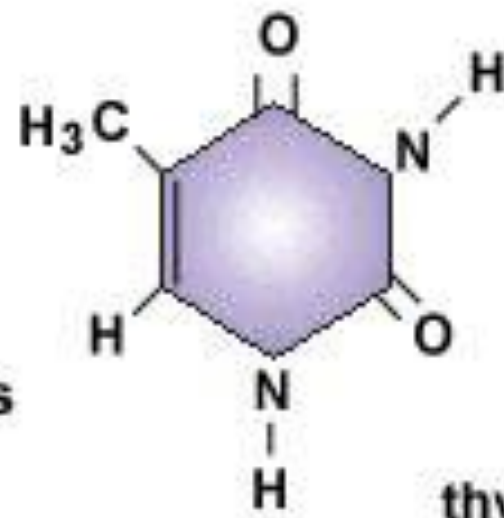
guanine



purines

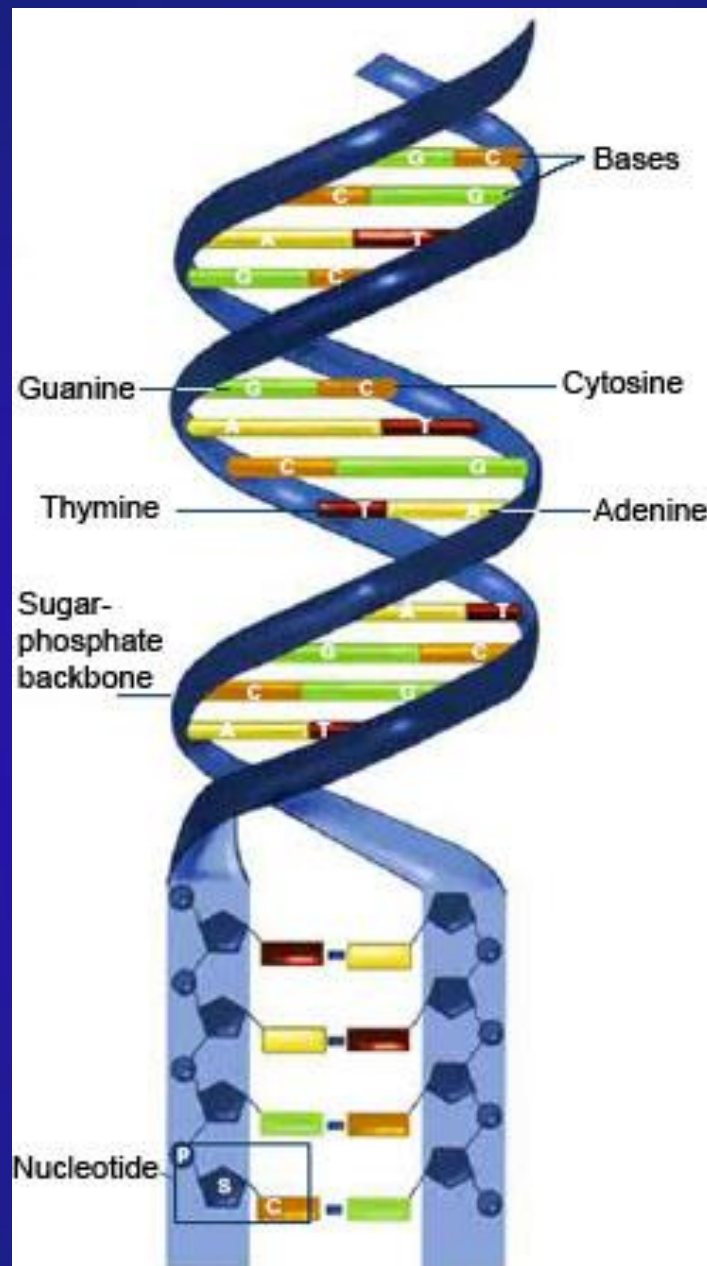


cytosine



thymine

pyrimidines



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

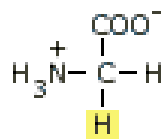
- The strands are complementary – kind of like “mirror images”
- Separate the two strands, and each can be used as a template for rebuilding of the opposing strand – DNA replication
- How does DNA replication occur?

<http://www.youtube.com/watch?v=zdDkiRw1PdU&feature=related>

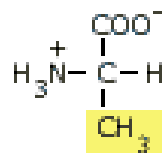
Genes and Proteins

- How does the nucleotide sequence contained in the genome (the full complement of DNA in an organism) direct cell function?
- 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 = 3 nucleotide base pairs per amino acid
 - Transcription & Translation = the processes for DNA-directed protein production

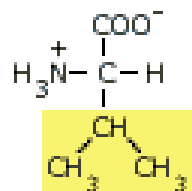
Nonpolar, alphabetical R groups



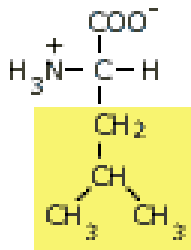
Glycine



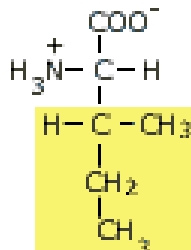
Alanine



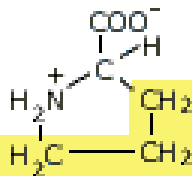
Valine



Leucine

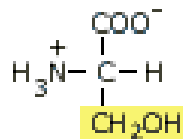


Isoleucine

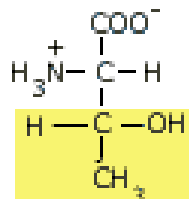


Proline

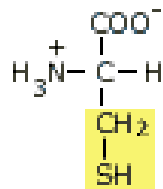
Polar, uncharged R groups



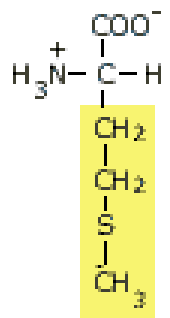
Serine



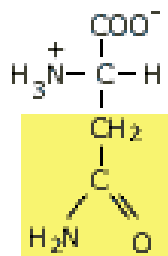
Threonine



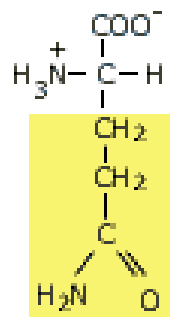
Cysteine



Methionine

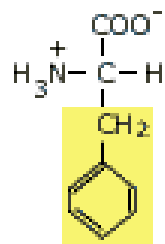


Asparagine

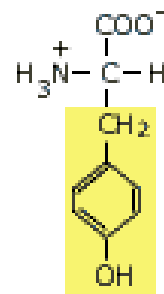


Glutamine

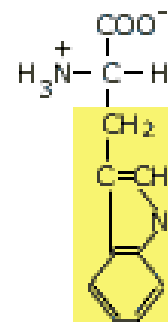
Aromatic R-groups



Phenylalanine

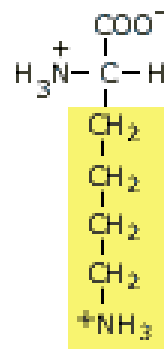


Tyrosine

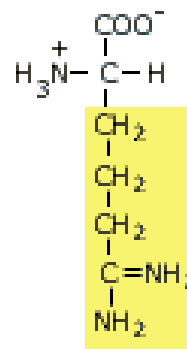


Tryptophan

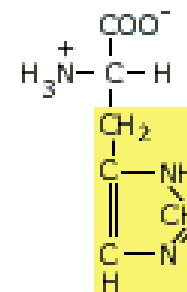
Positively charged R groups



Lysine

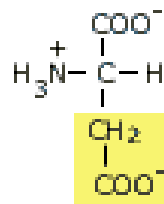


Arginine

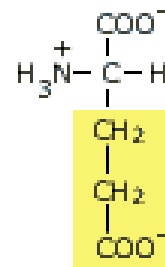


Histidine

Negatively charged R groups



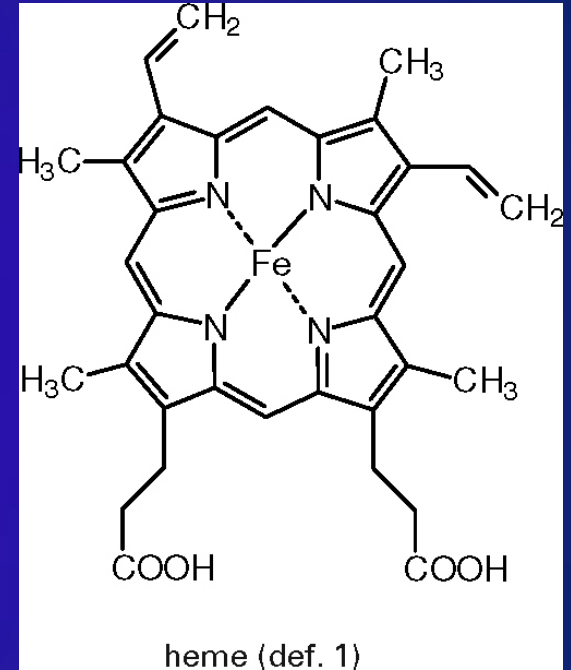
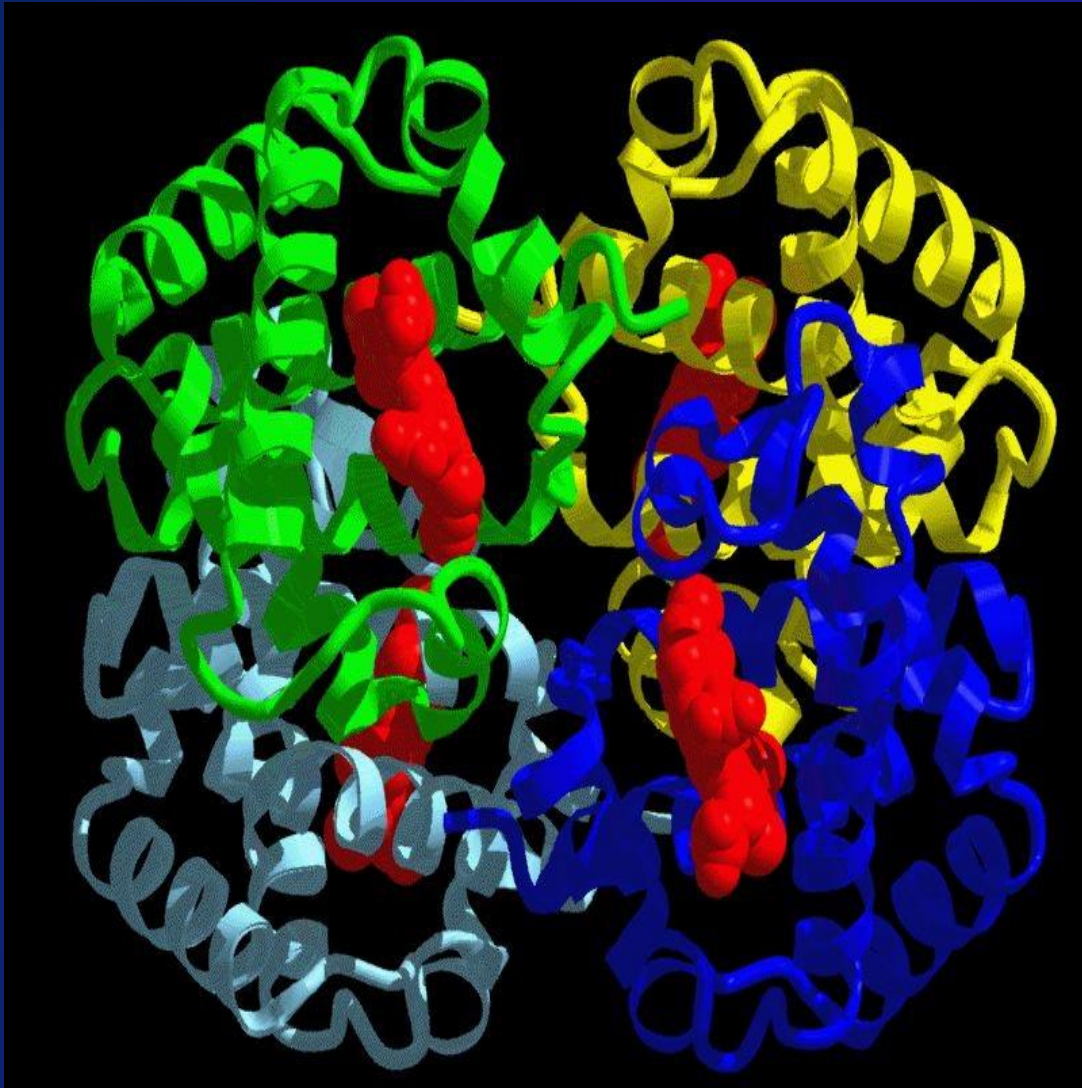
Aspartate



Glutamate

Transcription & Translation

<http://www.youtube.com/watch?v=983lhh20rGY&feature=related>



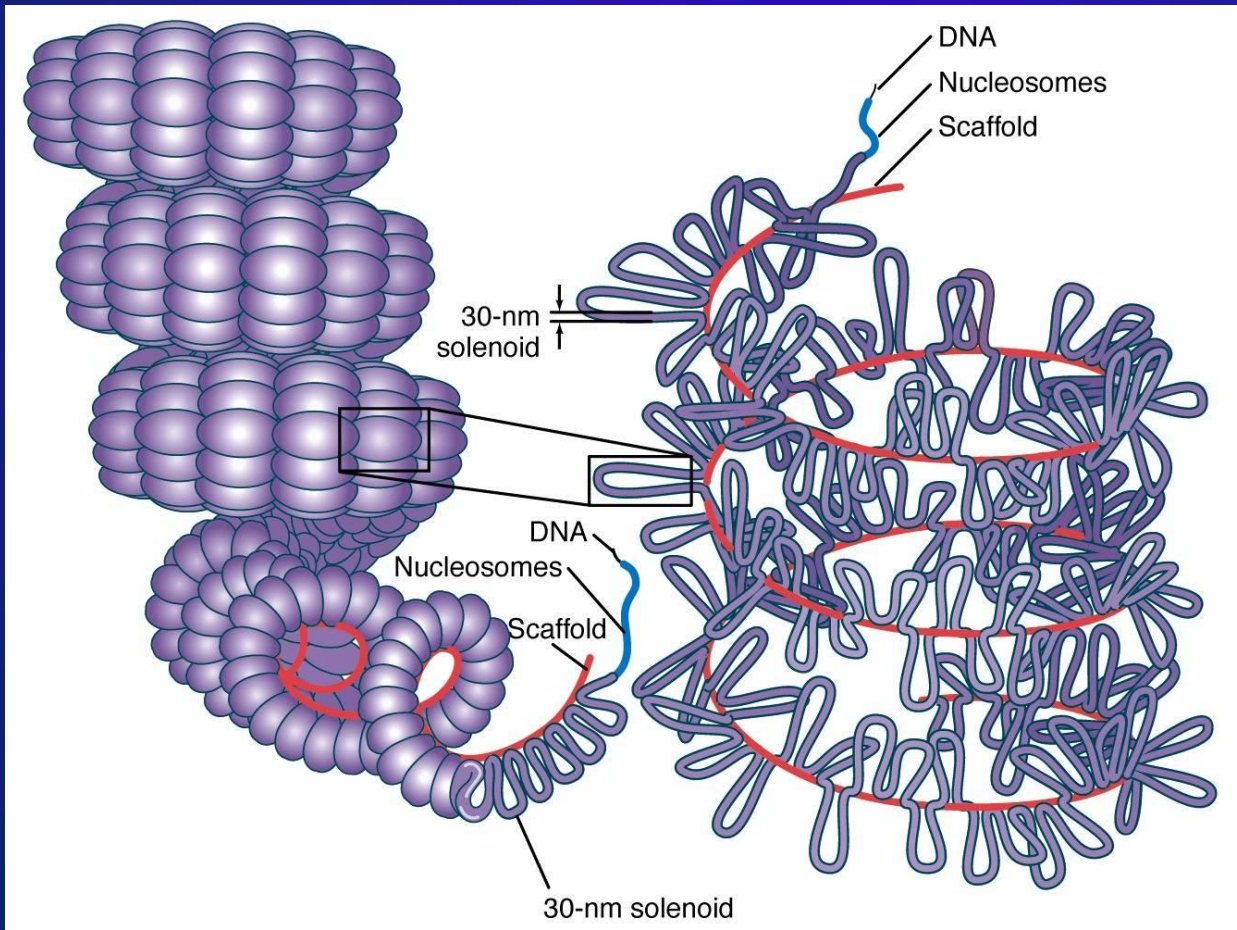
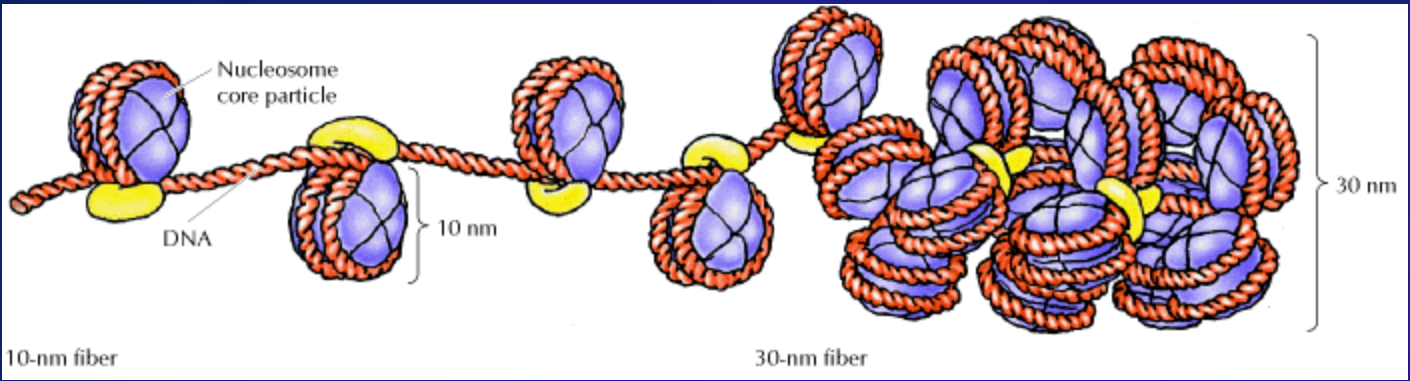
e.g., hemoglobin – $2\alpha + 2\beta$ chains, each which binds a heme-Fe complex (CO_2 and O_2 bind to the Fe)

But, need to understand:

- How DNA is organized within the cell nucleus?
- How DNA replicates, then divides the two copies between 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?
 - 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 = approx. 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 $6\mu\text{m}$ (0.006 mm) diameter nucleus, DNA is wound, folded and refolded
- Chromosomes are most tightly packaged just prior to cell division; more relaxed (chromatin) during normal cell function

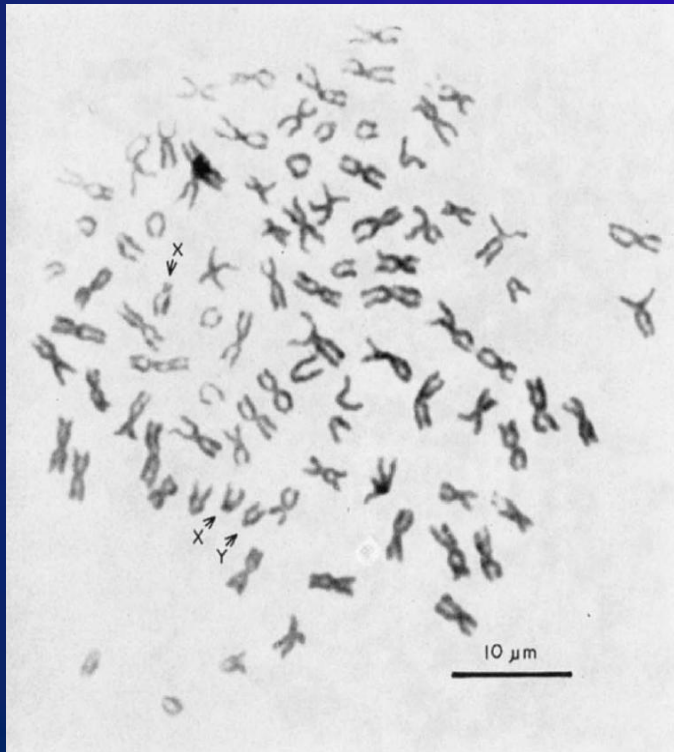
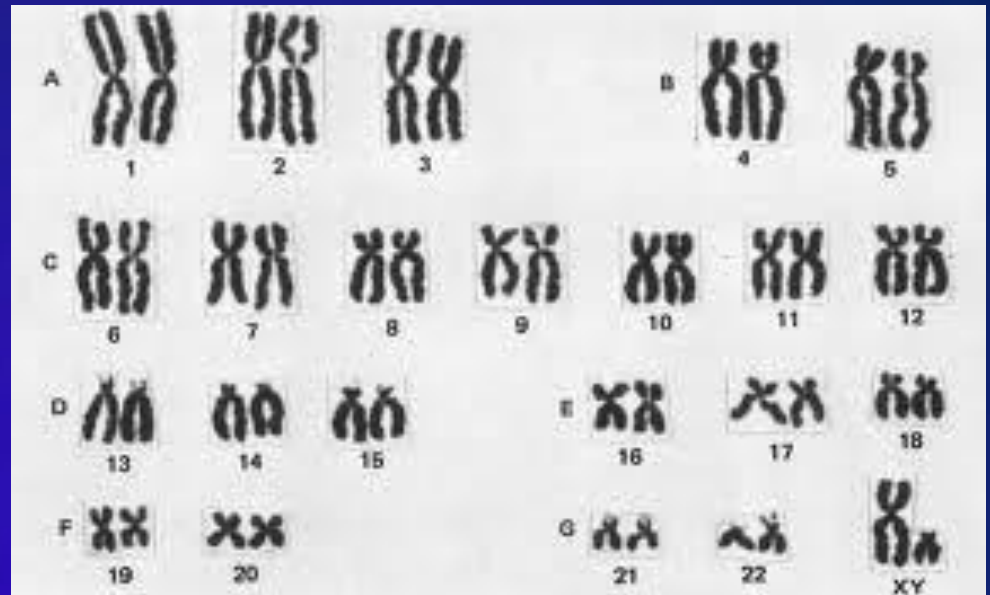


<http://www.youtube.com/watch?v=9kQpYdCnU14>

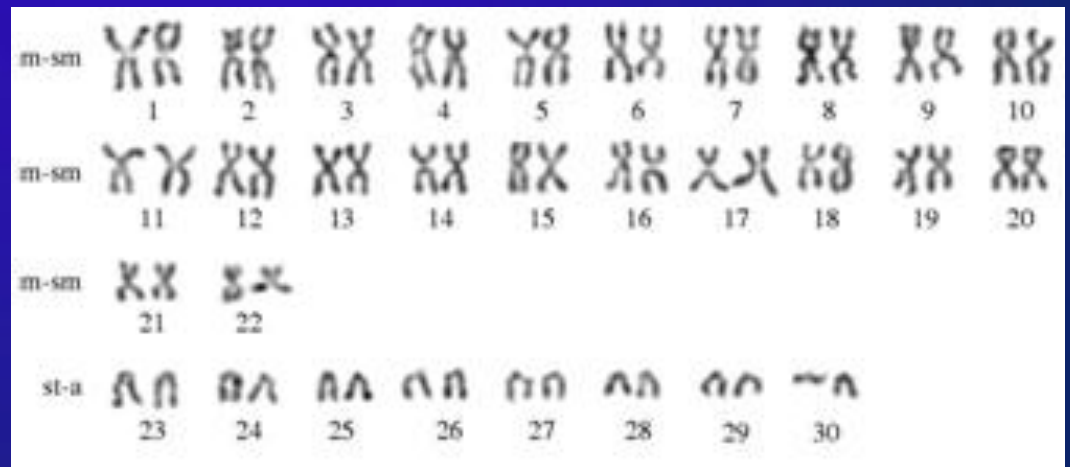
Chromosomes

- 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 from mom, one from dad)
- Karyotype - image of chromosome pairs at the most condensed stage (following replication – paired chromatids, and 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

<u>Genus / Species</u>	<u>2N Number</u>	<u>Genus / Species</u>	<u>2N Number</u>
<i>Salmo</i>		<i>Oncorhynchus</i>	
<i>salar</i> (Atlantic salmon)	58, 60	<i>tshawytscha</i> (Chinook salmon)	68
<i>trutta</i> (brown trout)	80	<i>kisutch</i> (coho salmon)	60
<i>Salvelinus</i>		<i>nerka</i> (sockeye salmon/kokanee)	56, 58
<i>confluentus</i> (bull trout)	78	<i>gorbuscha</i> (pink salmon)	52
<i>malma</i> (Dolly Varden)	82	<i>keta</i> (chum salmon)	74
<i>fontinalis</i> (brook trout)	84	<i>mykiss</i> (steelhead/rainbow trout)	58, 60
<i>namaycush</i> (lake trout)	84	<i>clarki</i> (cutthroat trout)	
<i>alpinus</i> (Arctic charr)	78		
<i>Homo sapiens</i> (us!)	46		

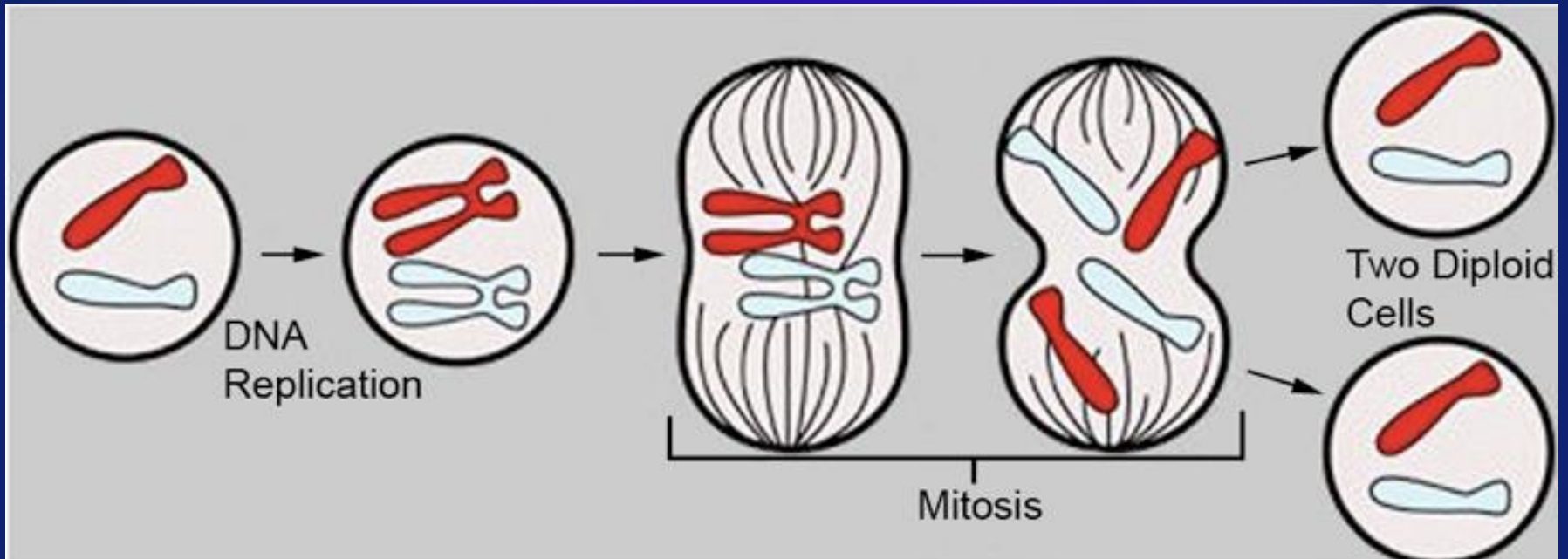
Mitosis

Process by which a cell duplicates its DNA and divides into two identical cells

Steps in mitosis

- Replication of chromosomes (paired chromatids)
- Condensation 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/user/ppornelubio#p/a/u/0/VIN7K1-9QB0>

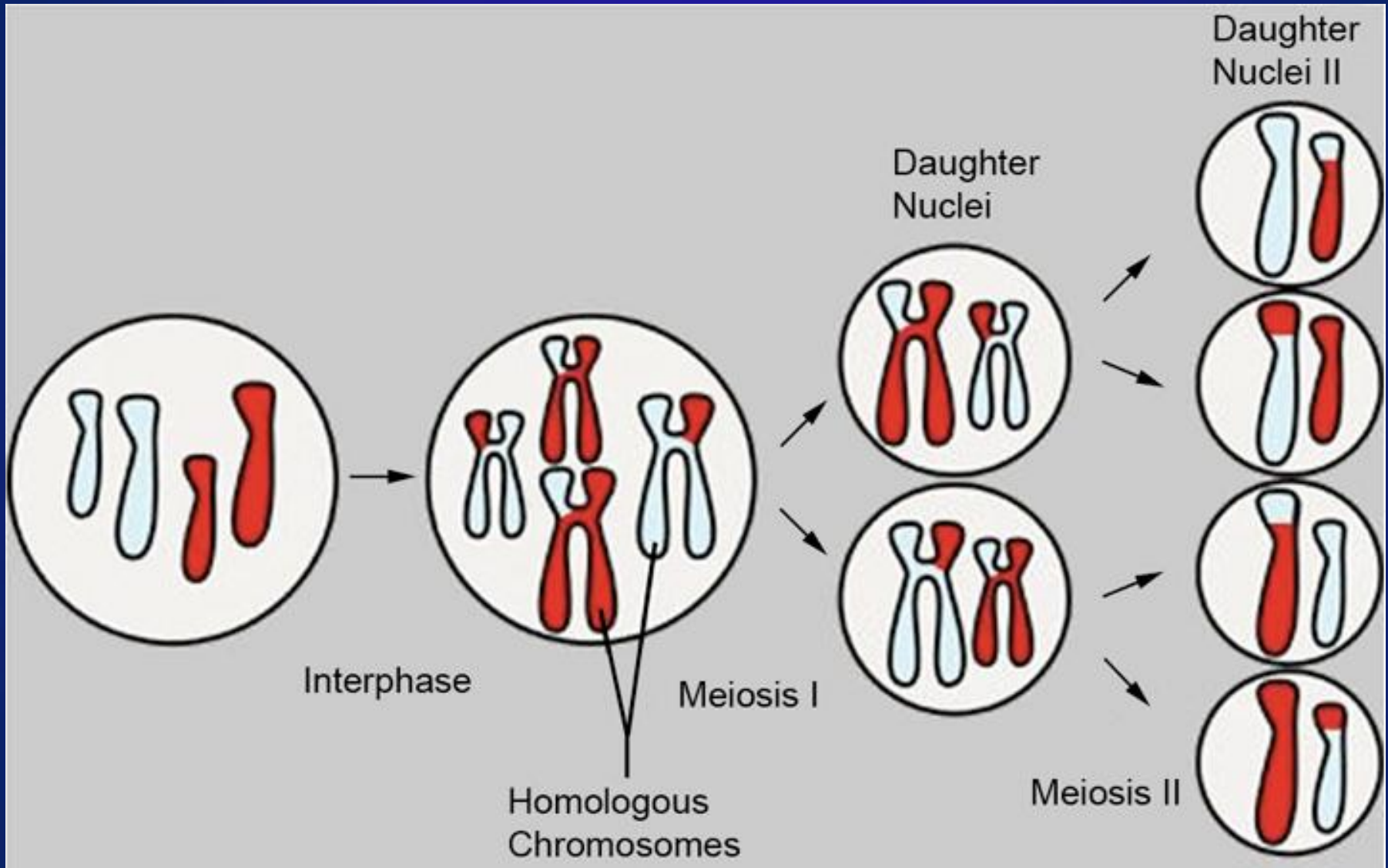
Meiosis

Process by which a germ cell (oocyte, spermatocyte) produces mature gametes (eggs or sperm), each containing only a single (haploid) set of chromosomes

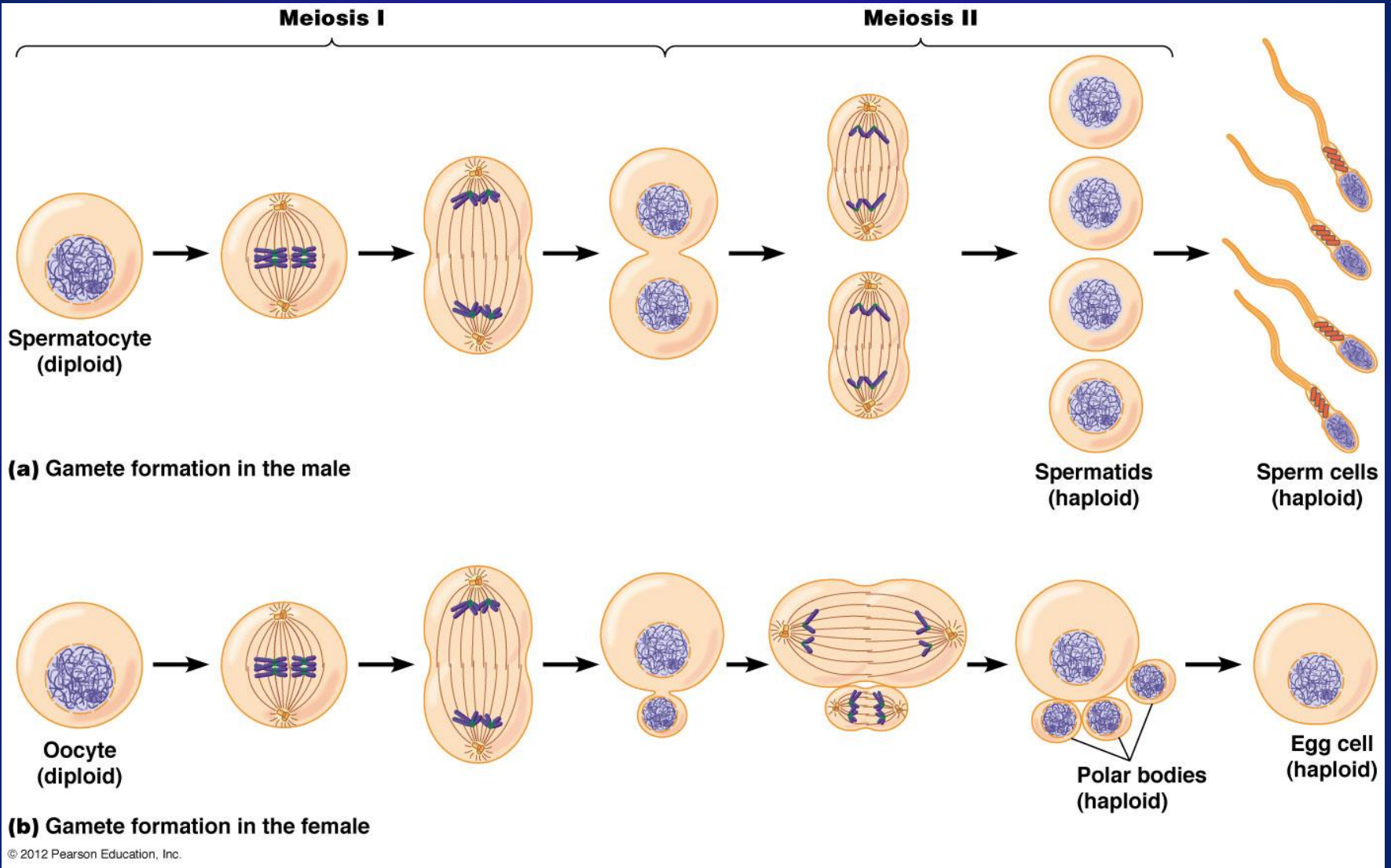
Steps in meiosis:

- 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 1st polar body (egg)
- Meiosis II – random segregation of chromatids
- Cytoplasmic division (sperm), or formation 2nd polar body (egg)

Meiosis



Gametogenesis



Genetic Variation / Mutation

- DNA replication is very efficient, but occasional mistakes do occur, producing changes in nucleotide sequence (mutations)
- A mutation within a gene (coding DNA) may result in a change in the amino acid sequence of the protein, and the change may alter protein character or functionality (or, render it totally non-functional)
- Different functional forms (alleles) of a protein (allozymes) can sometimes be observed and used as “markers/tags” with which to genotype individuals, and allele frequencies can characterize populations

Coding (Genes) versus Non-Coding DNA

- If gene mutations reduce, or nullify, protein functionality, will be (very strongly) selected against
- Therefore, there is limited DNA variation within genes (and even less within their proteins) among individuals, populations, and even species
- ...But, only a very small % of genome is actually made up of genes – most (98% ?) DNA is non-coding (sometimes, naively, referred to as “junk DNA”)
- A mutation within non-coding DNA has lesser (or no) fitness implications

<http://www.youtube.com/watch?v=ZvnhZI-GZS4&feature=plcp&context=C35708eeUDOEgsToPDskluC-Hhgeu6UebgCCzlyyCE>

Coding (Genes) versus Non-Coding DNA

- Therefore, changes to non-coding DNA can accumulate, and variation in the nucleotide sequence can be observed among individuals within a population, among populations within a species, among species within a genus, etc.
- Locations (loci) with sequence differences (alleles) are what we exploit as “markers/tags” with which to characterize individuals, and populations/stocks
- Types of DNA markers, and how can they be observed and the data applied to fisheries management will be discussed in the subsequent presentations

Genotypes to Phenotypic Traits

Qualitative Traits

- “Mendelian” traits
- trait controlled by (a mutation to) a single gene
- 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



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 and 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 ray 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
- fright response

- Production

- growth rate
- feed-conversion rate
- smoltification size/age
- 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

Tipping, J. A. and C. A. Busack. 2004. The effect of hatchery spawning protocols on coho salmon return timing in the Cowlitz River, Washington. North American Journal of Fisheries Management . 66:293-298.

- Cowlitz Salmon Hatchery – coho program 1967 to 2001
- Management objective - delay coho return to avoid by-catch of Chinook in coho fishery (... avoid work in winter)
- Percent in natural escapement vs. hatchery broodstock:

<u>Run Timing</u>	<u>natural</u>	<u>hatchery</u>
Early (Aug to mid-Oct)	40%	10%
Middle (mid-Oct to Nov)	33%	80%
Late (Dec to Feb)	27%	10%

Tipping, J. A. and C. A. Busack. 2004. The effect of hatchery spawning protocols on coho salmon return timing in the Cowlitz River, Washington. *North American Journal of Fisheries Management* . 66:293-298.

