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Bio 107/207
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Lecture 12
Molecular population genetics. I. Neutral theory

Classical versus balanced views of genome structure

- like many controversies in evolutionary biology, the so-called neutralist-selectionist controversy has had a long history.
- this controversy is actually the continuation of a previous controversy that was waged at the phenotypic rather than the molecular level.

- this controversy began in the 1920's with the development of two schools of genetics.
- the first of these camps were the **Naturalists** who studied natural populations.
- the second were the **Mendelians** whose research was exclusively in the laboratory.
- leaders of the latter school include Thomas Hunt Morgan, and his students Sturtevant, Bridges and Muller.
- the natural population school was initiated by the Russian geneticist Timofeeff-Ressovsky, Dobzhansky, and Mayr.

- these two research disciplines gave rise to what is known as the "Classical" and "Balanced" schools of genome structure.

Classical	Balanced
+ + + + + + + + + + + +	A_1 B_2 C_3 D_1 - F_4 G_6 H_2 I_3 J_4
+ + - + + + + + + + - + +	A_2 B_5 C_1 D_3 E_7 F_2 G_1 H_2 I_6 J_3

- the classical school viewed the genome as homozygous for "wild-type" alleles.
- interspersed throughout the genome are deleterious recessive alleles (-) present at low frequencies.
- this view originated from isolation of mutants in *Drosophila* – most of the genome is homozygous for wild type alleles.
- the small school of population geneticists studying natural populations developed a completely different view of the genome.
- they believed that natural populations harbored a large quantity of genetic variation.
- this view was shaped by considerable variation discovered to be underlying most morphological characters.
- as experiments were performed in the laboratory examining the fitness of chromosomes extracted from natural populations, this view was strengthened.
- individual chromosomes made homozygous were always found reduce fitness in individuals.
- this group developed the view that there was no such thing as a "wild-type" alleles - the majority of loci possessed two or more alleles and the most fit state was heterozygous not homozygous.

- these are two extreme representations - the classical school would not deny the existence of small numbers of loci experiencing balancing selection, nor would the balanced school deny that many loci harbor deleterious recessive alleles at low frequencies.
- although this distinction may seem to be of minor importance, many fundamental issues flow from these two views:

	Classical	**Balanced**
Speciation	Difficult (mutation-limited)	Easy (opportunity-limited)
Predominant mode of selection	Purifying	Balancing
Population variation	inter > intra	intra > inter
Polymorphism	transient, short-lived	balanced, long-lived

- according to the classical school, speciation would be a rather difficult process to accomplish, since there is little raw material available for natural selection.
- the balance school would predict that speciation would be much easier to achieve - all that is required is the proper environmental circumstances.
- the classical school holds that natural selection acts primarily in a “purifying” fashion, acting to remove deleterious alleles from populations once they arise by mutation.
- the balanced school believed that although purifying selection does occur, the primary type of selection acting in nature was balancing.

- differences also exist in how the two schools view variation within and among populations.
- the classical school views inter-populational differences as being far more important than intra-population differences.
- this is because most populations harbor little variation.
- because most populations harbor little variation, most of the genetic diversity within a polymorphic species would be inter-populational.
- the balanced school contends that most populations have an enormous reservoir of variation and thus any differences that exist between different populations are less important.

- the final difference between the two schools concerns how they view polymorphism.
- according to the classical school, polymorphism is transient.
- mildly deleterious or neutral alleles wander through populations by random drift, eventually being lost or going to fixation.
- the balanced school contends that polymorphisms are balanced and thus remain in populations for longer periods of time than predicted by the classical school.
- remember that modes of balancing selection, such as frequency-dependent selection, result in the establishment of stable equilibrium states.
- the frequencies of alleles are maintained at the equilibrium states and thus the alleles remain segregating in the population for longer periods of time than they would if they were neutral.

- the discovery of substantial levels of protein polymorphism by the technique of gel electrophoresis in the 1960's appeared to vindicate the balanced school because polymorphism was present at individual genetic loci at levels far above that predicted by the classical school.
- however, the proponents of the classical school had a very simple way to explain this excess variation
- it is "neutral" in its effect on the phenotype.
- since it has no effect on fitness, most loci have the equivalent of homozygosity for wild-type alleles.

Arguments leading to the development of neutral theory.

- the neutral theory was first proposed in its modern form by Motoo Kimura in 1968, although both Sewall Wright and Haldane had sketched out its form previously.
- what evidence led Kimura to propose the neutral theory?

1. The segregational load.

- when large amounts of protein variation were reported in *Drosophila* and humans, Kimura felt that this would impart too severe a "segregational load" if all this variation was adaptive.
- the segregational load refers to the fact that if balanced polymorphisms are maintained in a species population then each generation less fit genotypes are maintained.
- consider an overdominant locus such as sickle cell anemia.
- in each generation “less fit” homozygotes are produced by matings between the most fit heterozygotes.
- this will act to reduce the population fitness and generate what is called a segregational load.

- how great is this load?
- consider the example of sickle cell anemia.

Genotype	Hb^AHb^A	Hb^AHb^S	Hb^SHb^S
Fitness	1-s	1	1-t
	s = 0.12		t = 0.86

Segregational load = st/(s + t).

- for the sickle-cell polymorphism, the load is about 11%.
- in other words about 11% of the population dies every generation because of this polymorphism!
- clearly, a population can not have too many subject to strong overdominant selection like the sickle cell alleles - it simply would not have the reproductive capacity to maintain stable populations.
- if balancing selection is incapable of maintaining such a large number of loci at polymorphic equilibria, then the only viable alternative is that the majority of polymorphic loci are neutral in their effects on the phenotype.

2. The molecular clock

- first proposed by Zuckerkandl and Pauling in 1965.
- the approach used to document the clock was rather simple:

1. Obtain homologous amino acid sequences from a group of taxa.
2. Estimate divergence times (from the fossil record).

3. Assess relationship between protein divergence and evolutionary time.

- many proteins were found to exhibit a constant rate of amino acid divergence over evolutionary time – they followed a "molecular clock".
- the "clock" was observed to "tick" at different rates for different proteins – some evolved very quickly (e.g. alpha-globin) compared to others (e.g., histones).

- Kimura viewed the clocklike evolution of proteins like the alpha and beta globins, cytochrome c and myoglobin to be inconsistent with the action of natural selection.
- the constant rate of amino acid substitutions per year seemed to be more easily explained by the origin and fixation of neutral mutations.
- how can natural selection possibly account for the regular substitution of advantageous alleles?

- the molecular clock is a topic that has generated considerable controversy.
- some have argued that the claimed "constancy" of molecular evolution is nothing more than a confusion between a constant and an average.
- when we're dealing with the adaptive evolution of molecules over hundreds of millions of years, we would expect the rate to vary considerably depending on patterns of climatic change, competition with other species, etc.
- when we look at the **average** rate of evolution over long periods of time it appears to be constant, even though it has fluctuated considerably on both a daily and seasonal basis.
- for example, if we measure the average temperature in Santa Cruz in the last 50 years and compare it to the average temperature over the previous 50 years, the two should be virtually identical.
- this doesn't mean that the temperature is fluctuating considerably within these two time periods.

- Kimura doesn't think that this is a valid argument.
- he feels that the constancy of the molecular clock reflects a constancy of the rate of neutral evolution for specific types of proteins
- in other words, the rate of evolutionary change is more dependent on the properties of the molecule than the environment.

Main features of the neutral theory

1. The rate of protein evolution is roughly constant per site per year

- this is the "molecular clock" hypothesis originally proposed by Zuckerkandl and Pauling in 1965.
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- let us further assume that subsitution of an amino acid over evolutionary time periods follows a Poisson distribution
- a Poisson distribution is a discrete frequency distribution that describes the number of times a rare event occurs.
- for an event to be distributed in a Poisson fashion two features must be met.

1. the Poisson variable must exhibit a small mean relative to its maximum possible rate in a given sampling period.
- an amino acid substitution in a protein is indeed a rare event.
- we do not expect an new amino acid to be fixed at a particular site in a protein too frequently.
- for alpha-globin, it has been estimated that a new substitution occurs within a species every 7 million years.
- this has to be a small fraction of the rate that alpha-globin could evolve.

2. the occurrence of the event must be independent of past events in the sampling period.
- thus, the substitution of an amino acid in an evolutionary line must occur independently of past substitutions.
- these two conditions mean that "rare and random" events should be distributed in a Poisson fashion.

- let us assume that we have sequenced the homologous protein in two taxa.
- we then align the proteins and can estimate **K_{aa}, the mean number of amino acid substitutions per site**, as follows:

let N = no. of amino acids compared

d_{aa} = proportion of different amino acid sites

- if our model of molecular evolution follows a Poisson distribution then the probability of no substitution at any site is:

$$\Pr(0 \text{ subst.}) = e^{-K_{aa}}$$

and $$\Pr(1 \text{ or more subst.}) = 1 - e^{-K_{aa}}$$

if we assume $$d_{aa} = 1 - e^{-K_{aa}}$$

then $$K_{aa} = -\ln(1 - d_{aa})$$

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- it must be stressed that the clock is constant per site PER YEAR, not per site per generation, as predicted by the neutral theory.

- one would expect organisms with shorter generation times to exhibit faster rates of molecular evolution than species with longer generation times.
- how can this dilemma be solved?
- well, one possibility proposed is that an inverse relationship exists between generation and effective population sizes.
- these counterbalancing effects would act to standardize the rate of neutral evolution among species such that is constant on sidereal time.

2. Rate of substitution of neutral alleles equals the mutation rate to neutral alleles.

- let u equal the neutral mutation rate at a locus.
- the rate at which neutral alleles arise at a locus each generation is

rate of appearance = $2N \times u = 2Nu$.

- as we have seen before, the probability that an allele will become fixed by random genetic drift is equal to its frequency.
- if a new neutral allele enters a population, it will have an initial frequency of 1/2N.
- this initial frequency is equal to the probability of fixation of that allele.

probability of fixation = 1/2N

- therefore, the rate of evolution = $2Nu \times 1/2N = u$ (the neutral mutation rate!).

- this means that the fixation probability is unaffected by population size!

3. Heterozygosity levels determined by the "neutral parameter", $4N_eu$.

$$H = 4N_eu/(4N_eu + 1)$$

- if $4N_eu >> 1$ then heterozygosity will be high, if $4N_eu$ is $<< 1$ then heterozygosity will be low.
- in species with large, stable effective population sizes the amount of polymorphism can, in principle, approach 100%.

4. Rate of protein evolution varies with degree of selective constraint.

high constraint $\rightarrow$ low u $\rightarrow$ low H + slow rate of evolution

low constraint $\rightarrow$ high u $\rightarrow$ high H + fast rate of evolution

- the idea of Kimura is that most molecules we see today have reached some "optimal" state and that most changes have been variations on the theme,
- for example, globins are characterized by highly conservative three-dimensional structures despite the fact that the primary structure of the protein may have undergone substantial changes.

Modifications of the neutral theory.

- the neutral theory as originally proposed by Kimura in 1968 is not the version currently accepted by a wide spectrum of biologists.
- it is paradoxical that the main reason for modifying the neutral theory came about because levels of polymorphism were not too high, as originally proposed, but rather too low.

- let us examine this argument.
- according to the neutral theory, heterozygosity is determined by the product of $N_e u$.
- in species that have extremely large stable population sizes (e.g., *D. willistoni*) why don't heterozygosities approach 100%?
- why is the observed range of heterozygosity typically between 0.05 to 0.20?

- to account for the absence of species with high mean heterozygosities, Ohta modified the strict neutral theory to the **"nearly neutral theory".**
- in the nearly neutral theory, the majority of mutations that occur are not completely neutral, but rather subject to very slight negative (i.e., purifying) selection.
- under the nearly neutral theory selection coefficients on the order of $1/N_e$.
- if slightly deleterious alleles are subject to very weak selection in species with large effective population sizes, heterozygosities will be considerably below that predicted by the strict neutral theory.
- this is because selection is very efficient in these large populations to prevent these slightly deleterious alleles from increasing in frequency.

- the "nearly neutral" theory has an important consequence to molecular evolution.
- it predicts that the rate of neutral evolution will be greater in small populations than in large populations.
- this is because random drift will predominate in small populations and thus slightly deleterious alleles will behave as if they are neutral.
- these same alleles would be held at low frequencies in large populations and thus not contribute to either heterozygosity or polymorphism.
- the nearly neutral theory thus predicts that the molecular clock should be rather sloppy!

The neutralist-selectionist controversy

- the development of the neutral theory gave rise to the neutralist-selectionist controversy over the adaptive significance of protein polymorphism.
- in many respects, the discovery of substantial levels of protein variation in the 1960's provided the right answer to the wrong question.
- the question that required an answer at this time was not "How much genetic variation exists in natural populations" but rather "How much of the genetic variation that exists in natural populations is adaptive"
- the argument over the relative role that selection plays in determining levels and patterns of polymorphism was simply shifted to the allozyme level.

- the controversy has not been resolved - the interests of evolutionary geneticists have simply shifted to other issues.
- there has never been an argument among selectionists and neutralists that some polymorphisms may be neutral and others may be subject to balancing selection.
- the dispute concerns the **relative** importance of one type of natural selection over another.
- how do we go about solving this dilemma?

- two research schools developed.
- proponents of the selectionist school set about to prove, on the basis of biochemical and physiological evidence that certain polymorphic allozyme loci were subject to balancing selection.
- so far, a limited number of case studies have been done by this approach.
- this research program is extremely time-consuming and difficult.
- it involves basic research at many levels of biology including biochemistry, physiology, ecology to confirm the action of selection.
- some notable successes have been achieved.
- these examples include the *Lap* locus in the blue mussel *Mytilus edulis*, the *Pgi* locus in *Colias* butterflies, the *Ldh* locus in the killifish *Fundulus heteroclitus*.
- in each case, strong evidence favoring selection has been obtained.

- it is unfortunate that examples of balancing selection acting at particular allozyme loci has not been able to provide definitive refuting the neutral theory.
- most proponents of the neutral theory would simply say that these examples are exceptions to the general rule.
- what we require is not independent cases favoring selection in isolated species but an overall assessment of the role of selection **in one species**.
- so, for example, if 10 polymorphic enzyme loci were observed segregating in one species, how many of these are selectively maintained and what proportion are neutral?

- another way of testing the validity of the neutral theory has been to examine the patterns of variation in natural populations and see how close the data fits predictions of the neutral theory.
- these tests are rather weak in that selectionist interpretations of the same patterns are also possible.
- despite these drawbacks, this approach is capable of testing a large number of loci and a large number of species simultaneously and thus provide insights into this question of how many loci may be subject to selection and how many loci may be neutral.
- I will give two examples.

Global tests of the neutral theory.

Example 1. Relationship between enzyme structure and polymorphism.

- the neutral theory predicts that the main factor driving protein evolution is not environmental change but the neutral mutation rate.
- proteins evolve at faster rates because they have higher neutral mutation rates.
- higher neutral mutation rates are expected to lead not only to higher rates of evolution but also higher levels of polymorphism - the more neutral mutations arising at a locus the greater the chance that many will drift to appreciable frequencies.
- the rate of evolution of a protein is thus expected to correlate with its level of polymorphism.
- an important factor affecting a protein's neutral mutation rate is thought to be its subunit structure.
- the neutralist school has argued that the neutral mutation rate should be higher in monomeric enzymes (since these do not interact with other subunits), lower in dimers (since two subunits need to interact with each other at a number of residues) and lowest in tetramers.

- can subunit structure explain variation in polymorphism levels among allozymes?

- in a large study on allozyme data, 52% of the variation in allozyme heterozygosity levels in vertebrates (N = 648 species) can be accounted for by enzyme structure.
- 41 % of the variation in heterozygosity among 370 invertebrate species can be explained by enzyme structure alone!
- there is no *a priori* way for selection theory to account for this relationship.
- 41% is a rather high proportion but still leave considerable room for other factors.

Example 2. Relationship between heterozygosity and genetic distance.

- neutral theory predicts that a positive relationship should exist between H and Nei's D.
- the faster the neutral mutation rate, the greater the level of heterozygosity and the faster its rate of neutral evolution.
- in a large data set including just over 1500 species, Skibinski, Woodwark and Ward (1993) performed regression analyses examining the relationship between heterozygosity and D.
- this analysis allowed for an assessment of the total variation in protein heterozygosity and distance that can be accounted for by variation in neutral mutation rate among loci.
- their analysis showed that between 50 and 90% of the variation in genetic distance among taxa can accounted for by variation in H!!