**Introduction**

Remember that the **modern synthesis**, or neo-Darwinism, emerged in the 1930’s by integrating the laws of Mendelian inheritance with the principles of population genetics, the principles of Darwinian evolution by natural selection, and the surveys of variation in natural populations and the fossil record. The major tenets of the synthesis were:

1. populations contain genetic variation that arises at random via mutation and recombination
2. populations evolve by changes in allele frequencies
3. allele frequencies can change by mutation, migration, drift and natural selection
4. most mutations are deleterious
5. most adaptive phenotypic effects are small so changes in phenotype are slow and gradual
   a. some such changes (like certain color polymorphisms) can have large discrete effects
6. diversification occurs by speciation
   a. usually a gradual process
   b. usually by geographic isolation
7. population processes, continued for sufficiently long periods of time, give rise to changes of greater magnitude such as the divergence of genera, families, etc.

There was general agreement on most of these issues. Nevertheless, controversies persisted. The one most relevant to the field of molecular evolution questioned how much genetic variation could be maintained in a population. There were two schools of thought on this issue: (i) the classical school and (ii) the balance school.

The **classical school** held a view that went all the way back to Malthus, where natural selection was believed to be predominantly purifying or a negative force for population evolution. The school was modern; i.e., compatible with neo-Darwinism, in allowing positive natural selection to be the only population genetic force for adaptation. However positive Darwinian evolution was believed to be rare in the micro-evolutionary time-frame relative to purifying selection; i.e., it was believed that the vast majority of new mutations were deleterious, and the very rare beneficial mutants were fixed relatively quickly. Our models of directional selection are certainly consistent with this view. From this viewpoint, most individuals in natural populations were expected to be homozygous at the majority of loci.

The **balance school** held the view that polymorphism was common in natural populations, and important to adaptation. This school was championed by the naturalists, who began their careers collecting and cataloguing natural diversity. Remember the Victorian-era fascination with grand collecting expeditions such as those of Darwin and Wallace; the Neo-Darwinian naturalists were the academic descendents of that scientific tradition. They had extensive experience in the field collecting organisms, and they saw that natural populations contained very large amounts of phenotypic variation in characters that were thought to be relevant to an individual’s ability to reproduce and survive.

The balance school predicted that most populations would be heterozygous at most loci, and that such polymorphisms were maintained by different forms of balancing selection.

Both schools agreed on the importance of natural selection to adaptation. For quite some time there was no way to directly test their contradictory predictions about the levels of genetic polymorphism in natural populations; there were no ways to directly measure such genetic variation.
Note that neither school considered the possibility that selectively neutral variants made an important contribution to natural polymorphism.

“\textit{It is altogether unlikely that two genes would have identical selective values under all the conditions under which they may coexist in a population. ... cases of neutral polymorphism do not exist ... it appears probable that random fixation is of negligible evolutionary importance}”

—Ernst Mayr

\textbf{Protein electrophoresis and the rise of neutral theory}

\textit{Protein electrophoresis}

The ability to finally answer the questions about levels of natural variation was achieved in the late 1950’s and 1960’s with the development of two techniques in molecular biology.

First was the ability to directly sequence a protein. Although a slow and painful process, it did provide a technique for identifying a polymorphism. Because of its limitations it was used to look for variation between species, rather than population genetic polymorphisms. Nonetheless a surprisingly large amount of variation between species was revealed in many proteins.

Second, in the mid 1960’s Lewontin and Hubby (1966) discovered that by running a sample of proteins through a gel placed in an electric field (protein electrophoresis) and staining that gel, they could visualize proteins that differed by having nonsynonymous mutations that lead to amino acids with side groups that altered the charge or weight of the protein. Lewontin and Hubby were working on \textit{Drosophila} in the USA, the same technique was independently developed by Harris and Hopkinson (1966) in the UK who were working on human variation at UCL.

Compared with protein sequencing, electrophoresis was relatively fast, cost effective and easy to accomplish. Gel electrophoresis could only detect those changes that resulted in a change in a proteins charge or weight, whereas sequencing could detect all amino acid differences between
proteins. Nevertheless, electrophoresis allowed population geneticists to survey polymorphisms in natural populations at a scale that was previously impossible.

By using protein electrophoresis it was now possible to test the predictions of the classical and balance schools. Note that both schools were making very precise predictions. For example, Nobel Laureate H.J. Muller, a famous advocate of the classical school, openly predicted that only 1 in every 1000 human loci would be heterozygous. In contrast, Harris (1966) found 30% of human loci examined were polymorphic, with an average frequency of heterozygotes of 9.9%. In the same year Lewontin and Hubby (1966) reported their survey of 18 loci in five populations of *Drosophila*; there, about 30% of loci were polymorphic, with the mean heterozygosity of 11%. It was not long before similar levels of variation were uncovered in the natural populations of other species. Remember that these were underestimates, as not all amino acid substitutions will lead to a change in the electrophoretic mobility of a protein. In fact, we saw in an earlier topic that the genetic code appears to be organized in such a way as to minimize the effect of random DNA mutations on the physiochemical properties of the involved amino acids.

It became clear that the predictions of the balance school had been correct; levels of polymorphism in natural populations were high. However, just because their prediction was correct did not mean that their favoured mechanism, balancing selection, was correct. Advocates of the classical school had pointed out that there was a COST OF NATURAL SELECTION if high amounts of polymorphism were present in a population via a selective mechanism. Because it was so difficult to reconcile such high levels of polymorphism with balancing selection, Lewontin and Hubby argued that much of the variation might be selectively neutral.

**Population fitness and genetic load**

*Genetic load* is the extent to which the fitness of an individual is below the optimum for the population as a whole due to the deleterious alleles that the individual carries in its genome. In order to speak of the load associated with polymorphism in a population we define *genetic load* as the difference between the average fitness ($\overline{W}$) and the fitness of the best genotype (whose fitness is set equal to 1). Thus the load ($L$) is $L = 1 - \overline{W}$

<table>
<thead>
<tr>
<th>Genetic load on a population: an example</th>
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<tr>
<td>Imagine a population with two alleles (A and a) with frequencies $p = q = 0.5$. For simplicity let’s assume they only differ in their probability of surviving to reproduce.</td>
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<tr>
<td>AA = 40%</td>
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<tr>
<td>Aa = 50%</td>
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<tr>
<td>aa = 30%</td>
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<tr>
<td>We assign fitness of 1 to the Aa genotype. Hence, the relative fitness values are:</td>
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<tr>
<td>AA = 0.8</td>
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<tr>
<td>Aa = 1</td>
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<tr>
<td>aa = 0.6</td>
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<tr>
<td>The mean fitness of the population is:</td>
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<tr>
<td>$\overline{W} = \frac{0.25 \times 0.8 + 0.5 \times 1 + 0.25 \times 0.6}{1} = 0.85$</td>
</tr>
<tr>
<td>The fitness of the population is less than 1 because not all individuals in the population have the best genotype; in fact, exactly half possess a suboptimal genotype. The load of this population is:</td>
</tr>
<tr>
<td>$L = 1 - 0.85 = 0.15$</td>
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<tr>
<td>This means that for this population, averaged over all genotypes, the chance that an individual will die without breeding is 15%. Note that if every member of the population had the same genotype $\overline{W}$ would equal 1 and the load on the population would be zero.</td>
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</table>
Every individual whose fitness is less than maximal contributes to genetic load. What are the practical consequences of this in terms of the long term evolution of the population? Let's take an example of a single polymorphic locus maintained by balancing selection. Let's say the load on this population due to the long-term presence of homozygous genotypes is 0.1. If we assume the homozygous genotypes are deleterious under all conditions, then 10% of offspring born in each generation die before reproducing; this is sometimes called the SELECTIVE DEATH or GENETIC DEATH.

The cost of selection [sometimes called “Haldane’s dilemma”]:

The level of genetic load has implications for the maintenance of the population. Consider that natural populations produce more individuals than can possibly survive (remember the Malthusian principle). Let’s call this the excess reproductive capacity. The total load that can be tolerated by a population is bounded by the excess reproductive capacity of the population. When selective death exceeds the excess reproductive capacity, the population will decline.

Let’s continue to use the example above where L = 0.1. If every individual only produced one offspring (i.e., no excess reproduction), the population would decline by 10%. Assume a population of 500 individuals; with a load of 0.1, the reproductive population is 450. For the population to be maintained, the average family size for the 450 individuals must be at least 1/0.9 = 1.11 offspring (i.e., 450 × 1.11 = 500). The excess reproductive output is 1- (1/0.9) = 0.1111. In population terms, this is 450 × 0.111 = 50 individuals.

The famous British evolutionary biologist J. B. S. Haldane introduced the notion of the COST OF SELECTION (C) in terms of substitutional load (1957). Haldane defined the cost of selection as follows:

\[ C = \sum \frac{\text{proportion that die due to selection}}{\text{proportion that survive}} \text{ over all generations it takes to fix the allele} = \sum \frac{L/W}{1} \times N \]

As long as selection intensity remains the same, the ratio remains the same in each generation. However, if there is insufficient excess reproduction, the population size declines each generation until it goes extinct. What happens when we use Haldane’s formula for our above example? The cost of selection is 0.15/0.85 × 450 = 79.4; greater than the reproductive excess (50), so the population declines by roughly 29 individuals in one generation. Haldane takes the sum of this over all the generations it takes to fix an allele by selection; so, the cost is the total amount of excess reproduction to fix an allele. When the cost, in individuals, exceeds the population size, that population goes extinct.

Haldane estimated that a diploid population could tolerate no more that one substitution per 300 generations due to natural selection. Based on this estimate Haldane argued that molecular evolution was too fast to be explained by natural selection. Note there are some controversies about the assumptions that went into that estimate. The exact details of the cost are not the issue here, just that there can be a population cost to evolution by natural selection.

Regarding the cost of selection, Haldane famously remarked that “you can’t make an omelette without breaking some eggs.”
**Sources of genetic load and problems for the balance school**

The genetic load of a population can be partitioned into three sources: (i) **mutational load**; (ii) **substitutional load**; and (iii) **segregational load**. Let’s take them one at a time.

1. **Mutational load**: It seems reasonable to assume that most mutations are deleterious (remember the mutation accumulation experiments of T. Mukai!). Hence, every generation will experience an increase in the number of inferior genotypes by the process of mutation. We can work out a simple model of mutational load if we are willing to make some assumptions.

Let’s assume that all new mutations result in unconditionally deleterious alleles, and that all such alleles are recessive.

Remember we worked out an approximation of the equilibrium frequency of deleterious alleles given a certain mutation rate (\( \mu \)) and selection coefficient (\( s \)) [See population genetics, Topic 5 for a review]. The equilibrium frequency was

\[ q = (\mu/s)^{1/2} \]

Remember that population load is:

\[ L = 1 - \overline{F} \]

And remember that the average fitness under these assumptions was:

\[ \overline{F} = 1 - sq^2 \]

We can make substitutions:

\[ L = 1 - \overline{F} \]
\[ L = 1 - (1 - sq^2) \]
\[ L = 1 - (1 - s(\mu/s)) \]
\[ L = 1 - (1 - \mu) \]
\[ L = \mu \]

It is interesting that we estimate that the load is equal to the mutation rate. Because it suggests that the load is approximately independent of the reduction in fitness caused by the mutant (\( s \)).

We can understand this result by taking an example. Let’s say a mutant (M1) causes a reduction in survival by 10%. Now let’s compare it to a mutant (M2) that causes only a 1% reduction in survival. The frequency of M1 in the population will be 10% of the frequency of M2. Although the likelihood of survival is much lower for M1 (as compared with M2), the frequency of M1 in the generation is also lower (as compared with M2). Our model indicates that the result of this effect is that the allele frequency and likelihood of survival cancel out, giving a mutational load that is independent of \( s \).

We already considered genomic mutation rates in a wide variety of organisms (see **foundation topic 3**, and **population genetics topic 5** for a review). Let’s take the average rate: \( \mu = 10^{-6} = L \). The genomic mutational load is thus very small, and the required excess reproductive capacity to maintain the population is easily achieved by natural populations. Taken together with pervious consideration of equilibrium points (**population genetics topic 8**), we see that mutation-selection equilibrium at a single locus does not make a substantial contribution to population polymorphism nor to the population genetic load. However, if we sum over all mutations in a genome, where number of deleterious sites \( \times \) mutation rate \( >> 1 \), then it could be a significant source of population genetic load.
2. **Substitutional load**: Substitutional load is the reduction in average fitness that arises in a population in which natural selection is acting to replace one allele with another. Consider a case of directional natural selection (see Population Genetics Topic 6 for a review). Such a model is provided below.

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<th>Deleterious recessive</th>
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<tr>
<td>Genotype</td>
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<tr>
<td>Frequency</td>
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<tr>
<td>$W_{model}$</td>
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<td>$W$</td>
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Fixation of an allele under directional selection could take 1000 generations to complete. During these 1000 generations the population has a load because some individuals have inferior genotypes. In the example above, the homozygous recessive genotypes (aa) contribute to genetic load ($\overline{W} < 1$) until the process is complete when the $A$ allele becomes fixed in the population ($\overline{W} = 1$).

Haldane’s estimate of an upper bound of 1 substitution per 300 generations is a famous estimate of the effect of substitution load on the rate of evolution.

3. **Segregational load**: Segregational load arises when mendelian segregation generates selectively inferior genotypes. The principal case is when the heterozygote genotype has the highest fitness (overdominant selection). A general model is shown below.

<table>
<thead>
<tr>
<th>The model</th>
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<tbody>
<tr>
<td>Genotype</td>
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<td>Frequency</td>
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<td>$W$</td>
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Let’s look at the case of human sickle-cell haemoglobin where polymorphism is maintained by overdominant selection. Individuals who are homozygous for the sickle-cell allele, $Hb^s$, develop sickle cell anaemia. Selection against sickle-cell anaemia is intense, as roughly 80% die before reproduction. However, in some parts of the world individuals homozygous for the “normal” allele, $Hb^A$, are susceptible to infection by *Plasmodium falciparum*, the parasite that causes malaria. In these parts of the world heterozygotes for the normal and sickle-cell alleles have a selective advantage, as they are much less susceptible to malaria ($s_1 = 0.11$ and $s_2 = 0.8$). Because of this overdominant selection, $Hb^s$ reaches a frequency as high as 40% in some West African populations. These populations however suffer a high segregational load, as large numbers of normal and sickle-cell homozygotes are produced each generation.

Specific cases of overdominant selection are well known (e.g., $Hb^s$ in humans, and MHC in many species of mammals). Now let’s think about the consequences to segregational load of extending overdominant selection to many loci, as was suggested by the balance school. Let’s take humans as an example. Humans have around 30,000 genes, with an estimated 30% polymorphic (from Harris 1966). Let’s assume a very small load (0.001) at just half of the polymorphic human genes (4500). Although the fitness of individual loci would be 0.999, fitness over the whole genome would be $0.999^{4500} = 0.011$ (That’s the average fitness!). The load is about 0.989; this is a huge amount of selective death. If you’re not convinced, take a look at the cost of selection: 0.989/0.011 = 89. This is an impossible cost for humans; natural selection could not maintain polymorphism in the manner suggested by the balance school.
This is the type of calculation that Lewontin and Hubby (1966) were doing that led them to suggest that the level of polymorphism detected by protein electrophoresis could not be supported by overdominant selection. Another important point is that this level of load is consistent with extremely weak selection pressure (e.g., $s = 0.002$). Alleles subject to such weak selection pressure in finite populations are likely to be highly influenced by drift; i.e., they could behave as if they were neutral depending on the effective population size ($N_e$). More realistic levels of selection pressure imply a substantially higher cost of selection; i.e., requiring an excess reproductive capacity in the millions and billions.

Although the data generated by protein electrophoresis verified the predictions of the balance school, the data were incompatible with their hypothesized model of evolution. Such data heralded the demise of the balance school.

4. Other types of load: It is possible to frame the problem of genetic death in terms of other types of load such as arising from recombination, gene flow, drift, etc. Two types of interesting load are “incompatibility load” and “lag load”. On occasion mother and foetus can have incompatible genotypes (e.g., Rhesus blood groups), and the mother can mount an immune reaction against the foetus; this is called INCOMPATIBILITY LOAD. John Maynard Smith coined the term LAG LOAD to express the load that can arise when natural selection is out-of-step with the changes in the environment.

An important final note: All of the above genetic load arguments are based on models, i.e., intentional simplifications of reality, and there are many difficulties with the involved simplifications and assumptions. I will not go into the gory details, but please bear in mind that these arguments are not immune to criticism. It is possible to include a role for selection in population polymorphism if you invoke frequency dependent selection, or fluctuating selection pressures over time. This area is controversial, but has not seen much research since the advent of neutral theory.

**The neutral theory of molecular evolution.**

At the same time that Lewontin and Hubby were arguing that natural selection could not actively maintain high levels of polymorphism in Drosophila, the Japanese geneticist Motoo Kimura was drawing the same conclusions from his analysis of published amino acid sequences of proteins such as Haemoglobin. Zukerkandl and Pauling (1965) had already noted that divergence of proteins by amino acid substitutions appeared to be occurring at an approximately constant rate over time; this later came to be called the “molecular clock” hypothesis. Kimura realized that this phenomenon was incompatible with the classical and balance schools, and that this finding had very serious implications. Kimura took those protein data and extrapolated them as a means of estimating the number of substitutions per genome per year (for mammals); he obtained an estimate of 1 nucleotide substitution per 2 years! Remember that based on the cost of substitution load alone, Haldane put an upper limit on the human substitution rate of 1 per 300 generations! This finding led Kimura to consider the mathematical possibility a neutral theory of molecular evolution. The essentials of this theory were that much of what happened at the molecular level (DNA or protein) over the course of evolutionary time was independent of natural selection. One cannot overemphasize just how radical an idea this was at the time.

Kimura published a well developed mathematical theory of neutral molecular evolution in 1968. In the following year, Jack King and Thomas Jukes (1969) published the result of their work in which they independently arrived at the same conclusion as Kimura. King and Jukes published their work under the provocative title “Non-Darwinian evolution”. Collectively these articles initiated a longstanding controversy called the “neutralist-selectionist” debate.
The rate of evolution under neutral theory

It’s time to take a look at the theory itself. Neutral theory has an elegant simplicity. Let’s call the rate of nucleotide substitution at a site per year \( k \). This rate is simply the product of (i) the number of spontaneous mutations per generation and (ii) the probability that a mutation goes to fixation \( p \).

\[
k = \text{new mutations per generation} \times \text{probability of fixation}
\]

In a diploid population the number of mutations per generation is equal to the number of new mutations (the mutation rate \( \mu \)) multiplied over all the potential sites for such mutation (this is the effective population size; in diploids it is \( 2N_e \)).

\[
\mu \times 2N_e
\]

Next, we assume neutrality. Under neutrality, the fate of an allele is determined by genetic drift, and we already know that the probability of fixation \( p \) under drift is the reciprocal of the effective population size. For diploids this is

\[
p = 1/2N_e
\]

Hence, the **neutral rate** is given by:

\[
k = \mu \times 2N_e \times 1/2N_e
\]

Since \( 2N_e \times 1/2N_e \) cancel, we get the simple result:

\[
k = \mu
\]

This is one of the most important formulae in molecular evolution. It means that the rate of neutral molecular evolution is independent of effective population size (Well, as long as the allele is truly neutral, or its selection coefficient is so small that its fate is determined by drift). Now let’s think about this intuitively. In large populations the number of new mutants in each generation is high (because \( 2N_e \) is high), but the fixation probability is low \( (1/2N_e) \). In small populations the number of new mutations will be lower, but the fixation probability will be higher.

Note that the result only follows from the assumption of neutrality and a constant mutation rate. The result is not the same for selected mutants. For advantageous mutations under partial dominance, the probability of fixation is \( p = 2N_e s/N \). This gives a substitution rate of \( k = \mu \times 4N_e s \). Now the substitution rate depends on the effective population size \( (N_e) \) and selection coefficient \( (s) \) as well as the mutation rate.
Mutation, polymorphism and substitution under neutral theory

Prior to neutral theory, mutation, polymorphism, and substitution were thought to be largely separate processes; e.g., polymorphism was maintained by balancing selection, and substitution was driven by selection for advantageous mutants. Neutral theory connected these and advanced the hypothesis that polymorphism was simply one phase of molecular evolution.

1. The average time to fixation is $4N_e$ generations

Kimura and Ohta (1969) showed that it takes $4N_e$ generations for a neutral mutation to become fixed in a population of diploids by drift. Remember that genetic drift is a stochastic process, so $4N_e$ represents a long term average; in specific cases the time to fixation can differ greatly (see POPULATION GENETICS TOPIC 7 for a review of sampling error and genetic drift). What is clear is that the average time to fixation, let’s call this $t$, is controlled by the effective population size; smaller populations will, on average, fix neutral mutations at a higher rate than large populations.

![Diagram showing time to fixation for different effective population sizes](https://example.com/diagram)

A slice in time for each population is shown by a dotted vertical line (-----). Note that at such a slice in time the population with larger effective size is more polymorphic as compared with the smaller population.

2. The average time between consecutive neutral substitutions equals the reciprocal of the mutation rate.

This conclusion is a consequence of the conclusion above that the rate of neutral evolution is constant over time. If the substitution rate ($k$) is equal to the neutral mutation rate ($\mu$) then the long term average time between fixation events is $1/\mu$. Consider an annoying wrist watch that sounds a beep every hour of the day. The thing will “go off” 24 times each day, with the length of time between each “beep” being $1/24$ (1 hour).

In our case the fixation events (the “beeps” of our clock) are stochastic (see figure above), so the actual time between such events has random sampling errors. The expectation of $1/\mu$ comes from taking a long term average of the stochastic process.

Note that the average time between consecutive mutation events [unlike fixation events] is dependent on the effective population size (see figure below). Larger population sizes have
more genomes, and hence more opportunities for mutation events, so the mean time between events will be less.

3. The population will attain an equilibrium rate of substitution

Let’s return to the notion of a steady state. The rate of substitution does not depend on the effective population size: small populations drift to fixation at a higher rate, but this is compensated by the fact that there are fewer mutations in such populations to go to fixation; larger populations drift to fixation more slowly, but there are more mutations undergoing this process at any one time.

At mutation-drift equilibrium the mutations rate is equal to the substitution rate and the effective population size cancels out.
4. **The population will attain an equilibrium level of polymorphism**

Although new mutations are being lost or fixed due to drift, a new supply is being input by the process of mutation. These are opposing forces of evolution, so we can think in terms of an equilibrium, or STEADY STATE (See POPULATION GENETICS TOPIC 8 for a review of other types of equilibrium). The steady state in this case is the amount of polymorphism in the population; we measure it as the average frequency of heterozygotes in the population. The equilibrium heterozygosity is reached when the average number of new alleles from mutation is exactly balanced by the average number lost via drift. [Note we have assumed that each new allele is novel; this is called an infinite-alleles model.] A population at such a MUTATION-DRIFT EQUILIBRIUM has an expected heterozygosity ($H_e$) as follows:

$$H_e = 4N_e\mu(1+4N_e\mu)$$

It is clear that $H_e$ is dependent on the product $N_e\mu$. This quantity ($4N_e\mu$) is used over and over again in population genetics, and so it is given its own notation:

$$\theta = 4N_e\mu$$

Population geneticists seem to be obsessed with $\theta$. There is a good reason for this. Because it uses $N_e$, it solves many of the difficulties associated with using census size ($N$) in diversity calculations. Moreover, the expectations for various summary statistics used in population genetics [e.g., the difference between a pair of sequences, the number of segregating sites, the number of haplotypes] have a simple relationship with $\theta$. **The parameter $\theta$ is the single most important quantity in population genetics.**

The figure below illustrates the equilibrium level of $H_e$ as a function of $\theta$.

Let’s look at a specific example. Suppose a mutation rate of $10^{-6}$ per gamete. A population with an effective size of 1000 will have an equilibrium frequency of heterozygotes of 0.004. If the effective size was 250,000 (i.e., $\theta = 1$), the equilibrium frequency would be 0.50.

The figure illustrates that substantial levels of standing polymorphism can be maintained in populations without any genetic load. Thus “neutralists” suggest that this mechanism explains the population polymorphisms revealed by protein electrophoresis. The above figure also
illustrates another point: under neutral theory (i) the standing level of polymorphism (the equilibrium point) is dependent on effective population size, even though (ii) the rate of evolution is independent of effective population size.

**The neutralist-selectionist debate**

Neutralists and selectionists agree on many points

Neutral theory was a radical idea, and it sparked yet another debate: neutralism versus selectionism. Neutral theory was initially viewed as a challenge to Neo-Darwinism because it held that many (most) genetic differences between species were not adaptive. However, neutral theory is not a replacement for natural selection; rather it views the overall contribution of adaptive evolution to be much less than the selectionists. In fact, neutralists and selectionists agreed on many points. They agreed that morphological evolution was mainly driven by selective advantage, and that at the molecular level the vast majority of new mutants were deleterious and quickly removed from a population by purifying selection. The Figure below highlights the point that differences between neutralists and selectionists were mainly one of proportion.

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**The current controversies differ**

The early debate centred on the cost of natural selection; however, we now know that many of the assumptions used to calculate the costs were unrealistic. Some possible alternative views on genetic load are listed below:

1. Costs were considered as being completely independent, allowing multiplication across loci. We now know that this is an overestimate because the number of independent genotypes is less due to the complexity of metabolic and developments pathways.

2. Selection could act in response to a threshold level of polymorphism. Suppose that averaging a certain decrease in fitness over all polymorphic loci, regardless of the number of such loci in the genome is incorrect. Suppose that there is a threshold, say 10,000. In this model individual with more than 10,000 polymorphic loci have reduced fitness, and those with less than 10,000 polymorphic loci have high fitness. In this model natural selection acts on genomic levels of heterozygosity rather than each locus independently.
3. Early calculations assumed hard selection, where individuals with disadvantageous alleles die before reproduction. Alternatively, soft selection, where death before reproduction is due to non-genetic ecological reasons, reduces the overall cost of selection.

4. Genetic variation can be maintained by frequency-dependent selection with no segregational load.

Taking these observations together suggests a potentially greater role for natural selection in molecular evolution. The consensus opinion is that the initial arguments against selection based on genetic load are less conclusive than originally thought. Interestingly, there has been little recent work on genetic load under more realistic models, or on estimating the expected levels of population polymorphism under such models. However, even with a substantially greater role for natural selection in molecular evolution, no-one denies that neutral evolution must be granted a major role in molecular evolution. Neutral evolution probably underlies the majority of molecular substitutions.

The modern controversies no longer center on genetic load. Rather, the focus is on the predictions of neutral theory in specific cases of molecular evolution, and if the observed data in such cases fit the neutral expectations. We will return to this issue in the next set of notes.

Neutral theory has been immensely successful in molecular evolution, but not because of its arguments about genetic load. It is a successful theory because it provides a simple model that fits most data very well. Lastly, it provides a generally agreed upon null hypothesis for additional studies of molecular evolution.

### Misconceptions about neutral theory

There has been some confusion about what the neutral theory suggests, so it is worth trying to clear up some of the misconceptions.

**Myth 1:** Only genes that are unimportant can undergo neutral mutations. [Neutral theory only asserts that alternative alleles segregating at a locus are selectively equivalent. Such loci can, and do, encode genes that have important functional roles.]

**Myth 2:** Neutral theory diminished the role of natural selection in adaptation. [To the contrary, neutralists and selectionists both maintain that natural selection is the primary mechanism of adaptation, and that morphological evolution is primarily driven by natural selection.]

**Myth 3:** Nucleotide or amino acid sites that undergo neutral substitutions are not subject to natural selection. [Neutral theory does not preclude the possibility that adaptive mutations can occur at sites where neutral mutations occur. Neutral theory only asserts that adaptive mutations will be much less frequent and will go to fixation much more quickly; hence, most polymorphism observed in a population will be neutral.]

**Myth 4:** Neutral mutations have a selective coefficient of \( s = 0 \). [Because natural population sizes are finite, the fate of mildly deleterious alleles can be fixed due to drift. See POPULATION GENETIC TOPIC 8 for a review.]

**Myth 5:** Neutral mutations are always neutral. [Neutral theory makes no assertions about the stability of the environment. The selection coefficient will depend on the environment.]
Keynotes on neutral theory:

1. The fate of alleles is largely determined by random genetic drift. This does not imply strict neutrality, only that selection on most alleles is too weak to offset drift, with $|s| < 1/2N_e$.
2. The allele frequencies at any one time are in a transient state. They are either on their way to fixation or extinction, and their specific fate is governed by chance.
3. Substitution is an undirected process, with frequencies increasing and decreasing at random until fixation or extinction.
4. Unlike the substitution rate ($k$), the time to fixation ($t$) is dependent on the effective population size, with fixation taking a very long time in large populations ($t = 4N_e$ generations).
5. The neutral evolutionary process is governed by the continuous process of mutation.
6. Substitution and polymorphism are two facets of the same process.
7. Neutral theory does not preclude adaptation, it only suggest that the fate of the majority of new mutants is determined by chance rather than natural selection. It did assume that positive selection and balanced polymorphisms were extremely rare, but that assumption has been relaxed a little.
8. The rate of evolution should be inversely related to the degree of functional constraint [We will discuss this in the next topic].