BIOL2007 - MUTATION AS A SOURCE OF VARIATION

Genetic variation is essential for Darwin's theory of natural selection and all genetic variation must come, ultimately, from mutations. A **mutation** is any hereditary change in the DNA sequence or in chromosome number, form or structure. Most mutations arise from errors during DNA replication that fail to produce exact copies of parental DNA sequences. Mutations may include 1. **Base substitution**: the replacement of one base by another; 2. The **insertion** or **deletion** of single bases. 3. I**nversion** of a section of DNA. 4. **Duplication** or **deletion** of a section of DNA.
Background information about 1 to 4 is provided on the handout.

These mutations occur at different rates and are differentially affected by mutagenic agents. Some changes (e.g. inversions) may have *no discernible phenotypic effect* (if the chromosomal breaks occur in **non-transcribed** regions). Often mutations will be *non-functional or lethal* if they occur in an **essential** region of DNA.

There is *no constraint* at the level of the DNA on which mutations are possible. Non-functionality/lethality reflects constraints on what can survive (due to physiology and/or development) but not on what arises initially. **Any class of DNA sequence can arise by mutation.** *Variation produced by mutation is RANDOM with respect to the DIRECTION of adaptation. Natural selection imposes direction on evolution using undirected variation.*

In general terms, the **Neo-Darwinian** view is that evolution progresses by the gradual accumulation of advantageous mutations with individually small effects on the fitness of their carriers. Another view proposed by the so-called **catastrophists** (e.g. Goldschmidt, 1940's) - claimed that new species may suddenly arise through one or a few mutations of large effect ('macromutations' or 'hopeful monsters'). Their notion is that macromutations may be changes in regulatory genes that could affect many genes so as to produce a synchronized changes in many aspects of phenotypes. (We will come across these arguments in other contexts later in the course).

For today, we ask whether what we know about about mutation fits well with either of these views: **1. What is the natural rate of mutation? 2. What are the effects of mutation on fitness? 3. Is the mutation rate itself under genetic control? 4. Is evolution ever limited by the availability of new mutations?**

Question 1. What is the natural rate of mutation?
Note that different kinds of rate may be estimated and you should bear this in mind when you read around this topic and compare studies.
We can make estimates of different types of mutational rate. These include
1) The rate of base substitution. per base, per replication
2) The rate at which new mutations occur at a gene locus, per generation
3) The rate at which lethal or deleterious mutations accumulate on a chromosome
4) The rate at which new phenotypic variance is generated by mutation
We'll look at types 3) and 4) in the context of the remaining evolutionary questions about mutation.

A direct approach is to observe the rate at which a visible mutant phenotype arises in a laboratory population of a species. So the rate is found for a designated locus (or

loci) per generation. This approach has been used for many model systems such as bacteria, yeast, fruitflies and mice.

In mice, a classic method is the 'multiple locus technique'. Females that are homozygous for seven recessive mutations are crossed to males that are homozygous wild-type at those seven loci. The expectation is that almost all of the offspring are wild-type. Those progeny that aren't wild type are mutants. In one large scale experiment, over 500,000 progeny were examined and yielded estimates of the mutation rate per locus per gamete as 8×10^{-4}. In fruitflies – typical estimates of electrophoretically detectable mutation rates are ~ 4×10^{-6}. The table on your handout **('Table 2.2')** shows a range of examples. There is plenty of **variation** between case studies but an approximate **typical** figure for observed rates, by direct observation, of visible mutations is about $\mathbf{10^{-6}}$.

Question 2: effects of mutation on fitness.

Mutations can have a range of possible effects on fitness from lethal, through slightly deleterious to neutral. The class of mutations of small effect, **slightly deleterious**, are probably the **most frequent**. There are some ingenious ways in which it is possible to examine the effect of new mutations on fitness. Here we look at how to observe the rate of slightly deleterious mutations in experiments in which selection against them is minimised.

Some elegant findings come from Mukai's work with fruitflies examining the role of '**slightly deleterious**' mutations. One experiment (1964) used ~ **1.7 million flies** and examined the net effects of mutations at loci on the second chromosome. His work depended on the fact that *special breeding techniques* are available for fruitflies which allow the manipulation of entire chromosomes.

It is possible to 'extract' whole chromosomes from individuals and to maintain them subsequently in a controlled way. This is done by using 'BALANCER' chromosomes which have a set of unusual characteristics (see OVERHEAD).

1) Balancers include multiple overlapping inversions (as you'll know from other lectures such inversions will have the effect of *suppressing recombination* between homologous chromosomes)

2) Balancers are marked with mutant dominant alleles that alter the appearance (phenotype) of the fly

3) These dominant markers are lethal in homozygous condition.

The combination of these features means that we can make crosses between a population of interest and a balancer stock and extract specific chromosomes from the test population. This is shown on your HANDOUT. Note that the breeding scheme exploits the fact that, unusually, there is **no recombination in male fruitflies**.

MAINTENANCE SCHEME

Mukai maintained single wild type chromosomes in heterozygous condition with a balancer chromosome. He made 101 lines each of which carried an independently extracted wild type second chromosome. In each generation, these heterozygotes were crossed to the reference balancer stock and from the progeny he picked out the balancer heterozygotes (OVERHEAD). Repeated this procedure for **60 generations**.

What does this achieve? By running the experiment for many generations, Mukai allows time for mutations to occur on the wild type chromosomes. Importantly if mutations do occur they are, in effect, trapped on that wild type chromosome and over time new mutations are free to accumulate on the wild type chromosome.

The wild type chromosome is "**sheltered**" from the effects of a) recombination and b) selection. The special features of the balancer mean that **a) recombination is effectively suppressed between the pair of chromosomes**. So any combinations of newly arising mutations aren't broken up at meiosis and b) the wild type chromosome is kept in heterozygous state and any new recessive mutations are not exposed to natural selection. Selection operates on phenotypes.

ASSAY SCHEME

What happened to the **fitness** of individuals over successive generations? We expect fitness to **decline** because of the **accumulation** of mutations - mutations that would normally be eliminated by selection. Mukai tested the effect on fitness at regular intervals. Every so many generations he made the sheltered chromosomes **homozygous** by crossing together balancer heterozygotes and examined their fitness by measuring their **viability**.

Mukai's balancer chromosome carried **Curly wing** as a dominant marker. In this test, not all of the possible progeny types survive and we expect a 2:1 ratio of Curly winged and wild type wing phenotypes.

ANY DROP IN PROPORTION OF WILD TYPES INDICATES THE ACCUMULATION OF MUTATIONS LOWERING VIABILITY, THAT IS, LOWERING FITNESS.

Results?.

1) A **decline in viability** over the generations of mutation accumulation. After 40 generations, there was on average a **15% reduction** in the relative viability of the wild type homozygotes. After **60 generations** the reduction was of the order of **50%**. So the lines have become less fit reflecting their increasing mutational load.

2) How much variability in performance is there between the 101 independent lines? Does the data support the Neo-Darwinian or the Catastrophist view? Do they reflect the accumulation of deleterious mutations at many loci or the action of a relatively small number of mutations each of large effect?

a) if many mutations are involved, then expectation is that all the lines will respond in a similar way because they will each bear broadly similar numbers of mutations.

b) if few mutations are involved, then expect that some chromosomes will be free of mutations and others will have several. So there will be significant variation in the response in viability between the chromosomal lines.

Analysis of his data provided clear support for pattern a). There was relatively close agreement between the performance of the lines. Good news for Neo-Darwinism!

Mukai's experiments reveal what one commentator called the "destructive power" of mutation. Implies that natural selection can be a defence against chaos.

*As an aside, if you read about mutation and fitness, you may see the term "**mutation-selection balance**" (relevant to Jim's last lecture). The background algebra is given on your HANDOUT. The logic is to focus on the evolutionary fate of a deleterious allele. Selection will tend to operate against it to eliminate it from the population. But new copies of deleterious alleles will arise by mutation. So this can generate a balance between "what goes out" and "what comes in". – summarized by a simple equation relating gene frequency, mutation rate and selection coefficient. The HANDOUT gives some details of an example of a genetic disease which appears to be in mutation-selection balance.*

Question 3: is the mutation rate itself under genetic control?
Answer: Yes. Examples are genetic factors that may **increase** mutation rates (TRANSPOSABLE ELEMENTS). These are short sequences of DNA that have the capacity to move around the genome. They generally insert copies of themselves into recipient sites while the donor copy remains in place. **Frequently affect the function of genes at or near the site of insertion**, and SO HAVE A MUTATIONAL EFFECT.

Many examples are known for the fruitfly. A dramatic case is that of one family of transposons, the **P elements**, in a phenomenon called **HYBRID DYSGENESIS**. P elements characterise some strains but not others (M strains).

> If **M is introduced into a P background** there is no effect on the mutation rate. When **P-carrying chromosomes** are introduced into a strain with **M type cytoplasm**, the **offspring** of the hybrids have several problems. Usually **sterile**, and both gene **mutations** and chromosomal rearrangements appear **at high frequency**. These effects are temperature dependent.

Explanation is that in "dysgenic" crosses, the P element is mobilized, "hops" around the genome and has mutagenic potential in terms of disrupting gene function.

Question 4: is evolution ever limited by the availability of new mutations?
Answer: Yes. Evidence from other work on P element in fruitflies (Trudy Mackay).

She compared a population of flies derived from a **dysgenic cross** between **P element strains with a control (non-dysgenic) population**. Found substantial **increase in genetic variation** in a trait, bristle number, in the **dysgenic** population.

Evidence came from "**artificial selection"** (AS) experiment – a standard way of revealing underlying genetic variation in a trait of interest. In each generation only certain individuals can breed - usually those with extreme phenotypes. If the variation in the character has a genetic basis then artificial selection can drive the average trait value in the direction of selection from one generation to the next.

Mackay (HANDOUT) applied AS to a bristle phenotype in fruitflies in favour of "hairier" flies. For control non-dysgenic populations saw clear response to selection (solid lines). As the generations proceed, the average bristle score in the population is increasing. **BUT** when applied selection to populations founded from a dysgenic cross (dotted line) found a **faster** response to selection than the control.

The dysgenic cross is associated with elevated rates of mutation and the response to selection is steeper. So if more mutational variation is initially present see a faster response to selection in subsequent generations. **The general inference is that evolution is ultimately constrained by the availability of new mutations.**