Week 3 Highlights

Mutation Accumulation:

"if a given lot of individuals, known to contain no mutant genes at the start, is bred through a series of *n* generations (that is, to "F_n"), and one of the individuals of this last (*n*th) generation is then tested for mutant genes..., this test will reveal all mutant genes that arose in any of the preceding *n* generations." Muller (1928).

An approach that has been extensively used to investigate the rates and effects of spontaneous mutations is the mutation accumulation experiment. In a mutation accumulation experiment, sublines (mutation accumulation lines) derived from a genetically uniform ancestral line are allowed to independently accumulate spontaneous mutations for many generations. Each mutation accumulation line is kept at a small population size and under benign conditions during the period of mutation accumulation or after mutagenesis. In this way, the effects of natural selection are minimized, allowing all but the most deleterious mutations to accumulate. After the period of mutation accumulation, the fitnesses of the lines are measured in parallel with controls. The controls are often derivatives of the ancestral line, maintained in such a way that they can be assumed to be essentially mutation-free. These can be, for example, cryopreserved cells, embryos, eggs or dried seeds.

In general, mutation accumulation lines decrease in fitness as the experiment progresses and variance between lines increases 20. This pattern is consistent with a net accumulation of deleterious mutations, some of which are strongly deleterious: these generate most of the variance between lines. (Eyre-Walker and Kieghtley 2007 doi:10.1038/nrg2146).

Mukai (1964, 1972)

DURING the 1960s and 1970s Terumi Mukai and colleagues conducted some experiments that have had a major impact in population and evolutionary genetics. Their quest was to estimate the genomic rate and effects of deleterious mutations.

The first experiment to measure the fitness effects of a chromosome-wide accumulation of spontaneous mutations was carried out more than 30 years after Mueller's ideas by Mukai, working with lines of Drosophila at the National Institute of Genetics, Mishima, Japan (Mukai 1964). Mukai's design made use of the Cy/Pm balancer chromosome system.

Method:

He made lines that were homogeneous for second chromosome.

He then derived about 101 independent families (independent lines). In each of these, the second chromosome was made heterozygous over a balancer chromosome (ie., they had one wild-type second chromosome and one balancer second chromosome).

The lines were cultured for several tens of generations. Each generation, the lines were started with very few individuals. Thus, in the experiment, the wild-type second chromosome could accumulate mutations over generations. The balancer would protect the mutations from recombination. The hetereozygous condition and the population size protected muations from natural selection.

Every ten generations, Mukai measured viability of each of the 101 lines as a measure of fitness.

Results

Mean viability declined at the high rate of about 0.4% per generation. The rate of erosion in viability extrapolated to the haploid genome was in excess of 1% per generation, excluding lethals and severe detrimentals which were possibly lost.

Mukai's principal aim was to obtain information on the rates of mutations that cause the changes in mean and variance for viability. To do this, he turned to formulae of Bateman (1959) that relate the observed changes of mean and variance to the chromosome-wide mutation rate. Based on these, Mukai calculated that 0.14 mutations per generation with viability effects of greater than or equal to 3% was required to explain his experimental results.

Summary:

Mukai observed that

1. New mutations that affect viability arise at a rate of 1 per genome per generation.

2. Viability reduced by

    2% as heterozygotes and benign conditions of measurement

    6% as homozygotes and benign conditions of measurement

    100% when measured under harsh conditions

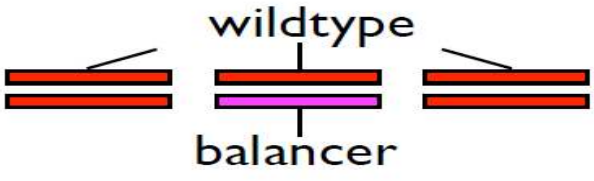
3. The effects of mutations on viability were used to estimate the rates of mutation (following Bateman 1959). Therefore, both these estimates are an under estimate because

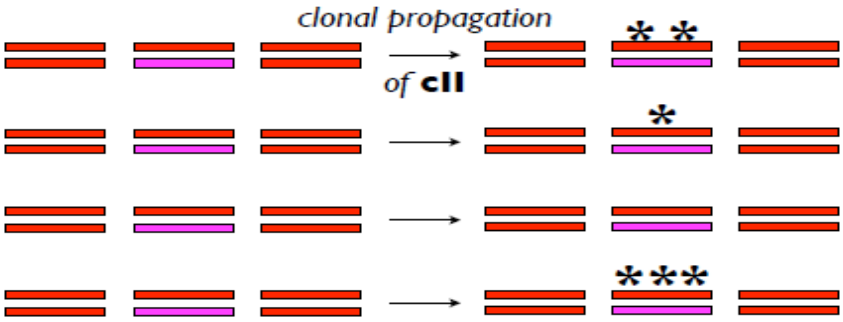
    All lethal mutations are lost

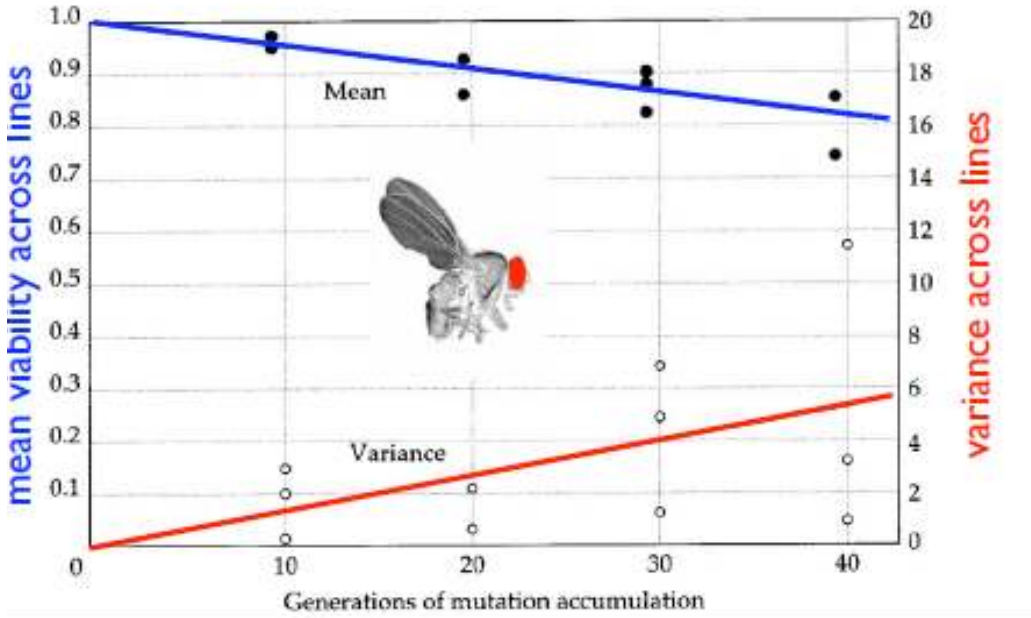
    Neutral mutations are not accounted for

    Viability is the only measure of fitness

    Most fitness measurements are done under benign conditions







Mutation Accumulation in *C. elegans*: Denver et al (2004)

The nematode Caenorhabditis elegans have both male and female gonads and can self-fertilize. This characteristic allowed Dee Denver and colleagues (2004) to set up 74 mutation accumulation lines from a single common ancestor and propagate them from a single worm each generation. Each line was maintained in the most benign environment possible, with optimal temperature and humidity, minimal crowding of individuals, abundant food, and no predators or parasites. This treatment insulated the worms as much as possible from natural selection.

Over a span of 214 C. elegans generations, the scientists periodically assessed the rate at which individuals in the mutation accumulation lines survived to adulthood. The red dots and best-fit line in the figure below show the data. As mutations piled up, the genetic quality of the worm population declined. Some lineages died out altogether.

The researchers simultaneously maintained control lines, founded from the same common ancestor but propagated from large numbers of individuals each generation. Any new mutants that appeared in these lines were in competition with nonmutant worms. Mutants with poor survival were less

likely to be represented in future generations. As shown by the black dots and best-fit line, this continuous natural selection maintained the genetic quality of the control lines.

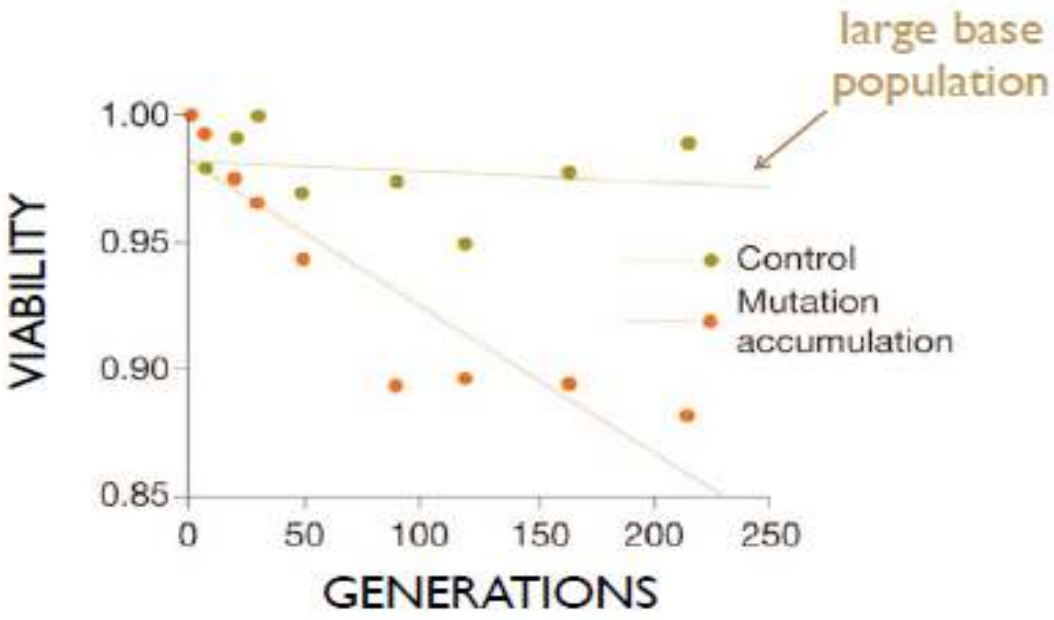
The scientists sequenced 10kb of DNA from mitochondria before and after mutation accumulation.

They found 13 transitions, 3 transversions, 10 multibase insertions and deletions.

Extrapolated to the 97Mb of the worm genome, this means 15 new mutations per genome per generation. In spite of this, the rate of mutation is very low, one per ten million bases.

This leads to the additional interesting observation that since lethal and deleterious mutations outnumber beneficial mutations, a population not experiencing natural selection will show declining average fitness over time.

An extension of the same experiment showed beneficial mutations do occur and natural selection quickly acts on it. For details, see (Freeman and Herron, page 187-188).



Mutation accumulation experiments using micro-organisms confirm the previous results.

Can Higher Mutation Rates be adaptive?

DNA polymerase varies heritably in its copy error rate. Therefore, mutation rates can be heritable.

Bacteria with higher mutation rates (3-100 times higher than wild type) are called Mutators.

Two experiments show the benefits and costs of higher mutation rates.

1. Giraud et al 2001 showed that higher mutation rates can have an advantage under strong slection for adaptation to a new environment.

Asexual populations should wait for new mutations to adapt to a new environment.

They inoculated axenic mice with mutator and non-mutator bacteria.

Mutator bacteria initially increased in frequency and showed strong competitive advantage over non-mutator bacteria.
Mutator genes “Hitch Hike” with the beneficial mutations that they produce.

In the long term, such competitive advantage disappeared.

Mutator bacteria, in the long term suffered costs- reduced transmission ability, reduced ability to colonise other mice gut, reduced fitness in other environments such as soil etc.

2. de Visser et al 1999 also showed that higher mutation rates can have an advantage under strong slection for adaptation to a new environment.
Asexual populations should wait for new mutations to adapt to a new environment.

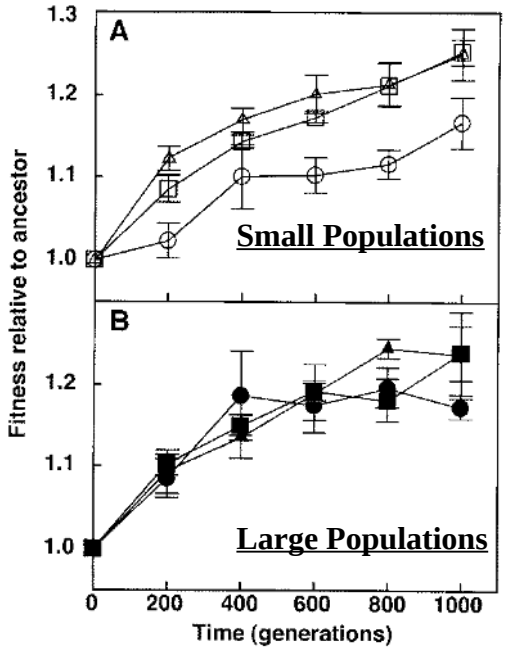
They inoculated mutator and non-mutator bacteria into minimal medium in large or small population sizes.

In small populations, mutator bacteria initially increased in frequency and showed strong competitive advantage over non-mutator bacteria.

Mutator genes “Hitch Hike” with the beneficial mutations that they produce.

In the large populations, such competitive advantage was not seen.

This shows a link between population size and advantage to mutating.



Circles are wild type (Non-mutator) while squares and triangles are mutator type bacteria.

Costs of being a mutator:

The mutators suffer a fitness disadvantage when selection is weaker (large population).

Once populations are adapted, lots of bad copies produced by mutators imposes a cost.

Origin of new Genes

Most mutations affect a single gene and form a new allele of an existing gene.

New genes are formed through various processes of which gene duplication is a very important process.

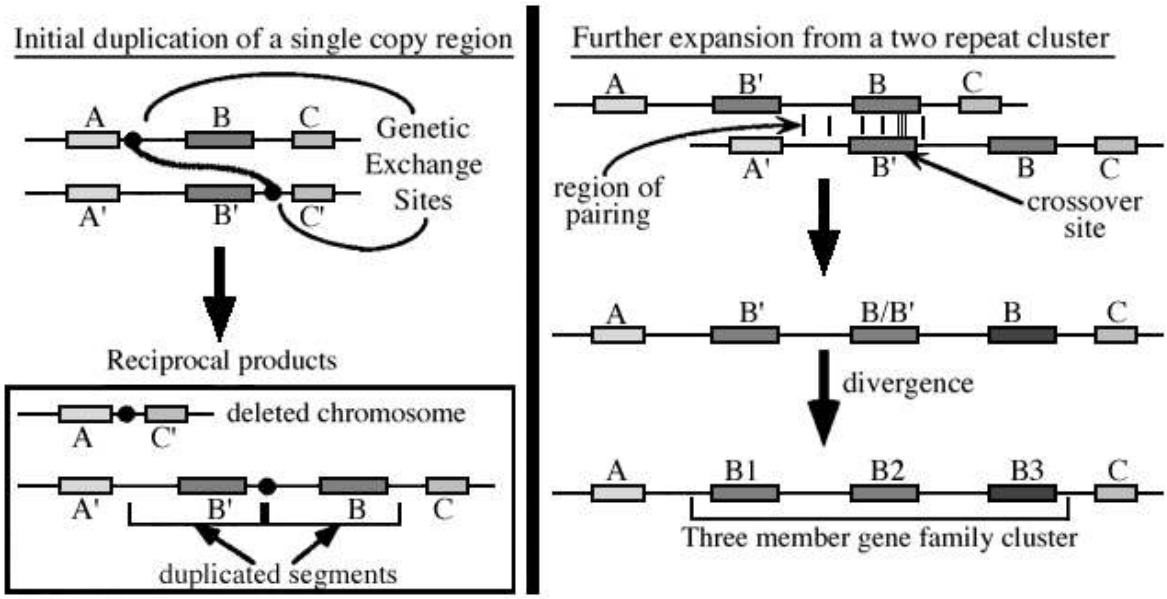
New gene origination is a driving force of evolutionary innovation in all organisms. Recent research has focused on identifying the mechanisms that generate new genes, and scientists have found that these mechanisms involve a variety of molecular events, all of which must occur in the germ line to be inherited by the next generation. After the germ-line mutational event, the new gene (e.g., a new gene duplicate located on human chromosome 2) will be polymorphic in the population; in other words, not all second chromosomes in the population will carry the duplication. Subsequently, the two most likely outcomes for the new gene are fixation (i.e., the new gene will reach a frequency of 100%) or extinction (i.e., the new gene will be lost).

Gene Duplication:

Gene duplication was the first mechanism of gene generation to be suggested (Ohno, 1970), and this process does indeed appear to be the most common way of creating new genes. Duplications are typically classified according to the size of the portion of the genome that is duplicated; thus, a duplication may be described as involving an entire genome, large segments of a genome, individual genes, individual exons, or even specific parts of exons (Betrán & Long, 2002).

The mechanisms that generate duplicate genes are diverse, and more details about these mechanisms are continually being discovered. These mechanisms include(a) whole genome duplications originating through nondisjunction, (b) tandem duplications originating through unequal crossover, (c) retropositions originating through the retrotranscription of an RNA intermediate, (d) transpositions involving transposable elements (Jiang *et al*., 2004; Morgante *et al*., 2005), and (e) duplications occurring after rearrangements and subsequent repair of staggered breaks (Ranz *et al*., 2007).

Duplications originating through unequal crossover:



Fate of a new duplicated gene:

1. The second copy may be conserved and the gene product may be amplified ie both copies produce the same product and hence enhance the quantity of teh gene product. Example: heat Shock Proteins (hsps).

2. The Second copy may accumulate mutations (sheltered from selection) and produce a different product and may be recruited to do new functions (Neo functionalisation). These closely related genes then form gene families. Threy can be identified as belonging the same family because of sequence and structural similarity. Example: Histones, globins etc.

3. The second copy may accumulate disabling mutations and may form no functional gene product. In this case, it would be called a Pseudogene.

Other ways in which new genes are formed:

Transposable Element protein Domestication:

Transposable elements (TEs) are so-called "selfish" segments of DNA that encode proteins that allow these segments to copy or move themselves within a genome. There are two types of TEs: DNA transposons and retrotransposons. DNA transposons are able to excise themselves out of the genome and be inserted somewhere else, whereas retrotransposons copy themselves through an RNA intermediate. Similar to viral insertions in the genome, TE insertions cause mutations and contribute to increased genome size, but they usually do not encode cellular proteins.

Interestingly, one way for a genome to acquire new genes is by recruiting transposable element proteins and using them as cellular proteins. Such events are called domestications of TE proteins.

Lateral (or Horizontal) Gene Transfer:

Scientists use the term "lateral gene transfer" to refer to the case in which a gene does not have a vertical origin (i.e., direct inheritance from parent to offspring) but instead comes from an unrelated genome. It is well known that this sort of transfer occurs between bacteria, and that it also has taken place between the genomes of the cellular organelles (mitochondria and chloroplasts) and the nuclear genomes (Roger, 1999). However, more recent transfer events between organelles and/or endosymbiont bacteria continue to occur (Bergthorsson *et al*., 2003; Hotopp *et al*., 2007). For example, large-scale sequencing efforts have revealed that much of the genome of the intracellular endosymbiont *Wolbachia pipentis* was integrated into *Drosophila* species (Hotopp *et al*., 2007). However, the mechanism for these transfers remains largely unknown, and the functional consequences of some of these transfers have yet to be explored.

Gene Fusion and Fission:

Existing genes can also fuse (i.e., two or more genes can become part of the same transcript) or undergo fission (i.e., a single transcript can break into two or more separate transcripts), thereby forming new genes. Interestingly, it has been observed that chimeric fusion genes sometimes involve two copies of the same gene (e.g., the alcohol dehydrogenase gene in *Drosophila*), and when that happens, the resulting genes undergo parallel evolution(Jones & Begun, 2005), in which they shift away from the functions of their parental genes.

***De Novo* Formation of Genes:**

New genes can additionally originate *de novo* from noncoding regions of DNA. Indeed, several novel genes derived from noncoding DNA have recently been described in *Drosophila* (Begun *et al*., 2007; Levine *et al*., 2006). For these recently originated *Drosophila* genes with likely protein-coding abilities, there are no homologues in any other species.

https://www.nature.com/scitable/topicpage/origins-of-new-genes-and-pseudogenes-835/#

Are there costs to new duplicated genes?

(**https://doi.org/10.1046/j.1365-2435.2000.00429.x**)

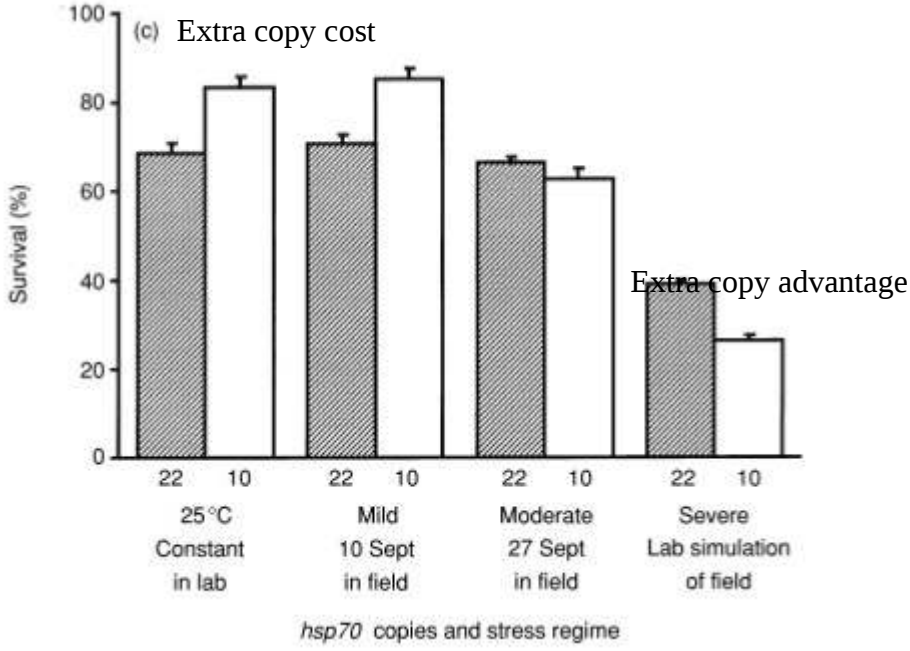
S. P. Roberts and M. E. Feder (2001) transgenically created two lines of *Drosophila melanogaster*- (a) one line containing the normal 10 copies of hsp 70 gene and (b) the other containing 22 copies (extra 12 copies) of hsp 70 gene.

The organisms were exposed to various temperature stress and their survival was measured.

Extra copies improved survival but only under extreme thermal stress.

Extra copies were disadvantageous under low stress conditions indicating that unnecessary production of hsp 70 is costly.

Evolution will strike a balance reflecting this trade-off in copy number.



Dark bars are for lines with 22 copies of hsp 70 and white bars are for lines with 10 copies of hsp 70.

A good example of gene duplication is Snake Venom genes

(http://statedclearly.com/videos/gene-duplications/)

Genetics

Science of heredity and Variation.

“Progeny resemble parents, but not completely”

Cause of resemblance between progeny and parents:

1. Shared Environments

2. Shared Biological Material
   Materials transferred to the progeny at the time of conception.
   Chromosomes, cytoplasmic material (mitochondria, plastids, rna, infectious agents etc), epigenetic markers.

Similarity between parents and offspring is NOT ONLY because of shared NUCLEAR GENES.

Pre-Mendelian Ideas About Heredity

1. Pangenesis
2. Preformationist Ideas- Spermists and Ovists
3. Epigenesis
4. Blending Heredity

Important features of Mendel’s Experiments:

- Variable traits
- Pure Breeds:

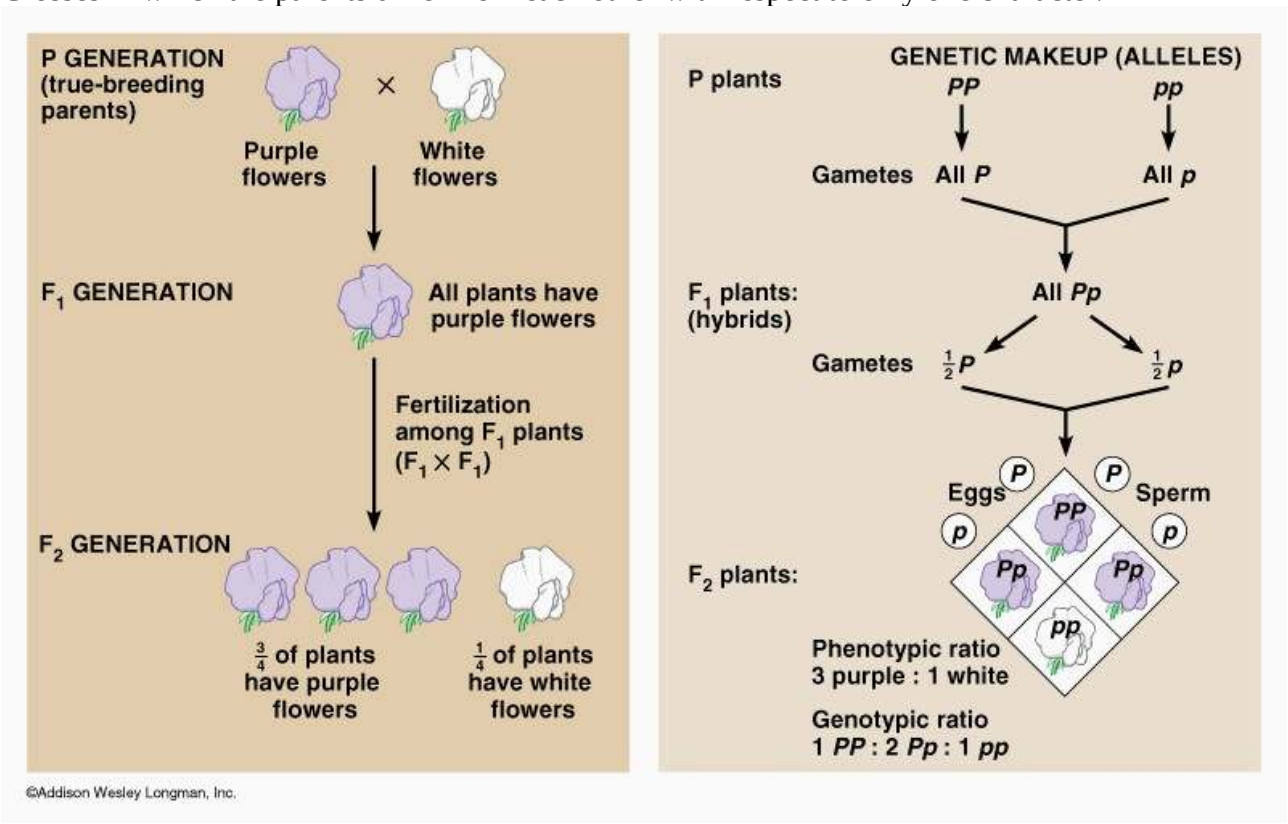
A **pure line** is a population that breeds true for (shows no variation in) the particular character being studied; that is, all offspring produced by selfing or crossing within the population are identical for this character.

- Quantitative analysis
- Peas allowed cross and self fertilization.

Cross-pollination is the transfer of pollen from the anther of one flower to the stigma of another flower on a different individual of the same species.

Self-pollination occurs when the pollen from the anther is deposited on the stigma of the same flower, or another flower on the same plant.

Monohybrid Crosses
Crosses in which the parents differ from each other with respect to only one character.



Mendel got a 3:1 ratio of purple to white…. (705:224)

Other monohybrid crosses yielded the following F2 phenotypic ratios:
Round (5474): Wrinkled (1850)
Yellow (6022): Green (2001)
Long (787): Short (277)

Mendel proposed 4 ‘postulates’ (hypotheses) to explain his data:
1) hereditary material is “particulate”
2) each organism has 2 particles governing each trait
3) if the particles differ, only one (‘dominant’) is expressed as the trait; the other is not expressed (‘recessive’).
4) during gamete formation, the two particles governing a trait SEPARATE and go into DIFFERENT gametes. Subsequent fertilization is RANDOM (these gametes are equally likely to meet with either gamete type of the other parent…and *vice-versa*). This is Mendel’s ***Principle of Segregation***

Test Cross:
Cross between (a) an individual with dominant phenotype but un-known genotype and (b) a homozygous recessive individual.

Mendel’s ideas rested on the hypothesis that the F1 plants were hiding a gene for ‘white’

Hypothesized Genotype = Aa
Based on the hypothesis of segregation, the F1 plant should produce two types of gametes at equal frequency.
F1 plant is mated with a homozygous recessive individual which can only give recessive alleles to offspring.
Therefore, the phenotypic ratio of the progeny of this cross will be the same as the proportion of the different types gametes produced by the F1 plant.

Mendel found a 1:1 phenotypic distribution of purple and white flowred plants among the progeny of this test cross. Thus, he proved that the F1 of the monohybrid cross was a heterozygote that produced two types of gametes in equal proportions.