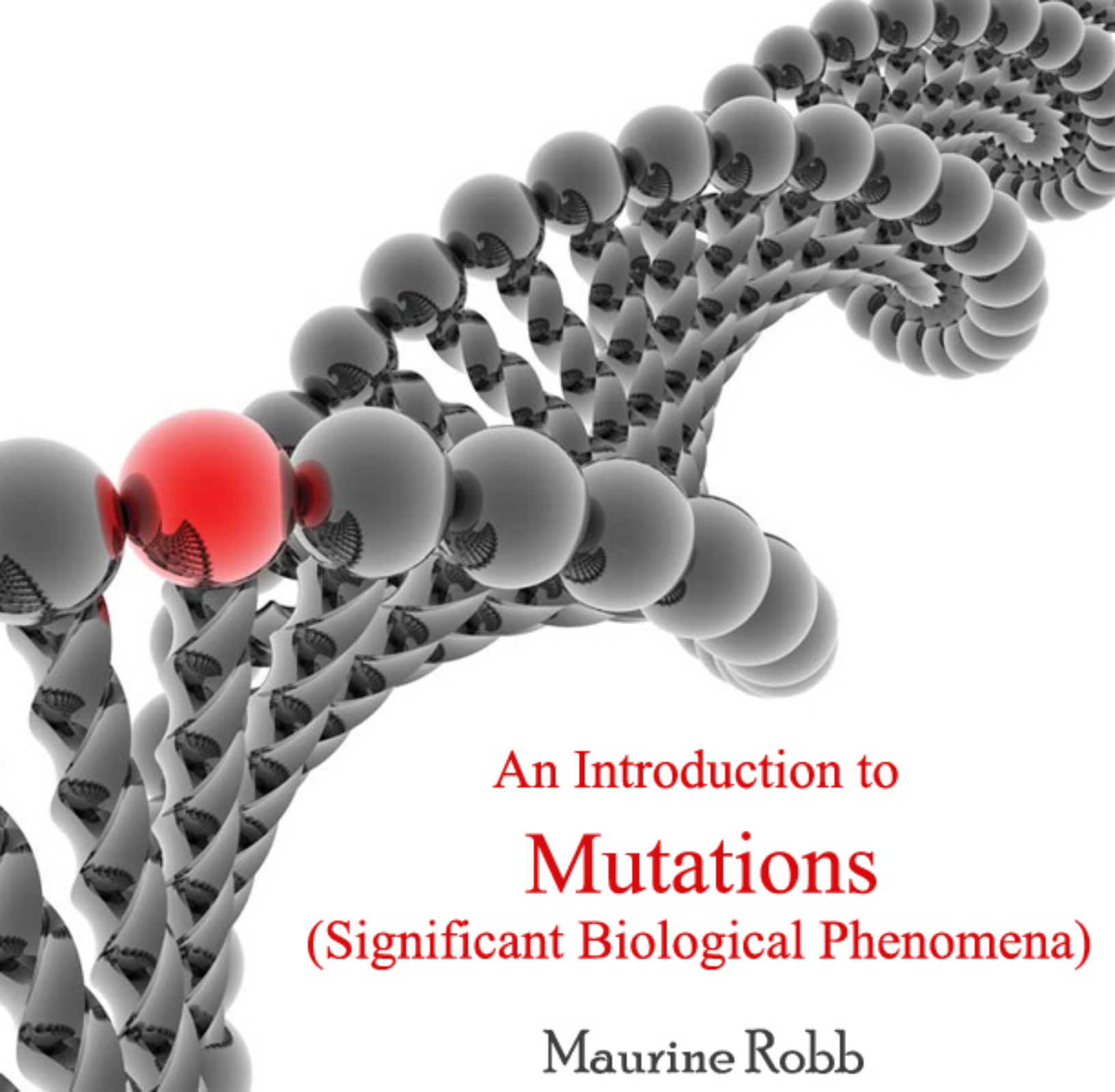

An Introduction to

Mutations

(Significant Biological Phenomena)

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Introduction to Mutations

In molecular biology and genetics, **mutations** are changes in a genomic sequence: the DNA sequence of a cell's genome or the DNA or RNA sequence of a virus. Mutations are caused by radiation, viruses, transposons and mutagenic chemicals, as well as errors that occur during meiosis or DNA replication. They can also be induced by the organism itself, by cellular processes such as hypermutation.

Mutation can result in several different types of change in DNA sequences; these can either have no effect, alter the product of a gene, or prevent the gene from functioning properly or completely. Studies in the fly *Drosophila melanogaster* suggest that if a mutation changes a protein produced by a gene, this will probably be harmful, with about 70 percent of these mutations having damaging effects, and the remainder being either neutral or weakly beneficial. Due to the damaging effects that mutations can have on genes, organisms have evolved mechanisms such as DNA repair to remove mutations.

Therefore, the optimal mutation rate for a species is a trade-off between costs of a high mutation rate, such as deleterious mutations, and the metabolic costs of maintaining systems to reduce the mutation rate, such as DNA repair enzymes. Viruses that use RNA as their genetic material have rapid mutation rates, which can be an advantage since these viruses will evolve constantly and rapidly, and thus evade the defensive responses of e.g. the human immune system.

Description

Mutations can involve large sections of DNA becoming duplicated, usually through genetic recombination. These duplications are a major source of raw material for evolving new genes, with tens to hundreds of genes duplicated in animal genomes every million years. Most genes belong to larger families of genes of shared ancestry. Novel genes are produced by several methods, commonly through the duplication and mutation of an ancestral gene, or by recombining parts of different genes to form new combinations with new functions.

Here, domains act as modules, each with a particular and independent function, that can be mixed together to produce genes encoding new proteins with novel properties. For example, the human eye uses four genes to make structures that sense light: three for color vision and one for night vision; all four arose from a single ancestral gene. Another advantage of duplicating a gene (or even an entire genome) is that this increases redundancy; this allows one gene in the pair to acquire a new function while the other copy performs the original function. Other types of mutation occasionally create new genes from previously noncoding DNA.

Changes in chromosome number may involve even larger mutations, where segments of the DNA within chromosomes break and then rearrange. For example, two chromosomes in the *Homo* genus fused to produce human chromosome 2; this fusion did not occur in the lineage of the other apes, and they retain these separate chromosomes. In evolution, the most important role of such chromosomal rearrangements may be to accelerate the divergence of a population into new species by making populations less likely to interbreed, and thereby preserving genetic differences between these populations.

Sequences of DNA that can move about the genome, such as transposons, make up a major fraction of the genetic material of plants and animals, and may have been important in the evolution of genomes. For example, more than a million copies of the Alu sequence are present in the human genome, and these sequences have now been recruited to perform functions such as regulating gene expression. Another effect of these mobile DNA sequences is that when they move within a genome, they can mutate or delete existing genes and thereby produce genetic diversity.

A mutation has caused this garden moss rose to produce flowers of different colors. This is a somatic mutation that may also be passed on in the germ line.

In multicellular organisms with dedicated reproductive cells, mutations can be subdivided into germ line mutations, which can be passed on to descendants through their reproductive cells, and somatic mutations (also called acquired mutations), which involve cells outside the dedicated reproductive group and which are not usually transmitted to descendants. If the organism can reproduce asexually through mechanisms such as cuttings or budding the distinction can become blurred.

For example, plants can sometimes transmit somatic mutations to their descendants asexually or sexually where flower buds develop in somatically mutated parts of plants. A new mutation that was not inherited from either parent is called a *de novo* mutation.

The source of the mutation is unrelated to the consequence , although the consequences are related to which cells were mutated.

Nonlethal mutations accumulate within the gene pool and increase the amount of genetic variation. The abundance of some genetic changes within the gene pool can be reduced by natural selection, while other "more favorable" mutations may accumulate and result in adaptive evolutionary changes.

For example, a butterfly may produce offspring with new mutations. The majority of these mutations will have no effect; but one might change the color of one of the butterfly's offspring, making it harder (or easier) for predators to see. If this color change is advantageous, the chance of this butterfly surviving and producing its own offspring are a little better, and over time the number of butterflies with this mutation may form a larger percentage of the population.

Neutral mutations are defined as mutations whose effects do not influence the fitness of an individual. These can accumulate over time due to genetic drift. It is believed that the overwhelming majority of mutations have no significant effect on an organism's fitness. Also, DNA repair mechanisms are able to mend most changes before they become permanent mutations, and many organisms have mechanisms for eliminating otherwise permanently mutated somatic cells.

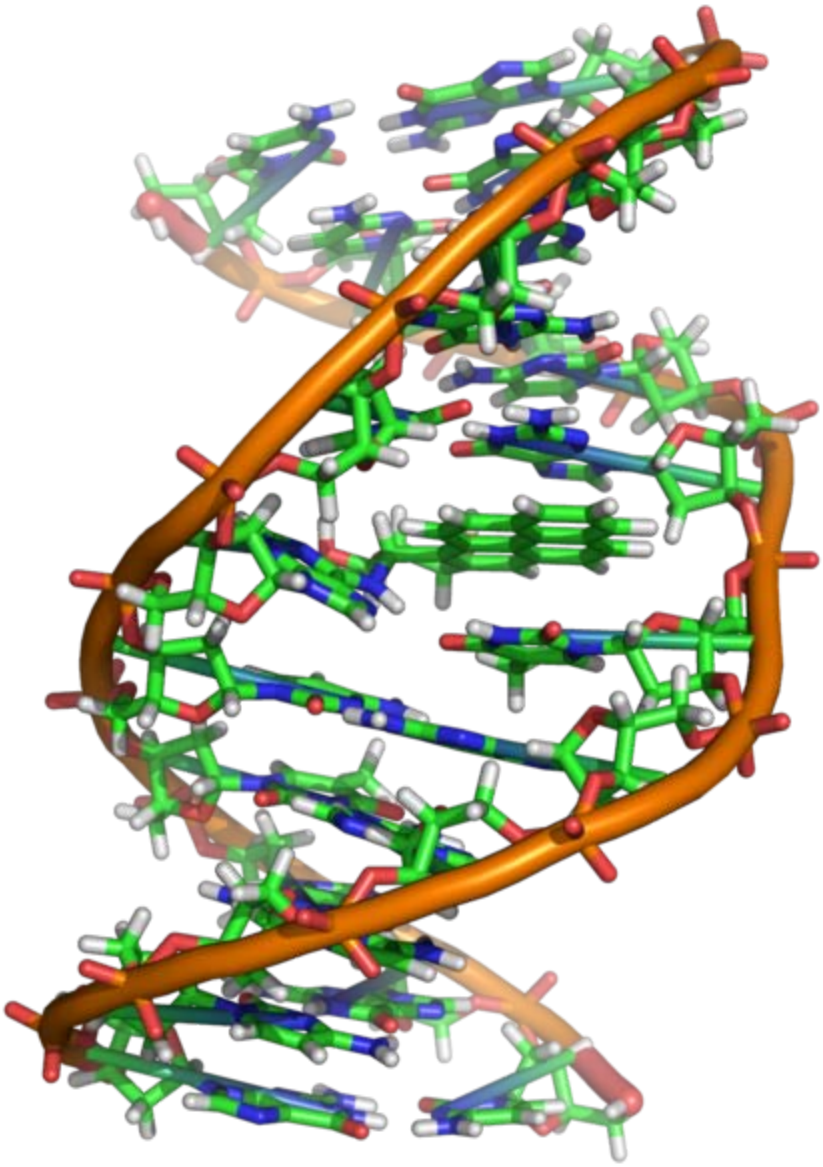
Mutation is generally accepted by biologists as the mechanism by which natural selection acts, generating advantageous new traits that survive and multiply in offspring as well as disadvantageous traits, in less fit offspring, that tend to die out.

Causes

Two classes of mutations are spontaneous mutations (molecular decay) and induced mutations caused by mutagens.

Spontaneous mutations on the molecular level can be caused by:

- Tautomerism – A base is changed by the repositioning of a hydrogen atom, altering the hydrogen bonding pattern of that base resulting in incorrect base pairing during replication.
- Depurination – Loss of a purine base (A or G) to form an apurinic site (AP site).
- Deamination – Hydrolysis changes a normal base to an atypical base containing a keto group in place of the original amine group. Examples include C → U and A → HX (hypoxanthine), which can be corrected by DNA repair mechanisms; and 5MeC (5-methylcytosine) → T, which is less likely to be detected as a mutation because thymine is a normal DNA base.
- Slipped strand mispairing - Denaturation of the new strand from the template during replication, followed by renaturation in a different spot ("slipping"). This can lead to insertions or deletions.



A covalent adduct between benzo[*a*]pyrene, the major mutagen in tobacco smoke, and DNA

Induced mutations on the molecular level can be caused by:

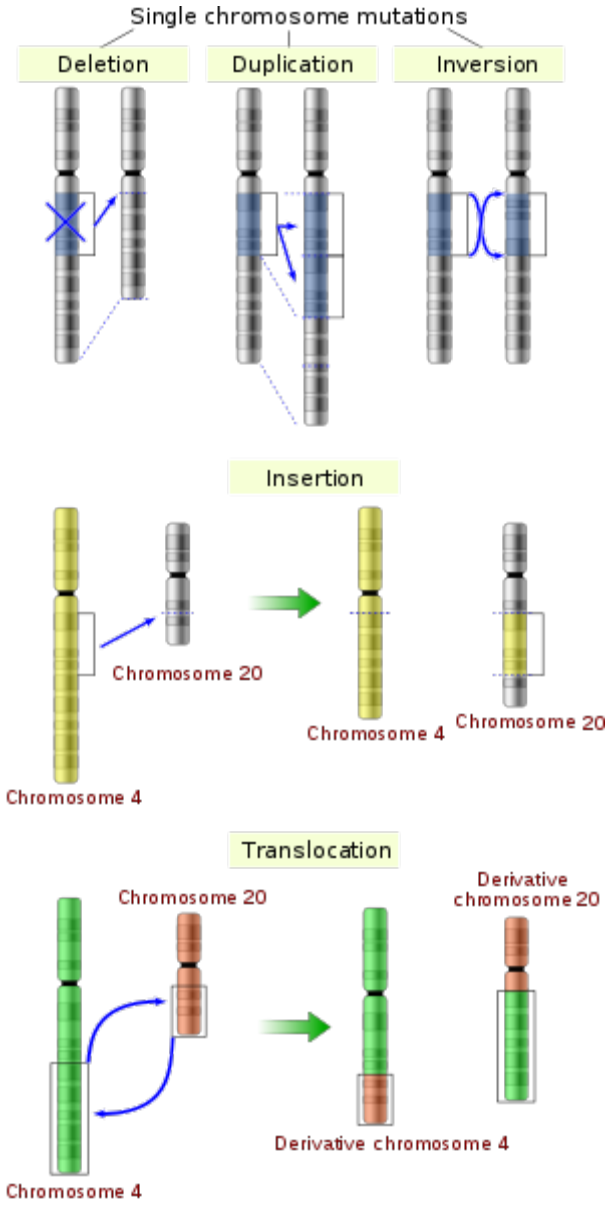
- Chemicals
  - Hydroxylamine NH_2OH
  - Base analogs (e.g. BrdU)
  - Alkylating agents (e.g. *N*-ethyl-*N*-nitrosourea) These agents can mutate both replicating and non-replicating DNA. In contrast, a base analog can only mutate the DNA when the analog is incorporated in replicating the DNA. Each of these classes of chemical mutagens has certain effects that then lead to transitions, transversions, or deletions.

    - Agents that form DNA adducts (e.g. ochratoxin A metabolites)
    - DNA intercalating agents (e.g. ethidium bromide)
    - DNA crosslinkers
    - Oxidative damage
    - Nitrous acid converts amine groups on A and C to diazo groups, altering their hydrogen bonding patterns which leads to incorrect base pairing during replication.
- Radiation
    - Ultraviolet radiation (nonionizing radiation). Two nucleotide bases in DNA – cytosine and thymine – are most vulnerable to radiation that can change their properties. UV light can induce adjacent pyrimidine bases in a DNA strand to become covalently joined as a pyrimidine dimer. UV radiation, particularly longer-wave UVA, can also cause oxidative damage to DNA.
    - Ionizing radiation
- Viral infections

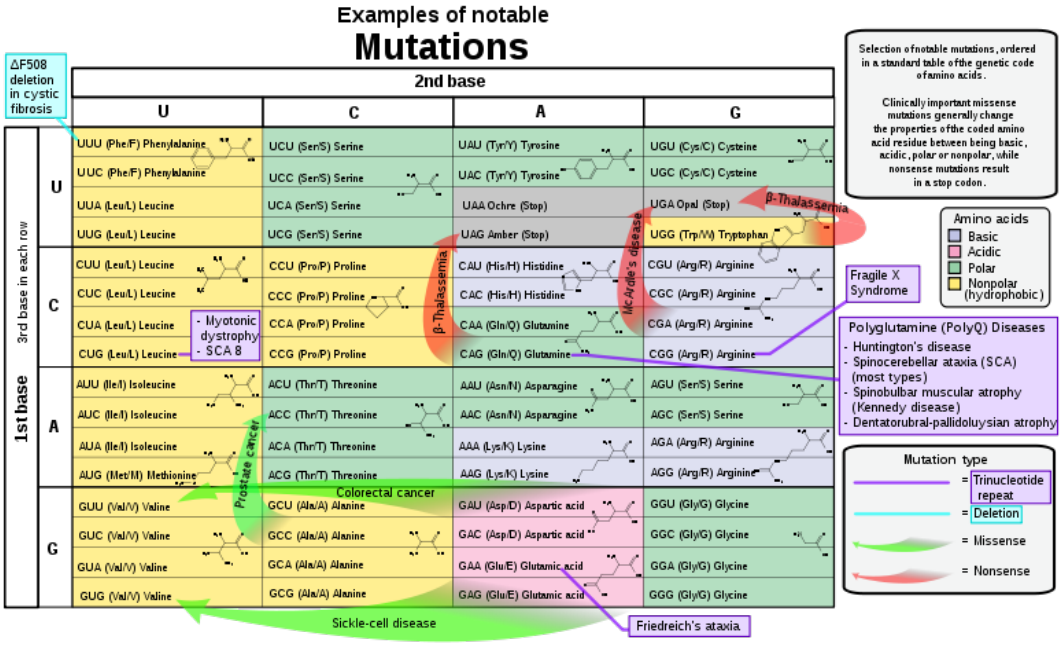
DNA has so-called hotspots, where mutations occur up to 100 times more frequently than the normal mutation rate. A hotspot can be at an unusual base, e.g., 5-methylcytosine.

Mutation rates also vary across species. Evolutionary biologists have theorized that higher mutation rates are beneficial in some situations, because they allow organisms to evolve and therefore adapt more quickly to their environments. For example, repeated exposure of bacteria to antibiotics, and selection of resistant mutants, can result in the selection of bacteria that have a much higher mutation rate than the original population (mutator strains).

Classification of mutation types



Illustrations of five types of chromosomal mutations.



Selection of disease-causing mutations, in a standard table of the genetic code of amino acids.

By effect on structure

The sequence of a gene can be altered in a number of ways. Gene mutations have varying effects on health depending on where they occur and whether they alter the function of essential proteins. Mutations in the structure of genes can be classified as:

- Small-scale mutations, such as those affecting a small gene in one or a few nucleotides, including:
  - **Point mutations**, often caused by chemicals or malfunction of DNA replication, exchange a single nucleotide for another. These changes are classified as transitions or transversions. Most common is the transition that exchanges a purine for a purine (A ↔ G) or a pyrimidine for a pyrimidine, (C ↔ T). A transition can be caused by nitrous acid, base mis-pairing, or mutagenic base analogs such as 5-bromo-2-deoxyuridine (BrdU). Less common is a transversion, which exchanges a purine for a pyrimidine or a pyrimidine for a purine (C/T ↔ A/G). An example of a transversion is adenine (A) being converted into a cytosine (C). A point mutation can be reversed by another point mutation, in which the nucleotide is changed back to its original state (true reversion) or by second-site reversion (a complementary mutation elsewhere that results in regained gene functionality). Point mutations that occur within the protein coding region of a gene may be classified into three kinds, depending upon what the erroneous codon codes for:
    - Silent mutations: which code for the same amino acid.
    - Missense mutations: which code for a different amino acid.

        - Nonsense mutations: which code for a stop and can truncate the protein.
    - **Insertions** add one or more extra nucleotides into the DNA. They are usually caused by transposable elements, or errors during replication of repeating elements (e.g. AT repeats). Insertions in the coding region of a gene may alter splicing of the mRNA (splice site mutation), or cause a shift in the reading frame (frameshift), both of which can significantly alter the gene product. Insertions can be reverted by excision of the transposable element.
    - **Deletions** remove one or more nucleotides from the DNA. Like insertions, these mutations can alter the reading frame of the gene. They are generally irreversible: though exactly the same sequence might theoretically be restored by an insertion, transposable elements able to revert a very short deletion (say 1–2 bases) in *any* location are either highly unlikely to exist or do not exist at all. Note that a deletion is not the exact opposite of an insertion: the former is quite random while the latter consists of a specific sequence inserting at locations that are not entirely random or even quite narrowly defined.
- Large-scale mutations in chromosomal structure, including:
    - **Amplifications** (or gene duplications) leading to multiple copies of all chromosomal regions, increasing the dosage of the genes located within them.
    - **Deletions** of large chromosomal regions, leading to loss of the genes within those regions.
    - Mutations whose effect is to juxtapose previously separate pieces of DNA, potentially bringing together separate genes to form functionally distinct fusion genes (e.g. bcr-abl). These include:
        - **Chromosomal translocations**: interchange of genetic parts from nonhomologous chromosomes.
        - **Interstitial deletions**: an intra-chromosomal deletion that removes a segment of DNA from a single chromosome, thereby apposing previously distant genes. For example, cells isolated from a human astrocytoma, a type of brain tumor, were found to have a chromosomal deletion removing sequences between the "fused in glioblastoma" (fig) gene and the receptor tyrosine kinase "ros", producing a fusion protein (FIG-ROS). The abnormal FIG-ROS fusion protein has constitutively active kinase activity that causes oncogenic transformation (a transformation from normal cells to cancer cells).
        - **Chromosomal inversions**: reversing the orientation of a chromosomal segment.
    - **Loss of heterozygosity**: loss of one allele, either by a deletion or recombination event, in an organism that previously had two different alleles.

By effect on function

- **Loss-of-function mutations** are the result of gene product having less or no function. When the allele has a complete loss of function (null allele) it is often called an **amorphic mutation**. Phenotypes associated with such mutations are most often recessive. Exceptions are when the organism is haploid, or when the reduced dosage of a normal gene product is not enough for a normal phenotype (this is called haploinsufficiency).
- **Gain-of-function mutations** change the gene product such that it gains a new and abnormal function. These mutations usually have dominant phenotypes. Often called a neomorphic mutation.
- **Dominant negative mutations** (also called **antimorphic mutations**) have an altered gene product that acts antagonistically to the wild-type allele. These mutations usually result in an altered molecular function (often inactive) and are characterised by a dominant or semi-dominant phenotype. In humans, Marfan syndrome is an example of a dominant negative mutation occurring in an autosomal dominant disease. In this condition, the defective glycoprotein product of the fibrillin gene (FBN1) antagonizes the product of the normal allele.
- **Lethal mutations** are mutations that lead to the death of the organisms which carry the mutations.
- A **back mutation** or **reversion** is a point mutation that restores the original sequence and hence the original phenotype.

By effect on fitness

In applied genetics it is usual to speak of mutations as either harmful or beneficial.

- A **harmful mutation** is a mutation that decreases the fitness of the organism.
- A **beneficial mutation** is a mutation that increases fitness of the organism, or which promotes traits that are desirable.

In theoretical population genetics, it is more usual to speak of such mutations as deleterious or advantageous. In the neutral theory of molecular evolution, genetic drift is the basis for most variation at the molecular level.

- A **neutral mutation** has no harmful or beneficial effect on the organism. Such mutations occur at a steady rate, forming the basis for the molecular clock.
- A **deleterious mutation** has a negative effect on the phenotype, and thus decreases the fitness of the organism.
- An **advantageous mutation** has a positive effect on the phenotype, and thus increases the fitness of the organism.
- A **nearly neutral mutation** is a mutation that may be slightly deleterious or advantageous, although most nearly neutral mutations are slightly deleterious.

In reality, viewing the fitness effects of mutations in these discrete categories is an oversimplification. Attempts have been made to infer the distribution of fitness effects using mutagenesis experiments or theoretical models applied to molecular sequence data.

However, the current distribution is still uncertain, and some aspects of the distribution likely vary between species.

By inheritance

- inheritable generic in pro-generic tissue or cells on path to be changed to gametes.
- non inheritable **somatic** (e.g., carcinogenic mutation)
- non inheritable post mortem aDNA mutation in decaying remains.

By pattern of inheritance The human genome contains two copies of each gene – a paternal and a maternal allele.

- A **heterozygous mutation** is a mutation of only one allele.
- A **homozygous mutation** is an identical mutation of both the paternal and maternal alleles.
- **Compound heterozygous** mutations or a **genetic compound** comprises two different mutations in the paternal and maternal alleles.
- A **wildtype** or **homozygous non-mutated** organism is one in which neither allele is mutated. (Just not a mutation)

By impact on protein sequence

- A **frameshift mutation** is a mutation caused by insertion or deletion of a number of nucleotides that is not evenly divisible by three from a DNA sequence. Due to the triplet nature of gene expression by codons, the insertion or deletion can disrupt the reading frame, or the grouping of the codons, resulting in a completely different translation from the original. The earlier in the sequence the deletion or insertion occurs, the more altered the protein produced is.

- A **nonsense mutation** is a point mutation in a sequence of DNA that results in a premature stop codon, or a *nonsense codon* in the transcribed mRNA, and possibly a truncated, and often nonfunctional protein product.

- **Missense mutations** or *nonsynonymous mutations* are types of point mutations where a single nucleotide is changed to cause substitution of a different amino acid. This in turn can render the resulting protein nonfunctional. Such mutations are responsible for diseases such as Epidermolysis bullosa, sickle-cell disease, and SOD1 mediated ALS (Boillée 2006, p. 39).

- A **neutral mutation** is a mutation that occurs in an amino acid codon which results in the use of a different, but chemically similar, amino acid. The similarity between the two is enough that little or no change is often rendered in the protein. For example, a change from AAA to AGA will encode arginine, a chemically similar molecule to the intended lysine.

- **Silent mutations** are mutations that do not result in a change to the amino acid sequence of a protein. They may occur in a region that does not code for a protein, or they may occur within a codon in a manner that does not alter the final amino acid sequence. The phrase *silent mutation* is often used interchangeably with the phrase *synonymous mutation*; however, synonymous mutations are a subcategory of the former, occurring only within exons. The name silent could be a misnomer. For example, a silent mutation in the exon/intron border may lead to alternative splicing by changing the splice site, thereby leading to a changed protein.

Special classes

- **Conditional mutation** is a mutation that has wild-type (or less severe) phenotype under certain "permissive" environmental conditions and a mutant phenotype under certain "restrictive" conditions. For example, a temperature-sensitive mutation can cause cell death at high temperature (restrictive condition), but might have no deleterious consequences at a lower temperature (permissive condition).

Nomenclature

Nomenclature of mutations specify the type of mutation and base or amino acid changes.

- Nucleotide substitution (e.g. 76A>T) - The number is the position of the nucleotide from the 5' end, the first letter represents the wild type nucleotide, and the second letter represents the nucleotide which replaced the wild type. In the given example, the adenine at the 76th position was replaced by a thymine.
    - If it becomes necessary to differentiate between mutations in genomic DNA, mitochondrial DNA, and RNA, a simple convention is used. For example, if the 100th base of a nucleotide sequence mutated from G to C, then it would be written as g.100G>C if the mutation occurred in genomic DNA, m.100G>C if the mutation occurred in mitochondrial DNA, or r.100g>c if the mutation occurred in RNA. Note that for mutations in RNA, the nucleotide code is written in lower case.
- Amino acid substitution (e.g. D111E) – The first letter is the one letter code of the wild type amino acid, the number is the position of the amino acid from the N terminus, and the second letter is the one letter code of the amino acid present in the mutation. Nonsense mutations are represented with an X for the second amino acid (e.g. D111X).
- Amino acid deletion (e.g. ΔF508) – The Greek letter Δ (delta) indicates a deletion. The letter refers to the amino acid present in the wild type and the number is the position from the N terminus of the amino acid were it to be present as in the wild type.

Harmful mutations

Changes in DNA caused by mutation can cause errors in protein sequence, creating partially or completely non-functional proteins. To function correctly, each cell depends on thousands of proteins to function in the right places at the right times. When a mutation alters a protein that plays a critical role in the body, a medical condition can result. A condition caused by mutations in one or more genes is called a genetic disorder. Some mutations alter a gene's DNA base sequence but do not change the function of the protein made by the gene. Studies of the fly *Drosophila melanogaster* suggest that if a mutation does change a protein, this will probably be harmful, with about 70 percent of these mutations having damaging effects, and the remainder being either neutral or weakly beneficial. However, studies in yeast have shown that only 7% of mutations that are not in genes are harmful.

If a mutation is present in a germ cell, it can give rise to offspring that carries the mutation in all of its cells. This is the case in hereditary diseases. On the other hand, a mutation may occur in a somatic cell of an organism. Such mutations will be present in all descendants of this cell within the same organism, and certain mutations can cause the cell to become malignant, and thus cause cancer.

Often, gene mutations that could cause a genetic disorder are repaired by the DNA repair system of the cell. Each cell has a number of pathways through which enzymes recognize and repair mistakes in DNA. Because DNA can be damaged or mutated in many ways, the process of DNA repair is an important way in which the body protects itself from disease.

Beneficial mutations

Although most mutations that change protein sequences are neutral or harmful, some mutations have a positive effect on an organism. In this case, the mutation may enable the mutant organism to withstand particular environmental stresses better than wild-type organisms, or reproduce more quickly. In these cases a mutation will tend to become more common in a population through natural selection.

For example, a specific 32 base pair deletion in human CCR5 (CCR5-Δ32) confers HIV resistance to homozygotes and delays AIDS onset in heterozygotes. The CCR5 mutation is more common in those of European descent. One possible explanation of the etiology of the relatively high frequency of CCR5-Δ32 in the European population is that it conferred resistance to the bubonic plague in mid-14th century Europe. People with this mutation were more likely to survive infection; thus its frequency in the population increased. This theory could explain why this mutation is not found in southern Africa, where the bubonic plague never reached. A newer theory suggests that the selective pressure on the CCR5 Delta 32 mutation was caused by smallpox instead of the bubonic plague.

Another example, is Sickle cell disease which is a blood disorder in which the body produces an abnormal type of the oxygen-carrying substance hemoglobin in the red blood cells. One-third of all indigenous inhabitants of Sub-Saharan Africa carry the gene,

because in areas where malaria is common, there is a survival value in carrying only a single sickle-cell gene (sickle cell trait). Those with only one of the two alleles of the sickle-cell disease are more resistant to malaria, since the infestation of the malaria plasmodium is halted by the sickling of the cells which it infests.

Prion mutation

Prions are proteins and do not contain genetic material. However, prion replication has been shown to be subject to mutation and natural selection just like other forms of replication.

Chapter- 2

Point Mutation

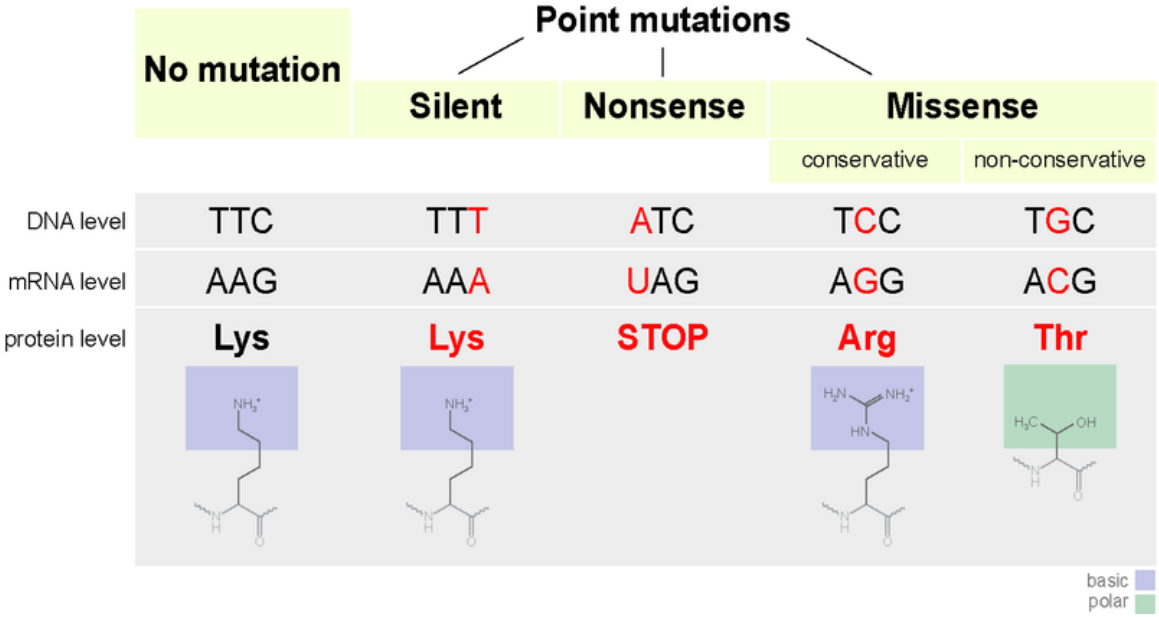


Illustration of three types of point mutations.

A **point mutation**, or **single base substitution**, is a type of mutation that causes the replacement of a single base nucleotide with another nucleotide of the genetic material, DNA or RNA. Often the term point mutation also includes insertions or deletions of a single base pair. One can categorize point mutations as follows:

- **transitions**: replacement of a purine base with another purine or replacement of a pyrimidine with another pyrimidine
- **transversions**: replacement of a purine with a pyrimidine or vice versa.

Transition mutations are about an order of magnitude more common than transversions. Point mutations can also be categorized functionally:

- nonsense mutations: code for a stop, which can translate the protein
- missense mutations: code for a different amino acid
- silent mutations: code for the same amino acid

- conservative mutations: result in an amino acid change; however, the properties of the amino acid remain the same (e.g. hydrophobic, hydrophilic, etc).
- non conservative mutations: result in an amino acid change that has different properties than the wild type.

Brief Description of Point Mutations: -

Nonsense mutation

In genetics, a **nonsense mutation** is a point mutation in a sequence of DNA that results in a premature stop codon, or a *nonsense codon* in the transcribed mRNA, and in a truncated, incomplete, and usually nonfunctional protein product. It differs from a missense mutation, which is a point mutation where a single nucleotide is changed to cause substitution of a different amino acid. Some genetic disorders, such as thalassemia and DMD, result from nonsense mutations.

Simple example

```
    DNA: 5' - ATG ACT CAC CGA GCG CGA AGC TGA - 3'shut
         3' - TAC TGA GTG GCT CGC GCT TCG ACT - 5'
   mRNA: 5' - AUG ACU CAC CGA GCG CGA AGC UGA - 3'
Protein:      Met Thr His Arg Ala Arg Ser Stop
```

Suppose that a nonsense mutation was introduced at the fourth triplet in the DNA sequence (CGA) causing the cytosine to be replaced with thymine, yielding TGA in the DNA sequence. Since TGA is transcribed-then-translated as UGA, the resulting transcript and protein product would be:

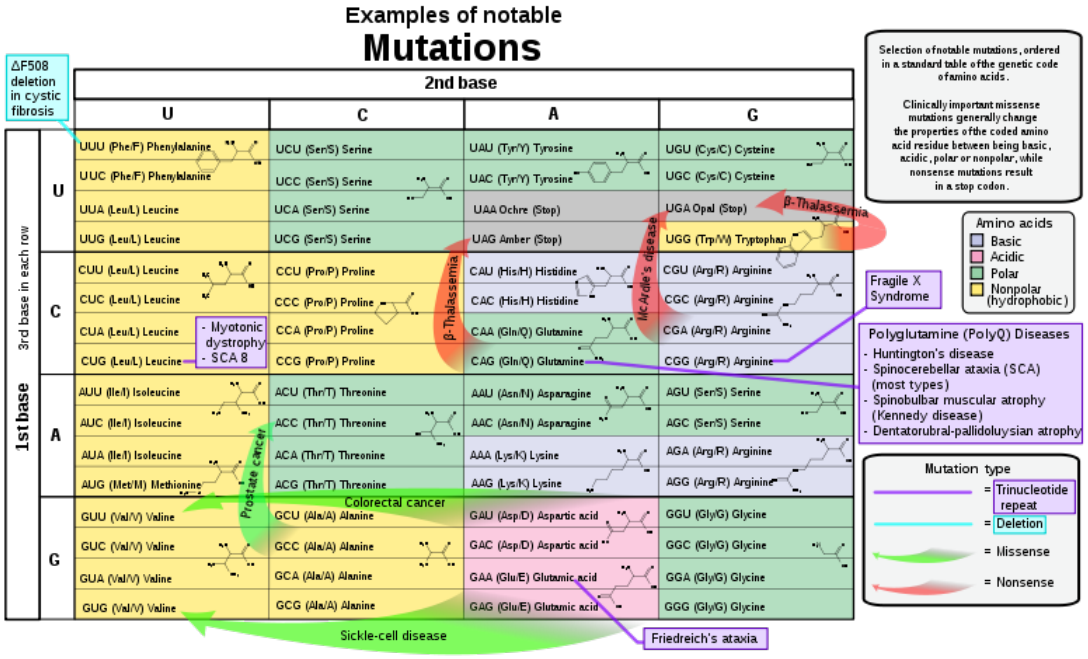
```
    DNA: 5' - ATG ACT CAC TGA GCG CGA AGC TGA - 3'
         3' - TAC TGA GTG ACT CGC GCT TCG ACT - 5'
   mRNA: 5' - AUG ACU CAC UGA GCG CGU AGC UGA - 3'
Protein:      Met Thr His Stop
```

The remaining codons of the mRNA are not translated into amino proteins because the stop codon is prematurely reached during translation. This can yield a truncated abbreviated protein product, which quite often lacks the functionality of the normal, non-mutant p-cell.

Nonsense-mediated mRNA decay

Despite an expected tendency for premature termination codons to yield shortened polypeptide products, in fact the formation of truncated proteins does not occur often *in vivo*. Many organisms—including humans and lower species, such as yeast -- employ a nonsense-mediated mRNA decay pathway, which degrades mRNAs containing nonsense mutations before they are translated into nonfunctional polypeptides.

Pathology associated with nonsense mutations



Selection of notable mutations, ordered in a standard table of the genetic code of amino acids. Nonsense mutations are marked by red arrows.

Nonsense mutations can cause a genetic disease by damaging a gene responsible for a specific protein, for example, dystrophin in Duchenne muscular dystrophy. The same disease may, however, be caused by other kinds of damage to the same gene. Examples of diseases in which nonsense mutations are known to be among the causes include:

- Cystic fibrosis (caused by mutations in the cystic fibrosis transmembrane conductance regulator gene).
- Duchenne muscular dystrophy (dystrophin)
- Beta thalassaemia (β-globin)
- Hurler syndrome

An experimental drug known as PTC124 may be useful in treating some cases of each of the above diseases (that is, the cases caused by a nonsense mutation). PTC124 was scheduled to enter the final phase of clinical trials in 2007.

Missense mutation

In genetics, a **missense mutation** (a type of **nonsynonymous mutation**) is a point mutation in which a single nucleotide is changed, resulting in a codon that codes for a different amino acid (mutations that change an amino acid to a stop codon are considered nonsense mutations, rather than missense mutations). This can render the resulting protein nonfunctional. Such mutations are responsible for diseases such as Epidermolysis bullosa, sickle-cell disease, and SOD1 mediated ALS (Boillée 2006, p. 39).

For example, in the most common variant of sickle-cell disease, the 20th nucleotide of the gene for the beta chain of hemoglobin found on chromosome 11 is erroneously changed from the codon GAG (for glutamic acid) to GUG (which codes valine), so the 6th amino acid is incorrectly substituted (after the initial methionine amino acid is removed).

Not all missense mutations lead to appreciable protein changes. An amino acid may be replaced by an amino acid of very similar chemical properties, in which case, the protein may still function normally; this is termed a neutral, "quiet", or conservative mutation. Alternatively, the amino acid substitution could occur in a region of the protein which does not significantly affect the protein secondary structure or function. When an amino acid may be encoded by more than one codon (so-called "degenerate coding") a mutation in a codon may not produce any change in translation; this would be a synonymous mutation (a form of silent mutation) and not a missense mutation.

Silent mutation

Silent mutations are DNA mutations that do not result in a change to the amino acid sequence of a protein. They may occur in a non-coding region (outside of a gene or within an intron), or they may occur within an exon in a manner that does not alter the final amino acid sequence. The phrase **silent mutation** is often used interchangeably with the phrase synonymous mutation; however, synonymous mutations are a subcategory of the former, occurring only within exons.

Because silent mutations do not alter protein function they are often treated as though they are evolutionarily neutral. However, many organisms are known to exhibit codon usage biases, suggesting that there is selection for the use of particular codons due to translational stability. Silent mutations may also affect splicing, or transcriptional control.

In molecular cloning experiments, it can be useful to introduce silent mutations into a gene of interest in order to create or remove recognition sites for restriction enzymes. (An online tool that can analyse a sequence of interest for possible mutations to create restriction sites is given in the External Links section.)

Recent results suggest that silent mutations can have an effect on subsequent protein structure and activity[1].

Transfer RNA

Transfer RNA(tRNA) availability is one of the reasons that a silent mutation might not be silent at all. For every codon there's a different tRNA molecule. So there's a tRNA specifically for the codon UCU and another specifically for the codon UCC (and so on for all the codons). Both of those tRNA molecules carry the amino-acid serine to the ribosome that is translating a mRNA molecule. However, if there's (for example) a thousand times less UCC tRNA then UCU tRNA, then the incorporation of serine happens a thoudand times slower when a mutation causes the codon to change from UCU to UCC. If it takes longer for the amino-acids to reach the ribosome, translation takes longer. This results in a lower expression of a certain gene with that 'silent' mutation. Also, if the ribosome has to wait too long, it might terminate translation prematurely.

Secondary Messenger RNA structure

Silent mutations change the secondary structure of RNA. Since RNA has a secondary structure that is not necessarily linear like that of DNA, the shape that goes along with the complementary bonding in the structure can have significant effects. For example, if the RNA molecule is not very stable, then it can be broken down quickly by enzymes in the cytoplasm. Alternatively, if the RNA molecule is too stable, and the complementary bonds are too strong for unpacking before translation, then the gene can also be under expressed.

Also, if the oncoming ribosome pauses because of a knot in the RNA, then the polypeptide can have time to fold into an unusual structure before the tRNA molecule has time to add another amino acid.

Examples

Steffen Mueller at the Stony Brook University designed a live virus vaccine in which the pathogen was engineered to have synonymous codons take the place of normally occurring ones in the genome. As a result, the vaccine was still able to infect and reproduce, albeit more slowly. Mice were vaccinated with this vaccine and they showed a resistance against the natural polio strain.

Mental disorders can be caused by silent mutations. One silent mutation causes the dopamine receptor D2 gene to be less stable and degrade faster, under expressing the gene.

Also, deviations from average pain sensitivity (APS) are caused by both an ATG to GTG mutation (nonsynonymous), and a CAT to CAC mutation (synonymous). Ironically, these two mutations are both shared by the Low pain sensitivity (LPS) and High pain sensitivity (HPS)gene. What distinguishes LPS from HPS is that LPS has an additional CTC to CTG silent mutation, while HPS does not and shares the CTC sequence at this location with APS.

LPS APS HPS
CAC CAT **CAC**
CTG CTC CTC
GTG ATG **GTG**

A silent mutation in the multidrug resistance 1 gene, which codes for a cellular membrane pump that expels drugs from the cell, can slow down translation in a specific location to allow the peptide chain to bend into an unusual conformation. Thus, the mutant pump is less functional.

For example, sickle-cell disease is caused by a single point mutation (a missense mutation) in the beta-hemoglobin gene that converts a GAG codon into GUG, which encodes the amino acid valine rather than glutamic acid. This is an example of a non-conservative (missense) mutation.

Point mutations that occur in non-coding sequences are most often without consequences, although there are exceptions. If the mutated base pair is in the promoter sequence of a gene, then the expression of the gene may change. Also, if the mutation occurs in the splicing site of an intron, then this may interfere with correct splicing of the transcribed pre-mRNA.

Sometimes the term *point mutation* is also used to describe insertions or deletions of a single base pair (which has more of an adverse effect on the synthesized protein due to the nucleotides' still being read in triplets, but in different frames: a mutation called a frameshift mutation).

A "point mutant" is an individual which is affected by a point mutation.

Causes

Point mutations may arise from spontaneous mutations that occur during DNA replication. The rate of mutation may be increased by mutagens. Mutagens can be physical, such as radiation from UV rays, X-rays or extreme heat, or chemical (molecules that misplace base pairs or disrupt the helical shape of DNA). Mutagens associated with cancers are often studied to learn about cancer and its prevention.

Chapter- 3

Insertion & Deletion (genetics)

In genetics, an **insertion** (also called an **insertion mutation**) is the addition of one or more nucleotide base pairs into a DNA sequence. This can often happen in microsatellite regions due to the DNA polymerase slipping. Insertions can be anywhere in size from one base pair incorrectly inserted into a DNA sequence to a section of one chromosome inserted into another.

On a chromosome level, an *insertion* refers to the insertion of a larger sequence into a chromosome. This can happen due to unequal crossover during meiosis.

N region addition is the addition of non-coded nucleotides during recombination by terminal deoxynucleotidyl transferase.

P nucleotide insertion is the insertion of palindromic sequences encoded by the ends of the recombining gene segments.

Trinucleotide repeats are sometimes classified as insertion mutations and sometimes as a separate class of mutations.

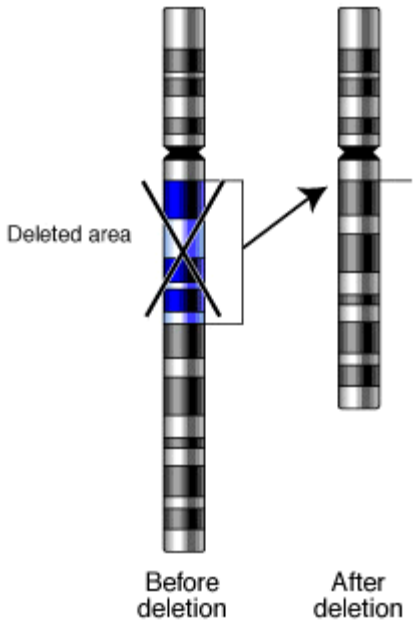
Effects

Insertions can be particularly hazardous if they occur in an exon, the amino acid coding region of a gene. A frameshift mutation, an alteration in the normal reading frame of a gene, results if the number of inserted nucleotides is not divisible by three, i.e., the number of nucleotides per codon. Frameshift mutations will alter all the amino acids encoded by the gene following the mutation. Usually, insertions and the subsequent frameshift mutation will cause the active translation of the gene to encounter a premature stop codon, resulting in an end to translation and the production of a truncated protein. These truncated proteins frequently are unable to function properly or at all and can possibly result in any number of genetic disorders depending on the gene in which the insertion occurs.

In-frame insertions occur when the reading frame is not altered as a result of the insertion; the number of inserted nucleotides is divisible by three. The reading frame

remains intact after the insertion and translation will most likely run to completion if the inserted nucleotides do not code for a stop codon. However, because of the inserted nucleotides, the finished protein will contain, depending on the size of the insertion, multiple new amino acids that may affect the function of the protein.

Deletion (genetics)



Deletion on a chromosome

In genetics, a **deletion** (also called **gene deletion**, **deficiency**, or **deletion mutation**) is a mutation (a genetic aberration) in which a part of a chromosome or a sequence of DNA is missing. Deletion is the loss of genetic material. Any number of nucleotides can be deleted, from a single base to an entire piece of chromosome. Deletions can be caused by errors in chromosomal crossover during meiosis. This causes several serious genetic diseases.

Causes

Causes include the following:

- Losses from translocation
- Chromosomal crossovers within a chromosomal inversion
- Unequal crossing over

- Breaking without rejoining

For synapsis to occur between a chromosome with a large intercalary deficiency and a normal complete homolog, the unpaired region of the normal homolog must loop out of the linear structure into a **deletion** or **compensation loop**.

Types

Types of deletion include the following:

- **Terminal Deletion** - a deletion that occurs towards the end of a chromosome.
- **Intercalary Deletion / Interstitial Deletion** - a deletion that occurs from the interior of a chromosome.

Effects

Small deletions are less likely to be fatal; large deletions are usually fatal - there are always variations based on which genes are lost. Some medium-sized deletions lead to recognizable human disorders.

Deletion of a number of base pairs that is not evenly divisible by three will lead to a frameshift mutation, causing all of the codons occurring after the deletion to be read incorrectly during translation, producing a severely altered and potentially nonfunctional protein.

Deletions are responsible for an array of genetic disorders, including some cases of male infertility and two thirds of cases of Duchenne muscular dystrophy. Deletion of part of the short arm of chromosome 5 results in a syndrome called Cri du chat, French for "cry of the cat" syndrome. It is found in approximately 1 in 50,000 live births. The surviving infants have a distinctive cry, severe mental retardation, and shortened life span.

Chapter- 4

Mutagenesis

Mutagenesis is a process by which the genetic information of an organism is changed in a stable manner, either in nature or experimentally by the use of chemicals or radiation. Mutagenesis as a science was developed especially by Charlotte Auerbach in the first half of the 20th century.

There are the following types of mutagenesis:

- Directed mutagenesis
- Insertional mutagenesis
- PCR mutagenesis
- Signature tagged mutagenesis
- Site-directed mutagenesis
- Transposon mutagenesis

Brief Description of Mutagenesis Types: -

Directed mutagenesis

Directed mutagenesis, also known as **directed mutation**, is a hypothesis proposing that organisms can respond to environmental stresses through directing mutations to certain genes or areas of the genome.

The hypothesis was first proposed in 1988 by John Cairns, of Harvard University, who was studying Escherichia coli that lacked the ability to metabolize lactose. He grew these bacteria in media in which lactose was the only source of energy. In doing so, he found that the rate at which the bacteria evolved the ability to metabolize lactose was many orders of magnitude higher than would be expected if the mutations were truly random. This inspired him to propose that the mutations that had occurred had been **directed** at those genes involved in lactose utilization.

Later support for this hypothesis came from Susan Rosenberg, then at the University of Alberta, who found that an enzyme involved in DNA recombinational repair, recBCD, was necessary for the directed mutagenesis observed by Cairns and colleagues in 1989.

The directed mutagenesis hypothesis was challenged in 2002, when John Roth and colleagues showed that the phenomenon was due to general hypermutability due to selected gene amplification, and was thus a "standard Darwinian process." Later research published in 2006 by Jeffrey D. Stumpf, Anthony R. Poteete, and Patricia L. Foster, however, concluded that amplification could not account for the adaptive mutation and that "mutants that appear during the first few days of lactose selection are true revertants that arise in a single step".

Insertional mutagenesis

Insertional mutagenesis is mutagenesis of DNA by the insertion of one or more bases.

Insertional mutations can occur naturally, mediated by virus or transposon, or can be artificially created for research purposes in the lab.

Signature Tagged Mutagenesis

This is a technique used to study the function of genes. A transposon, such as the *Drosophilla Melanogaster* P-element, is allowed to integrate at random locations in the genome of the organism being studied. Mutants generated by this method are then screened for any unusual phenotypes. If such a phenotype is found then it can be assumed that the insertion has caused the gene relating to that phenotype to be inactivated. Because the sequence of the transposon is known, the gene can be identified, either by sequencing the whole genome and searching for the sequence, or using the polymerase chain reaction to amplify specifically that gene.

Virus Insertional Mutagenesis

As mentioned in the introduction, insertional mutagenesis refers to mutation of an organism caused by the insertion of additional DNA bases into the organism's preexisting DNA. Because many viruses (not all of them) integrate their own genome into the genome of their host cells in order to replicate, mutagenesis caused by viral infections is a fairly common occurrence. Not all integrating viruses cause insertional mutagenesis, however.

It is important to note that not all DNA insertions will lead to a noticeable mutation. In fact, most will not. However, it is a common enough occurrence in viral DNA insertions that biologists researching gene therapy will avoid using viruses that integrate their DNA in the host genome when it is not necessary to do so, opting instead for viruses that transiently express their DNA (leave their DNA free-floating within the cell, rather than integrate it into the genome of the host). For those viruses that do integrate their DNA

into that of the host, the severity of any ensuing mutation depends entirely on the location within the host's genome wherein the viral DNA is inserted. If the DNA is inserted into the middle of an essential gene the effects on the cell will be drastic. Additionally, insertion into the promoter region of a gene can cause equally drastic effects. For instance, if the viral DNA is inserted into a repressor, the gene corresponding to that promoter may be over expressed - leading to an overabundance of its product and altered cellular activity. If the DNA is inserted into an enhancer region, the gene may be under-expressed - leading to relative absence of its product, which can significantly interrupt the activity of the cell.

Alteration of different genes will have varying effects on the cell. Not all mutations will significantly effect the proliferation of the cell. However, if the insertion occurs in an essential gene or a gene that is involved in cellular replication or programmed cell death, the insertion may compromise the viability of the cell or even cause the cell to replicate interminably - leading to the formation of a tumor, which may become cancerous.

Below is an example of a significant change in cell activity due to insertion of a viral gene into a portion of the hosts genome that controls replication.

Virus insertional mutagenesis is only possible with a replication competent virus. The virus inserts a gene (known as a viral onocogene) normally near the cellular myc (c-myc)gene. The c-myc gene is normally turned off in the cell, however when it is turned on it is able to push the cell into the G1 phase of the cell cycle and cause the cell to begin replication which allows the viral gene to be replicated. After many replications where the viral gene stays latent tumours begin to grow. These tumours are normally derived from one mutated/ transformed cell (clonal in origin). Avian leukosis virus is an example of a virus that causes a disease by insertional mutagenesis. Newly hatched chicks infected with Avian leukosis virus will begin to form tumours begin to appear in their bursa of fabricus (like the human thymus). This viral gene insertion is also known as a promoter insertion as it drives the expression of the c-myc gene. There is an example of an insertional mutagenesis event caused by a retrotransposon in the human genome where it causes Fukuyama-type muscular dystrophy .

Insertional inactivation

Insertional inactivation is a technique used in recombinant DNA engineering where a plasmid (such as pBR322) is used to disable expression of a gene.

The inactivation of a gene by inserting a fragment of DNA into the middle of its coding sequence. Any future products from the inactivated gene will not work because of the extra codes added to it. pbr322 mainly having two antibiotic sites that are amphicilin and tetracyclin region.

PCR mutagenesis

PCR mutagenesis is a method for generating site-directed mutagenesis. This method can generate mutations (base substitutions, insertions, and deletions) from double-stranded plasmid without the need for subcloning into M13-based bacteriophage vectors and for ssDNA rescue. The procedure involves a PCR reaction using a supercoiled plasmid vector as the template and two synthetic oligonucleotide primers containing the desired mutation with each complementary to the opposite strands of the vector. After PCR, the template (wild type) plasmid which is dam methylated in almost all E. coli is removed by digestion with Dpn I which is specific for methylated DNA. The new (mutant) DNA is not methylated and remains intact in the reaction. The reaction products are then transformed into competent E. Coli, where the linear double-stranded PCR product is ligated by the host cell, and propagated by appropriate selection.

Signature tagged mutagenesis

Often abbreviated to STM, **Signature-Tagged Mutagenesis** is a genetic technique used to study gene function. Recent advances in genome sequencing have allowed us to catalogue a large variety of organisms genomes, but the function of the genes they contain is still largely unknown. Using STM, a scientist may infer what function the product of a particular gene has by disabling it and observing the effect on the organism. The original and most common use of STM is to discover which genes in a pathogen are involved in virulence in its host, so that better medical treatments can be designed.

Basic Premise

The gene in question is inactivated by insertional mutation; a transposon is used which inserts itself into the gene sequence. When that gene is transcribed and translated into a protein, the insertion of the transposon affects the protein structure and (in theory) prevents it from functioning. In STM, mutants are created by random transposon insertion and each transposon contains a different 'tag' sequence that uniquely identifies it. If an insertional mutant bacterium exhibits a phenotype of interest, such as susceptibility to an antibiotic it was previously resistant to, then the scientist will sequence its genome and run a search (on a computer) for any of the tags used in the experiment. When a tag is located, the gene that it disrupts is also thus located (It will reside somewhere between a start and stop codon which mark the boundaries of the gene).

Scientists may use STM to discover which genes are critical to a pathogen's virulence by injecting a 'pool' of different random mutants into an animal model such as a mouse and observing which of the mutants survive and proliferate in the host. Those mutant pathogens that *didn't* survive in the host must have had an inactivated gene that was needed for virulence. This is hence an example of a negative selection method.

Site-directed mutagenesis

Site-directed mutagenesis, also called **site-specific mutagenesis** or **oligonucleotide-directed mutagenesis**, is a molecular biology technique in which a mutation is created at a defined site in a DNA molecule. In general, this form of mutagenesis requires that the wild type gene sequence be known.

Invention

Site-directed mutagenesis using oligonucleotides was first described in 1978. Michael Smith, its pioneer, shared the Nobel Prize in Chemistry in October 1993 with Kary B. Mullis, who invented polymerase chain reaction.

Basic mechanism

The basic procedure requires the synthesis of a short DNA primer containing the desired base change. This synthetic primer has to hybridize with a single-stranded DNA containing the gene of interest. The single stranded fragment is then extended using a DNA polymerase, which copies the rest of the gene. The double stranded molecule thus obtained is then introduced into a host cell and cloned. Finally, mutants are selected.

In 1987 Kunkel *et al.* introduced an improvement to this technique that eliminated the need for selection of the mutants. The plasmid to be mutated is transformed into an *E. coli* strain deficient in two enzymes, UTPase and uracil deglycosidase. The UTPase deficiency prevents the breakdown of UTP, a nucleotide that normally replaces dTTP in RNA, resulting in an abundance of UTP; the uracil deglycosidase deficiency prevents the removal of UTP from newly-synthesized DNA. As the double-mutant *E. coli* replicates the transformed plasmid, its enzymatic machinery incorporates UTP, resulting in a distinguishable copy. This copy is extracted, and then incubated with the Klenow fragment, dNTPs, DNA ligase, and an oligonucleotide containing the desired mutation, which attaches by base pairing to the complementary wild type gene sequence. The ensuing reaction replicates the UTP-containing plasmid using the oligonucleotide as primer, thus incorporating the desired mutation. This forms a chimeric plasmid, with one strand unmutated and containing UTP, and the other strand mutated and containing dTTP. When this plasmid is transformed into an *E. coli* strain with normal UTPase and uracil deglycosidase, the UTP-containing strand is broken down, whereas the mutation-containing strand is replicated, forming a plasmid lacking UTP but containing the desired mutation on both strands.

Cassette mutagenesis

Cassette mutagenesis involves the cleavage by a restriction enzyme at a site in the plasmid and subsequent ligation of an oligonucleotide containing the mutation in the gene of interest to the plasmid. Usually the restriction enzyme that cuts at the plasmid and the oligonucleotide is the same, permitting sticky ends of the plasmid and insert to ligate to one another.

PCR site-directed mutagenesis

The same result can be accomplished using polymerase chain reaction with oligonucleotide "primers" that contain the desired mutation. As the primers are the ends of newly-synthesized strands, by engineering a mis-match during the first cycle in binding the template DNA strand, a mutation can be introduced. Because PCR employs exponential growth, after a sufficient number of cycles the mutated fragment will be amplified sufficiently to separate from the original, unmutated plasmid by a technique such as gel electrophoresis, and reinstalled in the original context using standard recombinant molecular biology techniques.

For plasmid manipulations, this technique has largely been supplanted by a PCR-like technique where a pair of complementary mutagenic primers is used to amplify the entire plasmid. This generates a nicked, circular DNA which can undergo repair by endogenous bacterial machinery. However, this process does not amplify the DNA exponentially, but linearly. Yields are complicated by the fact that the product DNA must undergo the nick repair and is not supercoiled, resulting in reduced efficiency of bacterial transformation. Finally, the product DNA is of the same size as the plasmid. Therefore, the template DNA must be eliminated by enzymatic digestion with a restriction enzyme specific for methylated DNA. The template, which for this technique should be biosynthesized will be digested, but the mutated plasmid is preserved because it was generated *in vitro* and is therefore unmethylated.

Transposon mutagenesis

Transposon mutagenesis, or **transposition mutagenesis**, is a biological process that allows genes to be transferred to a host organism's chromosome, interrupting or modifying the function of an extant gene on the chromosome and causing mutation.

History

Transposon mutagenesis was first studied by Barbara McClintock in the mid-20th century during her Nobel Prize-winning work with corn.

Dynamics

In the case of bacteria, transposition mutagenesis is usually accomplished by way of a plasmid from which a transposon is extracted and inserted into the host chromosome. This usually requires a set of enzymes including transposase to be translated.

Chapter- 5

Budgerigar Colour Mutations

Anthracite budgerigar mutation

The **Anthracite budgerigar mutation** is an extremely rare mutation that occurs in the budgerigar. The mutation, similar to the Violet budgerigar mutation, causes a difference in the coloring of budgerigars. Anthracites have black or very dark gray feathers, possibly with some white depending on the budgerigar in particular. The mutation is believed to have started in Germany, and tends to be local to that area. Currently, most owners wishing to obtain an Anthracite need to import these budgerigars from Germany.

The description and genetic behaviour of the Anthracite and English Grey are identical, insofar as this can now be determined. It seems likely that the Anthracite is the re-emergence of the English Grey.

Appearance

A bird with two Anthracite factors has an extremely dark grey body, jet black markings and the cheek patches of the same very dark grey as the body. G W von Kamrath describes them as "jet black wing and tail markings and deep black cheek patches" .

A single Anthracite factor has a similar effect to the Dark mutation, causing a Skyblue to become Cobalt in appearance.

Historical Notes

The Anthracite mutation appeared in Germany in 1998 in the aviaries of Hans-Jürgen H Lenk, who successfully established the strain and continues to report on its development . Initially found only in Germany, by the end of 2008 descendents of this original mutation had been exported to America, Belgium, Canada, England, Finland, Holland, Italy, Norway, Sweden and Switzerland.

Genetics

The Anthracite mutation has an incompletely dominant relationship with its wild-type allele. That is, it shows a visible effect when present as a single factor (heterozygote or SF) and a different effect when present as a double factor (homozygote or DF).

In the green series varieties the SF Anthracite Light Green has one Anthracite allele and one wild-type allele at the Anthracite locus. This darkens the body colour to a shade somewhat deeper than a Dark Green. The DF Anthracite Light Green, with two Anthracite alleles, is a deep olive colour.

In the blue series varieties the SF Anthracite Skyblue has one Anthracite allele and one wild-type allele, with a body colour rather like a deep Cobalt. The DF Anthracite Skyblue with two Anthracite alleles, the true Anthracite variety, has a dark grey, almost black, body colour with mauve overtones.

Because the Anthracite factor is always visibly expressed no budgerigar can be split for Anthracite. The heterozygotes of Anthracite with just one Anthracite allele correspond to the splits of recessive mutations.

A single Anthracite factor has a similar effect to the Dark mutation, causing a Skyblue to become Cobalt in appearance - as was suspected with the English Grey. In fact, the similarity of the Anthracite to the English Grey is striking. The description is virtually identical, and the cobalt appearance of a Skyblue with a single Anthracite factor is exactly what was suspected for the English Grey.

The interaction of the Anthracite and Dark budgerigar mutations has not yet been investigated. It is expected that a combination of the two factors will deepen the body colour even more.

Australian Pied budgerigar mutation

The **Australian Pied budgerigar mutation** is one of approximately 30 mutations affecting the colour of budgerigars. It is the underlying mutation of the Banded Pied variety.

Appearance

All pied budgerigars are characterised by having irregular patches of completely clear feathers appearing anywhere in the body, head or wings. These clear feathers are pure white in blue-series birds and yellow in birds of the green series. Such patches are completely devoid of black melanin pigment. The remainder of the body is coloured normally.

Two young Australian Pieds, a Cobalt and a Yellow-face II Cobalt (?)

Opaline Cobalt Australian Pied cock

Cobalt Australian Pied hen

Cobalt Australian Pied cock with a Grey-Green cock

The Australian Pieds are very similar in appearance to the Clearflight Pieds, with a nape spot, clear areas on the wings and a clear area on the breast. They differ from the Clearflight Pied in the nape spot, which is not always present, and in their feet, which are usually pink. But the main point of difference is in the clear area of the body, which in the Australian Pied is located in the middle or lower breast, with the upper breast being always normally coloured, so that there is a clear division between the mask and the breast, just as in normal birds. In the Clearflight Pied the clear area on the breast, if present, is almost always adjacent to and running into the mask. Australian Pieds often have larger clear areas on the wings than Clearflight Pieds, with all primaries and many secondaries often clear, but this cannot be taken as a distinguishing feature as the pied areas are very variable in extent in both mutations. Australian Pieds have the usual white iris ring when adult, distinguishing them clearly from Recessive Pieds, which have no iris ring at any age.

In the Australian Pied the clear area often takes the form of a band running across the breast. Birds with such bands are highly prized, especially if the band is clear, sharp and symmetrical, and the feature is now quite common and distinctive due to selective breeding. Such birds comprise the **Banded Pied** variety.

Historical notes

Many isolated appearances of pied or variegated budgerigars were reported in Britain, in continental Europe and in Australia in the late 1920s and early 1930s, but reliable reports of breeding results and detailed descriptions of their appearance during that period are rare. One of the earliest reports of the appearance of a budgerigar which could have been an Australian Pied was of a bird owned by W G Bowden - it had a clear nape spot and its breeding behaviour clearly showed a dominant inheritance pattern. Mr Bowden obtained or possibly bred the bird in 1931 - he did not report its source. The bird, a cock, was basically a Light Green but it had 'a yellow patch on the back of the head, another on the base of the rump' and 'a yellow streak, about a quarter of an inch in width, from the left wing butt to halfway across the breast'. A number of its flights were reported to be white or yellow. When mated to an unrelated hen in 1933 this cock produced 14 young over three nests, of which 5 showed some clear feathers on the nape of the neck. This could have been the first report of a Clearflight Pied or an Australian Pied; which of these it was is now impossible to tell as the only description available matches both types of Pied.

Several similar pied birds were reported around the same time in Germany, bred by Herr Krabbe and separately by Herr Schucke, by Madame Lecallier in France, by G Wilson and T L S Dooley in England, in Holland and in Scotland, but detailed descriptions and the mode of inheritance are unknown.

The present-day Australian Pieds, including the Banded Pied variety, are believed to be descended from a strain first established in Sydney in 1935 by Keith Ings.

They were first imported to Britain in 1957/8, when Mr A M Cooper of Caerleon, South Wales, bought two such birds, a Pied Green and a Pied Grey, both cocks, from a dealer in Bristol. Most of the Australian Pieds in Britain are descended from the Cooper strain.

Genetics

The Australian Pied allele is dominant over its wild-type allele, although with less than 100% penetrance. The extent and distribution of the clear areas shown by both single-factor (SF) and double-factor (DF) Australian Pieds are variable. The range of variability of the two genotypes appears to be identical, so it is not possible to determine the genetic make-up by considering the extent of the clear areas. In both single- and double-factor birds this variability ranges from no clear feathers at all, via just one or two clear feathers, to over half the body area affected, although the clear areas in cocks tend to be larger than those of hens.

The Australian Pied gene is located on one of the autosomal chromosomes. There is no known linkage of this gene with any other mutation.

There is no universally accepted genetic symbol for either the locus or mutant allele, so the symbol Pa^+ for 'Pied, Australian' will be adopted here for the wild-type allele at this locus, and the symbol *Pa* for the Australian Pied mutant allele.

The factors governing the extent and distribution of the residual pigmentation are not known, although it is likely that at least some factors are sex-linked due to the different ranges in variability of the sexes .

Unlike the Clearflight Pied, the Australian Pied does not produce any Dark-eyed Clears when crossed with the Recessive Pied.

Blue budgerigar mutation

The **Blue budgerigar mutation** is one of approximately 30 mutations affecting the colour of budgerigars. It is part of the genetic constitution of the following recognised varieties: Skyblue, Cobalt, Mauve and Violet.

Appearance

The Blue mutation changes the colour of the body feathers, which are light green in the wild-type, to skyblue and the colour of mask and other parts which are yellow in the wild-type, to white. In the domesticated bird this mutation changes the Light Green variety into the Skyblue variety, the Dark Green into the Cobalt and the Olive into the Mauve.

Light Green cock

Skyblue cock

The green colouration of the wild budgerigar is due to the combined effect of a yellow pigment and an interference effect similar to that which gives colour to petrol on water, which in the budgerigar produces a blue colouration. Yellow pigment is present in the outer layer (cortex) of the cells forming the barbs of all feathers of the wild budgerigar with the exception of the cheek patches, although it is very weak in the outermost flight feathers. The distribution of the yellow pigment is clearly shown in the Lutino. The Blue mutation totally inhibits the production of this yellow pigment, and as far as is known, it has no other effect.

The yellow pigment in young budgerigars is paler than in adults, which makes green budgerigars in nest feather appear duller and Lutinos appear paler. A brighter and stronger yellow colouration appears after the first moult.

Variety	Pantone Code
Light Green	375
Skyblue	310
Cobalt	2915
Mauve	535
Violet	2727

The chemical nature of the yellow pigment in budgerigars and other psittacidae is unknown , and in the absence of a chemical name George A Smith coined the term "psittacins" to cover the yellow, orange and red pigments found in parrots and parrot-like birds. Psittacins impart a far more constant intensity of colour to feathers than do the more commonly found carotenoid pigments such as xanthophyll, the yellow pigment found in the canary. At each moult the canary extracts xanthophyll directly from its food, and the depth of colouration of the growing feathers is determined by the concentration of xanthophyll in its diet. Budgerigars cannot be colour-fed in this way, because they do not use xanthophyll as a pigment.

The Blue mutation provides a widely accepted division of domesticated budgerigars into two colour classes: the "Green series" and the "Blue series". Birds of the Green series exhibit yellow pigmentation, while birds of the Blue series lack yellow pigmentation. These names can be misleading, since some birds belonging to the Blue series, such as Albinos, are not blue; similarly, Lutinos belong to the Green series, yet are not green.

In combination with the Dark budgerigar mutation the body feathers become deeper shades of blue. A blue budgerigar with a single Dark factor is called a Cobalt, and one with two Dark factors a Mauve. The World Budgerigar Organisation has established precise standards for budgerigar body colours using the Pantone Codes, as shown to the right.

Historical Notes

The Blue mutation made its first recorded appearance in 1878 in the aviaries of M Limbosch of Uccle, a suburb of Brussels, but this strain died out, it is believed, in 1881. Blues appeared independently in Holland between 1881 and 1885, and a Mr Pauwels of Everberg, near Brussels, reintroduced them to Belgium from this Dutch strain .

The first Blues to be seen in England were some exhibited by Messrs Millsum and Pauwels at the Horticultural Hall in 1910 and the Crystal Palace in 1911 . Mr D Astley owned Blues in 1911 , and it is recorded that C Pelham Sutton of Putney bred a Blue in 1912 .

Blues remained quite rare until the 1930s, fetching up to £100 per pair in Japan around 1928, about the cost of a car at the time.

Genetics

The Blue mutation is recessive to its wild-type allele, so a bird possessing a single Blue allele (the heterozygote) is identical in appearance to the wild-type light green. That is, the presence of a single wild-type allele is sufficient to permit the full production of the yellow psittacin pigment. Among the budgerigar fancy such a bird is said to be a Light Green split blue, usually written Light Green/blue. In a bird which has two Blue alleles (the homozygote), the lack of the wild-type allele means the yellow pigment can no longer be produced, and so the body colour is blue — the Skyblue.

The locus of the Blue gene is situated on one of the autosomal chromosomes. The Yellowface Blue I mutation, the Yellowface II mutation form an autosomal co-dominant series of alleles with the Blue mutation .

The loci of the Dark budgerigar mutation and the Blue allelic series are situated on the same autosome, so the Dark mutation is linked to the Blue allelic series. The cross-over value (COV) or recombination frequency between the Dark and Blue loci is commonly stated to be about 14% , but some experiments have found much smaller values.

Cinnamon budgerigar mutation

The **Cinnamon budgerigar mutation** is one of approximately 30 mutations affecting the colour of budgerigars. It is the underlying mutation of the Cinnamon variety and, with Ino, a constituent mutation of the Lacewing variety.

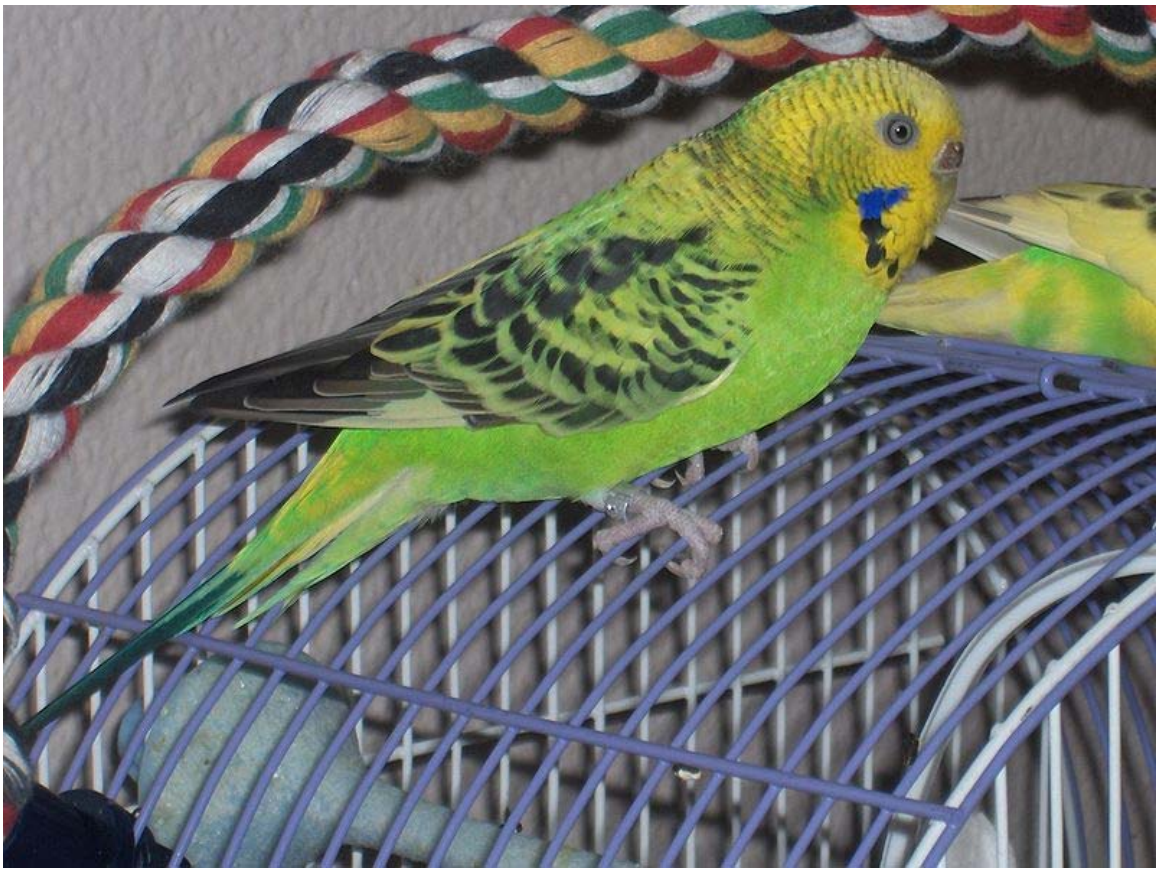
Appearance

All the markings which appear black or dark grey in the corresponding Normal appear brown in the Cinnamon, of a shade similar to that of white coffee. The Cinnamon markings on cocks tend to be considerably darker than on hens . The long tail feathers are lighter than Normals. The body color and cheek patches are much paler, being about half the depth of colour of the Normal. The feathers of Cinnamons appear tighter than Normals, giving a silky appearance . It is these quiet pastel shades and the sleekness of the plumage that give the variety its appeal.

The eyes of the newly-hatched Cinnamon are not black like the eyes of Normals, but deep plum-coloured . This colour can be seen through the skin before the eyes open , and immediately after opening a reddish-brown gleam can be seen. A few days later the eye darkens and is then barely distinguishable from the that of a Normal chick , but by this time the difference in down colour is visible: Normal chicks have grey down, but Cinnamon (and Opaline and Ino) chicks have white.

The skin of Cinnamon chicks is also redder than Normal's , and this persists into adulthood: the feet of Cinnamons are always pink rather than bluey-grey . The beak tends to be more orange in colour .

Superficially, the Cinnamon is very like the two types of Fallow, the German Fallow and the English Fallow, but the eye of the Cinnamon is the usual black with white iris (except for the first few days after hatching, when it is purplish or plum-coloured) whereas the eyes of both varieties of Fallow are red at all ages. The body colour of the Cinnamon is also a rather deeper shade of green or blue than that of the Fallows.



Opaline Light Green hen

Cinnamon Opaline Light Green cock

Historical Notes

Cinnamon specimens of many species have been observed in the wild. A stuffed Cinnamon Light Green budgerigar hen owned by Mrs Ellis of Cottenham, Cambridgeshire, in 1935 and said then to be at least 50 years old was thought by Cyril Rogers to be wild-caught when he examined it.

The first Cinnamon to be reported in Britain was a Cinnamon White Blue hen bred in 1931 from a pair of Light Green split blues by Miss M E J Hughes and her brother Mr G N Hughes of Hampton Hill, Middlesex. This bird was exhibited in 1931 and 1932,

although not described as a Cinnamon as that name had not then been adopted. The mutant hen and its sire died without further issue . Mr I J J Symes gave a description of what he called "the brown factor" in this bird, saying the wing markings varied from raw umber to burnt sienna.

Mr A D Simms, of Potter's Bar, also in Middlesex at the time, paired together several Dark Green split greywing siblings in 1931 bred from an Olive cock and a Greywing Light Green hen. Among other, eight Greywing Greens, all hens, were bred which showed a "rather peculiar colour in their nest feathers". These hens were probably Cinnamons or Greywing Cinnamons, but as the Cinnamon variety was not known at the time they were regarded as slightly strange Greywings.

Mr G F Porter of Codicote, near Hitchin, in Hertfordshire, obtained a pair of Dark Green split greywings from Mr Simms, and he too bred what he called Greywing Green hens. One of these he paired to a Cobalt split dilute cock and this pairing produced, among other progeny, a Cobalt cock which was later found to be split for Cinnamon and Dilute. This cock, paired to a Dark Yellow split blue hen, bred a Cinnamon Skyblue hen in early 1933. Other pairings of descendents from Mr Simms' Dark Green split greywings produced a Cinnamon Olive and a Cinnamon Cobalt for Mr Porter, also in 1933 . Towards the end on 1933 M Porter bred a Dark Green Cinnamon cock—the first Cinnamon cock to appear in Britain.

Mrs A Collier of Luton also bred two Cinnamon hens in 1933, a Mauve and an Olive , but as these were both from stock obtained from Mr Porter, these were almost certainly the same mutation. Mrs Collier was the first to report the characteristic plum-coloured eyes of the very young Cinnamon chick, perhaps being prompted to look for this as it was already a known characteristic of the Cinnamon Canary .

Further Cinnamons appeared in 1933 in the aviaries of Mr G Hepburn of Peterhead, Aberdeenshire. These Cinnamons were bred from a pair of Light Greens obtained from a dealer in Aberdeen, but the ring on the cock showed it came from a Mr Banham, who lived near Victoria Station in London. Mr Hepburn attempted to trace the origin of his birds but was unable to establish a firm link to Mr Simms' birds. Nevertheless, all three Cinnamon mutations, those of Messrs Hughes, Simms and Hepburn, originated within a circle of 15 miles radius and within two years of each other. This strongly suggests the importation of a single Cinnamon carrier cock into the Middlesex area around 1930.

Mr S E Terrill reported that the first Australian Cinnamon appeared about August, 1931, near Adelaide . In 1934 Mr Terrill said he had "four or five cock Cinnamons of two, probably three, generations and about 36 Cinnamon hens of at least three generations." Mr Schumacher, of Magdeburg, Germany, also bred budgerigars with brown wings in 1932, but he disposed of them the year after and it is not known if these were Cinnamons.

Towards the end of 1934 the Budgerigar Society recognised the Cinnamon variety for exhibition purposes and published its show standard.

Genetics

The Cinnamon mutation is sex-linked, the locus of its gene being carried on the X chromosome, and recessive to wild-type. This was determined first by Mr Cyril H Rogers working with Mr Simms and Mr Porter. It was reported in the Budgerigar Bulletin as early as August 1933, and in more detail in September 1934 . At the time of the first report a Cinnamon cock had never been bred. The first cock appeared late in 1933 as a result of a deliberate mating by Mr Porter of a Cinnamon hen and a split Cinnamon cock .

Cinnamon-like mutations are known in many other bird species, including the Canary, Greenfinch, Peach-faced Lovebird and Cockatiel. All these Cinnamon mutations are sex-linked recessives.

The gene locus has the symbol *cin*. The wild-type allele at this locus is notated cin^+ and the Cinnamon allele is notated *cin*.

Sex	Genotype	Phenotype
Cocks	cin^+/cin^+	Normal
	cin^+/cin	Normal (/cinnamon)
	cin/cin	Cinnamon
Hens	cin^+/Y	Normal
	cin/Y	Cinnamon

In birds, the cock has two X chromosomes and the hen has one X and one Y chromosome. So in hens whichever allele is present on the single X chromosome is fully expressed in the phenotype. Hens cannot be split for Cinnamon (or any other sex-linked mutation). In cocks, because Cinnamon is recessive, the Cinnamon allele must be present on both X chromosomes (homozygous) to be expressed in the phenotype. Cocks which are heterozygous for Cinnamon are identical to the corresponding Normal. Such birds are said to be split for Cinnamon, usually written '/cinnamon'.

The table on the right shows the appearance of all possible genetic combinations involving the Cinnamon mutation.

The Cinnamon gene is linked to other genes located on the X chromosome, i.e. to the genes of other sex-linked mutations. These sex-linked mutations include the Opaline, Ino and Slate mutations. The cross-over or recombination values between Cinnamon and these linked genes has not been measured accurately, but results collected by C Warner and T Daniels found 41 crossovers in 113 between Cinnamon and Opaline, giving a recombination ratio of 36±6%.

C H Rogers, reporting early breeding results in 1939 , notes the breeding of Cinnamon Slates by Mr G W Roderick, of Purley and Mr L Trevallion of Loughton, Essex. The

appearance of two Cinnamon Slates by 1939 suggests these two genes are not closely linked.

Cocks split for both Cinnamon and Opaline have one Cinnamon allele and one Opaline allele together with one each of the corresponding wild-type alleles. The linkage between the Cinnamon and Opaline genes gives rise to two types of split cinnamon opaline cocks, both visually identical.

- **Type I split cinnamon opalines cocks** are bred by mating Cinnamon Opalines to Normals and have the two mutant alleles on the same chromatid, symbolised as cin^{+}-op^{+}/*cin*-*op*. Geneticists call this 'coupling' rather than 'Type I'. Because of the linkage, the Cinnamon and Opaline alleles from Type I cocks tend to be inherited together in their progeny. When mated to Normal hens, Type I cocks produce predominantly Cinnamon Opaline and Normal hens, with Cinnamon and Opaline hens resulting rarely from a cross-over. Roughly one third of the hens will be Cinnamon-Opaline, one third Normal, one sixth Cinnamon and one sixth Opaline.

- **Type II split cinnamon opaline cocks** are bred by mating Cinnamons to Opalines and have the Cinnamon and Opaline mutant alleles on opposite chromatids, symbolised as *cin*-op^{+}/cin^{+}-*op*. Geneticists call this 'repulsion' rather than 'Type II'. Because of the separation, the Cinnamon and Opaline alleles from Type II birds tend to be inherited separately in their progeny. When mated to Normal hens, Type II cocks produce predominantly Cinnamon and Opaline hens, with Cinnamon Opaline and Normal hens resulting rarely from cross-overs. Roughly one third of the hens will be Cinnamon, one third Opaline, one sixth Cinnamon Opaline and one sixth Normal.

Hens cannot be split for any sex-linked gene, so only cocks exist in Type I and Type II form.

Clearflight Pied budgerigar mutation

The **Clearflight Pied budgerigar mutation** is one of approximately 30 mutations affecting the colour of budgerigars. It is the underlying mutation of the Continental Clearflight and Dutch Pied varieties. The Dark-eyed Clear variety results when the Recessive Pied and Clearflight Pied characters are combined .

Appearance

All pied budgerigars are characterised by having irregular patches of completely clear feathers appearing anywhere in the body, head or wings. These clear feathers are pure white in blue-series birds and yellow in birds of the green series. Such patches are completely devoid of black melanin pigment. The remainder of the body is coloured normally.

The Clearflight Pied has two main characteristics: a clear patch at the nape of the neck and, ideally, completely clear primary flight and long tail feathers. All other features are normal. However, few birds approach the ideal; most show considerable variation in the extent of the clear areas. The nape spot is almost always present, but it varies considerably in size, affecting just one or two feathers in some birds or extending well down the back and round into the breast on others. It is these latter birds, with extensive clear areas on the breast, that are known as Dutch Pieds. While well-marked Clearflight Pieds have all 10 primaries and both long tail feathers clear, many specimens show just a few clear flight feathers and occasionally none at all are affected .

Poorly marked Clearflight Pieds can look rather like Recessive Pieds, but they may be distinguished from them by the white iris ring, which is always present in adult Clearflights. Some specimens may also resemble Australian Pieds but may be distinguished from them by two characteristics. Firstly, Clearflights have normally coloured blue-grey feet (Dominant Pieds usually have pink feet), and secondly, if they possess extensive clear areas on the breast, these always extends down from the mask whereas the clear areas of a Dominant Pied are always lower down on the abdomen with an area of normal body colour immediately below the mask and separated from it by a sharp dividing line .

Dark-eyed Clears are a combination of the Recessive Pied and Clearflight Pied mutations, having two Recessive Pied alleles and either one or two Clearflight Pied alleles. They are completely clear yellow or white with no trace of the ghost markings often seen in Inos. The eye is a solid jet black (which can in some lights appear a deep plum colour) with no visible iris ring like Recessive Pieds. The cheek patches are silvery white, and the beak, cere and feet are also like those of the Recessive Pied.

Historical notes

Many isolated appearances of pied or variegated budgerigars were reported in Britain, in continental Europe and in Australia in the late 1920s and early 1930s , but reliable reports of breeding results and detailed descriptions of their appearance during that period are rare. One of the earliest reports of the appearance of a budgerigar which could have been a Clearflight Pied was of a bird owned by W G Bowden - it had a clear nape spot and its breeding behaviour clearly showed a dominant inheritance pattern. Mr Bowden obtained or possibly bred the bird in 1931 - he did not report its source. The bird, a cock, was basically a Light Green but it had 'a yellow patch on the back of the head, another on the base of the rump' and 'a yellow streak, about a quarter of an inch in width, from the left wing butt to halfway across the breast'. A number of its flights were reported to be white or yellow. When mated to an unrelated hen in 1933 this cock produced 14 young over three nests, of which 5 showed some clear feathers on the nape of the neck. This could have been the first report of a Clearflight Pied or a Dominant Pied; which of these it was is now impossible to tell as the only description available matches both types of Pied.

Several similar pied birds were reported around the same time in Germany, bred by Herr Krabbe and separately by Herr Schucke , by Madame Lecallier in France , by G Wilson

and T L S Dooley in England , in Holland and in Scotland , but detailed descriptions and the mode of inheritance are unknown.

L Raymaekers of Brussels was the first to establish a substantial strain of Clearflight Pied budgerigars, which he called White-flighted or Yellow-flighted budgerigars . These were all descended from a bird with a small clear head-patch which appeared in his aviaries in 1940 . Some birds, almost certainly from this strain, had been imported to England well before 1947, as F W Wait of Hemsby, near Yarmouth, advertised Whiteflights and Yellowflights for sale in that year .

Some variegated birds established in Holland also showed a clear head-patch but in addition they showed extensive clear areas down the back, on the breast (continuous with the mask) and on the wings. It was birds marked like this that became known as Dutch Pied . For many years this was believed to be a separate mutation from the mutation we now know as the Clearflight Pied, but by 1961 it was 'almost certain' that these were the same basic mutation, the difference between them being due to the selection of different modifying genes by breeders .

Reports that "Albinos and Lutinos with black eyes" were being bred appeared in Europe in the late 1940s. Initially these were believed to be a separate mutation, but after a time it was established they were a composite variety containing both the Clearflight Pied and the Recessive Pied mutations . When Clearflight Pieds were paired with Recessive Pieds and the resulting Clearflights/Recessive Pied young were paired back to Recessive Pieds, some birds were produced with one Clearflight Pied allele and two Recessive Pied alleles. These birds were not pied, but were entirely clear, either yellow or white , but unlike Inos these birds had a totally black eye. Later they became known as Dark-eyed or Black-eyed Clears.

Genetics

The Clearflight Pied allele is dominant over its wild-type allele, although with less than 100% penetrance. The extent and distribution of the clear areas shown by both single- and double-factor Clearflight Pieds are variable. The range of variability of the two genotypes appears to be identical, so it is not possible to determine the genetic make-up by considering the extent of the clear areas. In both single- and double-factor birds this variability ranges from no clear feathers at all, via just one or two clear feathers, to over half the body area affected, although the clear areas in cocks tend to be larger than those of hens .

Genotype	Phenotype
Pc^{+}/Pc^{+}	Normal
Pc^{+}/Pc	Clearflight Pied
Pc/Pc	Clearflight Pied

The Clearflight Pied gene is located on one of the autosomal chromosomes. There is no known linkage of this gene with any other mutation.

There is no universally accepted genetic symbol for either the locus or mutant allele, so the symbol Pc^+ for 'Pied, clearflight' will be adopted here for the wild-type allele at this locus, and the symbol *Pc* for the Clearflight Pied mutant allele. Both Taylor and Warner and Martin used just *P* for the Clearflight Pied locus, but as there are two dominant pied mutations a notation which treats them equally, distinguishing them with a second letter, seems preferable.

The factors governing the extent and distribution of the residual pigmentation are not known, although it is likely that at least some factors are sex-linked due to the different ranges in variability of the sexes .

The Dark-eyed Clear

The combination of one or two Clearflight Pied alleles with two Recessive Pied alleles produces the Dark-eyed Clear variety, with the appearance described above. This combination appears to result in the complete suppression of the melanin pigment in all the feathers, yet leaves the eyes jet-black. The two forms, with one or two Clearflight Pied alleles, are indistinguishable visually, but differ in their breeding behaviour.

Note: this combination of two pied mutations in the budgerigar should not be confused with the Black-eyed Clear recessive mutation found in some parrots .

Dark budgerigar mutation

The **Dark budgerigar mutation** is one of approximately 30 mutations affecting the colour of budgerigars. It is part of the genetic constitution of the following recognised varieties: Dark Green and Olive in the green series and Cobalt, Mauve and Violet in the blue series.

Appearance

Variety	Pantone Code
Light Green	375
Dark Green	369
Olive	371
Skyblue	310
Cobalt	2915
Mauve	535
Violet	2727

Budgerigars carrying the Dark factor are identical to the wild-type Light Greens or Skyblues in every respect except body colour and tail feathers. The body is darker in Dark Greens and Cobalts and darker still in Olives and Mauves, and the long tail feathers are darker in proportion. All these varieties have normal violet cheek patches.

The Dark Green's body colour is a rich shade of laurel green, and Cobalt's a deep blue, approximating to royal blue. The Olive is similar in shade to a Grey-green, but it may be easily distinguished by its cheek patch, which is violet in the Olive and grey in the Grey-green. The Mauve is rather a dull colour, quite different from the brilliant Violet and Cobalt. In nest feather the Mauve is a shade of lavender, almost grey, but the violet cheek patch, although somewhat darker than in other varieties, identifies it as a Mauve.

The Violet Cobalt (a composite of the Blue, Dark and Violet mutations) is a brilliant shade of violet, rather similar but not quite as deep as and rather bluer than the wild-type violet cheek patches.

The World Budgerigar Organisation has established precise standards for certain budgerigar body colours using the Pantone Codes, as shown to the right.

Light Green cock

Dark Green cock

Olive cock

Olive cock

Skyblue cock

Cobalt cock

Historical notes

The Dark mutation is common in the wild as Dark Green budgerigars have been observed in wild flocks on several occasions . One of the earliest to be seen was one captured during an expedition to Australia and exhibited in a London museum in 1847 . But the Dark mutation was not seen in the domesticated budgerigar until the summer of 1915 when a Dark Green was observed by Monsieur A Blanchard in his aviaries in Toulouse . At the time, Toulouse was the main commercial centre for budgerigar distribution in Europe, handling thousands of imported and aviary-bred birds each year. The origins of this first Dark Green are not known. Dark Greens were known initially as *Laurel Greens*, a name which remained popular throughout the 1920s.

Mon. Blanchard produced the first Olives from a pair of Dark Greens in the autumn of 1916 , and J D Hamlyn imported some of the early Olives to England from France in 1918 .

The first Cobalts were bred by Mon. Blanchard in 1920 , and by George F Hedges in 1923 while he was the aviary attendant for Madame Lecallier in France. These were initially called *Powder Blues* . Some of these latter Cobalts were purchased by Mrs Dalton Burgess and imported to England . She exhibited one (as a *Royal Blue*) in February 1924 at the Crystal Palace and later that year bred the first Mauves from them . She called the Mauves *French Greys* in nest feather, and when adult they were known as *Lilacs* or *Lavenders*.

The blue forms of the Dark mutation were far more popular than the Greens and commanded fantastic prices in the mid-twenties. In February 1927 Mauves and Cobalts were sold for £175 a pair, but by 1931 the price was down to £2 a pair, as more and more were quickly bred.

Genetics

The Dark mutation has an incompletely dominant relationship with its wild-type allele. That is, it shows a visible effect when present as a single factor (heterozygote) and a different effect when present as a double factor (homozygote). In the green series varieties the Dark Green has one Dark allele and one wild-type allele at the Dark locus and the Olive has two Dark alleles. In the blue series varieties the Cobalt has one Dark allele and one wild-type allele and the Mauve has two Dark alleles.

Because the Dark factor is always visibly expressed no budgerigar can be split for Dark. The heterozygotes of Dark — the Dark Greens and Cobalts — correspond to the splits of the recessive mutations.

The loci of the Dark mutation and the Blue allelic series are situated on the same autosome, so the Dark mutation is linked to the Blue allelic series. The cross-over value (COV) or recombination frequency between the Dark and Blue loci is often stated to be about 14% , but several careful measurements of this COV show quite widely varying results. Early measurements by Duncker and independently by Steiner obtained values of 14% and 7.6% respectively, and T G Taylor and C Warner collected results which showed only 5 cross-overs in 140 - a COV of 3.6% . Included in these were results from T G Taylor's own experiments, in which he found no cross-overs in 86 birds bred . It is now known that the environment and other genes can influence the COV, so some variability should be expected. A reasonable average of these measurements is a COV of 8%.

Dark Green/blues have one Dark allele and one Blue allele together with one each of the corresponding wild-type alleles. The linkage between the Blue and Dark genes gives rise to two types of Dark Green/blue birds, both visually identical.

- **Type I Dark Green/blues** are bred by mating Mauves to Light Greens and have the two mutant alleles on the same chromatid. Geneticists call this 'coupling' rather than 'Type I'. Because of the linkage, the Dark and Blue alleles from Type I birds tend to be inherited together in their progeny. When mated to Skyblues,

Type I birds produce predominantly Light Green/blue and Cobalt progeny, with Dark Green/blue Type II and Skyblues resulting rarely from a cross-over.

- **Type II Dark Green/blues** are bred by mating Skyblues to Olives and have the Dark and Blue mutant alleles on opposite chromatids. Geneticists call this 'repulsion' rather than 'Type II'. Because of the separation, the Dark and Blue alleles from Type II birds tend to be inherited separately in their progeny. When mated to Skyblues, Type II birds produce predominantly Dark Green/blue Type II and Skyblue progeny, with Light Green/blue and Cobalts resulting rarely from cross-overs.

Dilute budgerigar mutation

The **Dilute budgerigar mutation** is one of approximately 30 mutations affecting the colour of budgerigars. It is one of the constituent mutations of several recognised varieties: the Light, Dark, Olive, Grey and Suffused Yellows and the Grey and Suffused Whites.

Appearance

The Dilute mutation changes the body colour of the wild-type Light Green to yellow, with a variable amount of light green suffusion. The suffusion is deepest on the rump and around the vent. The spots and markings on the wing, head and neck, which are black in the wild-type, are pale grey. The cheek patches are pale lavender and the long tail feathers are pale bluey-grey. The eyes remain normal, with white irides when adult. There is considerable variation in the intensity of the green suffusion, but the best exhibition birds of the 1930s showed very little and also had very faint wing markings. These are the Dilute Light Greens, usually known as Light Yellows.

The green suffusion becomes progressively darker when single and double factors of the Dark mutation are present. These are the Dilute Dark Greens and Dilute Olives, usually known as Dark and Olive Yellows. When the suffusion is particularly heavy the bird is known as a Suffused Yellow.

In the blue series the absence of the yellow pigment turns the body colour to white, although this is usually suffused with blue, often quite heavily. When the suffusion is light, Dilute Skyblues are known as Whites; when it is heavy they are known as Suffused Whites. Dilute Cobalts and Mauves are usually known as Suffused Whites.

Historical Notes

Budgerigars were first introduced into Europe by the ornithologist and bird artist, John Gould, in 1840 , when he imported to England a pair which had been bred by his brother-in-law, Charles Coxon . They grew steadily in popularity. For the first thirty years of their domestication only the wild-type Light Green budgerigar was known, but in 1872 birds with greenish-yellow bodies and very pale wing markings were reported from Belgium (in both Brussels and Antwerp) and Germany (in both Kassel and Berlin) . This, now known as the Dilute mutation, was the first mutation observed in the domesticated budgerigar.

Joseph Abrahams obtained some of these new yellow birds from Belgium and bred the first Yellow, as it was called, in Great Britain in 1884 . These strains, in both Britain and Europe, laid the foundations for the very popular exhibition Light Yellow of the 1920s and 30s, which were often known by the alternative names of Buttercups or Buttercup Yellows. The popularity of the variety declined after the Lutino became available in the late 1930s.

In 1896, Mr Keartland of the Calvert expedition to Australia, observed a yellow budgerigar flying wild in a flock on three occasions . This suggests the Dilute mutation is relatively common in heterozygous form among the wild populations, as two such heterozygous individuals would need to mate in order to produce a visible Dilute.

Although the Blue mutation was first seen soon after the first Dilutes, in 1878, and had become established by 1890 in Europe, the first combination of the Blue and Dilute mutations in double homozygous form did not appear until around 1920, some 30 to 40 years later. This combination was the White (known as Silver in Australia), and it was first bred in England by H D Astley in September 1920 from a pair of Skyblues . A White was also reported in Paris in the same year . Whites were never as popular as the Yellows, as it was much harder to approach the exhibition ideal of a pure white bird.

Genetics

The Dilute mutation is recessive to its wild-type allele, so a bird possessing a single Dilute allele (the heterozygote) is identical in appearance to the wild-type Light Green. That is, the presence of a single wild-type allele is sufficient to permit the production of the normal number of melanin granules. Among the budgerigar fancy such a bird is said to be a Light Green split dilute, usually written Light Green/dilute, although Light Green/yellow has been used.

In a bird which has two Dilute alleles (the homozygote) the number of melanin granules is greatly reduced, to around 5% of the normal amount. This results in a much reduced intensity of the black markings, and less absorption of light which passes through the cloudy layer in the medulla of barbs. As this absorption of light is a necessary part of the process which generates the blue colouration the intensity of blue is also greatly reduced.

The Dilute mutation is one of a series of multiple alleles at the same locus, called dil^{+} in the wild-type. The others are the Clearwing (dil^{cw}) and Greywing (dil^{gw}) mutations. The Dilute allele (dil^{d}) is recessive to all other alleles at this locus, so for the Dilute character to be expressed in the phenotype the genotype must be homozygous for this allele.

Dominant Clearbody budgerigar mutation

The **Dominant Clearbody budgerigar mutation** is one of approximately 30 mutations affecting the colour of budgerigars. It is the underlying mutation of the Easley Clearbody variety.

Over the years many mutations have been reported which produce a (relatively) clear yellow or white body with normal black or dark wing markings, approximating to the beautiful painting of a (hypothetical) "laced Yellow" by R A Vowles shown in Dr M D S Armour's book, *"Exhibition Budgerigars"*. In an article published in *Cage and Aviary Birds* Dr T Daniels summarised those that were known in 1981. Many of these failed to become established, and others, reported separately, may have been the same mutation which appeared in different parts of the world. The Dominant Clearbody was one which was established successfully, probably twice, in both the USA and Australia.

Appearance

The mutation now known as the Dominant or Easley Clearbody was first described by its breeder, C F Easley. He said, "The body colour is changed from blue or green to white or yellow and the wing barring, flights and shaft feathers become jet black. Throat spots are black and the cheek patch is pale bluish or lavender." This describes perhaps the idealised variety, and only double factor Clearbodies approach this ideal. He goes on to say, "the clearness of the body and the darkness of the wing barrings depends on the strength of the factor in the individual bird", indicating the variability in residual suffusion and the intensity of the black markings.

Historical Notes

The alternative name of this mutation, 'Easley Clearbody', comes from the name of the breeder who discovered and established the mutation: C F Easley of Rialto, California. He has described in detail how this mutation, which he called 'Laced Clear', arose and was established. In January 1957 he paired an Opaline Dark Green cock to a Cobalt hen and bred a hen described as an "Opaline Greywing Dark Green with a yellow body and exceedingly dark grey wing markings". In January 1958 this hen was paired to a normal Dark Green cock and two further mutants were bred in a nest of four chicks, both cocks, with yellow bodies, black wing markings, black long tail feathers and pale violet (lavender) cheek patches, one in Opaline form and one Normal.

Mr Easley went on to establish a stud of over 200 Clearbodies. The early mutants showed considerable body suffusion but birds with much less suffusion were bred later. In a series of experiments, Mr Easley determined that the suffusion and depth of wing markings were variable, but the lavender cheek patch remained an unchanging characteristic. In establishing his stud, Mr Easley proved the mutation to be an autosomal dominant.

It appears Mr Easley did not dispose of any of his 'Laced Clears' until 1965, when he placed the first advertisement offering them for sale in the American Budgerigar Society Bulletin.

Mr Easley died in 1973, having previously disposed of all his stock, and no direct link to present-day Dominant Clearbodies has been established, but in the late 1970s Jeffrey Shultz of Highlands, Texas, had Clearbodies with lavender cheek patches which were due to a dominant mutation, so these were almost certainly descended from or related to the Easley Clearbodies. Around the same time Ben Pawlik of San Antonio, Texas, had birds he believed were of the Easley variety.

Ghalib Al-Nasser has described how Dominant Clearbodies came to Europe from the USA. Reinhard Molkentin imported two Dominant Clearbody cocks from California in 1990 and in 1992 Wilfried Kopp obtained some of their descendents from Herr Molkentin. Later, Dominant Clearbodies were imported to the UK when Gren and Pat Norris obtained an Opaline Cinnamon Grey Dominant Clearbody from Reinhard Molkentin in 2000 (who was by then in South Africa) and Ghalib Al-Nasser imported a Grey Green Dominant Clearbody cock from Wilfried Kopp in Germany in 2001, adding two further cocks later. It seems likely that all the Dominant Clearbodies in Europe, including the UK, are descended from the original two imported by Herr Molkentin from California in 1990.

However, it may be that the Dominant Clearbody made its first appearance in Beverley, South Australia, in the aviaries of Bob Hancock, who bred what he called Blackwing Yellows and Blackwing Silvers for many years. This mutation first made its appearance around 1933, when a Blackwing Silver hen and four Skyblues were bred from a pair of Skyblues. The Blackwing Silver had a white body with a faint blue suffusion on the rump, normal wing markings, a black eye, black long tail feathers and a silver-grey cheek flash. This hen, paired to a Dilute Skyblue cock, produced a Blackwing Silver cock and from these two all subsequent Blackwing Silvers and later Blackwing Yellows were bred. He established that the mutation is dominant and not sex-linked. The description of these two autosomal dominant mutations is so similar that it seems very likely that they are identical.

Genetics

The Dominant Clearbody allele is dominant over its wild-type allele, so a bird possessing a single Dominant Clearbody allele (the heterozygote or single-factor Dominant Clearbody) is converted from the wild-type Light Green to a Dominant Clearbody (SF)

Light Green as described in Appearance above. That is, the presence of a single Dominant Clearbody allele is sufficient to permit almost the full expression of the mutation.

The double-factor Dominant Clearbody, with two Dominant Clearbody alleles, is believed to have a clearer body and darker wing markings than the single-factor Dominant Clearbody.

The Dominant Clearbody gene is located on one of the autosomal chromosomes. There is no known linkage of this gene with any other mutation.

Dominant Grey budgerigar mutation

The **Dominant Grey budgerigar mutation**, often called the Australian Grey or simply Grey, is one of approximately 30 mutations affecting the colour of budgerigars. It is the basis of the Grey-Green and Grey standard varieties.

Appearance

The Dominant Grey mutation transforms the wild-type Light Green into the Grey-Green variety and the Skyblue into the Light Grey variety. The body colour of the Grey-Green is a dull mustard green and, compared to a Light Green, the mask is a slightly duller tone of yellow. The body colour of the Light Grey is an even, uniform, battle-ship grey.

Light Green cock

Grey-Green cock

In both the blue and green series birds the flights and long tail feathers are black. The pattern of black on the wing and tail markings is unchanged, but they are darkened to a jet black, resulting in high contrast between the black and yellow, which is particularly noticeable in the tail bar when the bird is in flight. The cheek patches are lilac-grey.

Variety	Pantone Code
Light Green	375
Grey-Green	398
Skyblue	310
Grey	428

When combined with the Dark mutation the body colour of both Greys and Grey-Greens becomes slightly darker, but the effect is much smaller than the effect of the Dark mutation on Light Greens and Skyblues.

As this is a dominant mutation the colour changes described above apply to both single factor (SF) and double factor (DF) Greys and Grey-Greens. The only difference between SF and DF birds is in the colour of the afterfeather and shaft of the contour feathers. In the SF Light Grey these are the normal white but in the DF birds the afterfeather is dark grey, with a black shaft.

The World Budgerigar Organisation has established precise standards for budgerigar body colours using the Pantone Codes, as shown to the right.

Historical Notes

The earliest recorded appearance of the Dominant Grey mutation was in 1934 , when Mrs S Harrison of Murrumbeena, Victoria, Australia, purchased a Grey cock from a dealer. The original breeder has not been identified. Early breeding results showed this Grey to be a Dark Grey (SF)/dilute, and Mrs Harrison went on to establish a substantial strain of Greys from this bird.

In 1936, it was reported that W F Shepherd of Kew, Victoria, also had Greys which he obtained from a colony breeder, and a Grey was also bred independently by R Hancock of Beverley, South Australia, in 1935.

Dominant Greys were first imported to Britain around 1937, one by Mrs R Brown of Morecambe for Mr Walter Higham , and one, from R Hancock's stock, by Tom Goodwin.

Genetics

The Dominant Grey allele is dominant over its wild-type allele, so a bird possessing a single Dominant Grey allele (the heterozygote or single-factor Dominant Grey) is converted from the wild-type Light Green to Grey-green as described in Appearance above. That is, the presence of a single Dominant Grey allele is sufficient to permit the full expression of the mutation.

The double-factor Dominant Grey, with two Dominant Grey alleles, is identical in appearance to the single-factor Dominant Grey, although there is some evidence that the colour of the breast afterfeathers is changed from white to grey in the double-factor bird.

The Dominant Grey gene is located on one of the autosomal chromosomes. There is no known linkage of this gene with any other mutation.

English Fallow budgerigar mutation

The **English Fallow budgerigar mutation** is one of approximately 30 mutations affecting the colour of budgerigars. At least three types of Fallow, the German, English and Scottish, all named after their country of origin, have been established, although none of these types is common. They are superficially similar, but adult birds may be distinguished by examining the eye. All have red eyes, but the German Fallow shows the usual white iris ring, the eye of the English Fallow is a solid red with a barely discernible iris and the iris of the Scottish Fallow is pink.

In an attempt to regularise the names of mutations across all psittacines, it has been proposed by Inte Onsman that the name *Pale Fallow* be adopted for this mutation. The

name *Dun Fallow* has also been proposed, and Terry Martin suggests *Beige Fallow* or *Grey-Brown Fallow*. But in Budgerigar circles the variety is commonly known as the English Fallow, and is the name retained here.

Appearance

In most respects English, Scottish and German Fallows are very similar. All resemble Cinnamons, but differ in having a much weaker body colour, which results in a rather attractive mustard-yellow breast shading to green on the rump (blue in the blue series). The depth of the green or blue suffusion varies in individual birds, but is always more intense towards the vent and on the rump. The throat spots, head and neck striations, and wing markings are a medium brown on a yellowish ground. The cheek patches are a lighter and duller shade of violet than normal. Cocks have a greyish-purple rather than the usual blue cere. The feet and legs are pink and the beak orange.

The most obvious distinction from Cinnamons is the red eye, which in the English Fallow is a clear bright red, without a white iris ring—a beautiful and attractive feature. The eye of the German Fallow is a deep ruby-red, like an Ino's but a shade darker, with the usual white iris ring when adult, and the Scottish Fallow has a pink iris ring. On hatching, young English Fallows have red eyes like Inos but young German Fallow chicks have plum-coloured eyes rather like Cinnamon chicks.

The Dark mutation deepens the body colour of the Fallow, but the difference between the Light Green, Dark Green and Olive Fallow is far less than that between the normal Light Green, Dark Green and Olive. The Olive Fallow is "a beautiful rich golden orange shade, and the chest is deep yellow olive - a truly lovely colouring", says Cyril Rogers in *The Fallows*.

Fallow Greys and Grey-Greens generally have darker wing markings. Opaline by itself lightens the body colour of Fallows, and in combination with Cinnamon produces a bird very similar in appearance to a Lacewing (i.e. a Cinnamon Ino), with virtually no body suffusion.

Historical Notes

The English Fallow first appeared in 1937 in the aviaries of F Dervan, of Luton. He was a beginner to aviculture, starting in 1934 with a pair of Skyblues. He bought a pair of Greens in 1935 and bred from the two pairs. In 1936 and 1937 he intermated the descendents from these two pairs very closely, and was surprised in 1937 when seven red-eyed Fallows appeared in the nests of two of the pairs.

These red-eyed birds were inspected by C H Rogers, who suspected they might be a new variety and advised Mr Dervan to mate one of his Fallows to a German Fallow to check. This pairing was made in 1938, and from three nests eight black-eyed youngsters were bred, proving the varieties were distinct.

In 1940 English Fallows of the Blue series were produced by Mr Dervan, and at that time he had 13 Fallows and 28 split Fallows .

W P Bland, writing in the Budgerigar Bulletin in 1962 , said he "... obtained some English Fallows and by 1939 had sixty". It seems unlikely that these birds were from Mr Dervan's strain if the date is correct. There is evidence that Scottish Fallows existed from the 1920s, and were originally called English Fallows , so it seems likely Mr Bland's were of this variety.

In the early 1960s C Warner and T G Taylor obtained English Fallows from two different sources, although allegedly from the same breeder. They found they bred only black-eyed young when cross-paired. One type had a faint iris ring while the other was completely devoid of iris pigmentation. Both varieties were distinct from the German Fallow, and they concluded that three distinct forms of Fallow existed at that time. The Fallows with the faint iris ring were good quality exhibition birds and became known as "Moffat" or Scottish Fallows after their owner, Jim Moffat.

In 1964 John Papin of California wrote that in America no less than five distinct Fallow varieties existed. These were

1. English Fallow, red eye, solid without ring
2. German, red eye with ring
3. Californian, similar to German, red eye with ring
4. Californian, a near solid red eye type with rather fine grey markings
5. Texas, a red eye with strong body colour

He said all were recessive and produce normals if intermated.

The numbers of all varieties of budgerigar in captivity declined dramatically during the war years and when aviculture restarted in earnest in the late 1940s English Fallows were very rare. They were originally quite small and were never very popular with breeders of exhibition birds. In the period from the 1960s to the 1980s they almost died out, but Dr Margaret Young of Rochester, Kent kept the variety alive almost single-handed, and now the variety is gaining steadily in popularity.

The name *Fallow* was first applied to the German Fallow by Herr Kokemüller after Dr Steiner, who examined some German Fallow feathers microscopically, wrote to him, "It would be better to describe this form as the fallow Budgerigar rather than cinnamon." At the time it was believed that Dr Steiner used the word by analogy with fallow or undeveloped land, to mean the melanin pigment was undeveloped , but as an alternative meaning for 'fallow' (and also for its German equivalent) is 'pale yellow' or 'light brown', it seems far more likely that it was this meaning that was intended . When the English Fallow appeared a few years later it was so similar in appearance to the German Fallow that for a time they were both called Fallows. Later, English, German and Scottish Fallows were proved to be distinct and separate mutations by test matings made independently by T G Taylor , Mrs Amber Lloyd of Walton-on-Thames and Frank Wait

, and qualified names were then introduced to distinguish them. It was found that birds of any two of the mutations produced only normal black-eyed young when paired together.

Genetics

The English Fallow is an autosomal mutation causing recessive changes to the form of the melanin pigment. There is no universally accepted genetic symbol for either the locus or mutant allele, so the simple symbol fe^+ will be adopted here for the wild-type allele at this locus, and the symbol *fe* for the English Fallow mutant allele, in keeping with the most widely used name in budgerigar circles.

In its visual effect, the English Fallow mutation is recessive to its wild-type allele, so a bird possessing a single English Fallow allele (the heterozygote, fe^+/fe) is identical in appearance to the wild-type light green. That is, the presence of a single wild-type allele is sufficient to permit the full production and normal distribution of the black melanin pigment. Among the budgerigar fancy such a bird is said to be a Light Green split English fallow, usually written Light Green/English fallow.

In a bird which has two English Fallow alleles (the homozygote, *fe*/*fe*), the lack of the wild-type allele means that normal black melanin pigment cannot be produced. Instead a pigment giving a brown appearance is substituted, resulting in brown markings where black would appear in the Normal.

Ino budgerigar mutation

The **Ino budgerigar mutation** is one of approximately 30 mutations affecting the colour of budgerigars. It is the underlying mutation of the Albino and Lutino varieties and, with Cinnamon, a constituent mutation of the Lacewing variety.

Appearance

In the green series the Ino is known as the Lutino, with pure yellow contour feathers, white or pale yellow flight feathers and tail feathers and silvery-white cheek patches. In some lights the body can show a very pale green sheen.

In the blue series the Ino is known as the Albino, and is pure white throughout. The cheek patches are almost the same colour as the body, but slightly more silvery. In some lights the body can show a very pale blue sheen.

Variety	Pantone Code
Lutino	102

The eyes of both the Lutino and Albino are red at all ages with white irides when adult, the beak is orange and the feet and legs are pink. The cere of an adult Ino cock is greyish-purple rather than blue.

The World Budgerigar Organisation has established precise standards for some budgerigar body colours using the Pantone Codes, as shown to the right for the Lutino.

The Ino mutation also induces changes in the nestling. The down is white rather than grey and appears only sparsely, never growing down the centre of the back. As the feathers appear, those down the spine and along the ventral centre line are late to develop.

The Ino gene masks the effect of virtually all other mutations, including Opaline, Dark, Dominant Grey, Dilute, and Clearwing. These genes, when present in an Ino in either heterozygous or homozygous form, cause no change in the appearance of the Ino. But the Ino gene does **not** entirely mask Cinnamon. A Cinnamon Ino, usually called a Lacewing, has pale brown or fawn spots, tail and wing markings. These markings are quite clear, but considerably fainter than the markings of a normal Cinnamon.

The Dark-eyed Clear has a similar body colour to the Ino, but has solid reddish-purple eyes without a white iris.

Cinnamon Dilute German Fallows, NSL Inos and Inos are all very similar and difficult to distinguish from each other, but the first two are so uncommon difficulties arise rarely in practice.

Albino hen

Albino hen and Lutino cock

Albino cock

Historical notes

The first known reference to the Ino mutation in the budgerigar was a report by Mr L van der Snickt, a Belgian fancier, in the German avicultural paper *Die Gefiederte Welt* (*The Feathered World*) in 1879. He wrote that he had seen that year nine Lutinos, all hens. (In fact, he called them Albinos, since the name Lutino did not then exist, but from his description and the fact that the Blue mutation was not established until the 1880s it is clear they were Lutinos.) One breeder of these birds was Mr Kessels, also of Belgium, who in 1881 bred 25 Lutinos, all hens.

A coloured picture of a Lutino appeared in the Brussels journal, *Acclimatation Illustrée*, in 1882, and it is thought they were being bred in Holland around 1885, while in England Mr C P Arthur of Melksham in Wiltshire bred what he believed was a pair of Inos around 1887. After the 1880s no mention seems to have been made in the press of Inos until the 1930s, when interest in budgerigar mutations suddenly increased.

In 1930/31, Lutino hens were owned by both Capt H S Stokes of Longdon, near Rugeley in Staffordshire, and Mrs Huntington of Warwick. In August 1932 Mr F J Mullis of Horsham, Sussex, bred an Albino hen. None of these led to an established strain.

In September 1931, Mr E Böhm of Bawerk in Germany bred, as the last of nine young from a pair of Cobalt split Dilutes, a snow-white red-eyed hen - the first recorded Albino. Almost exactly a year later, on 12 September 1932, a second Albino hen was bred by Mr Fischer of Honow in Germany from a pair of Skyblues. Both of these strains were established by the original breeders and also by others who acquired early stock

from them, in particular by Kurt Kokemüller of Arnum über Hanover, and Mr Schrapel, also of Hanover, who performed together the first genetic investigations into the Ino mutation and published the first correct pairing expectations in the German publication *Der Wellensittich* (*The Budgerigar*) in November and December 1933.

A third appearance of the Ino mutation occurred in Germany around 1933, when Mr Kuhlewein bred a Lutino hen in an uncontrolled breeding flight. This strain was also established.

Other Ino mutations also appeared in Europe in the early 1930s, and several British fanciers, including Walter Higham, Scott and Camplin, and Tod Boyd, had imported continental Lutinos by the mid-1930s. Some of these turned out to be of the non-sex-linked type and the unwitting mixing of the two mutations led to considerable confusion. All British Inos seem to have descended from these imported continental Inos.

In 1976, Dr T Daniels began a controlled programme of pairings to produce a Cinnamon Ino by deliberately crossing Cinnamons to Inos, and to estimate the cross-over value between these two mutations. The first Cinnamon Ino was produced in late 1979 and was identical in appearance to a Lacewing.

Genetics

The Ino mutation is a sex-linked recessive at the ino locus on the X chromosome. The wild-type genetic symbol is ino^+ and the ino mutant allele has the symbol *ino*. Its effect is to inhibit the production of the melanin pigment which is normally present in all feather barbs in either the medullary or cortical cells or both. The presence of black melanin pigment in the cortex of the barbs is necessary for the production of the black markings and in the medulla of barbs for the production of the blue colouration (which combines with the yellow pigment in birds of the green series to produce the green colouration), so this mutation removes all black and blue colourations resulting in a white bird in the blue series and a yellow bird in the green series.

Because the Ino mutation totally inhibits the production of normal melanin pigment it prevents the visible expression of all the other mutations which depend on the presence of melanin to show their effect. This is called epistasis, and Ino is phenotypically epistatic over many other mutations, including Dark, Grey, Opaline, and the Dilute series. It is not epistatic over the Blue mutation, so there are two forms of the albino budgerigar, one in the green series called the Lutino and one in the blue series called the Albino. Both these varieties may be masking many other hypostatic mutations, so the genotype of an Albino or Lutino with respect to these mutations cannot be determined visually. Nor is the Ino mutation epistatic over the Cinnamon mutation — see below.

Sex	Genotype	Phenotype
Cocks	ino^+/ino^+	Normal
	ino^+/ino	Normal (/ino)

Hens	*ino*/*ino*	Ino
	ino^{+}/Y	Normal
	ino/Y	Ino

In birds, the cock has two X chromosomes and the hen has one X and one Y chromosome. So in hens whichever allele is present on the single X chromosome is fully expressed in the phenotype. Hens cannot be split for Ino (or any other sex-linked mutation). In cocks, because Ino is recessive, the Ino allele must be present on both X chromosomes (homozygous) to be expressed in the phenotype. Cocks which are heterozygous for Ino are identical to the corresponding Normal. Such birds are said to be split for Ino, usually written '/ino'.

The table on the right shows the appearance of all possible genetic combinations involving just the Ino mutation.

The Ino mutation does not mask the Cinnamon mutation, these two genes being neither fully epistatic nor hypostatic to each other. When combined in doubly homozygous form (*cin-ino*/*cin-ino* in cocks or *cin-ino*/Y in hens) the Lacewing phenotype is produced. The Cinnamon markings are clearly visible, although considerably fainter than in a normal Cinnamon. For many years the Lacewing was thought by many to be a separate mutation but it was demonstrated in 1979 that it was simply a Cinnamon Ino when a Lacewing was deliberately produced by combining separate Cinnamon and Ino genes. Once brought together, these two genes are almost always inherited together due to the close linkage between them, giving the impression of being a single gene.

The Ino mutation is a member of a series of multiple alleles at the *ino* locus. Only one other member is known -- the Sex-linked Clearbody mutation.

The Ino gene is linked to other genes located on the X chromosome, i.e. to the genes of other sex-linked mutations. In addition to the Cinnamon mutation, these mutations include Opaline and Slate. The cross-over or recombination values between Ino and these linked genes has not been measured accurately, but some approximate measurements of the cross-over values have been made:

- **Cinnamon-Ino COV:** Breeding results collected by C Warner and T Daniels found just 1 crossover in 36 between Cinnamon and Ino. Other measurements found at least 1 cross-over in 18, so combining these the best estimate of the recombination value is ≥4±3%.

- **Opaline-Ino COV:** Only one direct measurement of the Opaline-Ino linkage has been reported. This found 3 cross-overs in 10, giving a recombination value of 30±17%. But since the ino locus is very close to the cinnamon locus the COV for Opaline-Ino must be very similar to that for Opaline-Cinnamon. The Opaline-Cinnamon linkage has been measured to be approximately 36±6%, so these two results are in agreement within the limited statistics.

Cocks split for both Cinnamon and Ino have one Cinnamon allele and one Ino allele together with one each of the corresponding wild-type alleles. The linkage between the Cinnamon and Ino genes gives rise to two types of split cinnamon-ino cocks, both visually identical.

- **Type I split cinnamon-ino cocks** are bred by mating Cinnamon-Inos (Lacewings) to Normals and have the two mutant alleles on the same chromatid, symbolised as cin^{+}-ino^{+}/*cin-ino*. Geneticists call this 'coupling' rather than 'Type I'. Because of the linkage, the Cinnamon and Ino alleles from Type I cocks tend to be inherited together in their progeny. When mated to Normal hens, Type I cocks produce predominantly Cinnamon-Ino (Lacewing) and Normal hens, with Cinnamon and Ino hens resulting extremely rarely from a cross-over. Roughly 48% of the hens will be Cinnamon-Ino (Lacewing), 48% Normal, 2% Cinnamon and 2% Ino.

- **Type II split cinnamon-ino cocks** are bred by mating Cinnamons to Inos and have the Cinnamon and Ino mutant alleles on opposite chromatids, symbolised as cin^{+}-*ino*/*cin-ino*$^{+}$. Geneticists call this 'repulsion' rather than 'Type II'. Because of the separation, the Cinnamon and Ino alleles from Type II birds tend to be inherited separately in their progeny. When mated to Normal hens, Type II cocks produce predominantly Cinnamon and Ino hens, with Cinnamon-Ino (Lacewing) and Normal hens resulting extremely rarely from cross-overs. Roughly 48% of the hens will be Cinnamon, 48% Ino, 2% Cinnamon-Opaline (Lacewing) and 2% Normal.

Hens cannot be split for any sex-linked gene, so only cocks exist in Type I and Type II form.

Opaline budgerigar mutation

The **Opaline budgerigar mutation** is one of approximately 30 mutations affecting the colour or appearance of budgerigars. It is the underlying mutation of the Opaline variety. When combined with the Yellowface II and Clearwing mutations the Rainbow variety is produced.

Appearance

The Opaline mutation is characterised by several features which are invariably present, although many show variations in the intensity of their expression. The most obvious effect is on the striations which extend from the top of the head down the neck to between the wings in the non-Opaline. In the Opaline these striations are very much reduced in intensity, being almost absent in many individuals, particularly in small birds of yellow (as opposed to buff) feather. The cap of the Opaline extends further back over the top of the head, gradually merging into an area the same colour as the body which continues down the back of the head to form a 'V' shape between the wings. The intensity

of the striations in this area is variable, but in the original mutations, particularly the Australian, the 'V' was very clear.

In the non-Opaline the wings show dark grey or black markings over a yellow or white ground, but in the Opaline the ends of the barbs of the wing coverts assume the same colour as the body, rather than the ground colour. This suffusion of body colour in the wings produces the opalescent effect which gave the mustation its name. The area of black pigmentation in each feather is reduced and in the original specimens the wing butts were particularly devoid of black pigment, resulting in a clear area often called the 'thumb-print'. These thumb-prints appear to be associated with a clear 'V', but are now seen less often, since the Budgerigar Standard calls for normal wing markings in the Opaline.

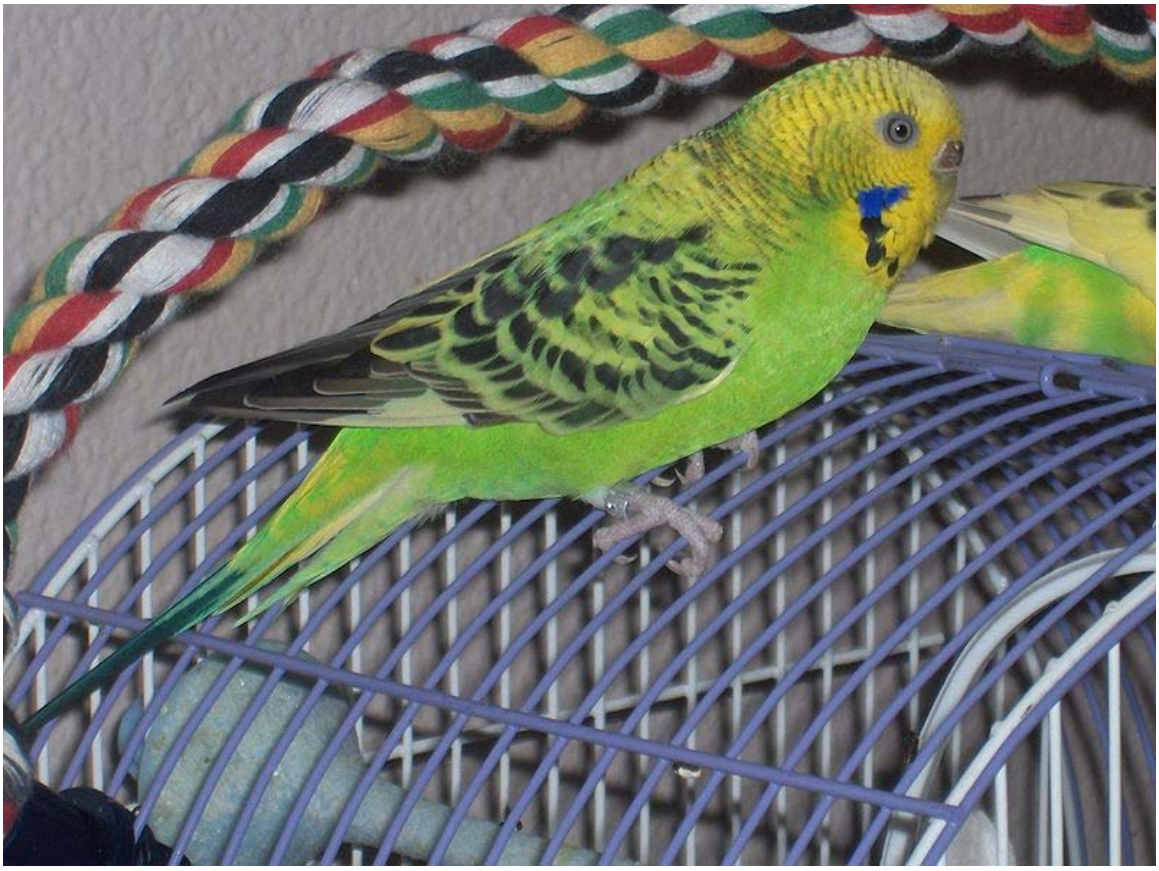
The flight feathers of the budgerigar consist of 10 primaries and 10 secondaries. These are dark grey with a clear central band across every feather from the 2nd primary to the 8th secondary. These clear areas are not visible in the folded wing, but form a prominent continuous band running right along the wing when it is stretched out. It is hidden from above by the coverts but is visible from beneath. In the Opaline this clear band is present on every flight feather and is much broader. Only the distal half of the flight feather is dark, with the clear zone extending from the mid-point to the shaft. Because it is broader it is visible in the primaries of the folded wing of the Opaline, just beneath the secondaries and primary wing coverts, as a small clear patch.

A similar effect occurs in all the wing feathers, most noticeably in the primary and secondary wing coverts, and also in the six tail feathers, which carry a similar clear band on feathers 2 to 6 in the non-Opaline. The first (longest) tail feather of the Opaline also carries a rather blotchy clear area of somewhat variable extent, and the suffusion of body colour present to a small degree in the non-Opaline is intensified in the Opaline.

Most Opalines show a brighter body colour than the corresponding non-Opaline, paricularly in nest feather and particularly in the rump area. This is due to a reduction in the melanin content of the barbules of the contour feathers.

The final characteristic of the Opaline (and the Cinnamon) is the colour of the down feathers of the young nestling. These are white instead of the usual grey, and this allows Opalines to be identified at a very early age.

Light Green cock



Opaline Light Green hen

Cobalt cock

Opaline Skyblue cock

Historical Notes

In 1933 Mr A Brown of Kilmarnock, Scotland, bred what was described as a 'pied' Cobalt hen from a perfectly normal Skyblue cock and Mauve hen. The parents came from a strain kept locally which had never produced anything unusual, and Mr Brown bred no more than the one mutant, even though the same pair bred many Cobalts in both 1933 and 1935.

Towards the end of 1933 Mr and Mrs Ashby of nearby Ayr purchased this 'pied' Cobalt, which they described as being "exceptionally large with a fine head and most excellent spots", although both parents were quite mediocre. The mutant's peculiarities were that the head, neck and nape were almost pure white with slight markings in places and nearly all the flight feathers, primaries and secondaries, were edged with cobalt in place of white, making the bird almost a 'Cobalt-wing'. The mutant was not a pied of any of the present-day types (these were not established in 1933), but an Opaline, although the variety was not to be known by that name until a few years later.

In 1934 the Ashby's paired the mutant hen to a quality Light Green split blue cock and Skyblues, Light Greens and a Dark Green of a perfectly normal appearance were bred. In 1935 one of the Skyblue cocks was mated back to the mutant hen, and the very first nest

produced two Opaline Cobalt cocks and an Opaline Skyblue hen. The Opaline mutation had been fixed.

Early in 1936 circumstances forced the Ashby's to dispose of all their Opalines, which at that time were known as 'Marbled', and the entire stock, with the exception of two pairs which went to Andy Wilson of Glasgow, went to Walter Higham of Blackburn, under the care of his aviary manager, Len Hillas. From these two studs came the vast majority of British Opalines, most of them carrying the wide head and large spots which first caught the attention of the Ashby's.

In Australia, also around 1933 (the exact date is uncertain), Mr S E Terrill discovered a mutant budgerigar, a Light Green hen in nest feather, among thousands of wild birds caught by trappers and sent to Adelaide market. He bought her, and described her special features as "... almost complete absence of barring on the back of the neck and mantle and its replacement by the body colour ... the mask being extended back, covering the top of the head ... the bars on the wing coverts reduced in number and intensity, their yellow margins being greatly enlarged and nuch suffused in green."

Mr Terrill, who lived near Adelaide, paired the hen to a Blue Silver (the Australian name at the time for the variety now known as Dilute Skyblue or White) and bred three cocks and a hen in 1934, all Light Green in appearance. About November 1935 the three cocks were paired up, one to a Cinnamon Light Green, one to a Cobalt and one to his mother. The first two pairings produced six Opalines, all hens, and the third several Opalines, both cocks and hens . The name 'Opaline' was suggested in 1936 by Mr R J Byfield of Hobart, Tasmania, on being particularly impressed by the vividness of colour shown by these young birds in nest feather . Mr Terrill adopted the name and after he suggested it in the Budgerigar Bulletin in September 1936 it rapidly gained universal acceptance throughout the world.

But maybe neither Mr Brown nor Mr Terrill were the first to breed an Opaline. In 1962 Mr J Riley of Yorkshire wrote, "In 1930 or 1931 a pair of my Light Greens produced a chick that was of good size and type with mask and spots that were a living dream; the only snag was that its wings were mismarked and grizzled, these markings extending over the bird's back." Mr Riley kept the bird and used it to try to improve the spots of his Light Greens, but further 'mis-marked' birds appeared. He disposed of them all soon afterwards and only a long time later did he see Opalines and realise that he had bred them first and cast them aside.

The Opaline appeared yet again in 1935, in the aviaries of L Raymaekers in Brussels. Mr Higham imported two Opaline Mauve cocks and one Opaline Greywing Mauve hen from Mr Raymaekers in 1937 and Cyril Rogers confirmed they were the same mutation as the Scottish one, although their wing barring seemed noticeably lighter.

Genetics

The Opaline mutation is sex-linked, the locus of its gene being carried on the X chromosome. It is recessive to wild-type. The gene locus has the symbol *op*. The wild-type allele at this locus is notated op^+ and the Opaline allele is notated *op*.

Sex	Genotype	Phenotype
Cocks	op^+/op^+	Normal
	op^+/op	Normal (/opaline)
	op/*op*	Opaline
Hens	op^+/Y	Normal
	op/Y	Opaline

In birds, the cock has two X chromosomes and the hen has one X and one Y chromosome. So in hens whichever allele is present on the single X chromosome is fully expressed in the phenotype. Hens cannot be split for Opaline (or any other sex-linked mutation). In cocks, because Opaline is recessive, the Opaline allele must be present on both X chromosomes (homozygous) to be expressed in the phenotype. Cocks which are heterozygous for Opaline are identical to the corresponding Normal. Such birds are said to be split for Opaline, usually written '/opaline'.

The table on the right shows the appearance of all possible genetic combinations involving the Opaline mutation.

The Opaline gene is linked to other genes located on the X chromosome, i.e. to the genes of other sex-linked mutations. These sex-linked mutations include the Cinnamon and Slate mutations and the two allelic mutations at the *ino* locus -- the Ino and the Sex-linked Clearbody. The cross-over or recombination values between Opaline and these linked genes has not been measured accurately, but results collected by C Warner and T Daniels found 41 crossovers in 113 between Cinnamon and Opaline, giving a recombination ratio of 36±6%. Since the *ino* locus is known to be very close to the *cin* locus, the recombination ratio between Opaline and both Ino and Sex-linked Clearbody must also be around 36%. The opinion has been expressed that there is a close link between Opaline and Slate.

Cocks split for both Cinnamon and Opaline have one Cinnamon allele and one Opaline allele together with one each of the corresponding wild-type alleles. The linkage between the Cinnamon and Opaline genes gives rise to two types of split cinnamon-opaline cocks, both visually identical.

- **Type I split cinnamon-opalines cocks** are bred by mating Cinnamon-Opalines to Normals and have the two mutant alleles on the same chromatid, symbolised as cin^+-op^+/*cin*-*op*. Geneticists call this 'coupling' rather than 'Type I'. Because of the linkage, the Cinnamon and Opaline alleles from Type I cocks tend to be inherited together in their progeny. When mated to Normal hens, Type I cocks produce predominantly Cinnamon-Opaline and Normal hens, with Cinnamon and Opaline

hens resulting rarely from a cross-over. Roughly one third of the hens will be Cinnamon-Opaline, one third Normal, one sixth Cinnamon and one sixth Opaline.

- **Type II split cinnamon-opaline cocks** are bred by mating Cinnamons to Opalines and have the Cinnamon and Opaline mutant alleles on opposite chromatids, symbolised as cin^{+}-*op*/*cin*-op^{+}. Geneticists call this 'repulsion' rather than 'Type II'. Because of the separation, the Cinnamon and Opaline alleles from Type II birds tend to be inherited separately in their progeny. When mated to Normal hens, Type II cocks produce predominantly Cinnamon and Opaline hens, with Cinnamon-Opaline and Normal hens resulting rarely from cross-overs. Roughly one third of the hens will be Cinnamon, one third Opaline, one sixth Cinnamon-Opaline and one sixth Normal.

Hens cannot be split for any sex-linked gene, so only cocks exist in Type I and Type II form.

Chapter- 6

Important Concepts of Mutations

Macromutation

Many biologists believe that adaptation occurs through the accumulation of small changes, such as the slight differences between parents and their children, some of which can result from mutations. However, an alternative that has been suggested for this process is **macromutation**, essentially when a sudden large-scale mutation produces a characteristic. This theory has generally been disregarded as the major explanation for adaptation, since a mutation on this scale is regarded as more likely to be detrimental than beneficial.

While macromutations appear to be the only explanation for differences such as the number of body segments among arthropods, at the genetic level where the original change occurs, very few changes to genes may actually be necessary to result in the large physical change. Some genes control other genes, where the higher the level of control, then the larger the change those genes can cause. Biologists make a distinction between changes to the genotype, and the resulting body structure resulting from those genes phenotype.

For example, polydactyl individuals have a large resulting change in their body structure (extra toes), but that change can result from a small change in their genotype. This is not the only possible cause of such changes. They can also result from errors during development, but such non-gene changes are not inherited by future generations.

Macromutation in Chromosomes

Instantaneous biological adaptation must occur when a non-deleterious or rarely useful change occurs in the number of chromosomes in the organism's phenome. This must have occurred when the ancestral ape produced offspring which possessed 46 instead of 48 chromosomes, leading to the eventual evolution of humanity.

This change cannot have taken place slowly as odd numbers and partial chromosomes in diploid creatures are not reproductively viable. Although the possibility of finding a

similarly endowed mate to enable reproduction is rather unlikely, the evolution from a single-celled organism to the complex multi-cellular beings found today was equally unlikely, making the possibility not entirely impossible and a rather viable rationale for the differences in chromosome number between the other Great Apes and humans.

This does not invalidate adaptation taking place as a result of slowly developing variation acted on by natural selection, as the genetic sequencing changes wrought by natural selection may lead to chemical instability of the genome concerned, causing fusion, splitting of chromosomes, usually deleterious, but very occasionally useful.

BRCA mutation

A **BRCA mutation** is a mutation in either of the genes BRCA1 and BRCA2. Hundreds of mutations have been identified, some of them cause increased risk of breast cancer, ovarian cancer and other cancers. Instead of a 12 percent lifetime risk of breast cancer, women with high risk BRCA1 or BRCA2 gene mutations may have a risk of up to 60 percent risk of developing breast cancer. The risk of developing ovarian cancer is about 55% for women with high risk BRCA1 mutations and about 25% for women with high risk BRCA2 mutations. The normal population of women experiences ovarian cancer at a rate of 1.8%.

Cancer risk

Both BRCA1 and BRCA2 are tumor suppressor genes and are involved in DNA repair of double-strand breaks. The BRCA2 protein also binds to and regulates RAD51 to fix DNA breaks. Mutations in BRCA1 and/or BRCA2 cause decreased stability of the human genome and result in dangerous gene rearrangements that can lead to hematologic cancers.

Patients carrying heterozygous germline mutations in either the BRCA1 or BRCA2 genes demonstrate highly penetrant breast and ovarian cancer phenotypes. The tumors arising in these patients exhibit loss of heterozygosity (LOH) at the wildtype allele.

Cancer prevention strategies

Women with a family history of breast and/or ovarian cancer are screened for mutations in their BRCA1 and BRCA2 genes. A number of investigation have been tried or are under investigation reduce cancer risk and improve survival rates.

Prophylactic surgery

Depending on further circumstances prophylactic mastectomy and/or salpingo-oophorectomy may yield a substantial reduction of breast and ovarian cancer risk in BRCA1 and BRCA2 carriers. .

For carriers of high risk BRCA1 mutations prophylactic oophorectomy around age 40 reduces the risk of ovarian and breast cancer and provides significant and substantial long term survival advantage. Earlier intervention does on average not provide any additional benefit but increases risks and adverse effects.

For carriers of high risk BRCA2 mutations oophorectomy around age 40 has only marginal effect on survival, the positive effect of reduced breast and ovarian cancer risk is nearly balanced by adverse effects. The survival advantage is more substantial when oophorectomy is performed together with prophylactic mastectomy.

The effect of preventive mastectomy on overall survival is very small when compared with intensive screening.

Other effects

There is likely little or no effect of a BRCA gene mutation on fertility.

Evolutionary advantage of BRCA mutations

Several theories assert that BRCA mutations have evolutionary advantages, such as higher intelligence. The Ashkenazi intelligence theory was proposed by Gregory Cochran and asserts that a defect in the BRCA1 gene might unleash neural growth.

Cat body type genetic mutation

Cats, like all living organisms, occasionally have mutations that affect their body type. Sometimes, these **cat body type genetic mutations** are striking enough that humans select for and perpetuate them. This is not always in the best interests of the cat, as many of these mutations are harmful; some are lethal in their homozygous form.

Scottish Fold, a cat breed with naturally occurring folded ears

This page gives a selection of cat body type mutant alleles and the associated mutations with a brief description.

Tail types

Example of naturally occurring curly tail in a domestic cat.

Jb = Japanese bobtail gene (dominant with incomplete penetrance). Cats heterozygous for this gene have abnormal tails, but unlike the Manx cat there are no associated skeletal disorders and the gene is not associated with lethality.

M = Manx gene (dominant). Cats with the homozygous genotype (MM) die before birth, and stillborn kittens show gross abnormalities of the central nervous system. Cats with the heterozygous genotype (Mm) show severely shortened tail length, ranging from taillessness to a partial, stumpy tail. Some Manx cats die before 12 months old and exhibit skeletal and organ defects. People have suggested that the Manx gene, because it was discovered in naturally occurring populations of cats, is a gene conferring some kind of selective advantage to the cats. The trait also occurred and died out in Cornwall (mainland England), but became fixed in the island population where outbreeding was not possible due to isolation.

A Japanese Bobtail's bobbed tail

There are numerous other bobtail types in the cat population, most of which are identical to the Japanese Bobtail or the variably expressed Manx mutation. However, some may be novel mutations that have not been investigated.

There are numerous types of curly-tailed cats whose tails loop over the back or form tight corkscrews. One such mutation has been developed into the American Ringtail but others have been regarded as curiosities and not perpetuated. The gene(s) responsible have not been fully investigated.

Limbs

Mk = Munchkin gene (dominant). Cats heterozygous for this gene (Mkmk) have shortened legs, but are not disabled. They have a ferret-like gait. The homozygous form (MkMk) may be lethal as litter sizes are smaller than average. Although there was initial

concern that Munchkin-type cats would have impaired mobility or spinal problems, this was based on comparison with dog breeds and proved to be unfounded due to the cat's more flexible spine. The mutation has occurred naturally in many locations and has also been perpetuated in feral cats without human intervention (Robinson's Genetics for Cat Breeders).

The mutation has proven not to be achondroplasia , but is most likely to be either hypochondroplasia or pseudochondroplasia which affect the long bones of the leg while leaving other bodily proportions, especially the head, unchanged.

Paws

Sh = Split Foot (Syndactyly). A dominant gene that reduces the number of toes resulting in a "lobster-claw" appearance. This is considered an undesirable mutation.

Polydactyl (extra-toed) cats. There are probably many genes, both dominant and recessive, that cause polydactyly in cats. Most cases of polydactyly in cats are perfectly harmless.

Pd = Thumb-cat polydactyly gene. The Pd gene (dominant with incomplete penetrance) causes the benign, pre-axial form of polydactyly where one or more extra toes occur near the dew claw. Often, the dew claw is converted into a thumb. There are occasional problems such as fused claws or claws facing in the wrong direction, but generally, this form of polydactyly is harmless.

On the other hand, the "hamburger-feet" polydactyly gene is associated with gene for radial hypoplasia (RH). The 1995 European Convention for the Protection of Pet Animals considers RH an impairing condition. In a scandal in the late 1990s, an experimental breeder in Texas tried to perpetuate this deformity as the "Twisty Cat" breed. Mild RH can cause the post-axial form of polydactyly – enlarged paws, extra three-jointed toes on the outer, little-toe side of the paws, and no thumb. X-rays can determine the structure of the extra toes and whether the cat has the gene for RH. Cats with the gene for RH should never be bred. Cats with severe RH have unusually short front legs. They move like a ferret and they tend to sit like a squirrel or kangaroo and are colloquially known as squittens. In some RH cats, the forelegs are twisted with the long bones either severely shortened or absent. All polydactyl cats are banned from German cat shows, possibly because of confusion with the impairing form of polydactyly associated with RH.

Polydactyl cats are relatively common in southwest Britain, Norway, Sweden, and the eastern coast of the USA and Canada, and some parts of Asia. Sailors thought they were lucky. There were, and are, many myths surrounding polydactyl cats:

- That they are superior mousers and ratters,
- That they have better balance on ships in stormy weather,
- That their paws are natural snowshoes,

- That their opposable thumbs (in the thumb-cat form of polydactyly) give them a survival advantage.

Ernest Hemingway supposedly collected polydactyl cats, and the reported descendants of his collection may still be found at the Ernest Hemingway House on Key West.

Ear types

Cu = American Curl gene (dominant). Cats with this gene have ears that start out normal, but gradually curl backwards. So far, no harmful defects have been associated with this gene.

Fd = Scottish Fold gene (dominant with incomplete penetrance). Cats with this gene have ears that curl forward. There are different degrees of folding, and more genes may be involved in the expression of the Fd gene. This gene is associated with bone and cartilage defects such as thickened tail and swollen feet. The homozygous form (FdFd) is probably lethal. However many breeders are careful in their breeding programs, so that such defects are kept to a minimun.

Australian Curl – a curl-eared mutation occurred in a female stray cat in Australia, but was not inherited by her offspring. When the original cat became ill, necessitating spaying, it was impossible to test-mate her sons back to her to identify a possibly recessive curled-ear mutation.

Sumxu – extinct Chinese Lop-eared cat breed reported between 1700 and 1938 around Peking, most descriptions are based on a specimen in a German museum. The mode of inheritance of its pendulous ears is not known.

Four Ears – CC Little reported a recessive mutation that produced four ears (more precisely four pinnae or ear flaps). In a group of four-eared cats studied in 1957, in addition to duplicated ears the eyes were reduced in size, the jaw was slightly undershot and the cats were relatively inactive and lethargic. Researchers believed that the functioning of the brain was affected. Breeding data indicated it was most often lethal with kittens dying in utero. The majority of recently reported four-eared cats have been healthy with various ear configurations suggesting other genes were involved or developmental abnormalities rather than hereditary factors.

Dynamic mutation

In genetics, a **dynamic mutation** is an unstable heritable element where the probability of mutation is a function of the number of copies of the mutation. That is, the replication product of a dynamic mutation has a different likelihood of mutation than its predecessor. These mutations, typically short sequences repeated many times, give rise to numerous known diseases including the Trinucleotide repeat disorders.

Robert I. Richards and Grant R. Sutherland called these phenomena, in the framework of dynamical genetics, dynamic mutations. Triplet expansion is caused by slippage during DNA replication. Due to the repetitive nature of the DNA sequence in these regions 'loop out' structures may form during DNA replication while maintaining complementary base paring between the parent strand and daughter strand being synthesized. If the loop out structure is formed from sequence on the daughter strand this will result in an increase in the number of repeats. However if the loop out structure is formed on the parent strand a decrease in the number of repeats occurs. It appears that expansion of these repeats is more common that reduction. Generally the larger the expansion the more likely they are to cause disease or increase the severity of disease. This property results in the characteristic of anticipation seen in trinucleotide repeat disorders. Anticipation describes the tendency of age of onset to decrease and severity of symptoms to increase through successive generations of an affected family due to the expansion of these repeats.

Common features

- Most of these diseases have neurological symptoms.
- Anticipation/The Sherman paradox refers to progressively earlier or more severe expression of the disease in more recent generations.
- Repeats are usually polymorphic in copy number, with mitotic and meiotic instability.
- Copy number related to the severity and/or age of onset
- Imprinting effects
- Reverse mutation - The mutation can revert to normal or to a premutation carrier state.

Founder mutation

In genetics, a **founder mutation** is a mutation that appears in the DNA of one or more individuals who are founders of a distinct population. Founder mutations initiate with changes that occur in the DNA and can get passed down to other generations.

Founder mutations originate in long stretches of DNA on a single chromosome—indeed, the original haplotype is the whole chromosome. As the generations progress, the proportion of the haplotype that is common to all carriers of the mutation is shortened (due to genetic recombination). This shortening allows scientists to roughly estimate the age of the mutation.

Frameshift mutation

A **frameshift mutation** (also called a **framing error** or a **reading frame shift**) is a genetic mutation caused by indels (insertions or deletions) of a number of nucleotides that is not evenly divisible by three from a DNA sequence. Due to the triplet nature of gene expression by codons, the insertion or deletion can change the reading frame (the grouping of the codons), resulting in a completely different translation from the original. The earlier in the sequence the deletion or insertion occurs, the more altered the protein produced is.

A frameshift mutation will in general cause the reading of the codons after the mutation to code for different amino acids, but there may be exceptions resulting from the redundancy in the genetic code. Furthermore, the stop codon ("UAA", "UGA" or "UAG") in the original sequence will not be read, and a stop codon could result at an earlier or later site. The protein being created could be abnormally short or abnormally long, and will most likely not be functional.

Frameshift mutations frequently result in severe genetic diseases such as Tay-Sachs disease. A frameshift mutation is responsible for the disabling of the CCR5 HIV receptor and some types of familial hypercholesterolemia (Lewis, 2005, p. 227-228). Frameshift mutations have also been proposed a source of biological novelty, as with the alleged creation of nylonase. However, a study by Negoro *et al* (2006) found that a frameshift mutation was unlikely to have been the cause and that rather a two amino acid substitution in the catalytic cleft of an ancestral esterase amplified Ald-hydrolytic activity.

Frameshifting may also occur during protein translation, producing different proteins from overlapping open reading frames, such as the gag-pol-env retroviral proteins. This is fairly common in viruses and also occurs in bacteria and yeast (Farabaugh, 1996).

A frameshift mutation is not the same as a single-nucleotide polymorphism in which a nucleotide is replaced, rather than inserted or deleted.

Thermodynamics

The effects of neighboring bases and secondary structure on the frequency of frameshift mutations has been investigated in depth. Fluorescently tagged DNA, by means of base analogues, permits one to study the local changes of a DNA sequence. Studies on the effects of the length of the primer strand reveal that an equilibrium mixture of four hybridization conformations was observed when template bases looped-out as a bulge, i.e. a structure flanked on both sides by duplex DNA. In contrast, a single-loop structure with an unusual unstacked DNA conformation at its downstream edge was observed when the extruded bases were positioned at the primer–template junction, showing that misalignments can be modified by neighboring DNA secondary structure.

Germline STAT 1 Mutation

Interferons induce the formation of two transcriptional activators: gamma-activating factor (GAF) and interferon-stimulated gamma factor 3 (ISGF3). A natural heterozygous germline STAT1 mutation associated with susceptibility to mycobacterial but not viral disease was found in two unrelated patients with unexplained mycobacterial disease.

This mutation caused a loss of GAF and ISGF3 activation but was dominant for one cellular phenotype and recessive for the other. It impaired the nuclear accumulation of GAF but not of ISGF3 in cells stimulated by interferons, implying that the antimycobacterial but not the antiviral effects of human interferons are mediated by GAF. More recently, two patients have been identified with homozygous STAT-1 mutations who developed both post–BCG vaccination disseminated disease and lethal viral infections. The mutations in these patients caused a complete lack of STAT-1 and resulted in a lack of formation of both GAF and ISGF3.

Splice site mutation

A **splice site mutation** is a genetic mutation that inserts or deletes a number of nucleotides in the specific site at which splicing of an intron takes place during the processing of precursor messenger RNA into mature messenger RNA. The abolishment of the splicing site results in one or more introns remaining in mature mRNA and may lead to the production of aberrant proteins. Several genetic diseases may be the result of splice site mutations. For example, mutations that cause the incorrect splicing of β-globin mRNA are responsible of some cases of β-thalassemia. Another Example is TTP (thrombotic thrombocytopenic purpura). TTP is caused by deficiency of ADAMTS-13. A splice site mutation of ADAMTS-13 gene can therefore cause TTP.

Chapter- 7

Adaptive Mutation

The central dogma of evolutionary theory is selection from random variation. In other words, mutagenesis occurs randomly, regardless of the utility of a genetic mutation to the organism. If it is beneficial,or neutral the organism will survive; if it is harmful, the organism will die. However, John Cairns has proposed that "[w]hen populations of single cells are subject to certain forms of strong selection pressure, variants emerge bearing changes in DNA sequence that bring about an appropriate change in phenotype." This suggests that there exists a particular physiological pathway that responds to a specific selective pressure to produce a mutation conferring the correct phenotype that will alleviate this pressure.

Such evidence was first produced by Cairns et al. in 1988. The original experiments involved a strain of *E. coli* that has a frameshift mutation in the lactose (LacZ) operon, inactivating the proteins needed for utilization of this sugar. The bacteria were then spread on an agar medium in which the only carbon source was lactose. This meant that a cell could grow only if a second mutation occurred in the lactose operon, reversing the effects of the mutation and therefore allowing the enzymes to be synthesized. Mutations with this effect appeared to occur significantly more frequently than expected, and at a rate that was greater than mutations in other parts of the genomes of these *E. coli* cells.

There is however a serious flaw in this experiment: Cairns does not distinguish between selection and detection of LacZ revertants. If he is testing to see if the presence of lactose as the selective agent causes mutations that confer the ability to eat lactose, then he should not detect this mutation with lactose present (i.e. looking for cells that grow with lactose as the only carbon source.) He will never know, in this case, if cells have acquired this ability without the presence of lactose - a possibility that his theory cannot reconcile. In fact, the acknowledgment of fundamental limitations on our ability to separate between mutation selection and detection has lead Vasily Ogryzko to suggest that for the proper description of the Cairns' experiments, the formalism of quantum theory would be required, with the phenomenon of adaptive mutations naturally following from such an approach.

Furthermore, when looking for additional mutations only in cells that have already reverted to Lac+, it would certainly be the case that other, unnecessary mutations would

be less numerous - especially since most are deleterious. However, Barry Hall has provided evidence that the mutation rate in bacteria under environmental stress increases across the board, most likely indiscriminately. When testing for tryptophan revertants (trp− → trp+, i.e. cells that have regained the ability to make tryptophan), he found that occurrence of auxotrophic mutants increased as well. Tryptophan revertants, which had been exposed to the environmental stress of lacking the amino acid, saw a 1.8% rate of auxotrophy for any other amino acid. When testing for auxotrophy in cells in non-stressed colonies, he found a rate below 0.01%. From these data, Hall hypothesized that cells under stress enter a "hyper-mutable state," where cells increase their general rate of mutation, increasing the overall probability that they will acquire a mutation conferring a phenotype that aids their survival. Hall later determined that his mutants were the result of transposon activity, and concluded that his results were in fact about transposon biology, and not mutagenesis.

Similar results have been observed in other experiments. These experiments suggested that mutations in bacteria are influenced by the selective pressures that the bacteria are placed under.

One possible explanation is that under conditions of stress the global rate of errors in DNA replication and repair mechanisms is increased, and hence the mutation rate is increased.

Another explanation stems from a similarity in cellular mechanisms underlying the acquisition of adaptive mutations in the bacterial stationary phase cells and in the mammalian tumor cells. In both cases the adaptive mutations arise in response to a sustained stress environment and are promoted by high rate of genomic mutations. Cellular processes leading to the mutations are also surprisingly similar between both organisms and include silencing of differentiation, cellular senescence, programmed cell death, and DNA repair on the one hand, and activation of the error-prone replication and transposons on the other. The similarity suggests that the adaptive mutations may be an output of activation in the stressed cell of a special survival strategy for quick adaptation to the stressful environment. This strategy that is also referred as the mutator phenotype is an alternative to other stress-induced strategies, such as senescence and programmed cell death, activated in majority of stressed cells. Continuing stress-induced proliferative and survival signaling may be an important prerequisite for epigenetic reprogramming of some cells to activate the mutator phenotype.

Adaptive evolution in the human genome

Adaptive evolution results from the propagation of advantageous mutations through positive selection. This is the modern synthesis of the process which Darwin and Wallace originally identified as the mechanism of evolution. However, in the last half century there has been considerable debate as to whether evolutionary changes at the molecular level are largely driven by natural selection or random genetic drift. Unsurprisingly, the forces which drive evolutionary changes in our own species' lineage have been of particular interest. Quantifying adaptive evolution in the human genome gives insights

into our own evolutionary history and helps to resolve this neutralist-selectionist debate. Identifying specific regions of the human genome that show evidence of adaptive evolution helps us find functionally significant genes, including genes important for human health, such as those associated with diseases.

Methods

The methods used to identify adaptive evolution are generally devised to test the null hypothesis of neutral evolution, which, if rejected, provides evidence of adaptive evolution. These tests can be broadly divided into two categories. Firstly, there are methods that use a comparative approach to search for evidence of function altering mutations. The dn/ds test detects adaptive evolution if dn (the number of nonsynonymous substitutions; fitness effect is either neutral, advantageous or deleterious) is greater than ds (the number of synonymous substitutions; fitness effect is assumed neutral) (Yang and Bielawski 2000). The McDonald-Kreitman (MK) test quantifies the amount of adaptive evolution occurring by estimating the proportion of nonsynonymous substitutions which are adaptive, referred to as α (McDonald and Kreitman 1991, Eyre-Walker 2006). α is calculated as: $\alpha = 1-(dsps/dnps)$, where dn and ds are as above, and pn and ps are the number of nonsynonymous (fitness effect assumed neutral or deleterious) and synonymous (fitness effect assumed neutral) polymorphisms respectively (Eyre-Walker 2006). Note, both these tests are presented here in basic forms, and these tests are normally modified considerably to account for other factors, such as the effect of slightly deleterious mutations. The other methods for detecting adaptive evolution use genome wide approaches, often to look for evidence of selective sweeps. Evidence of complete selective sweeps is shown by a decrease in genetic diversity, and can be inferred from comparing the patterns of the Site Frequency Spectrum (SFS, i.e. the allele frequency distribution) obtained with the SFS expected under a neutral model (Willamson et al. 2007). Partial selective sweeps provide evidence of the most recent adaptive evolution, and the methods identify adaptive evolution by searching for regions with a high proportion of derived alleles (Sabeti et al. 2006). Examining patterns of Linkage Disequilibrium (LD) can locate signatures of adaptive evolution (Hawks et al. 2007, Voight et al. 2006). LD tests work on the basic principle that, assuming equal recombination rates, LD will rise with increasing natural selection. These genomic methods can also be applied to search for adaptive evolution in non-coding DNA, where putatively neutral sites are hard to identify (Ponting and Lunter 2006). Another recent method used to detect selection in non-coding sequences examines insertions and deletions (indels), rather than point mutations (Lunter et al. 2006).

Amount of adaptive evolution

Coding DNA

Many different studies have attempted to quantify the amount of adaptive evolution in the human genome, the vast majority using the comparative approaches outlined above. Although there are discrepancies between studies, generally there is relatively little

evidence of adaptive evolution in protein coding DNA, with estimates of adaptive evolution often near 0% (see Table 1). The most obvious exception to this is the 35% estimate of α (Fay et al. 2001). This comparatively early study used relatively few loci (fewer than 200) for their estimate, and the polymorphism and divergence data used was obtained from different genes, both of which may have lead to an overestimate of α. The next highest estimate is the 20% value of α (Zhang and Li 2005). However, the MK test used in this study was sufficiently weak that the authors state that this value of α is not statistically significantly different from 0%. Nielsen et al. (2005a)'s estimate that 9.8% of genes have undergone adaptive evolution also has a large margin of error associated with it, and their estimate shrinks dramatically to 0.4% when they stipulate that the degree of certainty that there has been adaptive evolution must be 95% or more. This raises an important issue, which is that many of these tests for adaptive evolution are very weak. Therefore the fact that many estimates are at (or very near to) 0% does not rule out the occurrence of any adaptive evolution in the human genome, but simply shows that positive selection is not frequent enough to be detected by the tests. In fact, the most recent study I mention states that confounding variables, such as demographic changes, means that the true value of α may be as high as 40% (Eyre-Walker and Keightley 2009). Another recent study, which uses a relatively robust methodology, estimates α at 10-20% Boyko et al. (2008). I will comment on weaknesses in the methods in a subsequent section, but it is clear that the debate over the amount of adaptive evolution occurring in human coding DNA is not yet resolved. Even if low estimates of α are accurate, a small proportion of substitutions evolving adaptively can still equate to a considerable amount of coding DNA. Many authors, whose studies have small estimates of the amount of adaptive evolution in coding DNA, nevertheless accept that there has been some adaptive evolution in this DNA, because these studies identify specific regions within the human genome which have been evolving adaptively (e.g. Bakewell et al. (2007)). More genes underwent positive selection in chimpanzee evolution than in human), something I will examine later. The generally low estimates of adaptive evolution in human coding DNA can be contrasted with other species. Bakewell et al. (2007) found more evidence of adaptive evolution in chimpanzees than humans, with 1.7% of chimpanzee genes showing evidence of adaptive evolution (compared with the 1.1% estimate for humans; see Table 1). Comparing humans with more distantly related animals, an early estimate for α in Drosophila species was 45% (Smith and Eyre-Walker 2002), and later estimates largely agree with this (Eyre-Walker 2006). Bacteria and viruses generally show even more evidence of adaptive evolution; research shows values of α in a range of 50-85%, depending on the species examined (Eyre-Walker 2006). Generally, there does appear to be a positive correlation between (effective) population size of the species, and amount of adaptive evolution occurring in the coding DNA regions. This may be because random genetic drift becomes less powerful at altering allele frequencies, compared to natural selection, as population size increases.

Non-coding DNA

Estimates of the amount of adaptive evolution in non-coding DNA are generally very low, although fewer studies have been done on non-coding DNA. As with the coding DNA however, the methods currently used are relatively weak. Ponting and Lunter

(2006) speculate that underestimates may be even more severe in non-coding DNA, because non-coding DNA may undergo periods of functionality (and adaptive evolution), followed by periods of neutrality. If this is true, current methods for detecting adaptive evolution are inadequate to account for such patterns. Additionally, even if low estimates of the amount of adaptive evolution are correct, this can still equate to a large amount of adaptively evolving non-coding DNA, since non-coding DNA makes up approximately 98% of the DNA in the human genome. For example, Ponting and Lunter (2006) detect a modest 0.03% of non-coding DNA showing evidence of adaptive evolution, but this still equates to approximately 1 Mb of adaptively evolving DNA. Where there is evidence of adaptive evolution (which implies functionality) in non-coding DNA, these regions are generally thought to be involved in the regulation of protein coding sequences. As with humans, fewer studies have searched for adaptive evolution in non-coding regions of other organisms. However, where research has been done on Drosophila, there appears to be large amounts of adaptively evolving non-coding DNA. Andolfatto (2005) estimated that adaptive evolution has occurred in 60% of untranslated mature portions of mRNAs, and in 20% of intronic and intergenic regions. If this is true, this would imply that much non-coding DNA could be of more functional importance than coding DNA, dramatically altering the consensus view. However, this would still leave unanswered what function all this non-coding DNA performs, as the regulatory activity observed thus far is in just a tiny proportion of the total amount of non-coding DNA. Ultimately, significantly more evidence needs to be gathered to substantiate this viewpoint.

Variation between human populations

Several recent studies have compared the amounts of adaptive evolution occurring between different populations within the human species. Williamson et al. (2007) found more evidence of adaptive evolution in European and Asian populations than African American populations. Assuming African Americans are representative of Africans, these results makes sense intuitively, because humans spread out of Africa approximately 50,000 years ago (according to the consensus Out-of-Africa hypothesis of human origins (Klein 2009)), and these humans would have adapted to the new environments they encountered. By contrast, African populations remained in a similar environment for the following tens of thousands of years, and were therefore probably nearer their adaptive peak for the environment. However, Voight et al. (2006) found evidence of more adaptive evolution in Africans, than in Non-Africans (East Asian and European populations examined), and Boyko et al. (2008) found no significant difference in the amount of adaptive evolution occurring between different human populations. Therefore, the evidence obtained so far is inconclusive as to what extent different human populations have undergone different amounts of adaptive evolution.

Rate of adaptive evolution

The rate of adaptive evolution in the human genome has often assumed to be constant over time. For example, the 35% estimate for α calculated by Fay et al. (2001) led them to conclude that there was one adaptive substitution in the human lineage every 200 years

since human divergence from old-world monkeys. However, even if the original value of α is accurate for a particular time period, this extrapolation is still invalid. This is because there has been a large acceleration in the amount of positive selection in the human lineage over the last 40,000 years, in terms of the number of genes that have undergone adaptive evolution (Hawks et al. 2007). This agrees with simple theoretical predictions, because the human population size has expanded massively in the last 40,000 years, and with more people, there should be more adaptive substitutions. Hawks et al. (2007) argue that demographic changes (particularly population expansion) may greatly facilitate adaptive evolution, an argument that corroborates somewhat with the positive correlation inferred between population size and amount of adaptive evolution occurring mentioned previously. It has been suggested that cultural evolution may have replaced genetic evolution, and hence slowed the rate of adaptive evolution over the past 10,000 years. However, it is possible that cultural evolution could actually increase genetic adaption. Cultural evolution has vastly increased communication and contact between different populations, and this provides much greater opportunities for genetic admixture between the different populations (Hawks et al. 2007). However, recent cultural phenomena, such as modern medicine and the smaller variation in modern family sizes, may reduce genetic adaption as natural selection is relaxed, overriding the increased potential for adaptation due to greater genetic admixture.

Strength of positive selection

Studies don't generally attempt to quantify the average strength of selection propagating advantageous mutations in the human genome. Many models make assumptions about how strong selection is, and some of the discrepancies between the estimates of the amounts of adaptive evolution occurring have been attributed to the use of differing such assumptions (Eyre-Walker 2006). The way to accurately estimate the average strength of positive selection acting on the human genome is by inferring the distribution of fitness effects (DFE) of new advantageous mutations in the human genome, but this DFE is difficult to infer because new advantageous mutations are very rare (Boyko et al. 2008). The DFE may be exponential shaped in an adapted population (Eyre-Walker and Keightley 2007). However, more research is required to produce more accurate estimates of the average strength of positive selection in humans, which will in turn improve the estimates of the amount of adaptive evolution occurring in the human genome (Boyko et al. 2008).

Regions of the genome which show evidence of adaptive evolution

A considerable number of studies have used genomic methods to identify specific human genes that show evidence of adaptive evolution. Table 2 gives selected examples of such genes for each gene type discussed, but provides nowhere near an exhaustive list of the human genes showing evidence of adaptive evolution. Below are listed some of the types of gene which show strong evidence of adaptive evolution in the human genome.

- *Disease genes*

Bakewell et al. (2007) found that a relatively large proportion (9.7%) of positively selected genes were associated with diseases. This may be because diseases can be adaptive in some contexts. For example, schizophrenia has been linked with increased creativity (Crespi et al. 2007), perhaps a useful trait for obtaining food or attracting mates in Palaeolithic times. Alternatively, the adaptive mutations may be the ones which reduce the chance of disease arising due to other mutations. However, this second explanation seems unlikely, because the mutation rate in the human genome is fairly low, so selection would be relatively weak.

- *Immune genes*

417 genes involved in the immune system showed strong evidence of adaptive evolution in the study of Nielsen et al. (2005a). This is probably because the immune genes may become involved in a coevolutionary arms race with bacteria and viruses. These pathogens evolve very rapidly, so selection pressures change quickly, giving more opportunity for adaptive evolution.

- *Testes genes*

247 genes in the testes showed evidence of adaptive evolution in the study of Nielsen et al. (2005a). This could be partially due to sexual antagonism. Male-female competition could facilitate an arms race of adaptive evolution. However, in this situation you would expect to find evidence of adaptive evolution in the female sexual organs also, but there is less evidence of this. Sperm competition is another possible explanation. Sperm competition is strong, and sperm can improve their chances of fertilising the female egg in a variety of ways, including increasing their speed, stamina or response to chemoattractants (Swanson and Vacquier 2002).

- *Olfactory genes*

Genes involved in detecting smell show strong evidence of adaptive evolution (Voight et al. 2006), probably due to the fact that the smells encountered by humans have changed recently in their evolutionary history (Williamson et al. 2007). Humans' sense of smell has played an important role in determining the safety of food sources.

- *Nutrition genes*

Genes involved in lactose metabolism show particularly strong evidence of adaptive evolution amongst the genes involved in nutrition. A mutation linked to lactase persistence shows very strong evidence of adaptive evolution in European and American populations (Williamson et al. 2007), populations where pastoral farming for milk has been historically important.

- *Pigmentation genes*

Pigmentation genes show particularly strong evidence of adaptive evolution in non-African populations (Williamson et al. 2007). This is likely to be because those humans that left Africa approximately 50,000 years ago, entered less sunny climates, and so were under new selection pressures to obtain enough Vitamin D from the weakened sunlight.

- *Brain genes?*

There is relatively little evidence of adaptive evolution in genes linked to brain development (Voight et al. 2006), and where there is evidence, these genes are often associated with diseases, e.g. microcephaly (see Table 2). However, there is a particular interest in the search for adaptive evolution in brain genes, despite the ethical issues surrounding such research. If more adaptive evolution was discovered in brain genes in one human population than another, then this information could be misinterpreted as showing greater intelligence in the more adaptively evolved population. Researchers should be very careful in how they present and discuss such results.

- *Other*

Other gene types showing considerable evidence of adaptive evolution (but generally less evidence than the types discussed) include: genes on the X chromosome, nervous system genes, genes involved in apoptosis, genes coding for skeletal traits, and possibly genes associated with speech (Nielsen et al. 2005a, Williamson et al. 2007, Voight et al. 2006, Krause et al. 2007).

Difficulties in identifying positive selection

As noted previously, many of the tests used to detect adaptive evolution have very large degrees of uncertainty surrounding their estimates. It is beyond the purview to look at all the modifications applied to individual tests to overcome the associated problems. However, it will briefly discuss in general terms two of what may be the most important confounding variables that may hinder accurate detection of adaptive evolution. Demographic changes are particularly problematic and may severely bias estimates of adaptive evolution. The human lineage has undergone both rapid population size contractions and expansions over its evolutionary history, and these events will change many of the signatures thought to be characteristic of adaptive evolution (Nielsen et al. 2007). Some genomic methods have been shown through simulations to be relatively robust to demographic changes (e.g. Willamson et al. 2007). However, no tests are completely robust to demographic changes, and new genetic phenomena linked to demographic changes have recently been discovered. This includes the concept of "surfing mutations", where new mutations can be propagated with a population expansion (Klopfstein et al. 2006). A phenomenon which could severely alter the way we look for signatures of adaptive evolution is bias gene conversion (BGC) (Galtier and Duret 2007). Meiotic recombination between homologous chromosomes that are heterozygous at a particular locus can produce a DNA mismatch. DNA repair mechanisms are biased towards repairing a mismatch to the CG base pair. This will lead allele frequencies to change, leaving a signature of non-neutral evolution (Galtier et al.

2001). The excess of AT to GC mutations in human genomic regions with high substitution rates (human accelerated regions, HARs) implies that BGC has occurred frequently in the human genome (Pollard et al. 2006, Galtier and Duret 2007). Initially, it was postulated that BGC could have been adaptive (Galtier et al. 2001), but more recent observations have made this seem unlikely. Firstly, some HARs show no substantial signs of selective sweeps around them. Secondly, HARs tend to be present in regions with high recombination rates (Pollard et al. 2006). In fact, BGC could lead to HARs containing a high frequency of deleterious mutations (Galtier and Duret 2007). However, it is unlikely that HARs are generally maladaptive, because DNA repair mechanisms themselves would be subject to strong selection if they propagated deleterious mutations. Either way, BGC should be further investigated, because it may force radical alteration of the methods which test for the presence of adaptive evolution.

Table 1: Estimates of the amount of adaptive evolution in the human genome

(format of table and some data displayed as in Table 1 of Eyre-Walker (2006))

α or proportion of loci that have undergone adaptive evolution (%)	Locus type	Outgroup species	Method	Study
20	Protein	Chimpanzee	MK	Zhang and Li 2005
6	Protein	Chimpanzee	MK	Bustamante et al. 2005
0-9	Protein	Chimpanzee	MK	Chimpanzee Sequencing and Analysis Consortium 2005
10-20	Protein	Chimpanzee	MK	Boyko et al. 2008
9.8	Protein	Chimpanzee	dn/ds	Nielsen et al. 2005a
1.1	Protein	Chimpanzee	dn/ds	Bakewell et al. 2007
35	Protein	Old-world monkey	MK	Fay et al. 2001
0	Protein	Old-world monkey	MK	Zhang and Li 2005
0	Protein	Old-world monkey	MK	Eyre-Walker and Keightley 2009
0.4	Protein	Old-world monkey	dn/ds	Nielsen et al. 2005b
0	Protein	Mouse	MK	Zhang and Li 2005
0.11-0.14	Non-coding	Chimpanzee	MK	Keightley et al. 2005

4	Non-coding	Chimpanzee and Old-world monkey	dn/ds	Haygood et al. 2007
0	Non-coding	Old-world monkey	MK	Eyre-Walker and Keightley 2009
0.03	Non-coding	N/A	Indel	Ponting and Lunter 2006

Table 2: Examples of human genes which show evidence of adaptive evolution

Type of gene	Gene name	Phenotype produced by gene/Region where gene expressed	Study
Disease	ASPM	Microcephaly (characterised by small head and mental retardation)	Mekel-Bobrov et al. 2005
Disease	HYAL3	Cancers, tumour suppression	Nielsen et al. 2005a
Disease	DISC1	Schizophrenia	Crespi et al. 2007
Immune	CD72	Immune system signalling	Nielsen et al. 2005a
Immune	IGJ	Links immunoglobulin monomers	Williamson et al. 2007
Immune	PTCRA	Pre T-cell antigen receptor	Bakewell et al. 2007
Testes	USP26	Testes specific expression	Nielsen et al. 2005a
Testes	RSBN1	Protein structure of sperm	Voight et al. 2006
Testes	SPAG5	Sperm associated antigen 5	Bakewell et al. 2007
Olfactory	OR2B2	Olfactory receptor	Nielsen et al. 2005a
Olfactory	OR4P4	Olfactory receptor	Williamson et al. 2007
Olfactory	OR10H3	Olfactory receptor 10H3	Bakewell et al. 2007
Nutrition	LCT	Lactose metabolism	Williamson et al. 2007
Nutrition	NR1H4	Nuclear hormone receptor related to phenotypes including bile acid and lipoprotein	Williamson et al. 2007
Nutrition	SLC27A4	Uptake of fatty acids	Voight et al.

		2006
Pigmentation OCA2	Lightened skin	Voight et al. 2006
Pigmentation ATRN	Skin pigmentation	Willamson et al. 2007
Pigmentation TYRP1	Lightened skin	Voight et al. 2006