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It's All In The Genes....

One of the most fundamental concepts of coat colour and coat patterning in horses is that they are all caused by mutations at cellular level ie in the DNA structure and the majority happen at conception. Most of these mutations change single DNA building blocks (nucleotides) in the gene and can have far reaching implications in the resulting individual ranging from simple modification of skin and hair pigment to affecting the development of enteric nerves, eye tissue, inner ear function and so on.

Mammalian hair shafts exhibit a wide range of shades. The shades reflect variation in the production of eumelanin (black) and pheomelanin (red) pigments and give rise to colours that we perceive as black, red, yellow, gray, or white hair fibres.

Two of the most important proteins in melanogenesis are the melanocyte-stimulating hormone receptor (MC1R but more commonly known as Extension) and the agouti protein. Melanogenesis is of course the process by which melanocytes produce melanin. But first let's get the basics explained….

Amino Acids

Amino acids are a set of 20 different molecules used to build proteins – they are organised by the body's cells into long chains called polypeptides which are the building blocks of proteins. The codes for the sequences in which amino acids are 'strung' together to create the various types of proteins are encoded in the genes.

Proteins

Proteins are large, complex molecules that play many critical roles in the body. They do most of the work in cells and are required for the structure, function and regulation of the body's tissues and organs.

Proteins consist of long chains of **amino acids** called polypeptides. There are 20 different types of amino acids that can be combined to make a protein. The sequence of the amino acids in the polypeptides determines each protein's unique 3-dimensional structure and its specific function.

Proteins can be described according to their function in the body – they are specialists that only perform one particular task:

Cells

Cells are the smallest independent parts of organisms. A cell is like a miniature and very complex factory that can make all the parts needed to replicate itself, which happens when cells divide.

There is a simple division of labour in cells - genes give instructions and proteins carry out these instructions to perform tasks like building a new copy of a cell or repairing damage.

As each type of protein is a specialist that only does one job, cells have to create new proteins if they require new jobs to be done. Similarly, if a cell needs to do something faster or slower than before, it makes more or less of the protein responsible. Genes tell cells which proteins to make and in what amounts.

Genes

An individual inherits two copies of each gene, one from each parent.

A gene is the basic physical and functional unit of heredity. Its primary function, as mentioned above, is to provide the information required by the cells to manufacture proteins. Genes are made from a long molecule called DNA which is copied and inherited from generation to generation. DNA and its close cousin RNA are nucleic acids made up of long chains of nucleotides.

Nucleotides

A nucleotide is the basic building block of the nucleic acids RNA & DNA. It consists of one chemical nucleobase – either adenine (A), guanine (G), cytosine (C) or thymine (T) plus a phosphate molecule and a sugar molecule. In RNA however Thymine (T) is replaced by Uracil (U) because it is apparently more stable.

RNA (Ribonucleic acid)

RNA is a family of large biological molecules that perform multiple vital roles in the coding, decoding, regulation, and expression of genes. An RNA strand has a single backbone made of alternating sugar (ribose) and phosphate groups. Attached to each sugar is one of four nucleobases—adenine (A), uracil (U), cytosine (C) or guanine (G).

RNA primarily transmits the genetic information from DNA that is required by the cells to produce the various proteins. There are several different types of RNA in the cell: messenger RNA (mRNA), ribosomal RNA (rRNA) and transfer RNA (tRNA). Each of these has a specific function to carry out in the coding and decoding process.

DNA (Deoxyribonucleic acid)

DNA is a long molecule, made up of nucleotides, that stores information as a code built from the four chemical nucleobases in the nucleotides: adenine (A), guanine (G), cytosine (C), and thymine (T). These nucleotides pair up with each other, A with T and C with G, to form units called base pairs which are arranged in two long strands that form a spiral called a double helix. The structure of the double helix is somewhat like a ladder, with the base pairs forming the ladder's rungs and the sugar and phosphate molecules (each nucleotide also contains a sugar molecule and a phosphate molecule (*see above*)) forming the vertical sidepieces of the ladder.

The order, or sequence, in which these base pairs line up within a DNA molecule provides information via a genetic alphabet

called the genetic code, similar to the way in which letters of the alphabet appear in a certain order to create words and sentences which provide information. The genetic code allows the genetic 'machinery' to read the information in the genes, which it does in triplet sets of codons. This information is the blueprint for constructing and operating a living organism.

DNA is stored in structures called chromosomes. For the most part, chromosomes come in matched sets, one chromosome from each parent.

Codons

A **codon** (triplet nucleotide sequence) is a sequence of three nucleotides that codes for a certain amino acid or signals the termination of translation (stop or termination codon).

Introns and Exons

An exon is the portion of a gene sequence that codes for amino acids so is the part of the gene sequence that is expressed in the protein that is built from that gene sequence. An intron is a portion of a gene sequence that comes in between the exons and does not code for amino acids. In the cells of plants and animals, most gene sequences are broken up by one or more introns.

How genes direct the production of proteins….

Most genes contain the information needed to make specific proteins but a few genes do produce other types of molecules that help the cell physically assemble the proteins. The journey from gene code to functioning protein is complex and tightly controlled within each cell. It consists of two major steps: transcription and translation. Together, transcription and translation are known as gene expression.

Transcription & Translation or Gene Expression

During the process of *transcription*, the information stored in a gene's DNA is transferred to a similar type of molecule called RNA (ribonucleic acid) in the cell nucleus. RNA, like DNA, is made up of a chain of nucleotide bases but RNA has slightly different chemical and structural properties to DNA. Thymine (T) is replaced by Uracil (U) and RNA is single stranded, not double stranded. There are several different types of RNA which each play their own specific role in the gene expression process.

Messenger RNA (mRNA) is a single-stranded RNA molecule that acts as a messenger template by becoming a replica of a section of DNA. It carries this information from the DNA out of the nucleus of the cell into the cytoplasm where *translation*, the second step in getting from a gene code to a protein, takes place.

In the cytoplasm the mRNA interacts with a ribosome (a specialized complex consisting of **Ribosomal RNA** (rRNA) and proteins) that "reads" the sequence of mRNA bases and translates this genetic code into its coded amino acid. The genetic code is a series of codons that each codes for a specific amino acid or signals the termination of translation (stop or termination codon),

Transfer RNA (tRNA) then assembles the protein, one amino acid at a time according to the code that is translated by the ribosomes from the mRNA which copied it directly from the DNA. Protein assembly continues until the ribosome encounters a "stop" codon (a sequence of three bases that does not code for an amino acid).

Transfer RNA (tRNA) is a small RNA molecule with triplet nucleotide sequences that are complementary to the triplet nucleotide coding sequences of mRNA. Each tRNA molecule has two important areas: a trinucleotide region called the anticodon* and a region for attaching a specific amino acid. Their function is to bond with the right amino acids and transfer them to the ribosomes where proteins are assembled according to the genetic code carried from the DNA by mRNA. They do this by forming base pairs with their complementary triplet nucleotide sequences on the mRNA molecule to ensure that the correct amino acid is inserted into right spot in the protein.

***Anticodon** - the sequential set of three nucleotides in transfer RNA that interacts with its complementary codon in messenger RNA during the transfer of amino acids in the ribosome.

This flow of information from DNA to RNA to proteins is one of the fundamental principles of molecular biology. It is so important that it is sometimes called the "central dogma."

If the sequence of the nucleotides in a gene changes, the sequence of the amino acids in the subsequent protein it blueprints for could also change which may then change the way the protein functions. Likewise, if part of a gene is deleted, the protein produced is shorter and may not work any more or may work in a completely different way to the original.

DNA Replication

Another important property of DNA is that it can replicate, or make copies of itself. Each strand of DNA in the double helix can serve as a pattern for duplicating the sequence of bases. This is critical when cells divide because each new cell needs to have an exact copy of the DNA present in the old cell. Every time a cell divides into two new cells the genes it contains are also copied into each new cell – this process is called DNA replication.

DNA can be copied very easily and accurately because each piece of DNA can direct the creation of a new copy of its information. DNA is made of two strands that pair together like the two sides of a zipper. The nucleotides are in the center, like the teeth in the zipper, and pair up to hold the two strands together. Importantly, the four different sorts of nucleotides are different shapes, so for the strands to close up properly, an **A** nucleotide must go opposite a **T** nucleotide, and a **G** opposite a **C**. This exact pairing is called base pairing.

When DNA is copied, the two strands of the old DNA are pulled apart by enzymes that move along each of the two single

strands pairing up new nucleotide units and then zipping the strands (one old, one new) closed. This produces two new pieces of DNA, each containing one strand from the old DNA and one newly made strand. This process isn't perfect and sometimes the proteins make mistakes and put the wrong nucleotide into the strand they are building which causes a change in the sequence of that gene. These changes in DNA sequence are called mutations.

Mutations produce new alleles of genes. Sometimes these changes stop the gene from working properly. In other cases these mutations can change what the gene does or even let it do its job a little better than before. Mutations can also produce new traits, such as when mutations to an allele for black pigment produces a new allele that causes the production of red pigment instead. These mutations and the appearance of new traits are an important part, and one of the causes, of evolution.

Alleles

Alleles are essentially forms of the same gene with small differences in their sequence of DNA bases. These small differences contribute to each individual's unique physical features.

Genes control a particular characteristic and the **alleles** of that gene produce variations in those characteristics through mutations to the original genetic codes. For instance, all horses have a pair of extension genes that control which colour pigment their skin cells produce so **all** horses must produce either black or red coat pigment. Which one they produce though depends on what combination of the two 'versions' or alleles of the extension gene they carry. If the two versions are the same, the individual is homozygous for that gene. If they have one of each, the individual is heterozygous for that gene.

Chromosomes

A **chromosome** is an organized structure of DNA and protein found in cells. It is a single piece of coiled DNA containing many genes, regulatory elements and other nucleotide sequences. Horses have 64 chromosomes (or 32 pairs).

Locus (plural Loci)

A locus is the specific physical location of a gene or other DNA sequence on the chromosome, like a genetic street address.

Genetic mutations

A gene mutation is a permanent change in the DNA sequence that makes up a gene. Mutations range in size from a change to a single DNA building block (DNA base) to changes to a large segment of a chromosome.

To function correctly, each cell depends on thousands of proteins to do their jobs in the right places at the right times. Sometimes though mistakes made in the reading of the genetic code or in the construction of proteins prevent one or more of these proteins from working properly. By changing a gene's instructions for making a protein, a mutation can cause the protein to malfunction or to be missing entirely. When a mutation alters a protein that plays a critical role in the body, it can disrupt normal development or cause a medical condition. In some cases, gene mutations are so severe that they prevent an embryo from surviving until birth. These changes occur in genes that are essential for development and often disrupt the development of an embryo in its earliest stages. Because these mutations have very serious effects, they are incompatible with life.

Only a small percentage of mutations cause genetic disorders—most have no impact on health or development; for instance some mutations alter a gene's DNA sequence but do not change the function of the protein made by the gene.

Each cell also has a number of pathways through which enzymes recognize and repair mistakes in DNA so in many cases gene mutations that could cause a genetic disorder are repaired before the gene is expressed and an altered protein is produced. A very small percentage of all mutations do actually have a positive effect. These mutations lead to new versions of proteins that help an individual better adapt to changes in his or her environment and is an integral part of the evolution process.

Gene mutations occur in two ways: they can be inherited from a parent or acquired during a person's lifetime. Mutations that are passed from parent to child are called **hereditary mutations** or germline mutations (because they are present in the egg and sperm cells). This type of mutation is present throughout a person's life in virtually every cell in the body and in horses, this is the type of mutation that is responsible for the various coat colours and most of the coat patterns that we see.

Mutations that occur only in an egg or sperm cell, or those that occur just after fertilization, are called **new (de novo) mutations**. These mutations are responsible for some of the coat patterns we see like Dominant White, where a pure white foal is randomly born from two solid parents. Subsequent DNA analysis indicates that a spontaneous or de nova mutation has occurred in the genetic coding of that individual at conception or shortly thereafter resulting in a vastly altered phenotype (appearance) to that of the parents and in variations to the genetic material that was supplied by the parents. Some of these mutations have also been found to be unique to that particular individual and its subsequent offspring in that other unrelated individuals exhibiting a similar phenotype have been found to have a different mutation again. This is particularly so with the Dominant White coat patterns – to date some 15 odd different 'families' of dominant whites have been identified and each family carries its own unique mutation which can be directly traced back in most cases to a single individual in that family. Of course, once a de nova mutation exists it then gets passed on to subsequent generations as a hereditary mutation.

Acquired (or somatic) mutations occur in the DNA of individual cells at some time during a person's life. These changes can be caused by environmental factors such as ultraviolet radiation from the sun, or can occur if a mistake is made as DNA copies itself during cell division. Acquired mutations in somatic cells (cells other than sperm and egg cells) cannot be passed on to the next generation.

Mutations may also occur in a single cell within an early embryo. As all the cells divide during growth and development, the individual will have some cells with the mutation and some cells without the genetic change. This situation is called mosaicism.

Alteration of DNA sequences & types of mutations

The DNA sequence of a gene can be altered in a number of ways.

- Insertion - the introduction of foreign DNA sequences into a gene.
- Deletion - deletion of DNA sequences from the genetic material. Small deletions may remove one or a few base pairs within a gene, while larger deletions can remove an entire gene or several neighboring genes. The deleted DNA may alter the function of the resulting protein(s)
- Duplication - a duplication consists of a piece of DNA that is abnormally copied one or more times. This type of mutation may alter the function of the resulting protein.

Types of 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. Point mutations include:

- **Missense mutation** - a point mutation in which a single nucleotide is changed, resulting in a codon that codes for a different amino acid. This can render the resulting protein non-functional. A common example in horses is the missense mutation carried by red based horses in their MC1R, or Extension, gene which makes the protein produced by this gene (involved in triggering the production of black pigment in the melanocytes) non-functional.
- **Nonsense mutation** – a point mutation where an amino acid codon is changed into a termination codon. This causes the protein to be shortened because of the stop codon interrupting its normal code. How much of the protein is lost determines whether or not the protein is still functional. Several families of Dominant Whites have a nonsense mutation in one of the alleles on their KIT gene which affects the distribution of melanocytes during foetal development.
- A **frameshift mutation** is caused by the addition or removal of a number of nucleotides. Because nucleotides are grouped into triplet nucleotide sequences or codons (ie a sequence of three nucleotides that codes for a certain amino acid) adding or removing a 'non-three' number of nucleotides alters the grouping of the remaining nucleotides resulting in a completely different translation from the original. The earlier in the sequence the deletion or insertion occurs, the more altered the protein.

Nucleotide repeats are short DNA sequences that are repeated a number of times in a row. For example, a trinucleotide repeat is made up of 3-base-pair sequences, and a tetranucleotide repeat is made up of 4-base-pair sequences. A repeat expansion is a mutation that increases the number of times that the short DNA sequence is repeated. This type of mutation can cause the resulting protein to function improperly.

The 'dilute causing' mutations

When it comes to colour genetics there are two main types of genetic modifications – those that cause changes in coat colour and those that create coat patterns. Each is caused by distinctly different genetic processes although both are caused by the actions of alleles.

The alleles that affect coat colour modify either the production or the distribution of coat pigment but do not modify the pigment cells themselves.

The alleles that cause the various coat patterns modify the production, physical placement and distribution of pigment cells but do not alter coat pigment. This produces distinct patterns of unpigmented pink skin and corresponding white hair.

Melanocytes and Melanin

Melanocytes are the specialised cells that produce and contain the pigment called melanin. They are located in the bottom layer of the skin, in the light-sensitive tissue at the back of the eye (the retina) where they play a role in normal vision, in the inner ear and in various other organs. Melanin is the substance that gives skin, hair and eyes their colour ie pigment.

Melanocytes make two forms of melanin - eumelanin (black/brown) and pheomelanin (yellow/red). Which one they produce is controlled by the actions of the **melanocortin 1 receptor,** a protein that is produced by the MC1R or Extension gene. By default, melanocytes produce pheomelanin which is interesting given that the production of black pigment or eumelanin is actually the wild type and therefore the dominant process but more on that below.

Eumelanin & Pheomelanin

In animals melanin pigments are derivatives of the amino acid tyrosine. The most common form of melanin in most species is eumelanin, a **black / brown** polymer consisting of dihydroxyindole carboxylic acids and their reduced forms**.** Pheomelanin is the other common form of melanin and is a cysteine-containing **red / yellow** polymer of benzothiazine units.

The first step in the production of both eumelanins and pheomelanins is initiated by tyrosinase, a copper containing enzyme that stimulates the production of melanin (and other pigments) from the amino acid tyrosine through a process called oxidation. This is essentially the same type of process that turns things like peeled potatoes, apples etc black once they're exposed to air. The first two steps in the process of producing melanin are the same for both eumelanin and pheomelanin but after that there is a 'fork' in the road. Which fork the process takes depends, in horses, on what directions are received from another gene known commonly as the extension gene.

Step one - Tyrosine - DOPA - dopaquinone

If instructions to the contrary are not received dopaquinone will combine by default with cysteine (another amino acid) to produce benzothiazines and ultimately **pheomelanin.**

If alternative instructions are received, dopaquinone will undergo a slightly more complex conversion to produce the dihydroxyindole carboxylic acids that form **eumelanin**.

The Extension Locus

The Extension locus is the genetic 'address' for the MC1R or Extension gene.

The MC1R or Extension gene (alpha melanocyte stimulating hormone receptor)

The MC1R gene provides instructions for making a protein called the *melanocortin 1 receptor protein* that controls which type of melanin is produced by melanocytes. This receptor protein is located in the outer membrane of the skin cells (melanocytes) where it waits to be activated (or not) by a chemical called the *alpha-melanocyte-stimulating hormone* (á-MSH). This hormone is produced by the pituitary gland and when it comes into contact, or binds, with the MC1R receptor protein it activates that protein, triggering a series of chemical reactions inside the melanocytes that stimulate these cells to produce black-brown pigment (eumelanin). If the receptor protein is not activated or is blocked the melanocytes make yellow-red pigment (pheomelanin) by default instead of eumelanin. These two different processes are briefly outlined above.

Red based horses ie chestnuts carry a missense mutation in their MC1R gene which results in a 'loss of function' of the melanocortin 1 receptor protein produced by the gene (a missense mutation replaces one amino acid with another). Essentially this altered protein is not able to bind with the á-MSH and 'switch on' the production of black pigment (eumelanin) in the melanocytes so, in the absence of any instructions to the contrary, the melanocytes make yellow-red pigment (pheomelanin) instead by default.

The wild type or 'normal' version of the MC1R gene does not carry this missense mutation so produces a fully functional MC1R protein. Horses which carry either one or two copies of the wild type version are able to produce black pigment which means that in order for a horse to be totally unable to produce black hair pigment it must carry two copies of the defective version.

In most cases the wild type version of a gene is the dominant one and if carried will be expressed ie will be reflected in the phenotype of the individual. The altered copy of a gene is usually recessive so an individual will only express it if they carry two copies of an altered gene. This is known as recessive and dominant inheritance.

The Agouti Locus

The Agouti locus is the genetic 'address' for the ASIP or Agouti gene.

Agouti - the ASIP gene (agouti signalling peptide)

The ASIP or Agouti gene produces a paracrine signalling peptide which disables MC1R by attaching itself to the melanocortin 1 receptor protein molecules to block or nullify the actions of á-MSH. This prevents á-MSH from contacting and binding with the melanocortin 1 receptor protein molecules so those molecules remain 'dormant' and do not send off the required signals to instruct the melanocytes to switch to producing black pigment (they continue to make red pigment by default). However, in horses this disabling does not occur over the entire body but is restricted to certain areas. As this is the wild type or dominant version of the gene a horse that carries either one or two copies of this gene will present as a red bodied horse with black points in the absence of other modifers.

One of the alternative alleles of the wild type version of the ASIP gene carries a frameshift mutation caused by a deletion in the second exon that is thought to act as a 'loss of function' mutation. This means that this altered version of the gene is unable to produce a functioning peptide so when an individual carries two copies of this altered version they can't produce any functional peptide at all. These horses present as totally black with all over black pigmentation in the absence of other modifiers. Horses that carry one functioning version and one non-functioning version can still produce a functional peptide so will still present as a red bodied horse with black points in the absence of other modifiers.

The third version of ASIP produces the brown phenotype but the science behind this allele is as yet unpublished.

Red based horses can also carry any combination of the ASIP alleles but because they already have a defective MC1R gene that makes their receptor protein incapable of bonding with á-MSH anyway, ASIP has no effect.

***SLC45A2 (Solute Carrier 45 family A2)* - MATP gene (membrane-associated transporter protein)**

Transporter families

Several families of transporter proteins control and facilitate exchange of amino acids and their derivatives between cells and their environment, between cells and their organelles and between cells and other living entities.

SLC45A2 or MATP is one of these transporters; it codes a membrane transporter protein that assists in melanin synthesis. Membrane transporter proteins are like 'doors' located within cell membranes where they allow substances like ions, small molecules and other proteins to pass through the membrane. Each transporter protein is designed to recognize only one substance or one group of very similar substances and research has correlated defects in specific transporter proteins with specific diseases.

As the MATP protein is involved in melanin synthesis it plays an important role in the pigmentation process. Mutations that reduce or eliminate its functionality in this process inevitably lead to changes in skin, hair and eye colouration - in humans such mutations have been identified as a factor in the light skin of Europeans.

In horses a single base change in exon 2 of the MATP gene results in an aspartic acid to asparagine substitution. As with similar mutations in the human version of this gene, this affects the nature of the pigments produced by melanocytes in individuals that carry this mutation and the resulting 'dilution' effect is referred to as **cream**. *It is important to note that the skin, eyes and*

hair of horses with the cream mutation do not lack melanocytes, melanosomes or melanins but rather exhibit hypomelanism, a term that generally refers to a reduction in the amount of dark pigmentation in an animal. This is perceived as being an overall brightening which produces a more brilliantly coloured animal.

Cream is unique amongst the pigment dilution mutations in that it has an incompletely dominant mode of expression. In heterozygous form it dilutes only pheomelanin but homozygosity for the mutation results in extreme dilution of both pheomelanin and eumelanin.

The mutation's preferential effect on pheomelanin has not yet been explained. Cream also affects the mane and tail to a greater extent than the body coat, a feature most vividly illustrated in the palomino coat colour. This characteristic of the cream gene is also unexplained but given that the PMEL17 mutation found in silver horses also affects mane and tail pigment more than body pigment, my guess is that it most likely has something to do with the pigment properties of the mane and tail which allows for greater dilution.

Pearl is a mutation of cream but to date the scientific research that discovered this has not been made readily available.

SLC36A1 (Solute Carrier 36 family A1)

SLC36A family members are solute carriers and whilst other solute carrier families have been found to play a role in coat colour, SLC36A1 was not known to be likewise involved until very recently.

This particular gene encodes a member of the eukaryote-specific amino acid/auxin permease (AAAP) 1 transporter family. It has a pH-dependent electrogenic transport activity for small amino acids such as glycine, alanine and proline so an increase in pH is required before the tyrosinase (*this is the copper containing enzyme that stimulates the production of melanin (and other pigments) from the amino acid tyrosine through a process called oxidation*) can be activated. The cytosolic pH gradient must also be maintained for proper sorting and delivery of the other proteins required for melanosome development. Therefore it is possible that a mutation in this gene may alter the pH gradient of the cell and subsequently affect the correct sorting and delivery of other proteins required for normal melanosome development, leading in turn to changes in pigment intensity in hair and skin.

In *Champagne* horses a DNA base substitution in the second exon of this gene has been found to change an amino acid in the transmembrane domain of the protein from threonine to arginine. The phenotypic effect of this base change is a reduction or dilution of hair and skin colour intensity for both red and black pigment in horses so it is theorized that this substitution affects the pH levels required to stimulate the melanin production process which leads to a corresponding reduction in the intensity of the pigment produced.

Until this discovery it was not known that *SLC36A1* plays a role in the pigmentation process.

SILV or PMEL17 (melanocyte protein 17 precursor / premelanosome protein / silver locus protein homolog)

PMEL is a transmembrane glycoprotein that is expressed primarily in pigment cells of the skin and eye. The expression of the PMEL gene is regulated by the microphthalmia-associated transcription factor (MITF). The protein plays a central role in the biogenesis of the early stages of melanosomes, which are the organelles within melanocytes that directly produce pigment.

A missense mutation in PMEL17 on horse chromosome 6 results in the silver coat colour dilution in the horse. The mutation, located in exon 11, changes the second amino acid in the cytoplasmic region from an arginine to a cysteine. An additional mutation located in intron 9 also shows complete association with the Silver phenotype in research done to date.

In contrast to cream, the *Z* locus is fully dominant and affects only eumelanin causing little to no visible change in the amount of pheomelanin regardless of zygosity. The change in eumelanin is most apparent in the mane and tail where the black base color is diluted to white and gray.

To date the mutation that creates the dun dilutes has not yet been located although it is known where the mutatation arises.

This article has been compiled with the assistance of my good friends the Genetics Home Reference site & Wikipaedia as well as original papers produced during studies of the genes disccussed.

2013 membership renewals were emailed out early in December to members with a listed email address. Please advise the national office as soon as possible if you did not receive it. Members without an email address were posted a hard copy.