Scientists have discovered 3 different variations in DNA sequencing in the TBX3 gene. D, which is the original or wild type version of the gene, produces asymmetrical distribution of pigment in the hair shafts on certain parts of the body (the dilution part of dun) and allows intense symmetrical colouration on other parts of the body (dun factors). The 2 variations they've found are non-dun1 (nd1) and non-dun2 (nd2).

The nd1 variation, caused by an SNP mutation dubbed SNP1, has lost much of the asymmetrical pigment distribution coding but retained the coding that allows the intense symmetrical colouration on certain parts of the body. So nd1 horses, in the absence of D, present with strong 'dun factors' but do not have the dilution that comes from the asymmetrical distribution of pigment in the hair shaft. Therefore they cannot be considered true genetic duns and are not eligible for dun registration.

D and nd1 have been found in a prehistoric horse that predates domestication. By contrast nd2 has not been found outside the domestic population of horses or in any of the closest relatives to the ancestor of the modern horse (ie Przewalski's Horse), which indicates that nd2 is a fairly recent mutation that happened after domestication. This is supported by the fact that is very little nucleotide diversity in nd2 DNA, which is consistent with a new mutation. nd1 however showed quite extensive nucleotide diversity which again is consistent with an ancient mutation. Interestingly enough Dun also has very little nucleotide diversity in the modern horse but has quite extensive nucleotide diversity in Przewalski's Horse. It's believed this is most likely due to domestication where selective breeding for non-dun reduced the genetic pool of true Duns and caused a bottleneck effect.

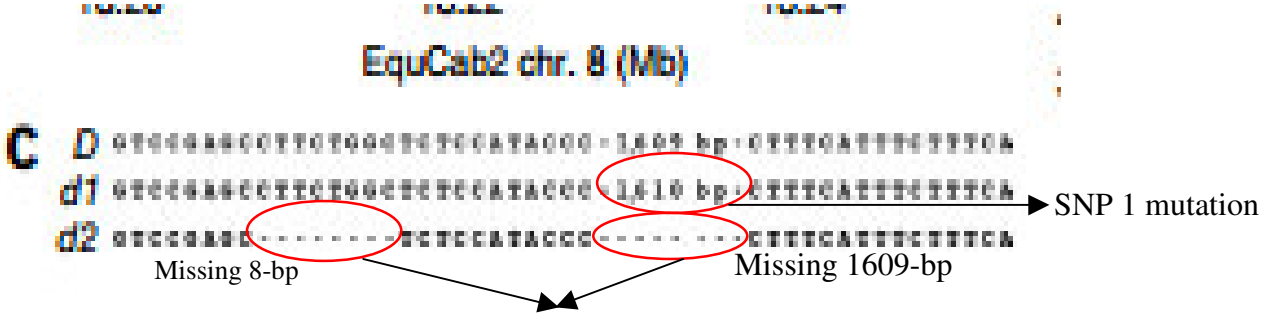
How did it come about?

At some point early on in evolution, an individual was born with a spontaneous single nucleotide polymorphism (SNP1) in the DNA sequencing in one of their TBX3 chromosomes that altered the asymmetrical pigment distribution coding and changed it to a more symmetrical distribution. A SNP is a single-nucleotide substitution of one base for another. The ~ 42,700 year old horse they tested was D/nd1 indicating that the SNP mutation occurred very early on in the horse's evolution. The ~4,400 year old horse was nd1/nd1.

Mendelian inheritance laws tell us that the original D/nd1 horse would have produced offspring that had a 50/50 chance of being DD or D/nd1 and then those D/nd1 offspring similarly produced DD and D/nd1 offspring until eventually 2 D/nd1 horses crossed with a 25% chance of producing an nd1/nd1 offspring and the first genetic non-Dun was born.

Somewhere further down the track, after or around the time the horse was domesticated, another spontaneous mutation occurred in an individual carrying at least one copy of nd1. (Haplotype comparisons and breed distribution suggest that the non-dun2 deletion arose on a non-dun1 chromosome and then increased in frequency, a history consistent with persistent selection during and after horse domestication for the non-dun phenotype; in fact, selection against camouflage colour is likely an important reason for changes in coat colour during animal domestication).

This individual was born with a deletion mutation, which essentially means a chunk of code was missing from its DNA, including the section containing the SNP1 mutation that caused nd1. Somewhere in this missing chunk of code are also the instructions for allowing the more intense symmetrical colouration on certain areas of the body indicative of D and nd1. So the nd2 allele had lost both the dilution and the ability to produce intense colouration in some parts of the body.



The Dun haplotype has 1,617 bp that is missing from the EquCab2 assembly, which is based on a non-dun horse. The missing sequence consists of nearly contiguous 1,609-bp and 8-bp segments.

We'll probably never know whether the individual in which the nd2 mutation first appeared was D/nd1 or nd1/nd1. Either way, the progress to intensely coloured, non-stripey nd2/nd2 individuals would have been similar to that which produced nd1/nd1 only aided and abetted by humans who decided they preferred the more intense and even colouring of the non-dun horse so began breeding for that attribute.

D is dominant over nd1 and nd2; nd1 is dominant over nd2.