A SERENDIPITY

WHY ARE BLUE RABBITS the same as LAVENDER CHICKENS

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Translated from Dutch
to English '25.

Serendipity is when you find something useful that you weren't looking for. This was the case with the article on the colour diluter 'd' in rabbits, which was published on 20 December 2008. While I was researching black diluters in chickens, I stumbled upon it by chance during my tour of the sites on which studies are published. My search was to find a replacement for the colour coffee latte (choc-based), which looked double-diluted, as if diluted by lavender. In 2008, I did not know what choc-lav looked like, so I thought it was darker than it actually was, as I later found out.

The term 'homology' is used when referring to the equal effect of a similar gene in both chickens and mammals. This indicates a common ancestor. This article discusses three gene mutations that cause the dilution of black and red pigments in hairs and feathers. In chickens, this is known as 'lavender', and in rabbits (in Europe), it is known as 'blue'.

The dilution of colour in rabbits is called 'dilution' (d), which is literally its meaning. In chicken genetics, this is known as 'lavender' (lav). In rabbit genetics, the diluter gene (d) is a single autosomal gene, meaning it is present in both sexes and is recessive.

In January 2008, the research paper 'A single point mutation within the melanophilin gene causes the lavender plumage colour dilution phenotype in chickens' was published. This paper explained how the lavender gene, as we know it, prevents 100% (wild-type) black or red pigment from entering the feathers. In 2012, Cor Tensen (NL) pointed out to me that his blue marten rabbits were similar to lavender chickens, which led to a discussion about comparing the colour genes of mammals and chickens. However, Leif Andersson (University of Uppsala, Sweden) had warned me that not everything was comparable, so I was sceptical of the claim. Lavender is actually the result of three altered genes, so the interaction is different and the pigment (black or red) does not reach the keratin in its usual maximum dose. The 2008 study already mentioned a mutation in the MLPH (melanophilin) gene that causes colour dilution. The effect in chickens is similar to that in quail: blue quail x lavender chicken produced hybrids with a diluted colour, meaning that blue in quail is the same as lavender in chickens (Testing Homology of Loci for Two Plumage Colours, 'Lavender' and 'Recessive White', with Chicken and Japanese Quail Hybrids, 2002). Although the list of animal species with the MLPH colour dilution is long, it has only really been substantiated in rabbits this year (2008).

In rabbits, dilution of black leads to blue and cream-brown, and dilution of red leads to yellow and cream-yellow. The gene d is also known as a colour diluter in cats, chickens, quails, mice, foxes and minks, although the list is much longer. Colour dilution also occurs in dogs and can be accompanied by unpleasant side effects such as baldness or patchy poor coat and skin quality (alopecia, blue dog disease) in diluted colours “fawn” (diluted red) and “blue” (diluted black).

There are specific breeds that are affected by this, and not every fawn or blue dog is affected. This is somewhat similar to wing patch and feather shredder in lavender chickens, which also does not occur in every lavender chicken. The study referred to in this article states that similar problems with the coat/feathers have not been reported other than in dogs. However, mentioning additional changes in the phenotype in dilution does give a different perspective on the feather shredder seen in many lavender chickens. Orpington breeders in Europe have mentioned that lavender is lethal when two lavenders are crossed. The breeders then spread the message: only cross two heterozygous lavenders (splits), as this reduces the risk of lethality. Theoretically, this would not matter if there was something lethal attached to the MLPH gene in its homozygous form. Breeding only with splits works if the problem is caused by another gene that is recessive and unrelated to lav. The (alleged) lethality of lavender has not occurred in other chickens, purebred or project...

I sent an email to Hanover with a few pages showing the variations in feather quality in the presence of lavender. The next day, I received an email saying that the university was willing to investigate the feather shredder in chickens raised on lavender. Follow-up: never heard from them again.

A condition that can be traced back to disturbed pigment deposition in the hair follicle also occurs in humans: Griscelli syndrome (Wikipedia, images), which also involves a dilution of hair colour and the accumulation of pigment in the hair shaft and pigment granules in the pigment-forming cells (melanocytes). A similar phenomenon occurs in cats, foxes and mice. In dogs with a diluted coat colour, there was a huge clumping of pigment in the hair shafts, hair follicles and hair bulb, as well as in the skin, both the epidermis and the dermis.

The coordination of the pigment formation process is determined by three events: melanosome capture (movement of the pigment granule from the nucleus of the pigment cell to the ends of the cell), melanosome transport and distribution. This requires three proteins (actually protein complexes), namely Rab27a (homologous in mammals, also associated with a family of cancer-causing genes), melanofilin (MLPH) and Myosin Va. In this trio, it is Rab27a that binds the pigment granules and also binds melanofilin, which in turn binds Myosin Va. Myosin Va acts on actin filaments (small proteins in the keratin cell), causing the pigment granule to be transported within the pigment cell so that it leaves the cell and moves to the keratin cell.

The above collaboration of this protein-coding trio can do more than dilute hair colour -> Griscelli syndrome in humans. Ultimately, the reason for studying chickens (or rabbits, mice, etc.) is to find out what the underlying cause of a mutation in humans is. Griscelli syndrome is interesting because, depending on which of the three genes is defective, the phenotypes (visible characteristics) differ. Griscelli syndrome is divided into three types depending on the defective gene in the trio. Type 1 is caused by a mutation in MYO5A. Dilution or complete absence of pigment production is often accompanied by neurological problems. Type 2 is caused by a mutation in the Rab27a gene and those who have it have a problem with their immune system in combination with a diluted hair colour.

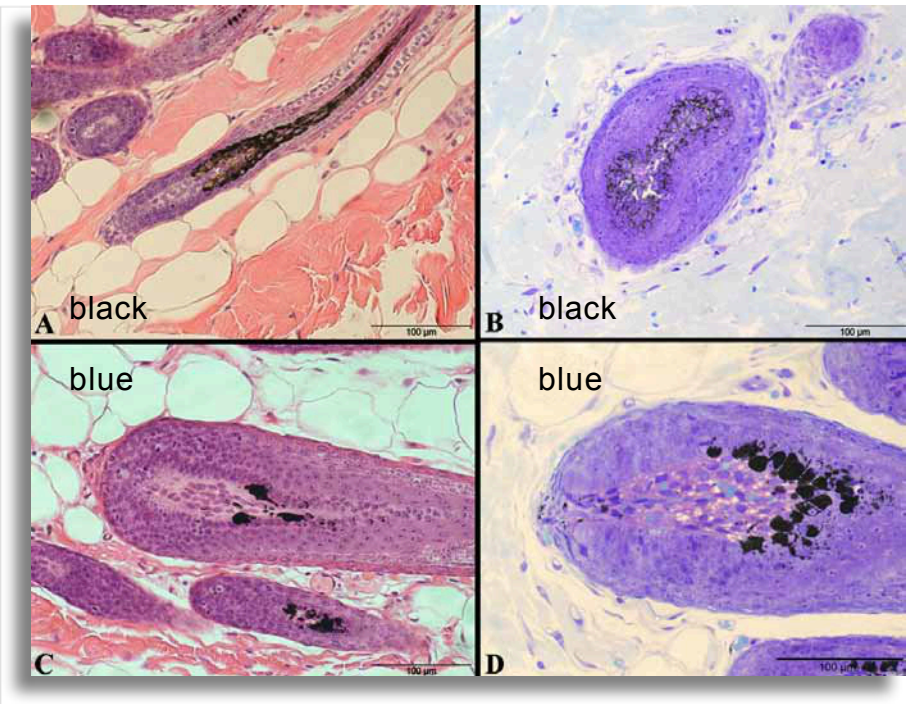
For those interested: Dilution of hair/feather colour occurs in many other species. In cats, this is caused by a deletion of one base pair in exon 2, leading to a premature stop codon 11 amino acids lower. This mutation results in a similarly truncated protein as in rabbits. Most other mutations that cause diluted colour in different species involve a single base exchange in the RAB27A binding domain. In lavender chickens and also in Griscelli syndrome type 3, the causative mutation is an R35W exchange. Dark blue-coloured mice show a deletion of 6 amino acids in positions 31-37. Other mutations have been described in dogs and quails. In dogs, a polymorphism of the last nucleotide of the first untranslated exon of MLPH can be completely associated with the diluted coat colour. In quails, the causative mutation is quite complex because it involves a number of chromosomal rearrangements that affect MLPH, but also three other genes. In conclusion, the diluted phenotype in rabbits is probably caused by two exon skipping (skipping a piece) of exon 3 and 4 and a frameshift of the open reading frame, causing a premature stop codon. The c.111-5C>A mutation located between intron 2 of MLPH is the most likely cause of this exon skipping and therefore the coat colour of these rabbits is diluted. The dilution also appears to be caused by another frameshift, but this was only found in one animal (c.585delG) that was homozygous for this. This deletion does not affect RAB27A binding as c.111-5C>A does. The mutations found in rabbits also occur in other species, providing additional insight into splicing mechanisms (in pre-mRNA).

Type 3 is caused by a mutation in MLPH (melanophilin) or the MYO5A gene, and clinical symptoms only occur if MLPH is defective. Griscelli syndrome in humans (types GS1, GS2 or GS3) corresponds to dilution (d) blue in mice, and GS1 also corresponds to lavender foal syndrome in (e.g. Arabian) horses. These animals have overstretched/cramped back muscles, causing them to lie stretched out/arched on the ground.

Because a mutation in MLPH has no negative physical consequences for the rest of the rabbit except for a dilution of the hair colour (the same applies to chicken feather colour; lavender chickens are otherwise healthy), this gene and the mutation have been studied in rabbits, as was previously done in chickens in 2008. A check was carried out for pieces of DNA from the melanofilin gene in various rabbit breeds. Three pieces were used (CA, delG, TC) and it was examined who was homozygous or heterozygous for what and what the phenotype was.

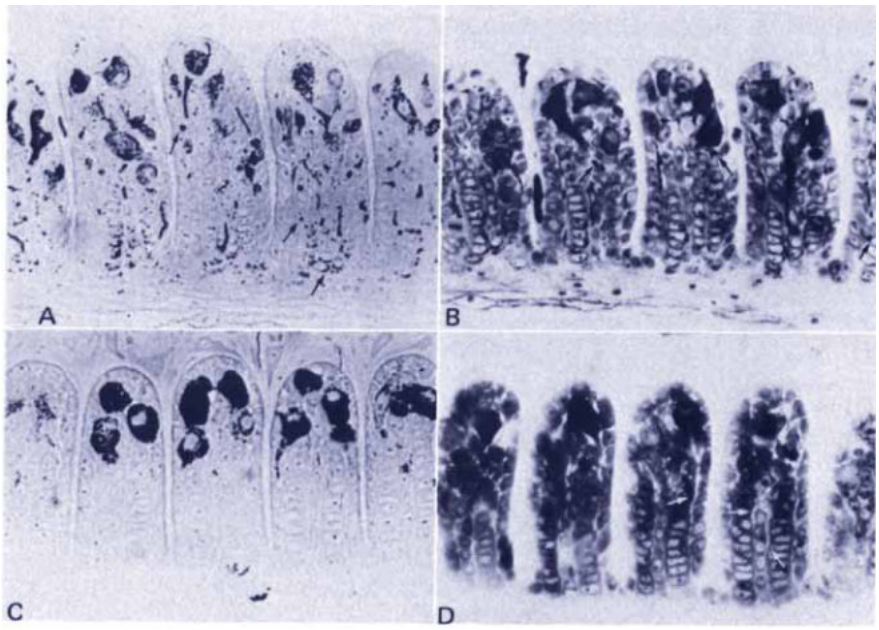
Why explain a homologous colour mutation, which also occurs in chickens, using rabbits? Because this recent article provides a much clearer explanation than the 2008 article on lavender in chickens, which provided far fewer comparisons and did not explain the specific mechanism, making it difficult for a layman to understand the full scope of the phenomenon, despite Wikipedia and other sources.

RABBIT: black and blue (d)



A and B black rabbit, C and D blue rabbit. Length of cross sections (A, C, D) and cross section B through the hair bulb. What is striking is the uneven distribution of pigment granules and clusters of melanin in the blue rabbit.

CHICKEN: lavender (lav) and black



Feather hairs from a feather that has been growing for two weeks. A and B are black, C and D are lavender. In the black feather, the pigment has penetrated into the dendrites, while in the lavender feathers, the pigment granules have clumped together and remain around the cell nucleus instead of being transported to the dendrites. Images: Brumbaugh 1981.

At the bottom, two animals are listed, one of which was heterozygous for SNP c.111-5C>A and the other for SNP c.585delG. These animals were dark blue compared to those that were homozygous for all tested pieces.

This darker colouring in blue rabbits is typical, as nothing similar is known in chickens. In chickens, the very light lavenders are those with blue or splash underneath. Returning to rabbits, the c111-5C>A polymorphism of the MLPH gene is the most likely cause of diluted colours in rabbits.

There is a third MLPH variant found in dogs (2020 doi: 10.3390/genes11060639.)

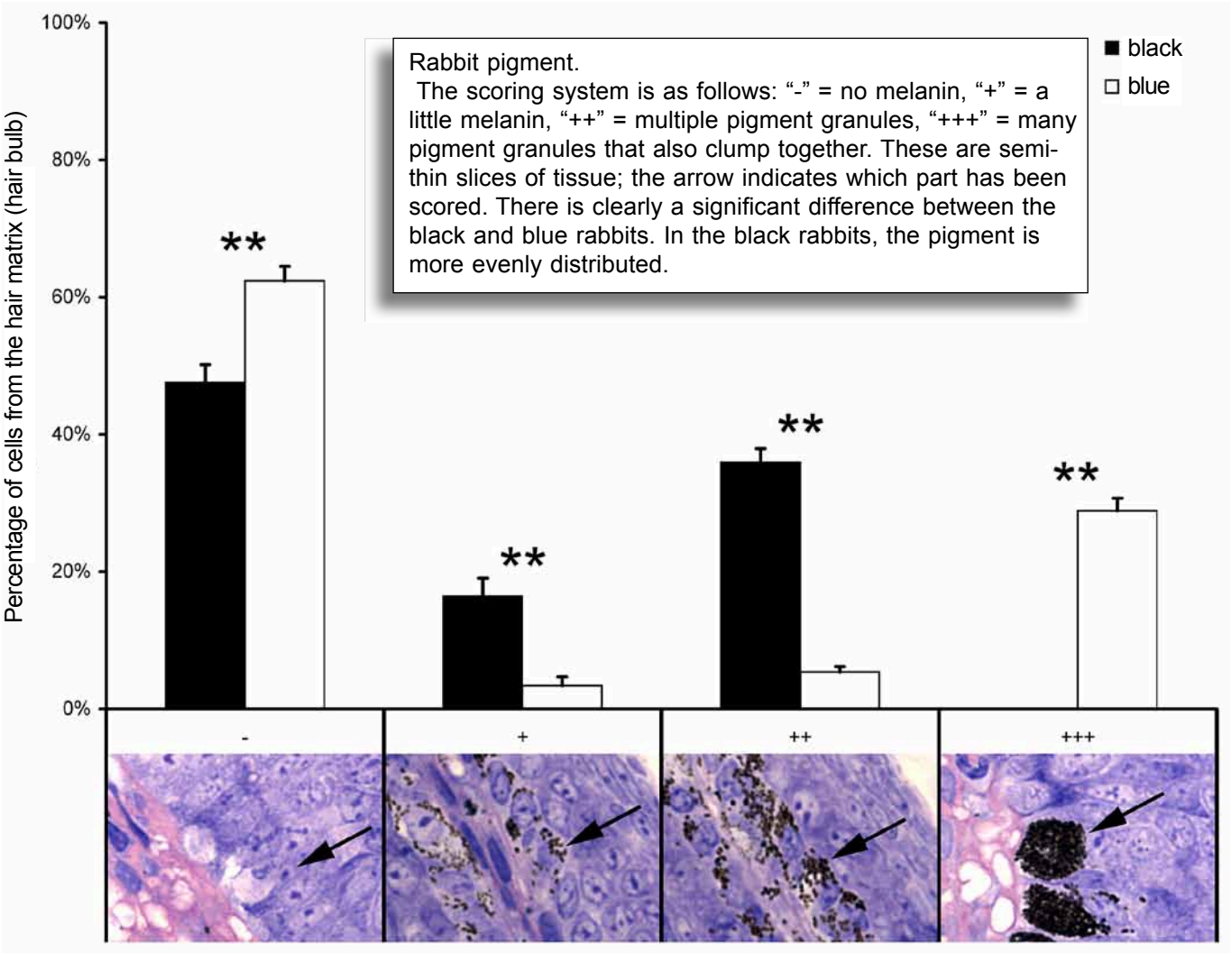
SNP - Single Nucleotide Polymorphism. Polymorphisms are one or two or more variants of a specific piece of DNA (sequence). The most common polymorphism is a variation on a single base pair. Polymorphisms can also be larger and contain long pieces of DNA. These are SNPs. They are studied e.g. to determine genotype and the resulting phenotype.

Below: Distribution of the melanophilin (MLPH) c.111-5C>A, c.585delG and c.953T>C mutations (alleles) among different breeds of rabbit. The dilution status and genotype correspond to the number of animals and their SNP genotypes. Two individuals that were much less diluted are shown in bold. These individuals had a different c.111-5C>A and c.585delG status, and their dilution status is called dark dilute.

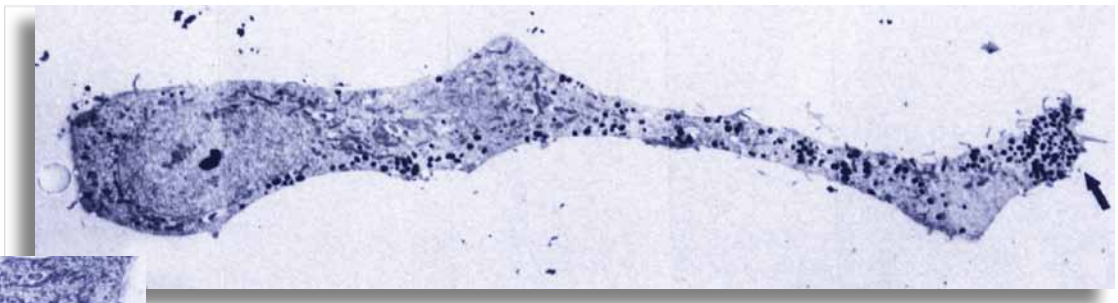
Rabbit breed	coat colour	Dilute genotype	amount animals	SNP c.111-5C>A			SNP c.585delG			SNP c.953T>C		
				C/C	A/C	A/A	w/w	w/del	del/del	T/T	C/T	C/C
Netherland Dwarf	Black	D-	11		x			x			x	
	Black	D-	2		x		x				x	
	Blackart	D-	1	x			x			x		
	Black	D-	1	x			x				x	
	Dilute	dd	4			x			x			x
	Dilute	dd	1			x			x		x	
Lionhead Dwarf	Black	D-	1		x			x			x	
	Black	D-	1		x		x			x		
	Black	D-	1		x		x				x	
Loh	Black	D-	2	x			x			x		
Netherl. Dwarf x Loh	Black	D-	5		x			x			x	
	Black	Dd	5		x			x			x	
	Dilute	dd	4			x			x			x
Vienna bl.	Dilute	dd	2			x			x			x
Dwarf Lop	Black	D-	2	x			x			x		
	Black	D-	1		x			x			x	
	Black	D-	1		x			x				x
	Dilute	dd	1			x			x			x
Giant Lop	Black	D-	1	x			x			x		
Checkered giant	Black	D-	1		x		x				x	
Rex	Black	D-	1		x			x				x
Angora	Dilute	dd	1			x	x					x
Lionhead Dwarf	**Dark-dilute**	**dd***	**1**		**x**				**x**		x	
Loh	**Dark-dilute**	**dd***	**1**			**x**		**x**				**x**

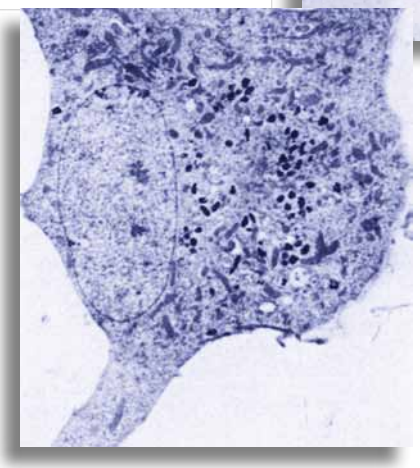
Below is another table showing the number of cells filled with pigment granules in the hair matrix layer or hair bulbs for black and blue rabbits. The clumping of the pigment granules is clearly visible in the blue rabbits.

Rabbit research: Two-exon skippng within MLPH is associatedwith coat color dilution in rabbits, 20 december 2013, DOI: 10.1371/journal.pone 0084525 https://pmc.ncbi.nlm.nih.gov/articles/PMC3869861/



Rabbit pigment.
The scoring system is as follows: "-" = no melanin, "+" = a little melanin, "++" = multiple pigment granules, "+++" = many pigment granules that also clump together. These are semi-thin slices of tissue; the arrow indicates which part has been scored. There is clearly a significant difference between the black and blue rabbits. In the black rabbits, the pigment is more evenly distributed.





Chicken pigment.
Above, the elongated thing is a pigment cell from a black chicken. The pigment is distributed throughout the cell and a group is already waiting in the dendrite to move to the keratin cell (arrow). On the left is a pigment cell from a lavender chicken. Most of the pigment remains around the cell nucleus and only a small number of granules pass through the dendrite to the keratin cell. The clumping occurs around the cell nucleus of the pigment cell or in the immediate vicinity. This is the same phenomenon that occurs in other animal species.