Text & photos: Sigrid van Dort, Jan 2023

**Anyone who knows basic chicken genetics is familiar with Ml (melanotic) which is black pigment that expands into areas that are ground coloured (gold or silver) in a wildtype situation.**

**The wildtype situation is defined by the basic e-allele, being ER (birchen), eb (asiatic partridge, hens without salmon-coloured breast), e+ (duckwing, hens with salmon-coloured breast) and/or eWh ( wheaten, hens with the least amount of black and where black has been replaced by red), without any other addition.**

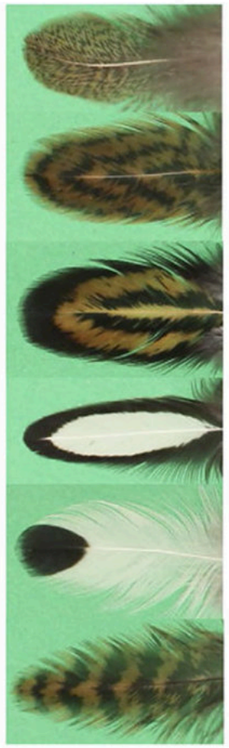
# MELANOTIC MAKE IT BLACKERDER!

## What Ml does to the feather pattern

**What Ml does**
Ml is part of several intricate feather patterns on the single feather, for example: spangled (black teardrop, half moon shape or v-shape at the end of the feather), autosomal barred (transverse bars or rows of 'corns'), double laced (black outerlace followed by one or more inner lacings), single laced (lacing around the outside of feather). For more on feather patterns and how they are constructed see the book Genetics of chicken colours, the basics at www.chickencolours.com

[Black can be also blue, dun, choc, lavender coloured by their respective genes added and altering the black colour.]

Ml causes black pigment to extend into regions where in wildtype ground colour is located. It is generally called a 'black enhancer' or 'extender'. Ml is incomplete dominant over wildtype (the amount of black from the chicken colour factory at delivery or of the e-allele in default). Incomplete dominant means: in one dose (Ml/ml+) so heterozygous, you can already see its influence. Complete in this respect is homozygous (Ml/Ml).
To make a pattern, Ml needs Pg, the pattern gene, for making lacings and



| Phenotype | Color locus | | | | |
|---|---|---|---|---|---|
| | *E/MC1R* | *PG/?* | *Ml/?* | *CO/?* | *DB/SOX10* |
| Stippled | + | + | + | + | + |
| Pencilling | *B* | *PG* | + | + | + |
| Double lacing | *B, Wh, Y* | *PG* | *ML* | + | + |
| Single lacing | *B* | *PG* | *ML* | *CO* | + |
| Spangling | *E, R* | *PG* | *ML* | + | *DB* |
| Autosomal barring | *E, R, BC* | *PG* | + | + | *DB* |

other shapes like spangles, corns, bars.

**Where to find Ml**

Having found the mutation (in GJA5) and location of it on chromosome 1, the scientists looked where Ml is first seen in the chicken, literally 'in' and not 'on'. They checked skin and feather follicles of a double laced Indian Game (Cornish) and of a partridge Plymouth Rock, both are hens because double laced and partridge express in hens, cocks do not they are wildtype'ish so black chest and ground coloured ornamental feathers.
In the Indian Game the expression of Ml was 9x more in the feather follicle compared to the partridge Plymouth Rock. A cross of Red Jungle Fowl x Sebright gave F1 Ml/ml+ offspring with a 5x larger expression of Ml.
Testing more chickens for the location of the Ml-mutation; it was found in black White crested Polish too.
Ml was mostly expressed in the collar of the feather follicle and in part where the vane is formed (the barbs or interlocked hairs of which the vane consists).

**The feather pattern depends on the e-allele**

In research papers the e-alleles are called MC1R-alleles (MC1R - melanocortin 1 receptor). FYI: the antagonist of MC1R is ASIP and 'causes' ground colour which is red pigment, sometimes made 'white' due to the silver mutation, but it still counts as pheomelanine. For feather patterns both MC1R and ASIP are working albeit in different cells.
We know that the e-allele is defining in how the pattern we aim for will work out. On the blackest E/E you don't get much of a pattern, there's too much black pigment and all columbian genes stacked, will not be powerful enough.

Best known is the difference between ER and eb when it comes to patterns and whether the tail of the cock is patterned too or that it is black. See the chicken colours book for explanations of the patterns on different e-alleles and photos of it.
Both the Indian Game and the Plymouth Rock were checked for their e-allele and both were eb based. F1 therefore showed double laced Ml/ml+ (not like we want to see it of course, however, it was double laced'ish and not multiple laced as in partridge with a ground coloured outer edge of the feather).
The conclusion is that, if there is an enough black containing e-allele or MC1R (Glu92Lys) mutation, eb here, in the F1, Ml expresses, also heterozygous (Ml/ml+) or 'incomplete'.

**Rumination as breeder1**

**In hobby breeding, Indian Game, some 15 years ago, could be both eb and eWh and also eb/eWh based, it didn't make much difference in the pattern of adult feathers is seemed. It was only visible in the chicks, the ones with stripes and the ones without and the ones with faint stripes. The wheaten way is not explained in this paper… Probably Ml has to be balanced when breeding on a black pigment hating e-allele and an impartial black-or-red e-allele as eb is. Possible even that the eWh/eWh chicks from this cross didn't make it to the show cage, idk because I've never bred this combination. It seems that the Ala137Thr mutation damps the Glu92Lys (<blackening sensitive) effect. This calls for more black (Ml) on wheaten to 'do' the pattern job. Could this be the reason for the expanding pattern on the cock's tail and the fuzziness of the lacing in, say, buff (black) laced Orpington? This is in contrast to the black tail of laced cocks < without additional Columbian genes. Idk, I've no x-ray eyes...**



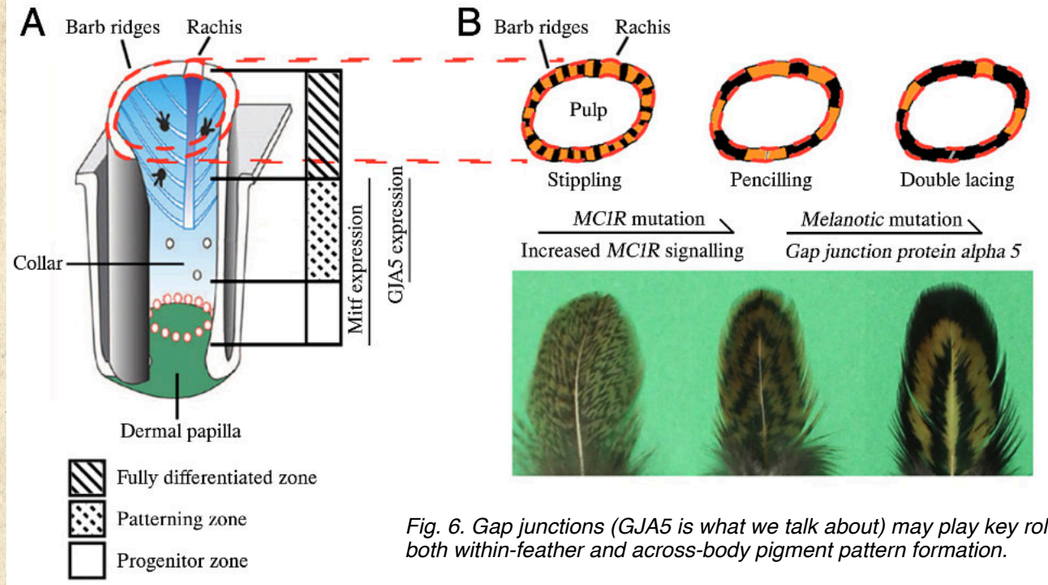
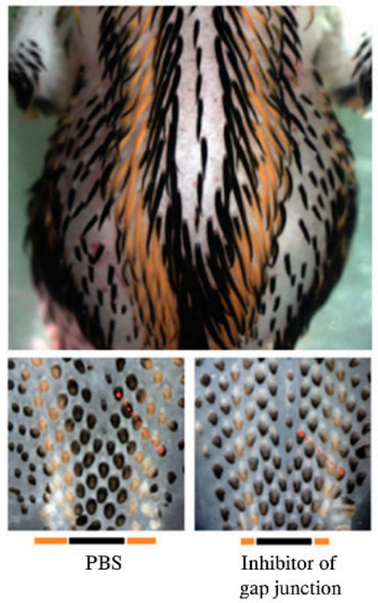
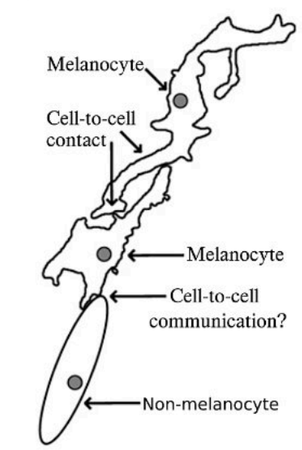



*Fig. 6. Gap junctions (GJA5 is what we talk about) may play key roles in both within-feather and across-body pigment pattern formation.*




*(A) Schematic drawing showing growing feather follicle structures [modified from Lin et al.], and the corresponding regions where MITF and GJA5 are expressed based on this study.*
*(B) Cross-section of growing feather follicles (Upper, schematic drawing) and the grown-up feathers (Lower, photos) for comparison between the three within-feather patterns and how mutations in MC1R and GJA5 act additively to enhance expression of eumelanin.*

*(C) Photos of Japanese Quail embryos [from Inaba et al.]. Dorsal view of an E10 embryo shows across-body pattern (Upper), which was affected by gap-junction activity (Lower, E7). The bottom bars show the width of the eumelanin and pheomelanin stripes. The top panel photograph was taken at 1x magnification while the two bottom panels were taken at 3× magnification on a Nikon SMZ 1500 microscope.*

*(D) Schematic drawing of possible ways for how gap junction proteins may be involved in cell-to-cell communication and thus affect pattern formations [modified from Inaba et al.].*

Although Ml is incomplete dominant, there is variation as mentioned above, you can say it has variable expression. There were Ml/ml+ chickens that showed what could be expected from a heterozygous pattern, but some of them also showed nothing melanotic at all in their feather pattern while being Ml/ml+.
For the effects on other parts of the chicken: Ml has no other effects on the chicken other than feather colour. If humans have the same mutation they would have atrial fibrillation, in mice this mutation causes atrial arrhythmias and conduction velocity. In the chicken the Ml mutation only affects the regulation of gene expression in the feather follicle and nothing else.

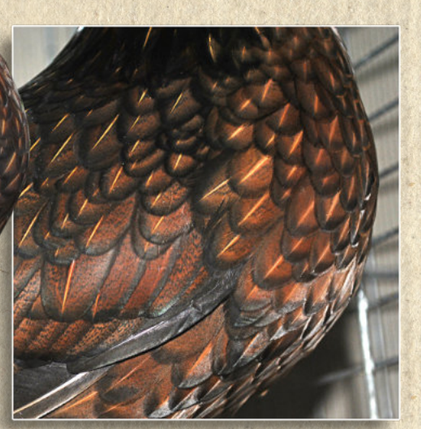
It is the altered expression of GJA5/connexin (forget this), that expands the black bands and the space between them if there is Pg present of course. The black bands become wider. The e-alleles (MC1R

extensions) must have Glu92Lys and/or Leu133GlnPro. These proteins can be found in all the 'blacker' e-alleles which are ER, eb.
But… but…. eWh is not part of this paper proteinwise and it doesn't have either of the two mentioned MI-sensitive proteins (apart from Pg).

**Rumination as breeder2**
In the list of 2014 (e-allele proteins) e+ (E*N) also has neither Glu and Leu. I've no idea whether this means that MI won't work on these alleles in the presence of Pg. MI does work on e+, as we have Wildfarbig, the MI-version of e+ in German bantams. Although it doesn't eliminate the salmon coloured breast in the hens, it makes e+ really dark. But they don't have Pg. This means that also the 'default' amount of black pigment in e+ (in the shape of stippling or peppering) is good enough to make MI do it's blackening work. I'm curious whether adding Pg to such a German Wildfarbige will make them double laced and how it looks. Maybe just as fuzzy laced as the buff laced Orpington?

*Indian Game come in different outfits if you look closely you see the ground colour differs (not only light conditions) also the double laced-way is different, apart from the different feather shapes. Yes, I know they are not bred for 'colour' but for type, however e-alele guesses are going on here.*





*When you put MI in e+, you get this...*



# FOR THE NERDS WHO WANT TO REALLY SEE IT

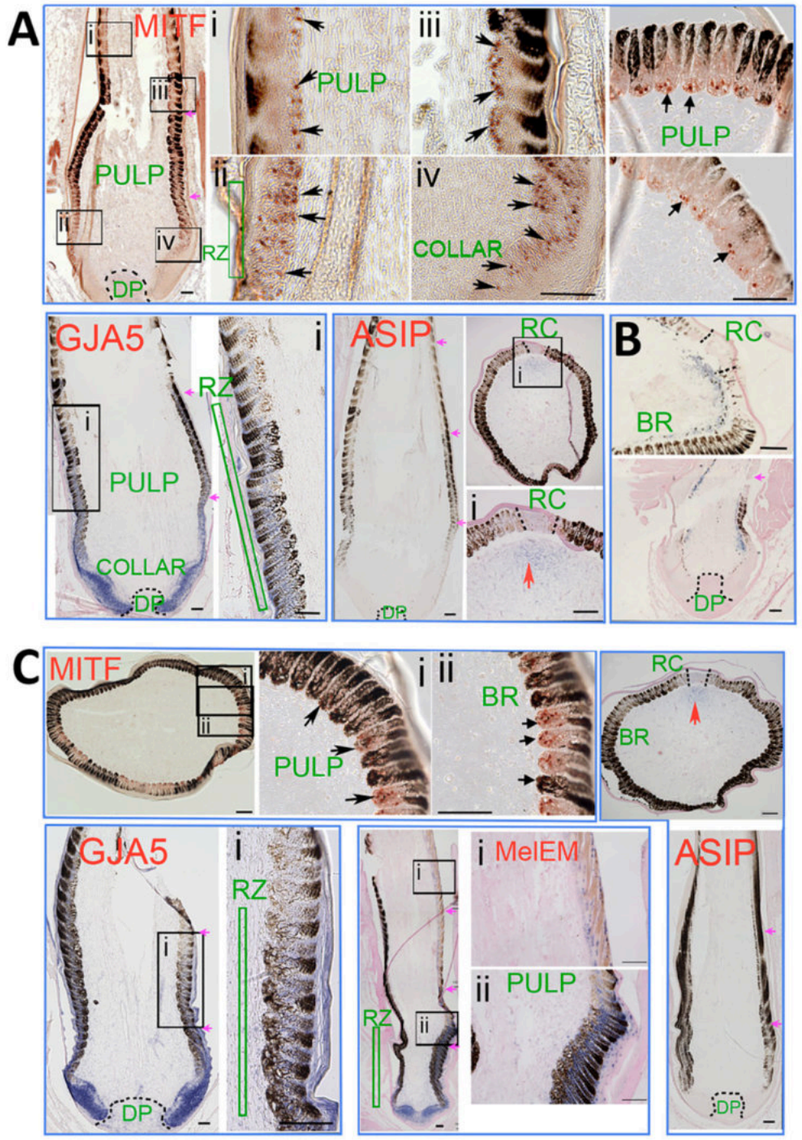



***Fig. 5. Molecular expression during feather formation. Partridge Plymouth Rock feather follicles (A), Fayoumi feather follicles (B), Dark Cornish feather follicles (C).***

*(MITF = Melanocyte inducing transcription factor, it helps control the development and function of pigment-producing cells called melanocytes.)*

*- **(A)** MITF+ cells (red nucleus staining) are present in the basal layer of the feather filament epidermis in longitudinal feather sections (arrows in A, Mitf-i, ii, iii, and iv) and cross-section (Right) of both eumelanin and pheomelanin regions. GJA5 is expressed (blue color) in keratinocytes in collar and ramogenic zones. GJA5 is also expressed in melanocytes in the ramogenic zone in both the eumelanin and pheomelanin zones, but with decreased expression in the more differentiated barb ridges. ASIP is absent in the pulp in longitudinal sections. Cross-sections show that ASIP is weakly expressed in the peripheral pulp adjacent to the rachis region.*

*- **(B)** Fayoumi chicken feathers with autosomal barring pattern (3) are shown for comparison. Both cross and longitudinal sections show lower ASIP expression in the peripheral pulp adjacent to the eumelanin region.*

*- **(C)** MITF immunostaining (Upper) shows positive melanoblasts in both eumelanin and pheomelanin regions in Dark Cornish feather follicles. GJA5 and ASIP expression patterns are similar to the expression patterns shown in Partridge Plymouth Rock feather follicles (A). MelEM (blue nucleus staining) is expressed in melanoblasts in the distal collar and ramogenic zone, with strong expression in the eumelanin region and weak expression in the pheomelanin region. For feather follicle components, please refer to Fig 6A, see next page. BR, barb ridge; DP, dermal papilla; RC, rachis; RZ, ramogenic zone.*
*(A) MITF, GJA5, ASIP panels; (B) Lower panel; and (C) GJA5, MelEM. Left panel, and ASIP panels are photomontages in which spliced junctions are indicated by purple arrows. (Scale bars in all panels, 100 µM.)*

**Used for references and figures:**
Association between polymorphism in the melanocortin 1 receptor gene and E locus plumage color phenotype (2014)
Cis-acting mutation affecting GJA5 transcription is underlying the Melanotic within-feather pigmentation pattern in chickens (2021)
The feather pattern autosomal barring in chicken is strongly associated with segregation at the MC1R locus (2021)