Melanin Pigmentation: Its Biological Roles, Inheritance and Expression in the Chicken

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Melanin, one of the most widespread natural pigments, plays a major role in the pigmentation of feathers, skin, shanks, eye and internal tissues of vertebrates. They are produced by highly specialized cells, termed melanocytes, that arise in the embryonic neural crest and migrate during early embryonic development to their ultimate sites of residence. In contrast, ocular melanocytes of the retinal and iridial pigment epithelium and the pecten arise from the outer layer of the embryonic optic cup. Migration from the neural crest by the undifferentiated melanoblasts proceeds rapidly as evidenced by their presence in the limb buds by 80-91 hours of incubation. Shortly after reaching their destination, they differentiate into melanin-producing melanocytes characteristic of the tissue in which they reside. Some, like those of the ocular choroid, testes and connective tissue membranes produce their pigment, and thereafter, remain melanogenically inactive. In contrast, melanocytes destined to pigment feathers, colonize reservoirs of melanoblasts located in the dermis near the dermal papillae of the developing feathers.
As pointed out by Proto (1981), melanins appeared early in evolutionary history and have remained largely unaltered to the present day. The two main types of melanins are the eumelanins (blacks and dark browns) and the pheomelanins (reds and yellows). The basic eumelanin synthesis pathway involves the oxidation of the amino acid, tyrosine, to dopa melanin and then dopaquinone by the enzyme tyrosinase. This is followed by a series of cyclizations and oxidations leading to the eumelanin polymer. Additional tyrosinase-related proteins (TRP1 and TRP2) are now known to be involved (see Hearing, 1993, for review), but their roles are not well understood at this time. Pheomelanin synthesis shares the same tyrosine dopa dopaquinone pathway but diverge with the addition of sulphydryl groups to form cisteinylldopa, and ultimately the red and yellow melanins of the feathers. Pheomelanins are found only in the feathers of chickens, all other melanins of the body being eumelanins.

The final pigment product involves the attachment of the melanin to a protein matrix to form a pigment granule or melanosome. Thus, melanin exists as cytoplasmic organelles within melanocytes, or in feather keratinocytes following transfer from melanocyte dendrites during feather formation. For a more detailed review and references on the subject of melanin pigment and melanocyte function, the reader is referred to several recent reviews: Bowers (1988); Smyth (1989) and Smyth (1990).

**Biological Roles of Melanin**

Obvious roles played by melanin include display and recognition, as well as protective coloration in females as natural ground nesters.
The absence, or greatly reduced levels of melanin during embryonic development has also been shown to lead to altered visual pathways connecting the neural retina and the brain in several species. Additionally, melanin appears to serve at the cell level as a scavenger for free radicals, e.g. superoxides. There is also strong evidence that melanin may be necessary for normal embryonic and early posthatch development in chickens. The latter is best illustrated by a study conducted at Massachusetts several years ago (Smyth et al., 1987) and described below.

In our study, we were interested in pleiotropic associations between hypomelanic (white feathered and/or albino) mutations and development of embryos and newly hatch chicks. The mutations included the $c$-alleles, recessive white ($c$) and complete albino ($c^a$); dominant white ($I$); sex-linked partial albinism ($s^a_l$); and pink eye ($pk$). All comparisons were made between mutant and normal phenotypes randomly segregating within a stock, although the mutations themselves were present in different stocks. The major negative relationships were found for the two albino types where 14 da. embryo weights and 14 da. posthatch weights were significantly depressed ($P<.01$) by the reduction in melanin. The pinkeye chicks showed a similar association but the two week posthatch weight difference was significant only at the $P < .05$ level. The two albinos also had significantly higher embryonic mortality ($P<.01$), as well as reduced bursa ($P<.01$) and yolk sac ($P<.05$) weights. Shorter down lengths ($P<.01$), naval protrusions ($P<.01$), and increased inflammation of the nares and hocks ($P<.05$) were also associated with $c^a$, $s^a_l$ and $pk$. Dominant and recessive whites did not differ significantly from their pigmented sibs, however, $c/c$ embryos and
chicks consistently fell between the means for their colored and albino hatchmates. Interestingly, this study suggests that the above developmental abnormalities are more prevalent in mutants with albinotic or near-albinotic eyes. For example, pink-eye birds have albino eyes but their feather color is diluted only from black to dark blue.

Earlier studies showed that recessive white ($c/c$) is associated with a reduced growth rate of approximately 30-40g to 6-8 weeks of age (see Fox and Smyth, 1985, for review). Significant growth depression was also demonstrated in broiler stocks. Consistent growth depression has not been demonstrated for dominant white and there appears to be no interaction between $I$ and $c$ so that the addition of $c/c$ to dominant whites does not further increase the recessive white effect (Fox and Smyth, 1985).

Inheritance of Plumage Color

Plumage color genetics is more complicated than hair color genetics for several reasons. First, the feather is a far more complex structure than a hair. Color genes may express themselves differently in the down, juvenile and adult plumages. Sex dichromatism is often present, particularly in association with the key E-locus genotypes. Furthermore, there appears to be no dosage compensation for the Z chromosome so that dosage effects for sex-linked genes are common. In spite of this, considerable information is available on the inheritance of plumage color. For more detail than is possible here, the reader is referred to the review by Smyth (1990).
In the chicken, both eumelanin and pheomelanin appear to be potentially present, their expression dependent on key genes in the genome. In other words, there is no specific gene for the production of pheomelanin. It is present where eumelanin is not, the black-red switch being controlled largely by the E-locus (see below).

Plumage pattern phenotypes result from a series of genetic alternatives:

1. Pigmented vs. non-pigmented (e.g. dominant white, recessive white, or albin)
2. If pigmented, is melanin diluted (altered) or not, e.g. blue, cream, lavender, etc.
3. Distribution of black pigment
   (a) Primary pattern (zonal distribution, e.g. columbian or pyle-zoned)
   (b) Secondary pattern (within feathers, e.g. barring, lacing, stippling, etc.
4. Color of non-black feather tissue, e.g. red, buff, silver, cream, dilute, etc.

The E-locus plays a major role in plumage color, controlling the primary pattern and interacting with other genes to contribute to the secondary pattern. This complex locus is represented by at least 9 alleles that vary from full black to solid salmon in color. The E-alleles and approximate dominance relationships are: E (self-black) > E_R (black-downed birchen) > E_RB (red-brown downed birchen); Wh (dominant wheaten, clear down, salmon female) > + (striped brown down, wild type-pyle zoned male and salmon-breasted female) > b (brown down; females are wild type without salmon breasts) s (speckled down; b-
like adults) > $e^{bc}$ (buttercup; wide striped down; $e^b$-like adults) - $e^y$
(recessive wheaten, clear down and salmon female). Only down and female coloration differs for the non-black and birchen phenotypes, all male patterns being pyle-zoned wild type. The birchen males both show limited pyle-zoning, the $e^{RB}$ effect approaching a dark wild type. Birchen females resemble the range in colors seen among black sex-link cross hens.

Once the phenotype associated with the E-locus is determined, it is subject to major modifications by other genes that also influence the distribution of black pigment. One group, the eumelanizers, enhance the spread of black and can even convert a wild type ($e^+$) bird into a solid black. These include melanotic ($M_l$) and charcoal ($Cha$), as well as other, as yet unidentified, modifiers with varying degrees of black pigment enhancement, some of which are recessive. In contrast, there are eumelanin inhibitors leading to columbian-like patterns, and even total elimination of eumelanin as in Buff Orpingtons or Buff Plymouth Rocks. The latter include $Co$ (columbian), $Db$ (also a columbian-like gene), $Mh$ (mahogany) and $Di$ (dilute), and undoubtedly, again, as yet unidentified alleles and other genes.

Note that no mention is made above of an $e$ allele to $E$ that incorrectly has been assumed to result in columbian patterns! Dunn's (1923) $e$ really represented the absence of $E$ in black-columbian crosses, where a wide range of phenotypes that were not black were lumped into one group. Various columbian-like phenotypes are actually produced by several genes working in concert. Some basic genotypes (probably bolstered by additional modifiers) include:
Standard Columbian (Buff Brahma) - e\textsuperscript{b}/e\textsuperscript{b} Co/Co

Black-Tailed Reds:

New Hampshire - e\textsuperscript{wh}/e\textsuperscript{wh} Co/Co Mh/Mh
Rhode Island Red - more likely e\textsuperscript{y}/e\textsuperscript{y} Co/Co Mh/Mh
Non-black buff - e\textsuperscript{Wh}/e\textsuperscript{Wh} Co/Co Mh/Mh Di/Di

Probably all colored plumage phenotypes result from multiple genes. For example, solid black phenotypes may be E, birchen (E\textsuperscript{R} or E\textsuperscript{RB}) or carry any other combination of E-alleles, if the appropriate eumelanizers are present to allow the total spread of black pigment. In other words, there are multiple routes to self black plumage. This becomes apparent when one examines the F\textsubscript{2} of crosses involving blacks. White plumages, except for the complete albino (c\textsuperscript{a}/c\textsuperscript{a}), also need the help of modifiers. For example, dominant white is almost completely ineffective against pheomelanin. This is why White Leghorns are generally E/E S/S B/B, and often Bl/Bl (blue-splashed white), all genes that help to mask red. Recessive whites, although less susceptible to the effects of pheomelanin, also accumulate supporting genotypes to insure whiteness.

Color Testing Problems

Poultry breeders, who wish to make test matings to determine color genes present in their stocks, are usually limited in available tester stocks. New Hampshires and Rhode Island Reds are commonly used and these are very poor choices because of the complexity of their genotypes as described above. Among other things, both contribute wheaten which can interact with some of the E-like alleles to produce a range of
misleading phenotypes some of which are capable of effectively masking the presence of E. It would be better to use e\textsuperscript{+}/e\textsuperscript{+} or e\textsuperscript{b}/e\textsuperscript{b} testers, or perhaps the Giant Jungle Fowl from the University of Arkansas.

Other genes that complicate color testing include dominant white (I) and sex-linked barring (B). In the case of I/I stocks, it is often necessary to produce an F\textsubscript{2} generations, which takes time and money. Sex-linked barring dilutes down colors and interacts inconsistently with some genotypes, e.g. B plus Co, both eumelanin inhibitors, results in an unexpected increase in black in the plumage.

**Pigment Problems in Non-feather Tissue**

Two undesirable pigment problems in meat chickens are melanin pigment in the shanks and/or the connective tissue fascia in the abdominal skin and membrane surrounding the fat pads in the same region. In general, shank and fascia pigmentation are associated, but this is not always true and occasionally either can appear alone. Most of our knowledge concerning dermal melanosis comes from studies made in the absence of dominant white, and less is known about the expression in these tissues in I/-birds (see Smyth, 1990, for review). Normally the sex-linked inhibitor of dermal melanin, Id will prevent the unwanted pigment, but some stocks may not be homozygous and no one is sure how many id\textsuperscript{+}-like alleles may be present. For example, McGibbon (1974) described a mutation (id\textsuperscript{c}) at the Id-locus that resulted in green shanks in homozygous dominant (I/I) White Leghorns. In addition, several other mutations have been proposed for the Id-locus suggesting a high degree of mutability. Such occurrences could explain occasional field problems. It is also possible that mutations at other loci may
intensify id⁺ or diminish the inhibiting ability of Id. For example, the extreme melanization of the fibromelanosis phenotype of the Silky fowl is due to a gene (Fm) whose only known function is to intensify the dermal melanization of id⁺. Could there be other mutations at the Fm-locus?

Certain plumage color genes have been found to enhance melanin deposition in both the shanks and the abdominal fascia. In general, these involve poorly understood, and often, seemingly illogical genetic interactions (see Smyth, 1990, for review). Some examples of these include:

1. Dominant white’s ability to suppress id⁺ is reduced in the presence of E. Here the shanks are clear at hatching but become pale green, or pale blue in white skin stock, over time.

2. Recessive white has little inhibiting effect on shank pigment with id⁺, and even less in the presence of E. Fascia pigmentation is frequently increased in the presence of c/c.

3. Dominant wheaten (eWh) affectively suppresses id⁺ expression in the shanks and fascia, but its look-alike e⁺ allele (recessive wheaten) has little or no effect.

4. Sex-linked barring (B) is also involved in some interesting interactions. Although it generally reduces shank pigmentation, Japp (1955) found B to result in dermal melanin in the shanks on a silver columbian background in the absence of id⁺. Barring has been found to reduce abdominal melanin in the presence of E, but to increase it when E is replaced by e⁺ or ebb, particularly in the presence of Co.
Color-Sex Crosses

Although the sex-linked partial albino gene (sa_l) yet may prove feasible for day-old sex determination using eye color (Silversides and Crawford 1991a, 1991b), most efforts at present involve the use of the sex-linked silver (S) and gold (s^+) alleles in the presence of I/I. Little new information has appeared on this since the work of Malone and Smyth (1979), with success depending on the presence of enough non-eumelanic down color to allow the expression of S and s^+ to be readily apparent (Smyth, 1990). The E-alleles, e^+, e^b or e^Wh all work well as long as the columbian gene, C_o, is present. The latter markedly enhances the differential expression of S and s^+, therefore, it improves the sexing accuracy. The maximum expression of S and s^+ is on a e^Wh/e^Wh C_o/C_o background as exemplified by the white-tailed red phenotypes of many brown egg-type crosses. Meat breeders might prefer a more restricted non-eumelanic area in the down (head region only), which will translate into less red pigment in the feathers at market ages.

Closing Thoughts

It would appear to be a fairly simple physiological procedure to add melanin pigment to feathers and other tissues. It has been my purpose to show herein that pigmentation is a complex biological function controlled by the products of numerous genes. I have always considered melanization as a model for a physiological system whose genetic control is little influenced by outside environmental factors. And what do we see? Essentially, a multigenic trait where many of the key contributors are individually identifiable and reveal various degrees of dominance and a wide range of genetic interactions. Although
simple individual selection for "darkness" or "lightness" of the overall plumage would reveal additive gene action and a fairly high heritability, we could make more progress if we understand and utilize the gene interactions. I would not be surprised to find that all biological processes have a similar genetic basis.

References


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