

Eggshell pigmentation: a review

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ABSTRACT

The variation in avian egg coloration, as a view from assuring animal reproduction successfully, has been explained to be a result of crypsis or mimetism, although other potential functions like filtering solar radiation has also been described. It is often a cultural preference for one color over another. The major reason which prompts the customer to select special egg color depends on customer's preference. Major pigments present in bird eggshells are protoporphyrin-IX, biliverdin-IX, and its zinc chelate. Biliverdin, a green pigment, is a byproduct of hemoglobin metabolisms and will eventually display a blue or green color in the eggshell. Protoporphyrines, in contrast to biliverdin, are an immediate precursor of the heme molecule, which gives eggshells a reddish or brown color. It is speculated that eggshell pigments are derived from disintegration of erythrocytes in the mucous layer of the shell gland. The blood and shell gland are considered as sites of biosynthesis of eggshell porphyrins. The timing for pigments secreted from epithelium cells of shell gland is varies among different species of birds. There are several factors such as stress, age, chemotherapeutic agents, diseases, and environmental factors, which are responsible for decreasing the intensity or almost total loss of eggshell color. But, egg color for different species varies mostly depending on different genetics or gene expression.

(Key Words: Eggshell pigment, Eggshell quality, Genetic study)

INTRODUCTION

Bird eggs show an enormous diversity of colors, from white, brown and deep blue (Figure 1). The possible functions of eggshell coloration include structural as well as sexually selection. The egg color is considered as a protection from harmful solar radiation (Lahti, 2008), reinforcement of eggshell structure (Gosler *et al.*, 2005), and thermal protection of developing embryos (Bakken *et al.* 1978). Recently, eggshell color is proposed as a sexually selected signal which a female displays her heritable phenotypic (and/or genetic) quality to her male partner and, thereby, promotes a higher allocation of parental care (e.g. through courtship feeding, nest defense, maintenance of nest hygiene, and brood-provisioning effort)

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(Moreno and Osorno, 2003). Gosler *et al.* (2005) indicated meanwhile that the egg speckling caused by protoporphyrin pigments might compensate for reduced eggshell thickness due to calcium deficiency.

There is still no evidence to show connection between eggshell color and egg quality. It is often a cultural preference for one color over another. The reasoning which prompts the customer to select a large, white egg or a smaller, heavily pigmented egg is quite different. The large egg represents value for money. The brown egg, on the other hand, will always manage to evoke Pavlovian images of farmyards and free-range feeding (Solomon, 1991). For example, in most regions of the United States, eggs are generally white. While consumers in some markets throughout the world prefer brown eggs over white eggs (United Kingdom, Italy, Portugal, Ireland, Southern Asia, Australia, and New Zealand) (Odabaşı *et al.*, 2007).



Figure 1 Chicken eggs show the variety of colors. (Liu, 2010)

1. Eggshell color and its quality

The eggshell strength is the main, but not the only factor that determines shell quality. Eggshell quality can be measured as: egg size, visual shell defects, specific gravity, shell color, shell breaking strength, percentage of shell (shell weight x 100/egg weight), shell thickness, and ultrastructure of the shell. Previous studies show that there is a correlation between shell color and shell quality in eggs of a single strain. The correlation coefficients were found: between eggshell color and eggshell strength was 0.393, between eggshell color and eggshell thickness was 0.372. But the correlation coefficient between

specific gravity and breaking strength was 0.748, which was higher than that between shell color and breaking strength do. The overall finding was that darker brown eggs had a higher shell quality than lighter brown eggs (Godfrey, 1949). The correlation between a measure of eggshell color and breaking strength was essentially 0.08 (Briggs *et al.*, 1974). The white egg layers have a significantly higher percent egg production than the blue egg layers; but no significant differences between white and blue egg layers were found for their shell quality, shell thickness, albumen weight, yolk weight, or yolk cholesterol content (Sadjadi *et al.*, 1983). Although heritability for external and internal egg quality are moderate to high, genetic correlations with eggshell colors were low, suggesting a minor relationship between shell color and physical attributes of the shell as well as internal egg quality in brown-egg dwarf layers (Zhang *et al.*, 2005). Furthermore, the measurement of shell color used in these studies is subjective and might be biased (Joseph *et al.*, 1999). It is determined that eggshell color is significantly correlated to shell quality, but not as accurate as is specific gravity or shell thickness (Ingram *et al.*, 2008).

However, eggshell color could possibly be used in conjunction with specific gravity in order to provide a more accurate estimator of eggshell quality. Yang *et al.* (2009) reported that some egg quality traits such as shell strength, shell thickness, shell weight, and shell ultrastructure can be accessed through the shell color. When comparing eggshell quality of different color phenotypes within blue eggshell and blue cross with White Leghorn stocks revealed that eggshell color have significant effect on eggshell quality. Darker eggshell and yellow-brown eggshell eggs have a better eggshell breaking strength than the lighter ones.

Although there is a widespread belief that pigmented eggs are stronger than white eggs (Godfrey, 1949; Briggs *et al.*, 1974; Yang *et al.*, 2009), conflicting evidence has also been reported (Wang *et al.*, 1997; Joseph *et al.*, 1999). We cannot conclude that eggshell color alone would be a sufficient estimator of eggshell quality.

2. Eggshell color and hatchability

While the scientific society debating if the relationship exists between egg color and hatchability, poultry producers have long held the belief that in typical brown egg laying breeds, light colored eggs will not hatch as well as those that are darker in color. Indeed, evidence shows that healthy and well-fed females lay more intensely colored eggs in certain songbirds (flycatchers). Hulet *et al.* (1978) investigated the relationship between eggshell color and hatchability using pheasant hens. It is found that olive and darker colored eggs had significantly greater hatchability over grey, light brown and blue eggs. Fertility of pheasant has been reported to be from 38.8 to 69.9% (Dorn *et al.*, 1982; Woodard *et al.*, 1983; Hulet *et al.*, 1985). Significant differences were observed in all aspects of eggshell ultrastructure with blue eggs having thinner shells with structural defects. Poor hatchability of blue eggs may reflect high rates of weight loss associated with a defective eggshell (Richards and Deeming, 2001). However, there is no significant difference in shell ultrastructure between the pigmented and unpigmented guinea fowl eggs. Both groups displayed a large amount of confluence, along with poor cap quality and late fusion, features associated with poor ultrastructure. There was a significant difference in both total shell thickness and

effective shell thickness with pigmented shells demonstrating higher values in both cases (Darnell-Middleton *et al.*, 1998).

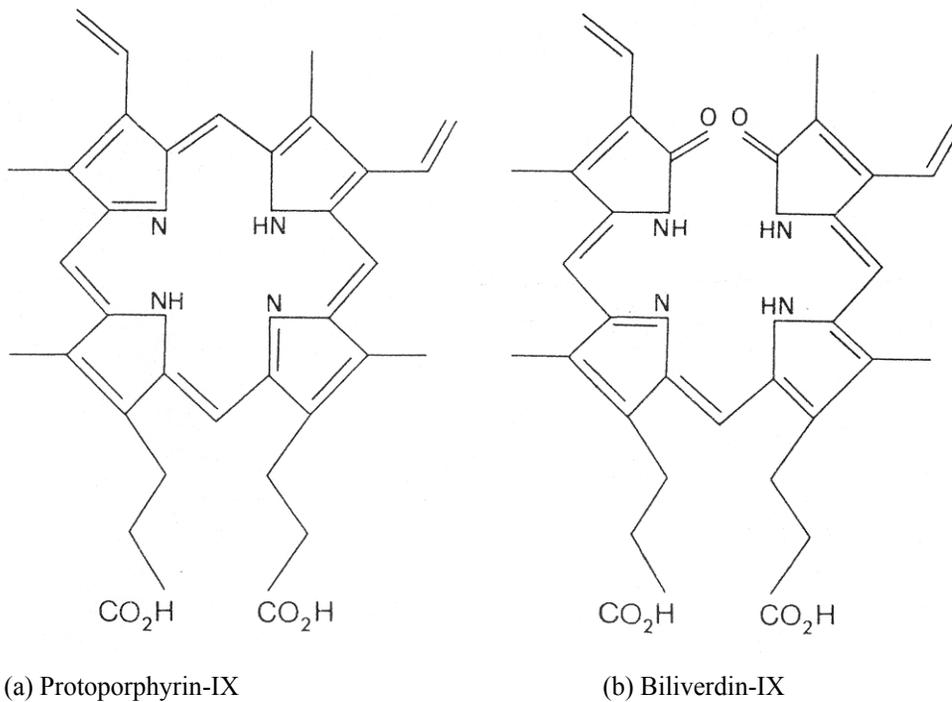
It is reported that the shade of brown pigment, intensity of light during incubation, and age of the breeder hens influenced the hatchability performance of embryos from brown eggs. Shafey *et al.* (2001) compared pigmented with non-pigmented eggshells and found that pigmentation influenced the spectral transmittance of light into the egg. High intensity of light during incubation reduced hatchability of light and medium brown eggs, but not the dark brown eggs (Shafey *et al.*, 2005).

It is known that biliverdin, responsible for the greenish blue pigment in eggshells, is a strong antioxidant. Therefore a female who is able to lay eggs containing this pigment is believed to have a higher capacity to control free radicals (Soler *et al.*, 2005), as they are able to remove antioxidants from their circulation via deposition in eggshells (Moreno and Osorno, 2003). But there is still no scientific evidence to support that blue eggshell color eggs has higher hatchability than white color eggs.

3. Eggshell pigments

The color of eggshell is the result of pigments deposition during egg formation within the oviduct. The type of pigments used greatly depends on the breed and is genetically determined (Kennedy and Vevers, 1973; van Brummelen and Bissbort, 1993). Eggshell color is determined by pigment released inside the shell gland during eggshell formation. The major pigment obtained from brown-shelled eggs was named as “oorhodeine” (Sorby, 1875, as cited in Lang and Wells, 1987). Later researchers recognized it as a porphyrin compound. “Oorhodeine”, as it was then referred to, was crystallized and identified as the hemoglobin porphyrin, protoporphyrin-IX. The green pigment of the shells for certain species, was believed to be identical to the bile pigment, biliverdin. In 1934, Lemberg confirmed its identity through isolating the compound in a crystalline form. Later, Kennedy and Vevers (1973) showed that the major pigments in the blue-green shell of the Araucano fowl were biliverdin, its zinc chelate, and protoporphyrin-IX. Significant amounts of uroporphyrin and coproporphyrin in addition to protoporphyrin were found in the brown shells from an undefined breed of domestic fowl (Kennedy and Vevers, 1973). However, the brown-shelled eggs layed by a modern hybrid, ISA Brown, have predominant protoporphyrin-IX with negligible amounts of other isolated porphyrins (Lang and Wells, 1987). Two extensive studies surveyed the shell pigments of 106 avian species, found that the pigments present in eggshells were mostly protoporphyrin-IX, biliverdin-IX (Figure 2), and its zinc chelate, sometimes traces of coproporphyrin-III (Poole, 1965; Kennedy and Vevers, 1976). Biliverdin, a green pigment, is a byproduct of hemoglobin breakdown and will eventually display a blue or green color in the eggshell. Protoporphyrines, in contrast to biliverdin, is an immediate precursor of the heme molecule, and will give eggshells a reddish or brown color (Kennedy and Vevers 1976; Mikšik *et al.* 1996).

The blood and shell gland are considered as sites of biosynthesis of eggshell porphyrins. It is speculated that eggshell pigments are derived from disintegration of erythrocytes in the mucous layer of the shell gland. Therefore, it is reasonable to think that the porphyrins of eggshell are secreted from erythrocytes which are known to synthesize porphyrins (Kennedy and Vevers, 1973). Even there is still



(Lang and Wells, 1987)

Figure 2 Structure of protoporphyrin-IX and biliverdin-IX.

lacking evidences to support these postulates (Lang and Wells, 1987). Polin (1957) indicated that uterine tissues produce twice as much porphrin from delta-aminolevulenic acid as from liver cells *in vitro*. Later experiments suggests that eggshell protoporphyrins are first synthesized in the shell gland, then secreted and deposited onto the eggshells in brown eggshelled hens (Baird *et al.*, 1975). The shell gland which secretes the cuticular layer is considered the final destination of pigment transportation (Yamada, 1972; Baird *et al.*, 1975; Solomon, 2002). Porphyrins are likely synthesized *de novo* in the shell gland where the process occurs. Recently, Zhao *et al.* (2006) demonstrated that birds lay blue shelled eggs might have the ability to synthesize biliverdin in the shell gland; a significant difference was observed in shell gland biliverdin concentrations between blue shelled (8.25 nmol/g) and brown shelled (1.29 nmol/g) chickens. This result suggests that blood is not the only site of biliverdin synthesis for eggshell pigmentation. In contract to results from chicken, Liu *et al.* (2010) study demonstrated that different eggshell colors in blue-shelled duck and white-shelled duck were influenced by the amount of biliverdin in uterus fluid and were not determined by the amount of biliverdin in the shell gland. Serveral lines of evidence reveals that biliverdin reflects the immunocompetence and antioxidant condition of female birds (Soler *et al.*, 2005; Morales *et al.*, 2006). Metabolism of the oviduct and shell gland were very active and 15.4% of cardiac output went to the oviduct during laying period (Boelkins *et al.*, 1973), consequently, a great quantity of reactive oxygen species were produced. So, the results of Wang *et al.* (2009) suggested that eggshell protoporphyrins and eggshell biliverdin were derived from a common precursor molecular, heme.

4. Time for pigment deposition

In the domestic fowls, pigments are deposited during the entire period of shell formation with acceleration at the last 3 to 5 hours before oviposition (Warren and Conrad, 1942). Tamura and Fujii (1967) demonstrated that porphyrin in eggs of Japanese quail and domestic fowl is distributed in the shell membrane, shell, and cuticle. Later, Lang and Wells (1987) reports that the eggshell color of fowls laying brown egg was due to the pigment associated with the cuticle. The pigment on the surface of quail eggshell is accumulated during the last 2 to 3.5 hours before oviposition (Poole, 1965; Soh *et al.*, 1993) (Figure 3). The eggshell pigmentation of China domesticated Dongxiang blue-shelled chicken was considered to reach a peak about 2 to 3 h before oviposition (Zhao *et al.*, 2006). The pigment granules are presented in the apical cells of the mucous epithelium in the shell gland, and its secretion is accompanied with the formation of the cuticular layer (Soh *et al.*, 1993), representing the final stage of eggshell formation. It is possible that onset of superficial pigment deposition and the cessation of calcium secretion are functionally related (Poole, 1965), because the superficial pigment is easily scrubbed from the eggshell surface, and shell calcification is nearly complete at the time of pigment deposition (Woodard and Mather, 1964). As for blue-shelled ducks, the biliverdin deposition pattern of eggshell was concurrent with the content of biliverdin in uterine fluid which increased from 12 to 23.5 h post oviposition (Liu *et al.*, 2010).

Soh *et al.* (1989) suggested that the ovulatory process was closely associated with the accumulation of superficial eggshell pigment in the shell gland of Japanese quail. However, administration of estradiol-17 and testosterone had no influence on pigment accumulation. But significant increases in the accumulated pigment 18 hours after oviposition of the preceding egg were found when progesterone was injected (Soh and Koga, 1994). There are some supporting reports that steroid hormones activate σ -aminolevulinic acid synthetase (Miller and Kappas, 1974) and σ -aminolevulinic acid dehydratase (Yamada, 1972), which synthesize porphyrins in the shell gland. The cuticular layer, which is simultaneously formed with superficial pigmentation on quail eggshells (Soh *et al.*, 1993), contains a remarkable amount of phosphorus as compared to the other shell layers (Quintana and Sandoz, 1978). Effects were seen in chickens (Ito and Hatano, 1964; Nys *et al.*, 1991) and the inorganic phosphorus concentrations in the shell gland fluid increased close to oviposition (Murakami *et al.*, 1991). Phosphate solution was demonstrated to stimulates the prostaglandin synthesis causing pigment secretion of the shell gland and premature oviposition in quail (Soh and Koga, 1999). Soh *et al.* (2000) hypothesized that increased inorganic phosphorus concentration of the shell gland fluid is involved in pigment secretion of the shell gland via stimulating effect on prostaglandin synthesis and there are some mechanisms to regulate oviposition.

5. Genetic study on eggshell pigmentation

The color of the eggshell is controlled by several genes encoding enzymes that regulate the production and deposition of pigments. Since only a few loci code for enzymes participating in the metabolic pathway of making brown shell color, it is a highly heritable trait (Etches, 1996). Brown

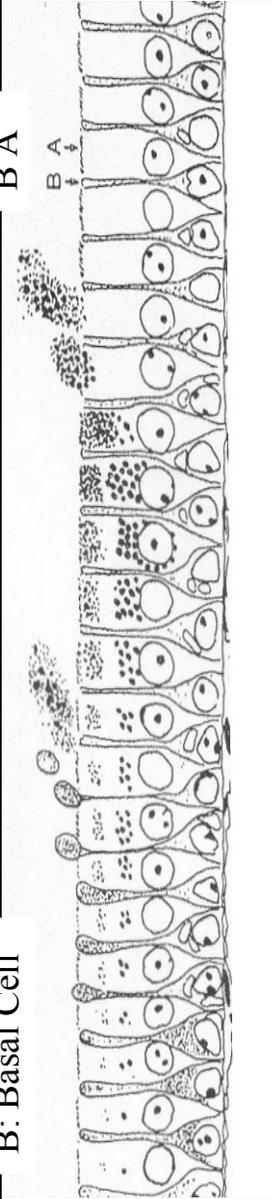
Stages	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	stage 6
Position of Egg	Magnum	Isthmus	Uterus	Uterus	Uterus	After Laying
Egg covered with	Albumen	Shell-membrane		Shell	Cuticle	
Lumen	B: Basal Cell					
Epithelium						

Figure 3 Changes in the secretion of the shell gland mucous epithelium of Japanese quail. A: Apical Cell, B: Basal Cell. (Modified from Lang and Wells, 1987)

eggshell color is a complex trait and many genes are proposed to account for the change of the shade (Punnett and Bailey, 1920; Hall, 1944). Analysis of brown egg strains and crosses among brown egg strains has shown a higher value for the dam's component of variance than for the sire's component (Hunton, 1962; Blow *et al.*, 1950). Major genes are probably segregating in these brown egg flocks, some of which may be acting in a dominant manner (Gowe *et al.*, 1965). Distribution comparisons indicated that two major autosomal loci affected the trait in these lines; one gene having incomplete dominance controls the amount of pigment deposition; the other completely inhibits pigment deposition when homozygous recessive (Wei *et al.*, 1992). Estimates of heritability for eggshell color in three Catalan poultry breeds were 0.49, 0.48 and 0.27, respectively (Francesch *et al.*, 1997). And in brown-egg dwarf layers, heritability for eggshell color was 0.46 (Zhang *et al.*, 2005).

Schwartz *et al.* (1980) indicated that genes of two mitochondrial enzymes, delta-aminolevulinic synthase (ALAS1) and ferrochelatase, may be located on chromosome one. However, the blue eggshell is controlled by a dominance gene, Oocyan (Punnett, 1933 as cited in Kennedy and Vevers, 1973). Poultry geneticists use *O* to designate the locus for the blue eggshell trait and the gene symbol for the recessive, wild-type gene is *O* or *O*⁺. When Zartman's (1973) study indicated that the locus for pea comb was on chromosome one, the blue egg locus was presumed to be on the same chromosome. Bruckner and Hutt (1939) had determined close linkage between pea comb and blue egg. The distance in centiMorgans(cM) between *P* and *O* in normal chromosome backgrounds has been reported as 6 (standard error not reported) (Bruckner and Hutt, 1939; n=35), 2.4±8.5 (Crawford, 1986; n=136), 4.3±1.3 (Bitgood *et al.*, 1983; n=210), 4.5±0.1 (Bartlett *et al.*, 1996; n=44), and 3.3 ± 1.4 cM (Bitgood *et al.*, 2000; n=152). The remarkable improvement of biochemical and molecular tools has now made it possible to identify the molecular nature of major genes, and to understand better their mode of action (Tixier-Boichard, 2002). A study by Wardecka *et al.* (2002) indicated that the microsatellite MCW0047 located on chromosome 4, and it was suggested to be linked to the genes affecting shell color at 33 weeks. And the loci affecting shell color at 53 weeks may be located at chromosome 5 (Wardecka *et al.*, 2003). Quantitative trait loci were detected for redness of eggshell color (using the L*a*b color system) on chromosome 11 (Sasaki *et al.* 2004). Huang *et al.* (2006) also demonstrated that the DNA fingerprints between green and white-shelled ducks were different. The recombination rate of chicken chromosome 1 is 3.9 cM/Mb, and the *O* locus is 2.3 cM from ALEV1. BLAST results of the genes coding for the enzymes in the porphyrin pathway, including ALAS1 in chromosome 12 and heme oxygenase 1 (HO1) in chromosome 1, showed no corresponding locus was found to specify the *O* locus (Zhao *et al.*, 2007).

6. Factors affecting eggshell color

Although the avian shell color was determined by genetic, there are several other factors responsible for decreasing the intensity or almost total loss of eggshell color:

(1) Age of birds

The color of shells on eggs from a given flock turns paler with age of the flock (Lang and Wells,

1987). It was reported that the decline in eggshell quality with age of the hen was a result of an increase in egg size without a proportionate increase in calcium carbonate deposition in the eggshell (Roland, 1979). Odabaşı *et al.* (2007) study indicated that size of the egg was the major factor affecting the color of the eggshell. These findings quantified the observations that older hens lay lighter colored eggs due to an increase in egg size associated with no proportionate change in the quantity of pigment deposited over the shell surface. For the blue-shelled ducks, the redness of eggshell also decreased when duck aged (Liu, 2010).

(2) Stresses

The majority of pigments are colocalized within the cuticle which is secreted by epithelial cells lining the shell gland. Environmental stimuli taking place at the last three to four hours of rapid cuticle deposition would likely determine the outcome of both shell quality and color (Hughes *et al.*, 1986). Stressors in poultry flocks such as high cage density, handling, loud noises, and others may result in the release of stress hormones, especially epinephrine. This hormone, when released into the blood, is responsible for causing a delay in oviposition and the cessation of shell gland cuticle formation. The above stressors, which result in hen nervousness and shock, could cause pale eggshells. The paleness is often the result of amorphous calcium carbonate deposited on top of a pre-existing fully formed cuticle or of an incomplete cuticle caused by premature arrest of cuticle formation (Sykes, 1959; Solomon *et al.*, 1987). One of the more common stressors which cause eggshell pigment loss is fear. If a bird is scared, there is an accompanying release of epinephrine which prepares the animal for "fight or flight". In fact, the relationship between stress and the production of pale eggs by laying hens is so close that researchers have suggested that loss of shell pigment may provide a basis for a non-invasive method of assessing stress in hens (Butcher and Miles, 1995).

(3) Diseases

Viral diseases, such as New Castle disease and infectious bronchitis, affect egg production in poultry (Cook, 1986; McCartney, 1989). These viruses have a specific affinity for the mucus membranes of the respiratory and reproductive tracts. Because the viruses infect and damage the reproductive tract directly, the signs of disease are also manifested directly on the egg. Thus, total egg numbers decline, eggshells become thinner and abnormally pale, and have irregular contour. Internal quality is also adversely affected. These egg production and quality problems can persist for extended periods of time (Butcher and Miles, 1995).

(4) Chemotherapeutic agents

A rapid decline in shell pigmentation is common following the ingestion of certain drugs, such as sulphonamides (Cooke, 1978). Feeding hens with coccidiostat Nicarbazin at a dose of 5 mg/day, resulted in the production of pale eggs within 24 hours (Schwartz *et al.*, 1975). Higher doses of coccidiostat Nicarbazin lead to complete depigmentation of the eggshell cuticle (Langs and Wells, 1987).

(5) Environmental factors

Although shell color is mainly determined by genetics, the effect of strong sunlight and high

temperatures on the hens induces a fading effect on the shells. It is unclear why the strong sunlight would cause a bleaching effect. Still, it is a problem for egg producers and retailers. Research conducted in Australia showed that providing cold water (5°C) in hot season enabled hens to produce eggs with normal dark color. The shell was also heavier and has a stronger strength. The eggshell color is also strongly affected by housing system; lightness for the outdoor eggs is 52.33 ± 0.37 and for the cage group is 50.49 ± 0.39 in Dongxiang blue-shelled layers (Wang *et al.*, 2009).

CONCLUSION

Bird eggs show an enormous diversity of eggshell colors. Major pigments present in bird eggshells are protoporphyrin, biliverdin, and its zinc chelate, and mostly depending on different genetics or gene expressions. Even the origin of eggshell protoporphyrin is still unclear, blood and the shell gland are considered as the sites of biosynthesis of eggshell porphyrins. Further study of the regulatory mechanism underlying the differential expression of HO1 and the mechanism controlling the biliverdin transportation from the shell gland into uterine fluid may play a key role in regulating bird eggshell colors.

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家禽蛋殼顏色形成之探討

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摘要：就確保物種順利繁衍之角度而言，鳥類蛋殼具有不同顏色，主要除賦予鳥蛋偽裝效果外，並可能與蛋溫之調節有關。就經濟效益而言，不同蛋殼顏色之禽蛋價格，主要取決於消費者之偏好。禽蛋蛋殼顏色，因品種不同而異；就目前所知，構成禽類蛋殼顏色之重要色素成員包括兩大類：1. 原卟咯紫質(protoporphyrin)；2. 膽綠質(biliverdin)及其衍生物例如鋅-膽綠質螯合物(zinc-biliverdin chelate)等。原卟咯紫質乃血色質(heme)形成過程之一中間產物，其已知係構成棕色蛋殼之主要色素；而膽綠質則為血色質裂解後之產物，係構成青色蛋殼之主要色素。蛋殼色素雖係由殼腺之上皮細胞所分泌，但就其色素合成之場所而言，則有源自於血液或殼腺等兩種不同論證。蛋殼色素自殼腺上皮細胞釋出的時間，可因禽類品種之不同而異。諸多因素包括：緊迫、年齡、藥物投予、疾病、與環境因素等，均可能影響蛋殼顏色之呈現；惟鑑於蛋殼顏色因禽類品種有所差異，顯示蛋殼顏色不同與遺傳基因之表現有密切關係。

(關鍵語：蛋殼色素、蛋殼品質、遺傳研究)

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