Traits of eggshells and shell membranes of translucent eggs

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ABSTRACT Translucent eggshells negatively affect the appearance of eggs and decrease their economic value. Translocation and accumulation of water from the contents to the shells of eggs are frequent occurrences. Causes of translucent eggshell formation have been investigated, but the primary reason is uncertain. In previous studies, scientists have found that the thickness of the eggshell membrane was significantly different between translucent and opaque eggs. However, there are some conflicts among studies. We performed 2 experiments with 3 breeding flocks of chickens to target the reasons for egg translucence. In experiment 1, eggs of 1,024 Brown-Egg Dwarf Layers (DWL) were used. Approximately 1,600 eggs were collected over 2 consecutive days. They were stored for 3 days, and then 120 translucent and 120 opaque eggs were selected for measurement of egg quality traits and weight loss over several weeks. In experiment 2, we used DWL and White Leghorn pure line (WLL) for assessment of eggshell ultrastructure and membrane traits. We chose 120 translucent and 120 opaque eggs from 3,500 DWL eggs and 125 translucent and 125 opaque eggs from 5,028 WLL eggs. The results are as follows: (1) translucent eggs had greater eggshell strength and lower ultimate failure stress of shell membrane than opaque eggs in both DWL and WLL groups, (2) translucent eggs had thicker shells and thinner shell membranes than opaque eggs in DWL, (3) no significant differences were found in either gas pore or bubble pore traits between translucent and opaque eggs in either line, and (4) no significant differences were detected in internal egg quality or weight loss between translucent and opaque eggs in either line. In summary, the present study suggests that variations in both eggshells and shell membrane structures are implicated in the formation of translucent eggs.

Key words: translucent eggshell, eggshell strength, shell membrane, ultimate failure stress, pore

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INTRODUCTION

Eggshell translucence is a result of the transfer of moisture from the egg's contents through the shell membrane and its accumulation in the eggshell, leading to increased transmission of light (Solomon, 1991); a translucent shell can be easily penetrated by *Salmonella* (Chousalkar et al., 2010).

Previous studies have focused on variations in eggshell strength, shell thickness, and the structure of the shell mammillary layer. No differences have been found in either eggshell thickness or eggshell strength between translucent and opaque eggshells (Holst et al., 1932; Nie, 2013). Additionally, no significant statistical differences in eggshell ultrastructure variations, such as size of mammillary cones and quantity of type A and type B mammillary bodies, have been reported (Solomon, 1991; Chousalkar et al., 2010). Though eggshells have pores that may provide adequate space for visible moisture accumulation, no significant difference exists in pore distribution between translucent and opaque eggshells, according to previous studies (Nie, 2013; Ray and Roberts, 2013). An eggshell membrane is a biopolymer network (Torres et al., 2010) and is composed of randomly oriented individual fibers (Bellairs and Boyde, 1969). Among membrane fibers, there are a large number of meshes, resulting in shell membrane porosity of about 52 percent (Chousalkar et al., 2010). In recent years, research has shown that translucent eggshells have membranes that are significantly thinner (Liu et al., 2007; Nie, 2013) than those of opaque eggs.

Even though studies have been conducted to discover the differences between translucent eggs and opaque eggs, the primary reasons for translucence remain uncertain. As there are many factors that potentially affect eggshell translucence, very precise control

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of within-group variation is necessary for probing these factors to determine the differences between translucent eggs and opaque eggs. In this study, we systematically measured physical traits of eggshells and shell membranes in both translucent and opaque eggs to explore the relationship of translucence with eggshell and eggshell membrane traits.

MATERIALS AND METHODS

We designed 2 experiments, using a sufficient number of eggs from a single flock of chickens per breeding line to minimize within-group variations and to focus on eggshell qualities and membrane characteristics. Experiment 1 investigated the general differences in egg quality measurements between translucent eggs and opaque eggs in a single line. Experiment 2 aimed to confirm the results of the first experiment and to measure additional physical traits in translucent and opaque eggs of 2 lines of chickens. Protocols were approved by the Animal Care and Use Committee of China Agricultural University (permit number: SYXK 2007-0023).

Experiment 1

Hens A pure line of Brown-Egg Dwarf Layers (**DWL**) was used for this experiment; it was developed at the China Agricultural University (**CAU**) by 4 repeated backcrosses of male meat type dwarf ISA-Vedette to the female CAU brown egg layers (Yang et al., 1996). The DWL were reared by CAU Poultry Breeding Corp., Beijing, China. As of the end of 2014, when this experiment took place, the DWL had been bred to the 13th generation. In this experiment, eggs from 1,024 56-week-old DWL were collected and analyzed. All hens were reared in individual cages under identical conditions and were fed the same food throughout the experiment.

Samples and Measurement We collected 1,600 eggs over 2 consecutive days and stored them in a constant environment (temperature 20 to 25°C, RH 50 to 60%) for 3 days. All eggs were further identified using a conventional source of illumination (60 watt clear glass incandescent light bulb) (Solomon, 1991) and classified into 3 grades according to the degree of shell translucence (opaque, semi-translucent, and translucent) (Holst et al., 1932). Of these, 120 translucent and 120 opaque eggs were selected for further study following our selection procedure (Figure 1).

The selected eggs were weighed and divided into 4 groups (A, B, C, and D; n = 30 for each group); the groups were similar to one another and each included a range of weights from small to large. Groups A, B, and D were stored at controlled room temperature (20 to 25°C) and used for egg quality measurement at 1 wk, 2 wk, and 3 wk respectively. Group C was used for measurement of weight loss, shell thickness and membrane thickness.

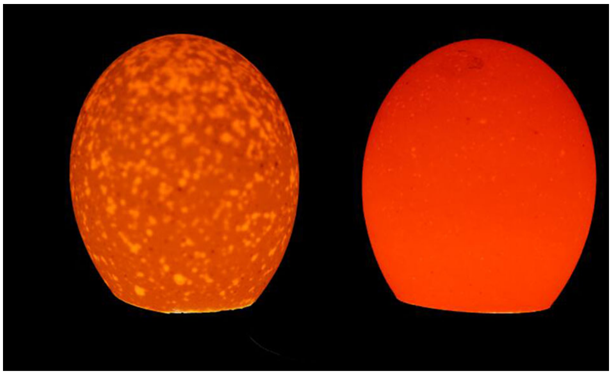

Figure 1. Translucent egg (left) and opaque egg (right) used in the experiment.

Egg quality traits include egg weight (**EW**), eggshell color (**ESC**), eggshell strength (**ESS**), eggshell thickness (**EST**), eggshell weight (**ESW**), yolk color (**YC**), yolk weight (**YW**), yolk ratio (**YR**), albumen height (**AH**), haugh unit (**HU**), and egg shape index (**ESI**). EW, ESW, and YW were measured with an electronic balance with an accuracy of 0.1 g within a range of 0 to 500 g. ESC was the average of 3 different shell areas (blunt end, middle end, and sharp end) measured with a portable spectrophotometer (CM-2600d, Konica Minolta, Inc., Tokyo, Japan). ESS was measured with an eggshell force gauge (Model-II, Robotmation Co. Ltd., Tokyo, Japan). EST was measured with a digital display micrometer gauge (395-741-10, Mitutoyo, Kawasaki, Japan). YC, AH, and HU were measured using an Egg Multi Tester (EMT-5200, Robotmation Co. Ltd.). ESI is the long diameter to short diameter ratio and was measured with an NFN384 egg quality analyzer (FHK, Tokyo, Japan). YR is the yolk weight to egg weight ratio. Eggshell and membrane thicknesses were measured at the blunt end, middle, and sharp end of the egg using a digital display micrometer gauge (Mitutoyo 395-741-10).

Experiment 2

Hens Two genetically distinct chicken lines, DWL and the White Leghorn pure line (**WLL**), were used in the experiment. Both lines were provided by CAU Poultry Breeding Corporation, Beijing, China. Experimental conditions were the same as described for experiment 1. Eggs from 1,356 42-week-old DWL hens (the generation after those used in experiment 1) and 1,130 50-week-old WLL hens were used in the experiment. We collected 3,500 eggs from DWL over 3 consecutive days and 5,028 eggs from WLL over 5 consecutive days and stored them in a constant environment (temperature 20 to 25°C, RH 50 to 60%) for 3 days. We selected 120 (40 × 3 d) translucent and 120 (40 × 3 d) opaque eggs from DWL, and 125 (25 × 5 d) translucent and 125 (25 × 5 d) opaque eggs from WLL for further measurements. Selection criteria were as described for experiment 1.

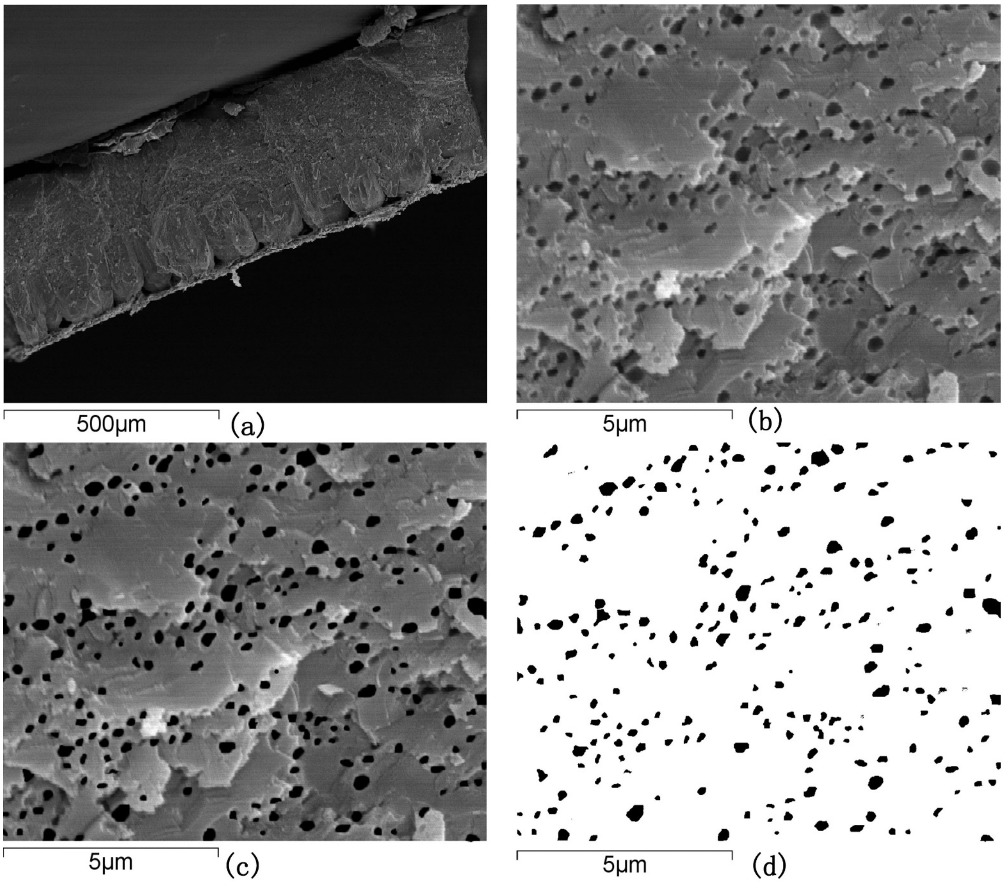

Figure 2. Eggshell thickness (a), bubble poles observed at 10,000× SEM (b), bubble pores were manually "painted" black using Photoshop software to increase the color contrast with other areas of the eggshell (c), bubble pores traits were measured by ImageJ software (d).

Eggshell Trait Measurements First, the eggshell strength of both translucent and opaque eggs was measured with an eggshell force gauge (Model-II, Robotmation Co. Ltd.). Albumen and yolk contents were removed through a hole drilled near the pointed end of each egg. The total shell membrane was separated mechanically from the air chamber and then washed with distilled water. 2 pieces (1 cm^2 each) of eggshell were taken from the equatorial region of the egg; one was used to count the quantity of gas pores (**QGP**) (Mehlum et al., 1987), and the other was used to count bubble pores and to measure the thickness of 3 shell layers (eggshell layer, mammillary layer, and effective layer). The eggshell pieces were dyed and the numbers of gas pores counted by optical microscopy using methods described in Mehlum et al. (Mehlum et al., 1987).

We used scanning electron microscopy (Panheleux et al., 1999; Zhou et al., 2011) at 200 times magnification to measure shell layer thicknesses (Figure 2a). Quantity of bubble pores (**QBP**), average area of bubble pores (**AABP**), area sum of bubble pores (**ASBP**), and an area ratio of ASBP to total area of eggshell (**ARBE**) in a cross section of the middle of the eggshell were measured at 10,000 times magnification. First, all shell samples were sputter-coated with gold to increase conductivity, then pictures of bubble pores were captured by SEM (Figure 2b). Next, each bubble pore was manually "painted" black using Photoshop software to increase the color contrast with other areas of the eggshell (Figure 2c). The area of each bubble pore and some other traits could be identified and measured automatically by ImageJ software (Figure 2d). In addition, by manually increasing the color contrast, traits of bubble pores could be accurately measured by ImageJ software. ASBP was calculated as the sum of the areas of all pores; AABP was calculated as ASBP divided by QBP; and ARBE was calculated as ASBP divided by the total area of the picture.

Membrane Traits After the membrane was separated from the eggshell and washed, 2 latitudinal direction and 2 longitudinal direction rectangular membrane samples (5 mm × 20 mm each) from the middle of the membrane were prepared for ultimate failure stress and maximum elongation measurements. A MARK-10 bench top tensile testing machine (Mark-10 Model MG, Mark-10 Corp., Hicksville, NY) equipped with a 5 N load cell was used for membrane tension tests at a testing speed of 2 mm/s.

A piece (1 cm × 1 cm) of eggshell membrane from the middle of the eggshell was taken and dried naturally for permeability testing. Water drop penetration time was measured by a contact angle analyzer (Model SL200K, Rycobel, Deerlijk, Belgium). Droplets were set at 2 μL. The middle thickness of membrane was fixated

(Morris, 1965) and then measured with a digital display micrometer gauge.

Statistical Analysis Outliers—values outside Mean ± 3 SD—were excluded. Data were tested against a normal distribution. The 2 groups' data were then analyzed using Student t tests (TTEST Procedure) in SAS9.2 (SAS Institute Inc., Cary, NC), assuming homogeneity of variances in the sample populations.

RESULTS AND DISCUSSION

Experiment 1

Summary information about the 4 groups of eggs (A, B, C, and D) is listed in Table 1. Egg quality traits of groups A, B, and D are listed in Table 2. In Table 1, we can see that initial differences among the 4 groups of eggs were well controlled, and the translucent and opaque eggs were similar in egg weight. In Table 2, we can see that average values of ESS, EST, and ESW of translucent eggs are significantly ($P < 0.01$) higher than those of opaque eggs; however, there were no significant ($P > 0.05$) differences in ESC, YC, YW, YR, AH, HU, or ESI between translucent and opaque eggs. The egg weight loss, shell thickness, and membrane thickness of group C are listed in Table 3. There was no significant difference in weight loss between translucent and opaque eggs after 4 weeks of storage ($P > 0.05$). Translucent eggshells are significantly thicker ($P < 0.01$) than opaque eggshells at the blunt end, middle, and sharp end, and membranes of translucent eggs are significantly thinner ($P < 0.01$) than those of opaque eggs in the middle and at the sharp end.

Overall, we found that there was no significant difference between translucent and opaque eggs in internal qualities; these results are consistent with prior research (Baker and Curtiss, 1958; Nie, 2013).

Eggshell strength is affected by both shell thickness and shell ultrastructure. Shells contain a mammillary layer, a palisade layer (an extension of the mammillary layer, formed after the mammillary layer has fused), and a vertical crystal layer (Lunam and Ruiz, 2000). The significant correlation between palisade layer width and puncture force (Carnarius et al., 1996) were confirmed. The quantity and species of shell matrix proteins influence calcite crystalline structure and types, which also greatly influence shell strength (Mann et al., 2003; Gautron et al., 2011; Hincke et al., 2012). In this study, eggshell weight is mainly affected by shell thickness and shell density, which can explain the greater shell strength, thicker shell, and greater weight of translucent eggs.

There was no significant difference in weight loss between the 2 kinds of eggs, even though translucent eggshells are thicker than those of opaque eggs. Water vapor exchange is dependent on the diffusive

Table 1. Basic groups' information of translucent and opaque eggs.

[1]Group	Weight (g, translucent)	N	Weight (g, opaque)	n
A	53.54 ± 2.03	30	54.04 ± 1.81	30
B	57.92 ± 0.98	30	57.78 ± 0.91	30
C	60.77 ± 0.74	30	60.82 ± 0.91	30
D	64.84 ± 3.45	30	64.66 ± 1.75	30

[1] Translucent eggs and opaque eggs were divided into 4 (A, B, C, D) groups respectively according to their weights.

Table 2. Difference of egg quality traits between translucent and opaque eggs for storage at 1, 2, and 3 weeks.

	Group A (1 wk)		Group B (2 wks)		Group D (3 wks)	
Egg quality	Translucent (n = 30)	Opaque (n = 30)	Translucent (n = 30)	Opaque (n = 30)	Translucent (n = 30)	Opaque (n = 30)
External egg quality						
EW (g)	51.86 ± 2.04	52.46 ± 1.81	54.16 ± 1.00	54.69 ± 1.04	61.59^{A} ± 3.37	59.66^{B} ± 1.92
ESC- L	70.33 ± 4.34	70.61 ± 4.36	70.2 ± 4.94	68.69 ± 4.04	69.14 ± 4.52	69.25 ± 4.20
ESC-A	11.82 ± 2.14	12.75 ± 2.76	12.63 ± 2.78	12.79 ± 1.94	12.25 ± 2.64	12.87 ± 2.54
ESC-B	25.06 ± 2.78	25.97 ± 3.06	26.53 ± 3.31	26.09 ± 2.07	26.02 ± 3.02	26.56 ± 3.31
ESS (kg/cm^2)	3.27^{A} ± 0.79	2.64^{B} ± 0.63	3.05^{A} ± 0.57	2.58^{B} ± 0.55	3.04^{A} ± 0.71	2.42^{B} ± 0.68
EST(μm)	337.1^{A} ± 31.4	312.7^{B} ± 25.3	333.4^{A} ± 26.2	299.6^{B} ± 27.8	339.6^{A} ± 15.9	310.0^{B} ± 26.1
ESW(g)	5.52^{a} ± 0.45	5.28^{b} ± 0.42	5.84^{A} ± 0.35	5.19^{B} ± 0.42	5.93^{A} ± 0.40	5.37^{B} ± 0.49
ESI	1.36^{A} ± 0.05	1.32^{B} ± 0.03	1.37 ± 0.04	1.37 ± 0.05	1.36 ± 0.05	1.35 ± 0.05
Internal egg quality						
YC	7.14^{a} ± 1.45	6.35^{b} ± 1.47	7.83 ± 1.09	8.33 ± 0.88	8.72 ± 0.65	8.39 ± 0.83
YW(g)	16.31 ± 1.13	16.13 ± 0.76	18.12 ± 1.42	18.06 ± 1.23	20.05 ± 1.46	19.87 ± 1.74
YR (%)	31.12 ± 1.85	30.80 ± 1.39	33.16 ± 0.02	33.85 ± 0.02	32.90 ± 0.02	33.32 ± 0.03
AH (mm)	6.14 ± 1.38	5.72 ± 1.33	5.36 ± 1.25	5.77 ± 1.64	5.06 ± 1.07	5.55 ± 1.23
HU	79.85 ± 9.40	76.69 ± 9.72	73.29 ± 8.96	73.74 ± 11.43	68.89 ± 8.47	72.96 ± 8.82

^{A,B}between translucent and opaque eggs for each trait, means without a common superscript differ ($P < 0.01$).

^{a,b}between translucent and opaque eggs for each trait, means without a common superscript differ ($P < 0.05$).AH = albumen height; ESC = eggshell colour; ESI = eggshell index; ESS = eggshell strength; EST = eggshell thickness; ESW = eggshell weight; EW = egg weight; YC = yolk colour; YR = yolk ratio; YW = yolk weight.ESI = long length of egg/short length of egg; YR (%) = YW/EW×100.

Table 3. Difference in weight loss, shell, and membrane thicknesses between translucent and opaque eggs of group C.

Traits	Translucent (n = 30)	Opaque (n = 30)
Initial weight (g)	60.77 ± 0.74	60.82 ± 0.91
Weight loss (g)		
0-1 wk	1.72 ± 0.39	1.59 ± 0.36
0-2 wk	3.34 ± 0.77	3.26 ± 0.60
0-3 wk	5.33 ± 1.24	5.20 ± 1.14
0-4 wk	7.13 ± 1.67	6.94 ± 1.32
Thickness of blunt end (μm)		
eggshell and membrane	$354.6^{A} \pm 19.1$	$324.4^{B} \pm 26.3$
eggshell	$329.4^{A} \pm 18.2$	$297.7^{B} \pm 24.9$
membrane	25.16 ± 9.47	26.65 ± 6.47
Thickness of middle end (μm)		
eggshell and membrane	$358.8^{A} \pm 18.46$	$333.8^{B} \pm 27.19$
eggshell	$336.8^{A} \pm 19.81$	$306.7^{B} \pm 27.28$
membrane	$22.12^{a} \pm 8.55$	$27.11^{b} \pm 6.57$
Thickness of sharp end (μm)		
eggshell and membrane	$365.4^{A} \pm 23.1$	$331.6^{B} \pm 32.3$
eggshell	$341.0^{A} \pm 21.7$	$300.4^{B} \pm 31.5$
membrane	$24.36^{A} \pm 7.31$	$30.82^{B} \pm 8.09$
Average thickness(μm)		
eggshell and membrane	$359.6^{a} \pm 15.9$	$330.1^{b} \pm 26.1$
eggshell	$335.7^{A} \pm 15.9$	$301.8^{B} \pm 25.4$
membrane	$23.84^{A} \pm 5.41$	$28.09^{B} \pm 4.72$

^{A,B} between translucent and opaque eggs for each trait, means without a common superscript differ ($P < 0.01$).

^{a,b}between translucent and opaque eggs for each trait, means without a common superscript differ ($P < 0.05$).

Average thickness = (blunt end+ middle end+sharp end)/3.

properties of gas pores compared to the resistance offered by the shell and shell membrane (Wangensteen and Rahn, 1971). For eggs stored in a constant environment where the eggs are neither fertilized nor strongly respiring, variations in eggshells may have little effect on vapor exchanges between the egg contents and the external environment. This result is consistent with the work of Holst et al.(Holst, Almquist and Lorenz, 1932) and Baker et al. (Baker and Curtiss, 1958). Previous reports have shown that the membrane of translucent eggshells is significantly thinner than that of opaque eggshells (Liu et al., 2007; Nie, 2013), and this is confirmed by the current experiment. Because the eggshell membrane is the first barrier that prevents egg contents from permeating to the outside, and it provides a non-mineralized platform for eggshell formation (Baláž, 2014), variations in the shell membrane may be important in formation of translucent eggshells.

In summary, in experiment 1 we first found a significant difference in shell thickness between translucent and opaque eggs. Second, the thinner membrane of translucent eggs was confirmed. Consequently, further comparisons in the details of eggshell porosity, eggshell ultrastructure, and shell membrane physical traits between the 2 types of eggs were carried out and discussed in experiment 2.

Experiment 2

Eggshell Layer Thicknesses We chose translucent and opaque eggs for comparing detailed shell traits and shell membrane characteristics; the results are presented in Table 4. In DWL, ESS, EST, and effective layer thickness of translucent eggshells were all significantly higher ($P < 0.01$) than those of opaque eggs. In WLL, ESS of translucent eggs was significantly higher ($P < 0.01$) than that of opaque eggs, whereas there were no significant differences in EST, effective layer thickness, or mammillary layer thickness ($P > 0.01$). Combining the results of the 2 pure breeding lines above, the only difference between the 2 kinds of eggs (translucent and opaque) was ESS, but EST, effective layer thickness, and mammillary layer thickness may be different in different lines. There are varying reports of differences between translucent and opaque eggs in EST, effective layer thickness, and mammillary layer thickness. Nie (2013) reported that the only difference between these 2 kinds of eggshells was the thickness of mammillary layer (where translucent was thicker than opaque). The EST and thickness of the effective layer showed no differences (Solomon, 1991; Nie, 2013). In terms of mammillary structure, both early (Chousalkar et al., 2010) and late fusion (Solomon, 1991) of the mammillary layer was observed in translucent eggshells; fusion timing is represented by low (early fusion) and high (late fusion) mammillary layer thickness. Other variations in the mammillary layer, such as mammillary cap arrangements, size of mammillary cones, and numbers of types A and B bodies, have been investigated (Solomon, 1991; Chousalkar et al., 2010). Therefore, the relationship between eggshell ultrastructure and translucence remains uncertain.

Eggshell Pores Gas pores and sub-microscale bubble pores are the 2 main pore systems of eggshells,

Table 4. Eggshell traits and eggshell membrane traits of translucent and opaque eggs.

[1]Traits	DWL				WLL			
	Translucent	n	Opaque	n	Translucent	n	Opaque	n
Eggshell								
EW(g)	48.20 ± 3.57	120	48.07 ± 3.69	120	56.18 ± 3.78	125	56.56 ± 3.57	125
ESS(kg/cm^2)	3.27^{A} ± 0.77	120	2.90^{B} ± 0.77	120	3.25^{A} ± 0.72	125	2.96^{B} ± 0.72	125
EST (μm)	327.6^{A} ± 19.7	30	289.7^{B} ± 35.2	30	304.5 ± 24.2	121	299.5 ± 22.4	125
MAT (μm)	90.96 ± 19.79	30	93.83 ± 18.87	30	75.84 ± 11.64	30	79.42 ± 12.00	30
EFT (μm)	227.9^{A} ± 27.0	30	190.7^{B} ± 35.3	30	226.7 ± 25.70	30	226.8 ± 21.40	30
Gas pores								
QGP	34.14 ± 3.62	30	34.96 ± 4.69	30	39.67 ± 6.33	30	40.60 ± 6.89	30
[2]Bubble pores								
QBP	149.3 ± 38.8	30	143.1 ± 43.0	30	127.9 ± 39.5	30	124.8 ± 39.5	30
AABP,(um^2)	0.03 ± 0.01	30	0.03 ± 0.01	30	0.04 ± 0.01	30	0.03 ± 0.01	30
ASBP (μm^2)	4.85 ± 1.38	30	4.82 ± 1.48	30	4.39 ± 1.23	30	4.21 ± 1.33	30
ARBE	4.36 ± 1.24	30	4.33 ± 1.33	30	3.96 ± 1.11	30	3.79 ± 1.20	30
Membrane thickness								
Middle end (μm)	19.53^{A} ± 5.55	80	22.54^{B} ± 6.66	80	18.09 ± 6.83	125	19.06 ± 8.76	125
Maximum elongation (%)								
Latitudinal direction	40.79 ± 13.08	120	39.83 ± 11.06	120	27.76 ± 8.17	75	27.58 ± 10.54	75
Longitudinal direction	44.52 ± 14.65	120	46.01 ± 19.80	120	33.41 ± 12.24	75	35.33 ± 12.13	75
UTS (N)								
Latitudinal direction	0.55 ± 0.18	120	0.54 ± 0.18	120	0.26 ± 0.09	75	0.30 ± 0.12	75
Longitudinal direction	0.64^{A} ± 0.20	120	0.72^{B} ± 0.24	120	0.41^{a} ± 0.17	75	0.47^{b} ± 0.16	75
WDPT(s)	50.40 ± 44.28	20	84.53 ± 79.84	20	91.19 ± 98.29	20	57.79 ± 77.13	20

DWL = Brown-Egg Dwarf Layers; WLL = White Leghornpure line;

[1] AABP = average areas of bubble pores; ARBE = areas ratio of bubble pores; ARSB = ASBP/areas sum of eggshell in picture; ASBP = areas sum of bubble pores; EFT = effective layer thickness; ESS = eggshell strength; EST = eggshell thickness; EW = egg weight; MAT = mammillary layer thickness; QBP = quantity of bubble pores; QGP = quantity of gas pores; UTS = ultimate failure stress; WDPT = Water Drop Penetration Time.

[2] Bubble pores are measured at 10,000 times magnification by scanning electron microscopy and visual size is 11.20μm × 9.10 μm.

^{A,B}between translucent and opaque eggs for each trait, means without a common superscript differ ($P < 0.01$).

^{a,b}between translucent and opaque eggs for each trait, means without a common superscript differ ($P < 0.05$).

and gas conduction is mostly regulated by adjusting the sizes and numbers of bubble pores in the palisade and mammillary layers (Zhou et al., 2011). Gas pores are formed by uncompelled fusion among mammillary knots and their columnar extension (Tullett, 1975), and bubble pores are very densely distributed small holes through the eggshell. According to previous reports (Holst et al., 1932; Solomon, 1991), there is more water in translucent eggs than in opaque eggs. Translocation and accumulation of water from the internal contents are common occurrences in eggshells. Deep open channels among mammillary knots, gas pores, and bubble pores may provide room for water accumulation. Physical characteristics of eggshell pores might provide clues for explaining the formation of translucent eggs. To evaluate porosity of eggshells, we measured QGP, QBP, AABP, ASBP, and ARBE. Comparisons of these indices can generally reflect the basic substructure variations of eggshell pores among eggs. As shown in Table 4, none of these traits showed significant differences between translucent and opaque eggshells in either of the 2 pure lines studied ($P > 0.05$). The QBP result is consistent with Nie's (Nie, 2013) report. The average diameter (0.20 μm, assuming bubble pores are circular) of bubble pores corresponded with Zhou et al.'s (2011) report of diameters less than 0.25 μm. Shell porosity is also expressed as weight loss of egg contents, and in a previous study no significant difference was found in shell porosity between translucent and opaque eggs (Talbot and Tyler, 1974). In this study, we demonstrated the similarity between translucent and opaque eggshells in the traits QGP, AABP, ASBP, and ARBE. Our results suggest that there may be no ultrastructural difference between these 2 kinds of eggshells, and therefore ultrastructural differences may not be the major reason for translucent egg formation.

Physical Traits of Eggshell Membrane We measured the eggshell membrane thickness at the middle of the egg, confirming the results of experiment one in DWL and detecting the same trend in WLL (Table 4). The shell membrane mainly consists of individual fibers and can be divided into 3 parts: the outer eggshell membrane, the inner eggshell membrane, and the limiting membrane (Baláž, 2014). Differences in membrane thickness may be affected by fiber arrangement and membrane compactness. Because translucent eggshells are a result of moisture accumulation in the eggshell (Solomon, 1991), and the innermost egg white surrounding the limiting membrane is thin (Hincke et al., 2010), and the porosity is very large (52.06%) (Torres et al., 2010), water drop penetration time was used to evaluate shell membrane permeability. Water drop penetration time of translucent eggs was not significantly different from that of opaque eggs in either line ($P > 0.05$). Drying the shell membranes greatly increases their permeability (Kutchai and Steen, 1971), so because of possible variations of membrane porosity due to the sample drying procedure, the test may cannot completely reflect the membrane permeability inside the eggs.

The ultimate failure stress value (longitudinal direction of eggshell membrane) of opaque eggshells is significantly ($P < 0.01$) larger than that of translucent eggshell membranes in both lines (Table 4). The inner membrane is a more compact structure than the outer, and the decrease in the diameters of the fiber from the outside to the inside of the eggshell membrane was confirmed (Zhou et al., 2011). The difference may be explained by variations in porosity, diameter of fibers, compactness of membranes, and orientation of individual fibers. Though individual fibers are randomly oriented (Bellairs and Boyde, 1969), the longitudinal direction of ultimate failure stress is larger than that of the latitudinal direction (Torres et al., 2010), as supported by current results. The shell membrane is a biopolymer network of fibers, and the behavior of the membrane structure is determined by both individual fibers and the interaction between them. The membrane is the first barrier preventing egg content from penetrating out, and ultimate failure stress can represent toughness and elasticity. From the difference in ultimate failure stress, we can deduce that the membrane of translucent eggs is more easily broken and that the vapors in the contents can be more easily penetrated in these eggshells.

These results show that eggshell strength and ultimate failure stress of the eggshell membrane are the 2 indices that consistently indicate the differences between translucent eggs and opaque eggs. However, the measured substructures of eggshells in this study did not show consistent differences. We recommend 2 points for future research. First, more ultrastructures of eggshells, beyond gas pores and bubble pores, should be investigated. The higher proportion of elemental nitrogen in translucent eggshells (Talbot and Tyler, 1974) may suggest protein infiltration from egg contents to the eggshell. Second, the reasons for ultimate failure stress of eggshell membranes require further study. Different ultimate failure stresses of shell membrane suggest a difference in cushioning capacity for thermal expansion and contraction and vapor exchange of egg contents. This could be caused by fiber numbers, chemical compositions, or something else.

Summary In this study of translucent eggshells, larger ESS and a smaller ultimate failure stress from longitudinal direction were found in DWL and WLL for the first time. Higher EST and lower eggshell membrane thickness were confirmed in DWL. The study suggests that both eggshells and shell membranes influence formation of translucent eggs, but the mechanism requires further exploration.

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