

Yet even more ways to dress eggs

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ABSTRACT Traditionally, it has always been assumed that the reason for the patterns on birds' eggs is essentially visual – chiefly for the purposes of avoiding predators (crypsis) or, in the case of cuckoos, to mimic the eggs of their host. This paper explores the idea that the patterning found on the eggs of many species that have no apparent need for their eggs to carry visual signals is essentially functional. Data from the long-term study of Great Tits *Parus major* in Wytham Woods, Oxfordshire, suggest that pigment acts as a structural adaptation for the eggshell, and that it compensates for thinner areas of the shell, which in turn relate to the availability of calcium prior to and during egg-laying. This paper was presented originally as the 54th Bernard Tucker Memorial Lecture, given to the Oxford Ornithological Society and the Ashmolean Natural History Society, in November 2004.

'Oology taken alone proves to be a guide as misleading as any other arbitrary method of classification, but combined with the evidence afforded by due study of other particularities, whether superficial or deep-seated, it can scarcely fail in time to conduct us to an ornithological arrangement as nearly true to Nature as we may expect to achieve.' (Sir Alfred Newton – *Dictionary of Birds* 1896)

There are many things for which we should thank Bernard Tucker. He was a founder member of both the Oxford Ornithological Society (OOS) and the Cambridge Bird Club, and played a key role in founding both the BTO and the Edward Grey Institute, at Oxford University. But for all his unique contribution, Bernard Tucker had little to say about eggs – unlike his great mentor at Oxford, the Rev. Francis (Frank) C. R. Jourdain (1865–1940). Jourdain and Tucker founded the OOS in 1921 and, Tucker said later, but for Jourdain he would probably have been a botanist! Jourdain was an oologist, but more than just an egg-collector; he was an astute and meticulous student of ornithology and one of the greatest authorities of his day on the reproductive biology of Palearctic birds (Tucker 1940). Both Tucker and Jourdain made important contributions to Witherby's five-volume *Handbook of British Birds*, and so while Tucker described the birds and wrote of their habits, Jourdain described their eggs and nests. Jourdain's importance in the old world of egg-col-

lecting was such that he gave his name to the oologists' club: the *Jourdain Society*, whose collections now reside at the Oxfordshire County Museum. Since the 1950s, egg-collecting has of course been illegal in the UK, and I don't doubt the importance of that to bird conservation and welfare. But looking, as I have, at thousands of clutches in the field, and at extensive museum collections, such as the national egg collection at the Natural History Museum, Tring, I cannot but feel some sympathy for those earlier ornithologists who were simply struck by the beauty of birds' eggs.

Apart from recognising them as a conveniently packaged meal, humans have long been captivated by the beauty and diversity of birds' eggs; there is a hint of perfection in these marvellous objects. Indeed, a recent (February–July 2004) exhibition of birds' eggs at the Walter Rothschild Zoological Museum (part of the Natural History Museum) was entitled 'The egg: the most perfect thing in the universe?' Well, exaggerated though this claim may be, if it is perfection that we seek, we should not let our

delight at the artistry of the patterns on eggshells distract us from the functional aspects of the egg's pigmentation, for I would suggest that here lies the real wonder; it is here that there is a real pointer to the perfection that evolution strives for. The patterning on eggshells is not a trivial, abstract creation. It serves a function, and in this paper I hope to show that, at least for some of these patterns (perhaps in the majority of species), the function is intimately and exquisitely integrated with the dynamic function of the eggshell itself. I shall also describe how this piece of science has developed with input from various sources, and how specific circumstances and observations can lead to specific conclusions that have sometimes required a complete change in the conceptual model we had of the systems under study (Gosler *et al.* 2005; Higham & Gosler *in press*).

The appearance of eggshells

Before looking at the function of eggshells, we should consider their variation in appearance. It is clear that, despite the attention that eggs had received, by the end of the nineteenth century the diversity of patterns displayed on their shells was considered bewildering. Sir Alfred Newton had faith that this diversity must reflect the evolutionary relationships of the species concerned (see p. 338). But that belief requires the assumption that either the patterns are essentially non-functional, and so free to vary over time in parallel with the evolution of new species, or such selection as might act upon eggshell patterning would direct their evolution in more similar trajectories between related than between unrelated species. In other words, in either case, the eggs of closely related species (those that have diverged more recently) should be more similar than those of more distantly related forms. However, if pigmentation patterns are functional, and adaptive, and if common problems require common solutions, then we should see recurrent characteristics cutting across taxa; i.e. species which are only distantly related may have similar eggs (compare the eggs of Great Tit *Parus major* and Water Rail *Rallus aquaticus*).

Indeed, this is essentially what I aim to suggest here.

All birds' eggs are basically similar in structure, although there are minor, interesting, and presumably adaptive, differences; for example, the fact that megapode *Megapodius* eggs lack an air space. So the most obvious variation lies in their size, shape and, of course, colours. Although, among vertebrates, birds are not unique in laying an egg with a calcified shell, they are unique insofar as that shell is often pigmented. Because it consists almost entirely of calcium carbonate, the unpigmented shell is basically white. We might, therefore, also consider the 'primitive' condition to be a white, unpigmented, egg, and apparently in support of this assertion is the fact that most non-passerine species lay white eggs. It might come as a surprise to learn this because we think of the wonderfully cryptic patterns on the eggs of Charadriiformes (waders, gulls, terns, etc.) as the classic case of eggshell pigmentation (plates 167 & 168). But the eggs of these, and other species which are mostly ground-nesting (and therefore under strong selection by predators), are atypical of non-passerines. Consider owls (Strigiformes), woodpeckers and their allies (Piciformes), parrots (Psittaciformes), kingfishers and their allies (Coraciiformes), pigeons and doves (Columbiformes) and swifts (Apodiformes), to choose but a few orders represented on the British List out of many possible examples. All species in these orders lay pure-white



166. Clutch of Great Tit *Parus major* eggs, Wytham Woods, Oxfordshire.

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167 & 168. Nest with eggs of Ringed Plover *Charadrius hiaticula* (same nest in both photos), Kent, May 1984.

eggs, and in many other orders some, if not all, species lay unpigmented eggs. Even within the Cuculiformes, so well known for their evolution of eggshell pigmentation that mimics that of their host species (usually a small insectivorous passerine), the typical eggshell colour of non-parasitic species is plain white. It is tempting then to assume that all these forms have white eggs simply because they have not evolved the mechanism to pigment their eggs, i.e. for one reason or another they simply haven't had to.

This idea has been around a long time; Alfred Newton suggested it in 1896. But I think that it is only partly correct. Kennedy & Vevers (1976) showed that trace amounts of protoporphyrin, what we recognise as the reddish eggshell pigments, occur in the eggshells of all bird species, even all those non-passerines that lay apparently pure white eggs. So while it is probably correct that these species haven't needed to evolve more overt patterns of pigmentation, for example because they do not suffer predation by visual (typically avian) predators, it may be wrong to think that the mechanism to pigment their eggs does not exist in these species. If this is right, it means that selection for pigmentation, for example by increased avian predation, could evolve a patterned egg very quickly, much more rapidly than if the actual mechanism had to evolve from scratch in every branch of the evolutionary tree.

This brief survey of non-passerine eggs therefore indicates a number of important things. First, while cryptic coloration is clearly

important for many ground-nesting species such as waders, nightjars (Caprimulgidae) and sandgrouse (Pteroclididae), this is actually the exception rather than the rule, but note that it is typically open-nesting species that lay pigmented eggs. Many of the non-passerines that lay white eggs also nest in holes, and while this behaviour might reduce the eggs' vulnerability to predators and obviate the need for cryptic pigmentation of the eggs, their appearance might also help the female to find the clutch in the hole. Even within the Charadriiformes, there are hole-nesting species that lay white eggs (e.g. Crab-plover *Dromas ardeola* and Puffin *Fratercula arctica*). Second, while offering a wonderful opportunity to study a dynamic evolutionary process, the eggs of parasitic cuckoos are exceptional in many ways (Davies 2002). The presence of gentes in the Common Cuckoo *Cuculus canorus* supports the view that pigmentation can evolve rapidly. This suggestion is also supported by the fact that non-parasitic Cuculiformes typically lay unpigmented eggs, but this fact also implies that the mechanism is probably more ancient than a superficial consideration of non-passerines might suggest. The intricate story of the Common Cuckoo was beautifully told by Nick Davies in his transcript of a previous Bernard Tucker Memorial Lecture, to which readers should refer (Davies 2002).

Concentrating now on passerine species (and remember that 60% of all bird species are passerines), there are some species that lay unpigmented eggs (the white eggs of Black Redstart *Phoenicurus ochruros* are somewhat sur-

prising) but the majority of passerines lay eggs with at least some pigment. The patterns on eggshells (of all species) are produced by pigmentation with biliverdin, producing greens and blues, and protoporphyrins, producing the reds, browns and black markings. While the former is a bile pigment, produced in the breakdown of haem (the essential component of haemoglobin), the latter is produced in haem synthesis (in mitochondria) within the cells. A notable difference in these pigments is in the ways that birds seem to use them. Whilst biliverdin pigments always form a ground colour to the egg, upon or within which there might then be speckling, protoporphyrins can appear as either a ground colour (e.g. the familiar brown domestic chicken egg) or as spotting – maculation. This also reflects a difference in the distributions of pigments within the shell, so that while the blue biliverdin pigments permeate the whole eggshell, protoporphyrins (even when a ground colour) tend to be concentrated in distinct layers within the eggshell or upon it. These compounds also have a number of interesting properties (see below).

A striking aspect of passerine eggs is that there appears to be as much diversity of pigmentation in this one order as there is across all the non-passerines put together (fig. 1). There are cryptically coloured eggs in the ground-nesting larks (Alaudidae) and pipits (Motacillidae), and eggs that appear to be cryptic, such as the uniformly dark brown eggs of Cetti's Warblers *Cettia cetti* and nightingales *Luscinia*. There are also, apparently, cryptic eggs in the corvids that are reminiscent of raptor eggs, but given that their *nests* are so conspicuous, it is perhaps debatable whether the pigmentation does indeed serve that function. There are also many examples of blue eggs in passerines. Biliverdin and protoporphyrin both have antioxidant properties. Recent research suggests that the blue biliverdin pigment might also act as an anti-viral agent, which might protect the egg and also indicate the condition of the female, though whether

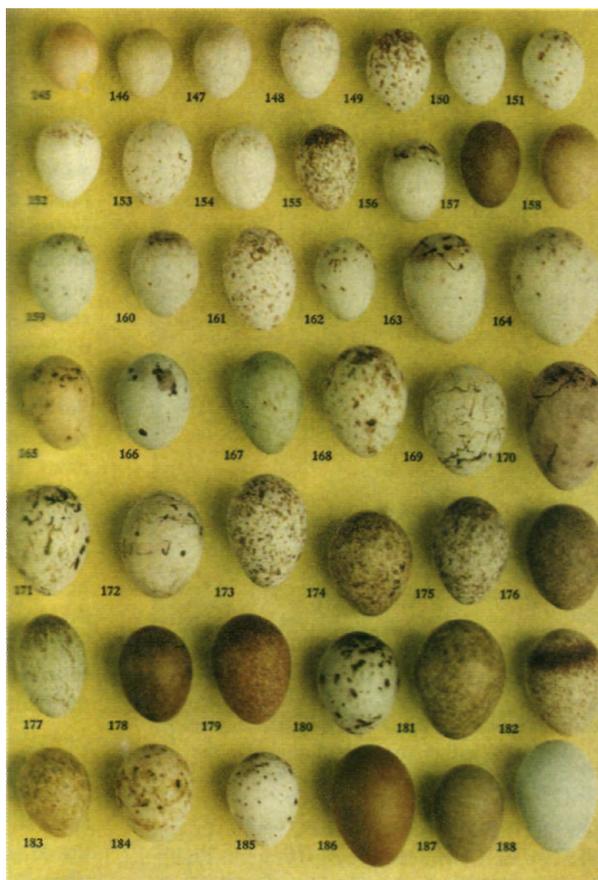


Fig. 1. Plate showing some of the diversity of passerine eggs. Note especially the concentration of speckling at the crown of the egg, forming a distinct corona ring in many (e.g. Wood Lark *Lullula arborea*, 182). Reproduced from Hoeher, S. (1974), *The Pocket Encyclopaedia of Birds' Eggs and Nesting Habitats*, Blandford Press, London.

this condition indication is a function of the pigment is unclear (Moreno & Osorno 2003). Interestingly, blue eggs seem to be especially common in species with complex mating systems, which supports the view that they might have a signalling role (Soler *et al.* 2005).

But these explanations (crypsis, cuckoos, sexual selection) are unconvincing in respect of the most-frequently encountered pattern found in passerines (and indeed in many non-passerines), and especially small passerines, throughout the world, in both tropical and temperate regions. This pattern consists of a small, plain white egg speckled with reddish spots, typically forming a ring around the blunt end of the egg – a 'corona ring' surrounding the 'crown' of the egg. Such eggs are seen in species covering a great range of taxonomic and ecological situations (for example, in Britain alone



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169. The author, checking nestboxes in the Wytham Woods study area, Oxfordshire.

there are tits (Paridae), nuthatches (Sittidae), treecreepers (Certhiidae), wrens (Troglodytidae), swallows (Hirundinidae), warblers (Sylviidae) and some finches (Fringillidae), in hole-nesters and open-nesters, in resident and migrant species, in insectivores and granivores, and in cuckoo hosts and species not parasitised by cuckoos.

Sources of variation in eggshells

In the late 1980s, while inspecting hundreds of Great Tit nests, year after year in Wytham Woods, near Oxford, I began to wonder what might be the purpose of these pigment speckles. At that time, the only convincing explanations for eggshell pigmentation on the eggs of *any* species were either crypsis or to make them mimic host eggs, i.e. to avoid predators or to avoid them being detected by cuckoos, respectively. So it was natural to try to fit all eggshell pigmentation into one or other of these paradigms. But tit eggs clearly do not fit. Although many ornithologists have tried (Lack 1968), and even still try, nobody could argue convincingly that the small reddish marks on tit eggs made them less conspicuous in the nest. Sir Alfred Newton had considered this back in 1896, and

rejected the notion (citing Hewitson 1838) saying:

‘In regard to the almost countless cases of spotted eggs in holes... the only supposition... would be that the species... have taken to hiding their treasures in times comparatively recently, and have not yet got rid of the ancestral habit of secreting and depositing pigment. ...no more can be added on this subject, interesting as it is, and worthy of much more investigation than it has received.’

This was, of course, pure speculation and given that it would seem remarkable that so many species, both related and not, would have to be getting ‘rid of the ancestral habit’ at exactly the same rate, I found this unconvincing. Since nothing *more* convincing had been published on the subject since then, I decided, in 1988, to accept Newton’s 100-year-old challenge and try to work out for myself what was going on. This paper presents some of the results of that decision.

It turned out that there were a number of good reasons for studying this subject in the Great Tit. One, practical, reason was already apparent: I was working within the context of the long-term Great Tit population study in Wytham Woods. Here, several hundred pairs of Great Tits, most of the local population, nest in nestboxes (of which there are about 1,000 in an area of woodland some 3.6 km² in extent). This means that the birds’ nests are readily accessible, and because the parents and their chicks are ringed as part of the long-term study, we are able to follow many generations of Great Tits within family lineages and ask, for example, whether the egg that a female lays is similar to the one from which she had hatched, or indeed the one from which her mother (or indeed father) had hatched.

This species was also very suitable for more theoretical reasons. The Great Tit is, of course, a hole-nester which lays one egg each day until its large clutch (typically about nine eggs in Wytham) is complete. Only then does the female incubate, although some late nesters may start incubating up to three days before the last egg is laid. The eggs are laid early in the morning, whereupon the female leaves the nest for the day. Before she leaves, she covers the eggs with moss or nest lining. This may serve to reduce evaporative water loss, but it seems

equally likely that it makes the eggs less conspicuous to any passing predator that comes across the nest hole. If this latter explanation is correct, it seems that the female herself doesn't appear to 'think' that her eggs are cryptic. Furthermore, the Great Tit is not a Cuckoo host, and neither does it generally dump eggs in other Great Tits' nests. So it is unlikely that the markings serve to identify the eggs, since the female does not have much need to be able to distinguish her own eggs. In fact, experiments with dummy eggs (Davies & Brooke 1989a,b) or which involve swapping eggs between nests (Pettifor *et al.* 1988; Gosler 1993) indicate that Great Tits are not good (in fact they seem to be hopeless) at spotting alien eggs in their nests; they will sit on pretty much anything you put in the nest once incubation has started. So the speckles on Great Tit eggs presented a real mystery.

Having looked at thousands of Great Tit clutches over the years, three things struck me about their speckles. First, I was pretty certain that the first egg was usually less marked than later eggs, but I was unaware as to whether subsequent eggs in a clutch varied in pigmentation in any systematic way. Second, it was clear that there was huge variation within the Wytham Great Tit population, with some birds laying immaculate (pure white) or very lightly speckled eggs, while others laid heavily dark-spotted eggs, but I was unaware of any particular geographical factor that might be causing that variation. Third, there was that 'corona' ring – what was the significance of that? It was possible to conceive of a system that might produce such a pattern, but for what purpose? Again I had no idea that this might reflect an engineering problem for the egg, and in fact my conceptual 'model' of eggshell pigmentation didn't help. Like most people, I had assumed that the bird made an egg, encased it in a shell, and then put spots on it – what we might call the 'inkjet printer' model. Indeed, we are led to that course of reasoning by the standard description of how bunting (Emberizidae) eggs get their streaks (e.g. Newton 1896, p.186): i.e. by the egg rotating in the eggshell gland while pigment is applied (I wonder whether that is

actually correct – do we *really* know?). I later realised that it was inconceivable that such a model would be able to produce the patterns that colleagues, students and I had discovered, and which I shall describe shortly.

So, having obtained a feel for the variation in speckling over a number of years, in 1988 I invented a simple method of scoring the spot patterns. With a little practice, I found that I could assess the average score for a clutch in a few seconds and even interpolate 'half' scores (e.g. 2.5, 3.5) repeatably. The scoring system is based on the recognition that there were really three aspects to the variation that I saw among clutches. This became known as the 'IDS' system because eggshells varied in pigment intensity (I), from white (score 0) or lightly spotted (score 1), to very dark-spotted (score 5); they varied in pigment distribution (D), from all spots concentrated at one (usually the broad) end (score 1) to evenly spotted all over the egg (score 5); and they varied in spot size (S), from small (score 1) to large (score 5). A series of sketches in the back of my notebook (fig. 2) each year helped me to maintain consistency in the general scoring method from one year to the next, and scoring clutches (blind – i.e. not referring back to previous scores) during incubation, allowed me to assess my consistency of recording – the 'repeatability' of the scores. Then from 1988 onwards, I scored every clutch in the 300 nestboxes that I monitor (about 60–150 Great Tit clutches each year).

I have now recorded the patterns on more than 1,500 clutches in this way. Since it turns out that the three scores are somewhat corre-



170. Incubating Great Tit *Parus major*, Wytham Woods, Oxfordshire.

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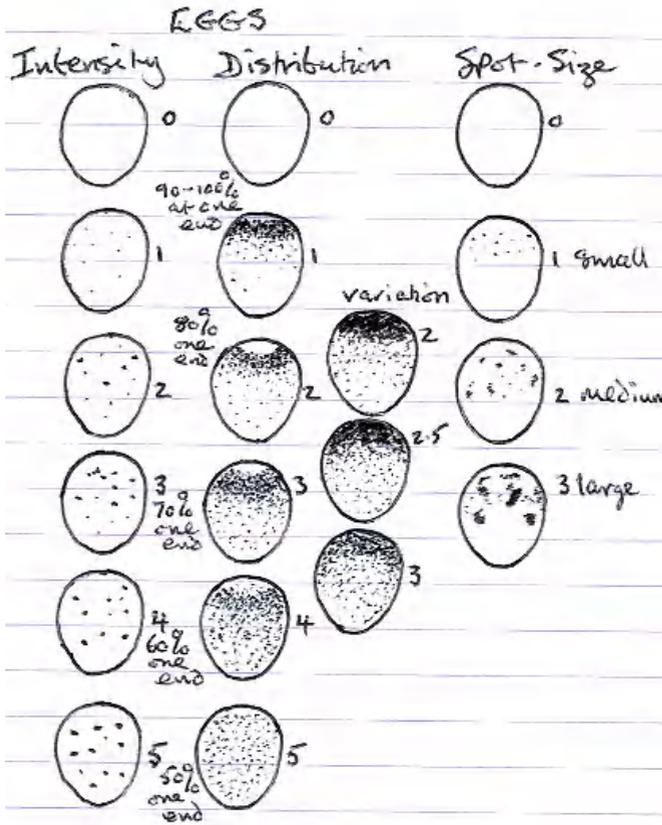


Fig. 2. Back page from author's 2002 field notebook, showing egg sketch diagrams used to maintain consistency in scoring 'IDS' (Intensity, Distribution and Spread – see text) across years.

lated (i.e. eggs with darker spots also tend to have larger spots more concentrated at one end, while paler speckles tend to be more evenly distributed), I also calculated two statistics (Principal components pc1 and pc2) that summarise the variation. These summary statistics have turned out to be very informative, and I called them 'darkness' (pc1) and 'spread' (pc2), as this most nearly expresses what they represent in terms of the eggshell's pattern (Gosler *et al.* 2000, 2005).

So what can we do with data of this kind? The first thing was to check on my repeatability from several scores made of the same clutches on different days, and then to test the repeatability of known (ringed) females in different years, and to see if it made any difference whether they were in the same box or a different box, or with the same male or a different male. In this way, I could see whether females were consistent in the patterns of eggs they laid, and start to get an idea of whether the environ-

ment affected the patterns. For example, if females laid eggs that were more similar in pattern when they nested in the same box than when in different boxes, there must be an environmental effect. Using the extensive ringing data from the Wytham Great Tit project, which allow us to find relatives, I also collated the egg-pattern data for mothers and daughters and for maternal and paternal grandmothers, to see whether there was evidence for a genetic basis to these eggshell patterns.

What I found (Gosler *et al.* 2000) was, first, that observer repeatabilities were tolerably high; eggs that I considered pale or spotty one week were regarded as more or less equally pale or spotty (respectively) the next (table 1). In fact, part of the difference was probably caused by the change in egg appearance between when they were fresh, and partially translucent (pinkish), and when incubated, when the developing embryo renders

them opaque (the white shell appears whiter and the speckling appears more contrasting). Although there was little 'box effect' or 'male effect' (i.e. a female's egg-pattern was not influenced by who she was mated with), there was a strong 'female effect', meaning that individual females were highly consistent in their eggshell patterns over the years. I was later to find that the result for the 'box effect' was somewhat misleading because when a given female changed boxes between years, she tended to stay in the same general area or even territory, so that key environmental effects (such as the food available to her) did not change as much as if she had moved to another part of the wood. The higher repeatabilities for females recorded over consecutive years reflects the fact that they are more likely to nest in the same nestbox.

Then I looked at the similarities of eggs between descendant relatives. Female Great Tits laid eggs that were essentially similar to those from which they had hatched a year or two

Table 1. Great Tit *Parus major* eggshell pigment pattern repeatabilities represented as percentages; for example, if all females laid identical eggs, irrespective of where or with whom (different males), the score would be 100%. Thus box repeatability is the effect of nestbox location on the egg trait. I = Intensity of eggshell pigment, D = Distribution of spots, S = Spot size (see text). Source: Gosler *et al.* (2000).

Group	I	D	S	No. clutches
Observer	77%	87%	76%	192
Box (different pairs)	4%	0%	0%	312
Male (different females)	10%	0%	7%	142
Female (different males)	66%	49%	52%	362
Females (all years)	67%	46%	54%	551
Females (consecutive years)	76%	58%	57%	162

Table 2. Summary of Great Tit *Parus major* eggshell pigment-pattern similarities between relatives (heritability). I = Intensity, D = Distribution, S = Spot size (see text). Note that while the darkness of the eggshell pigmentation appears partly to have a genetic basis, the spread seems not to, i.e. the origin of the variation must be environmental. Similarity between relatives' egg-patterns: none –, moderate *, strong **, very strong ***. Source: Gosler *et al.* (2000).

Relatives	I	D	S	Darkness	Spread
Daughter/mother	***	**	*	***	–
Daughter/maternal grandmother	**	*	**	**	–
Daughter/paternal grandmother	–	–	–	–	–

earlier (table 2). But more interesting than this, they were also similar to the eggs from which their mothers had hatched (i.e. the maternal grandmother's egg), but totally unlike the ones from which their fathers had hatched (the paternal grandmother's egg). These results have a number of interesting and important implications. First, they provide evidence that there is some genetic basis for the eggshell patterns. Second, and perhaps more interesting, is the fact that whatever genes the female carries that are relevant to this process, they are not inherited from the male, because if they were, there should be some resemblance between a female's eggs and those of her *paternal* grandmother: the nearest egg-laying ancestor on the male's lineage. Since in birds (unlike mammals) there is a female sex-specific chromosome (W, females being ZW), the opposite of the situation in mammals (including humans), in which there is a male-specific chromosome (Y, males being XY), this finding suggested that in birds the genes for the eggshell-speckling system are on the female's W chromosome (it is unlikely that the Great Tit has a unique system of genetic determination for eggshell speckling!). That this might be so had been suggested many years earlier in relation to Common Cuckoos (Punnett 1933) because, if otherwise, it is difficult to see how the host-specific genes could be maintained as distinct genetic lineages. If this

were not so, the genes for laying, say, Reed Warbler *Acrocephalus scirpaceus*-type eggs, would be 'diluted' over time because male Cuckoos are not thought to be choosy about whom they mate with, at least not in terms of what host species raised their mate. Hence my study of Wytham Great Tit eggs seemed to be confirming what had long been suspected for cuckoos (for logistic reasons it would be pretty-well impossible to do such a study with cuckoos themselves), and also indicating that, if both cuckoos and tits had such a system, it must surely be widespread in birds.

The function of eggshells and their pigments

Interesting though all of this was, it went no way towards explaining why the pigments were there. In seeking an answer to that question, I never doubted that the explanation would be adaptive, i.e. the spots would have some function, and serve some purpose. They were not random (for example they form a ring), and however elusive that purpose might be, it would be intimately tied to the specific functions of the eggshell. So what are those functions? The eggshell provides a semi-permeable barrier between the aqueous, internal environment that the chick requires to grow and develop, and the relatively dry (potentially desiccating) environment of the outside world. It allows gas exchange (oxygen in, carbon dioxide and water

out) but must prevent excessive water loss. It maintains the egg's internal environment with a stable shape, and presents a barrier to pathogens: bacteria, viruses and fungal attack. It may also protect the egg by camouflaging it. So the eggshell has many structural functions, and it was entirely possible that pigments served some function completely unrelated to their appearance.

It was at about this time that I started discussing eggshells with a colleague, Jim Reynolds, who was working on the problems that small birds face in finding enough calcium for breeding, principally because of the large amounts of calcium carbonate required for the formation of eggshells. Part of the problem is one of scale. Although for a large bird, the egg is relatively small compared with its body size (for a chicken, the egg is about 3% of body weight), for a small bird the eggs are relatively large (10% of body weight for a Great Tit). This means that while large birds can accumulate calcium over a relatively long period within the skeleton, to be drawn as required for eggshell

formation (Sugiyama & Kusuhara 2001), a small bird cannot; it must seek calcium daily during egg formation. Much of the research on birds' eggs involves chickens, and Jim had read Prof. Sally Solomon's work at Glasgow (Solomon 1987, 1997), which suggested that brown hens' eggs might be stronger than white. Solomon had studied protoporphyrins and commented that, like the eggshells themselves, they had a semi-crystalline structure. As they were similar in structure to phthalocyanin dyes, which were also used as lubricants in engineering, she wondered whether they might act like solid-state lubricants (e.g. like putting pencil graphite on a metal zip to make it run smoothly) between the calcite crystals of the shell. Such a lubricant might then act as a shock absorber within the shell, and so make the shell more resilient to impact, and thus stronger.

We were intrigued by this, and by the implication that if pigment strengthened eggshell, then perhaps localised pigment (spots) might compensate for local flaws in eggshell structure by strengthening the shell in those places. What

might cause such flaws? Given Jim's interest in calcium metabolism, and knowing that Great Tit females have to spend a lot of their time during egg formation looking for calcium, in the form of small snails (Gastropoda), to form eggshell (Graveland *et al.* 1994; Graveland & Berends 1997; Graveland & Drent 1997), it was obvious to think about calcium deficiency. Perhaps the variation in pigmentation that I had found within Wytham reflected variation in calcium availability.

For years, I had had mixed feelings about the 100-m altitude difference between the top and bottom of my 100-ha study area on the north side of Wytham hill. But I was about to discover that it was a blessing! The reason for the elevation, indeed the reason that the hill exists at all, is because it

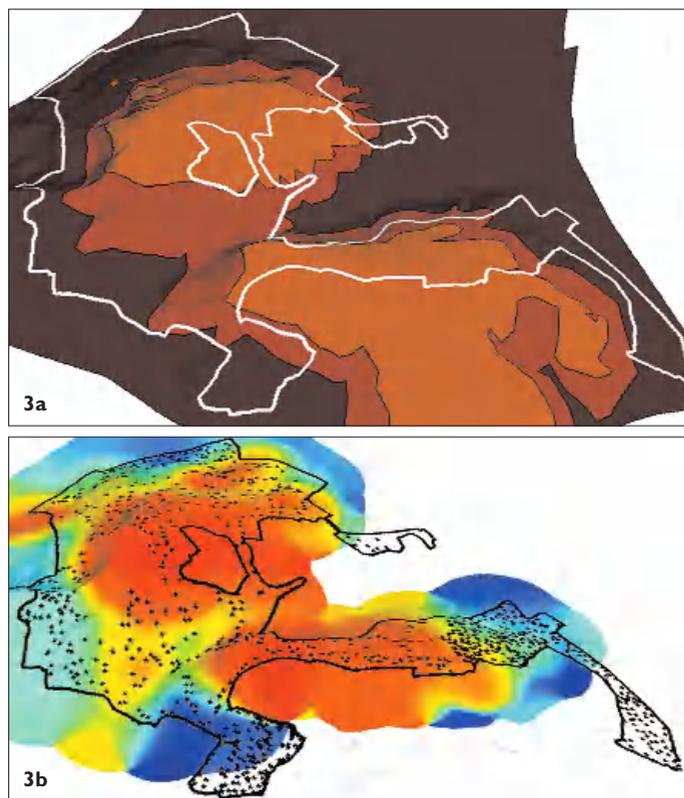


Fig. 3. GIS plots of Wytham Woods, Oxfordshire (from south). 3a shows main soils: clay (dark red), sands (light red) and limestone soils (brown). 3b shows interpolated soil calcium content from low (blue) to high (red).

is capped by Jurassic Corallian limestone. This rock is harder and more resistant to erosion than the sands that lie below it, and the clays that form the Thames floodplain beneath those, and which are exposed to the north of Wytham. Thanks to another colleague, Robin McCleery, I was able to obtain the soil survey data for Wytham, which included analysis results for the soils' calcium content sampled on a regular grid in 1974 by the Commonwealth Forestry Institute, Oxford University.

The soil survey figures showed that while soils on the limestone contained up to 27% calcium, those at the bottom of the hill could contain as little as 0.065%, which is actually less than is found in the average peat-bog. So there was something like a 415-fold range of variation in soil calcium in my study area. To see whether eggshell pigmentation might be related to calcium availability, I averaged the pigment scores for all clutches I'd recorded over the years for each nestbox, and plotted that against the average calcium value for the four or five soil samples taken nearest to that nestbox (fig. 4). Although soil calcium explained only about 4% of the variation among nestboxes in pigment darkness, the relationship was significant statistically ($P = 0.021$). Given how much 'noise' there must be in this analysis, and that we already knew that a large part of the variation among females had a genetic basis, this was an amazing, and very exciting result; from hunch to supporting evidence in a few clicks of a mouse – a real (if small in the great scheme of things) eureka moment! Clutches on high-calcium soils were in fact paler, less spotted, than those on low-calcium soils. A snail survey has subsequently shown that there are substantially more, and larger, snails on the limestone than on the sands and clays (Jubb *et al.* 2006), and this is undoubtedly how the soil effect is translated into a bird-eggshell effect.

It is worth considering for a moment just how important to this discovery,

and what follows, were the specific geographical details of our study site. It is pure serendipity that the Wytham estate presents such a range of soil conditions. Had I worked in any one of dozens of other tit study-populations across Europe that lie on relatively uniform geology and soils, I should have seen less variation in eggshell patterning, and certainly could not have ascribed what variation I did see to the diversity of the local environment. In other words, in this case, our ability to figure out what was going on depended on the environment in which we worked.

So this analysis suggested that pigments might indeed be related to eggshell function, in some way related to calcium availability. We assumed that this must be related to shell thickness, but to test that we would have to collect some eggs. Another aspect of eggshell function that is affected strongly by eggshell thickness is the rate of water loss from the egg, especially during incubation. Birds' eggs typically lose about 18% of their weight through incubation as a result of water loss. This is a consequence of the normal physiology of the egg and the growing embryo within it. However, the eggshell is the only obstacle to total desiccation: too thick, and gas and water-vapour loss are too restricted; too thin, and water is lost too rapidly.

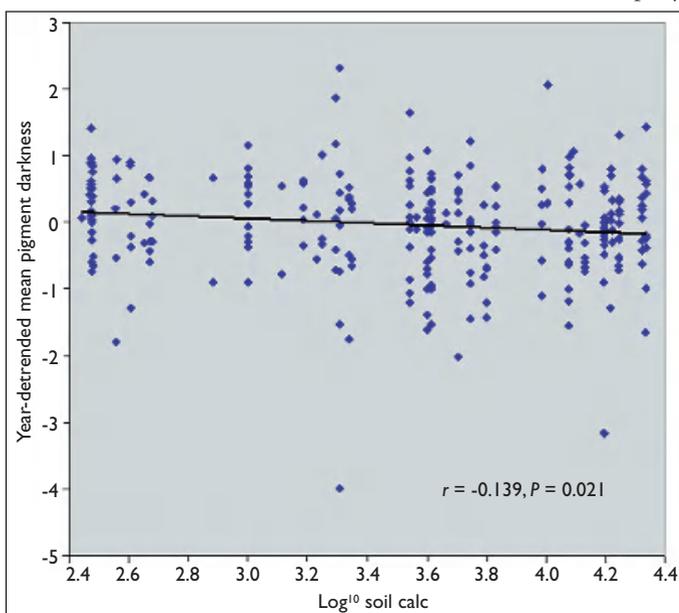


Fig. 4. The relationship between Great Tit *Parus major* eggshell pigment darkness and local soil calcium in the north Wytham study area. Each point represents a nestbox for which its value is the average of several clutches over 12 years (data from some 1,400 clutches and 267 nestboxes are represented in the figure). The correlation between them is statistically highly significant.

Either way, the embryo would die (Ar *et al.* 1974, 1979). Bakken *et al.* (1978) showed that protoporphyrins had another interesting characteristic: they reflected strongly in the infrared, i.e. they reflect heat. Bakken and colleagues were interested in why the eggs of ground-nesting species such as gulls (*Laridae*) didn't literally roast in hot sunshine when the parent bird was off the nest for any length of time. But if protoporphyrins prevented gull eggs from frying in the sun, maybe they could act to reduce the rate of water loss from the (supposedly) thinner shells of more spotted eggs. In 2002, I offered this question in the form of an MSc project in the Oxford University Zoology Department. The idea was to weigh eggs individually during incubation to monitor the rates of water loss, and to see whether this was reduced by the presence of pigment. At that time, we still believed that pigment was deposited on the surface of the eggshell after its formation (the 'inkjet model'), whereupon it might physically block pores in the eggshell, and so reduce water loss; however, what we found then, and over the next three breeding seasons radically altered our view.

James Higham took up the challenge of the MSc project, and he knew that we were still in the early stages of working out what was going on. The field was wide open and, wisely, he was going to make no assumptions. We agreed that we should not weigh whole clutches repeatedly, but individual eggs; and that we should know which egg was which in the laying sequence because we believed that if females were calcium-stressed, they might find it harder to find calcium for the eggshell as egg-laying progressed over 6–12 days. So we (mostly James) visited 30 nests, chosen for their location with respect to soil calcium, every day to number the day's eggs. Each egg was weighed repeatedly during incubation using a digital balance that weighed reliably to 0.002g in the field. My main job at this time was to record pigment score (IDS) for every egg in these clutches. This was

when we made the first discovery. James noticed it first: the eggs became spottier through the clutch (fig. 5). This was obvious to us visually when the eggs were laid out in sequence, but the IDS scores confirmed it absolutely (Gosler *et al.* 2005).

At this point, it seemed that we might be right about the reduction in shell thickness through the clutch, but we knew that to nail this one properly we would have to collect eggs. I obtained licences from English Nature to take, over two years, some fresh (unincubated) eggs for analysis and any deserted (unincubated) clutches that later became available. For the fresh eggs, we made a point of taking them in such a way that the female continued to lay, and in fact all the females whose eggs were taken later raised and fledged broods of their own in the same season. I would add at this point, that although it might seem that our regular nest-checks were quite intrusive to the birds, this was not actually the case. During the laying period, females rarely visit the nest after they have laid the day's egg, so the daily visits to number eggs could be made without risk of encountering their mother. Two visits made during incubation were sufficiently infrequent to prevent desertion and indeed no birds deserted the nest as a result of these nest visits.

From the collected eggs, we discovered a number of important things, of which five are listed here. Together, these have led to a radical rethink of how eggshell pigmentation works. First, the eggshell thickness is not constant across the surface of the egg. In particular, the thickest shell is found at the broad end of the egg (the 'crown'), while the next thickest is the region (the 'waist') between the widest point and the pointed end (the 'foot'). The small 'foot' region has the thinnest shell, but between the crown and the waist there is a band of relatively thin shell. This corresponds, more or less, both to the broadest part of the egg (the 'shoulder') and to the region in which the pigment corona, when it occurs, is found. Second, within each

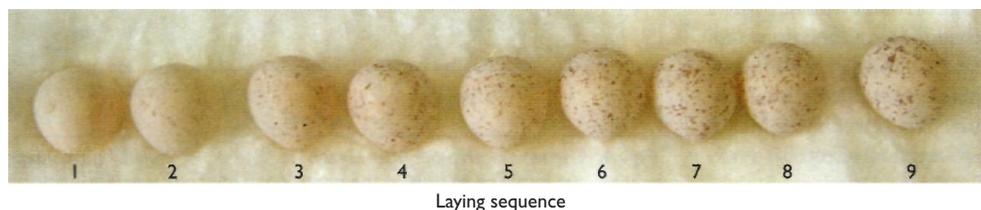


Fig. 5. Variation in eggshell pigmentation through the laying sequence of a clutch of nine Great Tit *Parus major* eggs from Wytham Woods in 2002.

region (crown, shoulder, waist, foot), the shell is thinner, sometimes substantially so (up to 50%), within a pigment spot than it is immediately outside the pigment spot. In other words, the pigment marks 'pits' or regions of thinner shell. Third, the pigment darkness is related to the difference in thickness between the pigmented and unpigmented shell (the depth of 'pit'), so darker spots mark out deeper pitting of the shell surface, and a more spotted eggshell is a less consistent eggshell in terms of thickness. This is also interesting because the long-term data showed that there was a strong genetic basis to pigment darkness, so also suggesting that there might be a genetic basis to how consistently the female can form the eggshell. Fourth, the pigment spread was strongly related to the shell thickness at the shoulder (less so at the crown, and not at all at waist or foot), such that thinner-shelled eggs had pigment more concentrated towards the blunt end of the egg. Fifth, after reducing the shell to ash at 800°C for 8 hours in a laboratory furnace to drive off any organic compounds, and correcting for egg size, we found that the total shell mass was strongly related to the soil calcium values near to the nest (Gosler *et al.* 2005).

So there we had it in terms of the structural relationships between the eggshell and its pigmentation. Birds nesting in lower-calcium areas laid more heavily pigmented eggs because they were thinner-shelled, and the spots corresponded to thin areas of shell. But the 'inkjet model' now seemed ridiculous. How could the female bird possibly make an eggshell and then find the thin-shelled places to put pigment on them? Clearly there is a more sophisticated mechanism operating here. Our data suggest that at some fine, maybe cellular, scale, a shortage of calcium results in protoporphyrin being deposited instead. Solomon (1997) pointed out in her work on chickens that these pigments were deposited with the calcium carbonate as an integrated unit, and even suggested that the protoporphyrins and calcium might share the same protein carrier to cross the cell membrane. It now looks as though that might be right and that while this carrier 'prefers' calcium, it might take protoporphyrin instead when the former is scarce.



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171. The importance of snail (Gastropoda) shell to the laying female is highlighted by the contents of this Great Tit *Parus major* nest in Wytham on 1st June 2006: two unhatched eggs and a large piece of snail shell. The latter was almost certainly taken into the nest by the female for her own consumption.

That birds use pigments to strengthen tissues is of course well established. Birders, and especially bird ringers, will be familiar with the fact that light-coloured tips to feathers wear more readily than feathers or feather areas darkly pigmented with melanin. Plate 172 shows an example of feather-tip wear in a Great Spotted Woodpecker *Dendrocopos major*, photographed in spring, in which it is clear that the pale spots on the tips of the outer secondaries



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172. Great Spotted Woodpecker *Dendrocopos major* wing showing feather weakness of pale spots at tips of secondaries.

have worn more than the darker feather vane. Two similar examples are perhaps more familiar. Many otherwise pure-white species nevertheless have black primaries, for example Northern Gannet *Morus bassanus* and Snow Goose *Anser caerulescens*. Many finch, bunting and sparrow species show, especially in the males, buff tips to body feathers when they are fresh in the autumn. This makes them more cryptic and displays fewer sexual signals at an inappropriate time of year. These pale, less resilient, tips wear away through the winter to reveal the full breeding plumage in familiar species such as House Sparrow *Passer domesticus*, Common Chaffinch *Fringilla coelebs*, Brambling *F. montifringilla* and Reed Bunting *Emberiza schoeniclus*.

Pigment and water relations of eggshells

So what of the water-loss studies that James Higham was working on? It turned out that there was indeed a relationship between the rate of water loss and the egg's pigment darkness, but this relationship was far from simple, because it actually changed through the laying sequence of the clutch. So although in later eggs in the sequence (generally after about the sixth egg) the rate of water loss was reduced in darker eggs, in early eggs (especially the first two or three) in the sequence, darker eggs actually lost more water. Furthermore, all this interacted with the local calcium availability so that on low-calcium soils darker eggs in a clutch tended to lose less water, while the reverse was the case on high-calcium soils (Higham & Gosler in press). I have to say that we struggled for months to work out what might be going on here, but the answer surely lies in relation to our other findings.

Our thoughts now run something like this. We believe that the primary function of the protoporphyrins is to compensate, in terms of strength, for eggshell thinning caused by calcium deficiency. They can also help to reduce the permeability of the eggshell, and thus also water loss. However, while they might be a good solution to the first (primary) problem, they are not a perfect solution to the second. The actual mechanism by which pigment is deposited as a result of localised calcium deficiency suggests that the bird cannot apply more pigment than that which is directly proportional to the calcium shortage, but that might not be enough to compensate perfectly for shell-thinning in

terms of water loss. So, generally, lightly spotted eggs (early in the laying sequence, or on high-calcium soils) carry enough pigment to compensate in terms of strength for small areas of eggshell-thinning, but not enough to compensate in terms of water loss, while heavy pigmentation can compensate for both (Higham & Gosler in press).

But these findings suggested something else. The idea that protoporphyrins might represent a good adaptive solution to the problem of shell strength, but a less-than-perfect one for the problem of water loss, seemed to me less remarkable than would be the finding that the one compound offered a perfect solution to both problems. But if the pigment system was in fact a compromise, maybe incubating females had to adapt their incubation behaviour to compensate for the fact that a combination of factors outside their control resulted in a clutch of eggs with not-quite-perfectly adapted properties in terms of their heat transmission and water conductance.

With this question in mind, James Higham designed and executed an experiment in 2002, which I repeated in 2003 to increase the sample size, to test the proposition that the incubation environment of a nest was in some way related to the eggshell pigmentation of the clutch (fig. 6). If this were the case, the rate of water loss from eggs would be specific not just to the clutch, but to the female incubating it. So the solution to test this would be to weigh each of the eggs in a clutch and to swap these for a few days during incubation with similarly weighed eggs in another nest, more or less matched for size and timing (clutch size, lay date and estimated hatch date). We would then swap them back and reweigh each of the eggs to determine their rate of water loss while they were incubated by another female, who probably had a different incubation regime. We could then see whether the difference in water loss between the swapped eggs and their siblings (controls) left in the original nest was correlated with the difference in mean pigment darkness between the two nests in the pair. We also placed temperature probes, recording temperature to 0.1°C every minute, in the nest cups of a few of the nests to see if we could detect any differences.

What we found was really remarkable (fig. 7). There was a strong correlation between the difference in weight loss between swapped and control eggs, and the difference in darkness

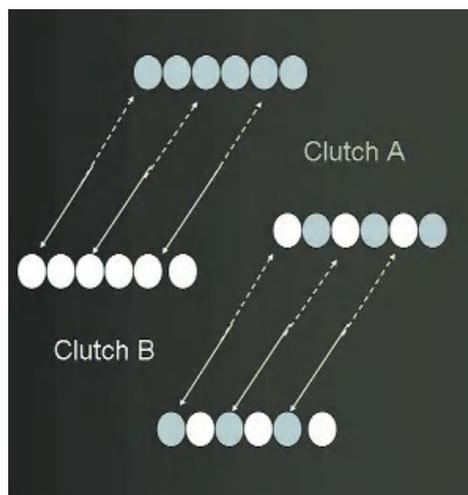


Fig. 6. Experimental design for partial clutch swaps during incubation. Clutches A and B each have six eggs. Swaps were conducted so that swap and control eggs were not biased relative to the laying sequence. Before swapping, and after replacement, eggs were individually weighed to 0.002 g. The mean weight of swapped eggs was compared with that of controls, and the difference plotted against the difference in mean pigment darkness between the two clutches. See fig. 7.

between the two clutches. But more than that, in both years, the line showing the 'best-fit' relationship to the data passed through the origin, indicating that nests with identically pigmented eggs would have identical incubation environments. This confirmed the suspicion that differences in incubation environment between nests, which are determined chiefly by the female's behaviour (e.g. time spent on or off the nest, were related to the pigmentation of her clutch. From the two pairs of nests that we were able to monitor with temperature probes, we found evidence that females incubating darker clutches maintained higher temperatures in the nest, but while this is as we should predict, it is too small a sample on which to base a definitive statement.

What all this means is that we should think of the bird sitting on her eggs in the nest as a highly integrated 'unit'. The nest is built by the female to certain specifications, eggs are constructed so as to compensate for local environmental conditions, and the female's incubation behaviour is supremely adapted to compensate for remaining deficiencies in the whole system. How the female might achieve this fine-tuning awaits further research, but my guess would be that she monitors nest-cup humidity – too

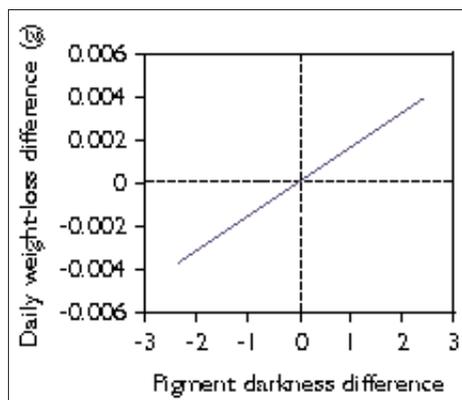


Fig. 7. Summarised results of cross-fostering experiment showing that the rate of weight loss experienced by Great Tit *Parus major* eggs during incubation depends on the specific nest environment, and that this in turn depends on the pigmentation of the clutch. See text and fig. 6 for further explanation.

much and she stands in the nest to reduce the heat to the eggs.

Further adaptation?

There is one further aspect to this tale, concerning the function of these pigments as a structural adaptation for the eggshell, and it has to do with the fact that the shell at the shoulder is thinner than that at the crown or waist. There is some reason to think that this might be a design constraint. That is, that for some reason it is difficult to produce the perfectly ovoid shape of the egg while maintaining a constant shell thickness. The evidence for this is that the difference (or ratio) in thickness between the shoulder and crown shell is itself correlated with the length-to-breadth ratio of the whole egg: the nearer this ratio is to 1 (i.e. a perfect sphere), the nearer the shoulder:crown shell-thickness ratio was to 1 (i.e. the same thickness). But there might be an adaptive reason also why the shoulder shell is thinner, indeed maybe the egg is 'ovoid' in order to produce this reduction in shell thickness in one latitude (recognised as the shoulder) of the egg. Note that this thinning is true of unpigmented shell, it is not just that the corona ring spotting is found there, and these spots mark thinner shell. The adaptive function then comes from the fact that the region of the shoulder and corona ring is also where the chick hatches. Some weakening of the shell in this region may help the tiny chick to break out.

In fact, we can go further in thinking about

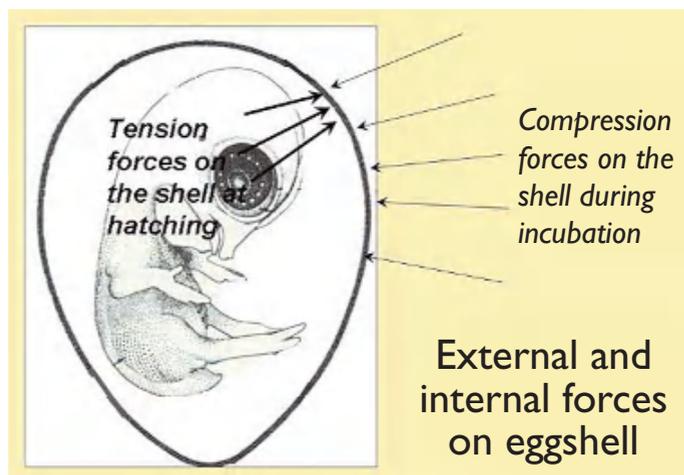


Fig. 8. Opposing compression and tension forces on the 'arch' of the eggshell. The arch analogy might be misleading because the crystalline 'bricks' of this arch are extremely fine-grained; but this may serve as a useful starting point.

this. We can think of the broad end of an egg as being structurally similar to an arch, and we can think of the forces acting on either side of the shell (outside and inside), as being like the forces acting upon an arch (fig. 8). An archway holds together because of the compression forces of masonry above it forcing it together. From outside the egg, the forces acting upon the shell are, similarly, compression forces. A weak arch can be demolished by removing a single brick, especially if punched out from beneath it, because this action applies a tension force that opposes the compression force. Similarly, the hatching chick applies a kind of tension force to the arch of the egg at hatching. Here the 'lubricating crystals' of the protoporphyrins could have an additional benefit, because the very compounds (pigment) that act as an intercrystalline shock absorber when under compression from without, should act to weaken the shell when under tension from within, so aiding the chick's liberation.

Summing up

We had always assumed that any pattern on an egg was a signal, i.e. that its purpose was principally visual. Since, in the cases for which the purpose of markings was known (chiefly waders and cuckoos) that purpose was visual, we assumed that the function of all spots, when found, would also be some sort of signal. Indeed, the very word 'pigment' implies such a function. From discussions that I have had with ornithologists and behavioural ecologists, I have the impression that many will continue to make

that assumption despite our findings. It is not inconceivable that, since the pigmentation we have studied can be used to indicate eggshell quality, birds might use the markings as some sort of signal. But this is not the principal reason. It is like suggesting that the reason that blood is red is so that you know when you are bleeding, so ignoring the principal function of haemoglobin in oxygen transport! Following this analogy for a moment, I would offer another, more specific to these eggshells.

Considering these specific pigments in terms of the visible patterns they display without considering their structural function is like looking at the reticulate pattern formed on a wall by the mortar between the bricks and thinking 'I wonder why the builder put that pattern there'. Our evidence suggests that the protoporphyrins are a very sophisticated mortar holding together the calcite 'bricks' of the eggshell wall. Any pattern they produce is secondary to that.

Nonetheless, there are almost certainly other things going on concerning the ground colour of eggshells. For a start, their genetic determination may be different (there is a male genetic component to eggshell colours of chickens, brown versus white), and again there can be no doubt that the eggs of certain, generally ground-nesting, species are highly cryptic, or that there are genes of Common Cuckoos whose eggs are adapted in colour to certain specific passerine hosts.

So where do we go from here? There is much to do, but I believe that these findings offer insight and opportunities in many areas. First, we need to find out how general are our findings. About 60% of bird species are passerines, and perhaps 30%–50% of these have spotted eggs like those of the Great Tit, or similarly speckled but with a ground colour. If it turns out that eggshell thickness, and thickness consistency (i.e. quality) can be deduced from their spottiness (i.e. without breaking them), we have a powerful tool for both pure and applied research in ornithology. For example, we know that man-made pollutants such as DDT still persist in the

environment and that they cause eggshell thinning in birds. Might eggshell pigmentation provide a means to assess the effects of such pollutants by simply photographing the eggs?

Finally, I should like to return to the quote from Sir Alfred Newton's 1896 *Dictionary of Birds* with which I started this account. Nineteenth-century ornithology was overwhelmingly preoccupied with systematics (naming and classifying), and that was right given that new species were being discovered almost weekly in the newly opening lands of the Empire. Newton's account of eggs (about 5,400 words over 11 pages) is largely concerned with the inability of ornithologists to find any general taxonomic value in eggshell patterning. Indeed, if you've ever tried to identify an egg from a book, without having seen the source (bird or nest), it often seems near impossible. There are certainly general themes (e.g. take a look at the plates of warbler eggs in *BWP*), but the overwhelming functional constraints on eggs mean that they are highly conservative or convergent in form. For this reason, the answer to the challenge that Newton posed, over 100 years ago, is likely to be both complex and fascinating, but maybe worth the wait.

Acknowledgments

I hope that my account of this work indicates that I have benefited greatly from discussion and input from many people. I am grateful to them all: to colleagues Rhys Green, Robin McCleery, Jim Reynolds, Jorn Scharlemann and Steve Wyatt; students Phil Barnett, James Higham, Morgan Tingley and Teddy Wilkin; and especially to Chris Perrins and Ben Sheldon, former and current Directors of the EGI respectively, who have, variously, supported my exploits in Wytham Woods since 1981. Teddy Wilkin kindly supplied the GIS maps shown in fig. 3.

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