

Modelling eggshell maculation

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ABSTRACT

The eggshells of many avian species are characterised by distinctive patterns of maculation, consisting of speckles, spots, blotches or streaks, the spatial-statistical properties of which vary considerably between (and often within) species. Understanding the mechanisms underlying the production of eggshell maculation would enable us to explore the costs and constraints on the evolution of maculation patterns, but as yet this area is surprisingly understudied. Here I present a simple model of eggshell maculation, which is based on the known biology of pigment deposition, and which can produce a range of realistic maculation patterns. In particular, it provides an explanation for previous observations of maculation heterogeneity and diversity, and allows testable predictions to be made regarding maculation patterns, including a possible signalling role.

Keywords: random field, pigmentation, protoporphyrin, shell-gland membrane

1. INTRODUCTION

The great diversity of avian eggshell pigmentation and its possible adaptive significance has fascinated biologists for over a century (Poulton, 1890). Ancestrally, avian eggshells were most likely homogeneously white and immaculate (Wallace, 1889; Kilner, 2006), although not necessarily devoid of pigment (Kennedy and Vevers, 1975). Since then, however, they have evolved remarkable variation in both the basal ground colour and in the presence and patterns of superficial pigmentation, or maculation, which can include speckles, spots, blotches and streaks (Kilner, 2006; see illustrative examples in Cassey *et al.*, 2011; Brulez *et al.*, 2015 and references therein; Figure 1). A number of hypotheses have been proposed to explain the functional significance of eggshell maculation (reviewed in Underwood and Sealy, 2002; Kilner, 2006; Reynolds *et al.*, 2009; Maurer *et al.*, 2011; Brulez *et al.*, 2015). These include, but are not limited to, crypsis to avoid predation (e.g. Götmark, 1993) and brood parasitism (e.g. Davies and Brooke, 1989), egg recognition (e.g. Pike, 2011),

thermoregulation (e.g. Bakken *et al.*, 1978), signalling maternal health (De Coster *et al.*, 2012), increasing eggshell strength (Gosler *et al.*, 2005), and providing defence against bacterial infection (Ishikawa *et al.*, 2010). However, despite renewed interest in the function of eggshell maculation (Brulez *et al.*, 2015), we still have a relatively poor understanding of the underlying causal mechanisms, even though this is crucial to understanding the evolution of maculation patterns and the factors constraining their production.

The various layers of the avian eggshell are formed sequentially as the immature egg rotates within the shell gland (or uterus) (Weiner and Addadi, 1991; Lavelin *et al.*, 2000). Eggshell formation ceases after the deposition of a surface crystal layer, and is completed by the formation of a thin cuticular layer of eggshell accessory material (Board and Sparks, 1991). Eggshell pigments are deposited during the latter stages of eggshell formation and so typically occur either within the upper layers of the eggshell or superficially on the eggshell's surface (Poole, 1965; Breen and De Bruyn, 1969; Soh *et al.*, 1993; Wang *et al.*, 2007;

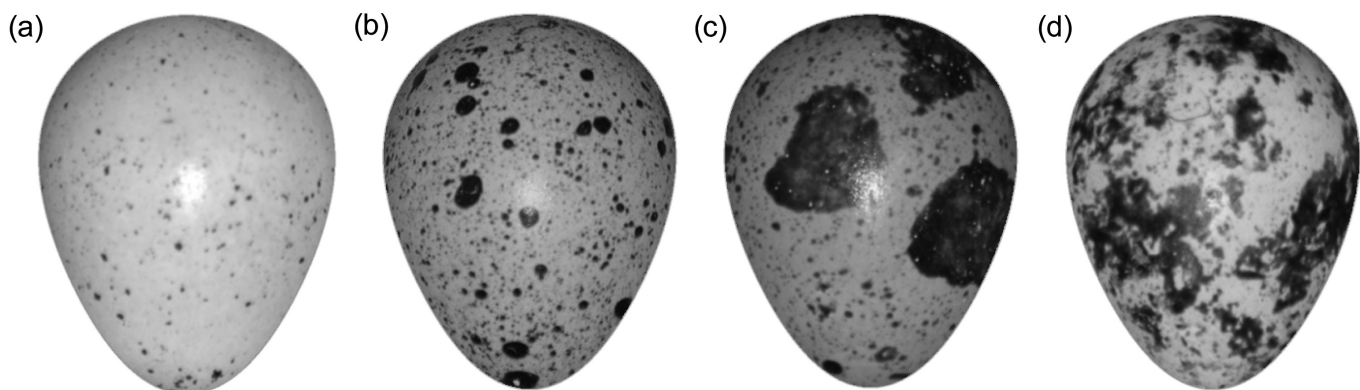


Figure 1 (a–d) Japanese quail (*Coturnix japonica*) eggs showing examples of different maculation patterns, varying in spot size, distribution and ‘smoothness’.

Samiullah and Roberts 2013). There are two main types of pigments responsible for the colouration and patterning on eggshells: biliverdin IX α produces a blue-green hue, while protoporphyrin IX produces brownish hues (Kennedy and Vevers, 1975). Homogeneously deposited pigment is responsible for the underlying base colour of the egg, while distinct patterns of maculation result from the heterogeneous deposition of relatively large amounts of pigment, typically protoporphyrin IX (Sparks, 2011). During the production of maculation, pigment granules in the apical cells of the shell gland epithelium (Tamura and Fujii, 1966; Poole, 1967) are secreted into the lumen shortly before oviposition (Poole, 1965; Tanaka *et al.*, 1977; Soh *et al.*, 1989; Tamura and Fujii, 1966) where they gather to form pigment masses between folds of the mucous membrane lining the shell gland (Soh *et al.*, 1989). Contraction of the shell gland membrane then deposits these accumulated pigments onto the eggshell's surface (Soh *et al.*, 1993), resulting in the speckles, spots, blotches and streaks, if the egg is in rotation (Solomon, 1987; Sparks, 2011), characteristic of the particular species or individual.

The aim of this paper is to present a simple model of eggshell maculation that is consistent with our biological understanding of the process, and which can account for the majority of maculation patterns observed in the eggs of real birds. Specifically, the model is based on the known morphology and functionality of the shell gland membrane, and explores the effects of varying each of the different stages (from membrane structure through to pigment accumulation and deposition) on the generation of maculation patterns. It is hoped that this will provide a foundation for future work on the evolution and function of maculation, by allowing the construction of models to generate maculation patterns for further theoretical or empirical exploration, and in the development of testable hypotheses relating to the costs and constraints of maculation production.

2. MODEL

2.1 Overview

I start with a model of the shell gland membrane, consisting of a continuous spherical surface comprising a 'landscape' of peaks and troughs with defined spatial-statistical properties (Figure 2a). It is assumed that this membrane secretes pigment, which accumulates in the troughs of the folds before being transferred to the eggshell surface during contraction of the shell gland membrane. These latter two processes are simply modelled by 'filling up' the troughs in the membrane with pigment to a predefined relative height (Figure 2b), quantising the membrane into pigmented and unpigmented regions (Figure 2c), and wrapping this quantised membrane around a sphere, which is then reshaped to resemble an egg (Figure 2d).

Variation in the spatial-statistical properties of the membrane (e.g. the depth, size and/or number of troughs) and/or the characteristics of the pigment filling the troughs in the membrane can produce a wide range of maculation patterns encompassing a large majority of the variation observed in real birds' eggs. These are explored in more detail below.

2.2 Varying the spatial-statistical properties of the shell gland membrane

The shell gland membrane is modelled as a spatially correlated (spherical) random field (*i.e.* a continuous spherical surface with a known spatial correlation structure) using unconditional Gaussian simulation, a common procedure in geostatistics (Diggle and Ribeiro, 2006). The particular, spatial-statistical properties of this random field are defined by an associated semivariogram model, which describes the average squared difference in membrane surface height between two given points as a function of the distance between those points (Olea, 1999). Semivariograms are widely used in geostatistics and there are a large number of texts dealing with their construction and interpretation, and readers are referred there for further information (e.g. see Olea, 1999 for an accessible introduction to the topic). Of particular importance here is that semivariogram functions typically show an initial increase with distance (because the surface heights at two spatially proximate locations tend to be more similar than those at two distant locations) before asymptoting at a distance, known as the range (r [where $0 \leq r \leq 180$], measured here in degrees), after which the heights at any given spatial locations cease to be correlated (Figures 3a and 4a). This relationship can be approximated using a variety of functions, of which the Gaussian and exponential functions were found to be useful for producing eggshell maculation patterns. Gaussian functions produce membranes with a smooth transition between peaks and troughs, and hence result in 'rounded' regions of maculation (Figure 3a; see also Figure 1b); in contrast, exponential functions result in 'jagged' areas of maculation (Figure 3a; see also Figure 1d). By varying the value of r it is possible to control the degree of spatial correlation: specifically, increasing the value of r (*i.e.*, the distance at which the function asymptotes) results in random fields with increasingly 'coarse' autocorrelation, resulting in wider spaced and larger regions of pigmentation (Figure 3b; see also Figure 1a,b,c).

A further characteristic of many species' eggs is the skewed distribution of maculation along the long axis of the egg, such that maculation occurs predominantly at one pole or the other (e.g. see Figure 1 in Gosler *et al.*, 2000 for examples in great tit [*Parus major*] eggs). This can be explicitly modelled by including a linear trend along one axis of the random field (specifically by varying the value of the trend coefficient, β ; Olea, 1999), and can therefore

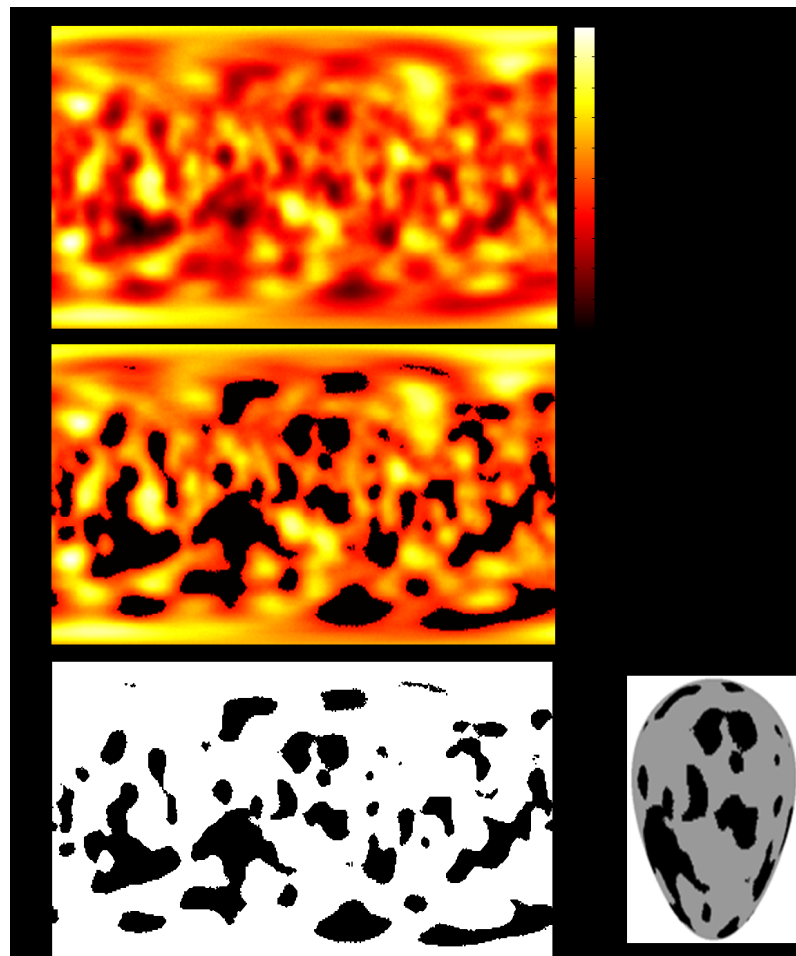


Figure 2 Modelling eggshell maculation. (a) The shell gland membrane is modelled as a spherical random field, here ‘spread out’ and represented by its equirectangular projection, with colours denoting the relative elevation of peaks and troughs among folds in the membrane. The membrane is simulated with a spatial resolution of 1 degree. (b) The membrane showing pigment (homogeneous black regions) partially filling the troughs. (c) The quantised membrane showing pigmented and non-pigmented regions. (d) The quantised pigment pattern mapped onto a three-dimensional model of an egg, following ‘contraction’ of the shell gland membrane. I assumed the egg had an underlying base colour (ranging from black [0] to white [1]), here set to 0.6. The shape of the egg was calculated following Todd and Smart (1984) assuming the following shape coefficients: $c_1 = 0.7$, $c_2 = 0.2$, $c_3 = 0.0$ and $c_4 = -0.05$, and a height to breadth ratio of 1.3. In this example, the membrane was modelled using the following parameters: Gaussian semivariogram function; $r = 10$; $q = 0.4$; $d = 1$; $\beta = 0$.

simulate maculation with varying degrees of polarisation: when $\beta = 0$, there is no trend and maculation is distributed evenly along the long axis of the egg; increasing values of β result in increasingly strong trends, and hence increasingly polarised maculation (Figure 4a,b).

By combining random fields with different spatial-statistical properties it is possible to create more complex membrane configurations, for example combining two or more patterns with differing spatial-statistical properties (e.g. spots combined with larger patches of pigmentation; Figure 4j,l).

2.3 Varying pigment deposition

I consider that pigment can vary in both quantity (q , as a proportion of overall membrane height, from a complete absence of pigment [$q = 0$] to where the membrane is

filled with pigment to the top of the highest peak [$q = 1$] and optical density (d , from transparent [$d = 0$] to opaque [$d = 1$]). Varying q affects how deeply the troughs in the membrane are filled, allowing membranes with the same spatial-statistical structure to produce very different patterns of maculation (Figure 4c,d,e,f). Varying d can produce maculation that is heterogeneously coloured, with areas of both heavy and weak pigmentation: when $0 < d < 1$ deeper troughs, containing more pigment, will produce darker regions of maculation than shallower troughs; similarly, deeper (typically central) regions of troughs will produce darker maculation than shallower (typically peripheral) regions (Figure 4g; see also Figure 1c,d).

By ‘rotating’ the egg during or shortly after the deposition of the pigment it is possible to simulate the effects of streaking (Figure 4h), and by applying pigment to the eggshell more than once (for example before and

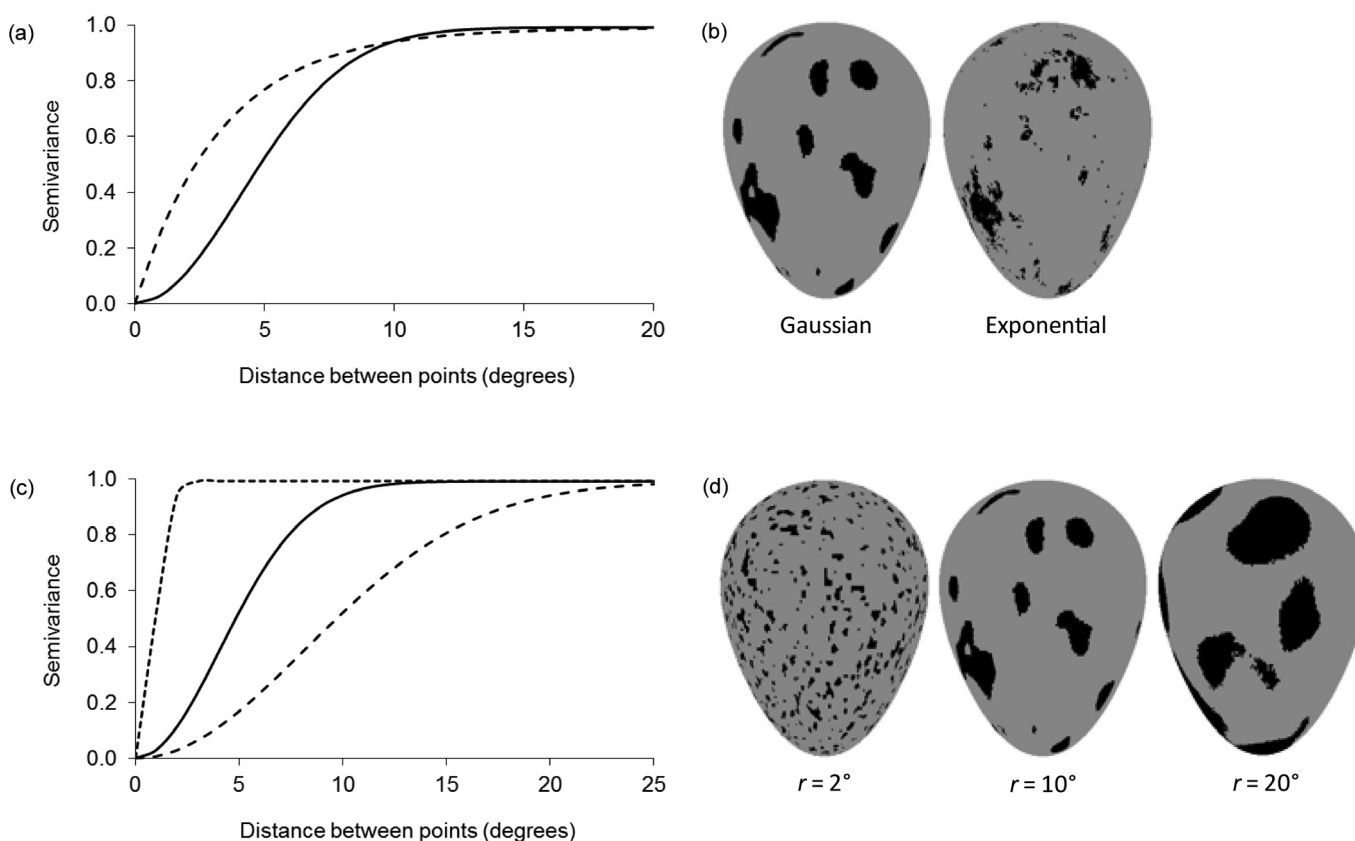


Figure 3 The effect of varying the type and range parameter (r) of the semivariogram function on eggshell maculation. The semivariogram function models the average (squared) difference of the membrane surface values between two given points (the semivariance) as a function of the distance between those points, and hence defines the spatial-statistical properties of the eggshell membrane. (a) Gaussian (solid line) and exponential (dashed line) semivariogram functions, both with a fixed $r = 10^\circ$, and (b) representative realisations of resulting maculation patterns. (c) Gaussian semivariogram functions with $r = 2^\circ$ (dotted line), $r = 10^\circ$ (solid line) and $r = 20^\circ$ (dashed line) (note that for both Gaussian and exponential semivariograms, r is defined as the distance at which the semivariance first reaches 95% of the asymptotic height; Olea, 1999), and (d) representative realisations of resulting maculation patterns. In all cases the membrane was modelled using the following additional parameters: $q = 0.4$; $d = 1$; $\beta = 0$, using a semivariogram with a fixed asymptotic value (known as the sill) and y-axis intercept (nugget) (*sensu* Olea, 1999).

after rotation of the egg) it is possible to generate complex patterns, for instance, simulating maculation within a distinct eggshell layer combined with superficial eggshell-surface maculation (Figure 4i) or combining spots and streaks (Figure 4k).

2.4 Implementation

The model was implemented in Matlab (Mathworks, Natick, MA) with calls to the 'predict.gstat' function in the gstat package (Pebesma, 2004) for R (version 2.15.2) to generate the spherical random fields. All code is available on request from the author.

3. DISCUSSION

Here I present a simple model of eggshell maculation, which is based on the biological mechanisms underlying the process of pigment deposition. Specifically, by

varying the spatial-statistical properties of the folds in the shell gland membrane, along with the amount of pigment secreted by the membrane epithelium, the model can simulate the majority of maculation patterns that have been observed in real birds' eggs (e.g. Brulez *et al.*, 2015) and can produce patterns that are statistically indistinguishable from those generated naturally. For example, there is no statistical difference between the maculation patterns present on real Japanese quail eggs (from Pike, 2011) and simulated patterns that appear, at least to human observers, to provide good species-typical matches (Figure 5), strongly suggesting that the model generates biologically plausible maculation patterns. Differences in membrane characteristics between species may therefore account for much of the inter- and intra-specific variation in maculation patterns observed, while also explaining phenomena such as within clutch consistency in maculation patterns, the heritability of maculation, and observed links between maculation and the laying female's health and physiological status.

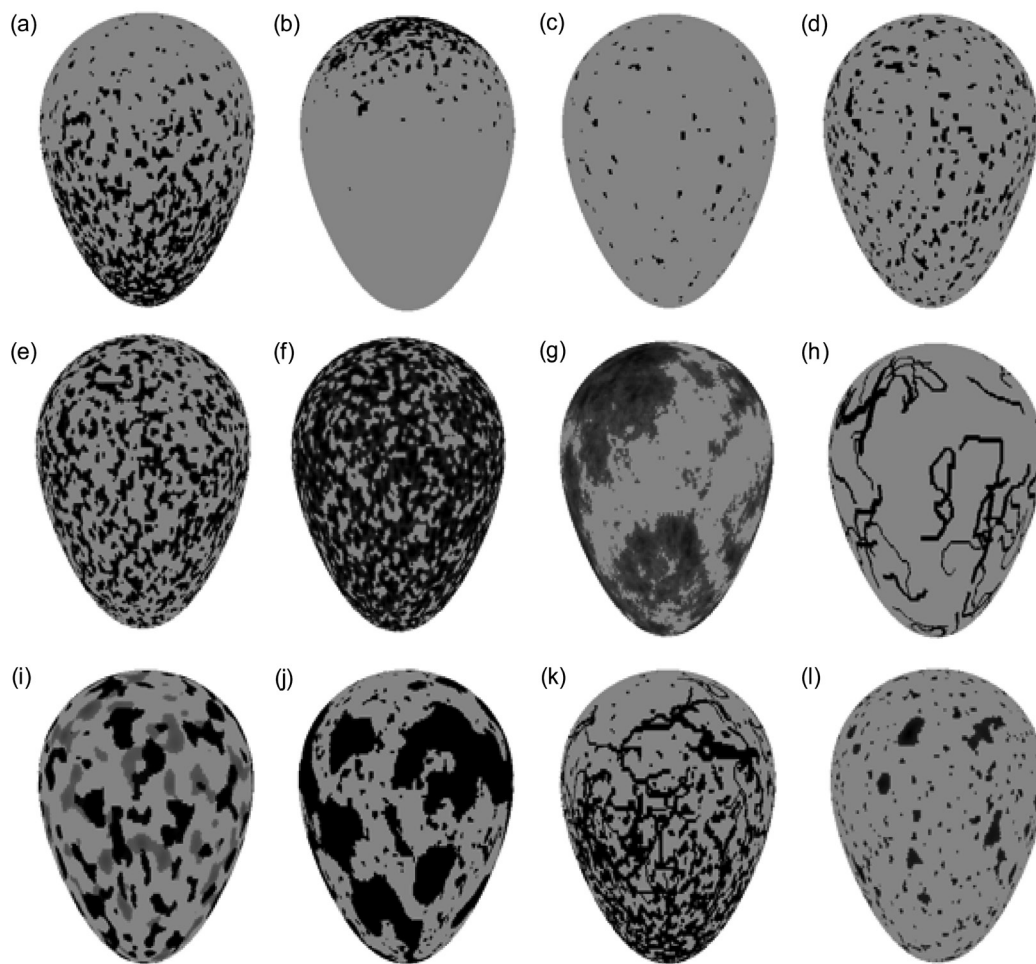


Figure 4 Examples of simulated maculation patterns. Please refer to the text for full details. Maculation was modelled using the following parameters: (a) Gaussian semivariogram function; $r = 2^\circ$; $q = 0.4$; $d = 1$; $\beta = 0.02$; (b) Gaussian semivariogram function; $r = 2^\circ$; $q = 0.4$; $d = 1$; $\beta = 0.05$ (and the resulting membrane flipped along the long axis of the egg); (c) Gaussian semivariogram function; $r = 2^\circ$; $q = 0.3$; $d = 1$; $\beta = 0$; (d) Gaussian semivariogram function; $r = 2^\circ$; $q = 0.4$; $d = 1$; $\beta = 0$; (e) Gaussian semivariogram function; $r = 2^\circ$; $q = 0.5$; $d = 1$; $\beta = 0$; (f) Gaussian semivariogram function; $r = 2^\circ$; $q = 0.6$; $d = 1$; $\beta = 0$; (g) exponential semivariogram function; $r = 10^\circ$; $q = 0.6$; $d = 0.6$; $\beta = 0$; (h) Gaussian semivariogram function; $r = 2^\circ$; $q = 0.3$; $d = 1$; $\beta = 0$; streaking was simulated by ‘rotating’ the egg around a static random field according to a correlated random walk with equal step lengths of 1° and directions chosen from a uniform distribution on $[-20^\circ, 20^\circ]$; (i) Gaussian semivariogram function; $r = 5^\circ$; $q = 0.4$; $d = 0.3$ and 1 ; $\beta = 0$; pigment was applied twice, with a 90° rotation between applications; (j) Gaussian semivariogram function; $r = 2^\circ$ and 10° ; $q = 0.4$; $d = 1$; $\beta = 0$; two membranes with different spatial-statistical properties were combined by overlaying them and retaining the lowest value at each spatial location; (k) Gaussian semivariogram function; $r = 2^\circ$; $q = 0.4$ and 0.2 ; $d = 1$; $\beta = 0.02$; pigment was applied twice, with rotation and streaking on the second application; (l) Gaussian semivariogram function; $r = 2^\circ$ and 5° ; $q = 0.4$; $d = 1$; $\beta = 0$; two membranes combined as in (j).

Although there are marked between-species differences in maculation patterns, many species of birds have evolved within-clutch uniformity as well as individual distinctiveness in maculation in order to aid distinguishing between an individual’s own eggs and those of a conspecific or a brood parasite (Baker, 1913; Davies and Brooke, 1989; Cherry *et al.*, 2007; Pike, 2011). Japanese quail, for instance, exhibit remarkably low within-clutch variation in the spatial-statistical properties of maculation patterns in their eggs compared with the variation between females (Pike, 2011). Assuming that pigmentation patterns are determined by the spatial-statistical properties of the shell gland membrane, consecutive eggs in a clutch

would be exposed to the same membrane, albeit with small conformational changes resulting from the precise position of the egg within the gland, or slight variation in the strength and distribution of uterine contractions, and so would be expected to exhibit maculation with similar (but not identical) spatial-statistical properties. This is certainly consistent with previous observations (e.g. Pike, 2011), although to my knowledge has not been addressed experimentally. This potential lack of flexibility in the ability to physiologically control the gross pigmentation of their eggs may explain why some species, such as Japanese quail, have evolved behavioural strategies to minimise the conspicuousness of their eggs (Lovell *et al.*, 2013).

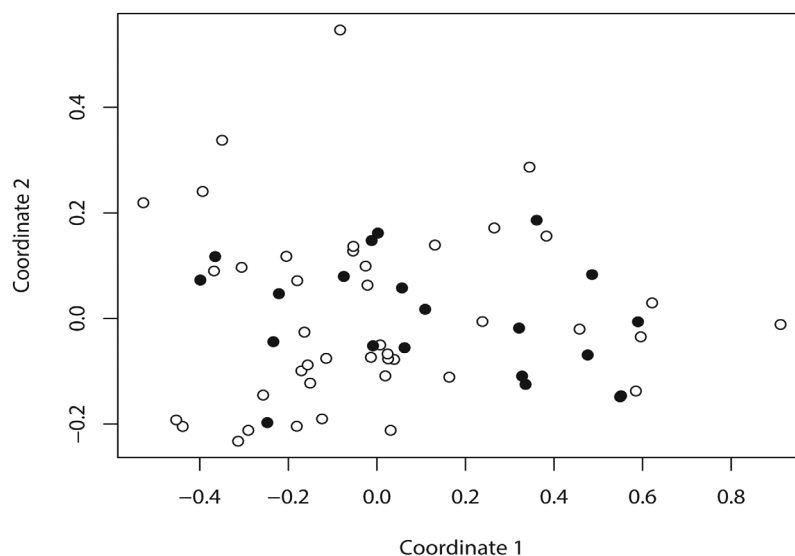


Figure 5 Non-metric multidimensional scaling (MDS) plot showing the locations of the 42 Japanese quail eggs described by Pike (2011) (white points) and 20 eggs simulated using the model described here (black points) in 'maculation-space' (i.e., a multidimensional phenotypic space describing various statistical properties of eggshell maculation patterns, as described in detail in Pike (2011)). The locations of the two sets of data points did not differ significantly (non-parametric MANOVA: $F_{1,61} = 1.43$, $P = 0.229$).

Eggshell maculation has been shown to be genetically female sex-linked in great tits, being inherited from mother to daughter (Gosler *et al.*, 2000; but see Mahler *et al.*, 2008), although as is the case for within-clutch uniformity the mechanism of maculation heritability has never been explored. If there was a genetic component to the spatial-statistical structure of the shell gland membrane, this could explain the heritability of eggshell pigmentation patterns.

The model presented here also allows us to make predictions linking maculation patterns to variation in the condition or physiology of the laying female. In humans, characteristics of the mucous membrane lining the respiratory tract (which shares many similarities with the avian shell gland membrane; Sevoian and Levine, 1957), such as membrane flexibility and the extent of folding, are known to be affected by certain diseases (Wiggs *et al.*, 1997), while respiratory diseases in birds have been shown to affect the shell gland and the deposition of pigment (Sevoian and Levine, 1957). It could therefore be predicted that condition- or health-related variation in the shell gland membrane impacts on the maculation patterns produced. Indeed, in various species body condition and health parameters are known to correlate with eggshell maculation (Martínez-de la Puente *et al.*, 2007; Sanz and Garcia-Navas, 2009; Duval *et al.*, 2014), although why this should be has not been explored. It is also noteworthy that certain characteristics of the shell gland membrane (Olson *et al.*, 1978) and the production of pigment (Soh and Koga, 1994) are known to be under hormonal control, which may provide a mechanistic link between the physiological status of a laying female and the maculation of her eggs. If an empirical link can be demonstrated between eggshell maculation, condition or health, and the underlying mechanism of maculation production, this would provide

evidence for a possible signalling role of maculation patterns (De Coster *et al.*, 2012).

In summary, the simple model of eggshell maculation presented here, which is based on the known biology of pigment deposition and which can produce a range of realistic maculation patterns, provides an explanation for previous observations of maculation heterogeneity and diversity, and allows testable predictions to be made regarding maculation patterns, including a possible signalling role.

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