



Individual quail select egg-laying substrate providing optimal camouflage for their egg phenotype

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Running head: active substrate selection for egg camouflage

Summary

Camouflage is conferred by background matching and disruption, which are both affected by microhabitat [1]. However, microhabitat selection that enhances camouflage has only been demonstrated in species with discrete phenotypic morphs [2, 3]. For most animals phenotypic variation is continuous [4, 5]; here we explore whether such individuals can select microhabitats to best exploit camouflage. We use substrate selection in a ground-nesting bird (Japanese quail, *Coturnix japonica*). For such species threat from visual predators is high [6] and egg appearance shows strong between-female variation [7]. In quail, variation in appearance is particularly obvious in the amount of dark maculation on the light-coloured shell [8]. When given a choice, birds consistently selected laying substrates that made visual detection of their egg outline most challenging. However, the strategy for maximising camouflage varied with the degree of egg maculation. Females laying heavily maculated eggs selected the substrate that more closely matched egg maculation colour properties, leading to camouflage through disruptive coloration. For lightly maculated eggs, females chose a substrate that best matched their egg background colouration, suggesting background matching. Our results show that quail “know” their individual egg patterning and seek out a nest position that provides most effective camouflage for their individual phenotype.

Highlights

- Maculation on eggs provides disruptive colouration.
- Individuals vary in maculation but lay where camouflage is most effective.
- For high maculation, birds lay on a substrate matching the maculation of the egg.
- Where maculation is low, the birds adopt a background matching strategy.

Results

Chromaticity analysis

When female quail were given a choice of 4 differently coloured substrates upon which to lay their eggs we found that birds chose substrates that most closely matched the chromaticity of their maculate regions, but contrasted with their background colour (Figure S1). There was a significant interaction between substrate and egg region (background or maculation), suggesting that that quail select laying backgrounds according to the appearance of their eggs ($F_{1,14} = 23.6$, $p < 0.0001$, Figure 1, Table S1). On chosen substrates ΔE values (Euclidian difference in colour/luminance) were significantly lower for maculated regions of the egg when compared to non-chosen substrates, i.e. there was a better colour/luminance match between the egg maculate and the chosen substrate compared to the other potential laying substrates (Tukey $q = 8.1$, $p < 0.01$). However, the converse was true for egg background regions, where ΔE values for non-chosen substrates were significantly lower, suggesting that birds chose to lay on substrates that contrasted with their egg background (Tukey $q = 4.2$, $p < 0.01$). We found no effect of simulated egg predation (taking eggs away) on laying choices ($F_{1,14} = 0.5$, $p = 0.490$).

Distribution of laying choice analysis

We also detected differences in the optimality of laying choices. The distribution of laying ranks (rank 1 = most camouflaging/smallest ΔE , rank 4 = least camouflaging/largest ΔE : see supplemental information for further details) was significantly skewed for all the dependent variables (VisRat: the ratio of the amount of the egg outline detected divided by quantity of other edges found in the substrate (false-alarms)(see experimental procedures), ΔE maculation and

ΔE background). When considering both VisRat and ΔE maculation ranks we found significantly more eggs ranked at position 1 (i.e. laid on the substrate offering the highest degree of camouflage) and fewer ranked at position 4 (i.e. laid on the substrate offering least effective camouflage) than expected ($\chi^2 = 81.55$, d.f. = 3, $p < 0.0001$ and $\chi^2 = 56.7$, d.f. = 3, $p < 0.0001$, respectively; Figure 2, Table S1). However, we found the reversed relationship in the background ΔE scores, with more scores ranked at position 4 and fewer at position 1 ($\chi^2 = 57.6$, d.f. = 3, $p < 0.0001$; Figure 2). These data suggest that birds chose to lay eggs on substrates that tended to conceal their eggs outline and match the colour of their maculation, but that contrasted significantly with their egg background colour.

As our population laid eggs with a wide range of maculation levels (percentage of egg surface) and there was a high degree of variation in laying substrate chosen (Figure S2) we also investigated the interaction between egg maculation level and camouflage. When these distributions were subdivided by the amount of maculation (into 4 percentiles) we revealed a significant interaction between maculation level and our three dependent variables (VisRat, ΔE maculation and ΔE background). Maculation level did not affect the distributions of VisRat or ΔE maculation optimality ranks ($\chi^2 = 13.2$, d.f. = 9, $p = 0.15$, $\chi^2 = 13.8$, d.f. = 9, $p = 0.12$; Figure 3, Table S1): within each maculation percentile, the distributions were significantly skewed towards rank 1, suggesting all birds chose to lay on substrates that maximised camouflage through reduced edge detection and colour matching of the maculate to the substrate. Conversely, ΔE background scores were significantly affected by maculation levels ($\chi^2 = 23.2$, d.f. = 9, $p = 0.006$): eggs in maculation percentiles 2, 3 and 4 (the 75% of eggs with greatest maculation, having between 26-66% maculation on their surface, Table S2) showed significant distribution skews towards optimality rank position 4 (Figure 3), as in the analysis described above, suggesting a background-contrasting laying strategy. However, the 25% of eggs with the

lowest maculation (percentile 1, 19% maculation, Table S2) showed a flat distribution, suggesting a mixed strategy, with some choosing substrates that matched the egg background colour, whilst others used the same strategy as birds with higher maculation. It should be noted that although some eggs in the fourth percentile may exhibit maculation that represents the majority of the egg surface (maculation level > 50% egg surface), eggs in all other percentiles show much less maculation (Table S2).

Discussion

Predation is a strong and pervading selection pressure throughout the animal kingdom. Camouflaged appearance is a much studied counter-adaptation to predation risk, but the effectiveness of camouflage is very much dependent on the appearance of the background against which the individual is viewed [9-11]. Thus for many animals that are highly mobile and live in a heterogeneous habitat, individuals might be expected to select their microenvironment so as to enhance the effectiveness of their appearance for camouflage. Within many populations there is strong inter-individual variation in appearance, and this should mean that there will be strong inter-individual variation in how they select microhabitats, so that each individual can select the microhabitat that maximises camouflage for their individual appearance phenotype. Our data suggest that individual females can indeed select the microhabitat that provides best camouflage for their particular egg phenotype during breeding. Surprisingly, other evidence for this is very limited [2, 3], perhaps because of the challenge in field situations of controlling for confounding selection pressures, since different microhabitats will likely differ in other ecologically-relevant attributes as well as how they influence camouflage. These problems were avoided in our study by using the eggs of ground-nesting birds. These often show strong between-female variation in appearance [7], predation pressure on such eggs can be very strong [12, 13], and the simple ecology of the egg life-history stage allows us to rule out many other potentially confounding factors. Further, we used a laboratory situation where we can

control all variables other than the appearance of the substrates on which individual females can lay.

Camouflage can be attained via two main mechanisms: background matching and disruptive colouration [14]. Background matching relies simply upon the patterning or colouration of an animal or object matching the background or substrate. Disruptive colouration however suggests that contrasting patterns around the edge of the animal serve to break up the outline of that animal, reducing the edge detection abilities of the predator. Further Cott [14] also proposed two classifications of disruption: 'differential blending', where colour patches either match or contrast with the background and 'maximal disruptive contrast', where adjacent patches are contrasting in tone or colour and only some match the background. Both of these theoretical components break up the continuity of the surface and suggest to the viewer multiple distinct objects or they simply prevent detection of otherwise salient body edges and hence the object at all. Our results showed a very strong behavioural effect, with females laying highly maculated eggs upon darker backgrounds resulting in crypsis through reduced edge detection (VisRat) and also colour matching of maculated egg regions to the substrate. If birds were simply background matching then all eggs with less than 50% maculation should be laid on lighter substrates, where there is the best colour match (i.e. low ΔE background scores), however, the majority of our eggs showed less than 50% maculation and yet the majority were laid on darker substrates and exhibited high ΔE background scores. We therefore suggest that our birds gained maximal camouflage through disruptive colouration, acting via differential blending. This strategy changed for lightly maculated eggs, with some eggs being laid upon lighter substrates that matched the egg background, hence we suggest these birds were simply background-matching as the low amount of maculation reduced the amount of contrast patterning at the egg edges, rendering any disruption more challenging.

This work opens research avenues across predator-prey systems exploring how behaviour and appearance traits combine to give effective camouflage, and how fine-tuned behaviours can be between individual and ontogenic variation in appearance. More fundamentally, our results should encourage camouflage (and visibility and distinctiveness more generally) to be seen not simply as a function of the appearance of an organism, but as a function of both appearance and behavioural traits, which will be both tightly linked, and highly sensitive to individual-level variation.

Although there is mixed evidence linking quality of clutch camouflage to protection from predation in ground nesting birds [15, 16], several studies have suggested that microhabitat variability could affect crypsis [17-19]. Our results show that quail “know” their individual characteristic egg patterning and seek out a nest position that provides most effective camouflage for their individual egg phenotype, although we did not collect data on how these choices would translate into fitness benefits and more work is required to investigate this. More generally, our work suggests that the behavioural decision-making underlying camouflage can be more fine-tuned to phenotypic variation than previously appreciated. It has previously been demonstrated that some vertebrates can facultatively alter their appearance to maximise anti-predatory protection in different circumstances [20, 21]; however here we provide a demonstration of an organism facultatively changing behaviour (selection of microhabitat for egg laying in this case) according to whether its phenotype allows it to achieve most effective camouflage predominantly by disruption or by background matching. We also present empirical evidence of camouflage through disruptive coloration in avian eggs. The selection pressures on egg appearance are the subject of intense current research effort [7, 12], our work suggests that at least some species may show strong linkage between egg phenotype and behaviour that allows maximisation of camouflage.

Experimental procedures

Adult female quail ($n = 15$) were provided with four differently coloured sand substrates during two laying trials. During one trial eggs were removed on a daily basis, whilst in the other trial eggs were not removed and birds were allowed to lay a clutch of up to 7 eggs. The order in which birds experienced these 'predation' or 'no predation' trials was counterbalanced across the population. During each trial we recorded the substrate where each egg was laid and took calibrated photographs of individual eggs on a daily basis [22].

In order to achieve an unbiased evaluation of the degree of crypsis of each egg on each substrate we required a photograph of each egg upon each laying substrate. Rather than photograph each egg in each location, a procedure that cannot be done 'blind' we artificially constructed these images using each calibrated egg photograph from each bird. First an RGB mask image was created that delimited the area in each photograph that corresponded to the egg. We then created chimeric images by copying the parts of the egg photograph into the central area of photographs of the potential laying substrates (Figure S3). All construction of the test images was done automatically in CIELAB space, ensuring an unbiased process that preserved all chromatic values.

Once each chimeric image was constructed, we identified the area of the image that corresponded to the maculated and background parts of the egg. We calculated the chromaticity of each image region (egg maculation, egg background and substrate) by taking the mean CIELAB (L^* , a^* , b^*) [23] values for all pixels in these regions. We then computed chromatic differences between the substrate and egg regions by measuring the Euclidian distances between these averaged LAB values (ΔE). These ΔE values were calculated individually for each chimeric test image. While CIELAB provides a useful estimate of human sensitivities to luminance and chromatic differences, it may not be applicable to all potential

predators, which likely differ from humans in a number of aspects of vision. Ideally, analyses should be linked to the visual systems of the appropriate predator groups or be truly objective. However with such a wide range of potential predators and visual models unavailable for many, we used a more parsimonious route to obtain conservative estimates of ΔE values. We repeated all analyses [both edge-detecting and chromatic-difference scores] with the CIELAB luminance channel data alone or green channel information from the calibrated RGB values (substituting ΔE_L or ΔE_G for ΔE) and found that the results were consistent (Table S1). It is important to note that there are few isoluminant contours (having a coloured edge with no luminance edge) within the real-world, so chromatic signals of edges are almost always redundant. Any attempt to conceal shape needs to hide luminance-defined edges with the highest priority [24] .

The systematic assessment of crypsis in our test images required a model predator. As mentioned above, the likely predators for quail eggs are many and varied and for the sake of both parsimony and computational efficiency we adopted a relatively simple model that attempted to find the outline of the egg (Figure S3). Contours within the test images were detected using a standard computer-vision edge-detection algorithm [25]. However, we did not simply score the detection of contours along the outline of the egg, as this measure may ignore another cryptic strategy, choosing to lay in a visually noisy substrate [26]. We also took account of the egg's context by calculating the ratio of the contours found in the substrate and the amount of the egg's own contour that was found (termed the visibility ratio, VisRat).

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Figure legends:

Figure 1. ΔE values for chosen and non-chosen substrates when comparing both maculation and background regions of the egg. Error-bars represent ± 1 standard-error.

Figure 2. Distribution of edge camouflaging (VisRat), ΔE maculation and ΔE background ranks.

Figure 3. Quail laying choices split by their optimality for each measure of camouflage. Top row: regardless of the level of maculation, birds chose to lay on substrates that minimised VisRat, ensuring the egg outline was least visible, i.e. maximising crypsis. Middle row: for eggs with moderate to large amounts of maculation (25-75th percentiles) the chosen substrate was most often the best available match to the colour of the maculate. Bottom row: birds rarely chose the substrate that matched the egg background, however for the least maculated eggs (0-25th percentile) there was a significant shift to a mixed strategy, with more birds choosing substrates that matched their egg background colour.

Figure 1
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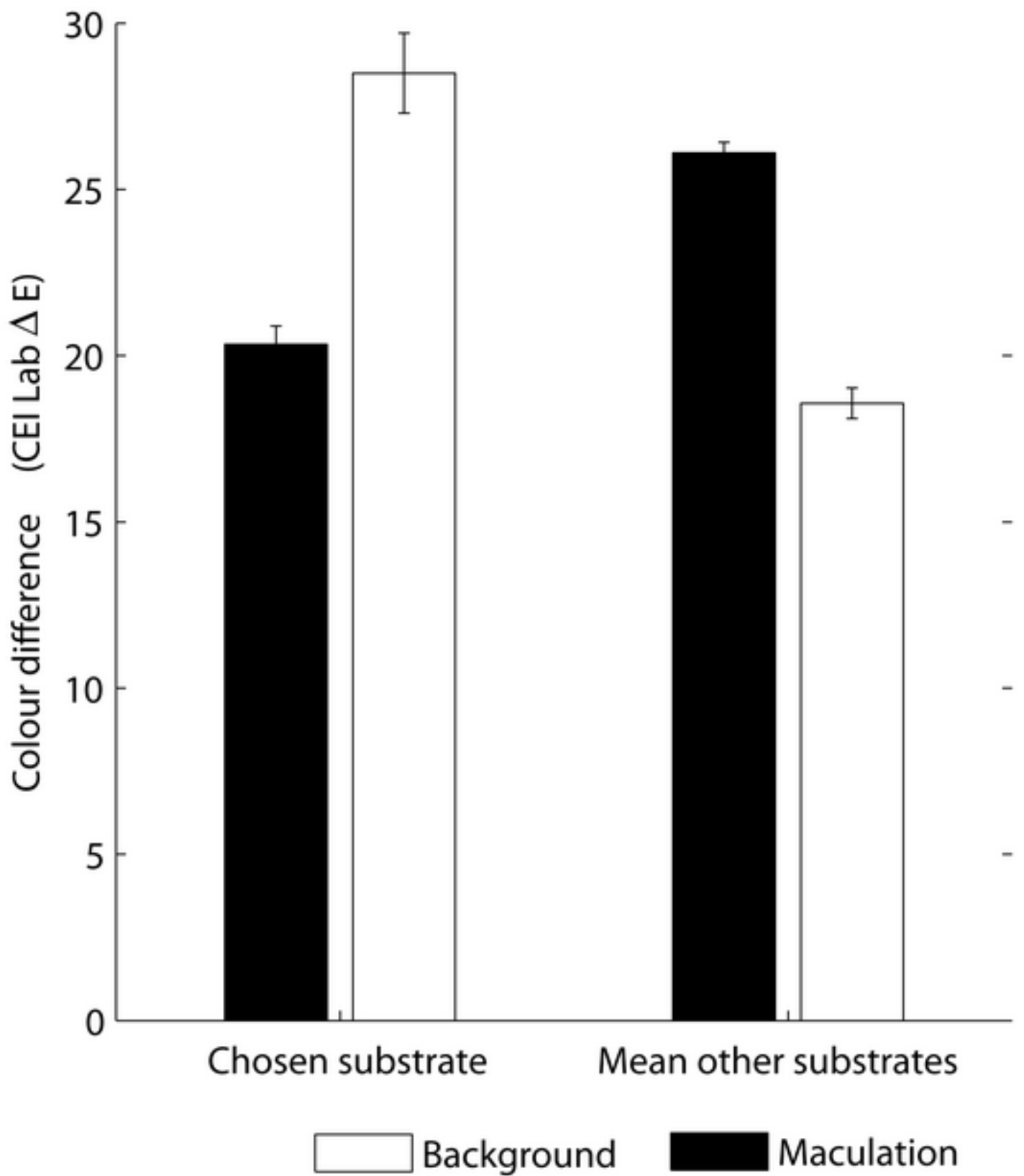


Figure 2

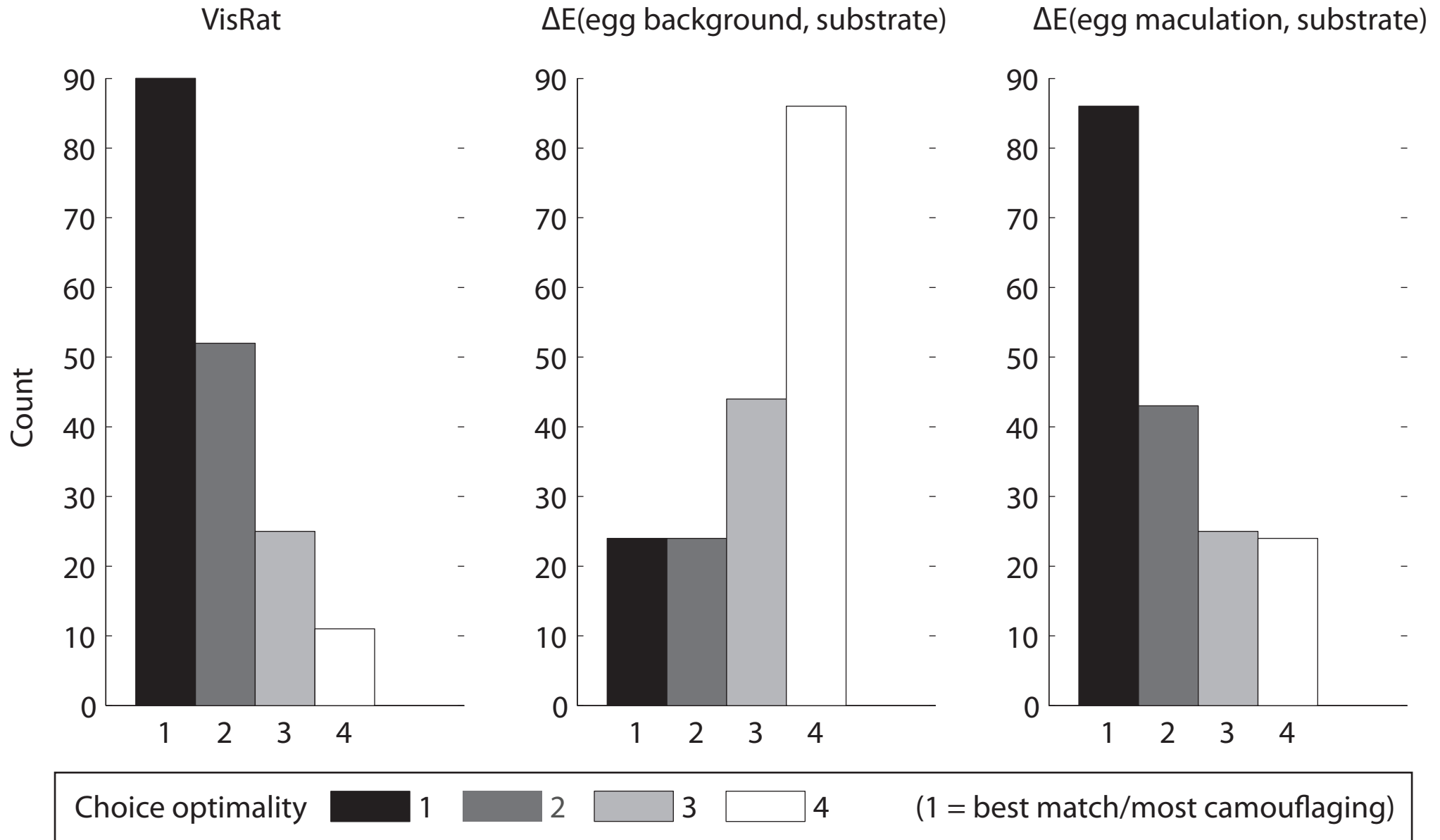
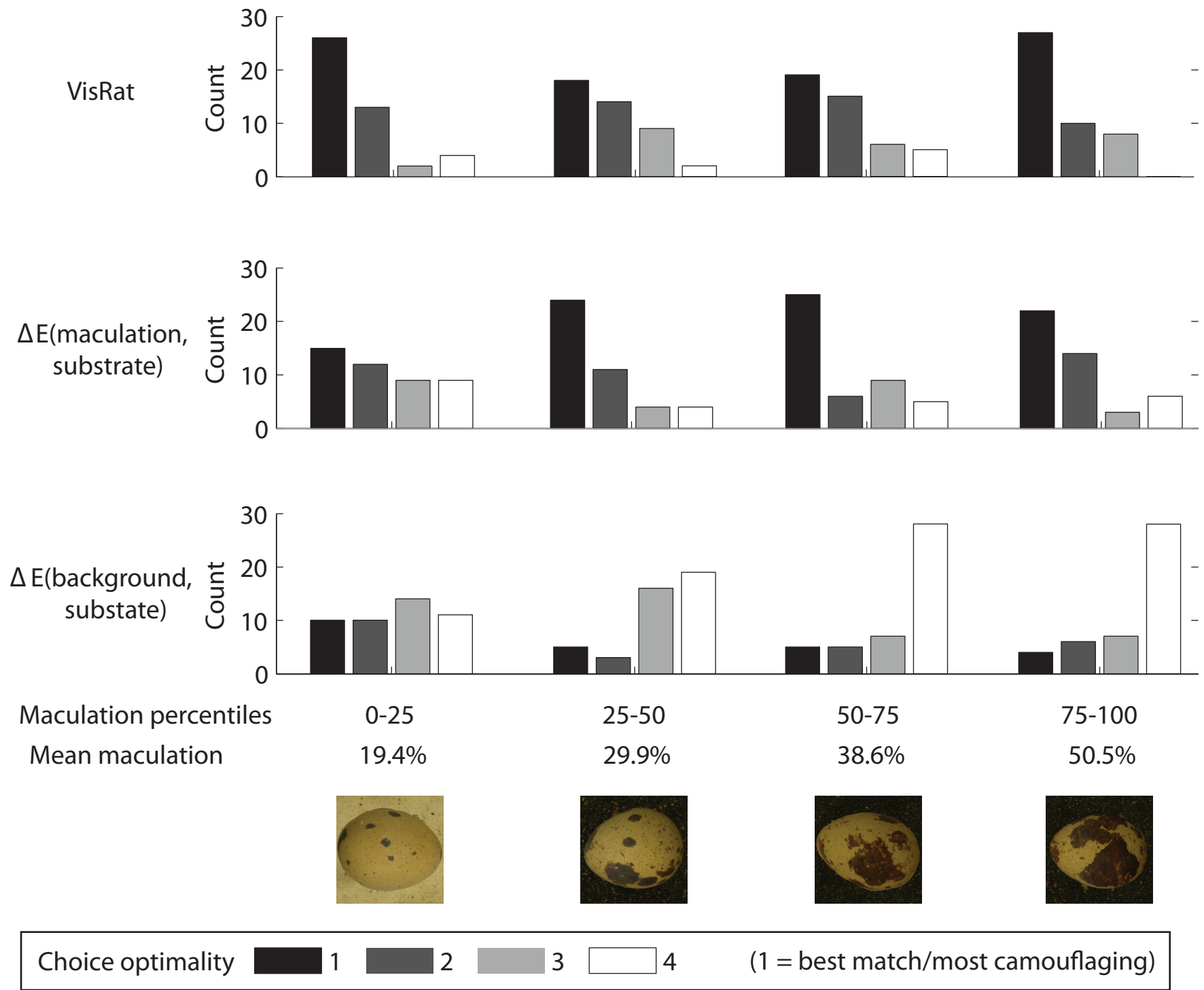


Figure 3



Inventory of Supplemental Information (in order of mention in main manuscript)

Figure S1 Normalised frequency plot of chromatic and visual difference measures, for chosen and non-chosen substrates. These data presented here relate to the analysis presented in Figure 1 and illustrate the raw difference values which are presented as ranks in Figure 2 of the main manuscript.

Table S1. Comparison of statistical results from models CIELAB variables L*a*b or Luminance alone. These data are related to Figures 1-3 in the main manuscript.

Figure S2 Stacked bar chart of laying positions split-by maculation percentile. This figure extends the information conveyed in Figure 3

Table S2. The amount of maculation in each percentile group. Relates to analysis of the interaction between maculation percentage and laying choices in the main manuscript.

Figure S3. Illustration of 'chimeric' egg photographs and original and a schematic illustration of the calculation of the visibility ratio (VisRat) for an egg. Relates to experimental methods section in main manuscript where descriptions of our photographic and edge detection protocols are placed.

Supplemental Experimental Procedures

A complete description of experimental procedures and analyses.

Supplemental references

References pertinent to the additional methods described above.

Supplemental Information section

Supplemental data

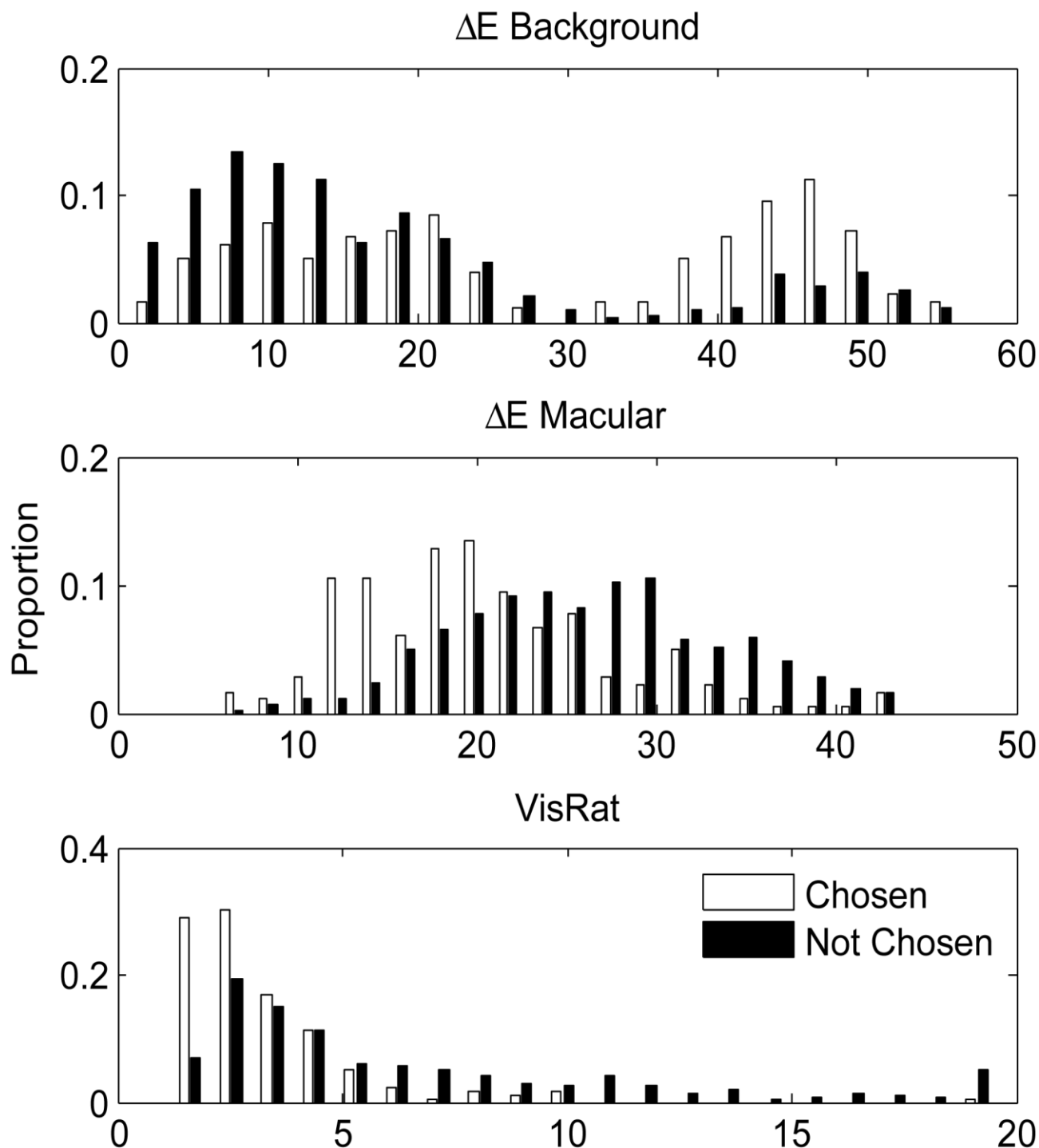


Figure S1 Normalised frequency plot of chromatic and visual difference measures, for chosen and non-chosen substrates. These data presented here relate to the analysis presented in Figure 1 and illustrate the raw difference values which are presented as ranks in Figure 2 of the main manuscript.

Table S1. Comparison of statistical results from models CIELAB variables L*a*b or Luminance alone: a) repeated measures GLM of $\Delta E/\Delta E_L/(\Delta E_G)$ for maculate and background regions of the egg, b) Chi square analysis of optimality rankings for each camouflage mechanism and c) Chi square analysis to determine the relationship between maculation level and each camouflage mechanism. Related to Figures 1-3 in the main manuscript.

Variable	d.f.	Significance for L*a*b analysis (ΔE)	Significance for L channel only (ΔE_L)	Significance for G channel only (ΔE_G)
(a)				
Substrate	1,14	F = 56.3, p <0.0001	F = 3.2, p = 0.09	F = 1.7, p = 0.210
Substrate*egg region	1,14	F = 23.6, p <0.0001	F = 26.7, p <0.0001	F = 28.2, p <0.0001
Predation	1,14	F = 0.5, p = 0.490	F = 0.37, p = 0.554	F = 0.28, p = 0.602
(b)				
VisRat	3	X ² = 81.6, p <0.0001	X ² = 109.2, p <0.0001	X ² = 86.0, p <0.0001
Δ Maculate	3	X ² = 56.7, p <0.0001	X ² = 22.0, p <0.0001	X ² = 22.1, p <0.0001
Δ Background	3	X ² = 57.6, p <0.0001	X ² = 50.1, p <0.0001	X ² = 50.3, p <0.0001
(c)				
VisRat	9	X ² = 13.2, p = 0.150	X ² = 9.4, p = 0.399	X ² = 14.2, p = 0.117
Δ Maculate	9	X ² = 13.0, p = 0.120	X ² = 8.9, p = 0.439	X ² = 8.9, p = 0.439
Δ Background	9	X ² = 23.3, p = 0.006	X ² = 20.4, p = 0.016	X ² = 20.3, p = 0.016

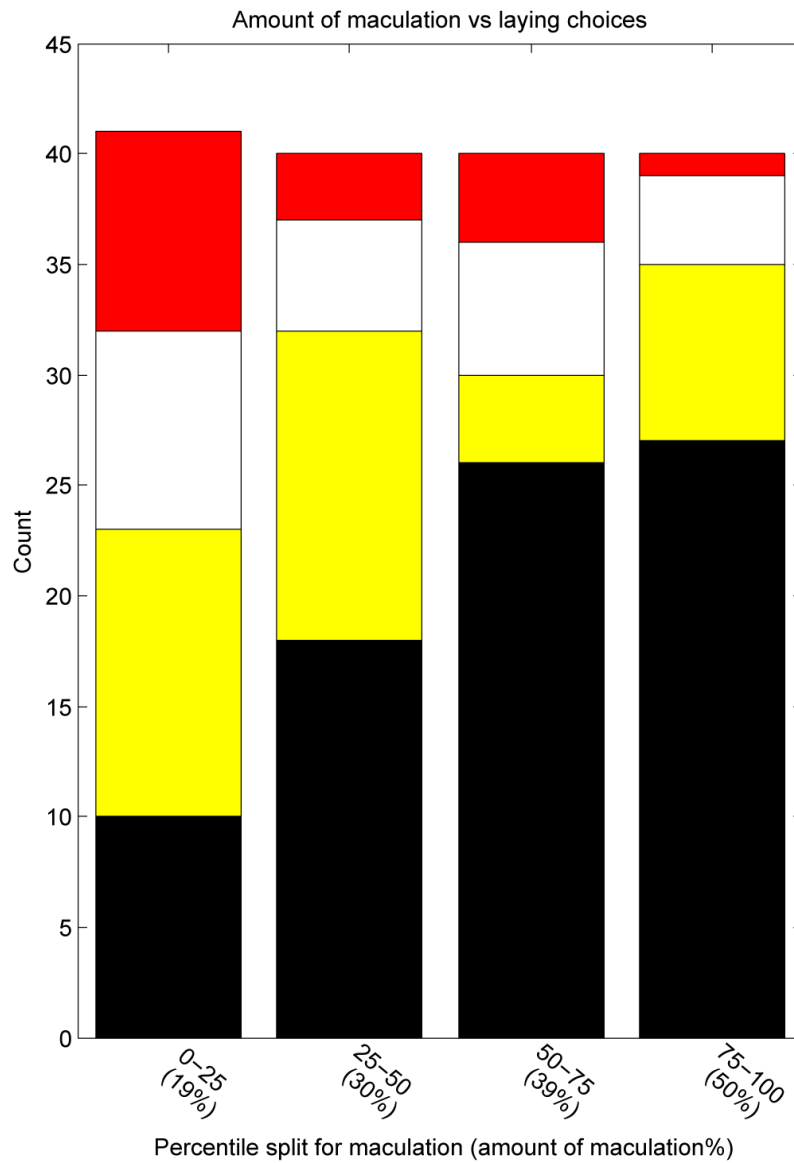


Figure S2 Stacked bar chart of laying positions split-by maculation percentile. The colours correspond to the four laying substrates available to the quail: black, red/brown (red), beige (yellow) and white. This figure extends the information conveyed in Figure 3 (main manuscript).

Table S2. The amount of maculation in each percentile group. Relates to analysis of the interaction between maculation percentage and laying choices in the main manuscript.

Percentile	Minimum	Mean	Maximum
1	6.3%	19.5%	26.0%
2	26.1%	29.9%	33.9%
3	34.1%	38.6%	43.5%
4	43.6%	50.5%	66.0%

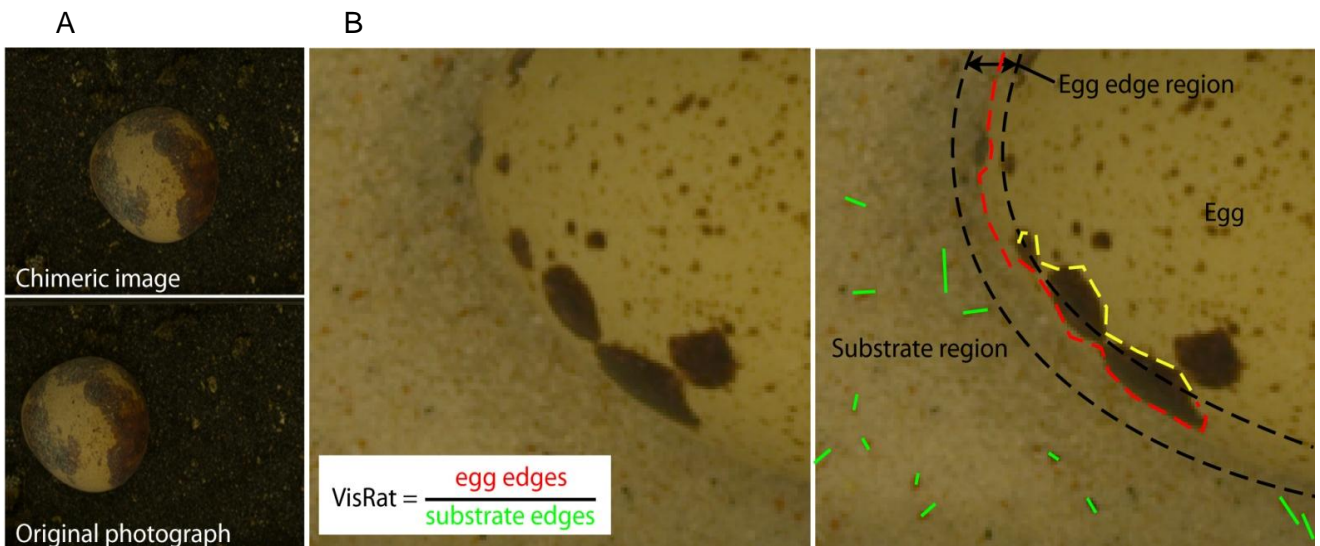


Figure S3. A (Top) constructed 'chimeric' egg photographs used in all subsequent analyses, (Bottom) original photograph of an egg upon the chosen laying substrate. Relates to experimental methods section in main manuscript where a description of our photographic protocols is placed. B. Schematic illustration of the calculation of the visibility ratio (VisRat) for an egg. Contours within the egg edge region (black dashed line) were scored as being part of a successfully detected egg contour, this value becomes the numerator. While those in the substrate region were summed and become the denominator in the VisRat calculation. In the current model the yellow contours were discarded. Relates to experimental methods section in main manuscript where a description of our edge detection protocols is placed.

Supplemental Experimental Procedures

Ethical note: All experiments were carried out with Ethical approval from the University of Glasgow and under Home Office Project Licence 60/4068 and personal licence 70/1364.

Animal housing and egg collection.

Adult female Japanese quail (*Coturnix japonica*) that had been bred and reared under standard feeding and housing conditions at the Cochno Research Farm, University of Glasgow were used in this study. Prior to experimental procedures all birds (n = 16) were housed in a deep litter arena (3m x 2.5m) at 19°C with a 14h:10h light:dark cycle and were provided with *ad libitum* food and water at all times. These environmental conditions were maintained throughout the experiment. Birds were then housed singly in arenas (97 x 43 x 65 cm) during each experimental laying period in full acoustic and visual contact with conspecifics at all times, allowing social contact. Within each arena birds were provided with four differently coloured sand substrates (Exo Terra, Rolf C Hagen Inc., Montreal, Canada) in plastic trays (30 x 20 cm). The position of each substrate was random within each arena and was rotated daily to control for any potential positional biases. In addition the surface temperature of each substrate was monitored regularly to ensure there were no thermal differences across substrates, which might affect laying decisions ($F_{3,93} = 0.33$, $p = 0.803$). Each female experienced two laying trials lasting 7 days, birds were given a rest period of 7 days between laying trials where they were communally housed in arenas 3m x 2.5m; during one trial eggs were removed on a daily basis, whilst in the other trial eggs were not removed from the arena and birds were allowed to lay a clutch of up to 7 eggs. The order in which birds experienced these 'predation' or 'no predation' trials was counterbalanced across the population. Birds laid between 2-7 eggs during each laying trial (5.6 ± 1.9 s.d.) and a total of 179 eggs were laid during both trials. One female did not lay any eggs in either trial and so was excluded from further analysis. During each laying trial we recorded on a daily basis the substrate where each egg was laid and took photographs of individual eggs (see next section).

Photography and calibration

All photographs were taken with a calibrated Nikon D60 dSLR camera with a Nikon lens (DX, AF-S NIKKOR 18-105mm). This enabled us to accurately translate the cameras RAW RGB values into XYZ colour space, we then convert the XYZ to CIELAB colour space using the Matlab image processing toolbox [1] . We adopted CIELAB because the values are perceptually uniform (for human vision) hence changes of similar numerical values in the L* (Luminance), a* (red-green) and b* (blue-yellow) axes will be perceived as having a similar perceptual difference. As a consequence we can estimate chromatic perceptual differences using 3D coordinates and by calculating the Euclidean distance (ΔE) between two CIELAB values [2].

Each egg was photographed twice, once upon the substrate upon which it was laid and a second time while placed upon a black card. Then each potential laying substrate was also photographed, with eggs removed, including the floor if the egg had been laid there. All photographs included a mini Gretag Macbeth (X-rite 50111) colour checker, this enabled the normalisation of estimated chromaticity values to the mean of the measured Macbeth tile values, thus controlling for any variation in illumination across cages and over time. If there was a previously laid egg near or touching the egg this was removed during the photography then both eggs were placed back in the positions they were found in.

In order to achieve an unbiased evaluation of the degree of crypsis of each egg on each substrate we required a photograph of each egg upon each laying substrate. Rather than photograph each egg in each location, a procedure that cannot be done 'blind' and without additional disruption to the quail, We artificially constructed these images. First an RGB mask image was created within Adobe Photoshop (Adobe Photoshop CS3 V10, Adobe Systems Incorporated) which delimited the area in each photograph which corresponded to the egg. We were careful to find the exact edge of each egg without including anything of the non-egg parts of the photograph. Then we were able to create chimeric images by copying the parts of the egg photograph into the central area of each of the

photographs of the potential laying substrates, a visual comparison of the chimeric images for the chosen substrates and the actual photographs of the laid egg confirms the effectiveness of this procedure, Figure S1. While the construction of the egg-mask was done with RGB values, all construction of the test images was done automatically within Matlab in CIELAB space, thereby ensuring an unbiased process which preserved all chromatic values.

Once each chimeric image was constructed we identified the area of the image that corresponded to the maculate and background parts of the egg. This was achieved by applying the k-means clustering algorithm (Matlab, with $k = 2$, giving a target of two centroids) to the CIELAB pixel values for the egg area of each image. This procedure is more reliable than simply hand masking the maculate area of the egg based upon visual inspection of luminances or chromaticities alone, because the maculate tends to differ in luminance and chromaticity from the egg background [3, 4].

Following the automatic segregation of the parts of the image corresponding to the egg maculate, the egg background and the substrate we could calculate the mean CIELAB values for each of these regions. We calculated the chromaticity of each image region (egg maculate, egg background and substrate) by taking the mean LAB values for all pixels in these regions. We then computed chromatic differences between the substrate and egg regions by calculating the Euclidian distances between these averaged LAB values (ΔE). These ΔE values were calculated individually for each chimeric test image.

The systematic assessment of crypsis in our test images required a model predator. The likely predators for quail eggs are many and varied and for the sake of both parsimony and computational efficiency we adopted a relatively simple model that attempted to find the outline of the egg. This is a useful measure of visibility as the egg outline could be hidden by one of two camouflaging techniques, namely background matching and disruptive colouration. The first solution hides the contour because there is no clear outline to be seen, while the latter solution hides the outline by offering alternative edges to those that correspond to the actual shape of the egg [5]. We also took

account of the egg's context by calculating the ratio of the contours found in the substrate and the amount of the egg's own contour that was found (termed the visibility ratio, VisRat), this process is illustrated in Figure S2. By taking a ratio of egg contour to background contour we modelled a further camouflaging technique, i.e. placing the egg into a cluttered substrate is more likely to hide the egg than placing it onto a clean piece of paper [6].

Contours within the test images were detected using a standard computer-vision edge-detection algorithm (Canny edge detector; implemented in the Matlab image processing toolbox, with settings: threshold = 0.2 and sigma = 3). Contour pixels were scored as part of the egg if they were in an area near the edge of the egg mask (4 pixels into the mask and 8 pixels beyond the mask; equivalent to a range of 1mm). Contour pixels were scored as part of the substrate if they were outside the egg mask and beyond the 1mm egg boundary area. Finally, we assumed that the egg boundary might be detected based upon chromatic or luminance edges, so the contour detection process was repeated for the L*, a* and b* values. We then took the most successful channel (that with the highest VisRat score) as the overall 'winner' and this score became our metric for the degree of egg visibility - we assumed that any evolved visual system would take the most informative cue towards detection rather than averaging together useful and uninformative cues to egg detection.

Statistical analyses

To determine how CIELAB colour variables differed between chosen and non-chosen substrates we ran a repeated measures GLM (SPSS Inc.) with CIELAB differences (ΔE) for the egg maculation and substrate and egg background and substrate [ΔE maculation; ΔE background respectively] for each female as the dependent variable and the following between subjects factors: predation experience (yes or no), egg position (maculate or background) and substrate (chosen or mean of non-chosen). We used a mean of all non-chosen substrates for each analysis. All data were checked for normality and homogeneity of variance. In order to elucidate the potential mechanisms

involved in laying choices we examined every laying choice to determine if it was optimal, i.e. the egg was laid on the most camouflaging substrate. We ranked each choice on a 1-4 scale with one being the most optimal (highest degree of camouflage) and 4 the least. This ranking was applied to the outputs from the edge detection algorithm (VisRat) and the CIELAB colour difference variables [both ΔE background and ΔE maculation. We then used Chi square analysis to determine differences in these three distributions, tabulating 'mechanism' against choice rank. Since eggs in this study varied substantially in the degree of maculation (6.3-65.9%) we also investigated how maculation level (proportion of maculation taken from photographs) influenced laying choices by running additional Chi Squared analyses comparing maculation proportion against choice rank for each mechanism [VisRat, ΔE (background) and ΔE (maculation)]. For these analyses maculation proportion was grouped into 4 percentiles (see table 2). Three sets of analyses (GLM and Chi square) were carried out, one using the mean CIELAB (L^* , a^* , b^*) values for all pixels in these regions, another utilising CIE luminance (L) data only, and the final set using the raw green pixel outputs from the camera (camera sensitivity peak=537nm, action spectra 71nm FWHM). We found no differences in biologically relevant variables across these three approaches (Table S1).

Supplemental References

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