

Manuscript Number: CURRENT-BIOLOGY-D-12-00766R3

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

Article Type: Report

Corresponding Author: Dr Paul George Lovell, Ph.D.

Corresponding Author's Institution: University of St Andrews

First Author: Paul G Lovell, PhD

Order of Authors: Paul G Lovell, PhD; Graeme D Ruxton, Ph.D.; Keri V Langridge, Ph.D.; Karen A Spencer, Ph.D.

Abstract: 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.

School of Psychology and Neuroscience,  
St Mary's College,  
University of St Andrews,  
Fife,  
United Kingdom.

14th December, 2012

Editor,  
Current Biology. Cell Press  
Elsevier Ltd.  
32 Jamestown Rd.  
London NW1 7BY  
United Kingdom

Dear Professor North,

We would like to re-submit the following manuscript, entitled "Individual quail select egg-laying substrate to provide optimal camouflage for their egg phenotype" to Current Biology. We have made the changes to the manuscript suggested by reviewer 1. We have addressed most of the concerns of Reviewer 2, however we don't feel a complete rewrite is appropriate at this stage.

You mentioned a potential shorter title in a previous email, I'm afraid I've misplaced that email, but we are happy for the shorter title that you suggested to be used.

If you require any further information, please do not hesitate to contact me.

Yours sincerely,

Dr Paul George Lovell

**Authors' responses to referees' comments on the resubmitted version are in bold throughout**

Reviewer #1

The authors have done an excellent job of dealing with the referee comments. This is a really exciting study with important findings. I have just a few very minor final suggestions.

Page 3: "We found no effect of simulated egg predation on laying choices ( $F_{1,14} = 0.5$ ,  $p = 0.490$ )." I think you need to give a little bit more information here because without reading the methods first this won't make sense to the reader.

**We have added "(taking eggs away)" to the text, we hope this is clearer.**

Likewise, on page 3 you should briefly say what the VisRat is.

**We've added a new sentence on page 3, after "Distribution of laying choices".**

Page 6: "Both of these theoretical components break up the continuity of the surface and suggest to the viewer multiple distinct objects." Or they simply prevent the detection of otherwise salient body edges and hence the object at all.

**We thank reviewer one for this suggestion and have added to the text.**

Page 7: "...but as a function of both appearance and behavioural traits, which will be both highly coevolved,..." Perhaps safer to say 'tightly linked' rather than 'highly coevolved' (or 'may be both highly coevolved...'), as strictly speaking coevolution involves reciprocal selection pressures, which might not be the case here.

**Once again, we thank reviewer 1 for the suggestion and have made the suggested alteration.**

Page 8: "...predator groups or be truly objective. However, with such a wide range of potential..."

**We have changed the sentence as suggested.**

Reviewer #2: To repeat my previous comments: this study shows that quail select backgrounds according to the patterning on the eggs (Graphical abstract). The observation is analogous to the finding that cuckoos lay eggs in the nests of birds whose eggs match their particular type. I am sure that this conclusion is justified (e.g. figure S2) however as far as I can see it is not possible to derive it from Figs 1 or 2.

By comparison the main conclusion in the Results is that quail choose backgrounds with low DeltaE values for the maculated regions. As the maculated regions have a fixed dark colour, all this means to me is that they prefer dark backgrounds.

**There is no outstanding disagreement about substance between ourselves and reviewer two. However, we hold slightly different views about which aspects of our**

data should be presented in order to convey our experimental findings to the readership. We agree that Figure one (and two) may illustrate a general preference for darker sands, which happen to match the eggs' maculate. We present these data as they are the main-effects of our study. Things become more interesting however when we examine the interactions between the birds' laying choices and the degree of maculation upon the eggs surface.

As we have already explained, both in our replies and in the paper, the nature of the birds' choices seem to vary as a function of the degree of maculation. The more maculated the egg, the more likely the bird is to choose a sand that matches the maculation colour. This is not the same as saying that quail simply "prefer dark backgrounds", if this were the case we would not expect to see a change in the pattern of laying choices (Figure 3), nor would we expect to see a change in the proportion of laying choices for the darkest substrate (see S2, kindly suggested by Reviewer 2). If the choice were for a darker substrate, then the majority of substrate choices would be for the black sand. However this is not the case, in the least maculated eggs fewer than 25% of eggs are laid upon this sand. In the more maculated eggs more than 50% of eggs are laid on the darker sand.

In a previous iteration of our review process Reviewer 2 suggested that "All that is needed is evidence that the distribution of eggs of a particular pattern differ significantly from the overall distribution of eggs.". We have included this (S1 and S2). However, our main results figures analyse the quality and a potential mechanism for evaluation of the match between each egg and the chosen laying substrate, we do this because we find it intuitive (and informative) that laying choices tend to consistently maximise camouflage (our VisRat measure, Fig 3, top-row) even though there seems to be a shift in the underlying mechanism as the degree of maculation varies (Fig 3, mid and bottom row). Again, if laying choices were simply favouring the darkest sand, then these patterns of laying choices would not be present.

The rest of the study concerning the 'optimality of laying choices' is contentious. Optimality is defined here for an artificial experimental situation and a sensible, but nonetheless speculative, model of camouflage. Do quail naturally nest on bare patches of fairly uniformly coloured fine sand? Is it valid to extrapolate from this situation to the 'optimality' of camouflage decisions in nature?

Certainly we feel that it is valid to discuss optimality even when perhaps the most appropriate camouflage isn't available. When a global minima for achieving maximal camouflage isn't available then a sensible animal will choose the best available alternative. This is exactly what we find in our data. As we already explain in the manuscript, we chose an experimental setting because we can control for all the other drivers that may influence laying choices, such as proximity to nutrition, predators, and other important aspects of an organisms life-history. We have previously answered the reviewers concerns regarding our choice of substrates in response to Reviewer 3 in the first version of this manuscript. For clarity we have quoted our original reply 'here: 'In the wild quail live in a range of habitats from dense grassland, to river edges to more open plains environments. Their nest consists of a small scrape in the soil substrate, sometimes lined with a small amount of grass. Sandy substrates provide an opportunity for the females to create a scrape, albeit without a grass covering. It should also be noted that Japanese quail have been bred in captivity for many generations and are normally provided with sawdust or a cage floor to lay on. Whilst sand is not their native substrate it does provide a naturalistic soil-like substance upon which to lay. Providing soil would not have been appropriate as in captivity moist bedding can cause significant foot problems and sourcing completely dry soil is not possible. Further, there would be ethical concerns about the

dust generated by use of completely dry soil. This would also not have allowed us to provide several different coloured backgrounds for the quail to choose between. Our data show that even in an artificial environment such as the lab using a domesticated animal it is still possible to show strong preferences for camouflaging substrates, indicating this is an innate behaviour. Further work on more naturalistic backgrounds with wild birds would probably yield even stronger preferences..

We have to be cautious with the interpretation of experiments with a limited range of artificial substrates, where the physical and visual texture and the illumination are not natural. For example can we rule out the hypothesis that the birds select a background that is \*appropriate\* (not necessarily matching) for the average colour of their eggs? The maculation would provide a disruptive effect against any background, and would moreover be cryptic against a visually textured background.

**This is a new issue that hasn't been raised in the previous three rounds of review. We already report that there is a significant match for maculation to substrate even when there is a relatively small amount of maculation – this is a main effect. We have already explained in previous responses, and within the text of the manuscript, that a mechanism favouring simple background matching to the majority colour on the eggs surface would result in the majority eggs being laid upon the yellow substrate. This only occurs in the very least maculated eggs. Remember, as we've stated already, that even in the most maculated eggs the degree of maculation is only 50% on average. In 2<sup>nd</sup> and 3<sup>rd</sup> percentiles the maculation level is less than 43.5%. So laying choices aren't based upon the majority colour. We aren't convinced that simply taking the average colour of the egg's surface would be a better account of our data, indeed such averaging would throw away information about patterning that is key to the appearance of these eggs. It is the patterning, with one part of the egg matching the background and the other contrasting with the background, that results in disruptive camouflage (a process described in the second paragraph of the discussion).**

Has the correct version of the ms been submitted? For example the response to my comments states that the text now asserts Figure 1 shows "that quail select backgrounds according to the appearance of their eggs". As far as I can see this text does not appear in the revised ms.

**We had revised the figure heading for Figure 1 and S1 to remove the ambiguity in our phrasing that we believed had confused Reviewer 2, we changed "egg position", to "egg region". We have now further clarified the relationship between our hypothesis, our data and our statistical results by including the sentence, below, in the results section.**

**"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)."**

I notice also that the 'mean maculations' for Figure 1 (19.4%. 29.9% etc) and figure S2 (6%, 26% etc) are very different. Were these different sets of eggs?

**Please accept our apologies, S2 was a new supplemental plot provided in response to the most recent review. There was a matlab scripting error - we were presenting the lower bound of each maculation percentile, in this particular plot, rather than the mean level of maculation, as is presented in the other plots. This has been corrected in S2.**

The parameter VisRat is used throughout the study but so far as I can tell is defined only in the supplementary material as 'the ratio of the contours found in the substrate and the amount of the eggs own contour that was found' (which is not English). Not only is this measure buried far into the supplementary material, but I cannot understand what it means. I thought the substrates were sand (e.g. Graphical abstract), so do not contain contours.

**The visRat measure is defined in the last paragraph of page 9. At the end of the “experimental procedures” section. However, as both reviewers have requested clarification we have added a brief definition upon the first use of the term, below <below heading “Distribution of laying choice analysis” page 3>.**

**We concur with Reviewer to that a uniformly flat sand would feature few visible contours. However, the sands weren't uniformly coloured, examination of the examples provided (S3 and the graphical abstract) reveals a gritty texture to each substrate. Also food pellets were distributed by the quail themselves, so there were contours present within the texture. As outlined in reference 26, the visibility of an object may be determined not just by its own visual characteristics but also by nearby context. We attempt to model this by measuring the contours in the surround and along the egg's edge. While there are few contours within the substrates, there are some and we are attempting to offer a model that could generalise to the evaluation of camouflage in a wider context.**

Overall this is an interesting and convincing finding (Figure S-2). The text is confusing, speculative and unconvincing. I suggest a rewrite that is accessible to non specialist readers.

**It appears that 2 of 3 reviewers agree that the paper is now readable. We have now added several points suggested by reviewer 2 and we feel that the paper is more readable to a general audience as a consequence. We do not feel the article would be any different with a rewrite, and significant revision would be likely to undermine all the suggestions made by the other reviewers.**

## Current Biology Conflict of Interest Form

Cell Press, 600 Technology Square, 5<sup>th</sup> Floor, Cambridge, MA 02139

If submitting materials via EES, please upload form with your final submission.

Otherwise, please email form as a PDF attachment to [currbioproofs@cell.com](mailto:currbioproofs@cell.com).

*Current Biology* requires all authors to disclose any financial conflict of interest that might be construed to influence the results or interpretation of their manuscript. Authors must declare any such conflict in the Acknowledgments section of the manuscript itself. As a guideline, any affiliation associated with a payment or financial benefit exceeding \$10,000 p.a. or 5% ownership of a company or research funding by a company with related interests would constitute a conflict that must be declared. This policy applies to all submitted research manuscripts and review material. Examples of statement language include: AUTHOR is an employee and shareholder of COMPANY; AUTHOR is a founder of COMPANY and a member of its scientific advisory board; this work was supported in part by a grant from COMPANY.

Please disclose any such interest below and sign on behalf of all authors on your manuscript.

---

Please check one:

None of the authors of this work has a financial interest related to this work.

Please print the following Disclosure Statement in the Acknowledgments section:

Signature: \_\_\_\_\_ Date: \_\_\_\_\_

Manuscript Number (if applicable): \_\_\_\_\_

First Author (please print): \_\_\_\_\_



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

P.George Lovell<sup>1,2\*</sup>, Graeme D. Ruxton<sup>3</sup>, Keri V. Langridge<sup>4</sup>& Karen A. Spencer<sup>2</sup>

1. Division of Psychology, Abertay University, Dundee, DD1 1HG, UK.
2. School of Psychology and Neuroscience, University of St Andrews, KY16 9JP, UK.
3. School of Biology, University of St Andrews, KY16 9TH, UK.
4. Department of Psychology, University of Exeter, EX4 4QG, UK.

\*Corresponding author

**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  $\Delta E$  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  $\Delta E$  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  $\Delta E$ , rank 4 = least camouflaging/largest  $\Delta E$ : 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),  $\Delta E$  maculation and

$\Delta E$  background). When considering both VisRat and  $\Delta E$  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  $\Delta E$  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,  $\Delta E$  maculation and  $\Delta E$  background). Maculation level did not affect the distributions of VisRat or  $\Delta E$  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,  $\Delta E$  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  $\Delta E$  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  $\Delta E$  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 ( $\Delta E$ ). These  $\Delta E$  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  $\Delta E$  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  $\Delta E_L$  or  $\Delta E_G$  for  $\Delta E$ ) 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).

***Acknowledgments:***

This project was funded by a BBSRC David Phillips Research Fellowship (KAS) and a grant to KAS and PGL from the University of Glasgow John Robertson Bequest Scheme. We thank staff from the Cochno Farm Research Centre, University of Glasgow for their help with animal

husbandry and Jane Robinson, Innes Cuthill and Nick Scott-Samuel for helpful discussions. We also thank three anonymous reviewers for constructive and thoughtful comments on earlier versions.

## References

1. Stevens, M., and Merilaita, S. (2011). *Animal Camouflage: mechanisms and function*, (Cambridge, UK ; New York.: Cambridge University Press.).
2. Jaenike, J., and Holt, R.D. (1991). Genetic-Variation for Habitat Preference - Evidence and Explanations. *Am Nat* 137, S67-S90.
3. Karpeštam, E., Wennersten, L., and Forsman, A. (2011). Matching habitat choice by experimentally mismatched phenotypes. *Evolutionary Ecology* 26, 893-907.
4. DeWitt, T.J., and Scheiner, S.M. (2004). *Phenotypic Plasticity: Functional and Conceptual Approaches*, (Oxford, New York: Oxford University Press).
5. Smith, M. (2011). *Phenotypic Variation: Exploration and Functional Genomics*, (Oxford University Press).
6. Caro, T. (2005). *Antipredator defences in birds and mammals*, (London: University of Chicago Press).
7. Kilner, R.M. (2006). The evolution of egg colour and patterning in birds. *Biological Reviews* 81, 383-406.
8. Pike, T.W. (2011). Egg recognition in Japanese quail. *Avian Biology Research* 4, 231-236.
9. Webster, R.J., Callahan, A., Godin, J.G., and Sherratt, T.N. (2009). Behaviourally mediated crypsis in two nocturnal moths with contrasting appearance. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* 364, 503-510.
10. Wang, Z., and Schaefer, H.M. (2011). Resting orientation enhances prey survival on strongly structured background. *Ecological Research* 27, 107-113.
11. Merilaita, S. (2003). Visual background complexity facilitates the evolution of camouflage. *Evolution* 57, 1248-1254.
12. Caswell Stoddard, M., Marshall, K.L.A., and Kilner, R.M. (2011). Imperfectly camouflaged avian eggs: artefact or adaptation? *Avian Biology Research* 4, 196-213.
13. Underwood, T., and Sealy, S. (2002). Adaptive significance of egg colouration. In *Avian Incubation: Behavior, Environment, and Evolution.*, D.C. Deeming, ed. (New York.: Oxford University Press.), pp. 280-298.
14. Cott, H.B. (1940). *Adaptive Colouration in Animals.*, (Methuen & Co. Ltd., London.).
15. Colwell, M.A., Meyer, J.J., Hardy, M.A., McAllister, S.E., Transou, A.N., Levalley, R.R., and Dinsmore, S.J. (2011). Western Snowy Plovers *Charadrius alexandrinus nivosus* select nesting substrates that enhance egg crypsis and improve nest survival. *Ibis* 153, 303-311.
16. Nguyen, L.P., Nol, E., and Abraham, K.F. (2003). Nest Success and Habitat Selection of the Semipalmated Plover on Akimiski Island, Nunavut. *The Wilson Bulletin* 115, 285-291.
17. Hockey, P.A.R. (1982). Adaptiveness of nest site selection and egg coloration in the African Black Oystercatcher *Haematopus moquini*. *Behav Ecol Sociobiol* 11, 117-123.
18. Lee, W.-S., Kwon, Y.-S., and Yoo, J.-C. (2010). Egg survival is related to the colour matching of eggs to nest background in Black-tailed Gulls. *Journal of Ornithology* 151, 765-770.
19. Sánchez, J.M., Corbacho, C., Muñoz del Viejo, A., and Parejo, D. (2004). Colony-site Tenacity and Egg Color Crypsis in the Gull-billed Tern. *Waterbirds* 27, 21-30.

20. Chiao, C.C., Chubb, C., Buresch, K., Siemann, L., and Hanlon, R.T. (2009). The scaling effects of substrate texture on camouflage patterning in cuttlefish. *VISION RES* 49, 1647-1656.
21. Stuart-Fox, D., and Moussalli, A. (2009). Camouflage, communication and thermoregulation: lessons from colour changing organisms. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* 364, 463-470.
22. Lovell, P.G., Tolhurst, D.J., Párraga, C.A., Baddeley, R., Leonards, U., Troscianko, J., and Troscianko, T. (2005). Stability of the color-opponent signals under changes of illuminant in natural scenes. *Journal of the Optical Society of America A* 22, 2060.
23. Commission International de l'Eclairage, C. (1976). CIE Colorimetry - Part 4: 1976 L\*a\*b\* Colour Space.
24. Stevens, M., and Cuthill, I.C. (2006). Disruptive coloration, crypsis and edge detection in early visual processing. *P Roy Soc B-Biol Sci* 273, 2141-2147.
25. Canny, J. (1986). A Computational Approach to Edge-Detection. *IEEE Transactions on Pattern Analysis and Machine Intelligence* 8, 679-698.
26. Troscianko, T., Benton, C.P., Lovell, P.G., Tolhurst, D.J., and Pizlo, Z. (2009). Camouflage and visual perception. *Philos Trans R Soc Lond B Biol Sci* 364, 449-461.

## Figure legends:

Figure 1.  $\Delta E$  values for chosen and non-chosen substrates when comparing both maculation and background regions of the egg. Error-bars represent  $\pm 1$  standard-error.

Figure 2. Distribution of edge camouflaging (VisRat),  $\Delta E$  maculation and  $\Delta E$  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  
[Click here to download high resolution image](#)

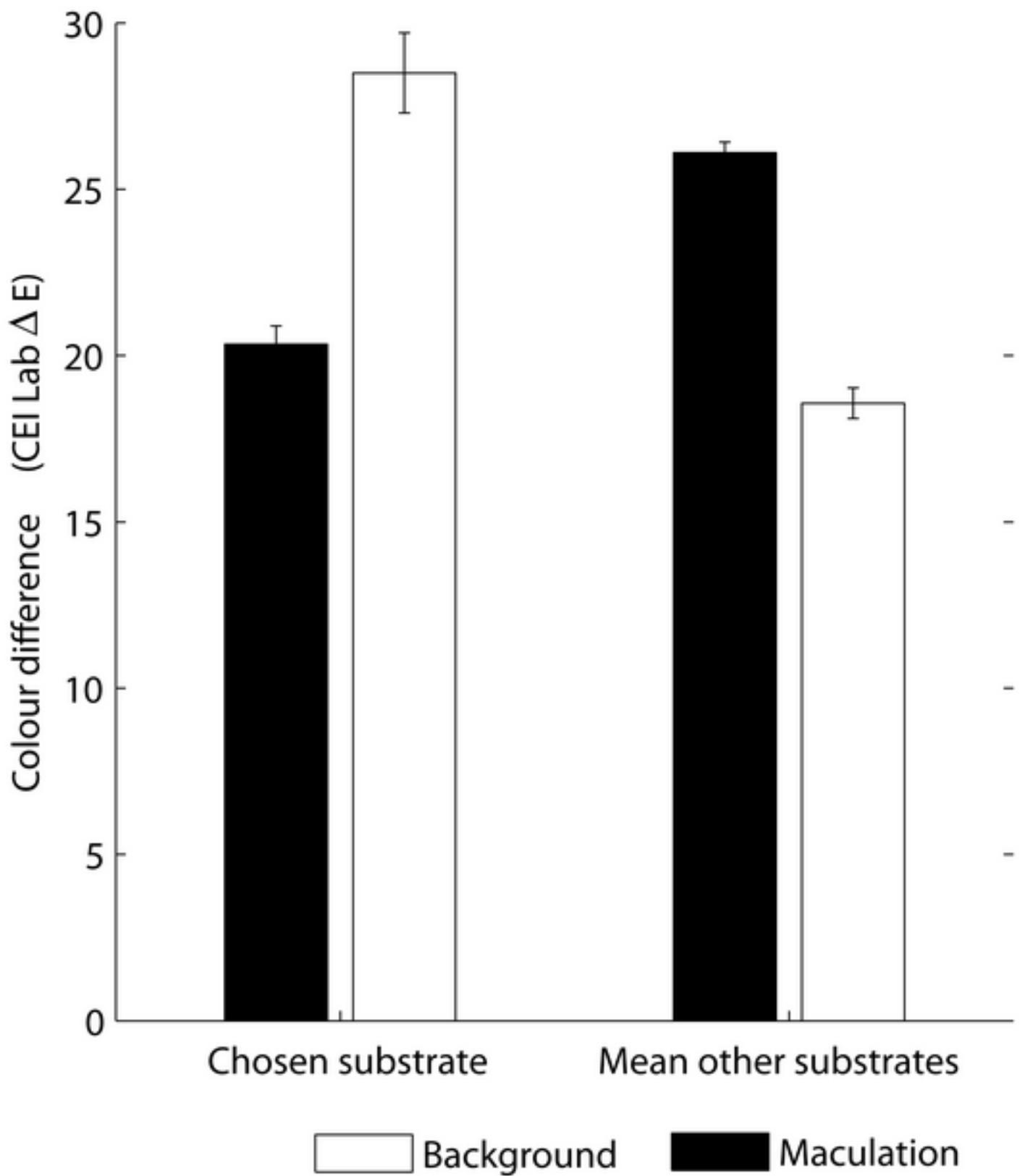
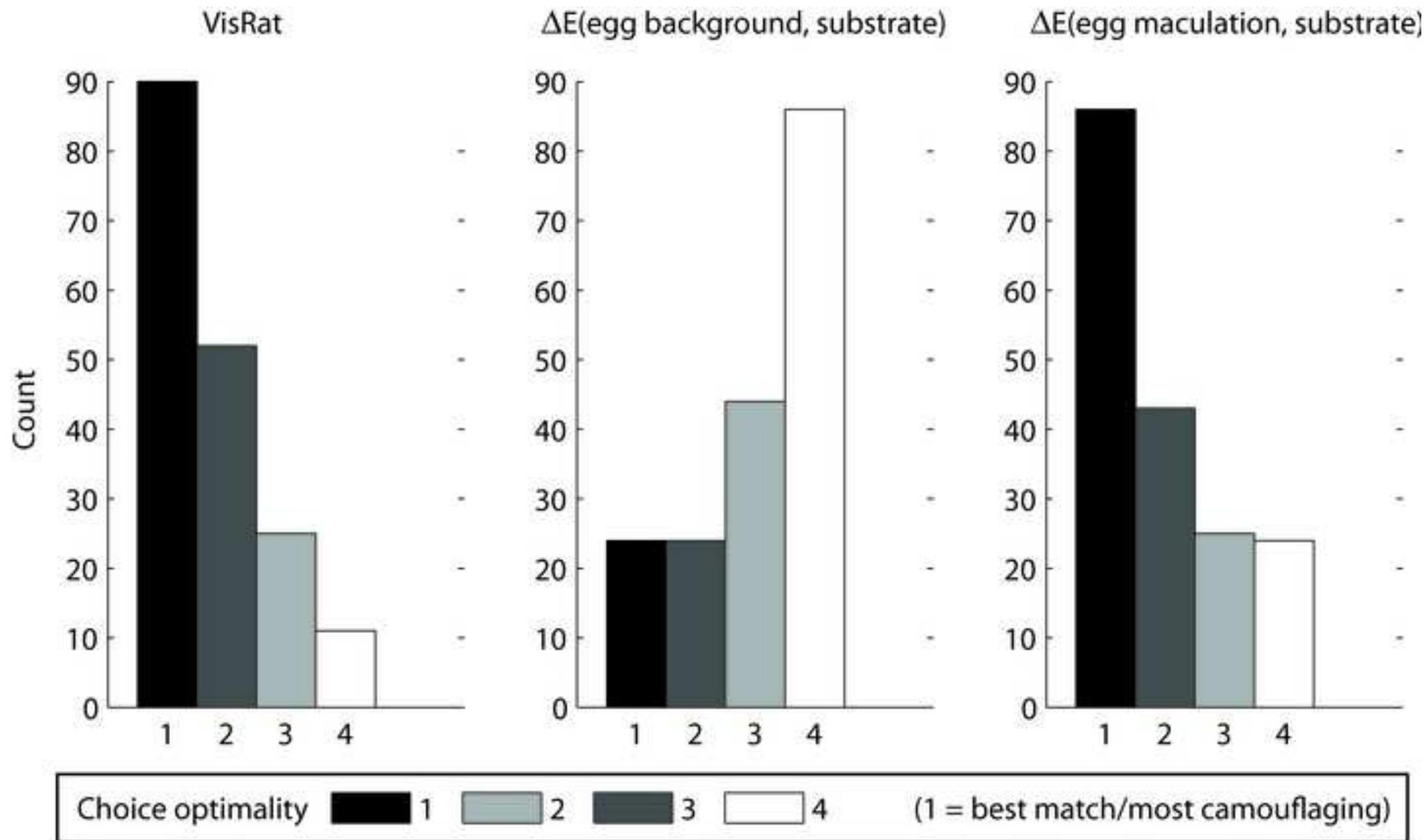
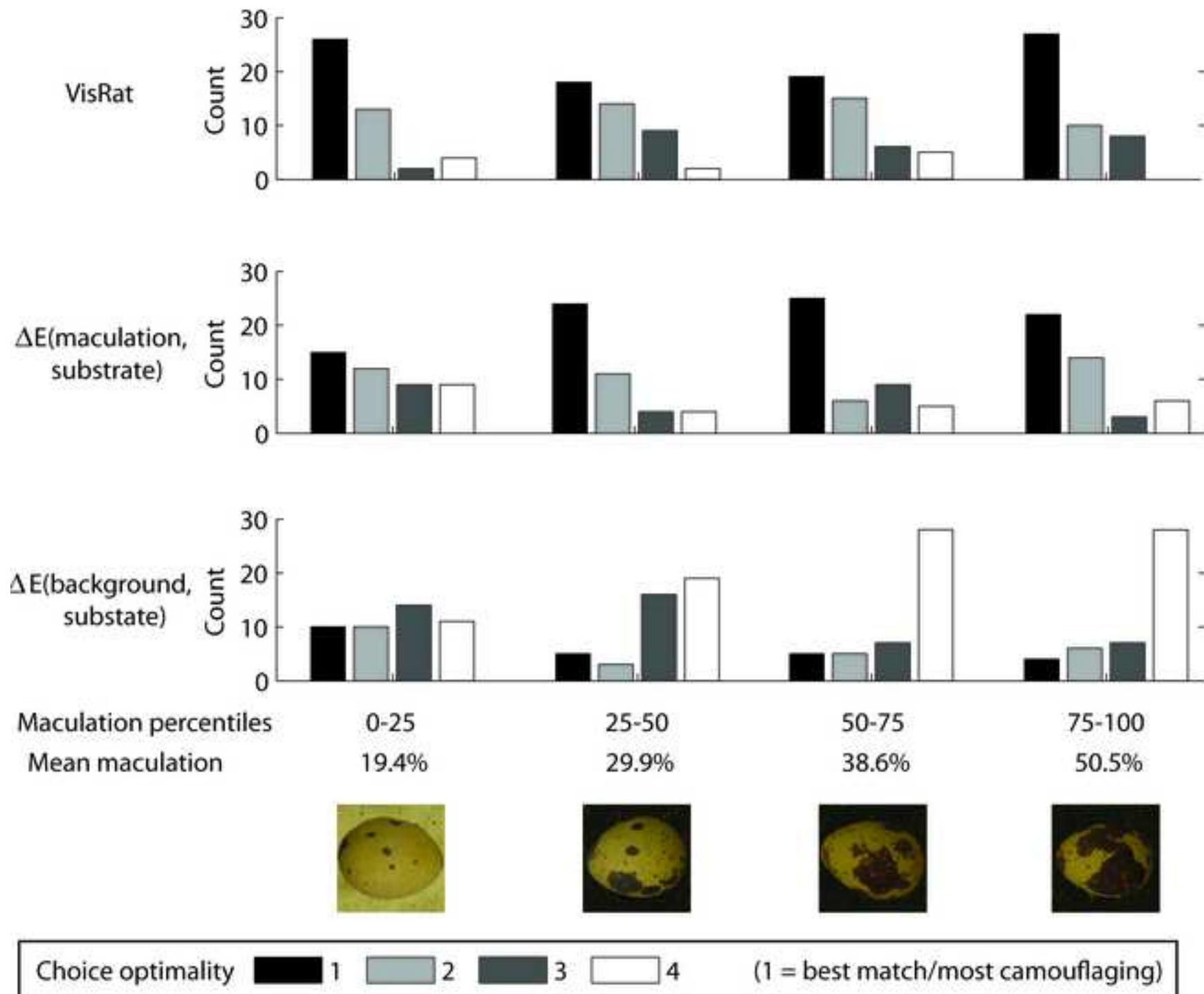


Figure 2  
[Click here to download high resolution image](#)



**Figure 3**  
[Click here to download high resolution image](#)



### **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**

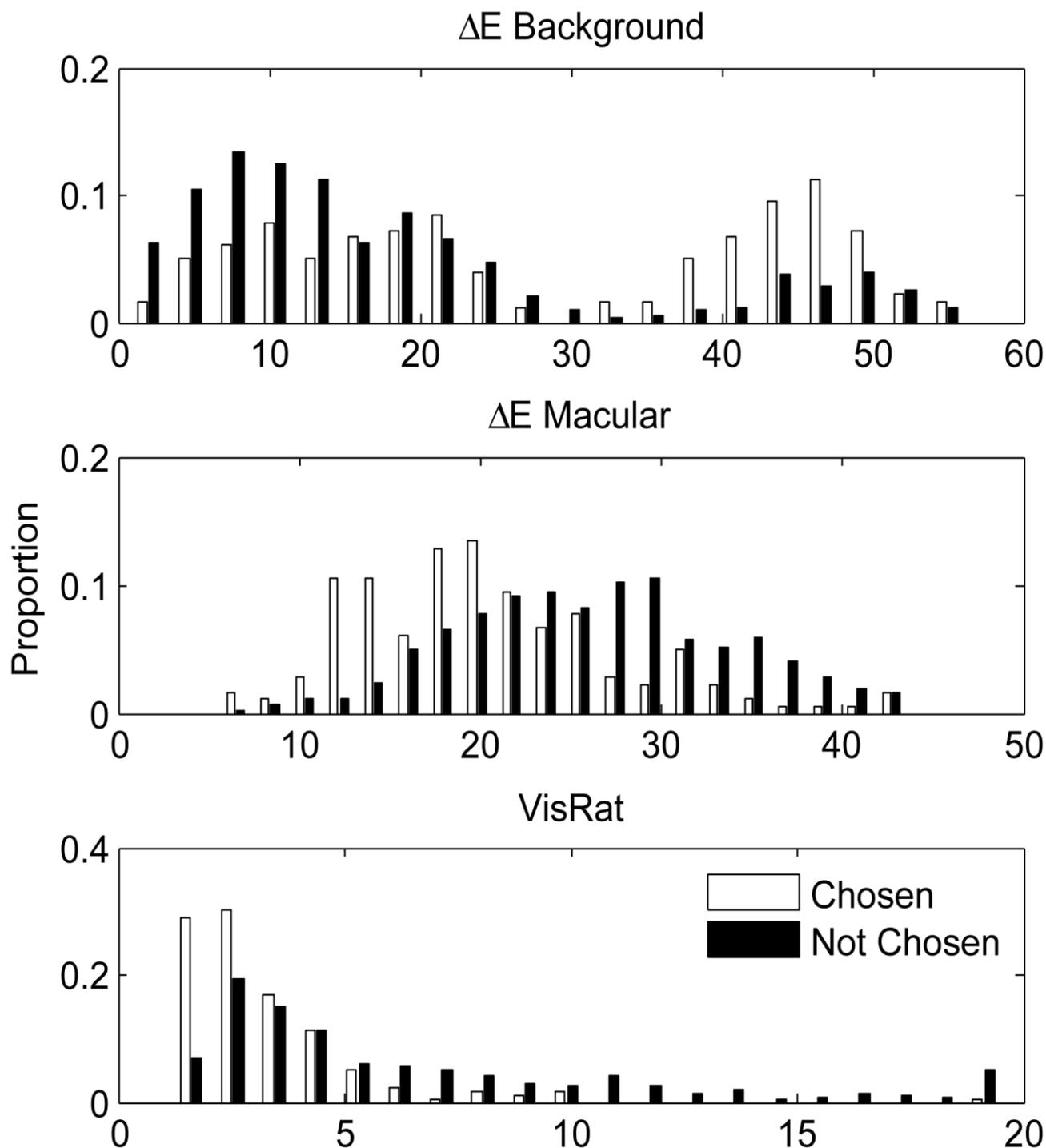


Figure S1 Normalised frequency plot of chromatic and visual difference measures, for chosen and non-chosen substrates. These data presented here relate to the analysis presented in Figure 1 and illustrate the raw difference values which are presented as ranks in Figure 2 of the main manuscript.

**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 ( $\Delta E$ )	Significance for L channel only ( $\Delta E_L$ )	Significance for G channel only ( $\Delta E_G$ )
(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 <sup>2</sup> = 81.6, p <0.0001	X <sup>2</sup> = 109.2, p <0.0001	X <sup>2</sup> = 86.0, p <0.0001
Δ Maculate	3	X <sup>2</sup> = 56.7, p <0.0001	X <sup>2</sup> = 22.0, p <0.0001	X <sup>2</sup> = 22.1, p <0.0001
Δ Background	3	X <sup>2</sup> = 57.6, p <0.0001	X <sup>2</sup> = 50.1, p <0.0001	X <sup>2</sup> = 50.3, p <0.0001
(c)				
VisRat	9	X <sup>2</sup> = 13.2, p = 0.150	X <sup>2</sup> = 9.4, p = 0.399	X <sup>2</sup> = 14.2, p = 0.117
Δ Maculate	9	X <sup>2</sup> = 13.0, p = 0.120	X <sup>2</sup> = 8.9, p = 0.439	X <sup>2</sup> = 8.9, p = 0.439
Δ Background	9	X <sup>2</sup> = 23.3, p = 0.006	X <sup>2</sup> = 20.4, p = 0.016	X <sup>2</sup> = 20.3, p = 0.016

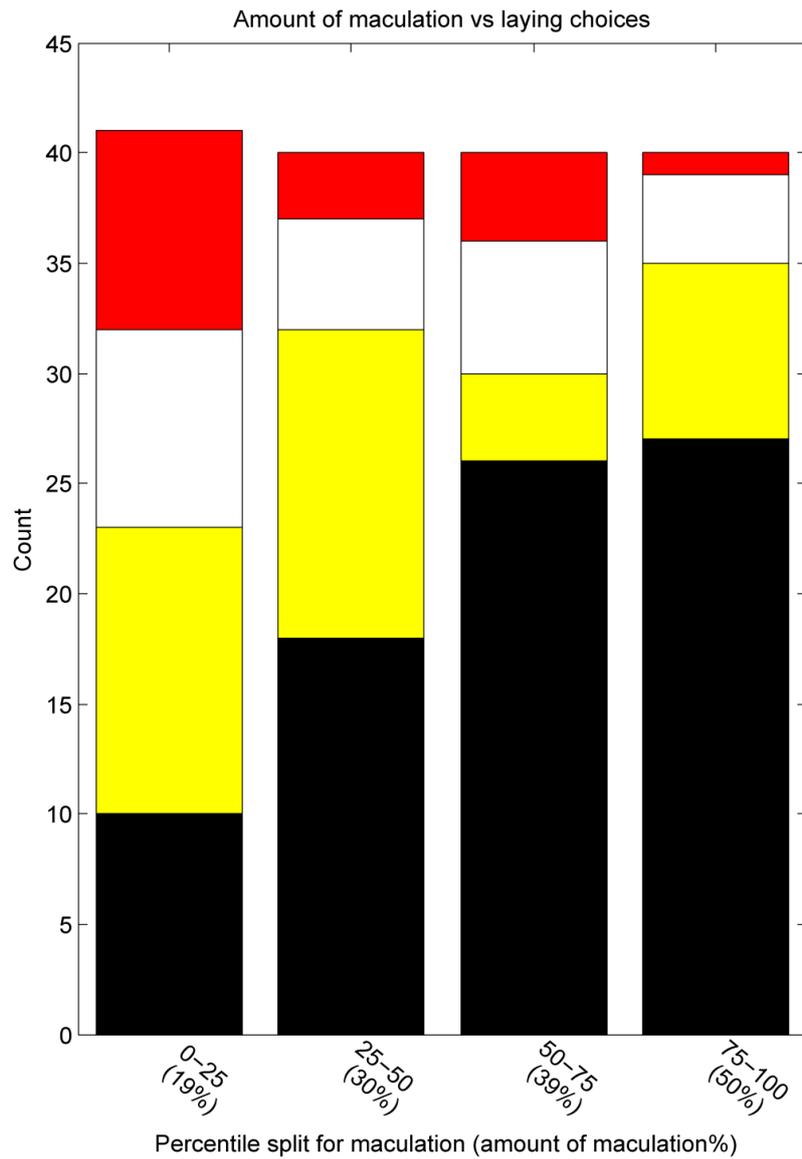


Figure S2 Stacked bar chart of laying positions split-by maculation percentile. The colours correspond to the four laying substrates available to the quail: black, red/brown (red), beige (yellow) and white. This figure extends the information conveyed in Figure 3 (main manuscript).

**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%

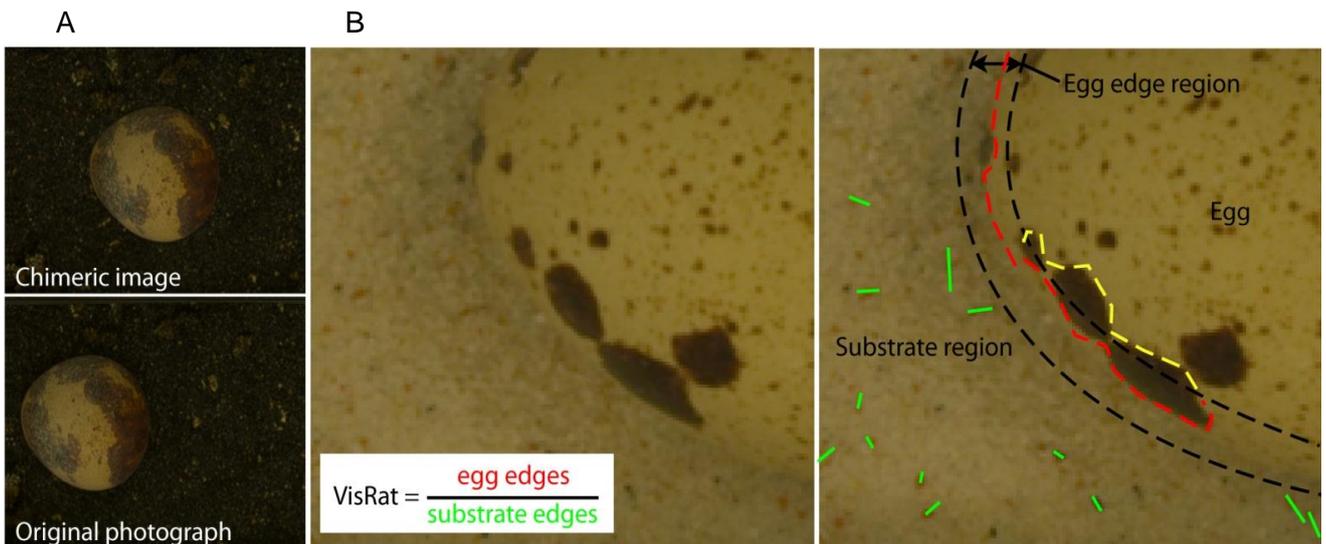


Figure S3. A (Top) constructed 'chimeric' egg photographs used in all subsequent analyses, (Bottom) original photograph of an egg upon the chosen laying substrate. Relates to experimental methods section in main manuscript where a description of our photographic protocols is placed. B. Schematic illustration 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 ( $5.6 \pm 1.9$  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 ( $\Delta E$ ) 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 ( $\Delta E$ ). These  $\Delta E$  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 ( $\Delta E$ ) for the egg maculation and substrate and egg background and substrate [ $\Delta E$  maculation;  $\Delta E$  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  $\Delta E$  background and  $\Delta E$  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,  $\Delta E$  (background) and  $\Delta E$  (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**

1. The MathWorks, I. (1984-2000). Matlab, 6 Edition, (Natick, MA. 01760-2098, USA).
2. Jain, A.K. (1989). Fundamentals of digital image processing (Prentice-Hall, Inc. Upper Saddle River, NJ, USA).
3. Cassey, P., Maurer, G., Lovell, G.P., and Hanley, D. (2011). Conspicuous eggs and colourful hypotheses: testing the role of multiple influences on avian eggshell appearance. *Avian Biology Research* 4, 185-195.
4. Cassey, P., Thomas, G.H., Portugal, S.J., Maurer, G., Hauber, M.E., Grim, T., Lovell, P.G., and Miksik, I. (2012). Why are birds' eggs colourful? Eggshell pigments co-vary with life-history and nesting ecology among British breeding non-passerine birds. *Biological Journal of the Linnean Society*.
5. Stevens, M., and Merilaita, S. (2009). Defining disruptive coloration and distinguishing its functions. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* 364, 481-488.
6. Dimitrova, M., and Merilaita, S. (2012). Prey pattern regularity and background complexity affect detectability of background-matching prey. *Behavioral Ecology* 23, 384-390.