

Report

Egg-Laying Substrate Selection for Optimal Camouflage by Quail

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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-colored 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 maximizing camouflage varied with the degree of egg maculation. Females laying heavily maculated eggs selected the substrate that more closely matched egg maculation color properties, leading to camouflage through disruptive coloration. For lightly maculated eggs, females chose a substrate that best matched their egg background coloration, 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.

Results

Chromaticity Analysis

When female quail were given a choice of four differently colored 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 color (Figure S1 available online). 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 and Table S1). On chosen substrates, ΔE values (Euclidian difference in color/luminance) were significantly lower for maculated regions of the egg when compared to nonchosen substrates, i.e., there was a better color/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 nonchosen 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 the Supplemental Experimental Procedures for further details) was significantly skewed for all the dependent variables (VisRat, or the ratio of the amount of the egg outline detected divided by quantity of other edges found in the substrate [false alarms] [see the 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 and 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 color of their maculation but that contrasted significantly with their egg background color.

Because 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 we subdivided these distributions by the amount of maculation (into four 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 and Table S1): within each maculation percentile, the distributions were significantly skewed toward rank 1, suggesting that all birds chose to lay on substrates that maximized camouflage through reduced edge detection and color 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 two three, and four (the 75% of eggs with greatest maculation, having between 26%–66% maculation on their surface; Table S2) showed significant distribution skews toward 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 one, 19% maculation; Table S2) showed a flat distribution, suggesting a mixed strategy, with some choosing substrates that matched the egg background color and others using the

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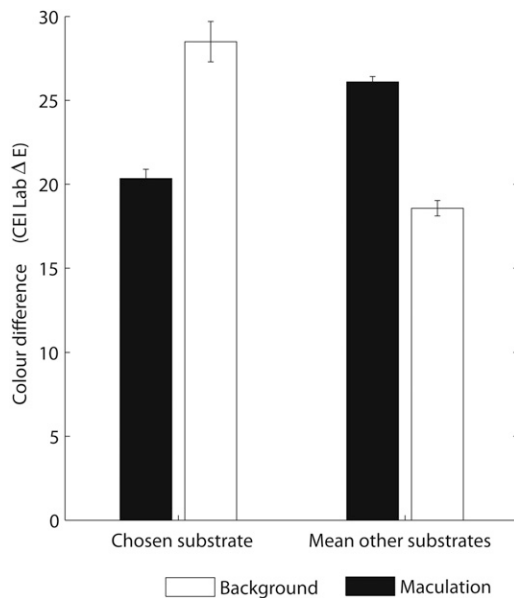


Figure 1. Chromatic Differences for Egg Regions and Chosen Laying Substrate

E values for chosen and nonchosen substrates when comparing both maculation and background regions of the egg are shown. Error bars represent ± 1 standard error. See also Figure S1 and Table S1.

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 counteradaptation 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 interindividual variation in appearance, and this should mean that there will be strong interindividual variation in how they select microhabitats, so that each individual can select the microhabitat that maximizes 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 in how they influence camouflage. We avoided these problems 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 in which we could 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 coloration [14]. Background matching relies simply upon the patterning or coloration of an animal or object matching the background or substrate. Disruptive coloration, 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,” in which color patches either match or contrast with the background, and “maximal disruptive contrast,” in which adjacent patches are contrasting in tone or color 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 behavioral effect, with females laying highly maculated eggs upon darker backgrounds, resulting in crypsis through reduced edge detection (VisRat) and also color matching of maculated egg regions to the substrate. If birds were simply background matching, then all eggs with

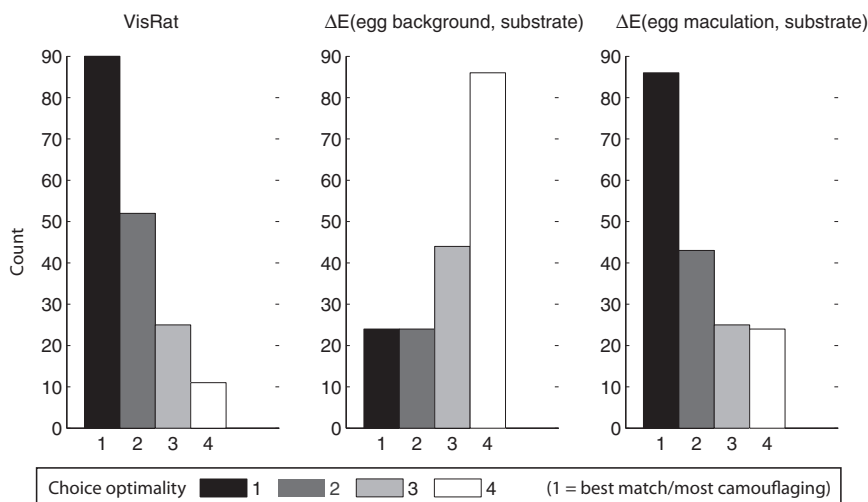


Figure 2. Distribution of Optimality Ranks for Camouflage and Chromatic Differences
Distribution of edge camouflaging (VisRat), ΔE maculation, and ΔE background ranks is shown. See also Figure S1 and Table S1.

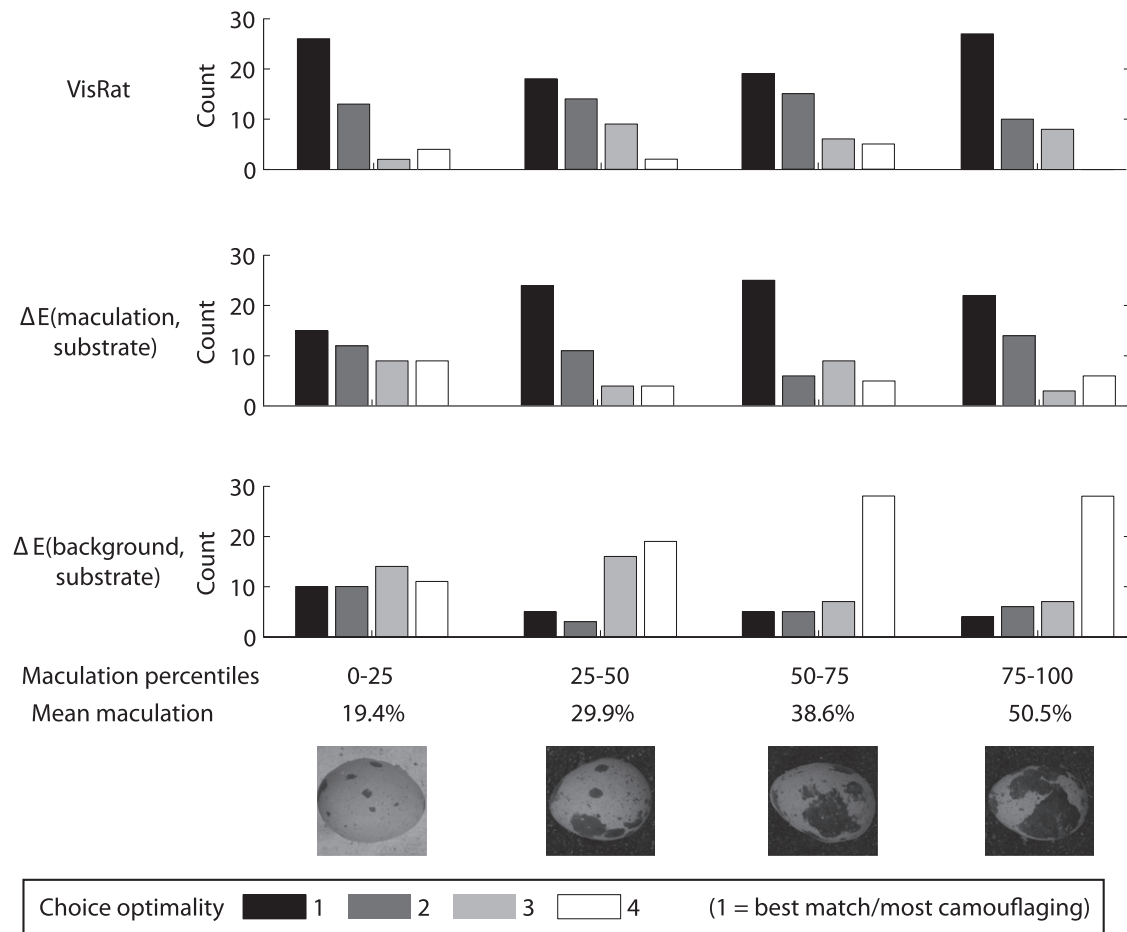


Figure 3. Quail Laying Choices Split by Their Optimality for Each Measure of Camouflage

Top row: regardless of the level of maculation, birds chose to lay on substrates that minimized VisRat, ensuring that the egg outline was least visible, i.e., maximizing crypsis. Middle row: for eggs with moderate to large amounts of maculation (25th–75th percentiles), the chosen substrate was most often the best available match to the color 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 color. See also Figure S2 and Tables S1 and S2.

less than 50% maculation should be laid on lighter substrates, where there is the best color 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 coloration, 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 that these birds were simply background matching because 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 behavior and appearance traits combine to give effective camouflage and how fine-tuned behaviors 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 behavioral 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 behavioral decision making underlying camouflage can be more fine-tuned to phenotypic variation than was previously appreciated. It has previously been demonstrated that some vertebrates can facultatively alter their appearance to maximize antipredatory protection in different circumstances [20, 21]; however, here we provide a demonstration of an organism facultatively changing behavior (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], and our

work suggests that at least some species may show strong linkage between egg phenotype and behavior that allows maximization of camouflage.

Experimental Procedures

Adult female quail ($n = 15$) were provided with four differently colored sand substrates during two laying trials. During one trial, eggs were removed on a daily basis, whereas 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]. All experiments were carried out with ethical approval from the University of Glasgow and under Home Office Project License 60/4068 and personal license 70/1364.

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. Although 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 colored 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 with 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).

Supplemental Information

Supplemental Information includes Supplemental Experimental Procedures, three figures, and two tables and can be found with this article online at <http://dx.doi.org/10.1016/j.cub.2012.12.031>.

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References

1. Stevens, M., and Merilaita, S. (2011). *Animal Camouflage: Mechanisms and Function* (Cambridge: 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. Karpestam, E., Wennersten, L., and Forsman, A. (2012). Matching habitat choice by experimentally mismatched phenotypes. *Evol. Ecol.* 26, 893–907.
4. DeWitt, T.J., and Scheiner, S.M. (2004). *Phenotypic Plasticity: Functional and Conceptual Approaches* (Oxford: Oxford University Press).
5. Smith, M. (2011). *Phenotypic Variation: Exploration and Functional Genomics* (Oxford: 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. *Biol. Rev. Camb. Philos. Soc.* 81, 383–406.
8. Wang, Z., and Schaefer, H.M. (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. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 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* (London: Methuen & Co. Ltd.).
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. *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. *J. Ornithol.* 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. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 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. *J. Opt. Soc. Am. A Opt. Image Sci. Vis.* 22, 2060–2071.

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. *Proc. Biol. Sci.* 273, 2141–2147.
25. Canny, J. (1986). A computational approach to edge detection. *IEEE Trans. Pattern Anal. Mach. Intell.* 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.