Avian vision models and field experiments determine the survival value of peppered moth camouflage

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Animal defensive coloration has long provided many important examples of evolution and adaptation. Of these, industrial melanism in the peppered moth is the classic textbook example of evolution in action, whereby dark and pale morphs suffer differential predation in polluted and unpolluted woodland based on their camouflage. Despite extensive work, a striking gap remains in that no study has ever objectively quantified their camouflage or related this directly to predation risk. Here we use image analysis and avian vision models to show that pale individuals more closely match lichen backgrounds than dark morphs. Artificial predation experiments in unpolluted woodland show 21% higher survival rates of pale than melanic individuals. Overall, we provide the strongest direct evidence to date that peppered moth morph frequencies stem from differential camouflage and avian predation, providing key support for this iconic example of natural selection.

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cross nature, many taxa have evolved camouflage to avoid detection or recognition. Animal defensive coloration has long provided flagship examples to illustrate and defend evolution and adaptation, including early work by Bates and Wallace on mimicry and camouflage. Arguably, the most important example of evolution in action is that of industrial melanism and the peppered moth (Biston betularia). During the Industrial Revolution (ca. 1760–1840), there was a rise of a new dark melanized (carbonaria) form in conjunction with a corresponding decline of the pale (typica) morph. The former is widely thought to have been well camouflaged against birds on trees where atmospheric pollution had killed off the lichen and soot particulates coated the bark, with typica effectively hidden on lichen-covered trunks and branches in unpolluted woodland. This was supported by the classic work of Kettlewell, who demonstrated strong selection against typica in polluted woodland and against carbonaria in unpolluted woodland where lichen persisted. Further work around this time supported Kettlewell and found that, despite persisting at frequencies as high as 80%, carbonaria still incurred a selective disadvantage in polluted areas. The introduction of the Clean Air Acts (1950s) provided further support, whereby pollution declined, lichen populations recovered, and there has since been a rise in the frequency of typica and a decline in carbonaria. Correspondingly, recent work has shown strong selection pressure against the melanic form, driven by differential avian predation. Therefore, this example provides key evidence for natural selection, showing the importance of a greater understanding of the ways in which anthropogenic activity influences defensive adaptations, and can provide further insight into both predator-prey dynamics and other anthropogenic impacts (e.g. climate change).

Despite the above, and considerable supporting work, this example of natural selection has been repeatedly attacked through unsupported claims of fraudulence on Kettlewell’s behalf. These claims have been firmly rebutted, but more importantly, the publication of scientific criticisms ultimately led to some doubts in the scientific community and furthered the promotion of an anti-evolution agenda from the non-scientific community. Most of these criticisms and uncertainties have since been largely addressed (for example, natural resting sites), and the validity of the original studies confirmed through further experiments providing reliable evidence indicating bird predation is the most important selective factor driving camouflage in Biston betularia. However, there remains a crucial gap in this example—remarkably, no study has quantified the camouflage of peppered moths, or related this to survival against predators in controlled experiments. This is crucial because humans and birds have visual systems differing in terms of number of receptor types, receptor sensitivity, and the ability of birds to perceive ultraviolet (UV) light. With typica appearing speckled under UV light (due to white wing scales strongly reflecting and black scales absorbing UV) and crustose lichen species on which typica rest, reflecting similar UV patterns, such natural backgrounds may consequently better conceal typica from its avian predators. In addition, for camouflage to work an object must closely resemble its background, and a fundamental criterion of camouflage theory is that the closer an object matches the background the less likely it is to be seen. To date, evidence that peppered moths are truly camouflaged has been indirect or subjective, being based on human assessment of either the moths directly or images of them.

Using museum specimens, including some of Kettlewell’s original collections, we used digital image analysis and models of avian vision to quantify the camouflage match for colour and luminance (lightness) of typica and carbonaria forms against lichen and plain tree bark (see Methods). We expected that typica would share greater similarities with crustose lichen backgrounds in comparison to plain bark backgrounds, whereas the reverse should be seen with carbonaria. Comparisons of 65 typica and 65 carbonaria individuals, each against a different sample of lichen and bark, allowed us to calculate discrimination values (just noticeable differences; JNDs) for colour and luminance of each moth to each background. Increasingly higher JND values indicate greater mismatch, and values close to 1.00 suggest camouflage so effective that colours cannot be distinguished between moths and their resting background.

Next, we performed predation experiments in unpolluted woodland with substantial lichen density (mostly in Cornwall, UK) to compare the likelihood of detection of typica and carbonaria morphs by avian predators. A widely used and powerful technique is to use artificial prey items designed to resemble real animals to predator vision (see “Methods” section); effective for monitoring survival over time when presented with an edible component and pinned to natural backgrounds in the field. We created artificial moths matching the appearance of typica and carbonaria forms using images of peppered moth museum specimens and measured predation over time for each morph, predicting that survival would be higher for models matching the typica morph. Overall, we show that, as predicted, to avian vision typica individuals of the peppered moth more closely match lichen covered bark, whereas carbonaria individuals more closely match plain bark. Furthermore, these differences translate into a strong survival advantage of typica individuals in unpolluted woodland.

### Results

#### Matches of morphs to different resting backgrounds

Peppered moth morphs differed significantly against lichen bark backgrounds ($F_{1,129} = 129.99$, $n = 130$, $p = 6.66 \times 10^{-14}$, Fig. 1), whereby typica morphs displayed low chromatic differences, or good camouflage (mean JND = 2.99 ± 1.17 standard error), and carbonaria had a poorer match to the background (8.03 ± 0.83 JND). There was no significant difference in the colour match between the morphs on plain bark backgrounds ($F_{1,129} = 1.66$, $n = 130$, $p = 0.19$); typica (9.81 ± 2.17 JND) and carbonaria (8.95 ± 1.53 JND). For luminance, there was also a significant difference in matching between morphs to lichen ($F_{1,129} = 196.9$, $n = 130$, $p < 2 \times 10^{-16}$), with typica (1.82 ± 0.67 JND) predicted to be close to indistinguishable in luminance to lichen, compared to carbonaria (6.64 ± 0.48 JND) that has greater achromatic contrast. Furthermore, there was a significant difference in luminance camouflage between morphs on plain bark ($F_{1,129} = 108.93$, $n = 130$, $p < 2 \times 10^{-16}$), with typica showing greater difference and worse camouflage (8.56 ± 1.03 JND) in comparison to carbonaria (3.07 ± 0.73 JND), which showed a close match. Overall, our results support the expectations that typica is a much closer match to crustose lichen, whereas carbonaria a closer match to plain bark (Fig. 1).

#### Survival of morphs in unpolluted woodland

We next performed predation experiments in unpolluted woodland. Artificial moths matching the appearance of typica and carbonaria forms with an edible pastry body were pinned to lichen covered tree trunks (confirmed as key resting sites for both morphs; Figs 2 & 3) in a randomised block design in different woodland areas. We expected that there would be a higher survival of typica than carbonaria targets. Survival was measured over a 48-h period across ten experimental blocks and significantly differed between typica and carbonaria ($X^2 = 22.23$, $df = 1$, $n = 500$, $p < 2 \times 10^{-16}$, Fig. 2). Survival probability estimates at 48 h were 0.43 ± 0.03 for carbonaria and 0.64 ± 0.03 for typica (Figs 2, 3). No morph-related censoring bias was observed for non-avian predation or disappearing targets: 20 typica and 17
carbomaria treatments required censoring. These results, overall, indicate that typica had an ~21% greater survival rate than carbomaria across the entire experiment.

Discussion

We have shown that the pale speckled form (typica) of the peppered moth has a close match to lichen covered bark for both colour and lightness, whereas the melanic (carbonaria) form is poorly matched and closer in lightness to plain bark. Therefore, to avian vision, the typica form is indeed better hidden against lichen covered trees than carbonaria. Crucially, this translates into a strong survival advantage, with replica models of typica being much less likely to be discovered by wild birds when on lichen covered backgrounds. These two components provide substantial direct support for the role of camouflage and differential avian predation in driving the rises and falls of polymorphic frequencies, and specifically the documented increases in typica during the post-industrial era. 9, 10

In our assessment of camouflage, the match of typica to lichen was very close using a model of avian vision, with values around 2–3 JNDs. Instead, carbonaria was a close match to plain bark for luminance, although not colour. In contrast, all moths had JNDs of around 7–9 for both colour and luminance against their incorrect background, indicating a substantially poorer match and diminished camouflage. These data are already in very close accordance with our predictions, but in addition, we used museum specimens and the resulting match between fresh
target appearance on detection / predation, and repeatedly shown no or minimal effect of body presence or appearance. These experiments have, for example, compared detection and attack rates on targets with camouflage or eyespot markings against birds, whereby an edible body (a dead mealworm larvae or pastry body, often mismatching the main target) was placed either directly on top of the wings or underneath, partly projecting out\cite{6,29,30,34}. In addition, studies have compared results of computer experiments with human subjects searching for computer-generated prey lacking edible bodies with work using the same stimuli types in field experiments\cite{26,34,36}. In all these studies, results are entirely consistent regardless of the placement and presence/absence of a body component. Finally, the detection of small targets and of texture is mediated primarily by luminance rather than colour contrast\cite{37,38}, and in this regard for both morphs the bodies were a close and similar match to the wings and natural background. Therefore, we are highly confident that our results are fully or largely explained by model wing appearance against the background.

Kettlewell\cite{39} showed that morphs of the peppered moth choose backgrounds to rest on that more closely resemble their appearance (e.g. \textit{carbonaria} on black stripes, \textit{typica} on white stripes). This was also confirmed in later work, although it is not straightforward as there can be variation among individuals, especially melanic ones, and the mechanisms underlying choices are unresolved\cite{40}. Work on other species has shown that individual moths have a remarkable ability to adjust their resting orientation and location to improve their own individual-specific, rather than species- or morph-specific, match to the background\cite{41}. Such behavioural approaches to facilitate camouflage have also been demonstrated in birds and lizards, among other taxa\cite{40,42,44}. Consequently, real live peppered moths may be able to further improve their match to appropriate backgrounds through behavioural responses to ultimately confer a greater survival advantage. While recent work has largely resolved the issue of where these moths rest (lower branch surfaces and trunks\cite{41}), more work is also needed on the fine-scale background resting locations of each morph, including with regards to lichen types. Current work, as here, has focused on crustose lichens rather than foliose species\cite{21,45}. This is partly because the use of crustose lichen is consistent with information on chosen resting positions of moths, and because crustose lichen has been shown to reflect UV in a similar manner to \textit{typica} moths, in comparison to foliose lichen, which absorbs UV creating a poor match with \textit{typica}\cite{4,21}.

While industrial melanism provides an important example of evolution, it is also an early demonstration of how anthropogenic changes affect species and their interactions with their environment. More recent work has investigated if and how camouflaged animals will be affected by climate change\cite{46}, with coral bleaching through ocean acidification already seemingly having an impact on the camouflage of some fish\cite{47}. It is possible that in industrially developing nations with high pollution levels, there are parallel examples to that of the peppered moth occurring at present. Advancing this understanding not only demonstrates evolution but illustrates the impact that humans can have on species interactions and fundamental biological processes, such as those between predators and prey.

**Methods**

**Museum collection photography.** Photographs of peppered moth (\textit{Biston betularia}) specimens were required for digital image analysis. Photographic data were obtained from three museums in the south of England: Bristol Museum and Art Gallery, the Exeter Ark of the Royal Albert Memorial Museum and Art Gallery, and Oxford University Museum of Natural History. The Oxford collections are noteworthy as they contain the specimens collected by Kettlewell for his original bird predation and breeding experiments. Access was permitted to the specimen

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**Fig. 3** A target matching a typica moth pinned to a lichen covered tree in woodland in the predation experiments.
drawers for selection of the most intact typica and carbonaria forms of both sexes for sampling, with selection based on wing completeness. Due to the age and fragility of the specimens in hand, care was taken to prevent damage to the original specimens.

We used a Nikon D7000 DSLR camera that had previously undergone a quartz conversion (Advanced Camera Services Limited, Norfolk, UK) by replacement of the internal UV filter with a transparent quartz sheet, as per previous studies. This allowed for full spectral sensitivity throughout the avian-visible spectrum. A CoastalOpt 105 mm UV Multi-APO lens with an internal diameter of 65 mm was used, as were 4 filters: UV block (transmitting 400–700 nm) and ultraviolet (UV) photographs were taken using a Baader UV pass filter (transmitting 315–400 nm). All images were taken using a 105 mm area within the well (70 mm) was selected as the minimal area of natural and artificial light so not to interfere with the lighting equipment, which consisted of an Iwasaki EYE Colour Arc lamp connected to a Ventronic ballast, mounted onto a PhotoPro photographic lighting stand with an attached Elnchrom umbrella. The arc lamp was left to warm-up for 15 min to allow the bulbs to reach at least 90% of its full light output. Prior to use, the UV-blocking filter applied over the arc lamp was removed using a steel brush bit as to allow for a full spectrum of wavelengths. A cylindrical sheet of PTFE was placed around the bulb, or around the specimen(s) to be photographed if accessible, to evenly diffuse the light.

If appropriate, a moth was removed from the specimen drawer and pinned into a neutral grey foam background. A maximum of three specimens per shot were photographed to ensure they were all uniform in size and wings were not out of the frame. To remove any effect of lighting variability, a light (95%) and dark (5%) reflectance standard (LabSphere, Congleton, UK) was included along with a scale bar positioned on the side closest to the specimens. The camera was positioned directly above using a copy stand or a tripod with a flexible head before being focused to obtain optimal sharpness. The exposure time was selected dependent on whether photographing with the visible or UV filter and optimised for correct exposure and to prevent saturation. A camera shutter remote was used to minimise unnecessary noise or movement.

**Natural resting background camouflage analysis.** Cryptic camouflage between the peppered moth and its natural resting backgrounds was explored by modelling avian vision to compare chromatic and achromatic contrasts. Woodlands in low-polluted areas were visited to obtain sites photographs of plain tree bark and photographs of bark possessing crustose lichen. Oak (Quercus robur) and ash (Fraxinus excelsior) trees were selected based upon their suitability as natural resting backgrounds as identified through previous peppered moth experiments. Selection of crustose lichen species, Lecidella elachosoma and Graphis scripta, as opposed to foliose lichen, was based upon previous work, and because previous experiments in both natural and modelled environments are present in lower sides of main branches, which are the natural resting locations most frequented by the peppered moth. Evidence against using foliose lichen is supported by ancestral populations found upon upper branches, whereas crustose lichens flourish underneath branches in unpolluted locations. 130 individual trees were selected according to the previous outlined suitability, photographing 65 plain bark and 65 lichen covered bark specimens under neutral light during daylight hours, using the same Nikon D7000 camera and lenses as per museum specimen collection. A light and dark reflectance standard and scale bar was positioned in each photograph to correct scaling and any lighting differences encountered during image analysis.

To prepare the images for analysis, each photo was converted into a multispectral image using the open-access software ImageJ with the Image Calibration and Analysis Toolbox plugin. A RAW image in both the UV and visible was selected for each specimen, and screened prior to this to check for overexposure. The light and dark reflectance standards were individually selected to normalise the channels and account for potential variation in light conditions. Manual alignment was then conducted on the visible RGB and UV channels of the multispectral image 32-bit stack for accurate colour measurements. For each sample of the two different background types, a single typica or carbonaria moth morph was selected for comparison by random selection or luminance (lightness) contrasted between the moth and its background.

For modelling avian predator vision, the blue tit (Cyanistes caeruleus) was selected because previous studies have shown them to attack the peppered moth, their foraging behaviour may be affected by UV cues, and they are the main avian predators of the moth models (see below). Therefore, our work is robust in accounting both for individual variation and in setting rigorous criteria, whereby models were only generated when colour and luminance matches to the real specimens were within 1.00 JND.

Once 50 individuals of each of the two morphs (n = 100) were correctly classified, they were scaled to size accurately matched real-life peppered moths; with each wing, on average, 221 mm² in surface area. Whatman Filter Paper No.1 12.5 cm stuck onto A4 plain paper. Using filter paper on which to print the targets was undertaken because this, unlike normal printer paper, reflects ultraviolet light and enabled us to match the UV appearance of typical moths even though printer ink is incapable of printing UV itself. Artificial wings were made waterproof using Plasti-Kote Matt Clear Acrylic spray paint, which permitted transmission / reflectance of UV.

An edible body was made of pastry, which has been shown in various previous experiments to be an effective measure of bird predation rates, including when attached to parts of birds. Following past protocols, lard and plain flour were mixed in a 1:3 ratio, before adding five drops of black food colouring for the carbonaria morphs, or a combination of approximately one red, one blue, three yellow and one black drop(s) for the typical morphs. White food colouring was used to add to the body mass of birds, to create 12 × 5 × 2 mm bodies, attached to the artificial wings using a 20-mm panel pin inserted into the centre of the pastry body, and left overnight in the freezer to set. The creation of the edible bodies for the targets was somewhat constrained by both available food colourings and of aiming to match both colour and luminance simultaneously to the moths.

We calculated, using the above methods and visual modelling, the colour and illumination contrast of 15 pantry bodies, of each morph, against the motifs themselves and natural resting backgrounds. For luminance, results for bodies to moths were: carbonaria (mean JND plus standard error: 3.838 ± 0.733) and typica (3.809 ± 0.731). For colour, matches were: carbonaria: (16.106 ± 1.477) and typica: (15.291 ± 0.641). The receiver operating characteristic (ROC) area under the curve for the body backgrounds for luminance were: carbonaria: (0.773 ± 0.348) and typica (1.660 ± 0.309). For colour, matches were: carbonaria: (13.721 ± 1.328) and typica (2.783 ± 0.550).

Using a different irradiance spectrum has been shown to have little effect on predicted cone catch values. Finally, to fully demonstrate that our modelling is robust for lighting, we compared the predicted cone catch values for 16 moths (eight typica and eight carbonaria) under both D65 and forest shade irradiance spectra. As expected, results were unchanged, with mean plus standard deviation cone catch values for forest as follows: carbonaria (forest = 0.27 ± 0.15, D65 = 0.27 ± 0.15), mediumwave (forest = 0.20 ± 0.13, D65 = 0.20 ± 0.13), shortwave (forest = 0.14 ± 0.10, D65 = 0.14 ± 0.10), ultraviolet (forest = 0.08 ± 0.06, D65 = 0.07 ± 0.06). In all cases, there was a very strong and significant correlation between the cone catch values under the two light conditions (Spearman’s rank correlation tests between forest and D65 for all results: ρ = 0.9998, r = 0.9998).

Visual discrimination (level of camouflage) was assessed according to the widely used Vorobyev and Osorio receptor noise discrimination model. On the basis of a recent detailed evaluation of estimates of receptor noise, a Weber fraction of 0.1 was selected for colour discrimination with single cone (UV, shortwave, mediumwave, longwave) The receptor ratio of 1.06:1 9.2:6.88:1 was natural, respectively, while a Weber fraction of 0.2 was utilised for luminescence. To quantify discrimination, JND values were calculated for both colour and achromatic contrast (luminance). JND calculations for the latter are based on double cones, and represent how discriminable two spectra are from one another under the assumption that visual discrimination is limited by receptor noise. A JND value of 1.00 is taken as the discrimination threshold for birds, whereby JND < 1.00 indicates two objects cannot be distinguished even under optimal viewing conditions, and as values increase > 1.00 this denotes increasing contrast and greater differences in distinguishability.

**Creation of artificial predation experiment targets.** Artificial moth targets for the avian predation experiments followed a wide range of past experiments using similar targets based either on natural backgrounds or designed to mimic real species. Targets were created using the multispectral images generated from the moth museum specimen photographs. As with a range of past work, images were converted to the predicted photon catches of the vision of the blue tit, under D65 lighting conditions, and then the output of a printer was calibrated in an iterative process such that the images colours (pixel values) converged to accurately match the real moths in terms of bird vision when reproduced. The match for every printed target was a JND value < 1.00, indicating indistinguishability from a real moth. Generally, most previous work has tended to, for example, simply mat the appearance of target wings to the average colour (in photon catches) of the substrate, e.g., tree bark (65/44). Past work matching artificial prey items to Lepidopteran models has also tended to ensure that matches of colour are based on targets falling within the range of photon catch values of the real animal model. Our work most closely follows other recent work on butterfly models, on which a visual discrimination model to create matches to the real butterflies, with matches chosen when colours fell within 1–3 JNDs. Our approach is in fact even more detailed in that, unlike past studies, we did not create all targets per treatment as identical and simply matching an average model colouration, but instead we included individual variation by matching different individual moth models (see below). Therefore, our work is robust in accounting both for individual variation and in setting rigorous criteria, whereby models were only generated when colour and luminance matches to the real specimens were within 1.00 JND.

Once 50 individuals of each of the two morphs (n = 100) were correctly classified, they were scaled to size accurately matched real-life peppered moths; with each wing, on average, 221 mm² in surface area. The specimens were printed using an HP LaserJet Enterprise 500 Colour M551 on Whatman Filter Paper No.1 12.5 cm stuck onto A4 plain paper. Using filter paper on which to print the targets was undertaken because this, unlike normal printer paper, reflects ultraviolet light and enabled us to match the UV appearance of typical moths even though printer ink is incapable of printing UV itself. Artificial wings were made waterproof using Plasti-Kote Matt Clear Acrylic spray paint, which permitted transmission / reflectance of UV.
follows a range of past work. Systems were used as a proxy for peppered moths to determine morph survival rate. Rips from >50% of the body and/or the wings were wholly or partially removed.

3. Wallace, A. R. Mimicry and Other Protective Resemblances Among Animals (Oxford University Press, 1898).

4. Majerus, M. E. N. Melanism: Evolution in Action. (Oxford University Press, Oxford, UK, 1998).

5. Kettlewell, H. B. D. Selection experiments on industrial melanism in the Lepidoptera. Heredity 9, 323–342 (1955).

6. Kettlewell, H. B. D. Further selection experiments on industrial melanism in the Lepidoptera. Heredity 10, 287–301 (1956).

7. Bishop, J. A. An experimental study of thecline of industrial melanism in Biston betularia (L.) (Lepidoptera) between urban Liverpool and rural North Wales. J. Anim. Ecol. 29, 209–242 (1970).

8. Lees, D. R. & Creed, E. R. Industrial melanism in Biston betularia: the role of selective predation. J. Anim. Ecol. 44, 67–83 (1975).

9. Clarke, C. A., Mani, G. S. & Wynne, G. Evolution in reverse: clean air and the peppered moth. Biol. J. Linn. Soc. 26, 189–199 (1985).

10. Cook, L. M., Mani, G. S. & Varley, M. E. Postindustrial melanism in the peppered moth. Science 231, 611–613 (1986).

11. Cook, I. M., Grant, B. S.,Saccheri, I. J. & Mallet, J. Selective bird predation on the peppered moth: the last experiment of Michael Majerus. Biol. Lett. 8, 609–612 (2012).

12. Zimova, M. et al. Function and underlying mechanisms of seasonal colour-moulting in mammals and birds: what keeps them changing in a warming world? Biol. Rev. https://doi.org/10.1111/brv.12405 (2016).

13. Hooper, J. Of Moths and Men: Intrigue, Tragedy and the Peppered Moth (Fourth Estate, London, 2002).

14. Coyne, J. A. Evolution under pressure: a look at the controversy about industrial melanism in the peppered moth. Review of Hooper 2002, Of Moths and Men: Intrigue, Tragedy and the Peppered Moth. Nature 418, 19–20 (2002).

15. Rudge, D. W. Did Kettlewell commit fraud? Re-examining the evidence. Public Underst. Sci. 14, 249–268 (2005).

16. Mikkola, K. On the selective forces acting in the industrial melanism of Biston and Oligia moths (Lepidoptera: Geometridae and Noctuidae). Biol. J. Linn. Soc. 21, 409–421 (1984).

17. Coyne, J. Not black and white. Nature 396, 35–36 (1998).

18. Majerus, M. E. N. in Insect Evolutionary Ecology (eds Fellows, M. D. E, Holloway, G. J. & Rollf, I.) 371–396 (CARI Publishing, Wallingford, UK, 2005).

19. Cathill, I. C. in Bird Coloration (eds Hill, G. E & McGraw, K. J.) 3–40 (Harvard University Press, Cambridge, MA, 2006).

20. Lytten, A., Lindström, L. & Mappes, J. Ultraviolet reflection and predation risk in diurnal and nocturnal Lepidoptera. Behav. Ecol. 15, 6 (2004).

21. Majerus, M. E. N., & Brunton, C. F. A., & Stalker, J. A bird’s eye view of the peppered moth. J. Evol. Biol. 13, 155–159 (2000).

22. Troscianko, J., Wilson-Aggarwal, J., Stevens, M. & Spottiswoode, C. N. Camouflage predicts survival in ground-nesting birds. Sci. Rep. 6, 19966 (2016).

23. Troscianko, J. & Stevens, M. Image calibration and analysis toolbox - a free software suite for objectively measuring reflectance, colour and pattern. Methods Ecol. Evol. 6, 1195 (2015).

24. Vorobyev, M. & Osorio, D. Receptor noise as a determinant of colour thresholds. Proc. R. Soc. B. 265, 351–358 (1998).

25. Marr, R. M. et al. Disruptive ecological selection on a mating cue. Proc. R. Soc. B. 279, 4907–4913 (2012).

26. Cathill, I. C. et al. Disruptive coloration and background pattern matching. Nature 434, 72–74 (2005).

27. Cathill, I. C. & Székely, A. Coincident disruptive coloration. Philos. Trans. R. Soc. B. 364, 489–496 (2009).

28. Liebert, T. G. & Brakefield, P. M. Behavioural studies on the peppered moth Biston betularia and a discussion of the role of pollution and lichens in industrial melanism. Biol. J. Linn. Soc. 31, 129–130 (1987).

29. Schafer, M. H. & Stobbe, N. Disruptive coloration provides camouflage independent of background matching. Proc. R. Soc. B. 273, 2427–2432 (2006).

30. Stevens, M., Cathill, I. C., Windsor, A. M. M. & Walker, H. J. Disruptive contrast in animal camouflage. Proc. R. Soc. B. 273, 2433–2438 (2006).

31. Stevens, M. et al. Field experiments on the effectiveness of ‘eyepots’ as predator deterrents. Anim. Behav. 74, 1215–1227 (2007).

32. Stevens, M., Hardman, C. J. & Stubbins, C. L. Conceivability, not eye mimicry, makes ‘eyepots’ effective anti-predator signals. Behav. Ecol. 19, 525–531 (2008).

33. Stevens, M., Winney, I. S., Cantor, A. & Graham, J. Object outline and surface disruption in animal camouflage. Proc. R. Soc. B. 276, 781–786 (2009).

34. Stevens, M. et al. Revealed by conspicuousness: distinctive markings reduce camouflage. Behav. Ecol. 24, 213–222 (2013).

35. Fraser, S., Callahan, A., Klassen, D. & Sherratt, T. N. Empirical tests of the role of disruptive coloration in reducing detectability. Proc. R. Soc. B. 274, 1325–1331 (2007).

36. Troscianko, J., Lown, A. E., Hughes, A. E. & Stevens, M. Defeating crypsis: detection and learning of camouflage strategies. Phil. Trans. R. Soc. B. 373, 37333 (2013).
37. Jones, C. D. & Osorio, D. Discrimination of orientated visual textures by poultry chicks. *Vis. Res.* 44, 83–89 (2004).
38. Osorio, D. & Vorobyev, M. Photoreceptor spectral sensitivities in terrestrial animals: adaptations for luminance and colour vision. *Proc. R. Soc. B* 272, 1745–1752 (2005).
39. Kettlewell, H. B. D. Recognition of appropriate backgrounds by the pale and black phases of Lepidoptera. *Nature* 175, 943–944 (1955).
40. Stevens, M. & Ruxton, G. D. The key role of behaviour in animal camouflage. *Birol. Rev.* https://doi.org/10.1111/bvr.12438 (2018).
41. Kang, C. K., Moon, J. Y., Lee, S. I. & Jablonski, P. G. Moths on tree trunks seek out more cryptic positions when their current crypticity is low. *Anim. Behav.* 86, 587–594 (2013).
42. Lovell, P. G., Ruxton, G. D., Langridge, K. V. & Spencer, K. A. Individual quail select egg-laying units by assessing egg’s coloration for their egg phenotype. *Curr. Biol.* 23, 260–264 (2013).
43. Marshall, K. L. A., Philpot, K. E. & Stevens, M. Microhabitat choice in island lizards enhances camouflage against avian predators. *Sci. Rep.* 6, 19815 (2016).
44. Stevens, M., Troschianko, J., Wilson-Aggarwal, J. & Spottiswoode, C. N. Improvement of individual camouflage through background choice in ground-nesting birds. *Nat. Ecol. Evol.* 1, 1325–1333 (2017).
45. Grant, B. S. Fine tuning the peppered moth paradigm. *Evolution* 53, 980–984 (1999).
46. Zimova, M., Mills, L. S. & Nowak, J. J. High fitness costs of climate change-induced camouflage mismatch. *Ecol. Lett.* 19, 3 (2016).
47. Grimsditch, G., Basheer, A. & Bryant, D. E. P. Extreme white colouration of frogfish *Antennarius maculatus* due to coral bleaching event. *Coral Reefs* 36, 1 (2017).
48. Stevens, M., Lown, A. E. & Wood, L. E. Colour change and camouflage in juvenile shore crabs *Carcinus maenas*. *Front. Ecol. Evol.* 2, 14 (2014).
49. Stevens, M., Alejandro Parraga, C., Cuthill, I. C., Partridge, J. C. & Troschianko, T. S. Using digital photography to study animal colouration. *Biol. J. Linn. Soc.* 90, 2 (2007).
50. Schneider, C. A., Rasband, W. S. & Eliceiri, K. W. NIH Image to ImageJ. *Nat. Methods* 9, 671–675 (2012).
51. Honkavaara, J., Koivula, M., Korpimaki, E., Sittari, H. & Vittala, J. Ultraviolet vision and foraging in terrestrial vertebrates. *Oxos* 98, 505–511 (2002).
52. Hart, N. S. & Hunt, D. Avian visual pigment characteristics, spectral tuning and evolution. *Am. Nat.* 169, 7–26 (2007).
53. Westland, S. & Ripamonti, C. *Computational Colour Science using MATLAB*. (Wiley, Chichester, UK, 2004).
54. Stevens, M. & Cuthill, I. C. Disruptive colouration, crypsis and edge detection in early visual processing. *Proc. R. Soc. B* 273, 2141–2147 (2006).
55. Pike, T. W. Using digital cameras to investigate animal colouration: estimating sensor sensitivity functions. *Behav. Ecol. Sociobiol.* 65, 849–858 (2011).
56. Endler, J. A. & Mielke, P. W. Comparing entire colour patterns as birds see them. *Biol. J. Linn. Soc.* 86, 405–431 (2005).
57. Stoddard, M. C. & Prum, R. O. Evolution of avian plumage color in a tetrahedral color space: a phylogenetic analysis of New World buntings. *Am. Nat.* 171, 755–776 (2008).
58. Langmore, N. E., Stevens, M., Maurer, G. & Kilner, R. M. Are dark cuckoo eggs cryptic in host nests? *Anim. Behav.* 78, 461–468 (2009).
59. Olsson, P., Lind, J. & Kelber, A. Chromatic and achromatic vision: parameter choice and limitations for reliable model predictions. *Behav. Ecol.* 29, 273–282 (2018).
60. Hart, N. S., Partridge, I. C., Cuthill, I. C. & Bennett, A. T. D. Visual pigments, oil droplets, ocular media and cone photoreceptor distribution in two species of passerine bird: the blue tit (*Parus caeruleus*) and the blackbird (*Turdus merula*). *J. Comp. Physiol. A.* 186, 4 (2000).
61. Vorobyev, M., Brandt, R., Petitsh, D., Laughlin, S. B. & Menzel, R. Colour thresholds and receptor noise: behaviour and physiology compared. *Vision. Res.* 41, 639–653 (2001).
62. Siddiqi, A., Cronin, T. W., Loew, E. R., Vorobyev, M. & Summers, K. Interspecific and intraspecific views of colour signals in the strawberry poison frog, *Dendrobates pumilio*. *J. Exp. Biol.* 207, 14 (2004).
63. Finkbeiner, S. D., Briscoe, A. D. & Reed, R. D. The benefit of being a social butterfly: communal roosting deters predation. *Proc. R. Soc. B* 279, 1739 (2012).
64. Cuthill, I. C., Stevens, M., Windsor, A. M. M. & Walker, H. J. The effects of pattern symmetry on detection of disruptive and background matching coloration. *Behav. Ecol.* 17, 828–832 (2006).
65. Seymourou, B. M. & Aiello, A. Keeping the band together: evidence for false boundary disruptive coloration in a butterfly. *J. Evol. Biol.* 28, 1618–1624 (2015).
66. Rowland, H. M. et al. Countershading enhances cryptic protection: an experiment with wild birds and artificial prey. *Anim. Behav.* 74, 1249–1258 (2007).
67. R. Core Team R. A Language and Environment for Statistical Computing. (R Foundation for Statistical Computing, Vienna, 2016).
68. Minitab. Minitab 18 Statistical Software. (Pennsylvania, USA: Minitab Inc.) (2010).
69. Bland, J. M. & Altman, D. G. Survival probabilities (the Kaplan–Meier method). *BMJ* 317, 7172 (1998).
70. Bewick, V., Cheek, L., & Ball, J. Statistics review 12: survival analysis. *Crit. Care* 8, 389–394 (2004).

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**Author contributions**

M.S. and O.W. designed and conceived the study. Fieldwork was conducted by O.W. Image analysis, vision modelling, and statistical analysis was conducted by O.W with input from M.S. and O.W. wrote the initial manuscript, which was reviewed and approved by all authors prior to submission.

**Additional information**

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