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Individual egg camouflage is influenced by microhabitat selection and use of nest materials in ground-nesting birds

Jesús Gómez[1] • Cristina Ramo[1] • Jolyon Troscianko[2] • Martin Stevens[2] • Macarena Castro[3] • Alejandro Pérez-Hurtado[3] • Gustavo Liñán-Cembrano[4] and Juan A. Amat[1]

✉ Jesús Gómez
j.gomez@ebd.csic.es (Jesús Gómez)

[1] Departamento de Ecología de Humedales, Estación Biológica de Doñana (EBD-CSIC), Calle Américo Vespucio 26, E-41092 Sevilla, Spain.

[2] Centre for Ecology and Conservation, College of Life and Environmental Sciences, University of Exeter, Penryn Campus, Penryn, TR10 9FE. UK.

[3] Departamento de Biología, Facultad de Ciencias del Mar y Ambientales, Universidad de Cádiz, Avenida Saharaui s/n, E-11510 Puerto Real, Spain.

[4] Instituto de Microelectrónica de Sevilla (IMSE-CNM), Consejo Superior de Investigaciones Científicas (CSIC) and Universidad de Sevilla, Calle Américo Vespucio 28, 41092, Sevilla, Spain.

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Abstract

Camouflage is a widespread strategy to avoid predation and is of particular importance for animals with reduced mobility, or those in exposed habitats. Camouflage often relies on matching the visual appearance of the background, and selecting fine-scale backgrounds that complement an individual's appearance is an effective means of optimising camouflage. We investigated whether there was an active selection of microhabitats and nest materials in three ground-nesting birds (pied avocet, Kentish plover and little tern) to camouflage their eggs using avian visual modelling. Plovers and avocets selected substrates in which their eggs were better camouflaged, and that choice was done at an individual level. Terns have lighter, less spotted eggs, and while they did select lighter background than the other species, their eggs were a poor match to their backgrounds. The worse matching of the tern eggs was likely due to a compromise between thermal protection and camouflage because they breed later, when temperatures are higher. Finally, the addition of nest materials improved egg camouflage in terms of luminance, although the materials reduced pattern matching, which may be associated with the different roles that the nest materials play. Active selection of substrates at an individual level may be crucial to improve nest success in species that nest in exposed sites.

Significance statement

Many bird species nest on the ground at sites with no vegetation cover where their nests are exposed to visual predators. We studied whether individual females chose nest substrates that improved the camouflage of their eggs. Using images of nests and avian vision modelling, we found that the choice of nesting substrates in two species was done at the individual level, so that egg camouflage was optimised. In addition, such species were even able to improve egg camouflage by the addition of materials into the nests. However, this pattern was not observed in another species with paler and less spotted eggs, which may reflect a trade-off between camouflage and overheating of eggs because it breeds later in the season. Although individuals may try to choose substrates in which their eggs are better camouflaged, other factors that compromise offspring survival may hamper this.

Key words: Crypsis • background matching • habitat choice • eggshells • pattern matching

Introduction

Evading detection is essential for individual survival, either for protection from predators or to go unnoticed by potential prey, making camouflage the most common coloration strategy in the animal kingdom (Stevens and Merilaita 2009). One way to avoid detection is by matching the background against which animals can be seen, which means that camouflage involves an interaction between the environment and the animal's phenotype (Endler 1978; Manríquez et al. 2009; Stevens and Merilaita 2011). However, habitats are not uniform even at small spatial scales, and this may favour individuals that choose specific sites in which their camouflage is enhanced according to the vision of their predators or prey. Many animals are also able to adaptively alter their phenotypic appearance to match their backgrounds (Ryer et al. 2008; Barbosa et al. 2012; Duarte et al. 2017), meaning that there are two potential main ways for animals to improve their individual-level camouflage: behaviourally electing microhabitats that enhance camouflage (Colwell et al 2011; Uy et al. 2017), and/or by using materials from the microhabitat to cover and decorate the animal’s body (Hölldobler and Wilson 1986; Stachowicz and Hay 2000; Hultgren and Stachowicz 2008; Lee et al. 2014; Mayani-Parás et al. 2015; Ruxton and Stevens 2015). Finally, another solution to improve crypsis is by manipulating the backgrounds, as for example some birds do covering the eggs with materials from the surroundings (Amat et al. 2012; Troscianko et al. 2016a). To date, relatively few studies have addressed whether behavioural selection of microhabitats and/or substrate modification by individuals in relation to their own appearance enhances camouflage (but see for example: Solís and de Lope 1995; Lovell et al. 2013; Kang et al. 2015), and even fewer studies have modelled the relevant visual systems (Marshall et al. 2016; Stevens et al. 2017).

The fitness of any ground-nesting bird depends on its own survival, and on that of its offspring, meaning that a number of factors influence their anti-predator behaviour and reproductive strategies (Fontaine and Martin 2006; Amat et al. 2017). Many Charadriiform birds (shorebirds and allies) nest at ground level in sites with little or no vegetation cover, because in such sites incubating adults are less vulnerable to predators (Amat and Masero 2004; Cunningham et al. 2016). However, when adults flush (flee) from nests because of predator disturbance, unattended eggs may remain vulnerable to both predation and overheating due to direct solar radiation (Montevecchi 1976; Grant 1982; Amat and Masero 2007; Gómez et al. 2016; Wilson-Aggarwal et al. 2016; Amat et al. 2017). One way with which ground-nesting birds in exposed sites may improve nesting success is by matching egg appearance to that of the laying substrates (Lee et al. 2010; Troscianko et al. 2016b). However, this may not be an easy task for shorebirds that breed in unpredictable sites, compelling the same individuals to breed in sites located hundreds of kilometres away, not only between breeding seasons, but also within the same breeding season (Stenzel et al. 1994; Figuerola 2007). In addition, at such sites shorebirds may encounter a variety of habitats, in which case they may use different strategies to match the appearance of their eggs with the nesting backgrounds. First, shorebirds might theoretically improve camouflage by laying eggs with similar coloration and patterning to those of the sites in which they breed. Given that egg coloration and patterning seem to be primarily genetically controlled (Gosler et al. 2000; see Fig. 2 in Skrade and Dinsmore 2013), it is unlikely that females may show plasticity to produce eggs that match the characteristics of their habitat. A different solution is to select microhabitats according to individual eggshell appearance, so that the eggs are better camouflaged, as Lovell et al. (2013) found under lab conditions in Japanese quail, *Coturnix japonica*. These authors concluded that the females were able to "know where to hide their eggs" because they matched the substrate luminance (perceived brightness) well. However, studies undertaken under natural conditions are needed, where multiple selective agents may play different roles at the same time (Underwood and Sealey 2002; Kilner 2006; Cherry and Gosler 2010; Maurer et al. 2011). Additionally, given that it may be difficult to find sites in which the eggs would be well camouflaged, another possibility for parents is to manipulate the nesting sites themselves by choosing nest materials that improve the camouflage of the clutch (Solís and de Lope 1995; Amat et al. 2012; Bailey et al. 2015; Troscianko et al. 2016a).

Here, we studied whether nest camouflage is improved through selection of microhabitat, not only at the species-level, but within species at an individual-level. Three ground-nesting bird species were chosen, all breeding in a similar habitat where their nests are exposed: Kentish plover *Charadrius alexandrinus*, pied avocet *Recurvirostra avosetta*, and little tern *Sternula albifrons*. In addition to species-level and individual-level matching of egg appearance to microhabitats, we predicted that egg camouflage should be improved by the addition of materials into the nest scrapes, and that this should be accomplished both at species- and individual-level. Finally, we expected that as the three species have to respond to different selective drivers that may compromise embryo survival (e.g., they may experience different thermal regimes), such drivers may compromise camouflage (Gómez et al. 2016), which would determine the apparent maladaptive choice of sites (poorer camouflage) by some species, in spite of nesting in the same area as the others.

Materials and methods

Study sites and species

Our study was conducted in 2014 in a 15 ha saltpan at Cádiz Bay Natural Park, southern Spain (36° 30' 53.4" N 6° 09' 23.3"W). We photographed 30, 37, and 18 nests of pied avocet, Kentish plover and little tern, respectively. These species make scrapes on the ground into which they add some materials (e.g., pebbles, mollusc shells, plant fragments; del Hoyo et al. 1996). It was not possible to record data blind because our study involved focal animals in the field.

At our study site, the pied avocet and the Kentish plover usually start nesting in late March−early April. The number of pied avocet nests increases rapidly early at the start of the breeding season and declines sharply; however, the number of Kentish plover nests increases slowly and continuously until a peak that is usually around mid-May. On the other hand, the little tern breeds later than the other two species (Fig. S1 Electronic Supplementary material [ESM]), facing more stressful hotter conditions (Fig. S2 ESM).

Eggshell colour and camouflage using digital image processing

Protocols for photography and image analysis closely followed previous studies (e.g. Troscianko and Stevens 2015; Troscianko et al. 2016a). Nests were photographed using a Nikon D7000 camera (fitted with a 105mm Micro-Nikkor lens, which transmits ultraviolet, UV) converted to full spectrum sensitivity by removal of its UV and IR blocking filter (Advanced Camera Services Limited, Norfolk, UK), replacing it with a quartz sheet to allow quantification of colour throughout the avian visible spectrum. Human-visible spectrum photographs were taken through a Baader UV-IR blocking filter (Baader Planetarium, Mammendorf, Germany), transmitting only visible spectrum light from 420 to 680 nm, and UV photographs were taken with a Baader UV pass filter, transmitting UV light from 320 to 380 nm. This resulted in five image layers: longwave (LW), mediumwave (MW), shortwave (SW) and two ultraviolet (UV) layers (from the camera's red and blue channels). Each image included a Spectralon reflectance standard (Labsphere, Congleton, UK) reflecting light with a flat spectral reflectance of 40% between 300 and 700 nm. All photographs were taken at f/8, ISO400, in RAW format between 09:00 and 11:00 h (GMT) to ensure that lighting conditions were comparable between photographs. The camera was mounted on a tripod 1.6 m high, and positioned at 1m from the focal nest.

Images were calibrated following Stevens et al. (2007) and Gómez and Liñán-Cembrano (2017). Briefly, we linearized and equalized the images using the toolbox released by Troscianko and Stevens (2015). We processed the images and transformed them to cone catch images so we were able to model the visual systems. Birds have four single cone types used in colour vision (sensitive to LW, MW, SW and UV light; Cuthill 2006), and additional double cones thought to be used in luminance vision (Osorio and Vorobyev 2005). Because the avocet, plover and tern are likely violet sensitive (VS) (Ödeen et al. 2010; Ödeen and Håstad 2013), we generated cone-catch images using peafowl sensitivities (*Pavo cristatus*; Hart 2002; a commonly used model for VS colour vision). Cone-catch images were generated using a widely used and tested image mapping approach, which is highly accurate for modelling cone catch responses (Stevens and Cuthill 2006; Stevens et al. 2007; Pike 2011; Troscianko and Stevens 2015), resulting in images corresponding to the LW, MW, SW, VS and double cone receptors. We inferred the visual system of the pied avocet, Kentish plover and little tern from that of closely phylogenetically related species following Ödeen et al. (2010). These authors did not quantify the absorbance of oil droplets, which may modify the outcome of models (Bowmaker 1977). However, colour discrimination in bright light, as in the site where the three species nest, is mostly dependent on the visual pigment (UVS or VS) and little on the ocular media (Lind et al. 2014).

Using Image J (Schneider et al. 2012) we selected three ROIs (regions of interest): the eggs, the nest, and the microhabitats. We manually selected all of the eggs that were in the image (1-4). After that, we created a circle that encompassed the eggs and grew it by 500 pixels to create our nest ROI (eliminating the eggs and including a small area around them). The remaining part of the picture was selected as the microhabitat (i.e., after removing the grey standard, nest, and eggs). All images were scaled using the width of the grey standard (32 mm) as a reference to properly compare pattern camouflage.

In the visual model we calculated luminance differences, colour differences, and pattern differences. Luminance (based on the double cones) and colour (single cones) differences were calculated using 'just noticeable differences' based on a widely-used model whereby receptor noise limits visual discrimination (JNDs; Vorobyev and Osorio 1998). Values of JND lower than 1 could not be distinguished by possible predators (Siddiqi et al. 2004), between 1- 3, small differences could be appreciated under good light conditions, and higher than 3 mean that two substrates could be easily differentiated by predators. Weber fractions were calculated based on peafowl cone ratios of (shortest to longest) 1 : 1.9 : 2.2 : 2.1 (Hart 2002), with a Weber fraction of 0.05 for the most dominant channel. Pattern differences were calculated from the average in absolute differences in bandpass energy spectra (for further details see Troscianko and Stevens 2015; Troscianko et al. 2016a, 2017). Differences were calculated between eggs and the nest, as well as between eggs and microhabitats. We used the visual system of the three species (avian VS) to model the perception of the incubating adults and how they select the laying substrates (nest materials and microhabitats). Although some nest predators have different visual systems to those of the species that we studied (e.g. gulls, which are UVS), we did not use the visual systems of nest predators because our study was not on nesting success, but on the choice of substrates and nest materials by plovers, avocets and terns according to their own perceptual visual system.

To describe how the eggs reflected more or less energy in the visible range of the sun spectrum we calculated the total reflectance of the eggs, nests and microhabitats using the reflectance images that were previously obtained (linearised and equalised). The total reflectance was measured by averaging the three camera bands in the visible (R-v, G-v and B-v). Moreover, within the egg (placed in the nest as it was projected in the image, not removing it) we differentiated between the pigmented maculation and the background colour using a thresholding algorithm. Separating eggs into maculated regions using simple thresholding inevitably leads to slightly shaded regions of the egg being erroneously classified as maculation. We therefore used a difference-of-Gaussians method that removed spatial information at a large scale (the scale of the whole egg), calculating the difference between this and the fine-scale image, then thresholding this image (code available by contacting with us). This allows identification of egg maculation without perfectly diffuse lighting conditions. All eggs were processed with the same threshold and Gaussian filter scales to ensure consistency. “Proportion of spottiness” was calculated as the total area of spots divided by the entire eggshell area and multiplied by 100 (projected in the image).

Chimeric experiment

As we could not exchange clutches between nests, we carried out a chimeric experiment (this process generated 870 chimerical nests of pied avocet, 1332 of Kentish plover and 306 of little tern). To test whether the choice of sites depended on the individual characteristics of eggs (luminance, coloration and patterning), we virtually placed the eggs of every entire individual clutch in the nests/microhabitats of the remaining individuals of the same species (Fig. 1). For example, we “placed” the Kentish plover eggs of nest 1 in the images of nests and microhabitats of each one of the remaining Kentish plovers (2-37), after “removing” the eggs of the latter nests.

Statistical analyses

General linear models (GLM) and General linear mixed models (GLMM) were used to test the hypotheses, with normal error distribution and using the identity link function. We used GLM to compare interspecific differences in total reflectance of eggs (all the eggs in every clutch as a unit) and substrates, and proportion of spottiness (mean of all the eggs in every clutch). Tukey post-hoc tests were used after for multiple comparisons between species. GLMMs and Tukey post-hoc tests were used to test differences in the three response variables related to camouflage (luminance, colour and pattern differences) among species and substrates (nest materials and microhabitat), with nest identity included as a random factor. Those response variables were transformed using the square root to improve residuals´ normality. GLMM models were also used to analyse intraspecific comparisons (see Chimeric experiment), including clutch identity and substrates (nest and microhabitat) identity as random factors to avoid pseudoreplication. All statistical analyses were carried out in R statistical software version 3.2.2 (R Core Team 2015) and significance level was set at 0.05.

Results

The eggs of the little tern had higher total reflectance (Table 1, $F_{2,82} = 12.33$, $p < 0.001$, M. R-sq: 0.2311, Adj. R-sq: 0.2124) and were less spotted ($F_{2,82} = 13.14$, $p < 0.001$, M. R-sq: 0.24, Adj. R-sq: 0.23), than those of the pied avocet and Kentish plover (see Fig. 2). The nest materials and microhabitats selected by the little terns were in accordance with the total reflectance values and had higher values than those selected by the other two species (Table 1).

In terms of background matching, the little tern had the least camouflaged eggs (Fig. 3; see ESM Table S1). In general, its eggs did not match the nest materials and the microhabitats as well as the other two species, and this was found for the three response variables (luminance, colour, and pattern, Fig. 3). In fact, little tern´s eggs were the most conspicuous in terms of coloration and they surpassed the threshold for which two objects would likely be perceptually differentiated (i.e., its colour JND > 3). On the contrary, the pied avocet and the Kentish plover selected nest materials and microhabitats in which their eggs were better camouflaged.

All three species added materials to the nest (e.g. small pebbles, shells, twigs, etc.). To test whether this addition of materials improved the camouflage of the egg, we made comparisons between the camouflage of eggs-nests and eggs-microhabitats (Table 2). The eggs of the pied avocet and the little tern were a better achromatic match to the additional nest materials than the microhabitat. In terms of coloration, the pied avocet also chose nest materials that more effectively matched their eggs in comparison with the microhabitat. On the contrary, the Kentish plover´s nest materials were a worse match to the eggs than their microhabitats, while there were no differences in the little tern. Regarding pattern matching, we found the opposite effects of luminance comparisons: the added nest materials were a worse match to the eggs than the microhabitats in all three species.

Lastly, in the chimeric experiment we found that eggs of individual pied avocets and Kentish plovers were better camouflaged against their own nest materials and microhabitats than if the eggs had been laid in the substrates selected by their conspecifics (Table 3). Differences appeared in the luminance and the chromatic variables rather than in the patterning, but individual Kentish plover also chose specific microhabitats that matched the patterning of its own eggs better. On the contrary, the eggs of the little tern were similarly camouflaged on their own nest as on nests of their conspecifics, suggesting that they do not select the substrates (either the microhabitat or the nest materials) at an individual level (Table 3).

Discussion

Pied avocet and Kentish plover eggs effectively matched the materials that adults added into the nest scrape, as well as the corresponding microhabitats, under the perceptual visual system of both species. In fact, the choice of microhabitats and nest materials was not only undertaken at the species level, but also at the individual level, which may be particularly important for nest survival (Lee et al. 2010; Troscianko et al. 2016a; but see Stoddard et al. 2016). This result supports the findings of Lovell et al. (2013) and Stevens et al. (2017), who found under laboratory and field conditions, respectively, that individual females selected substrates to lay their eggs based on egg coloration and patterning to improve camouflage. However, in our study the lighter and less spotted eggs of the tern did not as effectively match their nest microhabitats and nesting material, and their camouflage was worse than that of the other two species. Besides, adults did not choose the substrates based on the individual characteristics of eggs.

We found that nest materials did not improve all components of camouflage similarly. Overall, except in Kentish plover, the other species added materials that matched better the luminance of the eggs but this in turn decreased their pattern match, whereas for colour we did not find the same results for the three species (nest materials increased camouflage in the avocet, worsened it in the plover, but there were no differences in the tern). Matching the eggs in terms of luminance could be more advantageous than colour matching to reduce predation rates on eggs of ground-nesting birds (Troscianko et al. 2016b). However, nest materials may have multiple roles besides camouflage and they could still be advantageous for other functions, for example in maintaining an appropriate thermal environment in nests (Reid et al. 2002;

Mayer et al. 2009; Carroll et al. 2015), or they could keep the eggs above water if the scrape is flooded during rainy periods (Moreno et al. 1995). Therefore, the choice of optimal nesting materials could be affected by other factors in addition to camouflage and some of these may represent a compromise similar to the trade-offs known to affect eggshell coloration (Gómez et al. 2016). With these strategies (selection of microhabitats and addition of nest materials), together with other behavioural strategies to reduce egg detection (Amat et al. 2012; Ekanayake et al. 2015, Mayani-Parás et al. 2015; Wilson-Aggarwal et al. 2016; Amat et al. 2017), ground nesting birds could compensate for the lack of high levels of individual plasticity in egg characteristics (Gosler et al. 2000; Skrade and Dinsmore 2013) that other organisms exhibit to match specific habitats (Stevens et al. 2015; Polo-Cavia and Gomez-Mestre 2017; Edelaar et al. 2017). These behavioural strategies would facilitate the nesting of individual shorebirds in very distant sites, among which the coloration and pattern of the substrates may differ substantially, and in particular would facilitate camouflage within habitats that are visually variable.

How do egg characteristics contribute to camouflage in the three species? As well as in other animals (Ortolani 1999), several studies have shown the importance of the spottiness for egg camouflage in birds, given that more spotted eggs are better camouflaged (Montevecchi 1976; Kilner 2006; Gómez et al. 2016; Troscianko et al. 2016). Therefore, the lowest proportion of spottiness of tern eggs, as well as their lighter eggshells, may be a reason for their inferior camouflage. Interestingly, Stoddard et al. (2016) reported similar findings to ours, as they found that the camouflage of least terns *Sternula antillarum* eggs was worse than that of snowy plovers *Charadrius nivosus*. Obviously, phylogenetic factors could explain interspecific differences in egg coloration and spottiness, as the avocet and plover are more closely related (Baker et al. 2007). However, solar radiation could affect egg appearance not only at congeneric levels (Gómez et al. 2016) but also at a population level (Lathi 2008). Nevertheless, accounting for phylogenetic factors would likely not explain why little terns selected substrates that matched their eggs poorly, given that avoiding nest detection is so important for egg survival (Lee et al. 2010; Troscianko et al. 2016b). A plausible explanation could be related to the more stressful thermal conditions that the little tern presumably faces during its breeding season (ESM Fig. S2), so having lighter and less spotted eggshells, together with selecting lighter substrates, would be advantageous to reduce risks of overheating when the nests are left unattended by adults, even if this compromises egg camouflage (Montevecchi 1976; Mayer et al. 2009; Gómez et al. 2016). Additionally, it has been shown that little tern may adjust the distance at which the adults flush from the nest when a predator is approaching depending on the level of egg camouflage (Amat et al. 2017), so this behaviour may compensate for the poorer matching and reduce nest detection.

Our results support the findings of Lovell et al. (2013) on domestic quail, in that the selection of laying substrates was undertaken at an individual level according to the characteristics of eggshells, at least for Kentish plover and pied avocet. Our results are also in accordance with those of Stevens et al. (2017), which showed that individual ground-nesting birds improved the camouflage of their eggs by choosing appropriate backgrounds. They found differences among species, as we have found in this study, which indicates the importance of studying egg camouflage and habitat selection in different species and habitats to gain insights into the strategies used by different species. On the other hand, adding nest materials to the scrapes improved the achromatic camouflage but worsened the pattern camouflage of the eggs, which could be explained because the materials added are likely important for other functions in addition to camouflage. The apparent need to respond to other selective drivers that may compromise embryo survival, such as the risk of overheating, may explain why the camouflage of little tern eggs was worse than that of the other species. This highlights the importance of carrying out studies with wild species that face different stressful factors.

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Author contribution statement

JG designed the study and analysed the images. JT and MS contributed with materials and image analyses. JG, MC, AP-H, CR and JAA collected the field data. JG analysed the dataset, and wrote the manuscript. All authors contributed on later manuscript versions.

Compliance with ethical standards

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Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

All applicable institutional and national guidelines for the care and use of animals were followed and approved by Comité Ético de Bienestar Animal from EBD-CSIC (reference CEBA-EBD_2011_01).

Data availability

All relevant data are available from the CSIC Institutional Data (https://digital.csic.es/handle/10261/167693).

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Table 1 Apparent reflectances (estimated means ± standard errors) of eggshells, nests and microhabitats of pied avocet (A, n = 30), Kentish plover (Kp, n = 37) and little tern (Lt, n = 18). P-values (p) were calculated with a Tukey post-hoc test to the results of different general linear models (significant p-values are in bold)

	Total Reflectance (%)		
	EGGS	NESTS	MICROHABITAT
Pied Avocet	11.35 ± 0.53	14.39 ±.91	13.88 ± 0.92
Kentish plover	11.30 ± 0.48	16.74 ± 0.95	15.99 ± 0.95
Little tern	15.92 ± 0.97	22.80 ± 1.86	22.95 ± 1.96
p	Kp - A = 0.998 **Lt - A < 0.001** **Lt - Kp < 0.001**	Kp - A = 0.184 **Lt - A < 0.001** **Lt - Kp = 0.007**	Kp - A = 0,240 **Lt - A < 0.001** **Lt - Kp = 0.002**

Table 2 Model-adjusted means (±SE) of differences in camouflage (luminance, colour and pattern) between the eggs and the nest materials and the eggs and the microhabitats, and p-values (Tukey post-hoc test) for pied avocet (n = 30), Kentish plover (n = 37) and little tern (n = 18) according to the peafowl visual system. Significant p-values are in bold

		Luminance	Colour	Pattern
Pied Avocet	Egg-nest	0.39 ± 0.06	1.67 ± 0.25	5100 ± 317
	Egg-microhabitat	0.48 ± 0.06	1.82 ± 0.26	3488 ± 262
	p	**0.002**	**0.047**	**<0.001**
Kentish plover	Egg-nest	0.62 ± 0.05	2.43 ± 0.27	4725 ± 275
	Egg-microhabitat	0.61 ± 0.05	2.24 ± 0.26	3907 ± 250
	p	0.750	**0.017**	**<0.001**
Little tern	Egg-nest	0.61 ± 0.07	4.26 ± 0.51	6847 ± 399
	Egg-microhabitat	0.71 ± 0.07	4.45 ± 0.52	4948 ± 403
	p	**0.004**	0.196	**<0.001**

Table 3 Results of intra-specific comparisons for pied avocet, Kentish plover and little tern in a chimeric experiment (see material and methods). Model-adjusted means (±SE) of differences in camouflage (luminance, colour and pattern) between egg-nest and nest-microhabitat (GLMM) according to the peafowl visual system, and p-values (Tukey post-hoc test) are shown. Significant p-values are in bold

		Pied avocet		Kentish plover		Little tern	
		Original nest	Chimeric nest	Original nest	Chimeric nest	Original nest	Chimeric nest
Luminance	Egg-nest	0.39 ± 0.056	0.57 ± 0.049	0.62 ± 0.051	0.69 ± 0.044	0.61 ± 0.073	0.63 ± 0.063
		p < 0.001		**p = 0.007**		p = 0.56	
	Egg-microhabitat	0.48 ± 0.057	0.59 ± 0.050	0.61 ± 0.051	0.68 ± 0.044	0.71 ± 0.073	0.74 ± 0.063
		p < 0.001		**p = 0.006**		p = 0.57	
Colour	Egg-nest	1.53 ± 0.200	1.91 ± 0.220	2.10 ± 0.247	2.39 ± 0.248	3.76 ± 0.631	3.97 ± 0.59
		p < 0.001		**p = 0.024**		p = 0.515	
	Egg-microhabitat	1.68 ± 0.220	2.00 ± 0.230	1.87 ± 0.220	2.34 ± 0.242	4.07 ± 0.684	4.05 ± 0.604
		p = 0.006		**p < 0.001**		p = 0.953	
Pattern	Egg-nest	5100 ± 359	5102 ± 320	4680 ± 312	4828 ± 281	6847 ± 537	6917 ± 482
		p = 0.990		p = 0.322		p = 0.783	
	Egg-microhabitat	3488 ± 297	3607 ± 269	3878 ± 284	4195 ± 262	4949 ± 457	5119 ± 415
		p = 0.398		**p = 0.020**		p = 0.439	

FIGURES

Fig 1 Diagram representing the procedure employed for the chimeric experiment using photographs of nests of pied avocet (n = 30), Kentish plover (n = 37) and little tern (n = 18). The eggs of every clutch were virtually deposited in the nests of all other conspecifics. The camouflage of eggs was estimated with respect to the nests (N) and microhabitats (M). A grey standard was placed in every picture to standardise light conditions, but was not included in the microhabitat area in the analyses of digital images

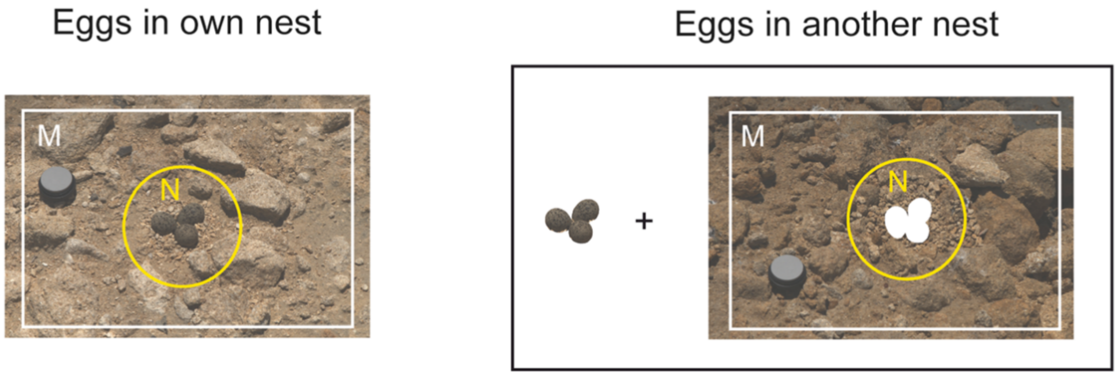

Fig 2 Proportion of spottiness (percentage of eggshell surface covered by spots, average of the eggs in every clutch) in three ground−nesting birds (sample mean, 95% confidence intervals, and minimum and maximum values; sample sizes are also shown). The images were linearised and standardised, using a grey standard, to control for the lighting conditions. After obtaining a reflectance image, contrast and exposure were changed equally for the three images to improve visualisation. Different letters denote significant differences

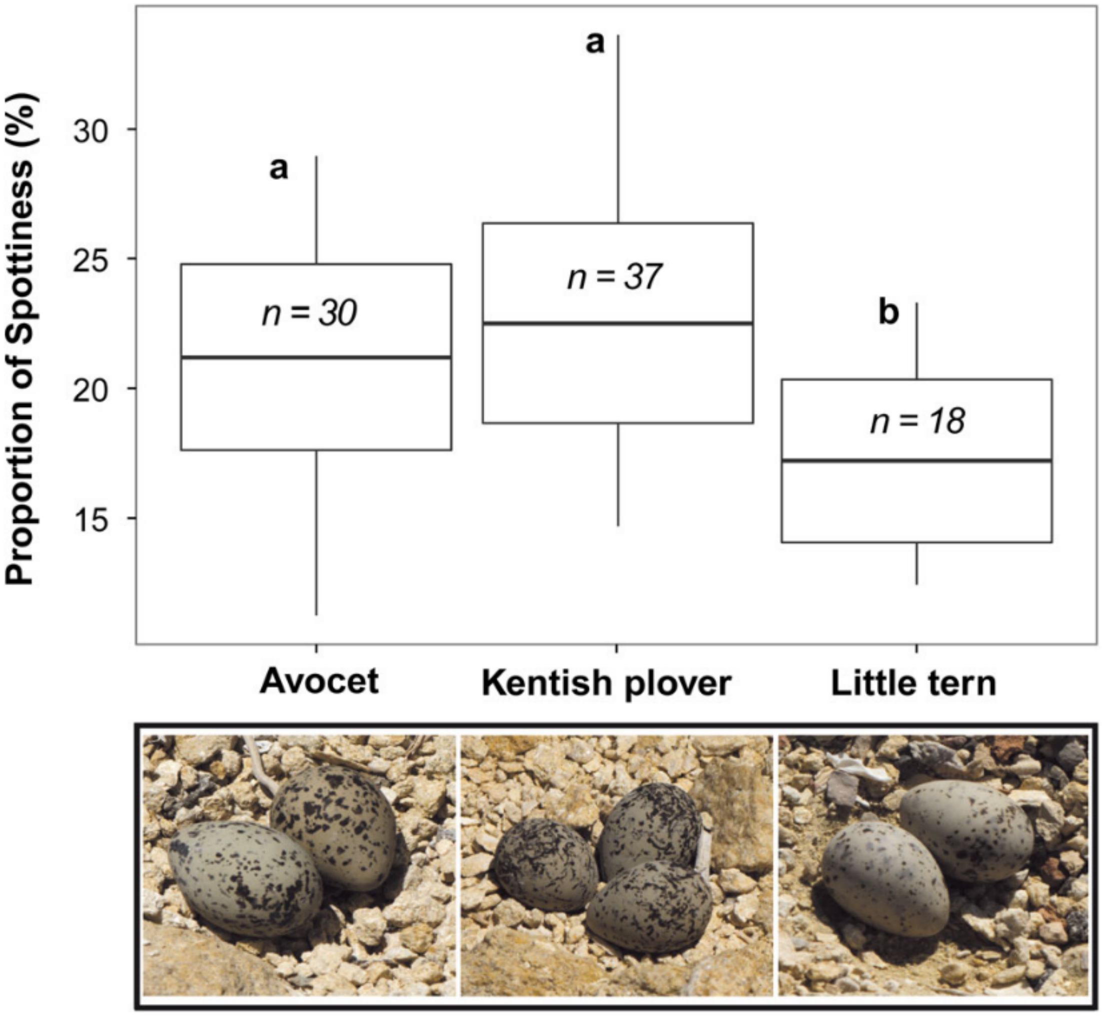

Fig 3 Differences in achromatic (luminance), chromatic (colour) and pattern camouflage between the eggs and the nest (left) and between the eggs and the microhabitats (right), in pied avocet (n = 30), Kentish plover (n = 37) and little tern (n=18), under the peafowl visual model. The higher the values (estimated means ± SE) the worse the camouflage. Values of differences lower than 1 (dashed line) in luminance and colour could not be distinguished by possible predators, between 1 - 3, small differences could be appreciated under good light conditions and higher than 3 (solid red line) mean that two substrates could be easily differentiated. Different letters denote significant differences