Short Communication

A non-destructive approach to collect nest material data using photographs

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The materials that birds use to build their nests have a profound effect on nest quality and consequently on the builder's reproductive success. Given that the common method to quantify nest materials by dismantling nests takes time and limits study species, we developed a non-destructive and much quicker method for quantifying nest materials using nest photographs. Using our photographic method, the proportions of the main materials in 45 Blue Tit *Cyanistes caeruleus* and 20 Dartford Warbler *Sylvia undata* nests, including grass, heather and moss, matched those found by dismantling the nests, but the proportions of rarer animal-derived materials differed between the two methods. Provided that there is an initial calibration with the dismantling method, the photographic method offers two key advantages: a reduction in the time it takes to quantify the major components of nests, and application to previously inaccessible data, including museum collections. Together, these advantages encourage further study of nesting materials and enable a better understanding of avian nest diversification.

Bird nests share the crucial function of protecting eggs and chicks. Despite this common function, nests are remarkably diverse within and among species in the size, structure and materials from which they are constructed (Collias & Collias 1984, Hansell 2000). Birds use various materials for their nests, including moss, grass, feathers and anthropogenic materials. Material composition has a profound effect on nest quality, which in turn can affect the nest builder's reproductive success. Nest materials can, for example, influence whether offspring recruit as breeding adults (Järvinen & Brommer 2020), affect a nest's insulative properties (Szentirmai & Székely 2004, Dawson *et al.* 2011) and reduce predation risks by better concealing the nest in its surroundings (Mulder *et al.* 2020). Furthermore, the addition of some materials can reduce ectoparasite levels (Gwinner *et al.* 2000, Peralta-Sanchez *et al.* 2010, Suárez-Rodríguez & Garcia 2017), and signal the builder's quality to its mate and to conspecifics (Brouwer & Komdeur 2004, Sergio *et al.* 2011, Trnka & Prokop 2011).

The quantification of nest materials is commonly done by dismantling the nest and weighing each of the different materials (e.g. Tomás *et al.* 2013, Biddle *et al.* 2017, 2018). Although the dismantling method provides reliable and exhaustive data, it has disadvantages. First, nest deconstruction is often time-consuming. Dismantling a nest consisting of fine materials requires gentle handling of individual pieces, which may take a few hours per nest. Secondly, the destructive nature of this method prevents access to the nests of rare species or historical nests from museum collections, thus limiting data coverage both taxonomically and temporally. Developing another method that overcomes these drawbacks would enable collection of larger samples of nest-material data, as well as allowing access to data from more species.

Here we propose a non-destructive, rapid approach to collect nest-material data using photographs of nests. To test whether the photographic method is practical, we photographed and deconstructed 45 nests made by Blue Tits *Cyanistes caeruleus* and 20 nests made by Dartford Warblers *Sylvia undata*, and compared the percentages of nest materials calculated from both dismantling and scoring photographs.

METHODS

We analysed 45 nests of Blue Tits, collected in 2018 from nestboxes around St Andrews, Fife, UK (for details, see Edwards 2020) and 20 nests of Dartford Warblers collected in 2009, 2015 and 2016 (the British National Nest Reference Collection at the Hunterian Museum, University of Glasgow; see Supporting Information for catalogue numbers). The nests were collected once the nesting attempt had finished. To killed any invertebrates left in the nests, we froze the Blue Tit

nests at −80 °C for 48 h or more, and dried them for an additional 48 h at 60 °C. We froze the Dartford Warbler nests at −30 °C for a week or more, and then air-dried them if they were damp.

Photographing nests

To score nest materials, we photographed each nest on a stage with a size reference, such as a ruler or a pattern with a known size (Fig. 1a and 1c). We chose to photograph the external wall of the nest rather than the interior, because top-down photographs of the interior would hugely overestimate the lining materials relative to the dismantling method. We photographed each nest from the side (using Olympus OM-D E-M10 and PEN Lite E-PL3 cameras, 13–42 mm and 40–150 mm lenses) at 20–30 cm from the lens. Due to the curved surface of the nest walls, when the camera focused on a particular section of the nest wall, other sections were more likely to fall out of focus, so we typically took multiple photographs per nest, and selected a single photograph in which the details of materials were best in focus for image analysis.

Scoring nest materials using photographs

We analysed each nest photograph by extracting a 4 × 2-cm quadrat (Fig. 1b and 1d). We chose 4 × 2 cm for the quadrat size as this was approximately the biggest size that fitted on the centre of the nests of both species. To process each picture, we placed the 4 × 2-cm quadrat in the centre of the nest photograph in ImageJ, using the ruler or the stage landmarks in the photograph as a reference (Fig. 1a and 1c). To create a single image for each category of nest materials (e.g. one for 'grass', one for 'moss', and so on), we isolated the area within the quadrat (Fig. 1b and 1d) and copied each material type. In each photograph we coloured a different material in black and calculated the number of pixels of each material within each quadrat, using ImageJ software (version 1.52a) and a custom code for ImageJ. The code first converts the photograph to an 8-bit image and sets the colour threshold to zero, allowing the program to calculate the number of pixels taken up by any region coloured in black. Materials were categorized as grass, heather *Calluna vulgaris*, moss, feathers, animal (e.g. hair, spider web, cocoon), other or unknown (i.e. the image was too dark or the material too ambiguous to score reliably). Heather was identified only in the Dartford Warbler nests. For the Dartford Warbler nests, we combined the feather and animal categories, as feather and animal categories together accounted for only 4.44% on average of the whole nest weight, whereas in Blue Tit nests, both materials constituted a larger part of the nest (feather: 9.94% and hair: 13.58% on average).
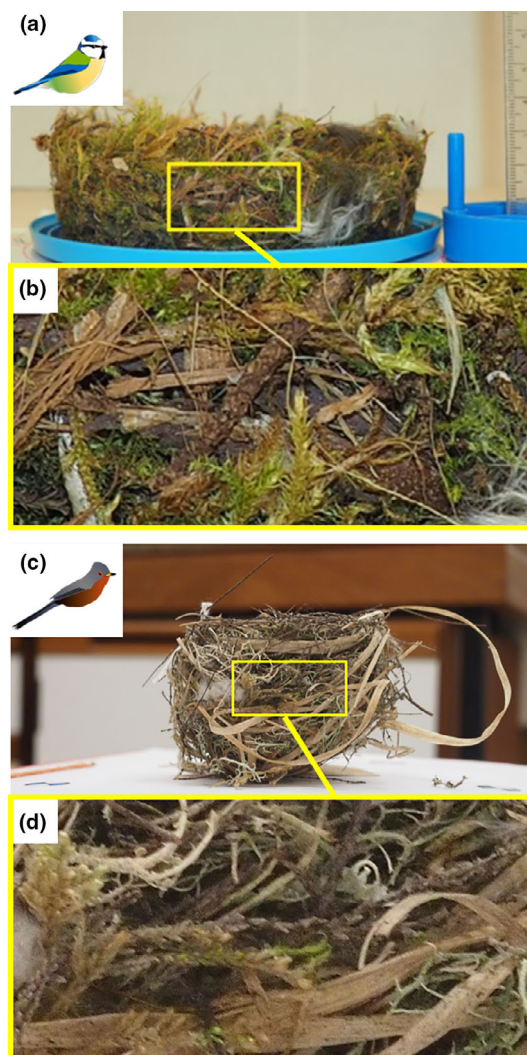

Figure 1. Photographs of a Blue Tit nest and a Dartford Warbler nest. (a) Blue Tit nests were photographed with a ruler as a reference length. (b) A 2 × 4-cm quadrat of the Blue Tit nest extracted for scoring nest materials. (c) Dartford Warbler nests were photographed with landmarks with a known size (i.e. circles on the left- and right-hand side of the nest). (d) A 2 × 4-cm quadrat of the Dartford Warbler nest extracted for scoring nest materials.

Once all the materials in each photograph had been quantified, we checked that the combination of all nest materials together was 90–110% of the total quadrat area. This helped to control for any lack of identification of materials, or pixels that were double-scored in separate images as different materials. For any photograph that did not meet these criteria, we repeated the analysis. The percentage of each material (*Mphoto*) was then calculated as:

Mphoto (%) = number of pixels of material × 100/ (number of pixels in quadrat − number of pixels of unknown areas).

Dismantling nests

Before dismantling a nest, we weighed it using a Scalix Precision Balance FGL-600. We then dismantled it by separating it into one of the material categories defined above, or another category 'fine material', which resulted from the larger materials disintegrating during the dismantling process. The category 'unknown' was not included for the dismantled materials, as we could examine materials closely. We calculated the weight of each material category by subtracting the weight of a polythene bag from that of the material in the bag. We calculated the percentage of each material (*Mdism*) as:

Mdism (%) = weight of material × 100/(weight of nest − weight of fine material).

The observer dismantling nests was blind to the nest-material composition measured from photographs.

Data analyses

To assess agreement between data from dismantling nests and scoring photographs, we calculated intra-class correlation (ICC) as a measure of agreement that accounts for systematic differences between two methods, unlike Pearson correlation (Watson & Petrie 2010). To test the level of agreement between two methods, we calculated ICC between the estimated percentages of each material, *Mphoto* and *Mdism*, using the 'icc()' function in the 'irr' package (Gamer *et al.* 2019). To test whether there was a consistent difference between the two methods (i.e. if one method tended to overestimate values relative to the other), we used paired *t*-tests using the 't.test()' function. All statistical analyses were run in R (R Core Team 2020), and statistical significance was assessed at α = 0.05.

RESULTS

In the Blue Tit nests, the average percentages of moss and grass were higher than those of feather and other animal-derived materials including hair (Fig. 2d). The percentages of all nest materials detected by the two methods were positively correlated (grass: ICC = 0.516, $P < 0.001$; moss: ICC = 0.464, $P = 0.016$; feather: ICC = 0.369, $P = 0.021$; other animal-derived materials: ICC = 0.521, $P = 0.004$; Fig. 2), although the degree of agreement (ICC: 0.369–0.521) was not very high (Watson & Petrie 2010). Specifically, the photographic method estimated higher percentages of grass ($t = -3.565$, df = 44, $P < 0.001$) and moss ($t = -5.631$,

df = 44, $P < 0.001$), and lower percentages of feathers ($t = 5.284$, df = 44, $P < 0.001$) and other animal-derived materials ($t = 4.701$, df = 44, $P < 0.001$) than did the dismantling method.

In the Dartford Warbler nests, the percentages of grass and heather were the highest, followed by those of moss and animal-derived materials (Fig. 2d). The two methods closely agreed for predominant materials including grass (ICC = 0.897, $P < 0.001$; Fig. 2a), heather (ICC = 0.714, $P < 0.001$; Fig. 2a) and moss (ICC = 0.596, $P = 0.001$; Fig. 2b), but not for rarer, animal-derived materials (ICC = 0.151, $P = 0.256$; Fig. 2c). The percentages estimated by the two methods for all materials did not differ (grass: $t = -1.612$, df = 19, $P = 0.123$; heather: $t = -1.476$, df = 19, $P = 0.156$; moss: $t = 1.612$, df = 19, $P = 0.123$; animal-derived materials: $t = -0.965$, df = 19, $P = 0.347$).

DISCUSSION

For both Dartford Warbler and Blue Tit nests, the percentages of the main nest materials estimated by the photographic and dismantling methods showed significant agreement. The level of agreement between the two methods, however, depended on the species and the types and distributions of nest materials.

For Dartford Warblers, there was close agreement for the major nest materials (grass, heather and moss), whereas for Blue Tits, we detected a higher proportion of grass and moss using the photographic method than by the dismantling method, probably because these materials were more abundant in the nest walls (which were photographed) than in the inner parts of the nest (which were not photographed). We also detected less animal-derived material using the photographic method than with the dismantling method. This is possibly because animal-derived materials are used for lining the inner layer of the nests we examined, and so might be less visible in the photographs of the external wall (Hansell 2000, Biddle *et al.* 2017).

Given that the correlation between methods varied between both species and material types, we have three suggestions for researchers who might consider applying the photographic method to a new species. First, we recommend a calibration of the photographic method before formal data collection. This calibration would involve measuring materials from a subset of the nests using both photographic and dismantling methods, thereby identifying differences between the methods, if any. Secondly, although we examined only the exterior layers of nests, it may be useful for species that build domed nests, or species that build nests with distinct layers made of different materials (e.g. the Common Blackbird *Turdus merula*; Biddle *et al.* 2018), to include photographs taken from another angle (e.g. inside view
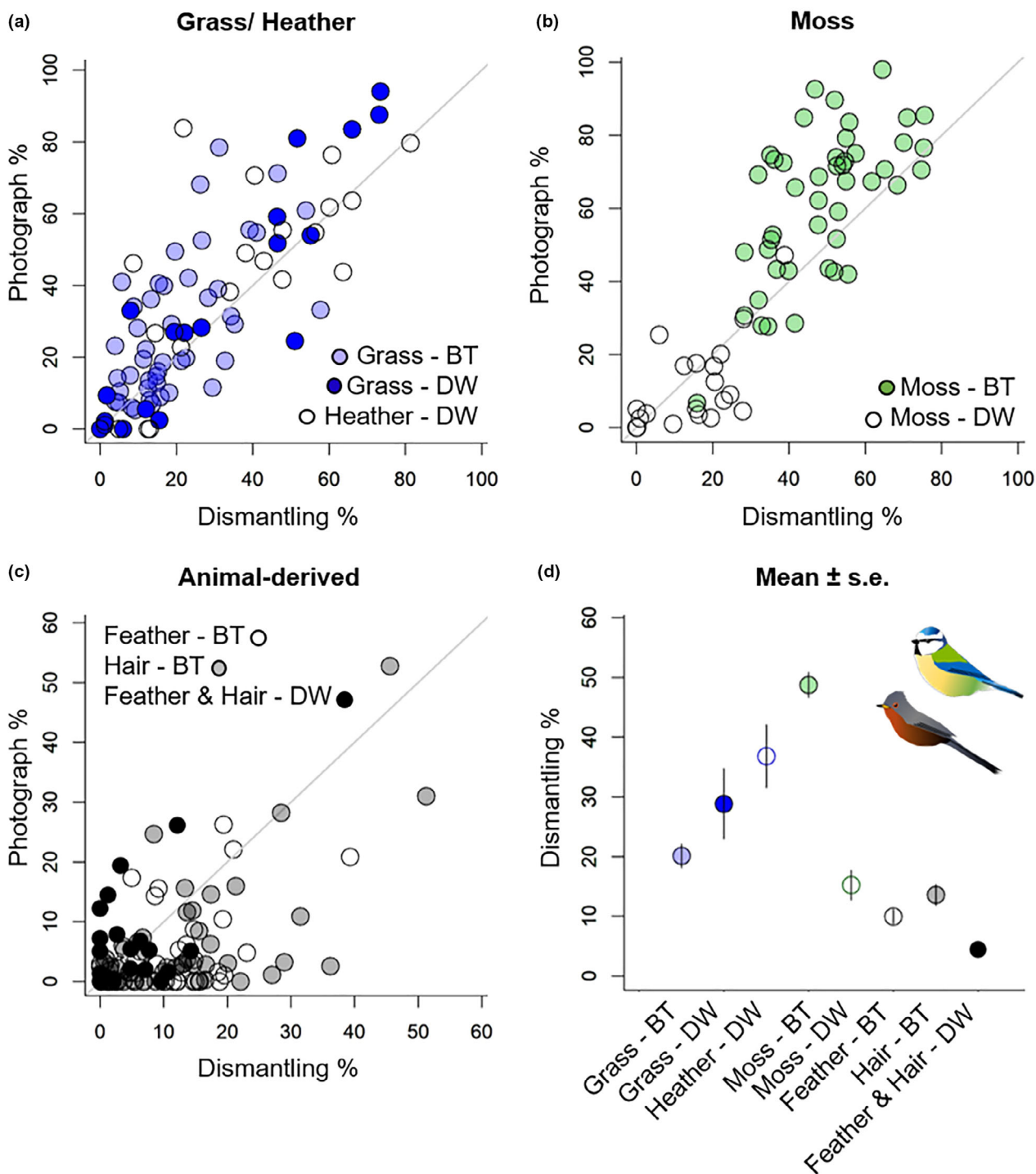
Figure 2. Agreement between photographic and dismantling methods in nest materials. Percentages estimated by our photographic method and the traditional dismantling method are shown for (a) grass and heather, (b) moss and (c) animal-derived materials including feather, hair and cocoons. BT = Blue Tit; DW = Dartford Warbler. Average percentage estimates of each material from the dismantling method are shown with standard errors in (d).

of domed nests; top-down view of nest cups, both of which would require additional lighting). Thirdly, although our method proved to be effective for quantifying the use of predominant materials such as grass, heather and moss, it should be used cautiously for quantifying rarer or lining materials, such as animal-derived materials, the detection of which depends on whether the material of interest is found in the photographed section. If a material is rare, then it might by chance not appear in a single photograph. In this case, combing through each part of a nest using the traditional dismantling method would be more appropriate.

When used with the appropriate precautions, a major advantage of the photographic method is its efficiency. In contrast to the dismantling method, which provides detailed and comprehensive data but is resource-consuming, it is relatively straightforward to train someone to score photographs. A scorer can then process a nest in less than half an hour, compared with the multiple hours that dismantling a nest can take. In addition, as this method does not require scanners, expensive cameras or professional software, it is accessible to a wide variety of bird nest enthusiasts (e.g. Weiner *et al.* 2020) with a smartphone to take photographs, and a laptop with the freely available ImageJ software to analyse the photographs.

This logistical benefit means that our photographic approach would be cost-effective for making large-scale comparisons of nest materials between populations and across time. This may be especially promising for species such as Great Tits *Parus major* and Blue Tits, for which long-term research projects are ongoing in multiple countries (Mainwaring 2017). Although these research projects do not always focus on nest building, possibly because of the effort involved in dismantling nests, the photographic method could open an opportunity to collect more nest-building data, by reducing time costs.

Our method can also increase the sample size of nests, for instance using historical nest collections. Although there are some museum collections that contain many nests, most of these old nests cannot be destroyed for data collection (Russell *et al.* 2013). Even with permits for dismantling, structural or morphological data from complete nests need to be acquired before dismantling, as such data would be lost afterwards. By applying our photographic method to historical nests in museum, we could take advantage of these relatively inaccessible resources, stretching the temporal windows of nest data by centuries (e.g. some of the nests in the Natural History Museum collection were collected in the 18th century; https://data.nhm.ac.uk/). This would be particularly useful to investigate trends that occur over long periods of time, such as those that may be caused by climate change. Long-term nest data could be used to reveal whether and how species responded to climate change by altering the composition of nest materials (Mainwaring *et al.* 2017, Edwards *et al.* 2020).

Moreover, the photographic method could broaden the taxonomic range of nest data, by encompassing species that are endangered (e.g. the Mangrove Finch *Camarhynchus heliobate*; Knutie *et al.* 2014) or even extinct species (e.g. the New Zealand Thrush *Turnagra capensis*; Hansell 2000). For these species, there are ethical problems in destroying any remaining nests in museum collections, making calibration for the photographic method difficult, but this could be overcome for instance by using nests of closely related species.

Finally, with further development, this photographic method could be adapted to automate the process of nest-material scoring using machine learning (Valletta *et al.* 2017), or to monitor addition/removal of materials at wild nests under construction, by taking top-down pictures *in situ* (e.g. NestWatch: https://nestwatch.org/). Such extensions could, for example, be useful for quantifying when artificial materials such as plastic (Sergio *et al.* 2011) or cigarette butts (Suárez-Rodríguez & Garcia 2017) are added during the building period, to identify how the use of novel material spreads in the population.

In summary, although this photographic method is unlikely to replace the dismantling method, we believe the photographic method and dismantling method occupy different 'niches'. Whereas the dismantling method affords a thorough analysis of nest materials, especially if the material is used in a small proportion in a nest, the photographic method would improve the efficiency of mapping material composition between populations or over time. Combining these methods should better equip us to explain the diversification of bird nest structure.

AUTHOR CONTRIBUTIONS

Shoko Sugasawa: Conceptualization (equal); Formal analysis (lead); Funding acquisition (equal); Visualization (lead); Writing-original draft (lead). **Sophie Edwards:** Funding acquisition (equal); Methodology (lead); Resources (lead); Writing-original draft (supporting). **Rowan Stanforth:** Data curation (equal); Writing-original draft (supporting). **Emily Bruton:** Data curation (equal); Writing-original draft (supporting). **Mike Hansell:** Resources (equal); Writing-original draft (supporting).

Maggie Reilly: Resources (equal); Writing-original draft (supporting). **Susan D. Healy:** Conceptualization (equal); Funding acquisition (equal); Supervision (lead); Writing-original draft (supporting).

Data Availability Statement

Input data, ImageJ code and R scripts are included as Supporting Information.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1. Input data, ImageJ code and R scripts.