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Pollen on feathers: unlocking the potential to study avian life histories through palynology

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ABSTRACT

Capsule: Bird-associated pollen can be effectively retrieved from single-use gloves worn during ringing to reveal patterns in habitat use and migratory ecology.

Aims: We compared abundance and richness of bird-associated pollen retrieved from ringers' gloves, bird bags, feather swabs and feather clippings from Pied Flycatchers *Ficedula hypoleuca* to establish optimal methods for avian palynological research. We also highlight real-world applicability through proof-of-concept analysis.

Methods: Thirty females from three sites were handled during routine ringing using new cotton gloves and bird bags. Feathers were swabbed and clipped under licence endorsement. Pollen was retrieved, identified and counted.

Results: More pollen (number of grains, greater richness) was retrieved from gloves, and bags were also effective, but other methods were poor. Most pollen was from plant taxa found at the breeding grounds, both arboreal and non-arboreal. Non-breeding-ground pollen (all arboreal) was sympatric with Pied Flycatcher wintering areas in West Africa and/or migratory routes. There were differences in UK and non-UK pollen assemblages, and between breeding sites, demonstrating the value of the approach for studying habitat and migratory connectivity.

Conclusion: Feathers are a rich source of pollen that can be quantified non-invasively by analysing ringer's gloves or bird bags, which has wide applicability both taxonomically and geographically.

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Studying avian life histories is challenging as no single research method offers comprehensive, continuous and long-term data on distribution, movement, habitat use and population dynamics. Accordingly, ornithologists use a range of methods, including field techniques (e.g. censusing, ringing), laboratory analyses (e.g. molecular taxonomy, microbiology) and computational approaches (e.g. population modelling, habitat suitability mapping) (Gilbert *et al.* 1998, Bibby 2000, Gosler 2004, Pettingill 2013). Indirect metrics can also be useful, for example, ptilochronology as a proxy for dominance (Grubb 2006), stable isotopes as a proxy for diet (Inger & Bearhop 2008) and first egg dates as a proxy for phenological responses to climate change (Crick & Sparks 1999).

Palynology includes the study of pollen from seed-bearing plants. Traditionally, palynology has been used in the reconstruction of climates and vegetation (Birks & Berglund 2018). Recently, however, the potential of palynology to support contemporary applied ecological

research has been increasingly widely recognized (Rull 2010, Buma *et al.* 2019, Goodenough & Webb 2022, Siggery *et al.* 2023). A recent review by Goodenough & Webb (2025) demonstrated the diversity of ornithological life-history research questions that can be answered through palynology. For example, research on bird-habitat associations by Holm & Svenning (2014) found that the European distribution of the Capercaillie *Tetrao urogallus* 12,000 years ago encompassed lowland areas with little arboreal pollen, suggesting that habitat requirements have changed temporally or the species has a broader ecological niche than is suggested by its current distribution. Palynology can also be a valuable tool for studying avian population dynamics when direct bird census data are not available. For example, Murray *et al.* (2023) analysed sediments from US lakes using Amaranthaceae pollen as a proxy for waterfowl abundance over the past 13,000 years, while Conroy *et al.* (2015) used American Mangrove (*Rhizophora mangle*) pollen to model 400 years of population change in Red-

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footed Booby (*Sula sula*) in Ecuador. Pollen can also be a useful method through which to study migration. For example, pollen from the bills of migratory birds, initially acquired during feeding, has been used to identify migratory routes, stopover sites and wintering grounds. Initial proof-of-concept work by Laursen *et al.* (1997) on birds in Denmark was followed by work by Wood *et al.* (2014) in the UK, finding that short-distance migratory warblers – Blackcaps *Sylvia atricapilla* and Chiffchaffs *Phylloscopus collybita* – carried more *Eucalyptus* pollen than long-distance migrants – Garden Warbler *Sylvia borin* and Willow Warblers *Phylloscopus trochilus* – which had more *Citrus* pollen.

Although there are several different sources of bird-associated palynological material, including sticky encrustations around bills as in the case of the migratory warbler research, feathers are perhaps most important (Groenman-van Waateringe 1998, Borgella Jr *et al.* 2001, Domínguez-Vázquez *et al.* 2021). Although partial pollen loss will occur with preening (and complete loss with moulting), feathers provide a ‘palynological time capsule’ for ornithological research. Thus, pollen retrieved from feathers of birds caught on their summer breeding grounds can reveal wintering habitat (or vice versa), as well as stopover sites (Cecere *et al.* 2010, 2011a, Karlioğlu Kiliç *et al.* 2023). Retention of pollen within feathers will partly depend on morphology of the exine (the durable sporopollenin coating protecting the microscopic reproductive cells), amount of pollenkit (the adhesive outer coating) and the electric charge of the pollen relative to the feather. For plants that are pollinated by animals, birds or insects, pollen grains typically have an ornate exine and also pollenkit. These features increase adhesion of pollen grains to, and facilitate retention on, the barbles and hooks of contour feathers (Cronk & Ojeda 2008, Chmel *et al.* 2021, Sengupta *et al.* 2021). These adaptations may increase the probability that animal-vectored pollen grains are recovered from feather samples relative to pollen that is dispersed primarily by wind, as those pollen grains generally lack the external ornamentation needed to adhere to external substrates (Moore *et al.* 1991).

Differences in the structure of exine are also key to visual identification of pollen grains, under a high-powered microscope, to species, genus or family level, dependent upon taxa. For avian researchers using palynology, the method used for collecting feather-based pollen in the field (e.g. direct methods such as feather clipping or swabbing, or indirect methods such as retrieving pollen from gloves worn during bird handling) must be efficient and effective. In addition to ensuring that as many pollen grains as possible are recovered, it is important that the pollen assemblage

retrieved from feathers is an accurate reflection of the pollen assemblage actually on the feathers. These taphonomic considerations have previously been investigated for differential pollen adherence to, and removal from, clothing fabric within forensic settings (Webb *et al.* 2018, Vichitnark *et al.* 2021, Küçük & Celenk 2025), which revealed highly variable retention on different fabrics, but have not been considered within the context of feather samples for avian research.

In this study, we compare four methods for sampling pollen from feathers of birds during routine ringing of 30 nesting adult female Pied Flycatchers, *Ficedula hypoleuca*: (1) single-use cotton gloves worn during bird handling, (2) single-use cotton bird bags used to hold birds between capture and ringing, (3) feather swabs and (4) feather clipping. The last two methods require samples to be taken from birds directly, which can increase handling time and require additional licence endorsement and ethical approval, while the first two methods are indirect approaches that can be built into standard bird-ringing practice, which are non-invasive and could be used at scale with minimal extra field effort or training. For each item type, we compare the pollen load (number of grains retrieved), taxonomic richness (number of taxa retrieved) and pollen assemblage, while also considering pollen origin (UK versus non-UK).

Although this is primarily a methodological paper, we also test whether it is possible to determine differences in UK breeding site (and non-UK wintering grounds and stopover sites) from feather pollen retrieved from Pied Flycatchers during breeding, as proof of concept that this approach has genuine applied research potential. We end by making methodological recommendations for future work to ensure palynology projects undertaken on any avian species anywhere in the world are maximally effective and that results are accurate, reliable and robust.

Methods

To ensure that laboratory methods were optimized, we calculated the efficiency of pollen retrieval from ringer’s gloves, bird bags, swabs and feather clippings. To do this, multiple replicates of each item, initially pollen-free, were spiked with pollen grains from one plant species in a laboratory setting to calculate percentage retrieved following standard processing. Additionally, we provided new and pollen-free cotton gloves, bird bags and swabs to a licenced ringer to be used during normal ringing activities to collect pollen from birds. We then applied our optimized retrieval laboratory methods to extract pollen from items used

in the field, before morphologically identifying pollen to family or genus (as appropriate) to establish the best approach for sampling pollen from avian plumage.

Optimizing pollen retrieval methods

To quantify pollen retrieval and ensure laboratory methods were effective, pollen spiking was undertaken on multiple replicates of each test item under laboratory conditions: (1) single new 100% cotton one-size ambidextrous gloves (MEDT, UK), (2) new pre-shrunk cotton bird bags (British Trust for Ornithology, UK), (3) viscose-tipped swabs that were pre-moistened with de-ionized water and protected with individual plastic containers (Scientific Laboratories Supplies, UK) and (4) contour feathers collected from around mist nets during standard ringing activities that were trimmed to 1–2 cm to replicate feather clippings from live birds.

Fresh pollen from Daisy *Bellis perennis* was harvested from anthers under controlled laboratory conditions. This species was selected due to easy availability and the echinate (densely covered with sharp, rigid points or bristles) morphological structure of its pollen, which means it adheres well to fabrics and skin and can thus be suitable for retrieval (Webb *et al.* 2018, Hunt & Morawska 2020). For each replicate of each test item, 500 ± 30 pollen grains were precisely counted onto a microscope slide under $100\times$ magnification and then transferred to the test item by rubbing the slide across the item. The slide was checked to ensure all grains had been transferred and the transfer process was repeated, if necessary, until all grains had been transferred.

The method for subsequently removing pollen from the test items varied. For gloves, the outside surface was aggressively scrubbed with a pollen-free clean toothbrush in warm water with mild detergent (Teepol™), as per Webb *et al.* (2018). The resultant suspension was centrifuged, the supernatant was removed, and the residual pellet was filtered (120 mm mesh) and screened under light optical microscopy on a standard glass slide. For bird bags, the bag was turned inside out before being treated in the same way. For swabs, 3 ml of diluted detergent was added to the individual swab container. Swab containers were resealed and agitated before the fluid was filtered and screened. For feathers, the feather was cut into small pieces and submerged in a centrifuge tube with 20 ml of filtered H₂O. The tube was Whirlymixed™ and agitated with a glass rod before centrifuging, filtering and screening.

Retrieval efficiencies, expressed as a percentage (e.g. 80% retrieval efficiency indicated that 80% of the

counted pollen grains added to a test item were successfully retrieved) with $\pm$ standard error were: glove = $94\% \pm 4.3\%$; bag = $96\% \pm 0.2\%$; swab = $95\% \pm 2.4\%$; feather = $80\% \pm 1.6\%$. Given the success rate of pollen retrieval on the test items when deliberately spiked with a known number of pollen grains, we were confident that the majority of pollen on the items used in field conditions could be retrieved using these protocols.

Field method

Birds were handled by a single ringer (co-author DGC) under licence number A3079 issued by the British Trust for Ornithology as part of normal ringing activities for the Re-trapping Adults for Survival (RAS) scheme. Special Methods Technical Panel endorsements permitted plumage swabbing (endorsement 12454) and clipping of up to 20 mm from one tail feather (endorsement 12260). In total, 30 adult female Pied Flycatchers were sampled from three locations on the English/Welsh border, UK (Crow Wood, Mansel Lacy and Paradise Farm; Figure 1) between 19 May and 7 June 2024, which was the maximum number of birds for which the endorsements were granted.

Pied Flycatcher was an ideal study subject because the species regularly breeds in nestboxes and was thus easy to sample on its breeding grounds. It was also a continuation of previous work by (Goodenough *et al.* 2017a) on Pied Flycatchers using the same three breeding sites. That work used stable isotope analysis of African-grown tertial feathers during the wintering period to successfully explore carry-over effects of wintering habitat choice on breeding success, but the isotopic signatures lacked the geographical specificity to determine wintering sites. More recently, work using light-level geolocators (e.g. Burgess & Hewson 2015) has helped refine wintering grounds used by specific breeding populations of birds in the UK and mainland Europe (Figure 1), but the Pied Flycatcher remains a candidate species for which more data on migratory ecology, wintering range and wintering habitat would be useful.

Birds were captured within nest boxes either during incubation, by lifting females from eggs, or during chick-feeding. For the latter, females were trapped inside nest boxes while delivering a food item, by remotely blocking the entrance. In all cases, the female was removed without disturbing the nest material using a new cotton glove, on the left hand, which had been protected from contamination until the point of use. The bird was then transferred into a new and contamination-free cotton bird bag during the

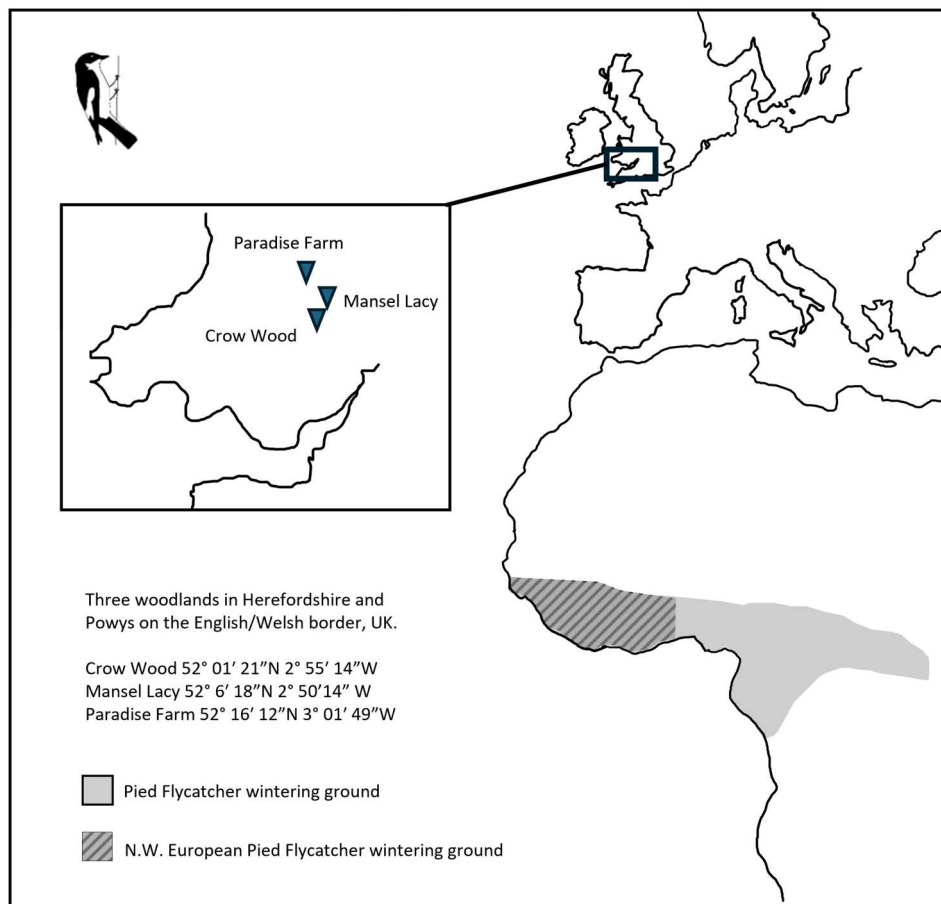


Figure 1. Breeding grounds of the Pied Flycatchers used in this study, and the wintering range in sub-Saharan Africa (After: *BirdLife* (n.d.) for known wintering grounds for all Pied Flycatchers; After: *Ouwehand et al. (2016)* for north-west European Pied Flycatcher wintering grounds).

preparation of ringing equipment with the ungloved right hand, before being removed again with the gloved left hand and held in the standard 'ringer's grip' (the bird's neck between the index and middle finger and the body held within the rest of the hand). If the bird was already ringed, the ring was checked and the number recorded before any other work was conducted; if the bird was un-ringed, she was ringed and the details recorded. To keep handling to a minimum before the swabbing and feather clipping processes were undertaken, no biometrics were recorded at this time.

The sterile swab was removed from its sterile plastic container with the ungloved right hand and used to sample plumage by moving the swab several times down the back of the bird from head to tail, before moving sequentially with the same swab over the left-wing mantle, right-wing mantle and breast feathers. The swab was then placed back in its container, labelled and refrigerated to limit fungal growth (*Goodenough et al. 2017b*). One innermost tail feather was then clipped to a maximum length of 20 mm and

stored in a labelled paper envelope. Choosing a contour (flight) feather was important to ensure hooks were present as part of the feather structure, as these would likely increase pollen retention; a tail feather was chosen rather than a wing feather as being potentially more difficult for the birds to preen and thus remove pollen. Finally, standard biometrics were taken and the bird was aged before being returned to its nest box immediately after processing. The ringer's glove was removed, and the glove and bird bag were stored in paper envelopes. Paper was chosen to retard the growth of fungus that might obscure the pollen.

All items ($n = 4 \text{ items per bird} \times 30 \text{ birds} = 120 \text{ items}$ overall) were processed as per the initial protocols developed in the laboratory when testing pollen retrieval. The resulting aliquots were processed to remove residues of cotton and the internal pollen genetic material via acetolysis (*Moore et al. 1991*), enabling the exine of the pollen to be clearly visualized under a light microscope. To further visually enhance the structures of the pollen, samples were stained with Grams Safranin before mounting

onto slides in glycerol jelly. Pollen grains were identified to family/genera taxonomic resolution using Moore *et al.* (1991) for UK pollen and Gosling *et al.* (2013) for West African pollen. The number of grains of each pollen type was counted. To avoid any potential for inter-observer variation, the pollen preparations, identification and counting for all items for all birds were undertaken by a single researcher (co-author TK), but a small number of slides were visualized by a second researcher (co-author JCW) for quality assurance, with no meaningful differences in grain counts per pollen type per item per bird identified.

Data analysis

To establish whether there were significant differences in pollen load and pollen taxonomic richness between the different pollen retrieval items, a repeated measures ANOVA framework was used. This meant that all differences were calculated on a per-bird basis to allow for repeated sampling of the same bird and avoid pseudoreplication. Six repeated measures ANOVAs were calculated: (1) overall pollen load, (2) UK pollen load, (3) non-UK pollen load, (4) overall pollen richness; (5) UK pollen richness and (6) non-UK pollen richness. The Greenhouse-Geisser method was used to compensate for a lack of sphericity in all analyses. Post-hoc testing was used for pairwise comparisons with a Bonferroni correction applied to allow for multiple comparisons. These tests were calculated in SPSS version 30 (IBM, USA) following guidelines by van den Berg (2026).

To establish whether differences in habitat on the breeding grounds could be detected from the bird-based pollen assemblage, a permutational ANOVA (PERMANOVA) was undertaken using Euclidian distance (Anderson 2017). Post-hoc pairwise tests were undertaken to compare each of the three sites to one another. Then, to establish whether birds at the different UK sites might have a different non-UK pollen assemblage (based on differences in wintering grounds and/or stopover sites if there were strong migratory connectivity at population level), a second PERMANOVA was undertaken on the non-UK pollen. These analyses were undertaken in the freeware package PAST (PAleontological STatistics) (Anderson 2017, Hammer 2022).

Results

Overall, 16,484 pollen grains were counted (30 birds; four samples per bird). The majority (15,947 grains;

96.7%) were from plant genera or families that occur in the UK. These were considered most likely to have been acquired in the UK and are henceforth referred to as the UK pollen assemblage, although the possibility that some pollen could have been retained from stopover sites if birds encountered plants in the same taxonomic group during migration cannot be discounted. The UK pollen assemblage was dominated by arboreal taxa (91.8%), especially oak *Quercus* spp., pine *Pinus* spp. and birch *Betula* spp., with the remaining 9.2% of the UK pollen assemblage being from non-arboreal taxa, dominated by grasses (Poeaceae) (Figure 2a).

The non-UK pollen assemblage (537 grains; 3.3%) encompassed six genera, all of which were arboreal (Figure 2b). Of these, four genera were sympatric with areas, shown in Figure 1, where Pied Flycatchers from north-west Europe are known to winter in West Africa: Bombax (*Rhodognaphalon* spp.; based on geographical range, likely East African Bombax *R. brevicuspe*), soap tree (*Tetrorchidium*; based on geographical range, likely *T. didymostemon* or *T. oppositifolium*), Christmas bush (*Alchornea*; could be one of four species in the genus that occur within Pied Flycatcher wintering range: *A. cordifolia*, *A. floribunda*, *A. hirtella* or *A. laxiflora*) and oil palm (*Elaeis*; based on range must be African Oil Palm *E. guineensis*). The other two non-UK genera, hackberry *Celtis* spp. and Fig *Ficus* spp. have species that occur in West African wintering ranges and in the Mediterranean Basin and thus could have been encountered on wintering grounds or stopover sites.

There was a significant difference in overall pollen load (i.e. number of individual pollen grains retrieved in total regardless of taxa or geographic location) between the different item types (repeated measures ANOVA: $F = 207.424$, d.f. = 1.740, $P < 0.001$; Figure 3a). Post-hoc testing revealed that there was no significant difference between the ringer's glove and the bird bag ($P = 0.759$) but the pollen load of samples from these items was significantly higher than either swab or feather clipping ($P < 0.001$ in both cases); pollen load was slightly but significantly higher on the swab compared to the feather clipping ($P = 0.027$). As expected, because most pollen was UK pollen, these findings were largely replicated when analysing pollen load for UK taxa only ($F = 205.027$, d.f. = 1.724, $P < 0.001$; gloves vs bag $P = 0.269$; glove/bag higher than swab/clipping $P < 0.001$; swab higher than clipping $P = 0.009$; Figure 3b). For non-UK pollen, sample sizes were notably smaller but there was a difference between item types ($F = 56.010$, d.f. = 1.229, $P < 0.001$; Figure 3c), with the pollen load on gloves being

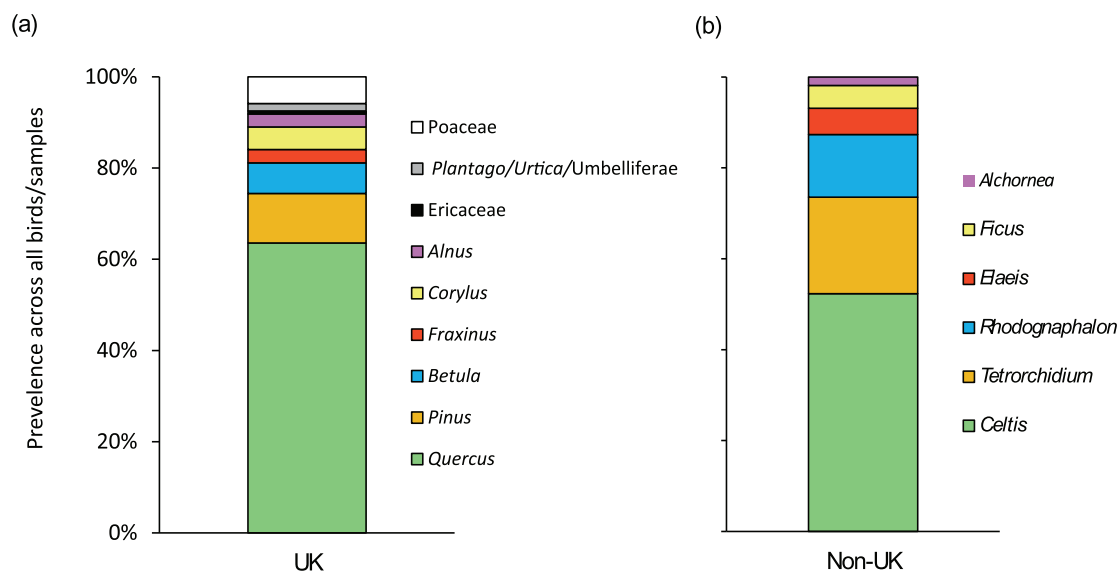


Figure 2. Pollen assemblage retrieved from all items and all birds combined showing: (a) UK-pollen and (b) non-UK pollen. Scientific names are used because several genera do not have common names.

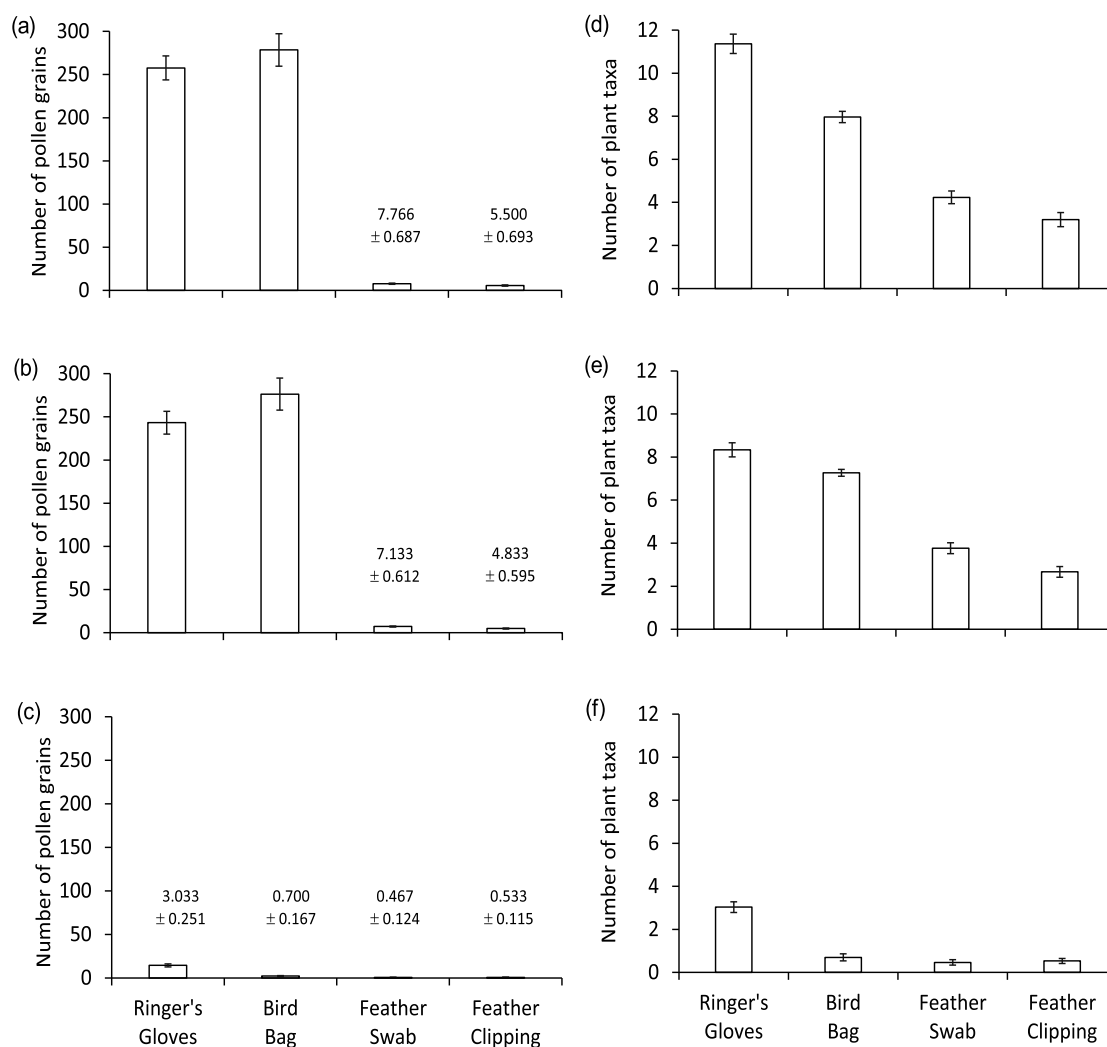


Figure 3. Pollen retrieved from items showing: (a) overall load, (b) UK load, (c) non-UK load, (d) overall taxonomic richness, (e) UK taxonomic richness and (f) non-UK taxonomic richness.

significantly ($P < 0.001$) higher than all other sample types, which did not differ significantly among themselves ($P \geq 0.115$).

There was a significant difference in overall taxonomic richness (i.e. number of taxa retrieved in total regardless of geographical origin) between the different sample types (repeated measures ANOVA: $F = 134.043$, d.f. = 2.715, $P < 0.001$; [Figure 3d](#)). Richness decreased between items in the order glove $\rightarrow$ bag $\rightarrow$ swab $\rightarrow$ feather clipping; all post-hoc contrasts were significant at $P < 0.001$ except for swab vs feather clipping, which did not differ ($P = 0.177$). This pattern was repeated for the richness of UK taxa (repeated measures ANOVA: $F = 125.450$, d.f. = 2.683, $P < 0.001$; [Figure 3e](#)), with all post-hoc contrasts being significant ($P \leq 0.028$). For richness of non-UK taxa, the significant overall difference ($F = 56.885$, d.f. = 2.242, $P < 0.001$; [Figure 3f](#)) was driven by more taxa being retrieved from gloves than all other items ($P < 0.001$), which did not themselves differ ($P \geq 0.897$).

Focusing on gloves only (joint best item for UK pollen load; best item for non-UK pollen load, UK pollen richness and non-UK pollen richness), a one-way PERMANOVA test was undertaken to compare pollen from Pied Flycatchers between the three UK sites on which they were caught while breeding. There was no overall statistically significant difference in the UK bird-based pollen assemblage retrieved from gloves in relation to UK breeding site (PERMANOVA: $F = 2.210$, d.f. = 2, $P = 0.082$); however, the post-hoc contrast for the Crow Wood versus Mansel Lacy sites was significant, while the other two post-hoc contrasts were non-significant ([Figure 4a](#)). This was driven by birds from Mansel Lacy having proportionally less oak pollen, more birch and pine pollen, and a greater richness of non-arboreal pollen than the Crow Wood site ([Figure 4a](#)). A second test to establish whether birds at the different UK sites might have a different non-UK pollen assemblage (based on differences in wintering grounds and/or stopover sites) was significant (PERMANOVA: $F = 6.801$, d.f. = 2, $P < 0.001$) with significant post-hoc contrasts for Crow Wood versus Mansel Lacy, and Mansel Lacy versus Paradise Farm, and a non-significant post-hoc contrast for Crow Wood versus Paradise Farm ([Figure 4b](#)). The differences were driven by Crow Wood birds having proportionally more hackberry and more African oil palm than those at Mansel Lacy, and Mansel Lacy birds having more soap tree than those at either Crow Wood or Paradise Farm ([Figure 4b](#)).

Discussion

Our research has shown that it is possible to retrieve valuable information from pollen collected from individual birds nesting in UK breeding sites. While UK arboreal and non-arboreal taxa were dominant in the pollen assemblage, non-UK pollen was also retrieved, albeit in low quantities, from migratory female Pied Flycatchers that had been in the UK for at least four weeks before being sampled. Two of the methods required no additional fieldwork or UK licence endorsements relative to standard ringing procedures, as they involved retrieving pollen from a ringer's gloves and bird-holding bags. Significantly more pollen, and a greater range of pollen, was retrieved from these items relative to feather swabs and feather clippings, both of which required a UK licence endorsement and increased bird handling and processing time.

This research agrees with previous studies that feathers are a rich source of palynological material (Cronk & Ojeda 2008, Chmel *et al.* 2021, Sengupta *et al.* 2021), and that pollen can be retained on plumage for several weeks (Cecere *et al.* 2011a). However, no previous research has empirically tested and compared sampling methods. The results of this study show for the first time that a meaningful pollen assemblage can be retrieved either from a new single-use cotton glove worn by a ringer when processing a bird, or from a new single-use cotton holding bag in which a bird is placed before being processed. Gloves were significantly better than bird bags in terms of the richness of pollen retrieved (UK and non-UK taxa) and in the amount of pollen retrieved (non-UK), with no difference relative to bird bags for amount of UK pollen. As gloves are also cheaper than bird bags, using gloves is recommended, if possible. If a ringer is not comfortable wearing gloves owing to reduction in dexterity, or if multiple people are involved in the ringing process (e.g. one ringer removing birds from mist nets and another ringer processing and releasing birds), bags are a very credible alternative. However, if ringers are not wearing single-use gloves when handling birds, and pollen is being retrieved from a bird bag, care should be taken that pollen on ringers hands does not transfer to the birds and contaminate bags before sampling (Hunt & Morawska 2020).

The more invasive and time-consuming methods of swabbing and feather clipping were substantially and significantly less effective than using gloves (or bags) and are thus not recommended. It is possible that wear at the tips of the 11-month-old tail feathers sampled in this study might have decreased their

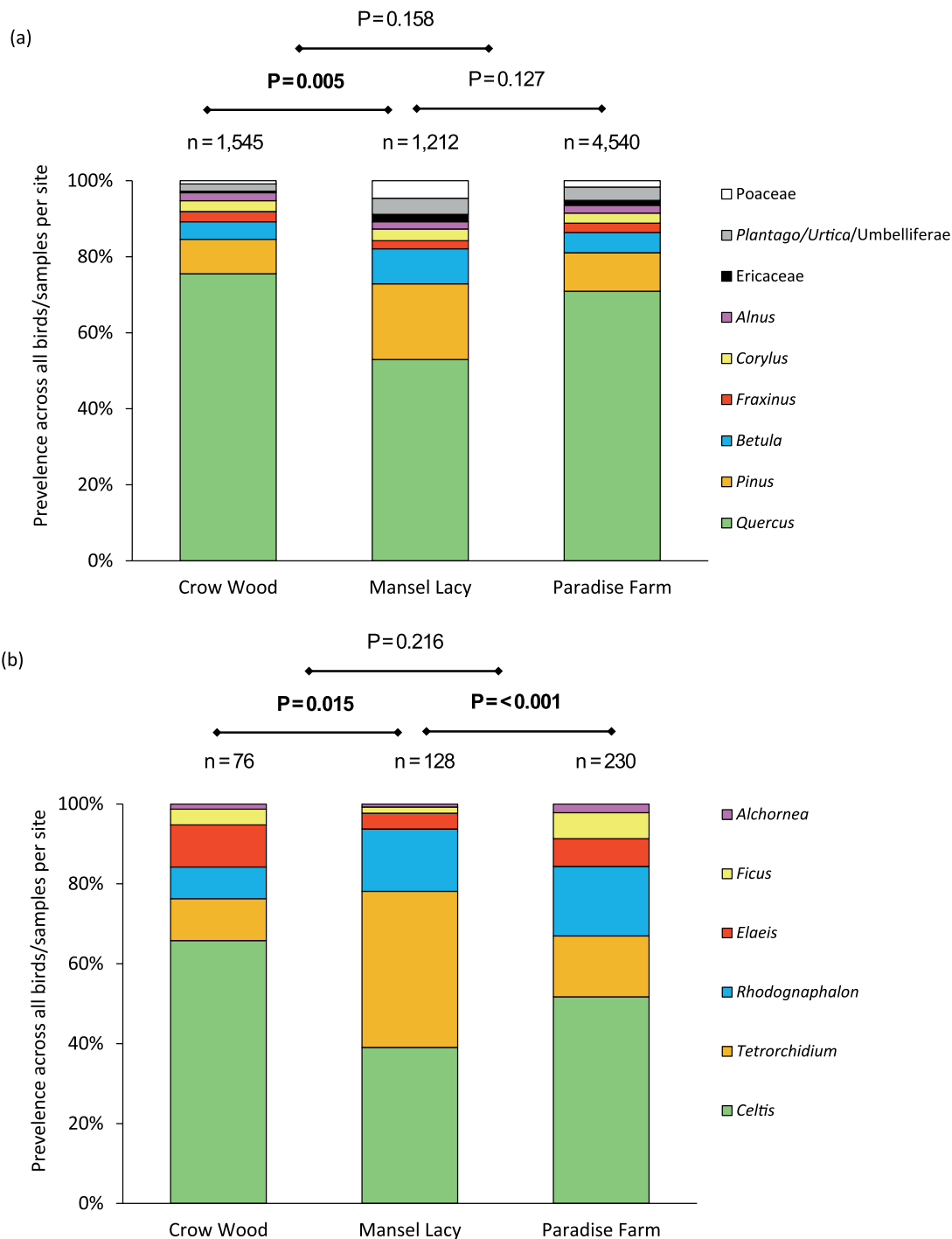


Figure 4. Pollen assemblage retrieved from gloves used to handle 30 female Pied Flycatchers from three different sites showing: (a) UK-pollen and (b) non-UK pollen. PERMANOVA post-hoc contrasts given above the stacked bars, with significant contrasts in bold.

ability to retain pollen, but the magnitude of the difference between feather clipping and gloves and bags was considerable.

Although this study did not compare avian palynological profiles with data from atmospheric pollen traps, several interesting patterns emerged nevertheless. From an avian biology perspective, this

proof-of-concept comparison of palynological profiles from birds breeding at three different UK sites (using gloves only, based on methodological results), revealed marked differences among sites. Pied Flycatchers have specialist habitat requirements, needing oak woodlands in which to breed – although not necessarily grazed oak woodlands (Goodenough 2014)

– so all three study sites were oak woodlands on the border of the Herefordshire/Powys counties. However, there were still differences in the UK pollen assemblages between two of the three sites, suggesting that palynology can reveal subtle differences in vegetation community.

Of even more interest was the difference in the non-UK pollen assemblage between birds breeding in different UK sites. This hinted at possible migratory connectivity between Pied Flycatcher populations on a much smaller spatial scale than revealed by Ouwehand *et al.* (2016), as the breeding sites we used were separated by only 20 km. Palynological investigation of birds in the UK was thus able to reveal spatial differences outside of the UK in ways not possible when stable isotope analysis of feathers was investigated for Pied Flycatchers at the same study sites (Goodenough *et al.* 2017a).

The main limitation in using palynology to answer ornithological research questions is reliance on microscopic morphological identification of pollen grains. Not only does morphological identification require specialist skills, but for the vast majority of pollen grains it is limited to genera or family, thus missing the taxonomic resolution needed to provide fine-scale spatial mapping. For example, some of the *Quercus* pollen found on female Pied Flycatchers here could have been *Q. robur* (Pedunculate Oak, found in the UK breeding grounds) but some could be *Q. suber* (Cork Oak, found on stopover sites). Similarly, pollen from the *Ficus* genus could be encountered on stopover sites or wintering grounds, but refining identification to specific *Ficus* species would give much more precise spatial understanding. One way to do this would be to use DNA identification of pollen grains to species level. This approach would be especially powerful if metabarcoding were performed on all pollen (Bell *et al.* 2016), with locations being refined to areas where all species co-occur. This has yet to be applied to birds, but there are several published studies using pollen metabarcoding to establish migration routes for Lepidoptera (Chang *et al.* 2018, Suchan *et al.* 2019, Jia *et al.* 2021). Such advances could allow identification of new stopover sites that are vital for species survival and ensure that such locations are appropriately protected to aid conservation efforts (Cecere *et al.* 2011b, Wood *et al.* 2014, Karlioğlu Kiliç *et al.* 2023).

While this research has studied one passerine species, with a focus on breeding habitat and migration ecology, the pollen retrieval methods herein tested could be used on any avian species anywhere in the world to answer questions specifically about migration or more

generally. This includes comparing intraspecific differences in habitat usage between males and females (or adults and juveniles) to study spatial resource partitioning to develop work by (Domínguez-Vázquez *et al.* 2021) on the endangered Black-pollled Yellowthroat *Geothlypis speciosa* in Mexico. This found that both sexes were more terrestrial than had been expected for a seemingly obligate water-associated species, but there was a much broader habitat niche for males versus females.

This approach could be expanded, where there is a substantial difference in local vegetation communities, to consider home ranges of other avian species, for which pollen data could provide information without the need for invasive tracking devices or the biases introduced by using patchy recapture or resighting data (Laver & Kelly 2008). Where there are differences in vegetation communities, for example between different islands in an archipelago, pollen could also be used to study patterns of natal dispersal without need for measurement of genetic parameters or (relatively scarce) data on individuals marked at their site of birth and recorded later elsewhere (van Noordwijk 1995). Feather-based palynology could also be used to compare dietary niche between sympatric species, building on the work of (Borgella Jr *et al.* 2001), which showed that different species had differing levels of food plant specificity, with some species being highly specialized and others being generalist (and thus more resilient to habitat loss and fragmentation).

Our hope is that the empirically-tested methodological protocols detailed in this paper provide robust foundations for future work on researching avian habitat, migration and dietary niches, and that the avian ecological analysis undertaken as proof-of-concept highlights the power of the approach and the range of ornithological research questions palynology can be used to answer.

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Author contributions

JCW: Conceptualization, Methodology, Project Administration, Visualization, Writing – original draft, and review and editing; **AEG:** Conceptualization, Formal Analysis, Methodology, Project Administration, Visualization, Writing – original draft, and review and editing; **TK:** Investigation, Writing – review and editing; **DGC:** Investigation, Writing – review and editing.

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