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Pre-Print Version

Exploring the prevalence and diversity of pollen carried by four species of migratory Old World warbler (Sylvioidea) on arrival in the UK

Matt J. Wood[1*], Peter J. Morgan[2], Julia C. Webb[1], Anne E. Goodenough[1], Frank M. Chambers[1] & Adam G. Hart[1]

[1] School of Natural and Social Sciences, University of Gloucestershire, Francis Close Hall, Swindon Road, Cheltenham, GL50 4AZ, UK.

[2] Portland Bird Observatory, The Old Lower Light, Portland Bill, Dorset, DT5 2JT, UK.

[*] Communicating author: mjwood@glos.ac.uk

Capsule Pollen encrusted around the bill of migrating warblers can reveal marked differences in foraging ecology between bird species.

Aims To examine patterns of the prevalence and diversity of pollen in four species of warbler, and explore the potential of pollen to act as an indicator of recent foraging behaviour.

Methods By isolating pollen from bill encrustations using laboratory palynological techniques and identification by light microscopy, we examined variation in the prevalence of the five most common pollen taxa, and variation in pollen assemblages in four species of warbler arriving on the south coast of England.

Results All samples contained abundant pollen, with 19 floral taxa identified. *Sylvia* warblers tended to carry *Prunus* and *Citrus* pollen, while *Phylloscopus* warblers mainly carried *Eucalyptus* pollen. Pollen assemblages varied markedly between bird species.

Conclusion Commercial and garden flowering trees are an important resource for migrating warblers. Pollen may be such a valuable resource that flowering plants might be included in the conservation management of stop-over sites. The use of pollen to resolve migratory routes may be problematic however, requiring detailed knowledge of both the distribution and flowering phenology of plants *en route*.

Migratory birds have breeding, stopover and wintering sites connected by migratory routes through different climatic zones. This makes them especially vulnerable to habitat and climate change, such that even small changes can have large population-level effects (Newton and Brockie 2008, Carey 2009). Recent declines in many UK migrant passerines, especially trans-Saharan migrants such as Willow Warblers (Morrison *et al.* 2010), emphasise this vulnerability. The geographical complexity of migration and the historical imbalance in the effort devoted to the study of birds that migrate between breeding grounds in northern European countries and southern wintering quarters (an imbalance especially marked in sub-Saharan migratory species) leaves much to be done to improve the patchy understanding of the migratory routes and ecology of migrant birds (Bairlein 2003, Newton and Brockie 2008).

To date, over 36 million birds have been marked with individually numbered rings in the UK (Newton 2010) but, despite the large numbers of migratory passerines ringed, few reliable connections have been made between breeding and wintering sites, although fascinating new insights have recently emerged from studies of species large enough to carry geolocators (McKinnon *et al.* 2013). For example, the Willow Warbler *Phylloscopus trochilus*, a sub-Saharan migrant commonly breeding in north-western Europe, has more than a million ringing records in the UK, yet only three [sic] birds ringed on breeding sites have been recovered on wintering grounds in central west Africa (Wernham *et al.* 2002). Knowledge of the wintering grounds of migrant passerines is, therefore, limited, particularly for birds wintering in inaccessible or remote habitats such as tropical rainforest. Informed conservation and management interventions for smaller species such as passerines (Goodenough *et al.* 2009) endorse the development of innovative empirical approaches (Bairlein 2003, Hobson 2008).

A proportion of migratory passerine birds are seen carrying pollen encrustations around the bill and forehead (Ash 1959, Laursen *et al.* 1997, Cecere *et al.* 2011a) presumably resulting from the exploitation of flowering plants as a fuel source on migration (Cecere *et al.* 2011b). Morphological differences between pollen from different plant taxa mean may therefore provide information on the plants the birds have used for foraging. The most comprehensive studies of pollen on migrant birds have focussed on a migration stopover site, the Italian island of Ventotene (Cecere *et al.* 2010, Cecere *et al.* 2011a, Cecere *et al.* 2011b). Behavioural observations showed that warblers foraged for nectar on locally available flowers, confirmed by palynology (the analysis of pollen) which also showed that stopover migrants carried pollen from plants not in flower on Ventotene Island, such as *Citrus* pollen, from foraging activity before arrival (Cecere *et al.* 2011a). A pollen encrustation, therefore, has the potential to act as a log of the previous behaviour of its bird 'vector'. Where a suitably diverse, or distinctive, pollen assemblage is identified, it may also be possible to use plant distribution maps to provide information on potential migratory routes, analogous to the use of pollen to provide information in forensic applications and reconstructing vegetation patterns in climate studies (Prentice and Webb 1998, Mildenhall *et al.* 2006). Thus, palynology has the potential to provide both ecological information on migrant bird foraging and geographical information on wintering grounds, migration routes and stop-over, albeit within the potentially broad geographical areas defined by the temporal and spatial distributions of pollen-bearing plants.

Avian palynology studies in migratory species have either examined pollen in the respiratory system, a lethal technique (Tamisier, cited in Laursen et al. 1997), or pollen clipped from the feathers around the bird's beak (Ash 1959, Ortega-Olivencia *et al.* 2005), which has the advantage of being non-lethal. This technique has been applied to warblers captured at sites in the UK and France (Ash 1959, Ash *et al.* 1961), Denmark (Laursen *et al.* 1997), in addition to more in-depth studies of opportunistic nectar feeders at stop-over sites in Italy (Cecere *et al.* 2010, Cecere *et al.* 2011a) and mechanisms of nectar uptake in Denmark (Holm and Laursen 1982). Bird ringers at Portland Bird Observatory on the south coast of England have become aware of the continued appearance in spring of apparently pollen-bearing migratory birds (P.J. Morgan & M. Cade, pers. obs.), first noted by John Ash at Portland in the 1950s (Ash 1959). We therefore screened apparent pollen encrustations using modern palynological techniques to assess the prevalence and diversity of pollen carried by four species of Old World warbler (Sylvioidea). By comparing two pairs of congeneric species with similar foraging behaviour but different migratory strategies (wintering in the Mediterranean versus sub-Saharan Africa), we aimed to examine how (i) the prevalence of pollen and (ii) pollen assemblages carried by warblers, varied between bird species, migratory strategy and foraging strategy.

METHODS

Study site and species

Pollen samples (encrustations attached to feathers around the bill) were clipped carefully from birds using clean, fine scissors. Sampled birds were a subset of those trapped in mist-nets and ringed by licensed ringers at Portland Bird Observatory (50° 31' 10.8"N, 2° 27' 4.1"W) between April 2006 and June 2008. The site is ideally located for the spring passage of migrant birds. It is situated on the southernmost tip of the Isle of Portland, a conspicuous outcrop of limestone 6km long by 2.4 km wide jutting lengthwise into the English Channel.

Two congeneric pairs of bird species frequently trapped and ringed at Portland were selected for study. The warbler species in each pair are closely related (Sibley and Ahlquist 1990, Beresford *et al.* 2005, Alström *et al.* 2006), and have similar foraging behaviours and bill and tongue morphology (Holm and Laursen 1982), but have contrasting migratory strategies: one of each pair is a sub-Saharan migrant, the other a Mediterranean migrant (Cramp and Simmons 1983). The *Phylloscopus* pair comprised Willow Warblers *P. trochilus* and Chiffchaffs *P. collybita*, and the *Sylvia* pair comprised Blackcaps *S. atricapilla* and Garden Warblers *S. borin*. Willow Warblers are thought to migrate from the UK to tropical west-central Africa, with Chiffchaffs wintering in the western Mediterranean (Wernham *et al.* 2002). When on breeding grounds, both *Phylloscopus* species feed on small insects from leaves of trees and shrubs. The *Sylvia* warblers forage in dense forest undergrowth, scrubland and woodland edges when on European breeding grounds, with Blackcaps migrating to the Mediterranean and Iberia and Garden Warblers migrating to sub-Saharan Africa, thought to be central west Africa (Cramp and Simmons 1983, Wernham *et al.* 2002).

Pollen screening

Samples (*n* = 113) were stored dry in air-tight Eppendorf tubes at 4°C, until processing and screening in the laboratory following standard protocols (Moore *et al.* 1991). Each sample was treated with cold 10% hydrochloric acid to disaggregate particles and to dissolve any carbonates. Excess sporopollenin and residual cellulose within the samples was removed by acetolysis (Erdtman 1960) to aid identification and comparison with type slides. Pollen grains were stained with basic fuchsin and mounted in glycerin jelly for microscopic examination. Ten traverses on each slide were scanned under a light microscope (Nikon Optiphot, x400 magnification) and the taxa present identified using Moore *et al.* (1991). Taxa present were recorded.

Statistical analysis

The presence or absence of each of the five most common pollen taxa on individual warblers was analysed in relation to the following predictors: (i) the 'host' bird species, (ii) sampling date, (iii) year, and their interactions. A global binary Generalized Linear Model was simplified using the information theoretic approach by the backward-stepwise elimination of non-significant predictors, beginning with higher order interactions at each step (i.e. beginning with species:date:year in this case). If the removal of a predictor resulted in a non-significant change in model deviance (i.e. $\delta\text{AIC} < 2$), the predictor was eliminated from the model (Crawley 2013). Global and final models were broadly similar, and the individual reinsertion of prior deleted predictors into the final model made no difference to the results of analyses. To summarise variation in the pollen assemblages identified among the birds sampled, a Principal Components Analysis

(PCA) was carried out on the presence/absence of all 19 pollen taxa. The resulting first two principal components were used, in separate analyses, as the dependent variable in generalized linear modelling. GLM analyses were conducted in R v3.01 (R Core Team 2013).

Where bird species was identified as a significant factor, three classes of orthogonal *a priori* treatment contrasts were introduced (Crawley 2013) to test hypotheses regarding the contributions of (i) bird genus (*Phylloscopus* vs. *Sylvia*), (ii) migratory strategy (Mediterranean vs. sub-Saharan) or (iii) bird species *per se* to pollen assemblage variation. These treatment contrasts are a useful analytical approach, because species, genus and migratory strategy could not be incorporated in a single model: in our case of two pairs of species, each with one sub-Saharan migrant and one Mediterranean migrant, 'species' would be equivalent to a 'genus:migratory strategy' interaction, and thus analytically intractable. The first class of treatment contrasts examined genus and migratory strategy (i.e. 'genus' contrast = Blackcap & Garden Warbler vs. Chiffchaff & Willow Warbler; migratory strategy contrast = Blackcap & Chiffchaff vs. Garden Warbler & Willow Warbler); and the second examined treatment contrasts between species. In summary, these orthogonal contrasts relate to the same final models (in which bird 'species' is retained as a factor), and simply vary the structure of the levels (i.e. contrasts) relating to that factor.

To establish whether pollen diversity might be useful to classify birds according to species, genus or migratory strategy, Discriminant Function Analysis (DFA) was undertaken using SPSS version 16.1, on the basis that a high level of classification accuracy was good evidence of substantial interspecific differences. A full DFA was run using presence/absence data for all pollen taxa, with classification power being ascertained using a jack-knife cross-validation procedure. The model was calculated repeatedly, each repetition involving the omission of a different individual case that is classified by the model and compared to the known outcome; this means that power was tested using a different data point to those that generated the model (Shaw 2003). This procedure was used because the sample size precluded the use of the preferred split-sample validation process (McGarigal *et al.* 2000). As sample sizes differed between species, classification accuracy was compared with prior probabilities calculated from the data. To determine the pollen genera that were the most important in creating pollen assemblage-level differences, a stepwise DFA was run (criterion to enter $\alpha = 0.05$; criterion to remove $\alpha = 0.10$). Separate full and stepwise DFAs were also run with bird genus (*Sylvia* or *Phylloscopus*) and migration strategy (Mediterranean or sub-Saharan) as binary classifying variables. The recommended case/variable ratio of 3:1 (Tabachnick and Fidell 2007) was met in all cases. All means are presented ±1 se.

RESULTS

Each of 113 suspected pollen encrustations collected from Willow Warblers, Chiffchaffs, Blackcaps and Garden Warblers contained abundant pollen. Nineteen different taxa were detected, varying considerably in prevalence (Table 1): the majority of samples (*n* = 70) contained *Eucalyptus* pollen, whereas other taxa were found in very few samples (e.g. Umbelliferae, *n* = 3; *Picea* (spruce) spp., *n* = 2).

Variation in pollen prevalence

We conducted separate analyses of the prevalence of the five most prevalent pollen taxa: *Eucalyptus*, *Citrus*, *Quercus*, *Prunus* and *Pinus* (Figs 1a-e). Warbler species was the only statistically significant predictor of the presence of the most prevalent pollen taxon, *Eucalyptus* (Fig. 1a: Wald $\chi^2 = 52.9$, df = 3, $P < 0.0001$), with Chiffchaffs carrying significantly more *Eucalyptus* than other warblers ($z = 4.2$, $P < 0.0001$). Migratory strategy and genus were found to be statistically significant species contrasts: Mediterranean migrants carried more *Eucalyptus* pollen than sub-Saharan migrants ($z = 4.1$, $P < 0.0001$), and *Phylloscopus* more than *Sylvia* warblers $z = 3.4$, $P < 0.001$). The presence of pollen from another commercial tree species, *Citrus* (Fig. 1b), also varied markedly between species (Wald $\chi^2 = 26.6$, df = 3, $P < 0.0001$), but differently in each year (species:year interaction, Wald $\chi^2 = 11.7$, df = 5, $P = 0.039$). Species contrasts indicated that Chiffchaffs ($z = -4.1$, $P < 0.0001$) and, to a lesser extent, Blackcaps ($z = -2.4$, $P = 0.004$) carried more *Citrus* pollen than Garden Warblers and Willow Warblers. Similarly, Mediterranean migrants carried less *Citrus* pollen than sub-Saharan migrants ($z = -4.4$, $P < 0.0001$).

Quercus pollen (Fig. 1c) also varied between warbler species contingent on year (species:year interaction, Wald $\chi^2 = 15.4$, df = 1, $P = 0.009$). Although species was not retained in the model as a main effect, species contrasts revealed that Chiffchaffs had significantly lower levels of *Quercus* pollen than other species (z = -2.2, $P = 0.028$). Contrasts of migratory strategy and genus were not supported ($P > 0.05$). *Prunus* pollen (Fig. 1d) varied significantly between warbler species (Wald $\chi^2 = 20.9$, df = 3, $P = 0.0001$), Blackcaps carrying *Prunus* more frequently than other species combined ($z =$

2.0, $P = 0.04$). Genus was the most preferred species contrast ($z = -3.2$, $P = 0.016$): *Sylvia* warblers carried more *Prunus* pollen than *Phylloscopus* warblers. The overall prevalence of *Prunus* pollen also varied between and within years (sample date:year interaction; Wald $\chi^2 = 6.3$, df = 2, $P = 0.044$). Lastly, *Pinus* pollen (Fig. 1e) showed a complex pattern of variation: *Pinus* prevalence varied between species and years (Wald $\chi^2 = 13.6$, df = 5, $P = 0.019$), and tended to be found earlier in samples from *Sylvia* warblers than *Phylloscopus* warblers (species:date interaction; Wald $\chi^2 = 8.65$, df = 1, $P = 0.034$).

Discriminant Function Analysis allowed individual cases to be correctly classified to species with 54.1% accuracy using presence/absence of the 19 different pollen taxa found in this study, compared to 37.8% accuracy *a priori* (i.e. by chance, using prior probabilities based on sample size). This increased to 56.8% when only the presence of *Eucalyptus* pollen was entered into the model, and to 62.2% when only *Eucalyptus* and *Prunus* were entered, reflecting the importance of species in the GLM analysis of these two pollen taxa (Figs 1a & 1d). Misclassifications in the full dataset arose mainly because Willow Warblers could not be differentiated from other species on the basis of pollen assemblage, but there was also some confusion between Blackcaps and Chiffchaffs. In the stepwise analyses, classifications of Blackcap and Chiffchaff were excellent (85.6%), but Willow Warblers still could not be distinguished. This was likely because this species had a similar prevalence of *Eucalyptus* to Blackcaps, and although Willow Warblers carried *Prunus* pollen far less frequently than Blackcaps and Garden Warblers, the overall prevalence of *Prunus* was only 16% (Table 1), such that this pollen taxon could not be used to differentiate (classify) all cases. In contrast to the PCA, birds could be classified to the correct genus using Discriminant Function Analysis with 74.3%

accuracy using the complete pollen dataset (compared to 51.4% *a priori*). This increased to 81.8% when only *Eucalyptus* and *Prunus* were entered. There was no bias as to where misclassifications arose in any analysis (ca.18% of *Sylvia* classified as *Phylloscopus* and vice versa).

Variation in pollen assemblages

Principal components analysis (PCA) of pollen assemblages (19 pollen taxa present) resulted in the first two component scores (PC1 and PC2) explaining 43.2% of total variance; if the presence/absences of the 19 pollen taxa were randomly distributed among the principal components, the first two PCs would have accounted for 15.4% of total variance. PC1 and PC2 were most strongly influenced by PC loadings with the five most prevalent pollen taxa (Table 1). The first component score (PC1) was significantly correlated with the two most prevalent pollen taxa: the presence of *Eucalyptus* and the absence of *Citrus* pollen (Table 1). Low values indicate the use of *Eucalyptus*, and high values the use of *Citrus*. PC2 was significantly correlated with the absence of (i) *Quercus* (oak spp.), (ii) the presence of *Prunus* pollen and (iii) the absence of *Pinus* (pine spp.) pollen, and non-significantly correlated with the absence of three less prevalent pollen taxa (Table 1). The ecological interpretation of PC2's five factor loadings is complex, but a focus on the three highest loadings indicates that PC2 can be thought of as an ordination of the exploitation of forest habitats: low values are associated with use of oak and pine.

Pollen assemblage variation (as described by PC1) varied significantly between warbler species (GLM: Wald $\chi^2 = 88.7$, df = 1, $P < 0.0001$; Fig. 1f). This pattern varied between years (species:year interaction; Wald $\chi^2 = 12.0$, df = 1, $P < 0.0001$). Garden

Warblers were distinct from other species in PC1 variation (*a priori* species treatment contrasts: z = -2.4, *P* = 0.019), having less *Eucalyptus* and more *Citrus* than other warbler species. Species treatment contrasts to examine variation by migratory strategy or genus were not found to be statistically significant descriptors of pollen assemblages. PC2 did not vary between warbler species as a simple main effect (GLM: Wald $\chi^2 = 2.0$, df = 1, *P* = 0.57): variation between species was contingent both on year (species:year interaction; $\chi^2 = 20.9$, df = 1, *P* = 0.0008) and sample date (species:date interaction; Wald $\chi^2 = 10.4$, df = 1, *P* = 0.015). The most preferred species contrast was between Chiffchaffs and other species (*z* = -2.8, *P* = 0.006); other contrasts between other warbler species, migratory strategy or genus were not supported (*P* > 0.05). These effects suggest complex variation in the prevalence of pollen taxa contributing to PC2.

Birds could not be classified to migratory strategy using Discriminant Function Analysis on the basis of their pollen assemblages at rates much above that expected by chance (full dataset = 74.3% accuracy; a priori chance = 73.0% accuracy). The stepwise analysis entered *Eucalyptus* and then *Quercus*, but classification accuracy was still low (78.4%). In all cases, the majority of Mediterranean migrants were classified correctly, but classification accuracy for Sub-Saharan migrants was low.

DISCUSSION

All 113 putative pollen samples collected from the encrustations at the base of the bills of migratory warblers at Portland Bird Observatory were found to contain abundant pollen. Pollen grains were identified from 19 different taxa, with marked differences in relative abundance across the samples. The phenology of flowering plants was not detected in our analyses, because sampling date did not predict the presence of pollen on warblers (likely due to the importance of warbler species as a predictor). Some of the taxa represented, including *Citrus*, were found in previous studies of pollen in migrant warblers (Ash 1959, Ash *et al.* 1961, Laursen *et al.* 1997, Cecere *et al.* 2010, Cecere *et al.* 2011a) and are consistent with birds passing through a southern European flora *en route* to the UK, while others were not reported in previous studies at Portland Bird Observatory (Ash 1959, Ash *et al.* 1961). We did not detect pollen taxa with sufficiently restricted range distributions to enable the inference of migratory routes. As in a previous study (Cecere *et al.* 2011a), short and long distance migrants varied in the pollen they carried: we found that Mediterranean migrants carried more *Eucalyptus* and less *Citrus* pollen than sub-Saharan migrants.

It is notable that pollen from *Eucalyptus*, a tree genus native to Australia, was especially prevalent in this study, present in 70 (61.9%) of 113 birds sampled. *Eucalyptus* species (the current study was not able to differentiate to species level) are now commonly cultivated in Africa and Iberia, where they are used for low-cost furniture and pulpwood (Doughty 2000). Indeed, the replacement of cork oak *Quercus suber* forests with commercial *Eucalyptus* has attracted conservation concerns (Doughty 2000). However, this study suggests that such non-native forests may be important as a foraging resource for some bird species. *Eucalyptus* is wind-pollinated, so to understand the significance of *Eucalyptus* pollen carried by migrant warblers it may be important to distinguish the accumulation of pollen dispersed over long distances, up to 1600m from an individual tree (Barbour *et al.* 2005), from that accumulated directly by foraging on

Eucalyptus flowers. Blackcaps and Chiffchaffs winter in the Mediterranean (Wernham *et al.* 2002) and are known to feed on the flowers of *Eucalyptus*, *Citrus* (J. Pérez-Tris, pers. comm.), and exotic garden plants (including *Aloe* spp. at Strait of Gibraltar Bird Observatory; C. Perez, pers. comm). Indeed, Laursen *et al.* (1997) and Cecere *et al.* (2011a) found that both *Eucalyptus* and *Citrus* were most prevalent in their studies, indicating the continued importance of arboriculture to warblers in more easterly Afro-Palaearctic migratory corridors. The *Phylloscopus* warblers carried the most pollen: the relatively light, hanging flowers at tips of branches found in *Eucaplytus* may suit the foraging techniques of these smaller birds.

Bird species was the strongest predictor of pollen prevalence and diversity, in both Discriminant Function Analysis and GLM approaches, with different bird species carrying different pollen assemblages. For example, *Phylloscopus* warbler pollen assemblages were dominated by *Eucalyptus*, while sub-Saharan migrant assemblages were dominated by *Citrus*. While *Eucalyptus* and *Citrus* are commonly found in spring migrant warblers (Laursen *et al.* 1997, Cecere *et al.* 2011a), we did not find the pollen of *Brassica* spp, Malvaceae and Cyperaceae found by Cecere *et al*. (2011a). Given that variation exists between species in pollen assemblages (as shown by the Principal Components Analysis), it is unsurprising that, overall, species should be an effective predictor in the univariate analyses of the five most common pollen taxa. In the PCA, the first component score (PC1) was significantly correlated with the two most prevalent pollen taxa: the presence of *Eucalyptus* and the absence of *Citrus* pollen (Table 1). PC1 might be considered, therefore, as an ordination of the exploitation of Iberian arboriculture by warblers: low values indicate the use of *Eucalyptus*, and high values the use of *Citrus*. PC2 was significantly correlated with the absence of (i) *Quercus* (oak spp.), (ii) the presence of *Prunus* pollen and (iii) the absence of *Pinus* (pine spp.) pollen, and non-significantly correlated with the absence of three less prevalent pollen taxa (Table 1). The ecological interpretation of PC2's five factor loadings is complex, but a focus on the three highest loadings indicates that PC2 can be thought of as an ordination of the exploitation of forest habitats: low values are associated with use of oak and pine.

Discriminant Function Analysis was successful in correctly classifying "unknown cases" to genus, suggesting that pollen assemblage differs substantially on this basis, but the same approach indicated that migratory strategy was not a good predictor of pollen assemblage. Overall, "species" was a more powerful predictor of pollen assemblages than either genus or migratory strategy. However, it is important to note that "species" as a factor in this model is, in effect, the factorial of "genus" and "migratory strategy".

A key development of this study would be the consideration of further warbler species, but further species carrying pollen are not seen at Portland Bird Observatory in sufficient numbers to allow comparison (e.g. we have only a handful of pollen samples from Whitethroats *Sylvia communis* and Lesser Whitethroats *S. curruca*). Despite this limitation, Discriminant Function Analysis was successful in correctly classifying the majority of "unknown cases" to genus, suggesting that pollen assemblage differs substantially on this basis. The GLM approach suggests that there are particularly strong between-genera differences in the amount of *Pinus* and *Prunus* pollen. Given that the two warbler genera studied here differ in their foraging behaviour (but that both pairs had a representative from each migratory strategy), pollen assemblages may be predicted by foraging behaviour rather than by migratory strategy. As a caveat to this, the ecology of a migrant at one stage in its migratory cycle (for example, foraging on breeding grounds) is not necessarily an indicator of its ecology at other stages, such as on wintering grounds just before migration or on stopover sites, when the pollen encrustations collected in this study were likely to be formed. According to Discriminant Function Analysis, migratory strategy was not a good predictor of overall pollen assemblage. However, there were significant differences in the occurrence of *Citrus* and *Eucalyptus* pollen between Mediterranean and trans-Saharan migrants.

The pollen found on different bird species indicates the foraging ecology of birds during their migration, via feeding on the nectar and/or pollen of the flowers of insect-pollinated plants such as *Citrus*, passing through the habitat of anemophilous (wind-pollinated) plants such as oaks and eucalypts, or insect-gleaning at flowers of both pollination strategies. Foraging resources exploited by migratory birds at this crucial stage in their life history are poorly understood, but studies of the importance of nectar feeding in the stop-over ecology of migrant warblers (Cecere *et al.* 2011b) indicate that the availability of flowering plants are a potentially important food source for migrant warblers. For example, in an experimental study of the drinking methods of warblers, examination of oral cavity morphology and estimation of fluid speed of a sucrose solution showed that the stomach of a Garden Warbler, with a volume of about 0.2 cm^3 can be filled in about 1 second, illustrating that feeding on nectar is a rapid and effective method of feeding when taking the energetic value of nectar into account (Holm and Laursen 1982). Cecere *et al.* (2011b) found that nectar uptake by warblers was not restricted to birds in poor condition, and may be an important water and energy source: uptake was followed by an increase in plasma glucose levels. The availability on migration of diverse and abundant flowering plants could play a role in the conservation management of long-range migratory warblers by providing strategically located stopover sites *en route*; the identification and support of these sites may be of considerable importance.

Pollen sampled from migrant warblers at the same site in the late 1950s (Ash 1959) found that all 11 putative pollen samples from whitethroats (*Sylvia communis, n* = 5), Blackcaps (*n* = 4), a Willow Warbler and a Chiffchaff contained *Citrus* pollen. The small sample sizes preclude statistical comparison with this study, but the absence of *Eucalyptus* is noteworthy. In a later study of warblers sampled in southern France, Ash *et al.* (1961) found that Blackcaps, Willow Warblers, Chiffchaffs and Orphean Warblers *S. hortensis* all carried pollen that contained mostly *Citrus*, with some pine *Pinus*, maple *Acer* and bog myrtle *Myrica*. Ash *et al.*'s (1961) data indicate that between 1.3% and 12% of Blackcaps carried visible pollen, with variation linked to date within a given year. Laursen *et al.* (1997), in a study at five sites in Denmark, found that warbler species differed in the prevalence of pollen (between 0.4% and 4.4%). Notably, samples collected from *Phylloscopus* warblers arriving in Denmark were dominated by *Eucalyptus* pollen (as was the case for *Phylloscopus* warblers sampled in this study). The majority of these previous studies were based on small samples of migratory birds analysed for pollen diversity (*n* = 3, Ash 1959; *n* = 44, Ash *et al.* 1961; *n* = 38, Laursen *et al.* 1997). With these relatively small sample sizes, we can only speculate on the potential role of changing vegetation distribution patterns in the intervening decades, but the detection of *Eucalyptus* pollen in later studies (Laursen *et al.* 1997; Cecere *et al.* 2011a; this study) might reflect an increase in the planting of *Eucalyptus* around the Mediterranean since the 1950s for use in paper production and in drainage projects (Doughty 2000).

At the outset of this study, the aim was to detect pollen from plant taxa that would allow location of wintering grounds. This was not possible, for three reasons. First, the taxonomic specificity of microscopic examination of pollen was not sufficiently detailed to allow use of plant distribution data to indicate bird distribution. More detailed microscopic examination may cast light here. The molecular identification of pollen to reveal hidden taxonomic diversity may also be useful, analogous with the cryptic molecular species diversity of parasites (Bensch *et al.* 2004). Second, the ranges of plants in potential wintering grounds are not mapped at a sufficiently fine geographic scale to be informative: a ‘ground-truthing’ restriction. Third, it remains unclear whether warblers carrying pollen on arrival in the UK are representative of the wider population. The absence of pollen on the majority of birds ringed at Portland Bird Observatory (~95%, P.J. Morgan & M. Cade, pers. comm.) may indicate that flowering plants are unavailable *en route* (unlikely given the timing of migration) or that most individuals do not need to land and take advantage of the high energy nectar and/or insect resource offered by flowering plants. It may be that those individuals collecting pollen are taking respite during bad weather or are individuals that need to “top up” reserves, i.e. individuals migrating sub-optimally. Palynologists know that some pollen taxa are more adhesive than others. For example, *Citrus* (F. M. Chambers, pers. comm) and *Eucalyptus* (Girijashankar 2010) are known to be sticky, explaining why they are most commonly found on the birds we sampled (Fig. 2). Little is known about the loss of pollen from subjects, certainly not birds (although Laursen *et al.* (1997) note that pollen encrustations may be retained from previous seasons until feathers are moulted), and so this is an area requiring further research in forensic palynology. Some pollen taxa may, therefore, be more useful than others as geographical markers. The search for African pollen taxa, a hope that tantalised earlier researchers (Ash 1959, Laursen *et al.* 1997), was unsuccessful in the current study, suggesting that pollen analysis might be useful only as an indicator of migratory behaviour in the short term, and not of migratory wintering quarters. This seems more likely to be a reflection of foraging ecology than a systematic short-coming of the technique, with pollen representing recent flower foraging activity when *en route*, rather than a record of overwintering foraging activity.

Whatever the experience of the minority of birds carrying obvious pollen encrustations, there remains the potential to study the remaining majority of 'clean' birds, by examining small amounts of pollen trapped in feathers. The microscopic structure of feathers appears to trap pollen (J.C. Webb, pers. obs.) potentially enabling the study of birds that may be more representative of the population and so increasing sample size. In order to yield sufficient pollen samples for analysis, this study was limited to two congeneric species pairs of warblers. Wider sampling of 'clean' feathers from a larger number of congeneric species pairs would be feasible, and may provide enough pollen to examine more robustly the contributions of foraging ecology (genus) and migratory strategy to pollen assemblages. Increased sample size may detect more uncommon pollen taxa, which when combined with vegetation distribution mapping, e.g. Atlas Florae Europeae (Jalas and Suominen 1988), may enable the identification of wintering grounds in a way similar to that used for forensics and the reconstruction of past vegetation patterns (Huntley and Birks 1983, Delcourt and Delcourt 1991).

Pollen encrustations on migratory warblers act as log of the migratory ecology of their avian carriers, revealing marked differences in foraging ecology and the potential importance of arboriculture as a resource on migration. But inferring migratory routes by the use of pollen studies remains elusive: significant advances in both field and laboratory are required, particularly for small passerines, to combine fieldwork focussed on suspected wintering areas with innovative analytical approaches.

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Figure 1.

The prevalence of pollen carried by four warbler species sampled at Portland Bird Observatory. Variation in the five most prevalent pollen taxa is shown: (a) *Eucalyptus*, (b) *Citrus*, (c) *Quercus*, (d) *Prunus* and (e) *Pinus* pollen, followed by (f) variation in pollen assemblages captured by Principal Component Analaysis (PC1, see main text). Warbler species legend: BC = Blackcap *Sylvia atricapilla*, CC = Chiffchaff *Phylloscopus collybita*, GW = Garden Warbler *Sylvia borin*, WW = Willow Warbler *Phylloscopus trochilus*.

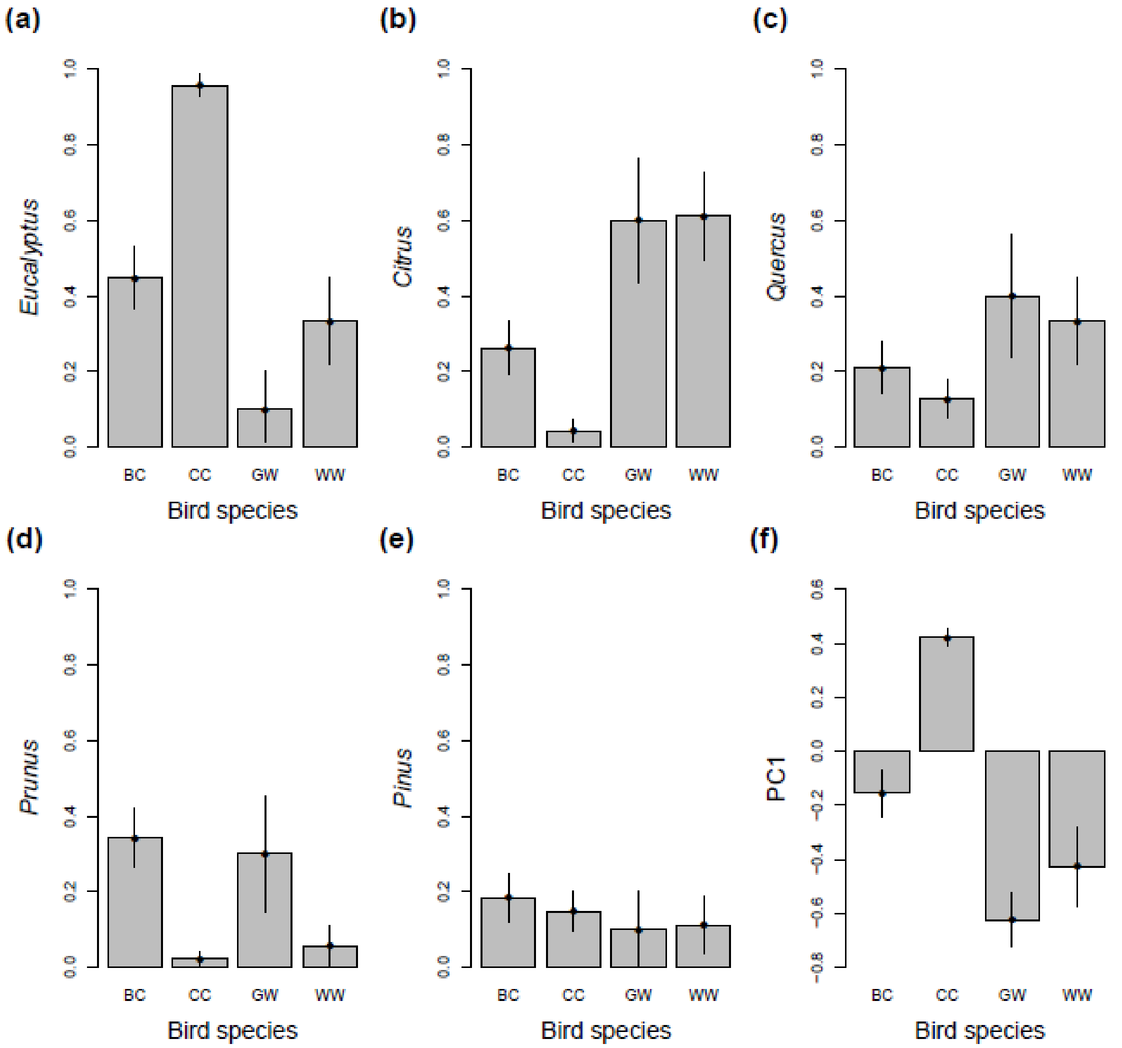

Figure 2.

Typical pollen encrustations found on warblers at Portland Bird Observatory. Blackcaps *Sylvia atricapilla* (left) are most commonly found with orange, waxy pollen encrustations, probably from *Citrus* (© Matt Wood), while (b) Chiffchaffs *Phylloscopus collybita* (right) typically carry brown-black pollen, probably from *Eucalyptus* (© Martin Cade).

Table 1.

Prevalence of pollen in four species of Old World warbler (Sylvioidea) on spring passage at Portland Bird Observatory. The 19 pollen taxa collected were found in 113 samples in Spring 2006-2008, they are shown with their Principal Component (PC) loadings. Prevalence is shown ± standard error (se), with statistical significance of correlations (Pearson's correlation coefficient, r_p) between the first two PCs and their loading variables (***$P < 0.001$, **$P < 0.01$, *$P < 0.05$). [†]The five most prevalent pollen taxa were analysed independently (Results; Figs b-f).

Pollen taxon		Prevalence	se	PC1	PC2
Eucalyptus[†]	Gum tree spp.	0.61	0.046	0.78***	-0.11
Citrus[†]	Orange, lemon, lime, grapefruit spp.	0.26	0.024	-0.60***	-0.10
Quercus[†]	Oak spp.	0.21	0.020		-0.77***
Prunus[†]	Cherry, blackthorn, plum, peach, almond spp.	0.16	0.015		0.21**
Pinus[†]	Pine spp.	0.15	0.014		-0.54***
Salix	Willow spp.	0.062	0.0058		
Poaceae	Grass family	0.053	0.0050		
Acer	Maple spp.	0.044	0.0042		
Alnus	Alder spp.	0.044	0.0042		
Myrtilus-Corylus	Myrtle spp. or Hazel spp.	0.035	0.0033		
Umbelliferae	Carrot family	0.027	0.0025		-0.15***
Betula	Birch spp.	0.018	0.0017		
Chenopodiaceae	Goosefoot family	0.018	0.0017		
Ericaceae	Heather family	0.018	0.0017		
Lychnis	Campion spp.	0.018	0.0017		
Picea	Spruce spp.	0.018	0.0017		
Corydalis	Corydalis spp.	0.0089	0.00083		
Fagus	Beech spp.	0.0089	0.00083		
Fraxinus	Ash spp.	0.0089	0.00083		