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

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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 (Sylvioidae). 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. $\delta$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 ($z =
Genus was the most preferred species contrast ($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 Eucalyptus 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 Analysis (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 < 0.01, * P < 0.05). † The five most prevalent pollen taxa were analysed independently (Results; Figs b-f).

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<th>Pollen taxon</th>
<th>Prevalence</th>
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