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

3

4 Matt J. Wood<sup>1\*</sup>, Peter J. Morgan<sup>2</sup>, Julia C. Webb<sup>1</sup>, Anne E. Goodenough<sup>1</sup>, Frank M.  
5 Chambers<sup>1</sup> & Adam G. Hart<sup>1</sup>

6

7 <sup>1</sup> School of Natural and Social Sciences, University of Gloucestershire, Francis  
8 Close Hall, Swindon Road, Cheltenham, GL50 4AZ, UK.

9 <sup>2</sup> Portland Bird Observatory, The Old Lower Light, Portland Bill, Dorset, DT5  
10 2JT, UK.

11 \* Communicating author: [mjwood@glos.ac.uk](mailto:mjwood@glos.ac.uk)

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

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

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

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

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

33

34 Migratory birds have breeding, stopover and wintering sites connected by migratory  
35 routes through different climatic zones. This makes them especially vulnerable to habitat  
36 and climate change, such that even small changes can have large population-level effects  
37 (Newton and Brockie 2008, Carey 2009). Recent declines in many UK migrant  
38 passerines, especially trans-Saharan migrants such as Willow Warblers (Morrison *et al.*  
39 2010), emphasise this vulnerability. The geographical complexity of migration and the  
40 historical imbalance in the effort devoted to the study of birds that migrate between  
41 breeding grounds in northern European countries and southern wintering quarters (an  
42 imbalance especially marked in sub-Saharan migratory species) leaves much to be done  
43 to improve the patchy understanding of the migratory routes and ecology of migrant birds  
44 (Bairlein 2003, Newton and Brockie 2008).

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

56 species such as passerines (Goodenough *et al.* 2009) endorse the development of  
57 innovative empirical approaches (Bairlein 2003, Hobson 2008).

58 A proportion of migratory passerine birds are seen carrying pollen encrustations  
59 around the bill and forehead (Ash 1959, Laursen *et al.* 1997, Cecere *et al.* 2011a)  
60 presumably resulting from the exploitation of flowering plants as a fuel source on  
61 migration (Cecere *et al.* 2011b). Morphological differences between pollen from different  
62 plant taxa mean may therefore provide information on the plants the birds have used for  
63 foraging. The most comprehensive studies of pollen on migrant birds have focussed on a  
64 migration stopover site, the Italian island of Ventotene (Cecere *et al.* 2010, Cecere *et al.*  
65 2011a, Cecere *et al.* 2011b). Behavioural observations showed that warblers foraged for  
66 nectar on locally available flowers, confirmed by palynology (the analysis of pollen)  
67 which also showed that stopover migrants carried pollen from plants not in flower on  
68 Ventotene Island, such as *Citrus* pollen, from foraging activity before arrival (Cecere *et*  
69 *al.* 2011a). A pollen encrustation, therefore, has the potential to act as a log of the  
70 previous behaviour of its bird ‘vector’. Where a suitably diverse, or distinctive, pollen  
71 assemblage is identified, it may also be possible to use plant distribution maps to provide  
72 information on potential migratory routes, analogous to the use of pollen to provide  
73 information in forensic applications and reconstructing vegetation patterns in climate  
74 studies (Prentice and Webb 1998, Mildenhall *et al.* 2006). Thus, palynology has the  
75 potential to provide both ecological information on migrant bird foraging and  
76 geographical information on wintering grounds, migration routes and stop-over, albeit  
77 within the potentially broad geographical areas defined by the temporal and spatial  
78 distributions of pollen-bearing plants.

79 Avian palynology studies in migratory species have either examined pollen in the  
80 respiratory system, a lethal technique (Tamisier, cited in Laursen et al. 1997), or pollen  
81 clipped from the feathers around the bird's beak (Ash 1959, Ortega-Olivencia *et al.*  
82 2005), which has the advantage of being non-lethal. This technique has been applied to  
83 warblers captured at sites in the UK and France (Ash 1959, Ash *et al.* 1961), Denmark  
84 (Laursen *et al.* 1997), in addition to more in-depth studies of opportunistic nectar feeders  
85 at stop-over sites in Italy (Cecere *et al.* 2010, Cecere *et al.* 2011a) and mechanisms of  
86 nectar uptake in Denmark (Holm and Laursen 1982). Bird ringers at Portland Bird  
87 Observatory on the south coast of England have become aware of the continued  
88 appearance in spring of apparently pollen-bearing migratory birds (P.J. Morgan & M.  
89 Cade, pers. obs.), first noted by John Ash at Portland in the 1950s (Ash 1959). We  
90 therefore screened apparent pollen encrustations using modern palynological techniques  
91 to assess the prevalence and diversity of pollen carried by four species of Old World  
92 warbler (Sylvioidea). By comparing two pairs of congeneric species with similar foraging  
93 behaviour but different migratory strategies (wintering in the Mediterranean versus sub-  
94 Saharan Africa), we aimed to examine how (i) the prevalence of pollen and (ii) pollen  
95 assemblages carried by warblers, varied between bird species, migratory strategy and  
96 foraging strategy.

97

## 98 **METHODS**

99

### 100 **Study site and species**

101 Pollen samples (encrustations attached to feathers around the bill) were clipped carefully  
102 from birds using clean, fine scissors. Sampled birds were a subset of those trapped in  
103 mist-nets and ringed by licensed ringers at Portland Bird Observatory (50° 31' 10.8"N, 2°  
104 27' 4.1"W) between April 2006 and June 2008. The site is ideally located for the spring  
105 passage of migrant birds. It is situated on the southernmost tip of the Isle of Portland, a  
106 conspicuous outcrop of limestone 6km long by 2.4 km wide jutting lengthwise into the  
107 English Channel.

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

123



124 **Pollen screening**

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

134

135 **Statistical analysis**

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

147 (PCA) was carried out on the presence/absence of all 19 pollen taxa. The resulting first  
148 two principal components were used, in separate analyses, as the dependent variable in  
149 generalized linear modelling. GLM analyses were conducted in R v3.01 (R Core Team  
150 2013).

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

166       To establish whether pollen diversity might be useful to classify birds according  
167 to species, genus or migratory strategy, Discriminant Function Analysis (DFA) was  
168 undertaken using SPSS version 16.1, on the basis that a high level of classification  
169 accuracy was good evidence of substantial interspecific differences. A full DFA was run

170 using presence/absence data for all pollen taxa, with classification power being  
171 ascertained using a jack-knife cross-validation procedure. The model was calculated  
172 repeatedly, each repetition involving the omission of a different individual case that is  
173 classified by the model and compared to the known outcome; this means that power was  
174 tested using a different data point to those that generated the model (Shaw 2003). This  
175 procedure was used because the sample size precluded the use of the preferred split-  
176 sample validation process (McGarigal *et al.* 2000). As sample sizes differed between  
177 species, classification accuracy was compared with prior probabilities calculated from the  
178 data. To determine the pollen genera that were the most important in creating pollen  
179 assemblage-level differences, a stepwise DFA was run (criterion to enter  $\alpha = 0.05$ ;  
180 criterion to remove  $\alpha = 0.10$ ). Separate full and stepwise DFAs were also run with bird  
181 genus (*Sylvia* or *Phylloscopus*) and migration strategy (Mediterranean or sub-Saharan) as  
182 binary classifying variables. The recommended case/variable ratio of 3:1 (Tabachnick  
183 and Fidell 2007) was met in all cases. All means are presented  $\pm 1$  se.

184

## 185 **RESULTS**

186

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

192

### 193 **Variation in pollen prevalence**

194 We conducted separate analyses of the prevalence of the five most prevalent pollen taxa:  
195 *Eucalyptus*, *Citrus*, *Quercus*, *Prunus* and *Pinus* (Figs 1a-e). Warbler species was the only  
196 statistically significant predictor of the presence of the most prevalent pollen taxon,  
197 *Eucalyptus* (Fig. 1a: Wald  $\chi^2 = 52.9$ ,  $df = 3$ ,  $P < 0.0001$ ), with Chiffchaffs carrying  
198 significantly more *Eucalyptus* than other warblers ( $z = 4.2$ ,  $P < 0.0001$ ). Migratory  
199 strategy and genus were found to be statistically significant species contrasts:  
200 Mediterranean migrants carried more *Eucalyptus* pollen than sub-Saharan migrants ( $z =$   
201  $4.1$ ,  $P < 0.0001$ ), and *Phylloscopus* more than *Sylvia* warblers  $z = 3.4$ ,  $P < 0.001$ ). The  
202 presence of pollen from another commercial tree species, *Citrus* (Fig. 1b), also varied  
203 markedly between species (Wald  $\chi^2 = 26.6$ ,  $df = 3$ ,  $P < 0.0001$ ), but differently in each  
204 year (species:year interaction, Wald  $\chi^2 = 11.7$ ,  $df = 5$ ,  $P = 0.039$ ). Species contrasts  
205 indicated that Chiffchaffs ( $z = -4.1$ ,  $P < 0.0001$ ) and, to a lesser extent, Blackcaps ( $z = -$   
206  $2.4$ ,  $P = 0.004$ ) carried more *Citrus* pollen than Garden Warblers and Willow Warblers.  
207 Similarly, Mediterranean migrants carried less *Citrus* pollen than sub-Saharan migrants ( $z$   
208  $= -4.4$ ,  $P < 0.0001$ ).

209 *Quercus* pollen (Fig. 1c) also varied between warbler species contingent on year  
210 (species:year interaction, Wald  $\chi^2 = 15.4$ ,  $df = 1$ ,  $P = 0.009$ ). Although species was not  
211 retained in the model as a main effect, species contrasts revealed that Chiffchaffs had  
212 significantly lower levels of *Quercus* pollen than other species ( $z = -2.2$ ,  $P = 0.028$ ).  
213 Contrasts of migratory strategy and genus were not supported ( $P > 0.05$ ). *Prunus* pollen  
214 (Fig. 1d) varied significantly between warbler species (Wald  $\chi^2 = 20.9$ ,  $df = 3$ ,  $P =$   
215  $0.0001$ ), Blackcaps carrying *Prunus* more frequently than other species combined ( $z =$

216 2.0,  $P = 0.04$ ). Genus was the most preferred species contrast ( $z = -3.2$ ,  $P = 0.016$ ): *Sylvia*  
217 warblers carried more *Prunus* pollen than *Phylloscopus* warblers. The overall prevalence  
218 of *Prunus* pollen also varied between and within years (sample date:year interaction;  
219 Wald  $\chi^2 = 6.3$ ,  $df = 2$ ,  $P = 0.044$ ). Lastly, *Pinus* pollen (Fig. 1e) showed a complex  
220 pattern of variation: *Pinus* prevalence varied between species and years (Wald  $\chi^2 = 13.6$ ,  
221  $df = 5$ ,  $P = 0.019$ ), and tended to be found earlier in samples from *Sylvia* warblers than  
222 *Phylloscopus* warblers (species:date interaction; Wald  $\chi^2 = 8.65$ ,  $df = 1$ ,  $P = 0.034$ ).

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

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

243

#### 244 **Variation in pollen assemblages**

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

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

262 Warblers were distinct from other species in PC1 variation (*a priori* species treatment  
263 contrasts:  $z = -2.4$ ,  $P = 0.019$ ), having less *Eucalyptus* and more *Citrus* than other warbler  
264 species. Species treatment contrasts to examine variation by migratory strategy or genus  
265 were not found to be statistically significant descriptors of pollen assemblages. PC2 did  
266 not vary between warbler species as a simple main effect (GLM: Wald  $\chi^2 = 2.0$ ,  $df = 1$ ,  $P$   
267  $= 0.57$ ): variation between species was contingent both on year (species:year interaction;  
268  $\chi^2 = 20.9$ ,  $df = 1$ ,  $P = 0.0008$ ) and sample date (species:date interaction; Wald  $\chi^2 = 10.4$ ,  
269  $df = 1$ ,  $P = 0.015$ ). The most preferred species contrast was between Chiffchaffs and  
270 other species ( $z = -2.8$ ,  $P = 0.006$ ); other contrasts between other warbler species,  
271 migratory strategy or genus were not supported ( $P > 0.05$ ). These effects suggest  
272 complex variation in the prevalence of pollen taxa contributing to PC2.

273         Birds could not be classified to migratory strategy using Discriminant Function  
274 Analysis on the basis of their pollen assemblages at rates much above that expected by  
275 chance (full dataset = 74.3% accuracy; a priori chance = 73.0% accuracy). The stepwise  
276 analysis entered *Eucalyptus* and then *Quercus*, but classification accuracy was still low  
277 (78.4%). In all cases, the majority of Mediterranean migrants were classified correctly,  
278 but classification accuracy for Sub-Saharan migrants was low.

279

## 280 **DISCUSSION**

281

282 All 113 putative pollen samples collected from the encrustations at the base of the bills of  
283 migratory warblers at Portland Bird Observatory were found to contain abundant pollen.  
284 Pollen grains were identified from 19 different taxa, with marked differences in relative

285 abundance across the samples. The phenology of flowering plants was not detected in our  
286 analyses, because sampling date did not predict the presence of pollen on warblers (likely  
287 due to the importance of warbler species as a predictor). Some of the taxa represented,  
288 including *Citrus*, were found in previous studies of pollen in migrant warblers (Ash 1959,  
289 Ash *et al.* 1961, Laursen *et al.* 1997, Cecere *et al.* 2010, Cecere *et al.* 2011a) and are  
290 consistent with birds passing through a southern European flora *en route* to the UK, while  
291 others were not reported in previous studies at Portland Bird Observatory (Ash 1959, Ash  
292 *et al.* 1961). We did not detect pollen taxa with sufficiently restricted range distributions  
293 to enable the inference of migratory routes. As in a previous study (Cecere *et al.* 2011a),  
294 short and long distance migrants varied in the pollen they carried: we found that  
295 Mediterranean migrants carried more *Eucalyptus* and less *Citrus* pollen than sub-Saharan  
296 migrants.

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



308 *Eucalyptus* flowers. Blackcaps and Chiffchaffs winter in the Mediterranean (Wernham *et*  
309 *al.* 2002) and are known to feed on the flowers of *Eucalyptus*, *Citrus* (J. Pérez-Tris, pers.  
310 comm.), and exotic garden plants (including *Aloe* spp. at Strait of Gibraltar Bird  
311 Observatory; C. Perez, pers. comm). Indeed, Laursen *et al.* (1997) and Cecere *et al.*  
312 (2011a) found that both *Eucalyptus* and *Citrus* were most prevalent in their studies,  
313 indicating the continued importance of arboriculture to warblers in more easterly Afro-  
314 Palaeartic migratory corridors. The *Phylloscopus* warblers carried the most pollen: the  
315 relatively light, hanging flowers at tips of branches found in *Eucalyptus* may suit the  
316 foraging techniques of these smaller birds.

317 Bird species was the strongest predictor of pollen prevalence and diversity, in  
318 both Discriminant Function Analysis and GLM approaches, with different bird species  
319 carrying different pollen assemblages. For example, *Phylloscopus* warbler pollen  
320 assemblages were dominated by *Eucalyptus*, while sub-Saharan migrant assemblages  
321 were dominated by *Citrus*. While *Eucalyptus* and *Citrus* are commonly found in spring  
322 migrant warblers (Laursen *et al.* 1997, Cecere *et al.* 2011a), we did not find the pollen of  
323 *Brassica* spp, Malvaceae and Cyperaceae found by Cecere *et al.* (2011a). Given that  
324 variation exists between species in pollen assemblages (as shown by the Principal  
325 Components Analysis), it is unsurprising that, overall, species should be an effective  
326 predictor in the univariate analyses of the five most common pollen taxa. In the PCA, the  
327 first component score (PC1) was significantly correlated with the two most prevalent  
328 pollen taxa: the presence of *Eucalyptus* and the absence of *Citrus* pollen (Table 1). PC1  
329 might be considered, therefore, as an ordination of the exploitation of Iberian  
330 arboriculture by warblers: low values indicate the use of *Eucalyptus*, and high values the

331 use of *Citrus*. PC2 was significantly correlated with the absence of (i) *Quercus* (oak  
332 spp.), (ii) the presence of *Prunus* pollen and (iii) the absence of *Pinus* (pine spp.) pollen,  
333 and non-significantly correlated with the absence of three less prevalent pollen taxa  
334 (Table 1). The ecological interpretation of PC2's five factor loadings is complex, but a  
335 focus on the three highest loadings indicates that PC2 can be thought of as an ordination  
336 of the exploitation of forest habitats: low values are associated with use of oak and pine.

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

343 A key development of this study would be the consideration of further warbler  
344 species, but further species carrying pollen are not seen at Portland Bird Observatory in  
345 sufficient numbers to allow comparison (e.g. we have only a handful of pollen samples  
346 from Whitethroats *Sylvia communis* and Lesser Whitethroats *S. curruca*). Despite this  
347 limitation, Discriminant Function Analysis was successful in correctly classifying the  
348 majority of “unknown cases” to genus, suggesting that pollen assemblage differs  
349 substantially on this basis. The GLM approach suggests that there are particularly strong  
350 between-genera differences in the amount of *Pinus* and *Prunus* pollen. Given that the two  
351 warbler genera studied here differ in their foraging behaviour (but that both pairs had a  
352 representative from each migratory strategy), pollen assemblages may be predicted by  
353 foraging behaviour rather than by migratory strategy. As a caveat to this, the ecology of a

354 migrant at one stage in its migratory cycle (for example, foraging on breeding grounds) is  
355 not necessarily an indicator of its ecology at other stages, such as on wintering grounds  
356 just before migration or on stopover sites, when the pollen encrustations collected in this  
357 study were likely to be formed. According to Discriminant Function Analysis, migratory  
358 strategy was not a good predictor of overall pollen assemblage. However, there were  
359 significant differences in the occurrence of *Citrus* and *Eucalyptus* pollen between  
360 Mediterranean and trans-Saharan migrants.

361         The pollen found on different bird species indicates the foraging ecology of birds  
362 during their migration, via feeding on the nectar and/or pollen of the flowers of insect-  
363 pollinated plants such as *Citrus*, passing through the habitat of anemophilous (wind-  
364 pollinated) plants such as oaks and eucalypts, or insect-gleaning at flowers of both  
365 pollination strategies. Foraging resources exploited by migratory birds at this crucial  
366 stage in their life history are poorly understood, but studies of the importance of nectar  
367 feeding in the stop-over ecology of migrant warblers (Cecere *et al.* 2011b) indicate that  
368 the availability of flowering plants are a potentially important food source for migrant  
369 warblers. For example, in an experimental study of the drinking methods of warblers,  
370 examination of oral cavity morphology and estimation of fluid speed of a sucrose  
371 solution showed that the stomach of a Garden Warbler, with a volume of about 0.2 cm<sup>3</sup>  
372 can be filled in about 1 second, illustrating that feeding on nectar is a rapid and effective  
373 method of feeding when taking the energetic value of nectar into account (Holm and  
374 Laursen 1982). Cecere *et al.* (2011b) found that nectar uptake by warblers was not  
375 restricted to birds in poor condition, and may be an important water and energy source:  
376 uptake was followed by an increase in plasma glucose levels. The availability on

377 migration of diverse and abundant flowering plants could play a role in the conservation  
378 management of long-range migratory warblers by providing strategically located  
379 stopover sites *en route*; the identification and support of these sites may be of  
380 considerable importance.

381         Pollen sampled from migrant warblers at the same site in the late 1950s (Ash  
382 1959) found that all 11 putative pollen samples from whitethroats (*Sylvia communis*,  $n =$   
383 5), Blackcaps ( $n = 4$ ), a Willow Warbler and a Chiffchaff contained *Citrus* pollen. The  
384 small sample sizes preclude statistical comparison with this study, but the absence of  
385 *Eucalyptus* is noteworthy. In a later study of warblers sampled in southern France, Ash *et*  
386 *al.* (1961) found that Blackcaps, Willow Warblers, Chiffchaffs and Orphean Warblers *S.*  
387 *hortensis* all carried pollen that contained mostly *Citrus*, with some pine *Pinus*, maple  
388 *Acer* and bog myrtle *Myrica*. Ash *et al.*'s (1961) data indicate that between 1.3% and  
389 12% of Blackcaps carried visible pollen, with variation linked to date within a given year.  
390 Laursen *et al.* (1997), in a study at five sites in Denmark, found that warbler species  
391 differed in the prevalence of pollen (between 0.4% and 4.4%). Notably, samples collected  
392 from *Phylloscopus* warblers arriving in Denmark were dominated by *Eucalyptus* pollen  
393 (as was the case for *Phylloscopus* warblers sampled in this study). The majority of these  
394 previous studies were based on small samples of migratory birds analysed for pollen  
395 diversity ( $n = 3$ , Ash 1959;  $n = 44$ , Ash *et al.* 1961;  $n = 38$ , Laursen *et al.* 1997). With  
396 these relatively small sample sizes, we can only speculate on the potential role of  
397 changing vegetation distribution patterns in the intervening decades, but the detection of  
398 *Eucalyptus* pollen in later studies (Laursen *et al.* 1997; Cecere *et al.* 2011a; this study)

399 might reflect an increase in the planting of *Eucalyptus* around the Mediterranean since  
400 the 1950s for use in paper production and in drainage projects (Doughty 2000).

401         At the outset of this study, the aim was to detect pollen from plant taxa that would  
402 allow location of wintering grounds. This was not possible, for three reasons. First, the  
403 taxonomic specificity of microscopic examination of pollen was not sufficiently detailed  
404 to allow use of plant distribution data to indicate bird distribution. More detailed  
405 microscopic examination may cast light here. The molecular identification of pollen to  
406 reveal hidden taxonomic diversity may also be useful, analogous with the cryptic  
407 molecular species diversity of parasites (Bensch *et al.* 2004). Second, the ranges of plants  
408 in potential wintering grounds are not mapped at a sufficiently fine geographic scale to be  
409 informative: a ‘ground-truthing’ restriction. Third, it remains unclear whether warblers  
410 carrying pollen on arrival in the UK are representative of the wider population. The  
411 absence of pollen on the majority of birds ringed at Portland Bird Observatory (~95%,  
412 P.J. Morgan & M. Cade, pers. comm.) may indicate that flowering plants are unavailable  
413 *en route* (unlikely given the timing of migration) or that most individuals do not need to  
414 land and take advantage of the high energy nectar and/or insect resource offered by  
415 flowering plants. It may be that those individuals collecting pollen are taking respite  
416 during bad weather or are individuals that need to “top up” reserves, i.e. individuals  
417 migrating sub-optimally. Palynologists know that some pollen taxa are more adhesive  
418 than others. For example, *Citrus* (F. M. Chambers, pers. comm) and *Eucalyptus*  
419 (Girijashankar 2010) are known to be sticky, explaining why they are most commonly  
420 found on the birds we sampled (Fig. 2). Little is known about the loss of pollen from  
421 subjects, certainly not birds (although Laursen *et al.* (1997) note that pollen encrustations

422 may be retained from previous seasons until feathers are moulted), and so this is an area  
423 requiring further research in forensic palynology. Some pollen taxa may, therefore, be  
424 more useful than others as geographical markers. The search for African pollen taxa, a  
425 hope that tantalised earlier researchers (Ash 1959, Laursen *et al.* 1997), was unsuccessful  
426 in the current study, suggesting that pollen analysis might be useful only as an indicator  
427 of migratory behaviour in the short term, and not of migratory wintering quarters. This  
428 seems more likely to be a reflection of foraging ecology than a systematic short-coming  
429 of the technique, with pollen representing recent flower foraging activity when *en route*,  
430 rather than a record of overwintering foraging activity.

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

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

451

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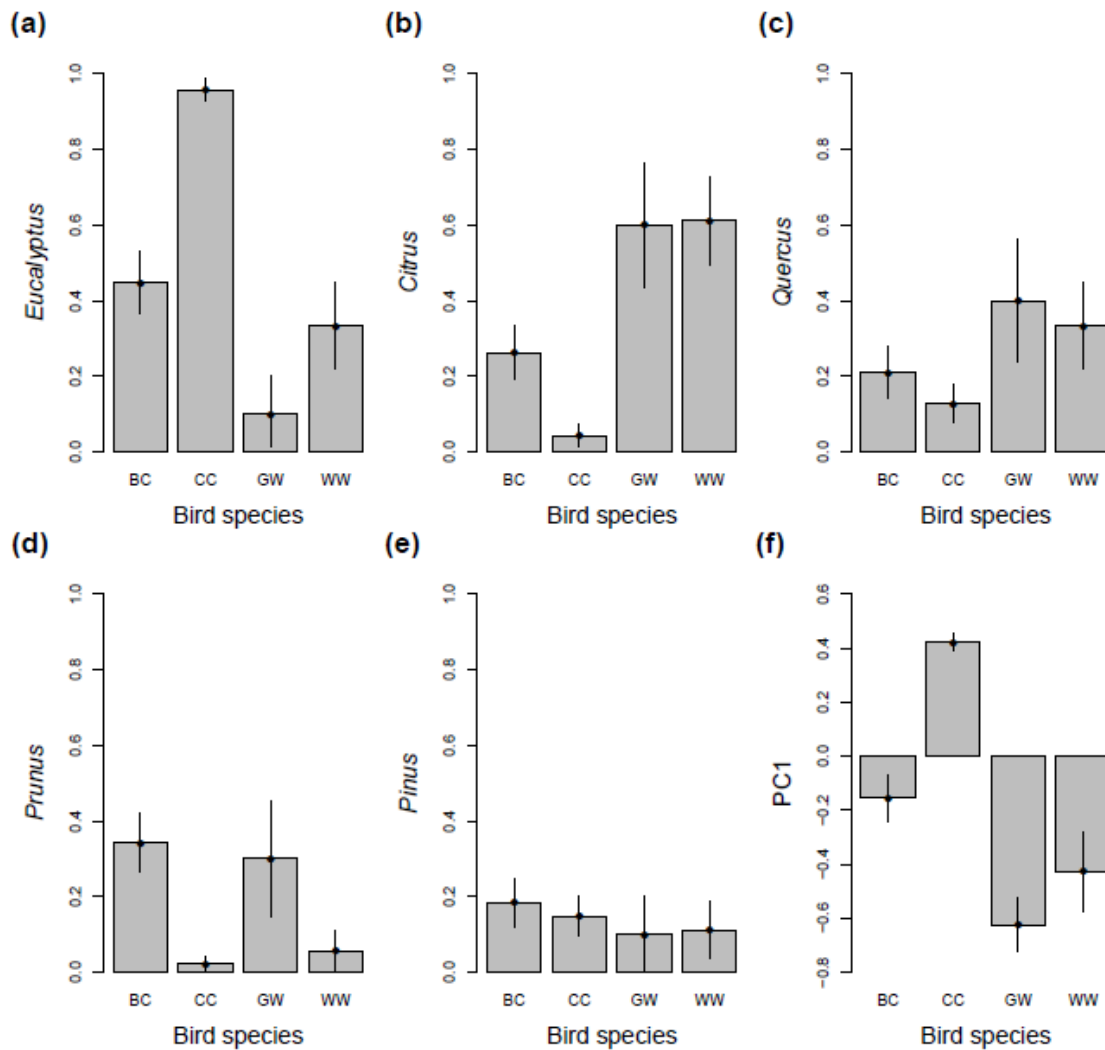
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547

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