



UNIVERSITY OF
GLOUCESTERSHIRE

This is a peer-reviewed, post-print (final draft post-refereeing) version of the following published document, This is an Accepted Manuscript of an article published by Taylor & Francis in Bird Study on 17th July 2014 available online:
<http://www.tandfonline.com/10.1080/00063657.2014.938017> and is licensed under All Rights Reserved license:

Wood, Matthew J ORCID logoORCID: <https://orcid.org/0000-0003-0920-8396>, Morgan, Peter J, Webb, Julia C ORCID logoORCID: <https://orcid.org/0000-0002-1652-965X>, Goodenough, Anne E ORCID logoORCID: <https://orcid.org/0000-0002-7662-6670>, Chambers, Frank M ORCID logoORCID: <https://orcid.org/0000-0002-0998-2093> and Hart, Adam G ORCID logoORCID: <https://orcid.org/0000-0002-4795-9986> (2014) Exploring the prevalence and diversity of pollen carried by four species of migratory Old World warbler (Sylvioidea) on arrival in the UK. *Bird Study*, 61 (3). pp. 361-370. doi:10.1080/00063657.2014.938017

Official URL: <http://www.tandfonline.com/doi/abs/10.1080/00063657.2014.938017>

DOI: <http://dx.doi.org/10.1080/00063657.2014.938017>

EPrint URI: <https://eprints.glos.ac.uk/id/eprint/1151>

Disclaimer

The University of Gloucestershire has obtained warranties from all depositors as to their title in the material deposited and as to their right to deposit such material.

The University of Gloucestershire makes no representation or warranties of commercial utility, title, or fitness for a particular purpose or any other warranty, express or implied in respect of any material deposited.

The University of Gloucestershire makes no representation that the use of the materials will not infringe any patent, copyright, trademark or other property or proprietary rights.

The University of Gloucestershire accepts no liability for any infringement of intellectual property rights in any material deposited but will remove such material from public view pending investigation in the event of an allegation of any such infringement.

PLEASE SCROLL DOWN FOR TEXT.

This is a peer-reviewed, pre-print (final draft post-refereeing) version of the following published document:

Wood, Matthew J. and Morgan, Peter J. and Webb, Julia C. and Goodenough, Anne E. and Chambers, Frank M. and Hart, Adam G. (2014). Exploring the prevalence and diversity of pollen carried by four species of migratory Old World warbler (Sylvioidea) on arrival in the UK. Bird Study, 61 (3) 361-370.

Published in Bird Study, and available online at:

<http://www.tandfonline.com/doi/abs/10.1080/00063657.2014.938017>

We recommend you cite the published (post-print) version.

The URL for the published version is

<http://dx.doi.org/10.1080/00063657.2014.938017>

Disclaimer

The University of Gloucestershire has obtained warranties from all depositors as to their title in the material deposited and as to their right to deposit such material.

The University of Gloucestershire makes no representation or warranties of commercial utility, title, or fitness for a particular purpose or any other warranty, express or implied in respect of any material deposited.

The University of Gloucestershire makes no representation that the use of the materials will not infringe any patent, copyright, trademark or other property or proprietary rights.

The University of Gloucestershire accepts no liability for any infringement of intellectual property rights in any material deposited but will remove such material from public view pending investigation in the event of an allegation of any such infringement.

PLEASE SCROLL DOWN FOR TEXT.

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^{1*}, Peter J. Morgan², Julia C. Webb¹, Anne E. Goodenough¹, Frank M.
5 Chambers¹ & Adam G. Hart¹

6

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

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

11 * Communicating author: mjwood@glos.ac.uk

12

13

14

15

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 ± 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 cm³
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

452 **ACKNOWLEDGEMENTS**

453 We thank the warden of Portland Bird Observatory and Field Centre, Martin Cade, and
454 its many visiting bird ringers for their support and assistance with sample collection, in
455 particular Emma Cockburn, Denis & Val Jackson, Hannah & Glenn Thomas, Liz Kerr
456 and Iain Dodd. We thank the British Ecological Society for funding from a Small
457 Ecological Project Grant (ref. 702/815). John R.G. Daniell carried out laboratory pollen
458 identification in the Centre for Environmental Change and Quaternary Research
459 (CECQR) at The University of Gloucestershire.

460

461

462 **REFERENCES**

- 463 **Alström, P., Ericson, P.G.P., Olsson, U. & Sundberg, P.** 2006. Phylogeny and
 464 classification of the avian superfamily Sylvioidea. *Mol. Phylogenet. Evol.*, 38,
 465 381-397.
- 466 **Ash, J.S.** 1959. Pollen contamination by birds. *Br. Birds*, 52, 421-426.
- 467 **Ash, J.S., Jone, P.H. & Melville, R.** 1961. The contamination of birds by pollen and
 468 other substances. *Br. Birds*, 54, 93-100.
- 469 **Bairlein, F.** 2003. The study of bird migrations – some future perspectives. *Bird Study*,
 470 50, 243-253.
- 471 **Barbour, R.C., Potts, B.M. & Vaillancourt, R.E.** 2005. Pollen dispersal from exotic
 472 eucalypt plantations. *Conserv. Genet.*, 6, 253-257.
- 473 **Bensch, S., Pérez-Tris, J., Waldenström, J. & Hellgren, O.** 2004. Linkage between
 474 nuclear and mitochondrial DNA sequences in avian malaria parasites: Multiple
 475 cases of cryptic speciation? *Evol.*, 58, 1617-1621.
- 476 **Beresford, P., Barker, F.K., Ryan, P.G. & Crowe, T.M.** 2005. African endemics span
 477 the tree of songbirds (Passeri): molecular systematics of several evolutionary
 478 'enigmas'. *Proc. R. Soc. London, Ser. B*, 272, 849-858.
- 479 **Carey, C.** 2009. The impacts of climate change on the annual cycles of birds. *Philos.*
 480 *Trans. R. Soc., B*, 364, 3321-3330.
- 481 **Cecere, J.G., Cornara, L., Mezzetta, S., Ferri, A., Spina, F. & Boitani, L.** 2011a.
 482 Pollen couriers across the Mediterranean: the case of migrating warblers. *Ardea*,
 483 99, 33-42.
- 484 **Cecere, J.G., Matricardi, C., Frank, B., Imperio, S., Spina, F., Gargallo, G.,**
 485 **Barboutsis, C. & Boitani, L.** 2010. Nectar exploitation by songbirds at
 486 Mediterranean stopover sites. *Ardeola*, 57, 143-157.
- 487 **Cecere, J.G., Spina, F., Jenni-Eiermann, S. & Boitani, L.** 2011b. Nectar: an energy
 488 drink used by European songbirds during spring migration. *J. Ornithol.*, 152, 923-
 489 931.
- 490 **Cramp, S. & Simmons, K.E.L.** (eds.) (1983) *The Birds of the Western Palearctic*,
 491 Oxford: Oxford University Press.
- 492 **Crawley, M.J.** 2013. *The R Book*, Chichester: John Wiley & Sons.
- 493 **Delcourt, H.R. & Delcourt, P.A.** 1991. *Quaternary Ecology : a Paleoecological*
 494 *Perspective*, New York; London: Springer.
- 495 **Doughty, R.W.** 2000. *The Eucalyptus: a Natural and Commercial History of the Gum*
 496 *Tree*, Baltimore: Johns Hopkins University Press.
- 497 **Erdtman, G.** 1960. The acetolysis method, revised description. *Svensk Bot. Tidskr.*, 54,
 498 561-564.
- 499 **Girijashankar, V.** 2010. Effect of *Eucalyptus* pollen isolation methods on pollen
 500 viability, debris content, quantity isolated and pollen density per stigma. *J. Plant*
 501 *Breeding Crop Sci.*, 2, 273-279.
- 502 **Goodenough, A.E., Elliot, S.L. & Hart, A.G.** 2009. The challenges of conservation for
 503 declining migrants: are reserve-based initiatives during the breeding season
 504 appropriate for the Pied Flycatcher *Ficedula hypoleuca*? *Ibis*, 151, 429-439.
- 505 **Hobson, K.A.** 2008. Using endogenous and exogenous markers in bird conservation.
 506 *Bird Conserv. Int.*, 18, S174-S199.

507 **Holm, E. & Laursen, K.** 1982. Observation and experiments on the oral apparatus and
508 its function in some warbler species (Sylviidae). *Zool. Anz. Jena* 209, 224-246.
509 **Huntley, B. & Birks, H.J.B.**, 1983. *An Atlas of Past and Present Pollen Maps for*
510 *Europe: 0-13000 Years Ago*. Cambridge: Cambridge University Press.
511 **Jalas, J. & Suominen, J.** (eds.) (1988) *Atlas Florae Europaeae. Distribution of Vascular*
512 *Plants in Europe*, Cambridge: Cambridge University Press.
513 **Laursen, K., Holm, E. & Sorensen, I.** 1997. Pollen as a marker in migratory warblers,
514 Sylviidae. *Ardea*, 85, 223-231.
515 **Mcgarigal, K., Cushman, S. & Stafford, S.G.** 2000. *Multivariate Statistics for Wildlife*
516 *and Ecology Research*, New York; London: Springer.
517 **Mckinnon, E.A., Fraser, K.C. & Stutchbury, B.J.M.** 2013. New discoveries in
518 landbird migration using geolocators, and a flight plan for the future. *Auk*, 130,
519 211-222.
520 **Mildenhall, D.C., Wiltshire, P.E.J. & Bryant, V.M.** 2006. Forensic palynology: why
521 do it and how it works. *Forensic Sci. Int.*, 163, 163-172.
522 **Moore, P.D., Webb, J.A. & Collinson, M.E.** 1991. *Pollen Analysis*, 2nd ed. ed. Oxford:
523 Blackwell Scientific Publications.
524 **Morrison, C.A., Robinson, R.A., Clark, J.A. & Gill, J.A.** 2010. Spatial and temporal
525 variation in population trends in a long-distance migratory bird. *Divers. Distrib.*,
526 16, 620-627.
527 **Newton, I.** 2010. *Bird Migration*, London: Collins.
528 **Newton, I. & Brockie, K.** 2008. *The Migration Ecology of Birds*, London: Academic
529 Press.
530 **Ortega-Olivencia, A., Rodriguez-Riano, T., Valtuena, F.J., Lopez, J. & Devesa, J.A.**
531 2005. First confirmation of a native bird-pollinated plant in Europe. *Oikos*, 110,
532 578-590.
533 **Prentice, I.C. & Webb, T.** 1998. BIOME 6000: reconstructing global mid-Holocene
534 vegetation patterns from palaeoecological records. *J. Biogeogr.*, 25, 997-1005.
535 **R Core Team** 2013. R: A Language and Environment for Statistical Computing. R
536 *Foundation for Statistical Computing, Vienna, Austria.* <http://www.R-project.org>.
537 **Shaw, P.J.A.** 2003. *Multivariate Statistics for the Environmental Sciences*, London:
538 Arnold.
539 **Sibley, C.G. & Ahlquist, J.E.** 1990. *Phylogeny and Classification of Birds: a Study in*
540 *Molecular Evolution*, New Haven: Yale University Press.
541 **Tabachnick, B.G. & Fidell, L.S.** 2007. *Using Multivariate Statistics*, 5th ed. ed.
542 London: Allyn & Bacon.
543 **Wernham, C.V., Toms, M.P., Marchant, J.H., Clark, J.A., Siriwardena, G.M. &**
544 **Baillie, S.R.** 2002. *The Migration Atlas: Movements of the Birds of Britain and*
545 *Ireland*, London: T. & A.D. Poyser.
546
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*.

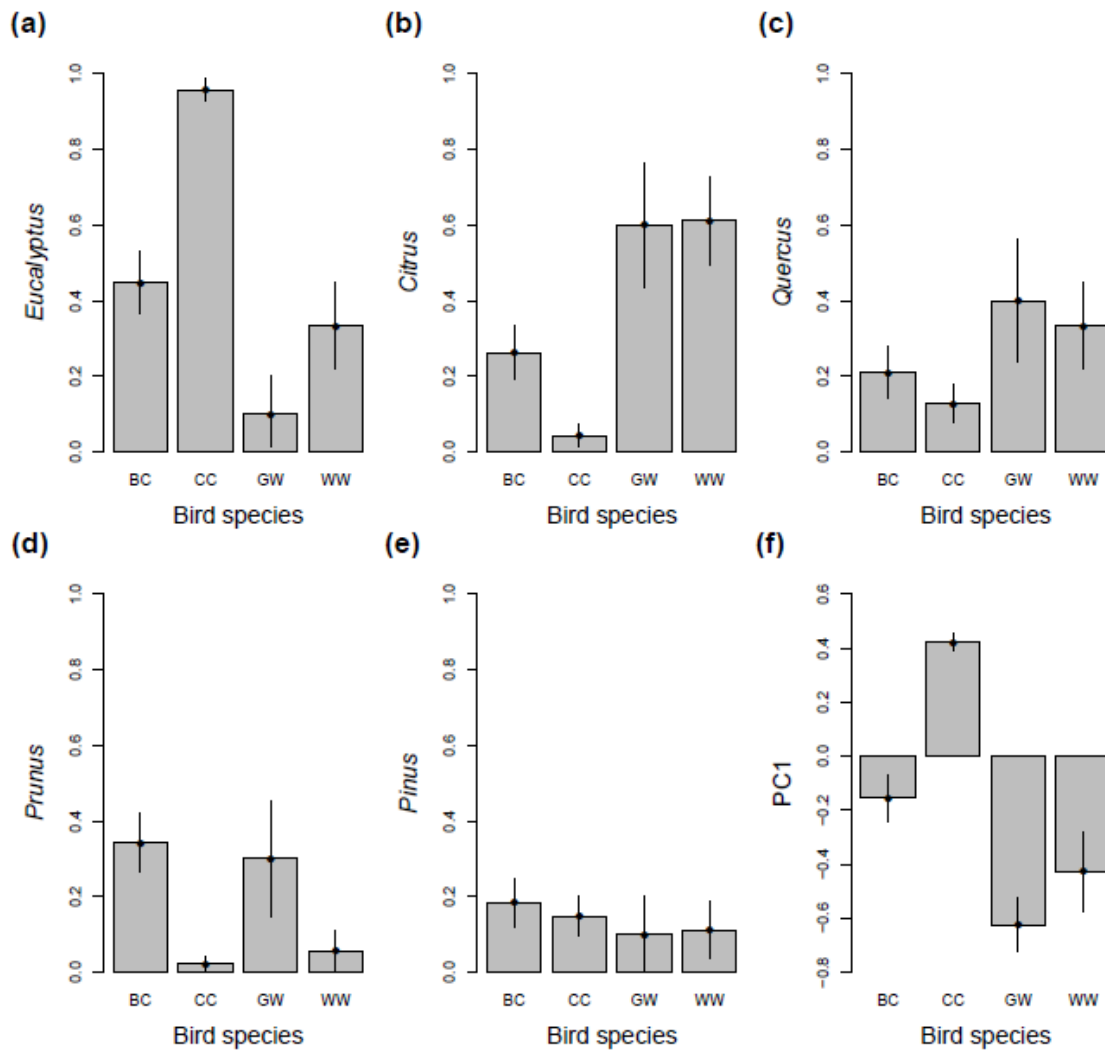


Figure 2.

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		