

1 **Bacterial degradability of white patches on primary feathers**
2 **is associated with breeding date and parental effort in a**
3 **migratory bird**

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14 We compared the in vitro bacterial degradability of the white patch and of the
15 melanized area of primary feathers of breeding male and female Pied Flycatchers
16 *Ficedula hypoleuca* and related it to laying date, brood size and brood mass. Bacterial
17 degradability of male and female white feather patches, but not of melanized ones, was
18 positively correlated with laying date. Male Pied Flycatchers, but not females, showed a
19 positive correlation of bacterial degradability of the white patch, but not of the
20 melanized patch, with brood size and brood mass. Feather degradability appears to be
21 negatively related to individual quality and positively to reproductive effort.

22 **Keywords:** Breeding phenology, feather-degrading bacteria, reproductive effort,
23 unmelanized feather tracts

24

25 Feathers are inert integumentary appendages of birds that wear down and break
26 gradually under natural conditions since their production during moult (Vágási *et al.*
27 2011, Vágási 2014). Physical abrasion, which mainly results from the impact of
28 airborne particles and/or the rubbing of different objects in the environment
29 (Barrowclough & Sibley 1980, Burt 1986, Bonser 1995), as well as feather lice have
30 received considerable attention and have been suggested as the cause of holes on
31 feathers, though this conclusion is still controversial (Pap *et al.* 2005, Vas *et al.* 2008,
32 Vágási 2014). Feather degrading bacteria (FDB) have only been seriously considered in
33 this context since the seminal work by Burt and Ichida (1999). Thus, certain damages
34 could be the consequence of bacterial degradation of feathers (Vágási *et al.* 2010).
35 Abiotic and biotic factors may operate simultaneously, complementarily and additively
36 in natural environments (Vágási *et al.* 2011), thereby partly driving the evolution of
37 feathers towards the production of more resistant structures, for instance through their
38 melanisation (Bonser 1995, Goldstein *et al.* 2004, Ruiz-de-Castañeda *et al.* 2012) or
39 through the application of uropygial oil during preening (Shawkey *et al.* 2003, Moreno-
40 Rueda 2011).

41 Arrival and subsequent breeding dates in long distance migrants are dependent on
42 general individual quality and condition (Møller *et al.* 1994, Kokko *et al.* 1999),
43 including feather quality which seems particularly important in this context (Møller *et*
44 *al.* 2004, Pap *et al.* 2005). Thus, we should expect late breeders to present a more
45 degraded plumage than early breeders. As breeding date is normally repeatable
46 (Lundberg & Alatalo 1992, Svensson 1997, Van der Heugd & McCleery 2002), the
47 period between moult and breeding in the next season should be equal for all
48 individuals, so this difference should not be due to variation in feather age since moult.

49 Given the stronger selection on males to arrive early, the association between feather
50 wear and breeding date should be especially marked in males.

51 By the end of the reproductive cycle feathers reach their poorest physical state and
52 are thus replaced during moult. Interestingly, feather wear does not increase linearly
53 throughout the annual cycle, but becomes disproportionately accentuated during the
54 breeding season when birds face a higher workload and elevated locomotor activity
55 (Vágási *et al.* 2011). Lucas *et al.* (2005) in a brood size manipulation experiment in
56 European Starlings *Sturnus vulgaris*, found that birds caring for enlarged broods had
57 more free-living bacteria on their chest feathers than birds with reduced broods. Life-
58 history theory predicts that allocation of time, energy and resources to feather protection
59 (e.g., preening) can conflict with parental care (Whittingham 1993, Merilä & Hemborg
60 2000).

61 The Pied Flycatcher *Ficedula hypoleuca* is a long-distance migrant with a sexually
62 dichromatic plumage, showing unmelanized patches on the head and wings at the base
63 of primaries and secondaries. The size of the patch in male *Ficedula* flycatchers is
64 relevant in territorial intra-sexual conflicts (Garamszegi *et al.* 2006, Hegyi *et al.* 2008),
65 and in sexual selection in males (Sheldon & Ellegren 1999, Sirkiä & Laaksonen 2009,
66 Sirkiä *et al.* 2010) and possibly in social selection in females (Morales *et al.* 2007).
67 Male Collared Flycatchers *Ficedula albicollis* with large white wing patches also
68 survive better than those with small patches (Török *et al.* 2003).

69 In a recent study using partly the same data set as the one considered here, we found
70 that the white wing patch of the fourth primary feather of pied flycatchers was more
71 degradable *in vitro* by *Bacillus licheniformis* than the contiguous melanized area within
72 the same feather (Ruiz-de-Castañeda *et al.* 2012). Although, previous studies had
73 already demonstrated that dark melanized feathers resist bacterial degradation more

74 effectively than white unmelanized feathers (Goldstein *et al.* 2004, Gunderson *et al.*
75 2008), our study confirmed for the first time this pattern at an intra-feather level. In
76 addition, we also found sex differences in bacterial degradability of the white feather
77 patch, males showing more degradable white patches possibly because they have been
78 subjected to stronger sexual selection than females. We have here focused on the
79 variation of *in vitro* bacterial degradability of the white feather patch in relation to
80 brood size and laying date. We assume that feathers in a poor state at the moment of
81 collection in the field may be subsequently more degradable by bacteria in *in vitro* tests.
82 Based on this assumption, we predict that: (1) Breeding date may correlate negatively
83 with feather quality and positively with *in vitro* bacterial degradability of the
84 unmelanized wing feather patch in males and females, as late breeders may have a
85 plumage of poorer quality; (2) Parental effort, estimated as brood mass and number of
86 nestlings, may correlate positively with *in vitro* bacterial degradability of the
87 unmelanized wing feather patch in males and females. This correlation may be mediated
88 by the costs of increased parental effort on the physical state of feathers in the field; (3)
89 Due to the higher resistance to bacterial degradation of melanized than of unmelanized
90 feather tracts, the previous predictions may apply less clearly to the melanized tracts of
91 the primary feathers.

92

93 **METHODS**

94

95 We conducted this study in 2009 on a population of Pied Flycatchers breeding in
96 nest-boxes in a montane oak forest in Valsaín, central Spain (see Ruiz-de-Castañeda *et*
97 *al.* 2012 for details). The Pied Flycatcher is a small migratory, hole-nesting passerine
98 that breeds in central Spain. In the study area egg laying typically occurs in late May,

99 and clutch sizes range from two to six eggs (Sanz et al. 2003). Both sexes feed the
100 young and fledging usually takes place within 14-16 days of hatching.

101 We here considered a subset of the individuals included in Ruiz-de-Castañeda *et al.*
102 (2012). A total of 60 nests were included (58 pairs plus two females). Birds were
103 captured when nestlings attained 13 days (hatching date = day 1). On the same day, we
104 recorded the number of nestlings and we weighed them individually to the nearest 0.1g
105 with a Pesola spring balance. We previously recorded the exact date when the first egg
106 was laid.

107 Aseptic feather sampling techniques and conservation conditions used here were
108 fully described in Ruiz-de-Castañeda *et al.* (2012), as well as the protocol followed to
109 obtain *in vitro* bacterial degradability data. For this study, we used 51 unmelanized and
110 51 melanized fragments from females, and 54 unmelanized and 44 melanized fragments
111 from males.

112 We constructed four different General Linear Models (GLM), two for females and
113 two for males, including *in vitro* bacterial degradability of the unmelanized and
114 melanized fragments as the normal dependent continuous variable. Laying date and
115 brood mass were included as continuous predictors. Brood mass at the age of 13 days
116 and brood size were highly correlated ($r = 0.96$, $p < 0.001$, $n = 60$), and these results
117 were equivalent. Here, we present only the results for brood mass. Note that we
118 controlled statistically in all the models for the length of the analyzed fragment by
119 including this variable as a continuous predictor.

120 Final models were obtained by a backward deletion procedure. We first obtained the
121 full model, including all possible predictors and then we sequentially removed variables
122 when the variance explained did not significantly improve the model ($\alpha = 0.05$).

123 For those predictors that were significantly correlated with bacterial degradability of
124 the unmelanized or melanized fragments in both males and females, we compared the
125 slope of the correlations between males and females using the module Homogeneity of
126 Slopes in *Statistica 7.0*.

127

128 **RESULTS**

129 For the unmelanized patch of males, laying date and brood mass were significantly
130 and positively correlated with bacterial degradability of the unmelanized feather patch
131 when controlling for length of the fragment (GLM, laying date: $F_{1, 51} = 7.70$, $p = 0.01$;
132 brood mass: $F_{1, 51} = 7.21$, $p = 0.01$; fragment length: $F_{1, 51} = 14.75$, $p < 0.001$; $r^2 = 0.32$).
133 In other words, late-breeding males (Fig. 1a) and males caring for large broods (Fig. 2)
134 presented primary feathers whose white tracts were more susceptible to bacterial
135 degradation. We tested the possible presence of influential observations using the
136 Cook's distance values (D_i) and found 5 relevant observations. Then, we excluded these
137 observations and ran our model to test the influence of these observations on the
138 estimated parameters of the model and the result was the same. For the melanized
139 fragment of males, only the length of the fragment was included in the final model
140 (GLM, $F_{1, 41} = 5.00$, $p = 0.03$).

141 For the unmelanized feather patch of females, only laying date was positively
142 correlated with bacterial degradability of the wing feather patch when controlling
143 statistically for length of the fragment (GLM, laying date: $F_{1, 51} = 5.78$, $p = 0.02$;
144 fragment length: $F_{1, 51} = 5.07$, $p = 0.03$; $r^2 = 0.17$). Late-breeding females had more
145 degradable white patches than early-breeding ones (Fig. 1b). In the case of the
146 melanized fragment in females, no variable was significantly associated with their
147 bacterial degradability (all $p > 0.11$). Furthermore, the slopes of the correlations

148 between bacterial degradability and laying date for males and females were not
149 significantly different ($F_{1, 102} < 0.001, p = 0.98$).

150

151 **DISCUSSION**

152 Our findings suggest that bacterial degradability of the unmelanized feather patch in
153 both adults increased with delayed laying dates, a good indicator of phenotypic quality.
154 Moreover, an increased parental effort resulted in greater *in vitro* bacterial degradability
155 of a socially and sexually selected unmelanized plumage trait. Males whose broods
156 were heavier and larger showed higher *in vitro* bacterial degradability of the
157 unmelanized white wing patch of primary feathers.

158 Bacterial degradability of the unmelanized wing feather patch in both males and
159 females in our population correlated positively with laying date, with the slope of these
160 correlations not being significantly different in both sexes. This supports the general
161 assumption that early breeding is associated with the best quality individuals (Møller
162 1994, Kokko 1991), including quality of their plumage and/or their protective
163 adaptations (Møller *et al.* 2004). For example, Pap *et al.* (2005) found that the number
164 of feather holes in Barn Swallows *Hirundo rustica* was positively correlated with arrival
165 dates, i.e late breeders showed an increased number of feather holes.

166 Activities associated with parental care can greatly deteriorate the quality of feathers
167 due to increased locomotion and prolonged stays in abrasive and/or infectious (sources
168 of lice and bacteria) environments and due to limited time, energy and resources
169 available for feather protection (Lucas *et al.* 2005, Vágási *et al.* 2011). Accordingly,
170 male pied flycatchers of our population that were in charge of larger broods possibly
171 could have had more structurally degraded feathers at the moment of collection in the
172 field, which could be accordingly also more degradable by bacteria in our latter *in vitro*

173 tests. Ramnani *et al.* (2005) using scanning electron microscopy found that *Bacillus*
174 *licheniformis* grew closely adhered to the barbules. Thus, it is possible that more
175 degradable feathers had already structurally impaired barbules which could be more
176 easily attacked by bacteria (Ruiz-Rodriguez *et al.* 2009). However, we cannot confirm
177 this assumption of our study as we did not measure feather degradation before *in vitro*
178 tests. As shown by Ruiz-de-Castañeda *et al.* (2012), the white wing feather patch in
179 female pied flycatchers is more resistant to *in vitro* bacterial degradation than in males,
180 possibly because males experience more intense sexual selection to produce honest
181 signals in the context of sexual signaling, which could explain these differences in
182 relation to brood mass. This may have precluded finding an association of degradability
183 with brood mass in females.

184 Neither brood mass nor laying date explained *in vitro* bacterial degradability of the
185 melanized area of the primary feathers in male and female pied flycatchers in our
186 population. This is in accordance with our previous study (Ruiz-de-Castañeda *et al.*
187 2012) where we showed that melanized areas were more resistant to *in vitro* bacterial
188 degradation than the white patches, and that males and females did not differ in the
189 degradability of the melanized part of the feathers. Because the largest part of the
190 primary feather surface in pied flycatchers is melanized, its resistance is probably
191 critical to facilitate flight. Thus, sensitiveness of these areas to reproductive costs may
192 have been minimized during evolution.

193 Our results suggest that breeding phenology seems a relevant predictor of bacterial
194 feather degradability in males and females in accordance with the fact that the best
195 individuals presumably breed earlier. Furthermore, we found that male pied flycatchers
196 in charge of larger broods may pay extra costs in terms of reduced feather condition
197 making their white wing feather patches more degradable by bacteria.

198
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209

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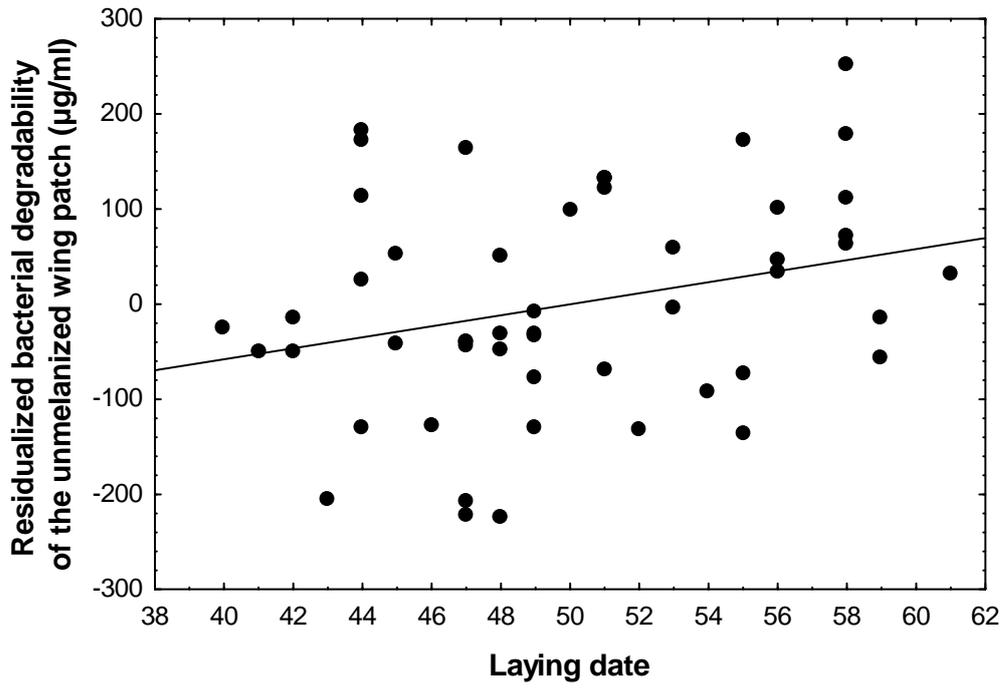
290 **Figure 1.** Bacterial degradability of unmelanized wing patches of the 4th primary in
291 males (a) and females (b) in relation to laying date (1 = 1st April) when controlling for
292 length of the wing patch. We present bacterial degradability residualized by size of the
293 wing patch (Males: $r = 0.28$, $p = 0.05$, $n = 51$; Females: $r = 0.32$, $p = 0.02$, $n = 51$).

294

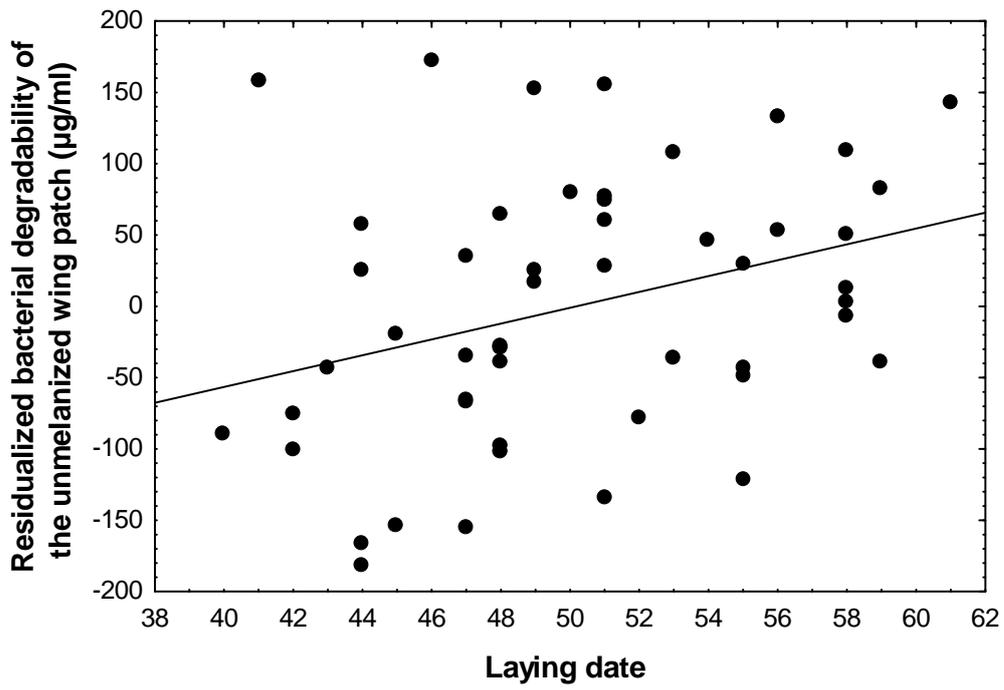
295 **Figure 2.** Bacterial degradability of the unmelanized wing patch of the 4th primary in
296 males in relation to brood mass when controlling for laying date and length of the patch.
297 We present bacterial degradability residualized by laying date and size of the wing
298 patch ($r = 0.47$, $p = 0.001$, $n = 46$).

299

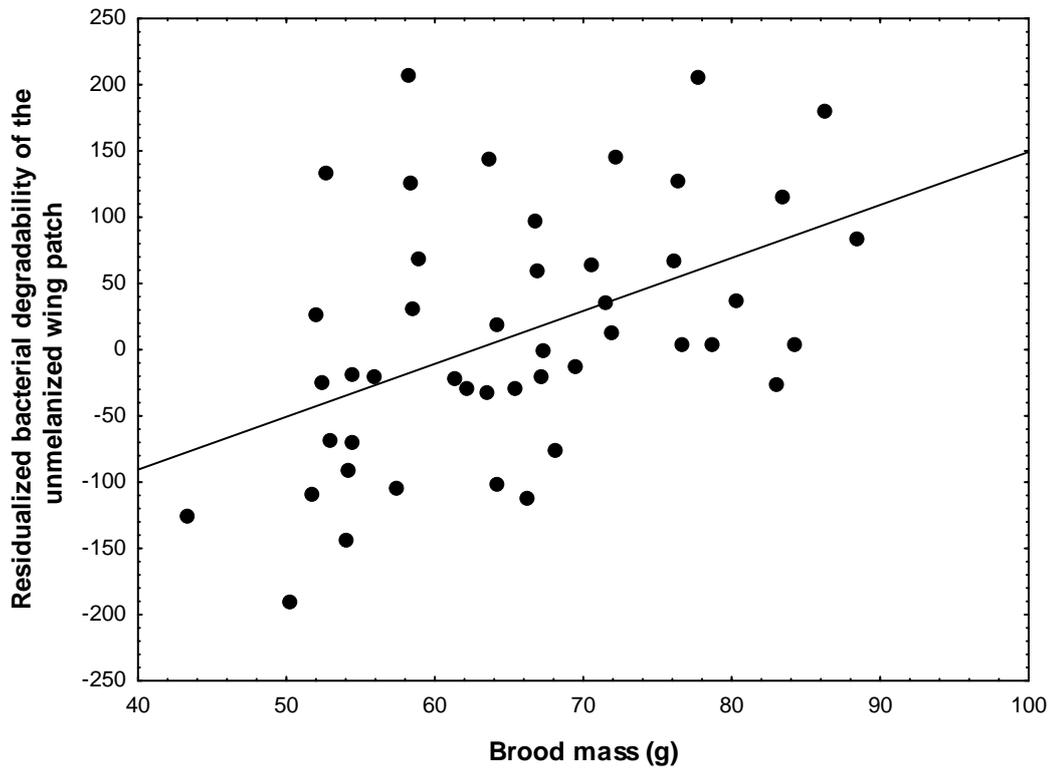
300 Fig. 1



301



302



304 Fig. 2