| 1 | Vertical transmission in feather mites: |
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| 2 | insights on its adaptive value |
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23 Abstract.

- 24 1. The consequences of symbiont transmission strategies are better understood than their
- 25 adaptive causes.
- 26 2. Feather mites are permanent ectosymbionts of birds assumed to transmit mainly
- 27 vertically from parents to offspring. We studied the transmission of Proctophyllodes
- 28 doleophyes Gaud (Astigmata, Proctophyllodidae) in two European pied flycatchers,
- 29 Ficedula hypoleuca Pallas (Passeriformes, Muscicapidae) populations.
- 30 3. We experimentally demonstrate the vertical transmission of this mite species with an
- 31 acaricide experiment. We also compared (for two distant populations during four years)
- 32 reduction in mite intensity in adult birds from egg incubation to chick rearing periods,
- 33 against the predictions of three hypotheses on the relevance of host survival prospects
- 34 and mite intraspecific competition driving feather mites' transmission strategy.
- 35 4. Results are congruent with previous studies and show that feather mites massively
- 36 transmit from parents to chicks.
- 37 5. The magnitude of the transmission was closer to that predicted by our hypothesis
- 38 based on intraspecific competition, while a bet-hedging strategy is also discussed.

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| 47 | Keywords: Analgoidea, bet-hedging, dispersion, host-parasite interactions, symbionts. |
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49 Introduction

Individual hosts are ephemeral islands for symbionts, making transmission essential for 50 51 symbiont biology, and shaping their ecology and evolution (Poulin 2011, Clayton et al. 52 2015). Mode of transmission (e.g. horizontal versus vertical, or phoresis versus 53 autonomous movement) is one of the best known life-history traits for many symbiont 54 species, and the ecological (e.g. disease spread) and evolutionary (e.g. parasite 55 virulence) consequences of transmission strategies are well studied. However, the 56 adaptive reasons by which symbiont species have evolved a particular transmission 57 strategy remain elusive, and thus ultimate drivers of the diversity of transmission modes 58 are poorly understood. 59 Permanent symbionts such as feather mites or feather lice living on bird feathers 60 are suitable models to study transmission under natural conditions because they can be 61 studied noninvasively (e.g. Harbison et al. 2008). Moreover, they may be vertically 62 transmitted from parents to offspring in the nest and horizontally in social species, and 63 they can be seen with the naked eye or slight magnification in the field, thus simplifying 64 their study (Clayton et al. 2015). While feather lice are bird parasites and feather mites 65 most likely commensals or even mutualists of birds (Blanco et al. 2001, Galván et al. 2012), some aspects of their ecology are similar enough to gain insight from their 66 67 mutual comparison (Jovani 2003). For instance, studies on feather lice suggest that the species locomotory capabilities as well as inter and intraspecific competition are the 68 69 main drivers of their transmission (Harbison et al. 2008, Bartlow et al. 2016): species 70 less able to move when off the host and competitively inferior species (e.g. who may 71 obtain benefits leaving a crowded host in search of a less crowded one) are more likely 72 to highly disperse through vertical and phoretic transmission (Bartlow et al. 2016).

73 However, additional factors are likely at play in the evolution of transmission strategies.

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| 74 | For instance, host survival and intraspecific competition have been suggested to pose a |
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| 75 | trade-off in vertical transmission from parents to offspring (Darolova et al. 2001, |
| 76 | Brooke 2010): while nestlings are hosts with low survival prospects, remaining in the |
| 77 | adult implies higher intraspecific and interspecific competition (Harbison et al. 2008, |
| 78 | Brooke 2010). |
| 79 | Feather mites (Acari: Astigmata: Analgoidea and Pterolichoidea) are among the |
| 80 | most abundant ectosymbionts of birds (Proctor 2003, Doña et al. 2016). Current |
| 81 | evidence indicates that they transmit from parents to offspring at the nest through body- |
| 82 | body contact during chick rearing (Mironov & Malyshev, 2002), or among birds in |
| 83 | close contact outside the nest (Blanco et al. 1997), and only anecdotally by phoresis |
| 84 | (Jovani et al. 2001, Proctor 2003). Indeed, they cannot survive off of hosts (Dubinin |
| 85 | 1951, Proctor 2003) and, as a consequence, have evolved adaptations for not falling off |
| 86 | them, such as flattened bodies, broad clasping "feet" (ambulacra) and hooking spines on |
| 87 | body and legs (Mironov 1999), and behavioural adaptations such as avoiding feathers |
| 88 | about to be moulted (Jovani & Serrano 2001). |
| 89 | After previous anecdotal data by Dubinin (1951), the only studies that directly |
| 90 | addressed feather mite transmission were those by Mironov (2000) and Mironov and |
| 91 | Malyshev (2002), who studied three feather mite species of the common chaffinch |
| 92 | (Fringilla coelebs). These studies assumed that all feather mites found on nestlings |
| 93 | came from their parents (as our experiment also supports, see below) and, thus, that the |
| 94 | reduction in the intensity of feather mites on breeding adults from egg incubation to |
| 95 | chick rearing periods is caused by this transmission. From Dubinin (1951), Mironov |
| 96 | (2000) and Mironov and Malyshev (2002) studies (and considering the results here |
| 97 | presented) one can extract three important patterns in the transmission of feather mites. |
| 98 | First, about three quarters of the mites on the female parent transmit to her offspring. |

| 99 | Second, adults and tritonymphs (the last juvenile stage before becoming adults) are the | |
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| 100 | main mite transmitting stages. Third, chicks are progressively occupied by mites during | |
| 101 | their stay on the nest (i.e. when their flight feathers grow), and most chicks had feather | |
| 102 | mites before leaving the nest. | |
| 103 | Such transmission may seem maladaptive because most of the chicks from a nest | |
| 104 | will never survive (see below) and, consequently, transmission from parents to offspring | |
| 105 | seems a likely dead end for feather mites compared to remaining in the adult host. Why | |
| 106 | then do feather mites have this mass transmission to nestlings? | |
| 107 | In this study, we experimentally studied the vertical transmission of feather mites | |
| 108 | from European pied flycatchers, Ficedula hypoleuca. In addition, we test three | |
| 109 | hypotheses on the adaptive value of feather mite vertical transmission by integrating | |
| 110 | information on adult and chick host survival and the proportion of mites transmitting | |
| 111 | from parents to nestlings and the distribution of mites among nestlings. From a long- | |
| 112 | term study (see Camacho et al. 2015 for an overview) in a Spanish population (22 | |
| 113 | breeding seasons, 4,673 adults and 9,901 chicks ringed) we calculated a 52% | |
| 114 | probability that breeding adults would reproduce again in the study population (51% for | |
| 115 | males, 52% for females; JP, unpubl. data). However, nestlings ringed at age 13 days | |
| 116 | (they leave the nest at age 14-20 days; Potti & Montalvo 1993) have only about 13% | |
| 117 | probability of becoming breeders in the studied population in future years (the life | |
| 118 | expectancy of flycatchers is ca. 7 years; Potti 2000). Even after accounting for a higher | |
| 119 | emigration of young birds, this shows that passing from an adult to a nestling bird | |
| 120 | carries a strong risk for feather mites | |
| 121 | From a mite point of view, the probability of leaving the adult bird host would | |
| 122 | be $r = 100 \times (1 - (\frac{adult survival}{adult survival} + chick survival}))$. This hypothesis (H1) thus proposes a | |

123 probabilistic (rather than a binary) nature of the decision of feather mites to transmit to a

| 124 | chick or remain on the parent bird. We propose that natural selection has shaped the |
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| 125 | sensitivity of mites to particular cues (such as food availability or feather structure) that |
| 126 | indicate adult-vs-chick survival prospects. Overall, if feather mites only take this into |
| 127 | account for transmission, and given the adults/chicks survival expectancies of |
| 128 | flycatchers used in this study (see above), H1 would predict (assuming that all feather |
| 129 | mites behave similarly in these terms) that reduction in feather mite intensity in bird |
| 130 | parents (r) would be of 20% from egg incubation to the rearing of fledglings. |
| 131 | This hypothesis, however, does not take into account intraspecific competition in |
| 132 | feather mites. For instance, inter and intraspecific competition have been found to be |
| 133 | highly relevant for the transmission of feather lice (Harbison et al. 2008). In addition, |
| 134 | current evidence suggests that competition is one factor explaining the habitat |
| 135 | partitioning of feather mites (Fernández-González et al. 2015). In fact, feather mites are |
| 136 | known to maximize their spread even among feathers (i.e. among those they are |
| 137 | specialized to live on) and to segregate between the two wings of the bird host; i.e. |
| 138 | rather than concentrating in one wing they spread as much as possible, and this is even |
| 139 | true for birds having only a few mites (Jovani & Serrano 2004, Fernández-González et |
| 140 | al. 2015). So, in mite infrapopulations (i.e. all the mites living on a given bird) |
| 141 | intraspecific competition could favour occupying a lower quality (i.e. in terms of |
| 142 | survival) habitat if mite density is lower there. Therefore, in a system where the habitat |
| 143 | patches (the hosts) are ephemeral and where individuals are so sensitive to habitat loss |
| 144 | (if the host dies the mites will likely die) but competition is present, we hypothesise that |
| 145 | the transmission strategy of feather mites may have evolved under a trade-off between |
| 146 | high host survival (prioritizing less ephemeral hosts: parent birds) and low intraspecific |
| 147 | competition (prioritizing less crowded hosts: fledglings). Thus, our H2 hypothesis |
| 148 | predicts that mites would distribute among all available chicks, but that their |

| 149 | distribution should be weighted by the relative survival expectancies of mites on the |
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| 150 | different hosts (adults vs. chicks). In other words, mites would colonize all chicks to |
| 151 | take advantage of the relatively lower competition for space (and maybe for food) on |
| 152 | the 'empty' feathers of the chicks, but they would prefer to stay in the parent bird |
| 153 | because of higher host survival. So, the number of mites passing would depend on a |
| 154 | balance between host survival and the number of available hosts. In this scenario, we |
| 155 | estimated r as for H1, but now, in the denominator, chick survival was multiplied by the |
| 156 | number of chicks in the nest (e.g. for a nest with five nestlings H2 predicts a 60% |
| 157 | reduction of feather mite intensity in the parent |
| 158 | bird): $r = 100 \times (1 - (\frac{adult survival}{(adult survival + (chick survival * number of chicks))}))$ |
| 159 | |
| 160 | Our third hypothesis (H3) does not take into account host survival but only |
| 161 | intraspecific competition as the main driver of feather mite vertical transmission. |
| 162 | Therefore, under our H3 hypothesis feather mite numbers would decrease in parents |
| 163 | according to the number of nestlings in the nest (i.e. by maximizing as much as possible |
| 164 | the distribution among available hosts) as they do between the two wings of a bird (see |
| 165 | above). We estimated the expected percentage of reduction by dividing 100 by the |
| 166 | number of chicks plus one (the parent) as $r = 100 - (\frac{100}{number of chicks + 1})$. This |
| 167 | hypothesis considers that mites leave each parent towards chicks, and there they spread |
| 168 | as much as possible. So, it does not contemplate the effect of the number of mites |
| 169 | present in the other parent as it is unlikely that both adults would coincide in the nest |
| 170 | during mites' transmission. |
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172 Materials and methods

173 Sampling

We investigated the feather mite from European pied flycatchers in two populations 174 from Central Spain (see above) and The Netherlands (De Hoge Veluwe, 52° 2' N, 5° 51' 175 176 W) during four years. In both study localities birds were ringed with metal and coloured plastic bands. Feather mites were counted from primary, secondary and tertial feathers 177 178 of the right wing with the naked eye or with the help of a 10x magnifying glass 179 exposing the wing against day light. Even though this count method could entail some 180 error (i.e. because of daily movement of mites or cast skins, Proctor & Owens 2000), it 181 generally gives good estimates of the number of mites in a given bird (Behnke et al. 182 1999, Jovani & Serrano 2004). Moreover, the same observer counted mites within each 183 study unit (e.g. for different chicks from a nest, or for the two measurements of feather 184 mite numbers for a given parent bird), thus avoiding problems because of inter-observer 185 differences. Proctophyllodes doleophyes, is the most repeatedly recorded plumicolous 186 feather mite species of pied flycatchers (Doña et al. 2016). Moreover, this was the 187 species identified in a previous study from Spanish European pied flycatchers, firstly by 188 Sergey Mironov (Zoological Institute, Russian Academy of Sciences) and afterwards 189 with molecular barcoding (Doña et al. 2015a, b). 190 A total of 243 individual adult flycatchers were examined for feather mites. In The Netherlands, during 2009 and 2010 breeding seasons, 55 adult birds were inspected 191 192 for feather mites twice (paired data): both prior to and after the hatching of their chicks. 193 Adult male flycatchers (n=13) were captured inside empty nestboxes in May using clap-194 traps when still unmated or when their primary female was incubating and they were 195 trying to attract a secondary female to another nestbox. Adult females (n=42) were captured by hand in the nestboxes during their second week of incubation. Then, adult 196 197 males and females were recaptured when chicks were 7-8 days old. In Spain, during

| 198 | years 2000 and 2001, a total of 188 adult birds was sampled either during incubation or | |
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| 199 | while caring for naked chicks (three days old, i.e. before feather mite transmission, | |
| 200 | Mironov & Malyshev 2002). 101 of these birds were recaptured when chicks were 12- | |
| 201 | 13 days old, thus providing paired data. To reduce the proportion of estimation errors | |
| 202 | derived from small values, the mean and the standard deviation calculations of the | |
| 203 | reductions were done over birds with more than 25 mites during the pre-hatching period | |
| 204 | resulting in birds being excluded. In addition, we studied feather mite loads for all | |
| 205 | nestlings in 24 nests (92 nestlings aged 13 days) from the Spanish population (a | |
| 206 | subsample of the control nests from the acaricide experiment, see below). | |
| 207 | | |
| 208 | Acaricide experimental design | |
| 209 | In 2000 and 2001, in La Hiruela, (41° 04' N, 3° 27' W, Madrid, Central Spain), we | |
| 210 | experimentally studied the vertical transmission of feather mites from European pied | |
| 211 | flycatchers. We selected 45 nests where at least one of the two parents had feather mites | |
| 212 | during the egg incubation period. We applied three treatments: 1) In 11 nests, we | |
| 213 | fumigated parent feathers with a common acaricide Tabernil® (Tetramethrin 0.175, | |
| 214 | Piperonyl butoxide 0.910, in gaseous form); 2) In 28 nests parents were given a sham | |
| 215 | treatment with water; 3) In the remaining six nests parents were not treated with | |
| 216 | Tabernil nor with water. Later, when chicks were 13 days old, we inspected the wings of | |
| 217 | most adult birds (15 and 46 from the acaricide and control treatments, respectively) and | |
| 218 | all nestlings from all nests for feather mite presence. Given the low sample size and the | |
| 219 | congruence of the results among control treatments (see below), adult reductions of | |
| 220 | these treatments are reported pooled. | |
| 221 | | |

222 Results

| 223 | In the acaricide-treated nests, almost all adults (i.e. 80%) completely lost their feather |
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| 224 | mites (Fig. S1), except for two birds that conserved a single mite. In comparison, only |
| 225 | 14% of adults in the control nests completely lost their feather mites (chi-square = |
| 226 | 23.81, $P < 0.01$; Fig. S1). As expected, we did not find any feather mite in chicks from |
| 227 | experimental parents, but we detected feather mites in nestlings from 88% of control |
| 228 | nests [Number of nests positive for feather mites presence at day 13: Acaricide |
| 229 | treatment: zero out of eleven; Control treatment one: 25 out of 28; Control treatment |
| 230 | two: five out of six; chi-square = 29.20, $P < 0.01$). Thus, this experiment supports the |
| 231 | hypothesis that feather mites found on nestlings come exclusively from their parents |
| 232 | and not by any other means (e.g. from feather mites coming attached on hippoboscid |
| 233 | flies, or feather mites remaining on the nest-box from a previous year, Jovani et al. |
| 234 | 2001). |
| | |
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| 247 | (paired: 71% of mean reduction; Wilcoxon, $V = 423$, $P < 0.01$; unpaired: Wilcoxon, $W =$ | |
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| 248 | 662.5, <i>P</i> < 0.01). | |
| 249 | Females had larger and consistent decreases in feather mite intensities (GLM: | |
| 250 | Z=8.57; $P<0.01$; Fig 1). Mite load reductions in males were more variable than in | |
| 251 | females (and some males even increased their feather mite load, Fig. 1) which led to a | |
| 252 | significant sex*country interaction on r (GLM: Z=-12.90; P<0.01; Fig. 1, 2). In | |
| 253 | addition, male and female parents had similar feather mite loads before hatching of the | |
| 254 | chicks (Spearman's correlation, $\rho = 0.51$, $P < 0.01$; Fig S2) and also while rearing | |
| 255 | chicks (Spearman's correlation, $\rho = 0.41$, $P = 0.02$; Fig S3). | |
| 256 | Mite load reductions in both male and female parents in both countries notably | |
| 257 | exceeded the predictions arising from the relative survival prospects of parents vs | |
| 258 | nestlings (our H1; Wilcoxon, all P <0.05; Fig.2) and even exceeded the prediction of our | |
| 259 | H2 (all $P \le 0.05$; Fig.2). Feather mite reductions were much closer to those predicted by | |
| 260 | our maximized distribution hypothesis (H3; Wilcoxon, for all except females in Spain | |
| 261 | P>0.05; Fig. 2). Nonetheless, even in this case the reductions exceeded (but by much | |
| 262 | less) those predicted by H3 and thus leading to obtain statistical significance in the | |
| 263 | group of females in Spain (Wilcoxon, V =1899.5, P<0.01). In fact, H2 and H3 equations | |
| 264 | likely overestimate mite reductions when both parents have mites. This is because if the | |
| 265 | other parent is also passing mites to nestlings, mite intraspecific competition would | |
| 266 | increase in nestlings and thus fewer mites would pass from parent to nestlings. | |
| 267 | Therefore, our data show that the transmission is closer to H3 predictions, but even in | |
| 268 | this case feather mite transmission seems more extreme than H3 predicts. | |
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270 Discussion

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| 271 | Results suggest that the answer to why feather mites transmit massively to nestlings (i.e. |
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| 272 | a large proportion of the mites leave the parents) may be that benefits of avoiding |
| 273 | interspecific competition exceed the risks of transmitting to a host with lower survival |
| 274 | probabilities (the nestling). Thus, the observed transmission patterns of feather mites |
| 275 | should be interpreted as if transmitting mites were trying to avoid intraspecific |
| 276 | competition by distributing as evenly as possible among available hosts, either adults or |
| 277 | nestlings. This scenario is reinforced by the high prevalence (i.e. among nestling in |
| 278 | those nests where parents had mites) and repeatable abundance of feather mites within |
| 279 | nests we found. However, the aggregated mite distribution observed within chicks of the |
| 280 | same brood suggests that there are other factors (e.g. food resources and/or chick age) |
| 281 | promoting among-sibling variation in feather mite loads. |
| 282 | It could be argued that transmission in this system may be the result of despotic |
| 283 | competition, where subordinate mites are forced to occupy habitats of lower quality |
| 284 | (nestlings instead of adults). However, according to Mironov and Malyshev (2002), |
| 285 | tritonymphal and adult mites (the largest stages, and thus likely competitively superior) |
| 286 | are the ones involved in transmission. Moreover, we found that the absolute mite |
| 287 | reduction in parents was proportional to the number of mites recorded in the same birds |
| 288 | before transmission (females, Spain: GLM Z=83.06, P<0.01; Netherlands, GLM |
| 289 | Z=83.53, P<0.01; males, Spain, GLM Z=21.63, P<0.01; Netherlands, GLM Z=51.82, |
| 290 | P < 0.01; Fig. 1). In other words, those birds with more mites also lost more mites. |
| 291 | However, the relative reduction (i.e. the percentage of mites lost) was quite constant and |
| 292 | unrelated to the number of feather mites before transmission (Spearman correlations, all |
| 293 | P > 0.05 except for males in Spain). This gives support to the idea that transmitting mite |
| 294 | stages are in a similar proportion in different bird parents (Mironov & Malyshev 2002). |

| 295 | It may seem paradoxical that the most competitive immature stages, because of |
|-----|---|
| 296 | their size, are the ones leaving the adult bird (Mironov & Malyshev 2002), that is, the |
| 297 | hosts with higher survival chances and probably the ones providing more food to mites |
| 298 | (Haribal et al. 2011). A potential explanation is that tritonymphs and adult mites are the |
| 299 | most able to transmit, and thus those whose costs of transmission may be lower |
| 300 | (Mironov & Malyshev 2002). However, a non-exclusive hypothesis may involve |
| 301 | inclusive fitness. This is supported by the way in which population dynamics of feather |
| 302 | mites matches the life cycle of birds: feather mites reduce their numbers in birds during |
| 303 | bird breeding because of vertical transmission (as shown here) and then increase |
| 304 | through the year, reaching a maximum close to the next bird breeding season (Dubinin |
| 305 | 1951, Pap et al. 2010, Haribal et al. 2011, authors unpublished data). Given the |
| 306 | presumed low chance of horizontal transmission of these mites, this may indicate that |
| 307 | most of the mites found in a given bird can be close relatives (Doña et al. 2015a) and |
| 308 | that infrapopulations may be close to their carrying capacity before bird reproduction |
| 309 | (Pap et al. 2010, Haribal et al. 2011). Therefore, negative density-dependence would |
| 310 | increase the benefits of transmitting to a new host not only because it is free of mites |
| 311 | (our H3) but because mites are competing for resources with close relatives and thus |
| 312 | decrease their own inclusive fitness by remaining in the adult bird. In fact, in a previous |
| 313 | study we found that mitochondrial genetic diversity of mite species is highly repeatable |
| 314 | between birds of a given species (Doña et al. 2015a). In other words, in a mite species |
| 315 | with a high genetic diversity, each bird had several haplotypes (note that an alternative |
| 316 | would be that each bird had a single haplotype and that the sum of all birds lead to a |
| 317 | high genetic diversity of the mite species). Thus, this may suggest that mites from a |
| 318 | given haplotype spread among the different nestlings of a given nest, and that they do |
| 319 | not concentrate on a single nestling. This encourages future studies on the trade-off |

| 320 | between intraspecific competition and host survival, but also incorporating inclusive | |
|-----|---|--|
| 321 | fitness as a likely relevant component for understanding symbiont transmission in | |
| 322 | genetically isolated systems. | |
| 323 | Even if mite lineages spread among hosts (e.g. because of intraspecific | |
| 324 | competition, H3), this leads to an interesting outcome for mite lineages because the | |
| 325 | survival probability of a lineage not leaving the adult host would be 52%, but by | |
| 326 | spreading to the (for instance) five nestlings of the nest, the feather mite lineage | |
| 327 | increases to 76% the probability that at least one of the hosts will survive until | |
| 328 | reproduction, and thus until the next transmission opportunity for mites. Thus, feather | |
| 329 | mite lineages increase their survival expectancies by spreading the risk of mortality | |
| 330 | among the available hosts, and if so, this could be understood as a bet-hedging strategy | |
| 331 | (Fenton & Hudson 2002). | |
| 332 | Constraints may also be involved in feather mite transmission. In fact, bottleneck | |
| 333 | genetic signatures have been found for most feather mite species studied to date (Dabert | |
| 334 | et al. 2015, Doña et al. 2015a). In this study, female pied flycatchers (i.e. the | |
| 335 | incubating/brooding sex and thus that having more direct contact with nestlings) had | |
| 336 | higher and consistent decreases in feather mite intensities. In addition, we found that | |
| 337 | pair mates had similar feather mite loads before the hatching of the chicks, suggesting | |
| 338 | either mite horizontal transmission between adult birds in the nests (even though in this | |
| 339 | species they rarely coincide) or assortative mating according to some individual trait | |
| 340 | linked to feather mite load (Blanco et al. 2001). Moreover, the few males that increased | |
| 341 | their mite load may suggest a much higher reproduction of mites on the adult male | |
| 342 | which surpassed the number of mites transmitted to nestlings (but little is known about | |
| 343 | the feather mites' generation times), or transmission of mites from the female (including | |

Commented [A3]: esto lo quitaría, por especulativo/desconocido o directamente inexacto o falso...Evidentemente, coinciden alguna vez (en la elección de pareja entran juntos a la caja, y el macho entra a veces a cebar a la hembra mientras incuba, y luego coinciden bastantes veces cebando a los pollos (según nuestros videos de begging de los pollos). No creo que quitarlo detraiga mucho del mensaje

| 344 | extra pair mates, see below) and also indirectly through short contacts with nest material |
|-----|--|
| 345 | to the adult male. |
| 346 | Overall, our findings support the view that variation in the behaviour of breeding |
| 347 | birds may constrain the opportunities for feather mite transmission. A previous study in |
| 348 | chaffinches found that feather mite loads did not decrease in male birds along the |
| 349 | breeding season (Mironov & Malyshev, 2002). Indeed, here we found that some males |
| 350 | increased their feather mite load (Fig. 1). These differences could be explained by the |
| 351 | differences in mating system as, unlike chaffinches, pied flycatcher males are frequently |
| 352 | polygamous and interact with several females (Cramp and Perrins 1994, Canal et al. |
| 353 | 2012). Further work is encouraged here. |
| 354 | The study of adaptive strategies of symbiont transmission is in its initial stage |
| 355 | but recent studies are promising by adopting concepts already tested in dispersal studies |
| 356 | of free-living organisms (e.g. by testing condition-dependent dispersal in symbionts, |
| 357 | Skelton et al. 2015). In fact, the results here reported for feather mites could be easily |
| 358 | extended to other vertically transmitted symbionts. Experiments modifying the intensity |
| 359 | of competition in symbionts would allow testing directly the effect of intraspecific |
| 360 | competition in the outcome of transmission. The contribution of inclusive fitness to |
| 361 | symbiont dispersal strategies also needs more attention. Further research along both |
| 362 | research agendas will be relevant to achieve a more complete picture of the ecology and |
| 363 | evolution of host-symbiont systems. |
| 364 | |
| 365 | |
| 366 | |

367 Acknowledgements:

Commented [A4]: tenemos varios papers sobre eso en esta misma población, 2 de los cuales firma to Roger! Si no hay límite de referencias, sugiero incluir Canal et al. (2012) [Canal, D., Jovani, R and Potti, J. (2012). Multiple mating opportunities boost protandry in a pied flycatcher population. Behavioural Ecology and Sociobiology 66: 67-76]

| 368 | JD, RJ and JP were supported by the Ministry of Economy and Competitiveness (SVP- | |
|-----|--|--|
| 369 | 2013-067939, Ramon y Cajal research contract RYC-2009-03967, and projects | |
| 370 | CGL2014-55969-P and CGL2015-70639-P) and IdH by the Department of Education, | |
| 371 | Universities and Research of the Basque Government (studentship BFI. 09-13). We | |
| 372 | thank three anonymous reviewers for their constructive comments. | |
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- 471 transmission among hosts. *Proceedings of the Royal Society of London B:*
- 472 *Biological Sciences*, **282**, 20152081.
- 473

- 474 Table 1. Descriptive statistics of the number and aggregation of feather mites in
- 475 flycatcher nestlings (Spain). Two nests containing only one nestling are not shown
- 476 because were not used for the aggregation analyses. Variance-to-mean ratio <1 means
- 477 that mites are evenly distributed, ~ 1 if the distribution is random and >1 if the mites are
- 478 aggregated (Shaw et al. 1998; Bjørn et al. 2011).
- 479

| Nest | Nes | stling | s (soi | rted b | y mit | e abu | ndanc | Variance-to-mean ratio | |
|------|-----|--------|--------|--------|-------|-------|-------|------------------------|-------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | |
| 1 | 14 | 10 | 4 | | | | | | 2.71 |
| 2 | 14 | 10 | 9 | 7 | 5 | | | | 1.28 |
| 3 | 26 | 7 | 5 | 4 | | | | | 10.32 |
| 4 | 2 | 1 | 1 | 0 | | | | | 0.67 |
| 5 | 14 | 11 | 8 | 8 | | | | | 0.80 |
| 6 | 42 | 25 | 24 | 20 | 14 | | | | 4.36 |
| 7 | 4 | 3 | 1 | 1 | 0 | | | | 1.50 |
| 8 | 17 | 12 | 12 | 11 | | | | | 0.56 |
| 9 | 5 | 3 | 2 | 1 | 1 | 1 | | | 1.18 |
| 10 | 12 | 11 | 6 | | | | | | 1.07 |
| 11 | 13 | 6 | 4 | 2 | | | | | 3.67 |
| 12 | 2 | 1 | | | | | | | 0.33 |
| 13 | 2 | 2 | 1 | 1 | 0 | | | | 0.58 |
| 14 | 1 | 1 | 1 | | | | | | 0 |
| 15 | 2 | 1 | 0 | 0 | 0 | | | | 1.33 |

| 16 | 1 | 0 | 0 | | | | | | 1 |
|----|----|---|---|---|---|---|---|---|------|
| 17 | 8 | 5 | 0 | 1 | | | | | 3.9 |
| 18 | 5 | 1 | 1 | | | | | | 2.29 |
| 19 | 12 | 4 | 2 | 1 | 0 | 0 | 0 | 0 | 7.21 |
| 20 | 10 | 9 | 5 | 2 | 1 | | | | 3.02 |
| 21 | 3 | 2 | 1 | 0 | | | | | 1.11 |
| 22 | 2 | 0 | 0 | 0 | | | | | 2 |

480 Figure Legends

| 481 | |
|-----|--|
| 482 | Figure 1. Feather mite intensity in the same individual adult <i>Ficedula hypoleuca</i> during |
| 483 | the incubation and chick rearing period. Each line represents an individual bird. Bottom |
| 484 | panels are detailed views of the lower part of the top panels (note change in y-axis |
| 485 | scale) for individuals showing initial mite loads ranging from 0 to 40 mites. |
| 486 | |
| 487 | Figure 2. Boxplots of real and hypothesized feather mite reductions during the breeding |
| 488 | season in parent <i>Ficedula hypoleuca</i> . See main text for explanation of H1, H2 and H3. |
| 489 | "sp" refers to Spain and "ned" to Netherlands. Spanish males showed a high dispersion |
| 490 | in mite reductions. The second predicted value for H1 (dashed line) considers that the |
| 491 | probability of survival of nestling is the same as that of adults (52%). While this may |
| 492 | seem unrealistic because nestling survival is expected to be much lower than that of |
| 493 | adults (but see main text), it helps to illustrate that feather mite transmission is not |
| 494 | explained by the difference in host survival prospects. Note that during the pre- |
| 495 | transmission period all birds with > 25 mites decreased their feather mite loads (see |
| 496 | main text). Birds with fewer than 25 mites (during the pre-hatching period) are not |
| 497 | shown for illustrative purposes (n= 5). Similarly, Netherlands' males were not included |
| 498 | due to the low sample size of males fitting this criterion ($n=2$, the median reduction for |
| 499 | these males was of 99%). |
| 500 | |
| 501 | |
| 502 | |

503 504