

1 **Evolution of nestling faeces removal in avian phylogeny**

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19 Nest sanitation-related traits have often been explained at the intraspecific level as
20 reducing the probability of infection or detection by predators and parasites, but its
21 evolution within the avian phylogeny is still poorly understood. We compiled detailed
22 information of such traits for more than 400 bird species and, by means of modern
23 comparative methodologies, we reconstructed the evolution of adults' contribution to
24 removing their offspring's faeces and the production of faecal sacs by nestlings.
25 Furthermore, because the functional hypotheses used to explain nest sanitation

26 behaviour assume potential effects of brood size, body mass, nestling period and diet,
27 we explored the association between these traits and those related to nest sanitation in a
28 phylogenetically controlled framework. Our results suggest that parental removal of
29 nestling faeces has driven the evolution of faecal sacs, while the ancestral states
30 involved birds with faecal sacs removed by parents. These results support the long-held
31 idea that faecal sacs facilitate the removal of faeces by parents. Moreover, we found that
32 animal diets and small body sizes have favoured the evolution of faecal sacs suggesting
33 the existence of some chemical and physical constraints in relation to the evolution of
34 the mucous covering. Our results highlight the importance of nest sanitation in the
35 evolution of birds and their life history characteristics.

36

37 Keywords: birds, faeces, faecal sacs, nest sanitation, parent–offspring relationships

38 Nest sanitation behaviour is an important and widespread behaviour in birds
39 that, despite being known for a long time (Blair & Tucker, 1941; Herrick, 1900; Skutch,
40 1976; Thomson, 1934), is still poorly understood, particularly regarding its evolution
41 (Gow, Wiebe, & Musgrove, 2015; Guigueno & Sealy, 2012; Lang, Straight, & Gowaty,
42 2002). This is surprising because strategies and traits related to nest sanitation, or even
43 the effort devoted to such activities, have been suggested to help infer levels of selection
44 pressures acting within species-specific nest environments (Ibáñez-Álamo, Ruiz-
45 Rodríguez, & Soler, 2014). Recent experimental studies have focused on investigating
46 adaptive values of removal by parents of nestling faeces, the most common form of nest
47 sanitation behaviour in birds (Guigueno & Sealy, 2012). Since faeces contain
48 potentially pathogenic microorganisms, its removal would reduce the probability of
49 infection (Ibáñez-Álamo, Ruiz-Rodríguez, et al., 2014). In addition, parasites and nest
50 predators might use chemical (i.e. odours) and/or visual cues of nestling faeces to
51 located active nests and, therefore, removing it from nests would reduce the probability
52 of nest predation (Ibáñez-Álamo, Ruiz-Raya, Roncalli, & Soler, 2014; Ibáñez-Álamo,
53 Sanllorente, Arco, & Soler, 2013; Petit, Petit, & Petit, 1989; Weatherhead, 1984) and
54 parasitism (Ibáñez-Álamo, Ruiz-Raya, Rodríguez, & Soler, 2016). Nestlings of many
55 species encapsulate faeces within a mucous covering forming faecal sacs (Blair &
56 Tucker, 1941; Herrick, 1900; Thomson, 1934; Weatherhead, 1984). This covering acts
57 as a physical barrier to impede microbial infections of birds (Ibáñez-Álamo, Ruiz-
58 Rodríguez, et al., 2014) and might facilitate manipulation and removal of faeces by
59 parents (White 1773, cited in Blair & Tucker, 1941; Blair & Tucker, 1941; Herrick,
60 1900; Pycraft, 1909; Thomson, 1934). Thus, the evolution of faecal sacs should be
61 associated with parental removal of faeces from nests, although this prediction has
62 never been tested. Either parental removal would more easily evolve in species with

63 nestlings producing faecal sacs, or the evolution of faecal sacs would be particularly
64 beneficial in species in which parents removed them.

65 Benefits associated with nest sanitation behaviour may depend on ecological and
66 life history characteristics. It is, for instance, possible that the costs associated with the
67 presence of nestling faeces in the nest depend on the volume and contents of faeces.
68 Everything else being equal, negative impacts of small faeces in terms of attracting nest
69 predators or vectors for potentially dangerous microorganisms/parasites would be lower
70 than those of larger faeces (Ibáñez-Álamo et al., 2016; Petit et al., 1989). Moreover, if
71 faeces are not removed and accumulate in the nest during nestling development, species
72 with longer nestling periods and larger broods would differentially suffer higher costs
73 than species with the opposite life history traits. Therefore, we expect species with
74 longer nestling periods or larger broods to be those with faeces removal.

75 Species-specific diet is another element that might have influenced the evolution
76 of nest sanitation-related traits (Guigueno & Sealy, 2012). Diet affects the prevalence of
77 potentially pathogenic microorganisms in chicken faeces (Ryu, Park, Bang, Kang, &
78 Hwangbo, 2016) and the presence of animal components in the food of livestock
79 produces faeces with more intense odours than those feeding only from plants (e.g.
80 Mackie, Stroot, & Varel, 1998). Consequently, there are good reasons to think that diet
81 characteristics could affect the costs of not removing faeces from nests in terms of
82 microbial infection or detectability by predators. We explored this possibility by
83 analysing the relationship between diet (i.e. animals or plants) and nest sanitation-
84 related traits (faeces removal and faecal sac production).

85 Previous studies on the subject have been focused on exploring within-species
86 variation in a small number of bird species (e.g. Herrick, 1900; Ibáñez-Álamo et al.,
87 2016, 2013; Ibáñez-Álamo, Ruiz-Raya, et al., 2014; Ibáñez-Álamo, Ruiz-Rodríguez, et

88 al., 2014; Petit et al., 1989; Quan, Li, Wang, & Goodale, 2015; Thomson, 1934;
89 Weatherhead, 1984), but exploring the interspecific associations among traits related to
90 nest sanitation and ecological and life history characteristics is essential to understand
91 the evolution of nest sanitation in birds (Gow et al., 2015). Trying to fill this gap, we
92 investigated these scenarios potentially affecting the evolution of nest sanitation using
93 information collected from the literature for more than 400 bird species (19 Orders).

94

95 **Methods**

96 *Data collection*

97 After checking reviews on the topic (Blair & Tucker, 1941; Guigueno & Sealy, 2012;
98 Thomson, 1934), we searched for related articles in the Web of Science and Google
99 Scholar by using the following keywords: 'sanitation', 'nest sanitation', 'nest cleaning'
100 and 'f(a)ecal sacs'. We also checked the *Handbook of the Birds of the World* (Del Hoyo,
101 Elliott, Sargatal, Christie, & de Juana, 2016) for information on nest sanitation-related
102 traits. The literature used for each species is listed in the Supplementary Material (Table
103 S1). For each species, we gathered information on (1) whether faeces are removed from
104 nests; we considered a species to show removal when adults and/or offspring in some
105 way avoided its accumulation in the nest. We noted (2) the presence of faecal sacs and
106 whether (3) parents and (4) offspring remove nestling faeces at least during part of the
107 nestling period. If parents removed nestling faeces, we also noted (5) which sex (male,
108 female or both) was responsible. For seven species (*Accipiter nisus*, *Ardea cinerea*,
109 *Buteo buteo*, *Chiroxiphia caudata*, *Falco peregrinus*, *Haliaeetus albicilla*, *Ocyrceros*
110 *birostris*), we completed information for these variables using video recordings
111 available on the Internet (ARKive.org). We did not include precocial species in our data

112 set given that we were interested in the evolution of removing nestling faeces from
113 nests.

114 We also collected data on (6) body mass, (7) brood size, (8) nestling period
115 duration, (9) diet (animals versus plants) and (10) nesting habits (hole, semihole and
116 open nesters) using the *Handbook of the Birds of the World* (Del Hoyo et al., 2016). We
117 considered the diet of a species to be of animal origin when the nestlings were fed
118 mainly with animals such as invertebrates (i.e. insects), vertebrates (i.e. birds) or
119 carrion. Those species whose nestlings were mainly fed with seeds, fruits or nectar were
120 classified as having plant diets. For those species without specific information on the
121 nestling diet, we used the main feeding source for the species. All collected information
122 is reported in Table S2.

123

124 *Statistical analyses*

125 Because the expected interspecific associations may have a strong phylogenetic
126 component (Harvey & Pagel, 1991) we used the phylogenetic association among the
127 bird species considered in our analyses. Phylogenetic relationships were estimated in
128 the Mesquite environment (Maddison & Maddison, 2015) as the consensus (i.e.
129 majority rules consensus) tree of 1000 phylogenetic trees downloaded from
130 <http://birdtree.org/> (Jetz, Thomas, Joy, Hartmann, & Mooers, 2012; Table S3). The
131 predicted associations were subsequently explored with phylogenetically controlled
132 analyses.

133 Most of the characters we considered are of binary nature, including the
134 contribution of adults and nestlings to removing faeces from nests. In most species (349
135 of 370) the removal of nestling faeces is only performed by parents, but in some,
136 nestlings also contribute (11 species) or even are the only ones in charge of this task by

137 directly disposing of their own faeces (10 species). Because of the limited number of
138 species that do not remove faeces from the nests, or with nestlings participating in this
139 task, we did not include more than one categorical independent factor in our model. As
140 we were mainly interested in exploring the evolution of nest sanitation behaviour of
141 adults in relation to nestling traits, we considered the binomial information of whether
142 adults remove nestling faeces without the help of their offspring. Information on
143 nestling diet was also compiled as binomial information (mainly animals versus mainly
144 plants). In addition to binomial variables, some statistical models also include
145 continuous independent factors, namely, body mass, nestling period and brood size.
146 Thus, given that all dependent factors were of binomial distribution and the need to
147 control phylogenetic relationships, we used a phylogenetic generalized linear mixed
148 model for binary data (binaryPGLMM; Ives & Garland, 2014; Ives & Helmus, 2011) as
149 implemented in the R (version 3.2.3. R Core Team, 2016) statistical environment with
150 the appropriate libraries ('ape'; Paradis, Claude, & Strimmer, 2004), 'MASS' (Venables
151 & Ripley, 2002) and 'mvtnorm' (Genz & Bretz, 2009). The binaryPGLMM package
152 performs linear regressions for binary phylogenetic data, estimating regression
153 coefficients (hereafter 'estimate') with approximate standard error. At the same time, it
154 estimates the strength of the phylogenetic signal in the residuals (hereafter 's') and gives
155 an approximate conditional likelihood ratio test for the hypothesis that there is no signal
156 (Ives & Garland, 2014; Ives & Helmus, 2011).

157 Additionally, we explored possible associations in character evolution and the
158 direction of changes along the phylogenetic tree of several pairs of traits: (1) between
159 parental contribution to removing faeces from nests (alone or not) and nestling
160 production of faecal sacs, (2) between nestling diet (animals or plants) and adult
161 contribution to removing faeces, and (3) between nestling diet and production of faecal

162 sacs. Briefly, we used Pagel's discrete method to test models of independent and
163 dependent evolution (Harvey & Pagel, 1991). This method compares the ratio of
164 likelihood of two models: one of the models where the rates of change in each character
165 are independent of the state, and a second model where rates of change depend on the
166 state of the other trait. Since likelihoods associated with each of the eight possibilities of
167 transition are estimated, this approach provides a good method to study evolutionary
168 pathways through estimations of transition rates between pairs of binary character states
169 (i.e. test for any effects in Mesquite 3.04). In addition, we also tested for the possibility
170 that character X depended on character Y, or that character Y depended on X. We
171 performed these analyses as implemented in Mesquite (Maddison & Maddison, 2015)
172 with 50 ML replicates over 1000 repeated simulations.

173

174 **Results**

175 *Faeces removal by adults and presence of faecal sacs*

176 We collected information on nest sanitation-related characters of 417 species of birds
177 from 19 different Orders. Within the 396 species that remove nestling faeces from their
178 nests (94.96% of all species analysed), reliable information on production of faecal sacs
179 and on adult and nestling participation in faeces removal was available for 370 species.
180 Most of these species (96.76%) produce faecal sacs, and in most of them (97.49%) only
181 adults remove faeces from nests. In nests of the 12 species with no faecal sacs, faeces
182 removal is mainly done by nestlings alone (83.33%) although adults contribute in two
183 of them. Another interesting result is that, when adults participate in faeces removal,
184 both male and female perform this behaviour in the majority of species (91.9%) and just
185 in a few of them either the female (6.8%) or the male (1.3%) do it exclusively.

186 The study of evolutionary associations among these traits along the phylogenetic
187 tree suggests that the evolution of parental contribution to faeces removal from nests
188 depends on the presence of faecal sacs. Nodes with no faecal sacs and only adults
189 removing nestling faeces, as well as those with faecal sacs and nestlings able to dispose
190 of their own faeces, evolved to nodes with faecal sacs and only adults removing faeces
191 more than expected by random (Fig. 1). Therefore, the evolutionary acquisition of
192 faecal sacs was more likely in nodes with adult removal and no nestling help (difference
193 in log-likelihood = 2.92, $P = 0.029$), while the trait that only adults remove faeces
194 depends on whether the ancestral species had faecal sacs (difference in log-likelihood =
195 17.92, $P = 0.047$; Fig. 1).

196

197 *Nest sanitation, body mass, brood size and nestling period*

198 Nestling faeces removal was not significantly affected by body mass, brood size or
199 nestling period (all binary PGLMM: $P > 0.16$). In contrast, faecal sacs were more
200 frequent in species of lower body mass (binary PGLMM: estimate (SE) = -3.48 (1.19),
201 $Z = 2.93$, $P = 0.003$; phylogenetic signal s^2 : 6.52, $P = 0.001$) and shorter nestling period
202 (binary PGLMM: estimate (SE) = -9.69 (3.80), $Z = 2.55$, $P = 0.011$; phylogenetic signal
203 s^2 : 7.12, $P = 0.0003$). Brood size did not predict the existence of faecal sacs (binary
204 PGLMM: $P = 0.5221$) and, when considering all three life history traits together, body
205 mass (binary PGLMM: $P = 0.027$), but not the duration of the nestling period (binary
206 PGLMM: $P = 0.160$) or brood size (binary PGLMM: $P = 0.408$), reached statistical
207 significance explaining faecal sac evolution. When body mass and parental contribution
208 to removing nestling faeces from nests (i.e. with no help from nestlings) were
209 considered together, the presence of faecal sacs tended to be more common in species in
210 which only adults remove faeces (binary PGLMM: $P = 0.07$), while the effect of body

211 mass reached statistical significance (binary PGLMM: $P = 0.025$). Moreover, nestlings
212 help parents to remove faeces from nests more often in large species (binary PGLMM:
213 estimate (SE) = 2.11 (0.62), $Z = 3.40$, $P = 0.0007$; phylogenetic signal s^2 : 4.64, $P =$
214 0.0001). Species that do not remove nestling faeces are similarly distributed among hole
215 (6.3%), semihole (1.9%) and open nesters (4.9%) suggesting that is unlikely that nesting
216 habits affected the evolution of this behaviour.

217

218 *Nest sanitation and nestling diet*

219 The behaviour of removing nestling faeces from the nest was not associated with
220 offspring diet (88.0% of the 50 species with plant diets removed faeces while 95.9% of
221 the 363 species with animal diets did so; binary PGLMM: estimate (SE) = 0.01 (0.83), Z
222 = 0.01, $P = 0.99$; phylogenetic signal s^2 : 3.56, $P < 0.0001$). Nestlings of species feeding
223 on plants more often produced faeces within a mucous covering than those eating
224 mainly animals (100% of the 32 species with plant diets produced faecal sacs while
225 96.2% of the 313 species with animal diets did so; binary PGLMM: estimate (SE) = -
226 7.56 (3.38), $Z = 2.24$, $P = 0.025$), even after controlling for the effect of body mass
227 (binary PGLMM: estimate (SE) = -4.31 (0.97), $Z = 4.45$, $P < 0.0001$; phylogenetic
228 signal of the model: $s^2 = 2.29$, $P < 0.0001$). In contrast, species-specific nestling diets
229 were not associated with parental removal of faeces (binary PGLMM: estimate (SE) =
230 1.88 (1.72), $Z = 1.09$, $P = 0.27$), but were associated with body mass (binary PGLMM:
231 estimate (SE) = 2.25 (0.65), $Z = 3.44$, $P = 0.0005$; phylogenetic signal of the model: s^2
232 = 5.013, $P < 0.0001$). Results from correlations of the evolution of the characters
233 considered showed that the production of faecal sacs (Fig. 2), but not parental
234 contribution to remove faeces from nests (differences in log-likelihood = 2.62, $P =$
235 0.23), tended to be associated with nestling diet. We found evidence supporting the

236 influence of nestling faecal sacs on diets (differences in log-likelihood = 5.76, $P <$
237 0.0001) and of diets on faecal sacs (differences in log-likelihood = 4.31, $P = 0.04$).
238 Thus, diet is likely to be responsible for the evolution of faecal sacs but probably not for
239 the role that adults play in nest sanitation behaviour.

240

241 **Discussion**

242 Adults of most altricial bird species remove their nestling faeces from their nests, and
243 these faeces are usually encapsulated in a mucous covering that reduces the probability
244 of bacterial infection (Ibáñez-Álamo, Ruiz-Rodríguez, et al., 2014) and, apparently,
245 facilitates faecal handling by parents (White 1773, cited in Blair & Tucker, 1941; Blair
246 & Tucker, 1941; Herrick, 1900; Pycraft, 1909; Thomson, 1934). Furthermore, there
247 seems to be no differences between males and females as both sexes perform this
248 behaviour in most species in which adults participate in the removal of nestling faeces.
249 Our results show that these traits (adult removal and faecal sacs) are almost completely
250 fixed in altricial birds and much more widespread than previously thought (Blair &
251 Tucker, 1941; Skutch, 1976; Thomson, 1934), which suggests that parental participation
252 in cleaning the nest is critical for offspring development. Nest sanitation is an essential
253 component of parental behaviour, but its functionality has only been studied
254 intraspecifically (Carere & Alleva, 1998; Dell’Omo, Alleva, & Carere, 1998; Gow et
255 al., 2015; Lang et al., 2002; Thomson, 1934). To our knowledge, this is the first
256 interspecific study on the topic and the only one looking at the evolution of nest
257 sanitation within the avian phylogeny. Our main findings are (1) the existence of
258 correlated evolution between adult removal of faeces and the production of faecal sacs
259 by nestlings and (2) the detection of significant associations between life history
260 characteristics (i.e. nestling period and body mass), the evolution of faecal sacs and

261 parental contribution to removing faeces from nests. Finally, we also found (3) evidence
262 suggesting a role of nestling diet on the evolution of faecal sacs. All these results
263 considered together suggest that nest sanitation behaviour of adults, together with
264 nestling diet, have influenced the evolution of the structure of nestling faeces,
265 particularly in relation to the presence of the mucous covering (faecal sacs). Below we
266 discuss possible evolutionary implications of our findings.

267 Altriciality is considered the derived character of precocial ancestors (Starck &
268 Ricklefs, 1998) and selection pressures favouring the evolution of nest sanitation would
269 be higher in altricial species whose nestlings develop within the nest environment
270 (Guigueno & Sealy, 2012). Because we were interested on the evolution of removing
271 nestling faeces from nests, we did not include precocial species in our data set (apart
272 from one semiprecocial species, *Antigone antigone*) and thus cannot discuss the
273 possibility of correlated evolution between nest sanitation and altriciality. However,
274 since adults removing nestling faeces and nestlings producing faecal sacs were the
275 ancestral states (Fig. 1), we speculate that these traits had already evolved in precocial
276 bird species. Nest sanitation behaviours occur not only during the nestling phase, but
277 also during the egg incubation stage (Guigueno & Sealy, 2012), for instance renovating
278 green materials (Petit, Hossaert-McKey, Perret, Blondel, & Lambrechts, 2002) or
279 removing broken eggs or eggshells from the nest (Soler et al., 2011; Tinbergen et al.,
280 1962). Associated benefits of nest sanitation should be higher during the nestling period
281 and thus these traits would rapidly become fixed in altricial bird populations. However,
282 that the presence of faecal sacs is the ancestral state and that we only found them in
283 altricial species in our literature search also suggest that the mucous covering might
284 have evolved in relation to altriciality.

285 We found evidence of correlated evolution between parental removal of faeces
286 and nestling production of faecal sacs. Faecal sacs mainly evolved in species in which
287 adults remove faeces from their nests without help of their nestlings, while faeces
288 removal exclusively by adults evolved mainly in species with faecal sacs (Fig. 1). These
289 associations would be explained by faecal sacs facilitating faeces removal by parents
290 (White 1773, cited in Blair & Tucker, 1941), an idea already mentioned in the 18th
291 century and now widely accepted by the scientific community (Blair & Tucker, 1941;
292 Herrick, 1900; Pycraft, 1909; Thomson, 1934) even though it has not previously been
293 demonstrated. Our results offer the first demonstration of this hypothesis and we
294 encourage future studies to investigate whether predictions of the evolutionary pattern
295 found here fit the ecological context by, for example, exploring intraspecific covariation
296 in faecal sac resistance and the efficacy of parents removing nestling faeces.

297 We found no evidence supporting the expected associations between nestling
298 faeces removal and life history characteristics (see above), but body size and duration of
299 the nestling period were negatively associated with the presence of faecal sacs. We
300 predicted the opposite direction, however, because nest sanitation tasks should be more
301 beneficial for larger species (i.e. higher production of faeces) and for those with longer
302 nestling periods (higher accumulation of faeces). When considering both variables in
303 the same model, only body mass reached statistical significance. Explanations of these
304 results might be related to physical constraints of the mucous covering: above a certain
305 weight it may not guarantee the integrity of faecal sacs during parental removal. New
306 data are necessary to test this and other possible scenarios explaining the detected
307 association between body mass and faecal sac production. Independently of the reason,
308 our results show that nestlings participated in nest sanitation tasks more often in large
309 species (see Results), which may be related to the difficulty adults have removing faeces

310 with a weak, or no, mucous covering. Thus, because of the adaptive value of nest
311 sanitation (Kölliker, Royle, & Smiseth, 2012), nestlings of large species should directly
312 dispose of their own faeces from nests more often than those of small species.

313 We also argued that nestling diet should have affected the evolution of nest
314 sanitation behaviour and we found partial support for this hypothesis because faecal
315 sacs are more likely to evolve in species with nestlings feeding mainly on vegetal
316 material (Fig. 2). Diet, however, does not seem to have affected the evolution of
317 parental sanitation behaviour. Moreover, faecal sacs appeared less often in species with
318 nestlings mainly fed with animals. This is the most common source of food in birds
319 (72.4%; calculated from Burin, Kissling, Guimarães, Şekercioğlu, & Quental, 2016),
320 and, above, we proposed various scenarios in which the mucous covering could provide
321 fitness advantages for species with animal diets. We found the opposite result, however,
322 since faecal sacs appear more often in species with vegetal diets. We could speculate
323 that animal diets favour faeces with some specific components or in such concentrations
324 (i.e. uric acids) that might negatively interact with the mucous covering, but our results
325 do not allow us to discuss this or other scenarios (i.e. detectability by predators and/or
326 parasites). Thus, although our results emphasize the importance of nestling diet for the
327 evolution of nest sanitation behaviour (i.e. faecal sacs), further investigation is
328 necessary to detect the underlying causes.

329 Summarizing, our comparative analyses support the hypothetical correlated
330 evolution of parent and offspring traits related to nest sanitation, and emphasize the
331 importance of nest sanitation in the evolution of birds in general and their life history
332 characteristics in particular. We hope that our findings encourage further research
333 directed to explore functional hypotheses of the detected evolutionary patterns.

334

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344

345 Supplementary Material

346

347 Supplementary material associated with this article is available, in the online version, at
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349

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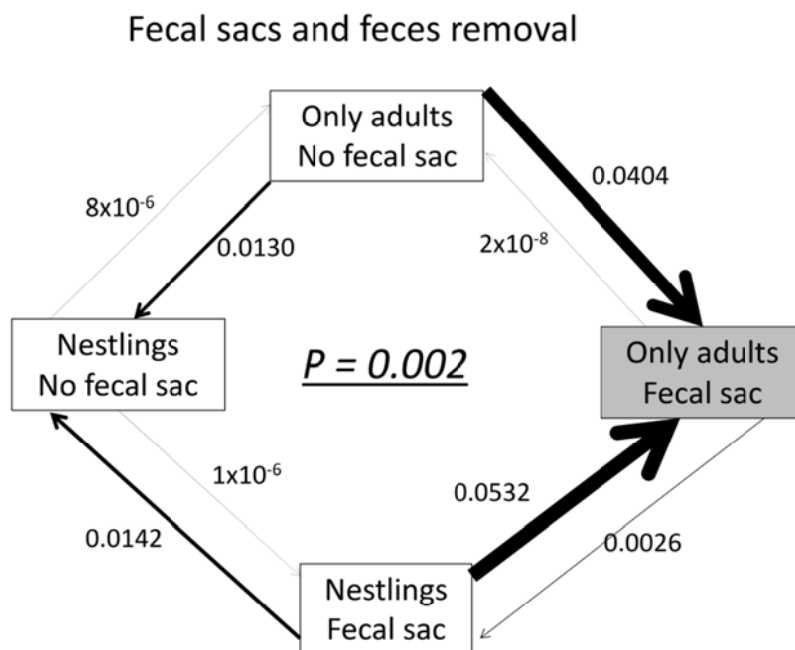
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462 Figure legends

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471 Fig 1: Flow diagram showing correlated evolution between the production of faecal sacs
472 by nestlings (faecal sac versus no faecal sac) and faeces removal by adults (only adults
473 versus nestlings). The number associated with each arrow represents the maximum
474 likelihood value and, together with arrow thickness, indicates which transition is
475 expected to be the most common. Probability (P) in the centre of the diagram indicates
476 the probability of correlated evolution between the binary traits after 10 ML replicates
477 and 1000 repeated simulations. Parsimony ancestral states of characters are shown in
478 the grey box.

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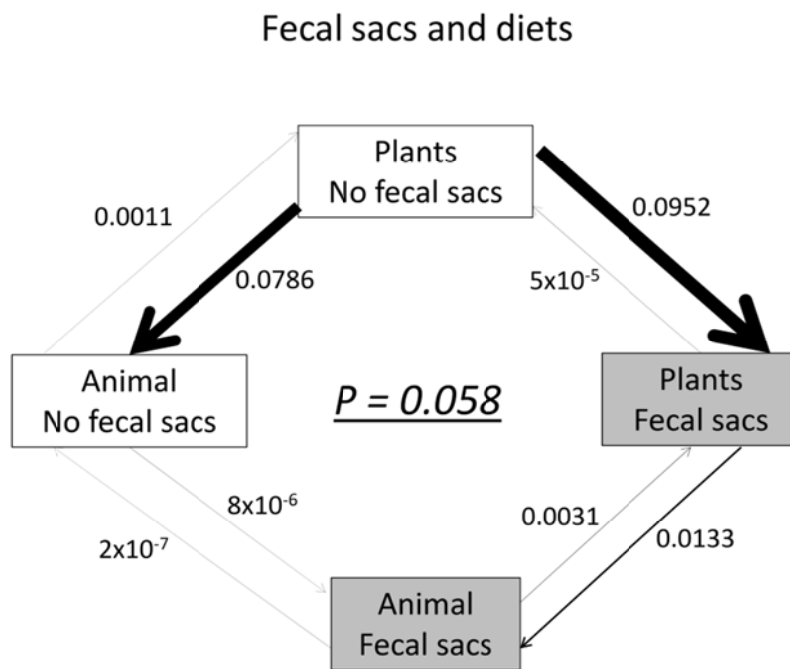


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480 Fig 2: Flow diagram showing correlated evolution between the production of faecal sacs
 481 by nestlings (faecal sac versus no faecal sac) and diet (animals versus plants). The
 482 number associated with each arrow represents the maximum likelihood value and,
 483 together with arrow thickness, indicates which transition is expected to be the most
 484 common. Probability (P) in the centre of the diagram indicates probability of correlated
 485 evolution between the binary traits after 10 ML replicates and 1000 repeated
 486 simulations. Parsimony ancestral states of characters are shown in grey boxes.

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