

1 **The impacts of predators and parasites on wild bumblebee**
2 **colonies.**

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35 **Abstract**

- 36 1. The study of wild bumblebee nests has been hindered by the difficulty in locating and
37 observing them. Here, 47 wild nests were located using a sniffer dog and volunteers.
38 The entrances to 32 nests were filmed continuously to identify successful nests (those
39 which produced gynes) and observe vertebrate species interactions.
- 40 2. Of the 47 nests, 71% and 21% produced gynes in 2010 and 2011, respectively.
- 41 3. A total of 39 vertebrate species were filmed at entrances but the majority did not
42 interact with the nests. Great tits (*Parus major*) depredated or attempted to depredate
43 bees on 32 occasions at the entrances to ten nests, something which has not previously
44 been described. Small mammals were very often recorded accessing entrances to
45 bumblebee nests, but whether they depredated bees was not known, and frequently-
46 visited nests were no less likely to produce gynes. Eight nests were entered by adult
47 wax moths, *Aphomia sociella*.
- 48 4. The faeces of 1,179 workers from 29 *Bombus terrestris* nests were screened
49 microscopically for parasites. *Crithidia bombi* infections were apparent in 49% of
50 worker bees, while *Nosema bombi* and *Apicystis bombi* were present in 5.5% and
51 0.68% of bees, respectively. Nests with a high prevalence of *C. bombi* infection were
52 less likely to produce gynes, the first evidence for a direct impact of this common
53 parasite on bumblebee colony reproduction in wild nests.
- 54 5. Overall, our data indicate that bumblebee nests are at the heart of a rich web of
55 interactions between many different predator and parasite species.

56 **Introduction**

57 Bumblebees are amongst the most abundant and important of pollinator species throughout
58 the temperate northern hemisphere, and some have undergone marked declines in abundance
59 and contractions of range in recent decades (Goulson et al. 2015). These declines have
60 stimulated much research, but the study of wild bumblebee nests has been somewhat
61 neglected as locating nests remains challenging (Kells and Goulson, 2003; Osborne et al.,
62 2008; Suzuki et al., 2009; Lye et al., 2009). Hence we still have a poor understanding of
63 bumblebee nesting and population biology (Osborne et al, 2008; Goulson *et al.*, 2010; Lye *et*
64 *al.*, 2012). This is significant as the nest is, arguably, the breeding unit; each nest normally
65 contains one breeding female, the queen (but see O'Connor et al. 2013).

66 Aspects of bumblebee ecology and behaviour have been studied using nests reared
67 from wild caught queens in the laboratory or obtained from commercial bumblebee rearing
68 companies, which are then placed in the field (e.g. Schmid-Hempel and Schmid-Hempel,
69 1998; Goulson and Stout, 2001; Goulson et al., 2002; Carvell et al., 2008; Whitehorn et al.,
70 2012). The outcomes of these experiments, whilst valuable, may not always provide an
71 accurate representation of wild bumblebees. Artificially reared bumblebee nests placed in the
72 field tend to be housed in constructed domiciles raised above the ground and with entrances
73 that are apparent (Lopez-Vaamonde et al., 2004; Carvell et al., 2008). They may thus be more
74 vulnerable to attack by predators and parasites than natural nests.

75 Bumblebees are thought to have a number of mammalian enemies in the UK; for
76 example, small mammals such as wood mice (*Apodemus sylvaticus*) and shrews (*Sorex* spp.)
77 are said to enter and depredate nests before the first brood of workers have emerged (Darwin,
78 1859; Sladen, 1912; Cumber, 1953; Pouvreau, 1973), or they may be excavated and eaten by
79 larger mammals such as badgers (*Meles meles*) (Sladen, 1912; Pouvreau, 1973; Alford, 1975)
80 and foxes (*Vulpes vulpes*) (Benton, 2006; Goulson, 2010). Bumblebee nests can also fall
81 victim to the larvae of the wax moth *Aphomia sociella* which consume the entire nest;
82 destroying comb and brood (Sladen, 1912; Pouvreau, 1973; Alford, 1975; Goulson, 2010). A
83 large proportion of our understanding of bumblebee nest predators originates from the
84 extensive work of Sladen (1912). Whilst his book formed the foundation for later bumblebee
85 research and many of the facts he presents are repeated in later texts, the reliability of some of
86 his observations are questionable. For example Sladen (1912) writes that ‘moles [*Talpa*
87 *europa*] and weasels [*Mustela nivalis*] also destroy nests’ yet later states that he has found
88 ‘no evidence for predation by any vertebrates other than mice and shrews’. Similarly in a
89 study of the life histories of 80 *Bombus pascuorum* (formally *Bombus agrorum*) nests,

90 Cumber (1953) documented that 17 were ‘destroyed by rodents, badgers, etc’ and 25 ‘died
91 out prematurely’, but no details on how this was determined is given. It is therefore unclear
92 how rodent predation was deduced as the cause of death, or what proportions of failed nests
93 were due to the different predators. Darwin (1859) quoted Col. Newman’s estimate that ‘Two
94 thirds of bumblebee nests are destroyed by field mice’ but again, methods for assigning mice
95 as the cause of failure are not given. Casual observation of bumblebee nests is unlikely to
96 produce useful data in this respect since vertebrate predators are likely to modify their
97 behaviour if a human observer is present, and most are nocturnal. Further clarification of the
98 predators of bumblebee nests and quantification of the rates of their destruction is needed to
99 advance understanding of bumblebee nest ecology and facilitate development of suitable
100 conservation strategies (Goulson, 2010; Winfree, 2010).

101 As with rates of predation, we also have poor data on the frequency with which
102 bumblebee nests survive to produce gynes or males. Data on wild nests in the UK is limited
103 to the study by Cumber (1953) who found 23 (28.8%) of 80 *B. pascuorum* nests produced
104 gynes. Experiments using artificially reared nests find varying levels of reproduction. For
105 example, 25 commercially reared *B. terrestris* colonies placed in the field resulted in a mean
106 of 13.7 gynes per nests (± 5.7) with 11/25 (44%) of nests failing to produce gynes
107 (Whitehorn et al., 2012). Of 36 laboratory reared *B. lucorum* nests, 5 (13.9% of nests)
108 produced gynes, ranging from 1 to 125 per nest and totalling 250 (Müller and Schmid-
109 Hempel, 1992), and in another study of 32 *B. lucorum* nests, 21.9% produced gynes (Imhoof
110 and Schmid-Hempel, 1999). Others reported lower success; for example none of 14
111 laboratory reared *B. terrestris* colonies placed in the field produced gynes (Otti and Schmid-
112 Hempel, 2008). It has been hypothesised that the majority of nest failures occur in the very
113 early stages when the founding queen is solely responsible for establishing a nest (Sladen,
114 1912; Free and Butler, 1959; Alford, 1975), so figures obtained from laboratory reared nests
115 or those followed in the wild after the first brood have hatched are likely to be overestimates.

116 Infection by internal parasites may also affect the survival and reproductive success of
117 bumblebee colonies (Durrer and Schmid-Hempel, 1995; Brown et al., 2003; Otti and Schmid-
118 Hempel, 2007), but the impact these parasites have on wild bumblebee nests has never been
119 quantified.

120 Here, we deploy cameras to film wild bumblebee nest activity, detect gyne
121 production, and to record visits by vertebrate predators, *A. sociella* or cuckoo bees
122 (*Psithyrus*). We also screen workers for internal parasites, providing a detailed account of the
123 factors affecting the fates of 47 bumblebee nests.

124

125 **Methods**

126 The work took place on the University of Stirling campus and nearby farmland in 2010 and
127 2011. A trained bumblebee nest detection dog and volunteers assisted in locating nests
128 (Waters et al. 2011; O'Connor et al., 2012). Searches were focussed on areas likely to have
129 bumblebee nests, particularly woodland and semi-natural grassland (Cumber, 1953; Alford,
130 1975; Svensson et al., 2000; Free and Butler 1959; Fussell and Corbet, 1992). Woodlands
131 comprised a mix of mature stands of oak (*Quercus robur*), ash (*Fraxinus excelsior*) and
132 beech (*Fagus sylvatica*) with some areas of younger deciduous trees and small areas of
133 planted coniferous woodland. Grasslands were semi-natural, ungrazed and characterised by
134 presence of tussocks of dead grasses and herbs. All sites had to be suitable for repeat visits
135 and for use of recording equipment, therefore areas of dense undergrowth, those prone to
136 water logging or next to roads and paths were not searched to avoid risk of equipment theft or
137 vandalism. On occasions, nests were found which were deemed too close to paths, and some
138 were reported by farmers in outbuildings. These were observed for a minimum of 20 min
139 twice each week and parasite samples were taken but they were not filmed.

140

141 **Cameras**

142 Ten camera recorders were designed and manufactured by N. Butcher at the Royal Society
143 for the Protection of Birds Headquarters, Sandy, UK. Each consisted of a black and white,
144 waterproof camera, (Misumi, MO-R430G-C) with a resolution of 240 T.V. lines. Six
145 infrared, no-glow bulbs were positioned around **each of the ten cameras** to facilitate night
146 filming. Infrared lighting was controlled by a digital timer, housed inside the weather proof
147 box. A metal hood fitted over and around the camera (and infrared bulbs) and measured
148 approximately 6 x 4 x 3 cm. This was connected to a metal stake 50cm in length. Both hood
149 and stake were painted with a green and brown pattern to camouflage the camera. The metal
150 stake was driven into the ground to hold the camera in position approximately 40-60 cm from
151 the bumblebee nest entrance. The camera was connected via a 4 m cable to a 12 Volt battery
152 and a MemoCam Digital Video Recording unit, (Video Domain Technologies Ltd., Petah
153 Tikva, Israel), which was housed inside a plastic weatherproof box (approximately 15 x 15 x
154 12 cm). The weather proof box and battery were wrapped in a waterproof sack and buried
155 inside a shallow pit, 4 m from the camera. The turf from the excavation was replaced above
156 the equipment to minimise disturbance and provide camouflage. The wire was also buried
157 just below the surface of the ground.

158

159 The MemoCam software package was designed for surveillance operations and has been used
160 for vertebrate observational studies (Bolton et al., 2007). The software allows the user to
161 specify an area of the filmed image to be movement sensitive. In this case the nest entrance
162 was selected. The software detected any movement at the nest entrance and recorded one
163 frame before this movement and the following five frames. This ensured there was no time
164 lag between the movement trigger and start of filming, as was found to be an issue with other
165 commercially available wildlife camera traps. Sensitivity was set so that movement of
166 anything greater than ~3mm in diameter would trigger recording (i.e. the very smallest
167 bumblebees were filmed, but diminutive flies were unlikely to trigger recording). Footage
168 was recorded onto 2 G.B. 'mini' S.D. memory cards. Batteries and memory cards were
169 replaced every two to three days. **When a nest expired, the camera was redeployed to a
170 newly-discovered nest as quickly as possible.**

171

172 *Video analysis*

173 Footage was viewed at x2 real time. Any events which were of interest were watched again at
174 slower speed to establish their exact nature. The number of bees entering and leaving nests
175 was recorded for one hour, from 12:00-13:00 hrs, each day and termed 'midday traffic'. In
176 some cases the nest was visited at midday by researchers, (changing batteries, S.D. cards,
177 etc.) and in these cases, bumblebee traffic for the hour nearest to midday was used. For every
178 day that a nest was filmed, a seven-day running mean of the midday traffic was calculated
179 **(the mean of the traffic on the day in question, plus the traffic on the previous and following
180 three days)**. The greatest value of seven-day mean midday-hour traffic was termed 'peak
181 traffic' and used as a proxy measure for the maximum size attained by each nest for statistical
182 analysis.

183 All vertebrates filmed within approximately 1m of the entrance were identified to
184 species and their behaviour was recorded. Behaviours were categorised as: no interaction
185 (where animals simply passed nest entrances); some interaction (sniffing at entrance, waiting
186 at hole); attempted predation (widening entrance, chasing bumblebee foragers) and predation
187 (bees killed); or entering or exiting the nest entrance. For each species, rates were calculated
188 for attempted predation/predation or use of nest entrance by dividing the total number of
189 events by the total number of days that the nest was filmed. Small mammals are more active
190 at night, with very few records during daylight hours. Their numbers were calculated for each
191 24 hr period beginning at 8am (instead of for example, midnight which would result in

192 nightly visits being split over two days). For small mammals which entered the hole, we
193 would expect a visit to consist of one record of entry followed by one record of exit, but this
194 was not always the case (presumably because some holes led to underground tunnel networks
195 with multiple exits). In this case, the number of entries or exits per 24 h period (whichever
196 was the greater) was used.

197

198 *Screening for internal parasites*

199 Faeces from *B. terrestris* workers from 29 nests were screened for the internal parasites
200 *Nosema bombi*, *Crithidia bombi* and *Apicystis bombi*. Faecal samples were collected from
201 five bees twice weekly from each nest where possible. Bumblebees were collected at their
202 nest in clean sample pots. They were released when they defecated or after 15 min. Faeces
203 were collected from the pot using a microcapillary tube which was then sealed at both ends
204 with PTFE tape, labelled and chilled on an ice block in the field before being refrigerated at
205 2-5 °C. Each bee was examined for signs of wing wear and assigned to one of four categories
206 (after Carter, 1992; Rodd et al., 1980; Müller and Wolflueller, 1993; Whitehorn et al.,
207 2011): 0= no wing wear; 1, some minor indentations; 2, most of margin with minor
208 indentations; 3, more than 5% wing surface missing. In the laboratory, samples were
209 transferred to a haemocytometer within 24 hours and examined under a light microscope at
210 x400 magnification. The presence of *N. bombi*, *C. bombi* and *A. bombi* was recorded and
211 numbers of each within 0.1 µL on the haemocytometer grid was counted. Counts of *C. bombi*
212 and *N. bombi* correlate with intensity of infection (Otterstatter and Thompson 2006; Otti and
213 Schmid-Hempel 2008). Bees were not marked after sampling, so it is possible that some
214 individual bees were screened more than once. Bees were caught as they entered or left the
215 nest; it is possible that these bees were intruders from another nest, but this is likely to be
216 very infrequent (O'Conner et al. 2013).

217

218 *Nest success*

219 We use gyne production as the measure of nest success, since the numbers of colonies in the
220 next generation depends upon the numbers of gynes (Chapman and Bourke 2001). In addition
221 to observation of video footage, gyne production can be detected by the presence of queen
222 cells in the nest and so once nest activity ceased we attempted to excavate them. However, it
223 was rarely possible to get to the nests (usually prevented by large tree roots), so these data are
224 not included.

225 The most common bumblebee species studied here was *Bombus terrestris*, in which
226 males and workers cannot be distinguished from camera footage, so detection of male
227 production was unreliable. Males could only be reliably distinguished for *B. pratorum* and *B.*
228 *lapidarius* of the species studied here.

229

230 *Statistical analysis*

231 Statistical analysis was carried out using R Statistical Software Version 2.12.2 (R
232 Development Core Team, 2011). Model fit was checked by visual examination of residuals.
233 Over-dispersion in the data was assessed and any points with Cook's Distance of greater than
234 1 were removed from analysis due to disproportionate influence on the data set (Zuur et al.,
235 2007).

236 A χ^2 test was used to compare the proportion of nests producing gynes in 2010 versus
237 2011, for all bumblebee species combined. This analysis included both filmed nests and those
238 that were observed bi-weekly. A General Linear Model (GLM) with binomial distributions
239 was used to assess the effect of 'peak traffic' and 'days filmed' (a proxy for nest duration) on
240 the likelihood of each nest producing gynes, using data from the filmed nests only. Data from
241 all bumblebee species were pooled as there were too few nests of species other than *B.*
242 *terrestris* for meaningful analysis (Table 1). However, the analysis was rerun excluding *B.*
243 *pratorum* (a species in which nests end early) in case this influenced the results.

244 Four separate GLMs were used to investigate the likelihood of *A. sylvaticus*, *Sorex*
245 spp., great tits (*Parus major*) and *A. sociella* visiting bumblebee nests. The response variable
246 used for each of these models was the total number of visits from the species of interest to
247 each nest, using 'year' as a fixed factors and 'peak traffic' as a covariate in the model. As
248 above, data from all bumblebee species were pooled. Models used quasi-Poisson distributions
249 to account for over-dispersion in the data. Some data points were removed from the analysis
250 (two nests each *A. sylvaticus*, *Sorex* spp. and *P. major* and one nest from the *A. sociella*
251 model) because these data were outliers (Zuur et al., 2007). There were too few nests visited
252 by bank voles (*Clethrionomys glareolus*) or field voles (*Microtus arvalis*) to allow statistical
253 analysis (four and three nests, respectively).

254 A GLM with a binomial distribution was used to assess the effect of visits from *A.*
255 *sylvaticus*, *Sorex* spp., *P. major* and *A. sociella* upon gyne production (binary response),
256 including 'peak traffic' as a covariate.

257 Two Generalized Linear Mixed Effects Models (GLMMs) were carried to identify
258 factors that influenced the likelihood of a *B. terrestris* worker bee carrying either a *C. bombi*

259 or a *N. bombi* infection. The model used ‘presence of infection’ (of either *C. bombi* or *N.*
260 *bombi*) as the binary response, with the following potential explanatory variables: ‘year’,
261 ‘habitat’, and ‘presence of other protozoan infection’ (i.e. either *C. bombi* or *N. bombi*,
262 whichever was not being used as response) as fixed factors in these two models. ‘nest’ (i.e.
263 the nest from which the worker was caught) was used as a random factor, and ‘day’ (i.e. day
264 on which the sample was taken; day one being the first day a nest was found in that year) as a
265 covariate. The interaction between ‘year’ and ‘day’ was also included.

266 To assess the impact of infections with either *C. bombi* or *N. bombi* on nest success,
267 i.e. gyne production, a GLM with binomial distributions was used to assess the likelihood of
268 *B. terrestris* nests producing gynes (the binary response), with the ‘proportion of infected
269 bees’ for *C. bombi*, *N. bombi* as covariates and presence or absence of *A. bombi* as a fixed
270 factor.

271

272 **Results**

273 A total of 47 bumblebee nests were found between 10 June and 25 August, and followed until
274 their demise. In 2010, 28 nests were located and 19 of these were filmed. In 2011, 19 nests
275 were found and 13 were filmed (Table 1). The majority were *B. terrestris* (34), with small
276 numbers of other species; *Bombus hortorum* (4), *Bombus lapidarius* (3), *Bombus lucorum*
277 (2), *Bombus pascuorum* (2) and *Bombus pratorum* (2).

278

279 ***Gyne production***

280 Across all 47 nests (i.e. all species and both filmed and observed nests) gyne production was
281 significantly greater in 2010 than 2011 with gynes successfully produced by 71.4% and
282 21.1% of nests in 2010 and 2011, respectively ($\chi^2_1 = 12.7$, $P < 0.001$; Figure 1a). **The pattern**
283 **remains similar if we use only the more reliable data for filmed nests (63% versus 23% of**
284 **nests produced gynes in 2010 and 2011, respectively).** Two nests (nests 27 and 29; Table 1)
285 failed on or soon after the day that they were found (i.e. >2 bees were seen to enter or leave
286 the entrance, but thereafter, either no or very few (<5) bees were seen. It is highly unlikely
287 that gynes could have been made by these nests, but as we have no estimations of peak
288 traffic, vertebrate species visits, etc, these two nests were not included in statistical analysis
289 of predator/moth visits etc.

290 Of the filmed nests suitable for analysis, (n=30) those with high ‘peak bumblebee
291 traffic’ were significantly more likely to produce gynes ($F_{1,28} = 40.3$, $P < 0.001$; Figure 1b).
292 The likelihood of nests producing gynes was not related to the duration of nest filming ($F_{1,28}$

293 = 0.80, $P = 0.379$; Figure 1c) and this was not affected by removing data for *B. pratorum*.

294 Therefore data were collected approximately equally for both nests that successfully
295 produced gynes and nests that failed to produce gynes.

296

297 *Species interactions with bumblebee nests*

298 Thirty-three vertebrate species were recorded at bumblebee nest entrances on at least one
299 occasion (Table 2) in addition to the wax moth *A. sociella*, cuckoo bumblebees (*Psithyrus*)
300 and other true bumblebees. The majority of large vertebrates filmed did not interact with the
301 bumblebees or their nests.

302

303 a) Mammals

304 The most commonly observed interactive vertebrates were rabbits (*Oryctolagus cuniculus*)
305 and grey squirrel (*Sciurus carolinensis*), both of which are very common in the study area.
306 Sometimes they dug in leaf litter near the nest entrance, but they did not appear intent on
307 gaining access to nests, did not attempt to interact with bees, and were regularly observed
308 carrying out similar behaviour away from nest entrances. *Erinaceus europaeus* were filmed
309 investigating entrance holes and enlarging the entrance in what appeared to be deliberate
310 access attempts on seven occasions, but they were unable to penetrate in to any of the nests
311 (Figure 2).

312 Small mammals were very frequently recorded entering and leaving nest entrances,
313 particularly *A. sylvaticus*. However, these events may indicate shared occupancy of the
314 burrow system rather than predation of bumblebee nests and these observations are therefore
315 difficult to interpret. However, at two nests, (nests 16 and 23; Table 1) wood mouse visits
316 peaked during a single night and no bumblebee traffic was seen thereafter (Figure 3). In these
317 instances, mice carried leaf litter into the entrances and in one case (Figure 4) excavation of
318 the tunnel revealed that the tunnel had been tightly blocked with leaf litter and more than 50
319 live but subdued adults and considerable numbers of pupae and larvae remained in the nest,
320 suggesting that the blockage had ended nest activity prematurely.

321 Visits from *A. sylvaticus* to nests were not influenced by year, ($F_{1,28} = 1.16$, $P = 0.291$)
322 or peak bumblebee traffic ($F_{1,28} = 1.23$, $P = 0.276$). Numbers of *Sorex* spp. visits to
323 bumblebee nests differed significantly between years ($F_{1,28} = 44.86$, $P < 0.001$; Figure 6) but
324 were not influenced by bumblebee traffic ($F_{1,28} = 0.020$, $P = 0.890$). There were too few nests
325 visited by bank and field voles to allow statistical analysis.

326 Neither *A. sylvaticus* nor *Sorex* spp. visits affected the likelihood of a nest producing
327 gynes ($\chi^2_1 = 0.48$, $P = 0.485$ and $\chi^2_1 = 0.32$, $P = 0.571$, for *A. sylvaticus* and *Sorex* spp.,
328 respectively).

329

330 b) Birds

331 A number of bird species were seen investigating nest entrances (Table 2), but only *P. major*
332 were observed to depredate bees. Foragers/males and gynes were observed being captured as
333 they departed from or returned to the nest. On a total of 32 occasions at six nests, the birds
334 pecked at walking bees, but also appeared to watch returning bees before they landed and
335 occasionally pursued bees into the air. Great tits were also filmed exhibiting 'stalking
336 behaviour' on 17 occasions at eight nests (i.e. remained at entrance holes, looked inside,
337 removed leaf litter from the entrance, etc.) but no bees were present at the time. Stalking,
338 predation attempts or successful predations took place at ten nests, in both years, at sites up to
339 4 km apart.

340 The number of *P. major* depredations were not significantly different in either year (χ^2
341 $_1 = 1.13$, $P = 0.470$). There appeared to be a trend for *P. major* to target larger nests with
342 greater peak bumblebee traffic than smaller nests with infrequent bumblebee traffic, but this
343 trend was not significant ($F_{1,28} = 7.94$, $P = 0.057$; Figure 5). *Parus major* attacks were
344 positively correlated with gyne production ($\chi^2_1 = 5.47$, $P = 0.019$, Figure 7a).

345

346 c) Invertebrates

347 *Aphomia sociella*, were filmed entering and leaving 8 of the 30 filmed nests, but the
348 likelihood was not affected by the year ($F_{1,28} = 0.92$, $P = 0.443$) or peak bumblebee traffic (χ^2
349 $_1 = 1.92$, $P = 0.279$). *Aphomia sociella* visitations were positively correlated with gyne
350 production ($\chi^2_1 = 3.88$, $P = 0.049$, Figure 7b).

351 A *Bombus sylvestris* (cuckoo bumblebee) female was filmed exiting a *B. pratorum*
352 nest 5th June 2011 (nest 20; Table 1). Within seven days the nest traffic was much reduced to
353 0-4 bees per hour. No other *Psithyrus* were observed.

354 One *B. lapidarius* nest was visited by 14 *B. terrestris* or *B. lucorum* workers (Figure
355 8) over six days. None of the *B. terrestris* visitors were carrying pollen and so cohabitation of
356 the burrow system seems doubtful.

357 A queen *B. terrestris* or *B. lucorum* entered a small *B. terrestris* nest on 8th July 2010
358 (nest 17; Table 1) and a queen exited the nest approximately twelve minutes later. The queen
359 walked around the entrance of the nest for some time, eventually walking out of view.

360 Whether this bee was the founding queen or an intruder is unclear as the nest was queenless
361 eight days later when it was excavated. Subsequent genetic analysis of the remaining twelve
362 workers showed that they were sisters (O'Connor et al., 2013). Similarly, a *B. terrestris* or *B.*
363 *lucorum* queen was filmed entering a *B. terrestris* nest (nest 10; Table 1) in early July, and
364 subsequent genetic analysis of nest mates showed that there were unrelated individuals in the
365 nest but the foreign queen was not found (O'Connor et al., 2013).

366

367 d) Internal parasites

368 In total 1,179 faecal samples from *B. terrestris* workers from 29 nests were examined for
369 infections of the three protozoan infections (682 and 497 collected in 2010 and 2011,
370 respectively). *Crithidia bombi* was far more prevalent (49.0%) than *N. bombi* (5.54%) and
371 only eight bumblebees (0.68%) were infected with *A. bombi* (bees from five nests, all
372 detected in 2010). All 29 nests contained at least one worker infected with *C. bombi*, while
373 62% of nests had at least one bee infected with *N. bombi*.

374 Infections of *C. bombi* were detected more frequently in the faeces of *B. terrestris*
375 with increased wing wear (assumed to be older bees) compared to unworn, younger bees (χ^2_3
376 = 60.89, $P < 0.001$; Figure 9). There was a significant 'year by day' interaction; *B. terrestris*
377 were less likely to present *C. bombi* infections towards the end of the summer and this
378 decline was more marked in 2011 ($\chi^2_1 = 11.00$, $P < 0.001$; Figure 10). Infection with *N. bombi*
379 was not (quite) significantly associated with *C. bombi* infection, though the relationship was
380 positive ($\chi^2_1 = 3.82$, $P = 0.051$). *Crithidia bombi* infections did not spread through all nest
381 mates in wild *B. terrestris* nests (Figure 11 shows two typical examples of sampled nests).
382 There were often uninfected and infected bees collected within the same sample, and
383 intensity of infections varied greatly.

384 The likelihood of a *B. terrestris* worker presenting a *N. bombi* infection was
385 significantly affected by 'year' ($\chi^2_1 = 15.16$, $P < 0.001$) with a far greater proportion of *N.*
386 *bombi* infections detected in 2010 (9.1% and 0.90% in 2010 and 2011, respectively).
387 Bumblebees infected with *C. bombi* were significantly more likely to be infected with *N.*
388 *bombi* ($\chi^2_1 = 11.34$, $P < 0.001$). The likelihood of a *B. terrestris* worker being infected with
389 *N. bombi* was not associated with bee wing wear ($\chi^2_3 = 0.27$, $P = 0.965$; Figure 9). There was
390 no relationship with 'day' ($\chi^2_1 = 0.1$, $P = 0.750$) and there was no 'day' by 'year' interaction
391 ($\chi^2_1 = 0.23$, $P = 0.630$).

392 The likelihood of a *B. terrestris* nest producing new gynes was not affected by
393 presence of *A. bombi* in at least one worker ($\chi^2_1 = 0.447$, $P = 0.580$), nor by the proportion of

394 workers infected with *N. bombi* ($\chi^2_1 = 0.217$, $P = 0.641$). The proportion of workers infected
395 with *C. bombi* was a significant negative predictor of the likelihood of a nest producing
396 gynes, ($\chi^2_1 = 7.433$, $P = 0.006$; Figure 12).

397

398 **Discussion**

399 The proportion of nests producing gynes varied between the two years of observations with
400 more nests producing gynes in 2010 than 2011. No obvious reason for this disparity was
401 observed in the field and the trend was not explained by rates of other species visitations to
402 nests. Indeed, the proportion of *B. terrestris* infected with *C. bombi* and *N. bombi* was
403 appreciably lower in 2011, but this is unlikely to have caused any reduction in gyne
404 production. Gyne production was positively predicted by peak traffic, suggesting that
405 intensive monitoring of nest traffic can provide useful data on the strength of bumblebee
406 nests. Bumblebee nests can utilize multiple entrance holes, sometimes metres apart (D.G.
407 pers. obs.), which might influence both measurements of traffic and of gyne production, but if
408 this did occur in our nests it was not sufficient to obscure the relationship between the two.

409 Perhaps surprisingly, gyne production was not predicted by colony duration, but it
410 should be noted that our estimate of colony duration (the length of time for which we
411 observed it) was crude, since we do not know when colonies were founded and hence how
412 long they had been in existence when we located them.

413

414 ***Interactions with mammals***

415 Despite many indications in the literature that large mammals such as *M. meles* and perhaps
416 *V. vulpes* are predators of bumblebee nests (Sladen, 1912; Pouvreau, 1973; Alford, 1975;
417 Goulson, 2010), no such events were recorded in our study. We have never discovered sets of
418 *M. meles* in the study area, so it is likely that they are locally absent. However, *V. vulpes* is
419 locally abundant and was captured four times on camera traps but showed no interest in the
420 nests. Furthermore, O'Connor (2013) surveyed studies of the diet of *V. vulpes*; of 2,617 scat
421 samples that have been dissected for identifiable prey parts, none contained fragments of
422 *Bombus*. Together, this suggests that Sladen (1912) may have been incorrect on this point.
423 *Erinaceus europeaus* were observed investigating nests, and had these been surface nests such
424 as those of *B. pascuorum* it seems plausible that they may have attempted to depredate them,
425 but they were unable to access these subterranean nests of *B. terrestris*.

426 Visits by smaller mammals were very frequent. All bumblebee nests were found in
427 networks of nests, runs and burrows which appeared to have been made by other animals,

428 which is a well-known trait of bumblebees (Sladen, 1912; Alford, 1975; Lye et al., 2012).
429 Camera footage indicated that the majority of these burrows were frequented by mice, shrews
430 and/or voles at the time of bumblebee occupation (Table 2). Early literature suggests that
431 these small mammals are major predators of bumblebee nests (Darwin, 1859; Sladen, 1912;
432 Cumber, 1953; Pouvreau, 1973), but we found no evidence for this. Visits by small mammals
433 were not targeted towards large nests, and nor did they predict subsequent gyne production.
434 *Sorex* spp. were more common in 2011, when fewer nests produced gynes, but we suggest
435 that this is unlikely to be causative since the nests visited by *Sorex* spp. did not have a
436 reduced likelihood of producing gynes.

437 So far as our data indicate, it seems most likely that small mammals are simply
438 sharing the burrows, using them at night when the bees are inactive. However, it is important
439 to note that Sladen (1912) suggests that small mammals may primarily depredate very young
440 nests, when only the queen is present. Our nests were detected by the presence of worker
441 traffic or by their smell, and all had workers present when located. Thus, we can infer nothing
442 about predation levels early in the season.

443 Filming inside bumblebee nest (perhaps using an endoscope) would be needed to
444 establish the actual relationships between small mammals and bumblebees. Such footage
445 would also facilitate examining interactions out-with the scope of this study (e.g. effects of
446 *Talpa europaea*). It would also be extremely interesting to film incipient nests as, in addition
447 to small mammal attacks, this is when most usurpations and nest failures are thought to take
448 place (Alford, 1975). However, finding and filming such nests in the wild poses a serious
449 challenge.

450

451 ***Interactions with birds***

452 *Parus major* were previously known to depredate vulnerable/walking bumblebees, including
453 bees feeding on *Rhododendron* spp. (Free and Butler, 1959) or *Tilia* spp. (Sladen, 1912;
454 Benton, 2006) which seem to have an intoxicating effect on bees, and also when infected
455 with *Sphaerularia bombi* (Bols; quoted in Benton, 2006). However, this is the first time that
456 *P. major* have been found to depredate ‘healthy’ bumblebees and identifies them as a
457 potentially significant predator of bumblebee nests, for almost a third of filmed nests were
458 targeted by them, and *P. major* is an abundant species across much of Europe and Asia. It
459 would be useful for further filming of nests to be carried out elsewhere to establish if this
460 behaviour is restricted to the region or is common elsewhere. *Parus major* tend to attack nests
461 with high traffic which were likely to produce (or be producing) gynes, and it seems likely

462 that this was simply because these nests were easier to locate. Since they were observed
463 capturing gynes it is possible that they are having a significant impact at the population level.

464 Other bird species such as *Corvus corone*, *Erithacus rubecula* and *Turdus merula* also
465 appeared to show interest in bumblebee nests.

466

467 ***Interactions with invertebrates***

468 Eight nests were entered by *A. sociella*, the larvae of which can be highly damaging to
469 bumblebee nests (Sladen 1912; Free and Butler, 1959; Pouvreau 1973; Alford 1975; Goulson
470 et al., 2002). As with *P. major*, visits were targeted at nests that were likely to go on to
471 produce gynes, perhaps because these nests were large and therefore more easily detected.
472 However, this positive relationship suggests that the moths may not have had a major impact
473 on nest success, perhaps because moth infestations that begin in summer are unlikely to cause
474 significant damage before the nest has produced new gynes. Of course, it may be that these
475 nests would have produced more gynes if not infested.

476 We recorded few other interactions with the larger invertebrates detectable with our
477 cameras. Only one *Psithyrus* was observed, a single *B. sylvestris* queen was recorded exiting
478 a *B. pratorum* nest. Nest traffic dwindled thereafter, but since the observation was made in
479 June, nests of *B. pratorum* (which is an early species) tend to be at the end of their natural life
480 at this time. We recorded no *Psithyrus* entering nests of *B. terrestris*, which is unsurprising
481 given that its main cuckoo bee associate is *Bombus vestalis*, which did not occur in Scotland
482 at the time (Benton, 2006). It should also be noted that, as with predation by small mammals,
483 *Psithyrus* are thought to attack mainly when their host nests are small, so it is likely that we
484 may have missed much of this activity.

485 We did record one instances of repeated entry of a *B. lapidarius* nest by *B.*
486 *terrestris/lucorum* workers, and it seems likely that they were stealing nectar as this has been
487 reported before (Free and Butler, 1959; Andrews, 1969). The *B. lapidarius* nest had already
488 produced gynes, but it seems likely that if a nest were invaded at an earlier stage, the effect
489 could be detrimental to the host colony, either through reducing food stores or horizontal
490 pathogen transmission. Intra-specific robbery may also occur, but we could not detect this
491 with our cameras.

492 It appeared that a failed usurpation attempt was recorded at one *B. terrestris* nest and
493 potentially a successful usurpation at another. Usurpation by true bumblebee queens is
494 thought to occur early in the season, (Sladen, 1912; Alford, 1975; Donovan and Weir, 1978;
495 Paxton et al., 2001) whereas the potentially successful usurpation occurred later in the season.

496 These were the only detected incidences of nest usurpation which is surprising considering
497 the frequency found in previous studies. For example, of 48 artificially reared *B. terrestris*
498 nests which were placed in the field in spring time, 18 colonies were invaded by a total of 30
499 wild *B. terrestris* queens (Carvell et al., 2008). However, these were not wild nests but were
500 lab-reared and placed in artificial boxes, which may be more easily detected.

501

502 *Effects of internal parasites*

503 Infections of *C. bombi* and *N. bombi* spread horizontally between nest mates consuming
504 contaminated nectar and pollen from stores in wax pots within nests, **via contact between**
505 **individuals or shared contact with larvae**, (Otti and Schmid-Hempel 2008; Erler et al., 2012;
506 **Folly et al. 2017**) or between foragers visiting flowers which have recently been
507 contaminated by an infected bumblebee (Durrer and Schmid-Hempel, 1994; Rutrecht et al.,
508 2007; Graystock et al. 2015). In broad accordance with earlier studies, *C. bombi* was found to
509 be the most common of the three parasites, followed by *N. bombi* at low prevalence and with
510 *A. bombi* being very scarce. Rates of infections of *C. bombi* and *N. bombi* have been found to
511 vary greatly between bumblebee species, populations and years (Otti and Schmid-Hempel,
512 2008; Popp et al., 2012), and we found that *N. bombi* prevalence was tenfold higher in 2010
513 (the year when gyne production was higher) compared to 2011.

514 Neither *N. bombi* nor *A. bombi* infections were associated with any measurable
515 negative impact on traffic or gyne production (though this does not of course demonstrate
516 that they are not harmful to their hosts). Interestingly, *N. bombi* infections were more
517 frequent in individuals that were also infected with *C. bombi*, which may be because the
518 presence of one parasite impairs the immune response, enabling attack by a second, or
519 alternatively may be because jointly infected bees possess behavioural traits that render them
520 more likely to be exposed to both parasites. For example, their preferred flower(s) may be
521 ones on which rates of pathogen contamination are high.

522 In accordance with previous studies we found that older bumblebees were more likely
523 to be infected with *C. bombi* and this is thought to be due to increased exposure and reduced
524 immune response (Shykoff and Schmid-Hempel, 1991; Otterstatter and Thompson, 2006).
525 However, in contrast to previous studies we also found that, having taken into account the
526 effects of bee age, prevalence dropped in late season, particularly in 2011. **We are unable to**
527 **explain this pattern.**

528 To our knowledge, ours are the first data on patterns of changing parasite prevalence
529 in truly wild bumblebee nests, and also the first to provide evidence that these parasites might

530 impact on the success of wild nests. We found that nests with a high prevalence of infection
531 of workers with *C. bombi* were less likely to produce gynes. Without experimental
532 manipulation (for example by inoculating some nests in early season) we cannot be sure that
533 this relationship is causative, however, and *C. bombi* is generally thought to have relatively
534 mild effects on its host (Brown et al., 2000) (though when combined with other stress is can
535 strongly impact on queen founding success, Brown et al. 2003).

536 Overall, our data provide a unique insight into the relationships between bumblebees
537 and their predators and parasites, identifying many new questions and avenues for further
538 research. It would be fascinating to continue this further; for example, we gathered
539 insufficient data on any species other than *B. terrestris* to be able to detect differences
540 between bee species in their susceptibility to predators, but such differences are likely. The
541 discovery of regular predation by *P. major* at the entrances to bumblebee nests suggests the
542 possibility of significant negative impacts at the population level that were hitherto
543 unsuspected. Interactions with small mammals are likely to be very frequent but require
544 further investigation to indicate what happens beneath the ground. It is clear that bumblebee
545 nests are at the heart of a complex web of interactions with diverse predators and parasites
546 which we are only just beginning to describe.

547

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554

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682

683 Figure 5. Total number of great tit attacks in relation to peak traffic of nests. Points 8 and 23 were
684 removed from the analysis as they had Cook's distance greater than 1 (i.e. they were overly influential
685 outliers; Zuur et al., 2007).

686

687 **Figure Legends**

688 Figure 1 (a) Total nests and presence or absence of new gynes, for all species. (b) Mean bee peak of
689 traffic for nests with and without new gynes (filmed nests only). (c) Mean of total days nests were
690 observed for. Error bars in b and c show standard errors of means.

691

692 Figure 2a. Great tit depredating *B. terrestris* (nest 1; Table 1); (b) Hedgehog ‘rooting’ in leaves at nest
693 entrance (nest 1; Table 1).

694

695 Figure 3. Wood mouse visits during 24hr and daily bumblebee midday hourly traffic at (a) nest 23 (*B.*
696 *hortorum*) and (b) nest 16 (*B. terrestris*). Breaks in lines indicate loss of footage. Wood mice
697 transported leaf litter into nest entrances during visits.

698

699 Figure 4 (a) Wood mice transported leaf litter into *B. terrestris* nest entrance (nest 16; Table 1). (b)
700 Nest tunnel and external entrance was blocked by leaves and sticks placed by wood mice several
701 hours later. Bumblebee traffic ceased.

702

703 Figure 5. Total number of great tit attacks in relation to peak traffic of nests. Points 8 and 23 were
704 removed from the analysis as they had Cook’s distance greater than 1 (i.e. they were overly influential
705 outliers; Zuur et al., 2007).

706

707 Figure 6. (a) More shrews were recorded visiting nests in 2011 than in 2010 (mean and standard
708 errors); (b) There was no relationship between shrew visits and peak bumblebee traffic. *Points 23
709 and 15 were removed from statistical analysis as they were overly influential on the data set (Cook’s
710 distance of >1; Zuur et al., 2007).

711

712 Figure 7. Mean great tit attacks (a) and wax moth events (b) to nests with and without gyne
713 production (error bars show standard errors).

714

715 Figure 8 (a) *B. lapidarius* nest (b) visited by *B. terrestris* or *B. lucorum* worker. The footage allows
716 identification from the different stripe patterns between some species. (The red tail of *B. lapidarius*
717 appears white.)

718

719 Figure 9. Proportion of *B. terrestris* infected with *C. bombi* and *N. bombi* within each age
720 class (0=no wing wear; 1=some indentations; 2=<5% of wing surface damaged; 3=>5% wing
721 wear absent).

722

723 Figure 10. Proportion of worker *B. terrestris* infected with *C. bombi*, throughout the experiment in (a)
724 2010 and (b) 2011.

725

726 Figure 11. Intensity of *C. bombi* infections in *B. terrestris* from two typical nests for the duration of
727 observations (Ref 16 and 26; Table 1; (a) and (b) respectively).

728

729 Figure 12. Mean proportion of *B. terrestris* workers infected with *C. bombi* from 29 nests, with and
730 without gyne production (with interquartile ranges, maximum and minimum values shown).

731

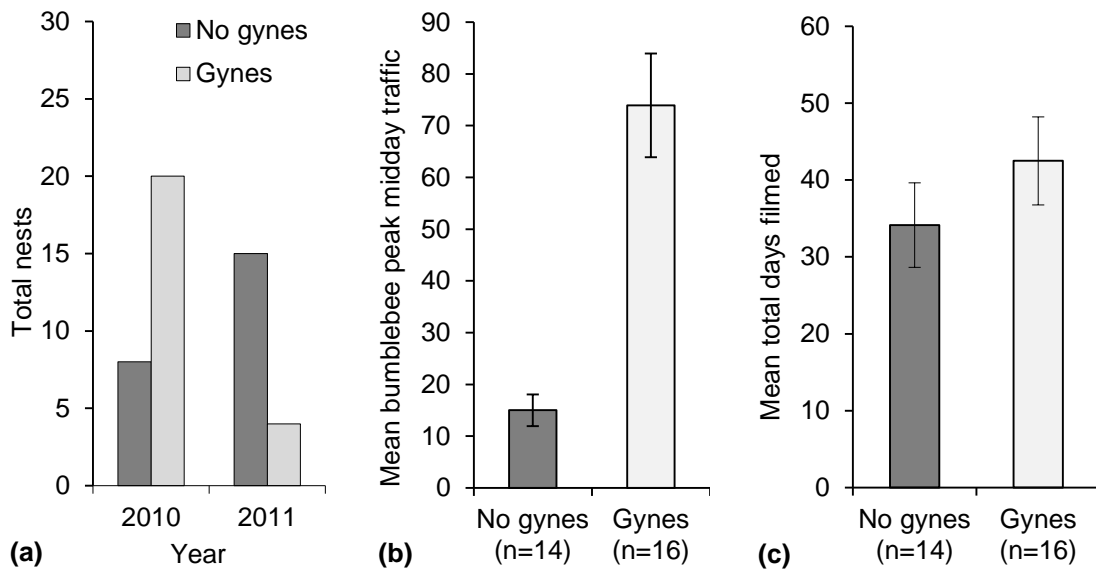
732 Table 1. Longevity, gyne production and the proportion of bees hosting *C. bombi* and *N. bombi*
 733 infections for filmed nests. *Nest which failed prior to filming; >2 bees were seen to enter or leave,
 734 but footage revealed few/no further bee traffic. These were excluded from predation analysis.

Nest details			Period of filming		Gynes Produced	Proportion of infected bees		
No.	Species	Habitat	Start	End		<i>C. bombi</i>	<i>N. bombi</i>	(n)
1	<i>B. terrestris</i>	Woodland	19/07/10	17/08/10	Yes	0.46	0.05	39
2	<i>B. terrestris</i>	Woodland	27/07/10	17/08/10	Yes	0.74	0.03	35
3	<i>B. terrestris</i>	Woodland	15/06/10	26/07/10	No	0.92	0.08	26
4	<i>B. terrestris</i>	Woodland	09/08/10	18/08/10	No	1.00	0.50	2
5	<i>B. terrestris</i>	Grassland	29/07/10	16/09/10	Yes	0.48	0.05	65
6	<i>B. hortorum</i>	Grassland	25/06/10	05/08/10	Yes	0.43	0.11	37
7	<i>B. hortorum</i>	Grassland	19/06/10	25/07/10	No	0.62	0.12	34
8	<i>B. lapidarius</i>	Woodland	27/07/10	10/08/10	Yes	0.50	0.00	6
9	<i>B. pratorum</i>	Grassland	10/06/10	08/07/10	No	0.80	0.07	15
10	<i>B. terrestris</i>	Grassland	13/06/10	28/07/10	No	0.34	0.00	29
11	<i>B. terrestris</i>	Woodland	22/06/10	30/07/10	Yes	0.50	0.06	34
12	<i>B. terrestris</i>	Woodland	09/08/10	20/08/10	No	0.29	0.00	7
13	<i>B. terrestris</i>	Woodland	18/08/10	22/08/10	Yes	0.60	0.20	5
14	<i>B. lucorum</i>	Woodland	16/06/10	29/08/10	Yes	0.51	0.03	63
15	<i>B. terrestris</i>	Woodland	22/06/10	27/07/10	Yes	0.65	0.18	55
16	<i>B. terrestris</i>	Woodland	13/08/10	06/09/10	Yes	0.28	0.01	80
17	<i>B. terrestris</i>	Woodland	16/06/10	16/07/10	No	1.00	0.00	9
18	<i>B. terrestris</i>	Woodland	29/06/10	16/08/10	Yes	0.39	0.07	61
19	<i>B. terrestris</i>	Woodland	19/06/10	03/09/10	Yes	0.69	0.21	94
20	<i>B. pratorum</i>	Woodland	31/05/11	20/06/11	No	-	-	-
21	<i>B. terrestris</i>	Woodland	01/06/11	08/08/11	No	0.55	0.02	60
22	<i>B. terrestris</i>	Woodland	01/06/11	29/08/11	No	0.27	0.01	138
23	<i>B. hortorum</i>	Woodland	01/06/11	08/08/11	No	0.38	0.00	13
24	<i>B. terrestris</i>	Woodland	02/06/11	11/07/11	No	0.71	0.00	24
25	<i>B. terrestris</i>	Grassland	02/06/11	29/06/11	No	0.50	0.00	10
26	<i>B. terrestris</i>	Grassland	06/06/11	02/09/11	Yes	0.38	0.02	112
27*	<i>B. terrestris</i>	Woodland	09/06/11	15/06/11	No	-	-	-
28	<i>B. terrestris</i>	Woodland	14/06/11	23/06/11	No	1.00	0.00	1
29*	<i>B. terrestris</i>	Woodland	23/06/11	24/06/11	No	-	-	-
30	<i>B. terrestris</i>	Woodland	27/06/11	01/09/11	Yes	0.27	0.00	75
31	<i>B. terrestris</i>	Woodland	12/07/11	28/09/11	No	0.38	0.00	58
32	<i>B. terrestris</i>	Woodland	22/07/11	10/09/11	Yes	0.33	0.00	51

736 Table 2. Interactions with animals observed on the cameras. Invertebrate observations were not
 737 recorded, with the exception of wax moths and their larvae.

Species (common name)	Events	Nests	Summary of interactions with nests (n=number of times observed)
Large mammals			
<i>Vulpes vulpes</i> (fox)	4	4	No interaction
<i>Mustela erminea</i> (stoat)	12	5	Enter and leave (1)
<i>Erinaceus europaeus</i> (hedgehog)	34	15	Attempts to gain access (7)
<i>Sciurus carolinensis</i> (grey squirrel)	157	22	Sniffed at or near entrance (32), looked in hole or dug at nearby leaves (7)
<i>Oryctolagus cuniculus</i> (rabbit)	207	22	Sniffed at entrance (34), entered hole (1)
<i>Lepus europaeus</i> (hare)	7	3	No interaction
<i>Capreolus capreolus</i> (roe deer)	8	4	No interaction
<i>Ovis aries</i> (sheep)	1	1	No interaction
<i>Felis catus</i> (cat)	6	3	No interaction
<i>Canis lupus familiaris</i> (dog)	1	1	No interaction
<i>Bos primigenius</i> (cow)	9	1	No interaction
Small mammals			
<i>Clethrionomys glareolus</i> (bank vole)	17	4	Enter and leave (8)
<i>Microtus arvalis</i> (field vole)	70	3	Enter and leave (21)
<i>Apodemus sylvaticus</i> (wood mouse)	1396	18	Enter and leave (837)
Unidentified small mammal	16	7	Enter and leave (16)
<i>Sorex</i> spp. (shrew species)	162	10	Enter and leave (56)
Reptiles and Amphibians			
<i>Lacerta vivipara</i> (common lizard)	1	1	No interaction
<i>Rana temporaria</i> (frog)	7	5	No interaction
<i>Bufo bufo</i> (toad)	5	3	No interaction
Birds			
<i>Anas platyrhynchos</i> (mallard)	1	1	No interaction
<i>Columba palumbus</i> (wood pigeon)	8	3	No interaction
<i>Corvus corone corone</i> (carrion crow)	16	4	Pecking at hole and widening entrance (5)
<i>Erithacus rubecula</i> (robin)	29	12	Investigation/waiting at nest (5) possible attempted predation of worker (1)
<i>Fringilla coelebs</i> (chaffinch)	20	6	Looking at or waiting at hole (3) no bee chases or kills
<i>Haematopus ostralegus</i> (oyster catcher)	12	1	No interaction; Investigating entrance (1)
<i>Turdus merula</i> (blackbird)	28	13	Investigating/waiting at hole (5) Possible attempted predation of worker (1)
<i>Turdus</i> spp. (thrush other)	10	6	Entrance investigated (1), no traffic and no predation
<i>Parus caeruleus</i> (blue tit)	1	1	No interaction
<i>Parus major</i> (great tit)	60	10	Predations (10) attempted predations (22) 'stalking' (17)
<i>Passer montanus</i> (tree sparrow)	6	4	No interaction
<i>Pica pica</i> (magpie)	1	1	No interaction
<i>Prunella modularis</i> (dunnock)	22	4	Investigating/waiting at entrance (6) no bee chases or kills
<i>Troglodytes troglodytes</i> (wren)	11	7	No interaction
Wax moth			
<i>Aphomia sociella</i>	19	8	Enter and leave (19)

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742 Figure 1

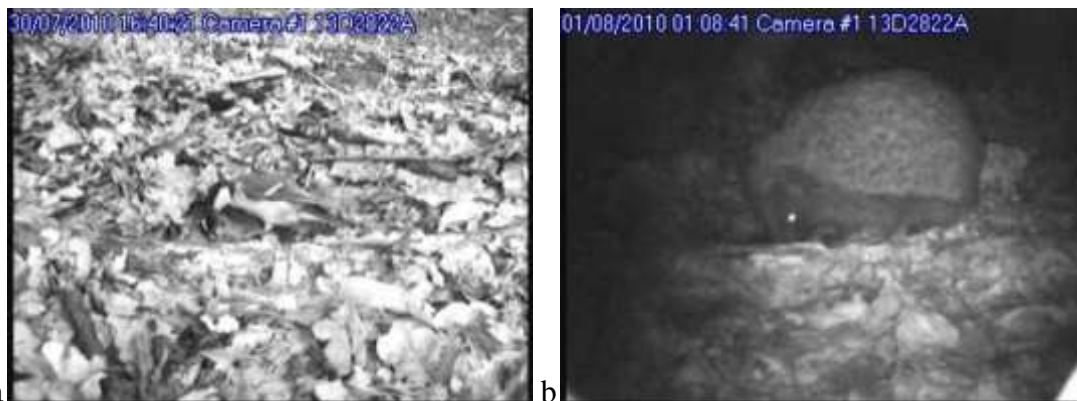
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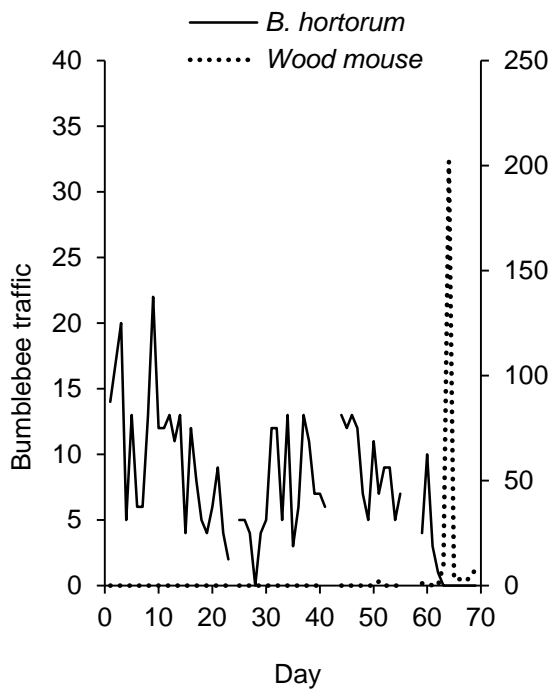


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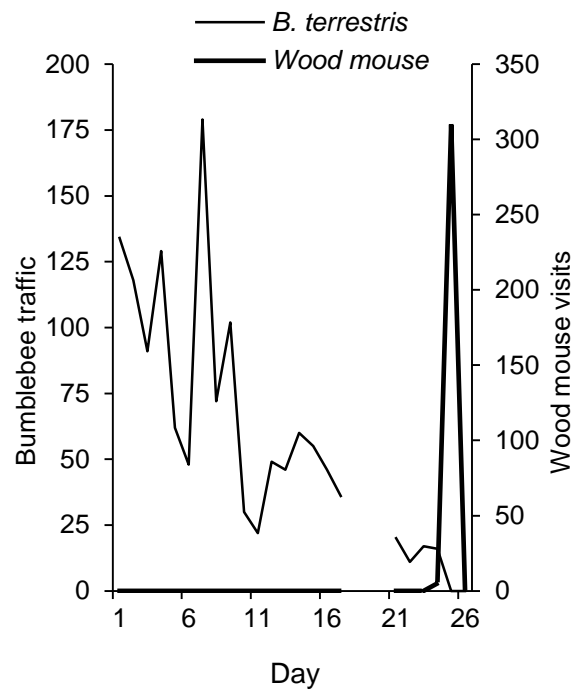
749 Figure 2

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751 (a)



(b)



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753 Figure 3

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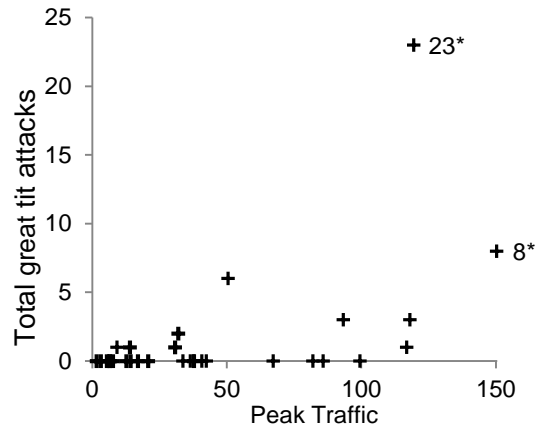


757 a

b

758 Figure 4

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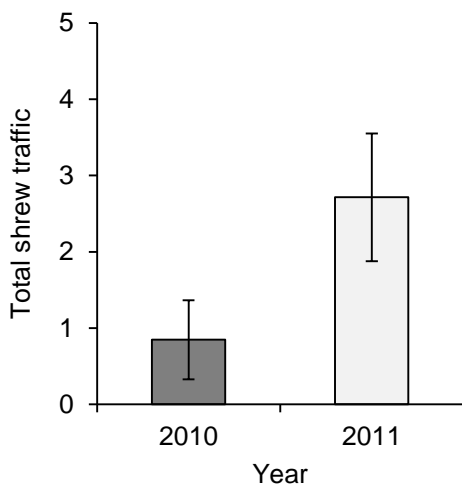
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761 Figure 5.

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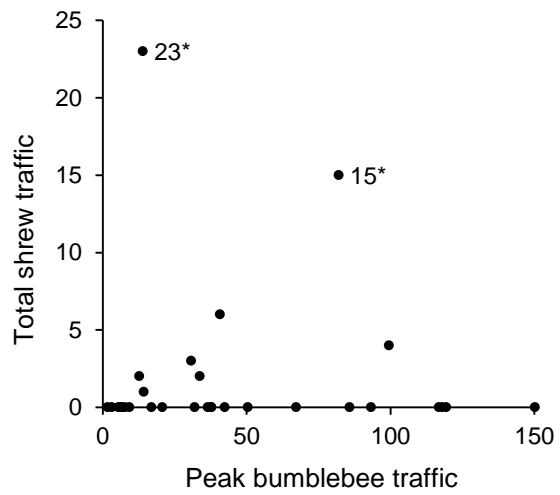
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765 (a)

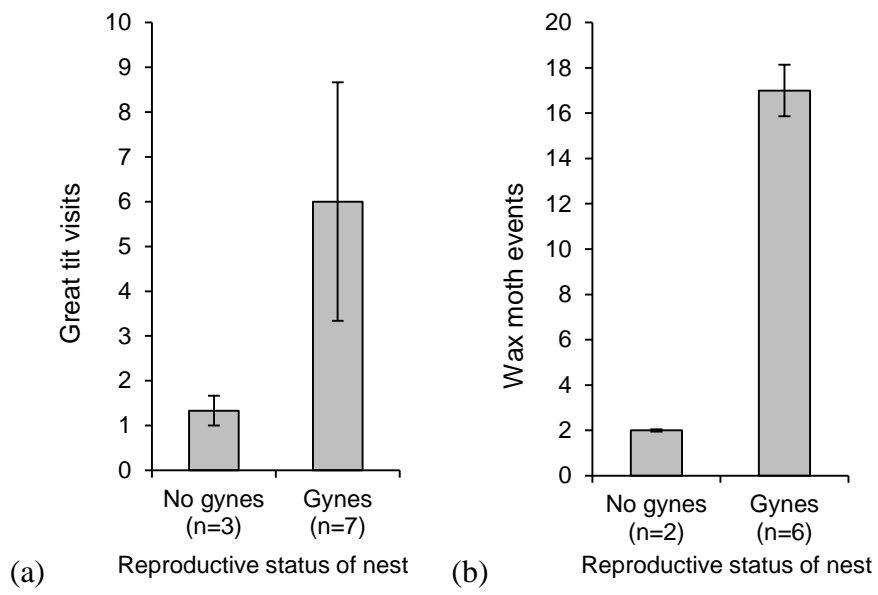


(b)



766
767 Figure 6.

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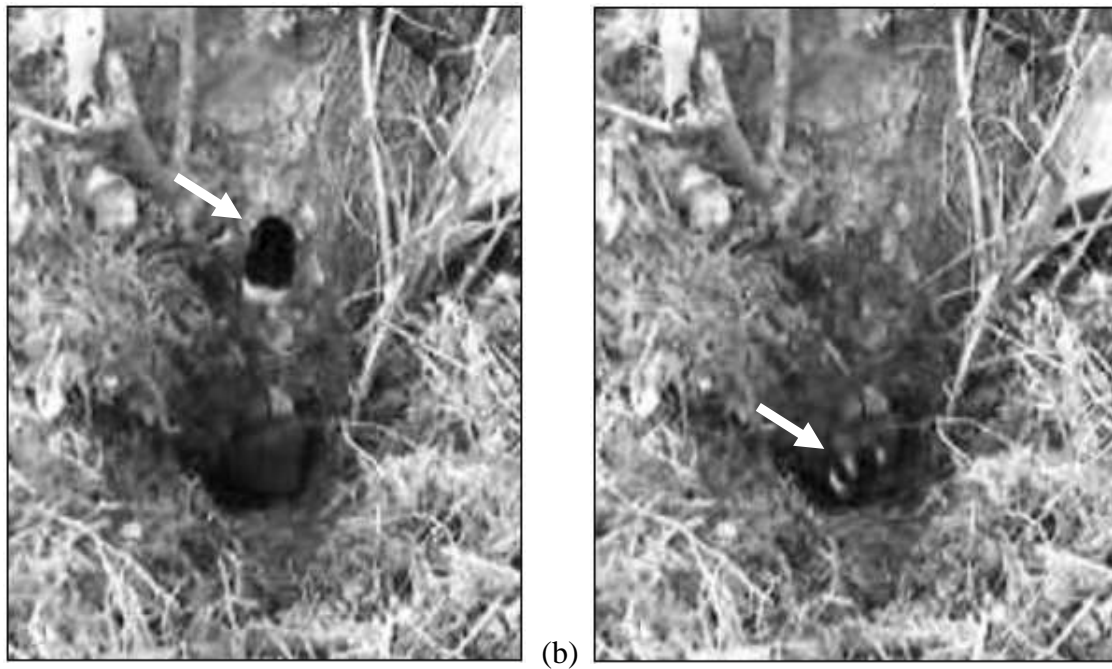


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771 Figure 7.

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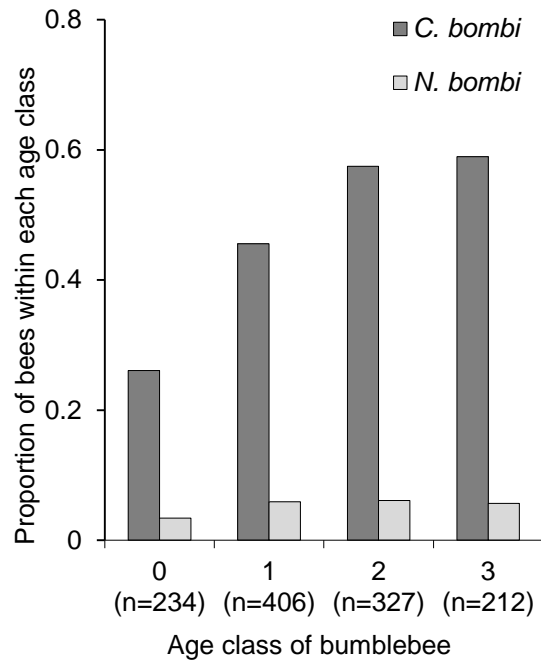
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775 Figure 8

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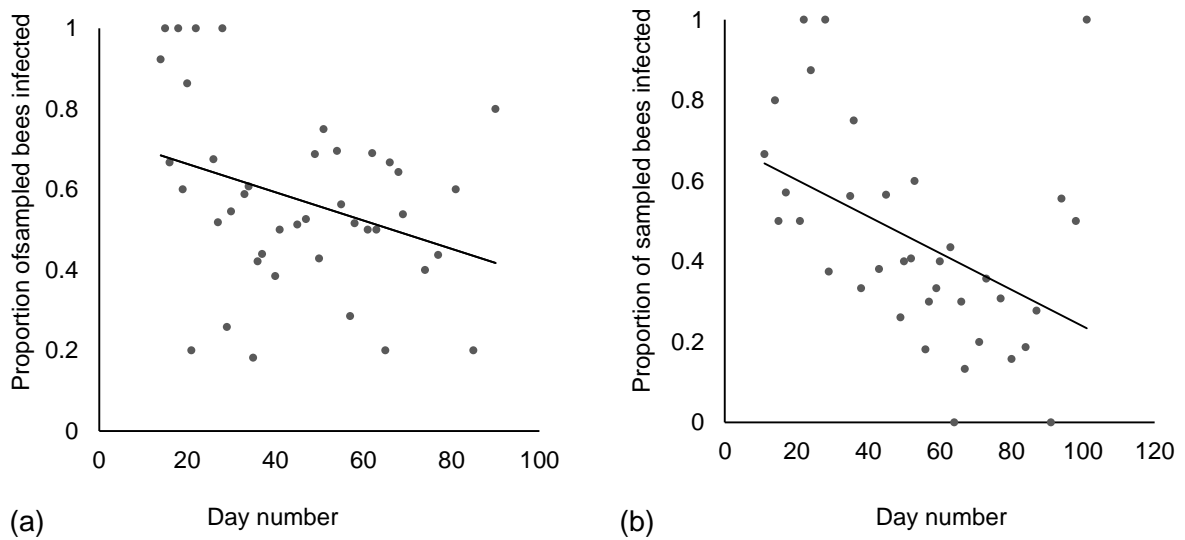
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778 Figure 9.

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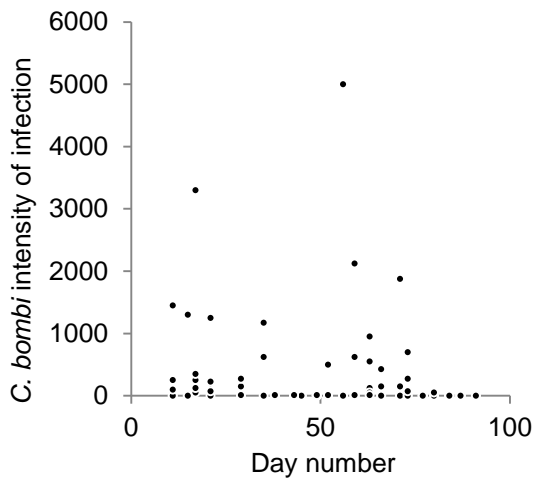
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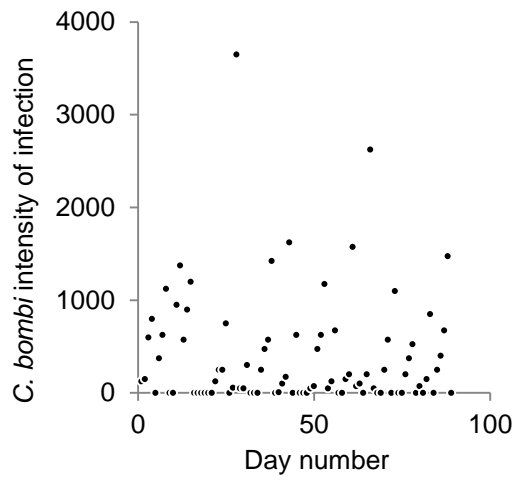
784 Figure 10.

785

786 (a)



(b)



787

788 Figure 11

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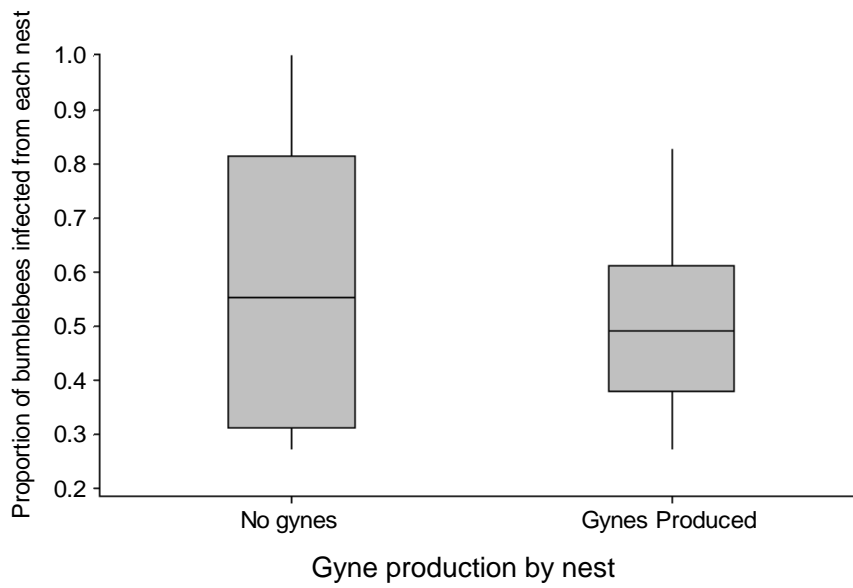
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796 Figure 12

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