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The relationship between managed bees and the prevalence of parasites in bumblebees

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15 **ABSTRACT**

16 Honey bees and, more recently, bumblebees have been domesticated and are now managed
17 commercially primarily for crop pollination, mixing with wild pollinators during foraging on
18 shared flower resources. There is mounting evidence that managed honey bees or
19 commercially produced bumblebees may affect the health of wild pollinators such as
20 bumblebees by increasing competition for resources and the prevalence of parasites in wild
21 bees. Here we screened 764 bumblebees from around five greenhouses that either used
22 commercially produced bumblebees or did not, as well as bumblebees from 10 colonies
23 placed at two sites either close to or far from a honey bee apiary, for the parasites *Apicystis*
24 *bombi*, *Crithidia bombi*, *Nosema bombi*, *N. ceranae*, *N. apis* and deformed wing virus. We
25 found that *A. bombi* and *C. bombi* were more prevalent around greenhouses using
26 commercially produced bumblebees, while *C. bombi* was 18% more prevalent in bumblebees
27 at the site near to the honey bee apiary than those at the site far from the apiary. Whilst these
28 results are from only a limited number of sites, they support previous reports of parasite
29 spillover from commercially produced bumblebees to wild bumblebees, and suggest that the
30 impact of stress from competing with managed bees or the vectoring of parasites by them on
31 parasite prevalence in wild bees needs further investigation. It appears increasingly likely that
32 the use of managed bees comes at a cost of increased parasites in wild bumblebees, which is
33 not only a concern for bumblebee conservation, but which may impact other pollinators as
34 well.

35
36 **Subjects** Entomology, Conservation Biology, Parasitology

37 **Keywords** pathogen spillover, pollinator conservation, honeybee, commercial bumblebee
38 production

39 INTRODUCTION

40 In recent years several bumblebee species as well as other pollinators have suffered range
41 declines in parts of Europe, the Americas and Asia (Biesmeijer et al. 2006; Cameron et al.
42 2011; Goulson et al. 2008; Potts et al. 2010). Changes in anthropogenic land-use is a major
43 contributing factor to these declines, with agricultural intensification reducing floral diversity
44 and nesting habitats from many pollinators (Goulson et al. 2005; Ricketts et al. 2008;
45 Vanbergen et al. 2013). This has left some bumblebee species fragmented, in small
46 populations with low genetic diversity, something which may make bees more vulnerable to
47 stresses such as parasites (Darvill et al. 2006; Ellis et al. 2006; Evison et al. 2013; Oldroyd
48 2007; Whitehorn et al. 2011).

49 In addition to the stresses of habit loss, pesticide exposure and natural parasites,
50 (Goulson 2003), the use of managed bees may place additional stresses on bumblebee
51 populations. Honey bees have been managed commercially for crop pollination and honey
52 production for centuries, and are often kept in commercial apiaries with tens to thousands of
53 colonies, substantially increasing the density of bees in an area. Bumblebees are also now
54 commercially produced and used mainly in greenhouses, but also sometimes in polytunnels
55 and open crops, in Europe, North America, South America, New Zealand and Asia to
56 enhance the yields of soft fruit crops (Velthuis & van Doorn 2006). Although the
57 greenhouses in which commercially produced bumblebees are most commonly used are
58 meant to be closed, the commercially produced bumblebees are frequently found foraging
59 outside the greenhouses, and wild bees have been found foraging inside them (Kraus et al.
60 2011; Morandin et al. 2001; Murray et al. 2013; Whittington et al. 2004). By freely mixing
61 with wild bumblebees, the deployment of commercially produced bumblebees effectively
62 increases the local density of bumblebees. Bumblebee parasites can be dispersed between
63 bumblebees following shared flower usage (Durrer & Schmid-Hempel 1994), and, as a result,

64 the rate of parasite transmission between bees will predictably rise with increased pollinator
65 density (Arneberg et al. 1998). In areas utilising commercially produced bumblebees, higher
66 parasite prevalence may be expected to be the result, due to either the spillover of parasites
67 from the commercially produced bumblebees, parasite spillback from wild bumblebees, or
68 stress related to the high pollinator density (Kelly et al. 2009; Power & Mitchell 2004;
69 Schmid-Hempel 2011).

70 The spillover of parasites from one host to another, either intraspecifically or
71 interspecifically, is well known for many organisms (Power & Mitchell 2004). There is now
72 good evidence that the honey bee parasites *Nosema ceranae* and deformed wing virus have
73 spilled over to bumblebees, with both being virulent and now widespread in their new
74 bumblebee host (Evison et al. 2012; Furst et al. 2014; Genersch et al. 2006; Graystock et al.
75 2013a; Plischuk et al. 2009). In addition, parasites may also spill over to wild bumblebees
76 from the commercially reared bumblebees used in greenhouses. Colonies of commercially
77 produced bumblebees have been shown in many studies to carry parasites (Colla et al. 2006;
78 Gegear et al. 2005; Manson et al. 2010; Meeus et al. 2011; Murray et al. 2013; Otterstatter &
79 Thomson 2007; Singh et al. 2010; Whittington & Winston 2003), with the most recent study
80 using sensitive molecular methods finding that three-quarters of the colonies investigated
81 were infected by at least one parasite and confirming that these parasites were in many cases
82 infectious (Graystock et al. 2013b). The introduction of commercially produced bumblebees
83 has been associated with the introduction of foreign parasites and correlated declines in
84 native bumblebee species in Japan, South America and North America, suggesting that the
85 spillover of parasites has occurred on multiple occasions (Arbetman et al. 2012; Colla et al.
86 2006; Goka et al. 2001; Meeus et al. 2011; Otterstatter & Thomson 2008; Szabo et al. 2012).

87 Although attention has focussed on parasite spillover, it is also possible that the use of
88 managed honey bees and commercially produced bumblebees may increase the prevalence of

89 parasites in wild bumblebees via parasite spillback or heightened stress from increased
90 competition when foraging. Managed honey bees or commercially produced bumblebees may
91 become infected with parasites carried by the wild bees, and their unnaturally high density in
92 apiaries or greenhouses may then result in them acting as a reservoir in which the prevalence
93 of parasites becomes high, from which the parasites can then spillback into wild bees (Kelly
94 et al. 2009). The increased competition for resources caused by the introduction of high
95 densities of managed honey bees or commercially produced bumblebees may also stress wild
96 bees due to the increased competition when foraging, which can have negative effects on
97 various fitness components including resistance to parasites (Brown et al. 2000; Elbgami et
98 al. 2014; Foley et al. 2012; Goulson & Sparrow 2009; Lafferty & Gerber 2002; Mallon et al.
99 2003).

100 The prevalence of parasites in wild bumblebees appears to be greater when the bees
101 are in proximity to greenhouses using commercially produced bumblebee colonies (Colla et
102 al. 2006; Murray et al. 2013; Otterstatter & Thomson 2008). However, whether this is due to
103 parasite spillover, parasite spillback, or stress, is not always clear. Here we investigate the
104 relationships between commercially reared bumblebees or managed honey bees and the
105 prevalence of a range of parasites in bumblebees. We first examine the relationship between
106 the prevalence of parasites in wild bumblebees and proximity to three farms in which
107 commercially reared bumblebees being used and two greenhouse farms in which they were
108 not being used. In addition, we examine the effect of proximity to honey bees on bumblebee
109 parasite prevalence, using bumblebee colonies located at two sites, either near or far from an
110 apiary.

111

112

113 **MATERIALS AND METHODS**

114 **The effect of proximity to commercially reared bumblebees**

115 To determine the prevalence of parasites at sites either using commercially produced
116 bumblebees or not, five greenhouse farm sites in England were selected. Sites were selected
117 based on the presence of large scale commercial fruit farms (ca. 50-75 ha) that utilised
118 greenhouses and/or polytunnels for crop growing. Sites were all of comparable size, located
119 in areas of open farmland with no other sites known to be deploying bumblebees within 10
120 km. Three of the sites in Cambridgeshire, Kent and Essex, were a focal greenhouse in which
121 commercially produced bumblebees were used for the pollination of the greenhouse crops (\approx
122 200-300 hives at each site), and two sites in Merseyside and Oxfordshire were a focal
123 greenhouse in which commercially produced bumblebees had not been used (all sites were at
124 least 70 km apart). Bumblebees were collected with a sweep net within 0.5 km of points 0.5,
125 3 and 5 km from the focal greenhouse sites, with approximately 50 bumblebees collected at
126 each of the three distances for each of the five sites. All bees were collected over a 16 day
127 period in the summer of 2011 (Cambridgeshire, Kent and Essex on 2nd July, 9th July and 11th
128 July respectively, Oxfordshire and Merseyside on 1st and 16th July respectively). A total of
129 471 bumblebees were collected from around the sites using commercially produced
130 bumblebees (222, 151 and 98 at the Cambridgeshire, Kent and Essex sites respectively) and a
131 total of 293 bumblebees from around the sites not using commercially produced bumblebees
132 (143 and 150 at the Merseyside and Oxfordshire sites respectively). The samples consisted of
133 *B. terrestris*, *B. hortorum*, *B. hypnorum*, *B. lapidarius*, *B. lucorum*, *B. pascuorum* and *B.*
134 *pratorum*, with most being either *B. terrestris* or *B. lapidarius* (40% and 25% of samples
135 respectively; see Table 1 for detail of the number of each species sampled at each distance at
136 each site). All of these 764 bumblebees were screened for parasites.

137

138 **The effect of proximity to managed honey bees**

139 Ten commercially produced *Bombus terrestris audax* bumblebee colonies (Biobest) with 80-
140 100 workers were used to determine the effect of proximity to managed honey bee colonies
141 on parasite prevalence within bumblebee colonies. The colonies were placed on a farm near
142 Tadcaster, West Yorkshire (53°52' N, 1°20' W). Five of the bumblebee colonies were
143 situated on the edge of an agroforestry field containing an apiary with 50, full-size honey bee
144 hives, and the remaining five bumblebee colonies were sited at the edge of a field 1 km away
145 from the apiary, with bees at both locations being in the same landscape with access to
146 similar floral resources (Elbgami et al. 2014). The colonies were placed in a row at the edge
147 of each site, with the same distance between hives in each case. The bumblebee colonies
148 remained at these sites for one month, during which they could forage freely. After this
149 period, 20 bumblebee workers were taken from each colony and screened for the presence of
150 the parasites.

151

152 **Molecular screening for parasite presence**

153 A ca. 0.5 cm³ sample of midgut, malpighian tubules and fatbody from each bee was
154 homogenised and DNA extracted from the homogenate using 5% Chelex. All DNA samples
155 were amplified for the *I8S* Apidae host control gene to confirm the quality of the DNA
156 extraction. Samples were then screened for the presence of the *Apicystis bombi*, *Crithidia*
157 *bombi*, *Nosema bombi*, *N. ceranae*, *N. apis* and deformed wing virus (DWV) parasites using
158 parasite specific primers and conditions (Chen et al. 2005; Gisder & Genersch 2013; Klee et
159 al. 2006; Meeus et al. 2010; Table S1). Products were run alongside a size standard on a 1%
160 agarose gel stained with ethidium bromide to confirm amplicon size. Each assay included a
161 negative and a positive control.

162

163 **Statistical analysis**

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164 The prevalence and richness of parasites was compared between sites in which greenhouses
165 did or did not use commercially produced bumblebees, and between the sites near to or far
166 from the honey bee apiary using generalized linear models (GLM) with the likelihood ratio χ^2
167 statistic. The parasite richness (number of parasite species detected in a single host) was
168 compared between sites using a negative binomial distribution and log link function and
169 changes in the prevalence of individual parasites with a binomial distribution and logit link
170 function. When looking at the effect of commercially produced bumblebees, site type
171 (greenhouses in which commercially produced bumblebees were or were not used), transect
172 distance, and site location nested within site type were included as factors, with the species
173 and sex of bumblebees sampled also included as factors. We did not include sampling date in
174 the these models because it covaried with site, but checked for temporal autocorrelation using
175 Box-Ljung tests and retested the GLM without site and instead including sampling date
176 (number of days after the first sample was collected) as a covariate. We checked for spatial
177 autocorrelation using Moran's I (Rogerson 2010). When looking at the effect of managed
178 honey bees, location (near to or far from the apiary), and colony nested within location, were
179 used as factors. Nonsignificant terms were removed stepwise based on log-likelihood ratio
180 tests in all cases to obtain the minimum adequate models (Table S2). All analyses were
181 carried out in PASW Statistics 20 (IBM, Armonk, NY, USA).

182

183

184 **RESULTS**

185 **The effect of commercially produced bumblebees on parasite prevalence in** 186 **wild bumblebees**

187 Overall, most wild bumblebees had either no infections (40.7%) or infection by a single
188 parasite species (40.3%), with cases of bumblebees infected by two or three parasite species

189 being rare (16.8% and 2.1% respectively). There was a significant interaction between the
190 distance from the greenhouses and whether they were or were not using commercially
191 produced bumblebees on the numbers of parasite species that infected bumblebees ($\chi^2 = 6.78$,
192 d.f. = 2, $P = 0.034$), and this was not affected by either the species or sex of the bumblebee
193 ($\chi^2 = 3.04$, d.f. = 6, $P = 0.804$ and $\chi^2 = 0.874$, d.f. = 1, $P = 0.35$, respectively). The numbers of
194 parasite species recorded decreased with distance from the greenhouses at sites which were
195 using commercially produced bumblebees, but were unaffected by distance at the sites which
196 were not using these bees (Fig. 1A). There was also a significant difference between
197 individual sites nested within categories of using or not using commercially produced
198 bumblebees ($\chi^2 = 29.0$, d.f. = 3, $P < 0.001$), but the bees sampled from around sites using
199 commercially produced bumblebees nevertheless had significantly more parasite species
200 overall when controlling for this ($\chi^2 = 23.2$, d.f. = 1, $P < 0.001$). The samples were collected
201 within a relatively short 16 day period and showed no temporal autocorrelation (Box-Ljung
202 tests all $P > 0.05$), with there being no effect of sampling date on the number of parasite
203 species found ($\chi^2 = 2.86$, d.f. = 1, $P = 0.091$). There was also no spatial autocorrelation
204 (Moran's I = 0.062; a value of 1 indicates perfect correlation and of 0 indicates random
205 dispersion). Of the individual parasites, the prevalence of both *A. bombi* and *N. ceranae* were
206 affected significantly by the interaction between distance and whether sites were using
207 commercially produced bumblebees or not ($\chi^2 = 44.5$, d.f. = 2, $P < 0.001$, and $\chi^2 = 7.98$, d.f.
208 = 2, $P = 0.019$, respectively). *A. bombi* was more common close to greenhouses when those
209 greenhouses were using commercially produced bumblebees but showing little effect of
210 distance when they were not (Fig. 1B). *N. ceranae*, in contrast, increased with distance from
211 the greenhouses at the sites not using commercially produced bumblebees but was little
212 affected by distance at the sites where they were (Fig. 1D). *Crithidia bombi* was more
213 prevalent in bumblebees caught from around sites using commercially produced bumblebees

214 than those not using them ($\chi^2 = 15.1$, d.f. = 1, $P < 0.001$ but displayed no proximity effect (χ^2
215 = 0.756, d.f. = 2, $P = 0.685$; Fig. 1C). *N. ceranae* was the only parasite to show a significant
216 effect of the species or sex of bumblebee sampled (Table S2), which was due to all 7 of the *B.*
217 *hortorum* bees sampled being workers that were infected by *N. ceranae*. The prevalence of *N.*
218 *bombi*, *N. apis* and DWV in bumblebees caught were all under 1% and were not affected by
219 any variables (Table S2; Fig. 1).

220

221 **The effect of managed honey bees on parasite prevalence within bumblebee** 222 **colonies**

223 The mean parasite richness varied between bumblebee colonies but was significantly higher
224 overall in colonies located in close proximity to honey bees ($\chi^2 = 5.66$ d.f. = 1, $P = 0.017$;
225 Fig. 2A). The average prevalence of *C. bombi* in bumblebee colonies near honey bees was
226 58%; significantly higher than the 30% found in colonies far from honey bees ($\chi^2 = 17.9$ d.f.
227 = 1, $P < 0.001$; Fig. 2B). The prevalence of *A. bombi* and *N. ceranae* in colonies located near
228 honey bees averaged 30% and 43%, respectively, which did not differ from the prevalence of
229 these parasites in colonies far from honey bees ($\chi^2 = 0.83$ d.f. = 1, $P = 0.36$; $\chi^2 = 0.27$ d.f. = 1,
230 $P = 0.61$). *N. ceranae* prevalence did, however, differ between colonies within sampling sites
231 ($\chi^2 = 25.07$ d.f. = 8, $P = 0.002$). *N. apis* was only found in bumblebee colonies located near to
232 honey bee hives, but had a very low prevalence and thus did not differ significantly between
233 the sites ($\chi^2 < 0.01$ d.f. = 1, $P = 0.993$). *Nosema bombi* and DWV were not detected in any of
234 the 200 bumblebees sampled.

235

236

237 **DISCUSSION**

238 Although the study involved only a very limited number of sites and must thus be interpreted
239 with caution, the results suggest that the prevalence of parasites in bumblebees may be
240 affected by the presence of managed bees. The prevalence of *A. bombi* and *C. bombi* was
241 respectively 12% and 15% higher in bumblebees near greenhouses at the three sites using
242 commercially produced bumblebees compared to the two sites not using these bees, and the
243 prevalence of *Apicystis bombi* was also much higher 0.5 km from the greenhouses compared
244 with 5 km away from them. The samples were collected during a relatively short 16 day
245 period and the differences between sites were not due to spatial or temporal autocorrelation.
246 Bumblebees in colonies located close to the managed honey bee apiary had higher levels of
247 the parasite *C. bombi* compared to bumblebees in colonies that were located 1 km away from
248 the apiary. Although data from more sites are obviously needed to draw firm conclusions, the
249 results suggest that the presence of managed colonies of either bumblebees or honey bees
250 may increase the prevalence of parasites in wild bumblebees.

251 A wide diversity of parasites were detected in the wild bumblebees collected near
252 greenhouses, including the bumblebee parasites *A. bombi*, *C. bombi* and *N. bombi*, and the
253 honey bee parasites *N. ceranae*, *N. apis* and DWV, all of which have also been identified in
254 commercially produced bumblebees (Graystock et al. 2013b). *N. bombi*, *N. apis* and DWV
255 were very rare (< 1% prevalence) but the other parasites were more common. In general, the
256 parasite richness within wild bumblebees increased with proximity to greenhouses utilising
257 commercially produced bumblebees and bumblebees caught from around such greenhouses
258 had a higher prevalence of *A. bombi* and *C. bombi* than those caught around greenhouses not
259 using commercially reared bumblebees. Whether through parasite spillover, parasite
260 spillback, or the stress of increased competition, commercially produced bumblebees appear
261 to be increasing the prevalence of parasites in local bumblebees. These findings support
262 previous, microscopy-based studies that found a higher prevalence of parasites near sites

263 using commercially produced bumblebees (Colla et al. 2006; Murray et al. 2013; Otterstatter
264 & Thomson 2008). The effect of greenhouses using commercially produced bumblebees on
265 the prevalence *A. bombi* appeared to be influenced by proximity to the focal glasshouse site.
266 This perhaps suggests either a recent introduction from the greenhouses or that the dispersal
267 of the parasite through the environment is relatively limited. There have been no studies of
268 the horizontal transmission of *A. bombi*, although it has been commonly found at a low
269 prevalence when bees are examined using less sensitive microscopy methods (Goulson et al.
270 2012; Shykoff & Schmid-Hempel 1991). Worryingly this parasite has been implicated in
271 bumblebee declines in South America (Arbetman et al. 2012). *Crithidia bombi* was also
272 found to be more prevalent at sites using commercially produced bumblebees. Unlike *A.*
273 *bombi*, there was no proximity effect found, but *C. bombi* is known to readily transmit
274 between bumblebees and may therefore disperse rapidly through the environment (Durrer &
275 Schmid-Hempel 1994). The prevalence of none of the other parasites investigated differed
276 between sites with or without commercially produced bumblebees. *Nosema ceranae* was
277 abundant at some sites but completely absent at other sites. *Nosema ceranae*, is an emergent
278 honey bee parasite that is implicated in the collapse of honey bee colonies in some, but not
279 all, areas (Fries 2010; Higes et al. 2008; Klee et al. 2007; Paxton 2010; Paxton et al. 2008;
280 Roberts & Hughes 2014), and which has been shown to be widespread and virulent in
281 bumblebees (Furst et al. 2014; Graystock et al. 2013a; Plischuk et al. 2009).

282 Although based on only two sites, the comparison of the site with honey bee hives and
283 the site 1 km from the hives suggested that proximity to managed honey bee colonies may
284 also have an effect on parasite prevalence in bumblebee colonies. Although the levels of *N.*
285 *bombi*, *N. apis* and DWV were too low for any conclusions, and *A. bombi* and *N. ceranae* did
286 not differ between the two sites, *C. bombi* was significantly more prevalent in bumblebee
287 colonies that were near to the honey bee hives. This effect could not be due to spillover,

288 because *C. bombi* is unable to infect honey bees (Ruiz-González & Brown 2006). It could,
289 however, be due to stress from competition leading to the bumblebees close to the honey bee
290 apiary being more susceptible to infection (Brown et al. 2000; Elbgami et al. 2014; Goulson
291 & Sparrow 2009; Lafferty & Gerber 2002; Mallon et al. 2003), or to the honey bees vectoring
292 *C. bombi*. The potential role of stress and parasite spillback in driving elevated parasite
293 prevalence in wild pollinators has been largely ignored and would warrant further
294 investigation.

295 Our results suggest that managed colonies of bees may increase the prevalence of
296 parasites in bumblebees. The results here are based on only very few sites and clearly further
297 studies are needed using far more sites to establish their generality. It will be important for
298 such studies to consider the potential for parasite spillback and stress-related effects, in
299 addition to parasite spillover. It is clear that as long as there is mixing between managed and
300 wild bees, there is the potential for wild populations to be at risk from the effects on host-
301 parasite dynamics. These effects could prove to be a major conservation threat to
302 bumblebees.

303

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309

310 **References**

- 311 **Arbetman M, Meeus I, Morales C, Aizen M, and Smaghe G. 2012.** Alien parasite
312 hitchhikes to Patagonia on invasive bumblebee. *Biological Invasions* **15**:489-494.
- 313 **Arneberg P, Skorping A, Grenfell B, and Read AF. 1998.** Host densities as determinants
314 of abundance in parasite communities. *Proceedings of the Royal Society B-Biological*
315 *Sciences* **265**:1283-1289.
- 316 **Biesmeijer JC, Roberts SPM, Reemer M, Ohlemuller R, Edwards M, Peeters T,**
317 **Schaffers AP, Potts SG, Kleukers R, Thomas CD, Settele J, and Kunin WE. 2006.**
318 Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands.
319 *Science* **313**:351-354.
- 320 **Brown MJF, Loosli R, and Schmid-Hempel P. 2000.** Condition-dependent expression of
321 virulence in a trypanosome infecting bumblebees. *Oikos* **91**:421-427.
- 322 **Cameron SA, Lozier JD, Strange JP, Koch JB, Cordes N, Solter LF, and Griswold TL.**
323 **2011.** Patterns of widespread decline in North American bumble bees. *Proceedings of the*
324 *National Academy of Sciences of the United States of America* **108**:662-667.
- 325 **Chen YP, Higgins JA, and Feldlaufer MF. 2005.** Quantitative real-time reverse
326 transcription-PCR analysis of deformed wing virus infection in the honeybee (*Apis*
327 *mellifera* L.). *Applied and Environmental Microbiology* **71**:436-441.
- 328 **Colla SR, Otterstatter MC, Gegeer RJ, and Thomson JD. 2006.** Plight of the bumble bee:
329 Pathogen spillover from commercial to wild populations. *Biological Conservation*
330 **129**:461-467.

- 331 **Darvill B, Ellis JS, Lye GC, and Goulson D. 2006.** Population structure and inbreeding in a
332 rare and declining bumblebee, *Bombus muscorum* (Hymenoptera: Apidae). *Molecular*
333 *Ecology* **15**:601-611.
- 334 **Durrer S, and Schmid-Hempel P. 1994.** Shared use of flowers leads to horizontal pathogen
335 transmission. *Proceedings of the Royal Society of London Series B-Biological Sciences*
336 **258**:299-302.
- 337 **Elbgami T, Kunin WE, Hughes WOH, and Biesmeijer JC. 2014.** The effect of proximity
338 to a honeybee apiary on bumblebee colony fitness, development, and performance.
339 *Apidologie* doi:10.1007/s13592-13013-10265-y.
- 340 **Ellis JS, Knight ME, Darvill B, and Goulson D. 2006.** Extremely low effective population
341 sizes, genetic structuring and reduced genetic diversity in a threatened bumblebee species,
342 *Bombus sylvarum* (Hymenoptera : Apidae). *Molecular Ecology* **15**:4375-4386.
- 343 **Evison SEF, Fazio G, Chappell P, Foley K, Jensen AB, and Hughes WOH. 2013.** Host-
344 parasite genotypic interactions in the honey bee: the dynamics of diversity. *Ecology and*
345 *Evolution* **3**:2214-2222.
- 346 **Evison SEF, Roberts KE, Laurenson L, Pietravalle S, Hui J, Biesmeijer JC, Smith JE,**
347 **Budge G, and Hughes WOH. 2012.** Pervasiveness of parasites in pollinators. *PLoS ONE*
348 **7**:e30641.
- 349 **Foley K, Fazio G, Jensen AB, and Hughes WOH. 2012.** Nutritional limitation and
350 resistance to opportunistic *Aspergillus* parasites in honey bee larvae. *Journal of*
351 *Invertebrate Pathology* **111**:68-73.

- 352 **Fries I. 2010.** *Nosema ceranae* in European honey bees (*Apis mellifera*). *Journal of*
353 *Invertebrate Pathology* **103**:S73-S79.
- 354 **Furst MA, McMahon DP, Osborne JL, Paxton RJ, and Brown MJF. 2014.** Disease
355 associations between honeybees and bumblebees as a threat to wild pollinators. *Nature*
356 **506**:364-366.
- 357 **Gegear RJ, Otterstatter MC, and Thomson JD. 2005.** Does parasitic infection impair the
358 ability of bumblebees to learn flower-handling techniques? *Animal Behaviour* **70**:209-215.
- 359 **Genersch E, Yue C, Fries I, and de Miranda JR. 2006.** Detection of Deformed wing virus,
360 a honey bee viral pathogen, in bumble bees (*Bombus terrestris* and *Bombus pascuorum*)
361 with wing deformities. *Journal of Invertebrate Pathology* **91**:61-63.
- 362 **Gisder S, and Genersch E. 2013.** Molecular differentiation of *Nosema apis* and *Nosema*
363 *ceranae* based on species-specific sequence differences in a protein coding gene. *Journal*
364 *of Invertebrate Pathology* **113**:1-6.
- 365 **Goka K, Okabe K, Yoneda M, and Niwa S. 2001.** Bumblebee commercialization will cause
366 worldwide migration of parasitic mites. *Molecular Ecology* **10**:2095-2099.
- 367 **Goulson D. 2003.** *Bumblebees: behaviour and ecology*. Oxford: Oxford Univ. Press.
- 368 **Goulson D, Hanley ME, Darvill B, Ellis JS, and Knight ME. 2005.** Causes of rarity in
369 bumblebees. *Biological Conservation* **122**:1-8.
- 370 **Goulson D, Lye GC, and Darvill B. 2008.** Decline and conservation of bumble bees. *Annual*
371 *Review of Entomology* **53**:191-208.

- 372 **Goulson D, and Sparrow K. 2009.** Evidence for competition between honeybees and
373 bumblebees; effects on bumblebee worker size. *Journal of Insect Conservation* **13**:177-
374 181.
- 375 **Goulson D, Whitehorn P, and Fowley M. 2012.** Influence of urbanisation on the prevalence
376 of protozoan parasites of bumblebees. *Ecological Entomology* **37**:83-89.
- 377 **Graystock P, Yates K, Darvill B, Goulson D, and Hughes WOH. 2013a.** Emerging
378 dangers: deadly effects of an emergent parasite in a new pollinator host. *Journal of*
379 *Invertebrate Pathology* **114**:114-119.
- 380 **Graystock P, Yates K, Evison SEF, Darvill B, Goulson D, and Hughes WOH. 2013b.**
381 The Trojan hives: pollinator pathogens, imported and distributed in bumblebee colonies.
382 *Journal of Applied Ecology* **50**:1207-1215.
- 383 **Higes M, Martín-Hernández R, C. B, Bailón EG, González-Porto AV, Barrios L, del**
384 **Nozal MJ, Bernal JL, Jiménez JJ, Palencia PG, and Meana A. 2008.** How natural
385 infection by *Nosema ceranae* causes honeybee colony collapse. *Environmental*
386 *Microbiology* **10**:2659-2669.
- 387 **Kelly DW, Paterson RA, Townsend CR, Poulin R, and Tompkins DM. 2009.** Parasite
388 spillback: A neglected concept in invasion ecology? *Ecology* **90**:2047-2056.
- 389 **Klee J, Besana AM, Genersch E, Gisder S, Nanetti A, Tam DQ, Chinh TX, Puerta F,**
390 **Ruz JM, Kryger P, Message D, Hatjina F, Korpela S, Fries I, and Paxton RJ. 2007.**
391 Widespread dispersal of the microsporidian *Nosema ceranae*, an emergent pathogen of the
392 western honey bee, *Apis mellifera*. *Journal of Invertebrate Pathology* **96**:1-10.

- 393 **Klee J, Tek Tay W, and Paxton RJ. 2006.** Specific and sensitive detection of *Nosema*
394 *bombi* (Microsporidia: Nosematidae) in bumble bees (*Bombus* spp.; Hymenoptera:
395 Apidae) by PCR of partial rRNA gene sequences. *Journal of Invertebrate Pathology*
396 **91**:98-104.
- 397 **Kraus FB, Szentgyorgyi H, Rozej E, Rhode M, Moron D, Woyciechowski M, and**
398 **Moritz RFA. 2011.** Greenhouse bumblebees (*Bombus terrestris*) spread their genes into
399 the wild. *Conservation Genetics* **12**:187-192.
- 400 **Lafferty KD, and Gerber LR. 2002.** Good medicine for conservation biology: The
401 intersection of epidemiology and conservation theory. *Conservation Biology* **16**:593-604.
- 402 **Mallon EB, Brockmann A, and Schmid-Hempel P. 2003.** Immune response inhibits
403 associative learning in insects. *Proceedings of the Royal Society of London Series B-*
404 *Biological Sciences* **270**:2471-2473.
- 405 **Manson J, Otterstatter M, and Thomson J. 2010.** Consumption of a nectar alkaloid
406 reduces pathogen load in bumble bees. *Oecologia* **162**:81-89.
- 407 **Meeus I, Brown MJF, De Graaf DC, and Smaghe GUY. 2011.** Effects of invasive
408 parasites on bumble bee declines. *Conservation Biology* **25**:662-671.
- 409 **Meeus I, Smaghe G, Siede R, Jans K, and de Graaf DC. 2010.** Multiplex RT-PCR with
410 broad-range primers and an exogenous internal amplification control for the detection of
411 honeybee viruses in bumblebees. *Journal of Invertebrate Pathology* **105**:200-203.
- 412 **Morandin LA, Lavery TM, Kevan PG, Khosla S, and Shipp L. 2001.** Bumble bee
413 (Hymenoptera : Apidae) activity and loss in commercial tomato greenhouses. *Canadian*
414 *Entomologist* **133**:883-893.

- 415 **Murray TE, Coffey MF, Kehoe E, and Horgan FG. 2013.** Pathogen prevalence in
416 commercially reared bumble bees and evidence of spillover in conspecific populations.
417 *Biological Conservation* **159**:269-276.
- 418 **Oldroyd BP. 2007.** What's killing American honey bees? *PLoS Biology* **5**:e168.
- 419 **Otterstatter M, and Thomson J. 2007.** Contact networks and transmission of an intestinal
420 pathogen in bumble bee (*Bombus impatiens*) colonies. *Oecologia* **154**:411-421.
- 421 **Otterstatter MC, and Thomson JD. 2008.** Does pathogen spillover from commercially
422 reared bumble bees threaten wild pollinators? *PLoS ONE* **3**:e2771.
- 423 **Paxton RJ. 2010.** Does infection by *Nosema ceranae* cause “Colony Collapse Disorder” in
424 honey bees (*Apis mellifera*)? *Journal of Apicultural Research* **49**:80-84.
- 425 **Paxton RJ, Klee J, S. K, and Fries I. 2008.** *Nosema ceranae* has infected *Apis mellifera* in
426 Europe since at least 1998 and may be more virulent than *Nosema apis*. *Apidologie*
427 **38**:558-565.
- 428 **Plischuk S, Martín-Hernández R, Prieto P, Lucía M, Botías C, Meana A,**
429 **Abrahamovich AH, Lange C, and Higes M. 2009.** South American native bumblebees
430 (Hymenoptera: Apidae) infected by *Nosema ceranae* (Microsporidia), an emerging
431 pathogen of honeybees (*Apis mellifera*). *Environmental Microbiology Reports* **1**:131-135.
- 432 **Potts SG, Biesmeijer JC, Kremen C, Neumann P, Schweiger O, and Kunin WE. 2010.**
433 Global pollinator declines: trends, impacts and drivers. *Trends in Ecology & Evolution*
434 **25**:345-353.

- 435 **Power AG, and Mitchell CE. 2004.** Pathogen spillover in disease epidemics. *American*
436 *Naturalist* **164**:S79-S89.
- 437 **Ricketts TH, Regetz J, Steffan-Dewenter I, Cunningham SA, Kremen C, Bogdanski A,**
438 **Gemmill-Herren B, Greenleaf SS, Klein AM, Mayfield MM, Morandin LA, Ochieng**
439 **A, and Viana BF. 2008.** Landscape effects on crop pollination services: are there general
440 patterns? *Ecology Letters* **11**:499-515.
- 441 **Roberts KE, and Hughes WOH. 2014.** Immunosenescence and resistance to parasite
442 infection in the honey bee, *Apis mellifera*. *Journal of Invertebrate Pathology* **121**:1-6.
- 443 **Rogerson PA. 2010.** *Statistical methods for geography: a student's guide*. London: SAGE
444 Publications Ltd.
- 445 **Ruiz-González MX, and Brown MJF. 2006.** Honey bee and bumblebee trypanosomatids:
446 specificity and potential for transmission. *Ecological Entomology* **31**:616-622.
- 447 **Schmid-Hempel P. 2011.** *Evolutionary parasitology: the integrated study of infections,*
448 *immunology, ecology and genetics*. Oxford: Oxford University Press.
- 449 **Shykoff JA, and Schmid-Hempel P. 1991.** Incidence and effect of 4 parasites in natural
450 populations of bumble bees in Switzerland. *Apidologie* **22**:117-125.
- 451 **Singh R, Levitt AL, Rajotte EG, Holmes EC, Ostiguy N, van Engelsdorp D, Lipkin WA,**
452 **de Pamphilis CW, Toth AL, and Cox-Foster DL. 2010.** RNA viruses in hymenopteran
453 pollinators: evidence of inter-taxa virus transmission via pollen and potential impact on
454 non-*Apis* hymenopteran species. *PLoS ONE* **5**:e14357.

455 **Szabo ND, Colla SR, Wagner DL, Gall LF, and Kerr JT. 2012.** Do pathogen spillover,
456 pesticide use, or habitat loss explain recent North American bumblebee declines?
457 *Conservation Letters* **5**:232-239.

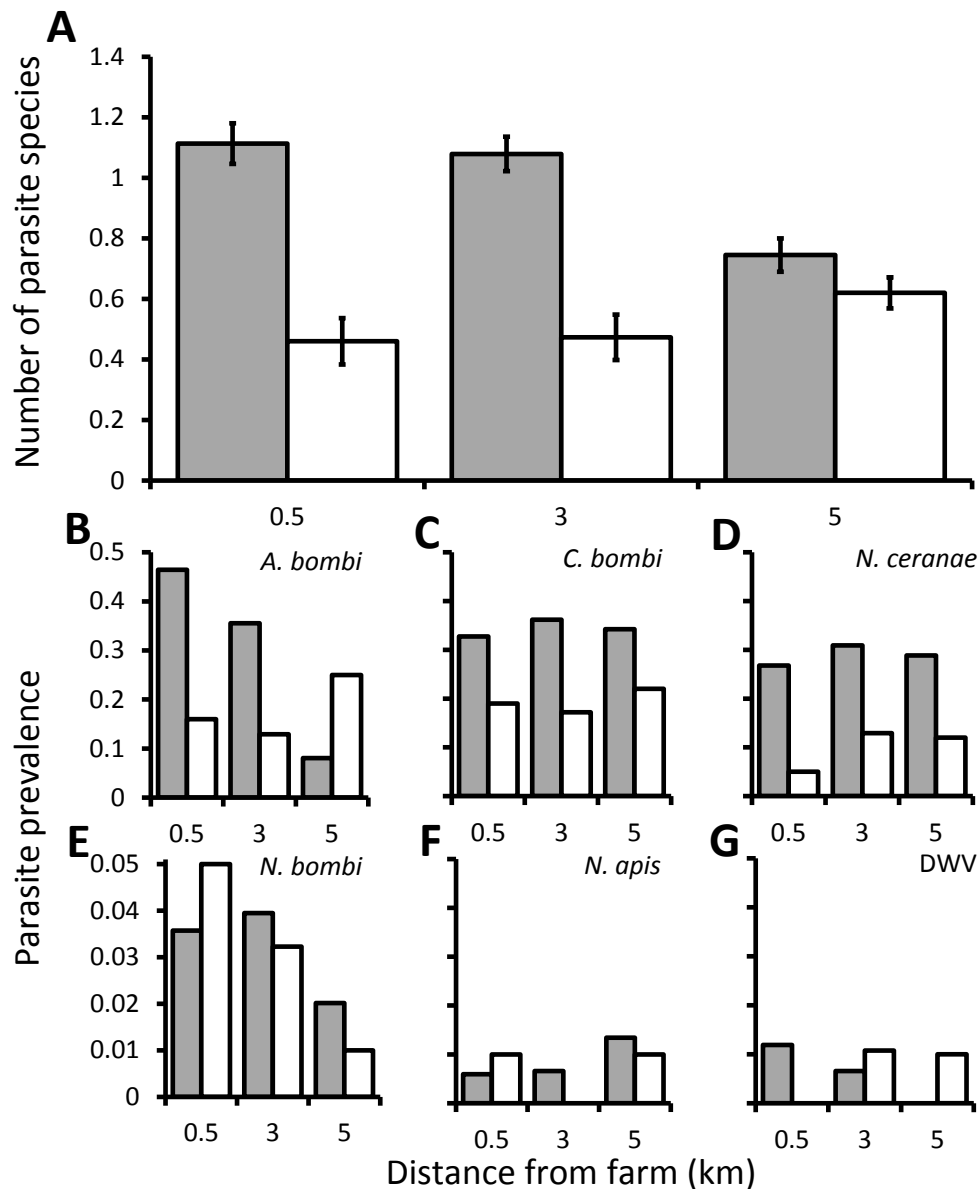
458 **Vanbergen AJ, Baude M, Biesmeijer JC, Britton NF, Brown MJF, Brown M, Bryden J,**
459 **Budge GE, Bull JC, Carvel C, Challinor AJ, Connolly CN, Evans DJ, Feil EJ,**
460 **Garratt MP, Greco MK, Heard MS, Jansen VAA, Keeling MJ, Kunis WE, Marris**
461 **GC, Memmott J, Murray JT, Nicolson SW, Osborne JL, Paxton RJ, Pirk CWW,**
462 **Polce C, Potts SG, Priest NK, Raine NE, Roberts S, Ryabov EV, Shafir S, Shirley**
463 **MDF, Simpson SJ, Stevenson PC, Stone GN, Termansen M, Wright GA, and Insect**
464 **Pollinators I. 2013.** Threats to an ecosystem service: pressures on pollinators. *Frontiers in*
465 *Ecology and the Environment* **11**:251-259.

466 **Velthuis HHW, and van Doorn A. 2006.** A century of advances in bumblebee
467 domestication and the economic and environmental aspects of its commercialization for
468 pollination. *Apidologie* **37**:421-451.

469 **Whitehorn PR, Tinsley MC, Brown MJF, Darvill B, and Goulson D. 2011.** Genetic
470 diversity, parasite prevalence and immunity in wild bumblebees. *Proceedings of the Royal*
471 *Society B-Biological Sciences* **278**:1195-1202.

472 **Whittington R, and Winston ML. 2003.** Effects of *Nosema bombi* and its treatment
473 fumagillin on bumble bee (*Bombus occidentalis*) colonies. *Journal of Invertebrate*
474 *Pathology* **84**:54-58.

475 **Whittington R, Winston ML, Tucker C, and Parachnowitsch AL. 2004.** Plant-species
476 identity of pollen collected by bumblebees placed in greenhouses for tomato pollination.
477 *Canadian Journal of Plant Science* **84**:599-602.



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479 **Figure 1 The effect of commercially produced bumblebees on parasite prevalence.**

480 Prevalence of parasites in bumblebees sampled within 0.5 km of locations that were 0.5, 3 or

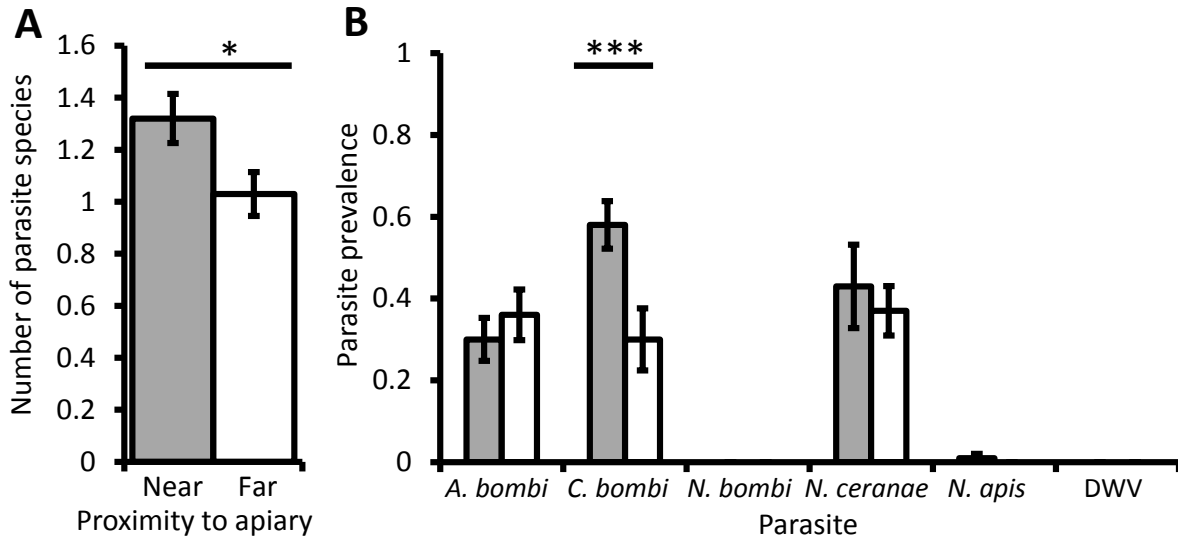
481 5 km from greenhouses that were either using (grey columns) or not using (white columns)

482 commercially produced bumblebee colonies. A) The mean \pm s.e. parasite richness (number of

483 species) infecting individual bees. B-G) The proportion of bumblebees sampled which were

484 positive for the *A. bombi*, *C. bombi*, *N. ceranae*, *N. bombi*, *N. apis* and deformed wing virus

485 (DWV) parasites.



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Figure 2 The effect of managed honey bees on parasite prevalence. The mean \pm s.e. parasite richness (number of species) per bumblebee (A), and the prevalence of six parasites per bumblebee colony (B), that were located either at a site near to (grey columns) or 1 km away from (white columns) a honey bee apiary. Asterisks indicate columns for which there was a significant difference between colonies located near to and far from the apiary (* $P < 0.05$; *** $P < 0.001$).

494 **Table 1** Samples collected to assess the prevalence of parasites in wild bumblebees sampled
 495 at three distances from greenhouses that either were or were not using commercially
 496 produced bumblebees.

Location	Species	N	Distance from greenhouses ± 0.5 km					
			0.5 km		3 km		5 km	
			F	M	F	M	F	M
Cambridgeshire Latitude: 52°18'0.79"N Longitude: 0° 3'2.46"W Area: ≈ 50 acre Number of hives: ≈ 200 ^a	<i>B. hortorum</i>	6	3	0	1	0	2	0
	<i>B. hypnorum</i>	9	3	0	3	0	3	0
	<i>B. lapidarius</i>	99	16	4	43	5	25	6
	<i>B. lucorum</i>	33	6	5	4	2	12	4
	<i>B. pascuorum</i>	15	5	0	3	0	6	0
	<i>B. pratorum</i>	16	0	5	5	2	3	2
	<i>B. terrestris</i>	44	15	5	6	0	16	2
Kent Latitude: 51°21'13.64"N Longitude: 1°17'8.00"E Area: ≈ 75 acre Number of hives: ≈300 ^a	<i>B. lapidarius</i>	21	6	1	5	1	8	0
	<i>B. lucorum</i>	12	4	0	3	1	4	0
	<i>B. pascuorum</i>	5	3	0	2	0	0	0
	<i>B. pratorum</i>	19	8	0	3	1	6	1
	<i>B. terrestris</i>	94	26	3	31	3	28	3
Essex Latitude: 51°56'0.67"N Longitude: 1° 0'18.17"E Area: ≈ 60 acre Number of hives: ≈240 ^a	<i>B. lapidarius</i>	30	6	0	5	9	7	3
	<i>B. lucorum</i>	2	0	0	2	0	0	0
	<i>B. pascuorum</i>	38	16	2	10	1	9	0
	<i>B. pratorum</i>	25	21	2	0	1	0	1
	<i>B. terrestris</i>	3	3	0	0	0	0	0
Merseyside Latitude: 53°30'40.61"N Longitude: 2°47'17.78"W Area: ≈ 75 acre	<i>B. hypnorum</i>	9	2	0	2	0	5	0
	<i>B. lapidarius</i>	17	6	0	2	1	8	0
	<i>B. lucorum</i>	30	5	0	11	1	11	2
	<i>B. pascuorum</i>	2	0	0	0	0	1	1
	<i>B. pratorum</i>	12	6	0	2	0	3	1
	<i>B. terrestris</i>	73	28	3	23	1	15	3
Oxfordshire Latitude: 51°40'10.01"N Longitude: 1°22'38.79"W Area: ≈ 50 acre	<i>B. lapidarius</i>	25	9	0	8	1	6	1
	<i>B. lucorum</i>	9	3	0	2	0	4	0
	<i>B. pascuorum</i>	12	5	0	4	0	3	0
	<i>B. pratorum</i>	16	5	0	2	3	6	0
	<i>B. terrestris</i>	88	27	1	29	1	28	2

497 ^a Numbers of hives estimated based on size of farm. As a general rule, producers recommend using 4 bumblebee
 498 hives/acre at the beginning of the season, then systematically introducing more hives as the original ones age.
 499 The estimates here are based on 4 hives/acre.
 500

501 **Supplementary Material**

502 **Table S1** PCR mixes and conditions for the detection of the various parasites.

Primers & source	Assay mix								Thermal cycling			Amplicon size (bp)
	DNTP (mM)	MgCl ₂ (mM)	5xbuffer (µl)	Taq (U)	Primer F (µM)	Primer R (µM)	Template (µl)	Total volume (µl)	1 Denaturing Min Temp	2 Replication Sec Temp	3 Elongation Min Temp	
<i>Nosema bombi</i> (Klee et al. 2006)	0.3	3.75	2	0.25	0.2	0.2	2	10	4 95	35x 60 95 60 50 60 72	4 72	323
* <i>Nosema apis</i> ^{Na} & <i>N. ceranae</i> ^{Nc} (Gisder & Genersch 2013)	0.2	1.5	2	2.5	0.2 ^{Na} 0.2 ^{Nc}	0.2 ^{Na} 0.2 ^{Nc}	1	10	4 95	35x 60 94 60 58 60 72	5 72	297 ^{Na} 662 ^{Nc}
<i>Apicystis bombi</i> (Meeus et al. 2010)	0.4	1.5	2	1.25	0.5	0.5	1	10	2 94	35x 30 94 30 60 45 72	3 72	260
<i>Apidae</i> ^A (internal) and <i>Crithida bombi</i> ^{Cb} (Meeus et al. 2010)	0.4	1.5	3	1.25	0.1 ^A 0.5 ^{Cb}	0.2 ^A 0.5 ^{Cb}	2	15	2 94	35x 30 94 30 57 45 72	3 72	130 ^A 420 ^{Cb}
RT-PCR	Probe (nM)	Taqman Fast Virus 1-step Master mix(µl)			Primer F (µM)	Primer R (µM)	Template (µl)	Total volume (µl)	1 Reverse transcription Min Temp	2 Denaturing Sec Temp	3 Annealing and elongation Time Temp	Amplicon size (bp)
Deformed wing virus (Chen et al. 2005)	200	5			0.65	0.65	2	10	5 50	20 95	40x 3 s 95 3 min 60	702

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505 **Table S2** Statistical models used to analyse the richness and prevalence of parasites in
 506 bumblebees sampled either 0.5, 3 or 5 km (\pm 0.5 km) from three farms using commercially
 507 produced bumblebee colonies and two farms not using commercially produced bumblebees.

TERM IN MODEL	χ^2	df	P
Overall number of parasite species:			
Commercial bumblebees presence	23.2	1	<0.001
Site nested within status	29.0	2	<0.001
Distance from greenhouses	0.112	3	0.946
Species of bumblebee sampled	3.04	6	0.804
Sex of bumblebee sampled	0.874	1	0.35
Commercial bumblebees presence*Distance from greenhouses	6.78	2	0.034
<i>Apicystis bombi</i>:			
Commercial bumblebees presence	1.92	1	0.166
Site nested within status	113.1	3	<0.001
Distance from greenhouses	14.1	2	0.001
Species of bumblebee sampled	6.59	6	0.36
Sex of bumblebee sampled	2.05	1	0.152
Commercial bumblebees presence*Distance from greenhouses	44.5	2	<0.001
<i>Crithidia bombi</i>:			
Commercial bumblebees presence	15.1	1	<0.001
Site nested within status	2.79	3	0.425
Distance from greenhouses	0.315	2	0.854
Species of bumblebee sampled	1.72	6	0.943
Sex of bumblebee sampled	0.019	1	0.89
Commercial bumblebees presence*Distance from greenhouses	0.756	2	0.685
<i>Nosema ceranae</i>:			
Commercial bumblebees presence	0.009	1	0.994
Site nested within status	146.1	3	0.104
Distance from greenhouses	4.53	2	<0.001
Species of bumblebee sampled	27.4	6	<0.001
Sex of bumblebee sampled	7.64	1	0.006
Commercial bumblebees presence*Distance from greenhouses	7.98	2	0.019
<i>Nosema bombi</i>:			
Commercial bumblebees presence	0.068	1	0.794
Site nested within status	1.45	3	0.694
Distance from greenhouses	3.41	2	0.182
Species of bumblebee sampled	4.72	6	0.58
Sex of bumblebee sampled	0.034	1	0.854
Commercial bumblebees presence*Distance from greenhouses	0.626	2	0.731
<i>Nosema apis</i>:			
Commercial bumblebees presence	0.009	1	0.994
Site nested within status	4.07	3	0.254
Distance from greenhouses	2.01	2	0.366
Species of bumblebee sampled	4.83	6	0.566
Sex of bumblebee sampled	0.245	1	0.62
Commercial bumblebees presence*Distance from greenhouses	0.935	2	0.626
deformed wing virus:			
Commercial bumblebees presence	0.001	1	0.994
Site nested within status	5.65	3	0.13
Distance from greenhouses	1.32	2	0.516
Species of bumblebee sampled	3.69	6	0.718
Sex of bumblebee sampled	1.53	1	0.216
Commercial bumblebees presence*Distance from greenhouses	4.87	2	0.088

508