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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 *Apicystis bombi* and *C. bombi* were more prevalent around greenhouses using
26 commercially produced bumblebees, while *C. bombi* was 18% more prevalent in bumblebees
27 from near to the honey bee apiary than those far from the apiary. Whilst these results are from
28 only a limited number of sites, they support previous reports of parasite spillover from
29 commercially produced bumblebees to wild bumblebees, and suggest that parasite prevalence
30 in wild bees may in addition be increased by the stress of competing with managed bees or
31 the vectoring of parasites by them. It appears increasingly likely that the use of managed bees
32 comes at a cost of increased parasites in wild bumblebees, which is not only a concern for
33 bumblebee conservation, but which may impact other pollinators as well.

34
35 **Subjects** Entomology, Conservation Biology, Parasitology

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

38 INTRODUCTION

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

47 In addition to the stresses of habit loss, pesticide exposure and natural parasites,
48 (Goulson 2003), the use of managed bees may place additional stresses on bumblebee
49 populations. Honey bees have been managed commercially for crop pollination and honey
50 production for centuries, and are often kept in apiaries of up to thousands of colonies,
51 substantially increasing the density of bees in an area. Bumblebees are also now
52 commercially produced and used mainly in greenhouses in Europe, North America, South
53 America, New Zealand and Asia to enhance the yields of soft fruit crops (Velthuis & van
54 Doorn 2006). Although these greenhouses are meant to be closed, the commercially produced
55 bumblebees are frequently found foraging outside the greenhouses, and wild bees have been
56 found foraging inside them (Kraus et al. 2011; Morandin et al. 2001; Murray et al. 2013;
57 Whittington et al. 2004). By freely mixing with wild bumblebees, the deployment of
58 commercially produced bumblebees effectively increases the local density of bumblebees.
59 Bumblebee parasites can be dispersed between bumblebees following shared flower usage
60 (Durrer & Schmid-Hempel 1994), and, as a result, the rate of parasite transmission between
61 bees will predictably rise with increased pollinator density (Arneberg et al. 1998). In areas
62 utilising commercially produced bumblebees, higher parasite prevalence may be expected to

63 be the result, due to either the spillover of parasites from the commercially produced
64 bumblebees, parasite spillback from wild bumblebees, or stress related to the high pollinator
65 density.

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

83 Although attention has focussed on parasite spillover, it is also possible that the use of
84 managed honey bees and commercially produced bumblebees may increase the prevalence of
85 parasites in wild bumblebees via parasite spillback or heightened stress. Managed honey bees
86 or commercially produced bumblebees may become infected with parasites carried by the
87 wild bees, and their unnaturally high density in apiaries or greenhouses may then result in

88 them acting as a reservoir in which the prevalence of parasites becomes high, from which the
89 parasites can then spillback into wild bees (Kelly et al. 2009). Alternatively, the increased
90 competition for resources caused by the introduction of high densities of managed honey bees
91 or commercially produced bumblebees may stress wild bumblebees, which can have negative
92 effects on various fitness components including resistance to parasites (Brown et al. 2000;
93 Elbgami et al. 2014; Goulson & Sparrow 2009; Lafferty & Gerber 2002; Mallon et al. 2003).

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

104

105

106 **MATERIALS AND METHODS**

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

108 To determine the prevalence of parasites at sites either using commercially produced
109 bumblebees or not, five greenhouse farm sites in England were selected. Sites were selected
110 based on the presence of large scale commercial fruit farms that utilised greenhouses and/or
111 polytunnels for crop growing. Sites were all of comparable size, located in areas of open
112 farmland with no other sites known to be deploying bumblebees within 10 km. Three of the

113 sites in Cambridgeshire, Kent and Essex, were a focal greenhouse in which commercially
114 produced bumblebees were used for the pollination of the greenhouse crops, and two sites in
115 Merseyside and Oxfordshire were a focal greenhouse in which commercially produced
116 bumblebees had not been used. Bumblebees were collected with a sweep net at points 1, 3
117 and 5 km from the focal greenhouse sites, with approximately 50 bumblebees collected at
118 each of the three distances for each of the five sites. All bees were collected within a three
119 week period in the summer of 2011. A total of 471 bumblebees were collected from around
120 the sites using commercially produced bumblebees and a total of 293 bumblebees from
121 around the sites not using commercially produced bumblebees. All of these 764 bumblebees
122 were screened for parasites.

123

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

125 Ten commercially produced *Bombus terrestris audax* bumblebee colonies (Biobest) with 80-
126 100 workers were used to determine the effect of proximity to managed honey bee colonies
127 on parasite prevalence within bumblebee colonies. Five of the bumblebee colonies were
128 situated in an apiary in Yorkshire, consisting of 50, full-size honey bee hives, and the
129 remaining five bumblebee colonies were sited 1 km away from the apiary, with bees at both
130 sites being in the same landscape with access to similar floral resources (Elbgami et al. 2014).
131 The bumblebee colonies remained at these sites for one month, during which they could
132 forage freely. After this period, 20 bumblebee workers were taken from each colony and
133 screened for the presence of the parasites.

134

135 **Molecular screening for parasite presence**

136 A ca. 0.5 cm³ sample of midgut, malpighian tubules and fatbody from each bee was
137 homogenised and DNA extracted from the homogenate using 5% Chelex. All DNA samples

138 were amplified for the *18S* Apidae host control gene to confirm the quality of the DNA
139 extraction. Samples were then screened for the presence of the *Apicystis bombi*, *Crithidia*
140 *bombi*, *Nosema bombi*, *N. ceranae*, *N. apis* and deformed wing virus (DWV) parasites using
141 parasite specific primers and conditions (Chen et al. 2005; Gisder & Genersch 2013; Klee et
142 al. 2006; Meeus et al. 2010); Fig. S1). Products were run alongside a size standard on a 1%
143 agarose gel stained with ethidium bromide to confirm amplicon size. Each assay included a
144 negative and a positive control.

145

146 **Statistical analysis**

147 The prevalence and richness of parasites was compared between sites in which greenhouses
148 did or did not use commercially produced bumblebees, and between the sites near to or far
149 from the honey bee apiary. The parasite richness (number of parasite species detected in a
150 single host) was compared between sites using a generalised linear model (GLM) with linear
151 distribution, logit link function and the likelihood ratio χ^2 statistic. Changes in individual
152 parasite prevalence were analysed using GLM with binomial distribution, logit link function
153 and the likelihood ratio χ^2 statistic. When looking at the effect of commercially produced
154 bumblebees, site type (greenhouses in which commercially produced bumblebees were or
155 were not used), transect distance, and site location nested within site type were included as
156 factors. When looking at the effect of managed honey bees, location (near to or far from the
157 apiary), and colony nested within location, were used as factors. Nonsignificant terms were
158 removed stepwise in all cases to obtain the minimum adequate models. All analyses were
159 carried out in PASW Statistics 20 (IBM, Armonk, NY, USA).

160

161

162 **RESULTS**

163 **The effect of commercially produced bumblebees on parasite prevalence in**
164 **wild bumblebees**

165 Overall, most wild bumblebees had either no infections (40.7%) or infection by a single
166 parasite species (40.3%), with cases of bumblebees infected by two or three parasite species
167 being rare (16.8% and 2.1% respectively). The pathogen richness per bee was higher at sites
168 at which commercially produced bumblebees were used, and within these sites, richness was
169 greater closer to the focal glasshouse ($\chi^2 = 60.18$, d.f. = 1, $P < 0.001$, and $\chi^2 = 21.11$, d.f. = 2,
170 $P < 0.001$, respectively; Fig. 1A). Driving this trend, *A. bombi* was found at a higher
171 prevalence in bumblebees near sites using commercially produced bumblebees ($\chi^2 = 14.14$,
172 d.f. = 2, $P < 0.001$), and within these sites displayed a proximity effect, infecting 46% of bees
173 collected < 1 km from the focal greenhouse and only 8% of bees collected 5 km from the
174 greenhouse ($\chi^2 = 44.46$, d.f. = 2, $P < 0.001$; Fig. 1B). *Crithidia bombi* was more prevalent in
175 bumblebees caught from around sites using commercially produced bumblebees than those
176 not using them (34% compared to 19%) but displayed no proximity effect ($\chi^2 = 19.22$, d.f. =
177 1, $P < 0.001$, and $\chi^2 = 0.844$, d.f. = 2, $P = 0.656$, respectively; Fig. 1C). The prevalence of *N.*
178 *ceranae* did not differ significantly between bumblebees caught from around sites using or
179 not using commercially produced bumblebees (28% and 19% respectively; $\chi^2 < 0.001$, d.f. =
180 1, $P = 0.995$; Fig. 1D), but the within-site variation in the prevalence of this parasite was very
181 large (range from 0% to 46% between sites; $\chi^2 = 151.1$, d.f. = 3, $P < 0.001$). The prevalence
182 of *N. bombi*, *N. apis* and DWV in bumblebees caught were all under 1% and displayed no
183 interaction between site and proximity to the greenhouse ($\chi^2 = 1.01$, d.f. = 2, $P = 0.602$, Fig.
184 1E; $\chi^2 = 1.03$, d.f. = 2, $P = 0.597$, Fig. 1F; $\chi^2 = 4.29$, d.f. = 2, $P = 0.117$, Fig. 1G;
185 respectively).

186

187 **The effect of managed honey bees on parasite prevalence within bumblebee**
188 **colonies**

189 The mean parasite richness varied between bumblebee colonies but was significantly higher
190 overall in colonies located in close proximity to honey bees ($\chi^2 = 5.66$ d.f. = 1, $P = 0.017$;
191 Fig. 2A). The average prevalence of *C. bombi* in bumblebee colonies near honey bees was
192 58%; significantly higher than the 30% found in colonies far from honey bees ($\chi^2 = 17.9$ d.f.
193 = 1, $P < 0.001$; Fig. 2B). The prevalence of *A. bombi* and *N. ceranae* in colonies located near
194 honey bees averaged 30% and 43%, respectively, which did not differ from the prevalence of
195 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,
196 $P = 0.61$). *N. ceranae* prevalence did, however, differ between colonies within sampling sites
197 ($\chi^2 = 25.07$ d.f. = 8, $P = 0.002$). *N. apis* had very low prevalence in general, and was only
198 found in bumblebee colonies located near to honey bee hives ($\chi^2 < 0.01$ d.f. = 1, $P = 0.993$).
199 *Nosema bombi* and DWV were not detected in any of the 200 bumblebees sampled.

202 **DISCUSSION**

203 Although the study involved only a very limited number of sites and must thus be interpreted
204 with caution, the results suggest that the prevalence of parasites in bumblebees is affected by
205 the presence of both commercially produced bumblebees and managed honey bees. The
206 prevalence of *A. bombi* and *C. bombi* was respectively 12% and 15% higher in bumblebees
207 near greenhouses at the three sites using commercially produced bumblebees compared to the
208 two sites not using these bees, and the prevalence of *Apicystis bombi* was also much higher
209 within 1 km of the greenhouses compared with 5 km away from them. Bumblebees in
210 colonies located close to the managed honey bee apiary had higher levels of the parasite *C.*
211 *bombi* compared to bumblebees in colonies that were located 1 km away from the apiary.

212 Although data from more sites are obviously needed to draw firm conclusions, the results
213 suggest that the presence of managed colonies of either bumblebees or honey bees may
214 increase the prevalence of parasites in wild bumblebees.

215 A wide diversity of parasites were detected in the wild bumblebees collected near
216 greenhouses, including the honey bee parasites *N. ceranae*, *N. apis* and DWV. Recently,
217 these three parasites, as well as the bumblebee parasites *A. bombi*, *C. bombi* and *N. bombi*,
218 have also been identified in commercially produced bumblebees (Graystock et al. 2013b).
219 *Nosema ceranae*, is an emergent honey bee parasite that is implicated in the collapse of
220 honey bee colonies in some, but not all, areas (Fries 2010; Higes et al. 2008; Klee et al. 2007;
221 Paxton 2010; Paxton et al. 2008), and which has been shown to be widespread and virulent in
222 bumblebees (Furst et al. 2014; Graystock et al. 2013a; Plischuk et al. 2009). Deformed wing
223 virus is almost ubiquitous in honey bee populations, with only heavy infections causing
224 significant colony collapse (de Miranda & Genersch 2010; Highfield et al. 2009). It has also
225 been found previously in bumblebees and, while its pathology and route of transmission in
226 bumblebees is unknown, it too is widespread and can have virulent effects (Evison et al.
227 2012; Furst et al. 2014; Genersch et al. 2006). Whilst *N. apis*, does not appear to be able to
228 infect bumblebees, it has been detected and found viable inside commercially produced
229 bumblebees (Graystock et al. 2013b), suggesting that it may be vectored by bumblebees even
230 if it cannot infect them.

231 In general, the parasite richness within wild bumblebees increased with proximity to
232 greenhouses utilising commercially produced bumblebees and bumblebees caught from
233 around such greenhouses had a higher prevalence of *A. bombi* and *C. bombi* than those
234 caught around greenhouses not using commercially reared bumblebees. Whether through
235 parasite spillover, parasite spillback, or the stress of increased competition, commercially
236 produced bumblebees appear to be increasing the prevalence of parasites in local

237 bumblebees. These findings support previous studies that found, albeit using less sensitive
238 non-molecular screening methods, a higher prevalence of parasites near sites using
239 commercially produced bumblebees (Colla et al. 2006; Murray et al. 2013; Otterstatter &
240 Thomson 2008). The effect of greenhouses using commercially produced bumblebees on the
241 prevalence *A. bombi* appears to be influenced by proximity to the focal glasshouse site. This
242 perhaps suggests either a recent introduction from the greenhouses or that the dispersal of the
243 parasite through the environment is relatively limited. There have been no studies of the
244 horizontal transmission of *A. bombi*, although it has been commonly found at a low
245 prevalence when bees are examined using less sensitive microscopy methods (Goulson et al.
246 2012; Shykoff & Schmid-Hempel 1991). Worryingly this parasite has been implicated in
247 bumblebee declines in South America (Arbetman et al. 2012). *Crithidia bombi* was also
248 found to be more prevalent at sites using commercially produced bumblebees. Unlike *A.*
249 *bombi*, there was no proximity effect found, but *C. bombi* is known to readily transmit
250 between bumblebees and may therefore disperse rapidly through the environment (Durrer &
251 Schmid-Hempel 1994). The prevalence of none of the other parasites investigated differed
252 between sites with or without commercially produced bumblebees. In the cases of *N. bombi*,
253 *N. apis* and DWV, the parasites were very rare (< 1% prevalence). *Nosema ceranae*,
254 however, was abundant at some sites but completely absent at other sites. Whilst
255 commercially produced bumblebee colonies have been found to contain *N. ceranae*
256 (Graystock et al. 2013b), it is reassuring that the prevalence of the parasite did not appear to
257 be primarily determined by the presence of commercially produced bumblebees, at least in
258 the case of the limited number of sites investigated here.

259 The proximity to managed honey bee colonies also had an effect on parasite
260 prevalence in bumblebee colonies. Although the levels of *N. bombi*, *N. apis* and DWV were
261 too low for any conclusions, and *A. bombi* and *N. ceranae* were not affected by proximity to

262 the honey bee hives, *C. bombi* was significantly more prevalent in bumblebee colonies that
263 were near to the honey bee hives. This effect on *C. bombi* prevalence cannot be due to
264 spillover, because this parasite is unable to infect honey bees (Ruiz-Gonzalez & Brown
265 2006). It could, however, be due to stress from competition leading to the bumblebees close
266 to the honey bee apiary being more susceptible to infection (Brown et al. 2000; Elbgami et al.
267 2014; Goulson & Sparrow 2009; Lafferty & Gerber 2002; Mallon et al. 2003), or to the
268 honey bees vectoring *C. bombi*. The results may suggest that the higher prevalence of *C.*
269 *bombi*, and potentially other parasites, near managed bees that have been reported previously
270 and considered to represent pathogen spillover (Colla et al. 2006; Murray et al. 2013;
271 Otterstatter & Thomson 2008), could to some extent be potentially due to stress or vectoring
272 resulting from the higher density of foraging bees in the area. This highlights the largely
273 ignored processes of density driven spillback and stress as other possible causes of elevated
274 parasite prevalence in wild bee populations in areas around managed bee.

275 Our results suggest that managed colonies of either bumblebees or honey bees may
276 increase the prevalence of parasites in bumblebees. The mechanisms may be three-fold: the
277 direct effects of spillover and spillback of parasites, most probably via shared flower use, and
278 the indirect effect of increased competition and stress. The results here are based on only very
279 few sites and clearly further studies are needed using far more sites to establish their
280 generality. It will be important for such studies to consider the potential for parasite spillback
281 and stress-related effects, in addition to parasite spillover. It is clear that as long as there is
282 mixing between managed and wild bees, there is the potential for wild populations to be at
283 risk from the effects on host-parasite dynamics. These effects could prove to be a major
284 conservation threat to bumblebees.

285

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289

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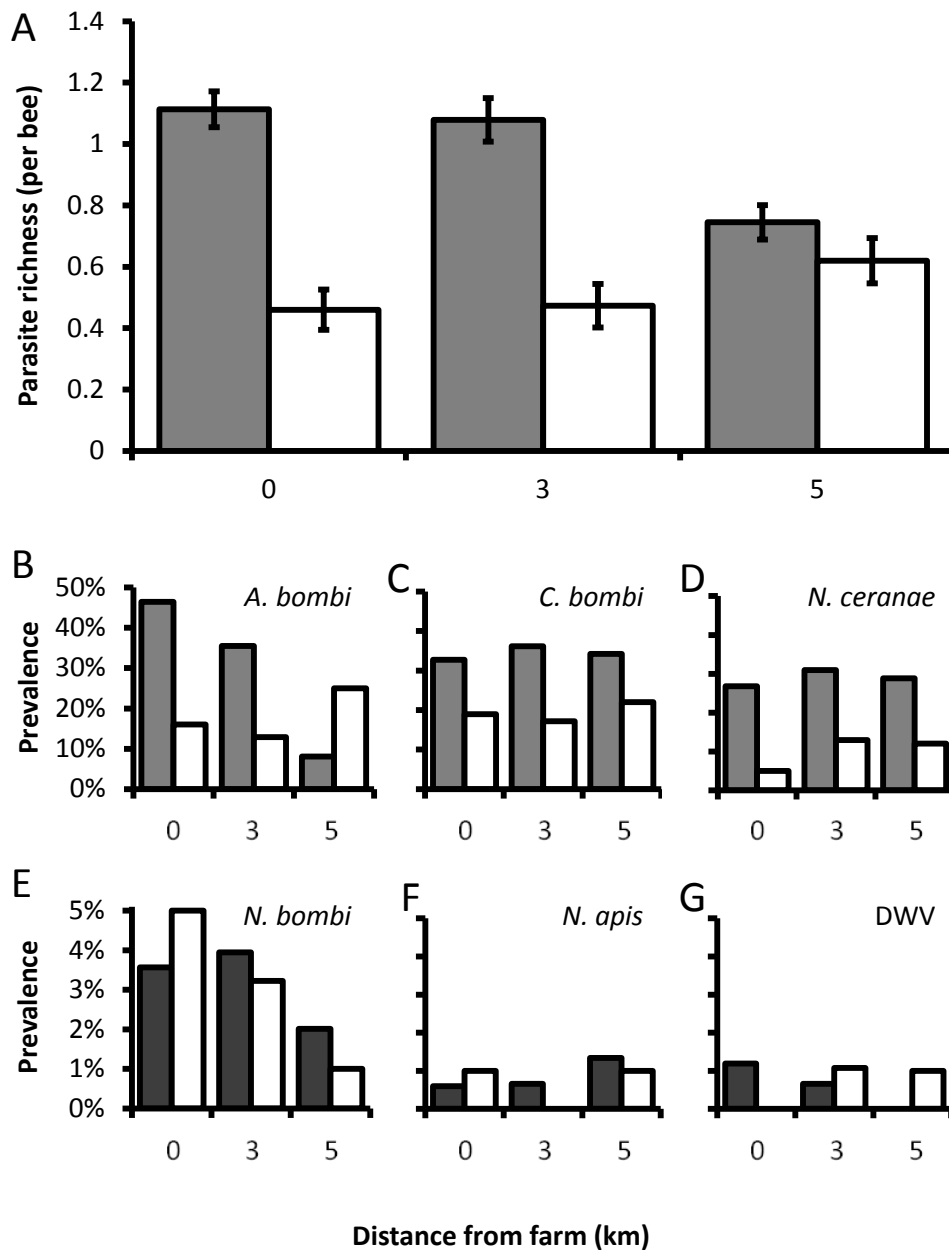
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447 **Figure 1 The effect of commercially produced bumblebees on parasite prevalence.**

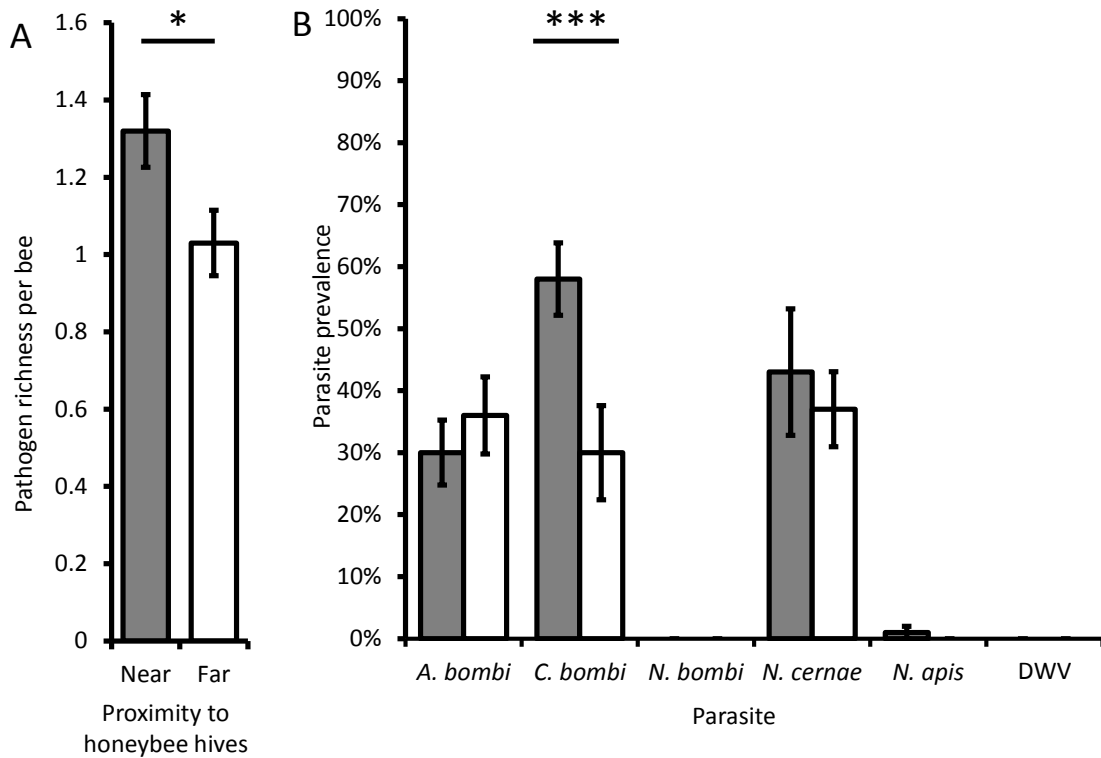
448 Prevalence of parasites in bumblebees sampled 0, 3 or 5 km from greenhouses that were
 449 either using (grey columns) or not using (white columns) commercially produced bumblebee

450 colonies. A) The mean \pm s.e. parasite richness (number of species) infecting individual bees.

451 B-G) The proportion of bumblebees sampled which were positive for the *A. bombi*, *C. bombi*,

452 *N. ceranae*, *N. bombi*, *N. apis* and deformed wing virus (DWV) parasites.

453



455

456 **Figure 2 The effect of managed honey bees on parasite prevalence.** The mean ± s.e.

457 parasite richness (number of species) per bumblebee (A), and the prevalence of six parasites

458 per bumblebee colony (B), that were located at two sites either near (dark grey bars) or far

459 (white bars) from the honey bee apiary. Asterisks and bars above columns indicate significant

460 pairwise differences (* when $P < 0.05$; *** when $P < 0.001$).

461

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

Primers & source	Assay mix								Thermal cycling			Amplicon size (bp)										
	dNTP ^a (nM)	MgCl ₂ (nM)	5sbuffer (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 <i>et al.</i> 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>Nbombi</i> -SSU-Jf (5-3): CCATGCATGTTTTGAAGATTATTAT																						
<i>Nbombi</i> -SSU-Jr1 (5-3): CATATATTTTTAAATATGAAACAATAA																						
<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 95 60 58 60 72	5 72	297 ^{Na} 662 ^{Nc}										
<i>Nosa</i> RNAPol-F2 (5-3): AGCAAGAGACGTTTCTGGTACCTCA																						
<i>Nosa</i> RNAPol-R2 (5-3): CCTTCAGACCACCCATGGCA																						
<i>Nosc</i> RNAPol-F2 (5-3): TGGGTCCCTAAACCTGGTGGTIT	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>Nosc</i> RNAPol-R2 (5-3): TCATCATGACCTGGTGTCTCTCT																						
<i>Apicystis bombi</i> (Meeus <i>et al.</i> 2010)																						
Universal: <i>NeoF</i> (5-3): CCAGCATGGAATAACATGTAAGG <i>NeoR</i> (5-3): GACAGCTCCAATCTCTAGTCCG	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										
Specific: <i>ApBF1</i> (5-3): CGTACTGCCCTGAATACTCCAG <i>ApUR2</i> (5-3): TTTCTCAITCTCAGATGATTGG																						
<i>Apidae</i>^A (host) and <i>Crithida bombi</i>^{Cb} (Meeus <i>et al.</i> 2010)																						
<i>ApidaeF</i> (5-3): AGATGGGGGCATTTCGTATTG <i>ApidaeR</i> (5-3): ATCTGATCGCCTTCGAACCT <i>SER</i> (5-3): CTTTGGTCGGTGGAGTGAT <i>SER</i> (5-3): GGACGTAATCGGCACAGTIT	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 56 45 72	3 72	130 ^A 420 ^{Cb}										
RT-PCR													200	5	0.65	0.65	2	10	5 50	20 95	40x 3 s 95 3 min 60	702
Deformed wing virus (Chen <i>et al.</i> 2005)																						
<i>DWV</i> -sense (5-3): ATCAGCGCTTAGTGGAG GAA <i>DWV</i> -antisense (5-3): TCGACAATTTTCGGACATCA																						

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