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

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ABSTRACT

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

Subjects Entomology, Conservation Biology, Parasitology

Keywords pathogen spillover, pollinator conservation, honeybee, commercial bumblebee production

INTRODUCTION

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

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

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

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

MATERIALS AND METHODS

The effect of proximity to commercially reared bumblebees

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

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

The effect of proximity to managed honey bees

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

Molecular screening for parasite presence

A ca. 0.5 cm^3 sample of midgut, malpighian tubules and fatbody from each bee was homogenised and DNA extracted from the homogenate using 5% Chelex. All DNA samples

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

Statistical analysis

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

RESULTS

The effect of commercially produced bumblebees on parasite prevalence in wild bumblebees

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

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

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

DISCUSSION

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

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

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

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

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

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

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

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

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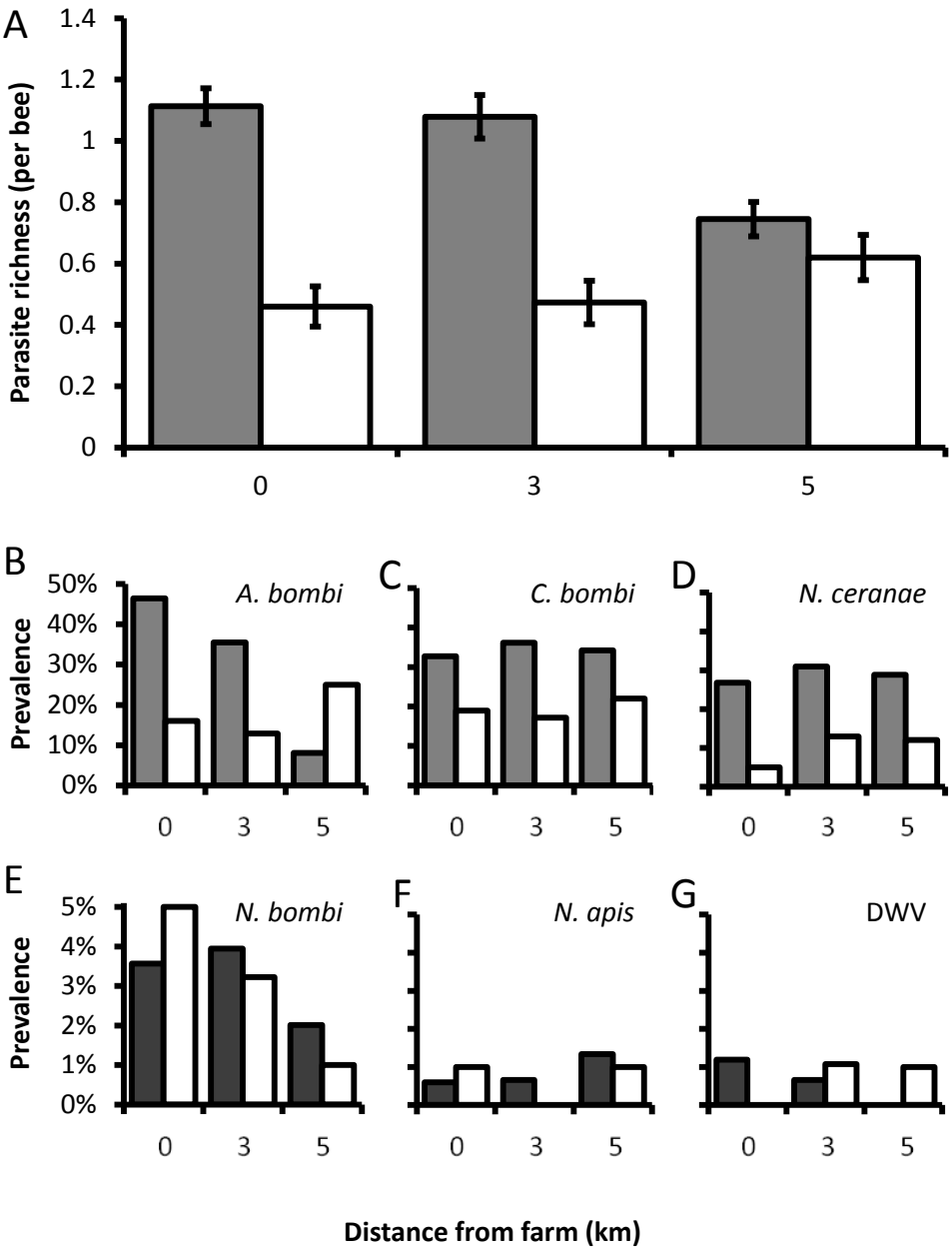

Figure 1 The effect of commercially produced bumblebees on parasite prevalence.

Prevalence of parasites in bumblebees sampled 0, 3 or 5 km from greenhouses that were either using (grey columns) or not using (white columns) commercially produced bumblebee colonies. A) The mean ± s.e. parasite richness (number of species) infecting individual bees. B-G) The proportion of bumblebees sampled which were positive for the *A. bombi*, *C. bombi*, *N. ceranae*, *N. bombi*, *N. apis* and deformed wing virus (DWV) parasites.

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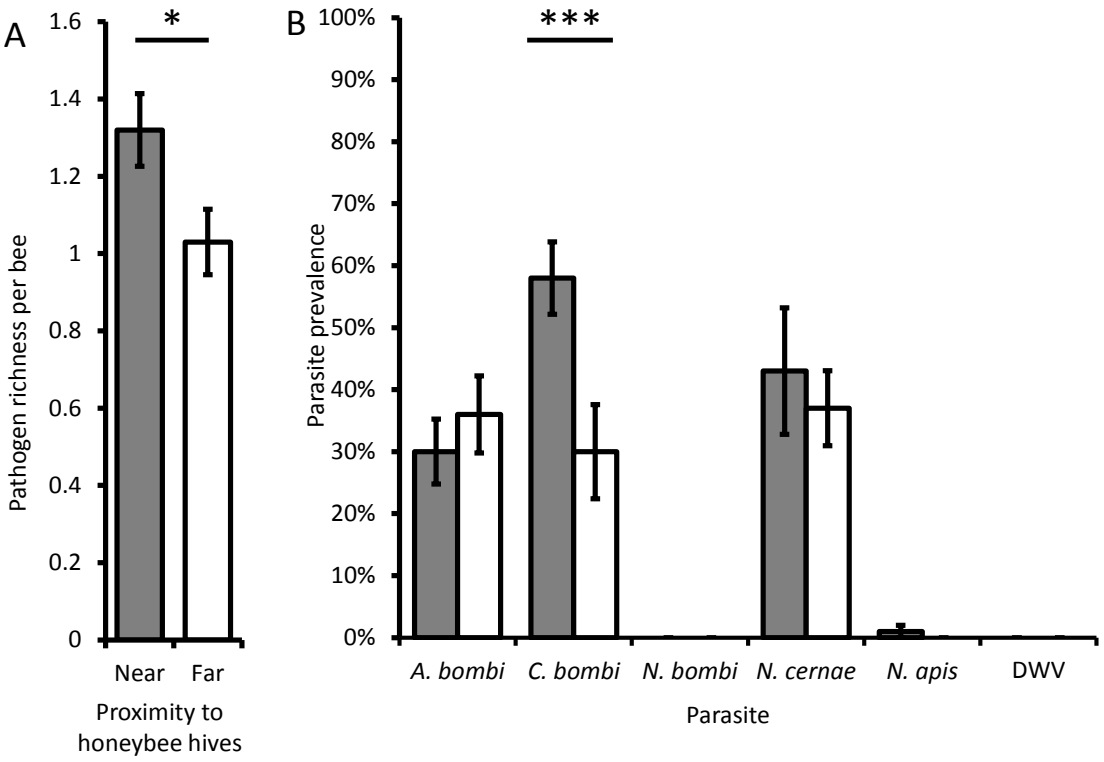

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456 **Figure 2 The effect of managed honey bees on parasite prevalence.** The mean ± s.e.

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 $P < 0.001$).

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

Primers & source	Assay mix: dNTP (nM)	Assay mix: $MgCl_2$ (nM)	Assay mix: 5xbuffer (µl)	Assay mix: Taq (U)	Assay mix: Primer F (µM)	Assay mix: Primer R (µM)	Assay mix: Template (µl)	Assay mix: Total volume (µl)	Thermal cycling: 1 Denaturing Min | Temp	Thermal cycling: 2 Replication Sec | Temp	Thermal cycling: 3 Elongation Min | Temp	Amplicon size (bp)
Nosema bombi (Klee *et al.* 2006) *Nbombi-SSU-Jf (5-3):* *CCATGCATGTTTTTGAAGATTATTAT* *Nbombi-SSU-Jr1 (5-3):* *CATATATTTTTAAAATATGAAACAATAA*	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
Nosema apis^{Na} & ***N. ceranae***^{Nc} (Gisder & Genersch 2013) *NosaRNAPol-F2 (5-3):* *AGCAAGAGACGTTTCTGGTACCTCA* *NosaRNAPol-R2 (5-3):* *CCTTCACGACCACCCATGGCA* *NoscRNAPol-F2 (5-3):* *TGGGTTCCCTAAACCTGGTGGTTT* *NoscRNAPol-R2 (5-3):* *TCACATGACCTGGTGCTCCTTCT*	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	297Na 662^{Nc}
Apicystis bombi (Meeus *et al.* 2010) *Universal:* *NeoF (5-3):* *CCAGCATGGAATAACATGTAAGG* *NeoR(5-3):* *GACAGCTTCCAATCTCTAGTCG* *Specific:* *ApBF1(5-3):* *CGTACTGCCCTGAATACTCCAG* *ApUR2(5-3):* *TTTCTCATTCTTCAGATGATTTGG*	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
Apidae^{A} (host) and ***Crithida bombi***^{Cb} (Meeus *et al.* 2010) *ApidaeF(5-3):* *AGATGGGGGCATTCGTATTG* *ApidaeR(5-3):* *ATCTGATCGCCTTCGAACCT* *SEF(5-3):* *CTTTTGGTCGGTGGAGTGAT* *SER(5-3):* *GGACGTAATCGGCACAGTTT*	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	**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 & elongation Time | Temp**	**Amplicon size (bp)**
Deformed wing virus (Chen *et al.* 2005) *DWV-sense (5-3):* *ATCAGCGCTTAGTGGAG GAA* *DWV-antisense (5-3):* *TCGACAATTTTCGGACATCA*	200	5			0.65	0.65	2	10	5 | 50	20 | 95	40x 3 s | 95 3 min | 60	702

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