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Plight of the bumble bee: Pathogen spillover from commercial to wild populations

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ARTICLE INFO

Article history:
Received 1 September 2005
Received in revised form
7 November 2005
Accepted 20 November 2005
Available online 3 January 2006

Keywords:
Bee conservation
Bumble bees
Crithidia bombi
Nosema bombi
Pathogen spillover

ABSTRACT

Pathogen spread or 'spillover' can occur when heavily infected, domestic hosts interact with closely-related wildlife populations. Commercially-produced bumble bees used in greenhouse pollination often have higher levels of various pathogens than wild bumble bees. These pathogens may spread to wild bees when commercial bees escape from greenhouses and interact with their wild counterparts at nearby flowers. We examined the prevalence of four pathogens in wild bumble bee populations at locations near and distant to commercial greenhouses in southern Ontario, Canada. Bumble bees collected near commercial greenhouses were more frequently infected by those pathogens capable of being transmitted at flowers (*Crithidia bombi* and *Nosema bombi*) than bees collected at sites away from greenhouses. We argue that the spillover of pathogens from commercial to wild bees is the most likely cause of this pattern and we discuss the implications of such spillover for bumble bee conservation.

1. Introduction

In order to effectively conserve wildlife, we need to understand the emergence and spread of pathogens (Daszak et al., 2000; Dobson and Foufopoulos, 2001; Logiudice, 2003). Indeed, pathogens have been recognized as a significant threat to biodiversity, and are implicated in the extinction or decline of various wildlife populations (reviewed by Altizer et al., 2003). The movement of pathogens from domestic to wild organisms can have detrimental effects on wild hosts (Daszak et al., 2000; Kat et al., 1995; Kennedy et al., 2000; Power and Mitchell, 2004). A process termed "pathogen spillover", occurs when pathogens spread from a heavily infected 'reservoir' host population to a sympatric 'non-reservoir' host population (Daszak et al., 2000; Power and Mitchell, 2004). For example, parasitic sea lice (*Lepeophtheirus salmonis*) spread into wild salmon (*Oncorhynchus* spp.) populations when commercially-reared fish escape from infested salmon farms (Morton et al., 2004); such spillover has been implicated in the demise of wild fish cohorts in both Canada (Morton et al., 2004) and Europe (McVicar, 1997; McVicar, 2004). Although pathogen spillover has been documented in vertebrates and plants (Power and Mitchell, 2004), its occurrence in wild invertebrates is currently unknown. Here, we consider pathogen spillover from commercial to wild bumble bees.

Bumble bees (*Bombus* spp., Hymenoptera, Apidae) are distributed naturally throughout North America and are of considerable importance as pollinators of native plants (Kearns and Thomson, 2001). Since the early 1990's, private companies have mass-produced and distributed colonies of native bumble bees (*Bombus impatiens* Cresson in the east and *B. occidentalis* Greene in the west, although more recently *B. impatiens* has also been shipped to the west) to large-scale commercial greenhouses for year-round pollination of tomato (*Lycopersicon esculentum*) (Whittington and Winston, 2004) and sweet pepper (*Capsicum annuum*) (Shipp et al., 1994). A

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doi:10.1016/j.biocon.2005.11.013

pollinating force of commercial *Bombus* can reach 23,000 bees per greenhouse (estimated from data presented in Morandin et al., 2001). However, foraging bumble bees regularly escape from greenhouses during the summer months and up to 73% of the pollen carried by returning workers is collected from plants outside the greenhouse (Whittington et al., 2004). The potential for contact between commercial and wild bumble bees near greenhouses is, therefore, substantial.

There is growing evidence that commercially-reared bumble bees have higher prevalences of various pathogens than their wild counterparts. Several studies have found that the intestinal protozoa *Crithidia bombi* Lipa and Triggiani (Kinetoplastida: Trypanosomatidae) and *Nosema bombi* Fantham and Porter (Microsporidia: Nosematidae), and the tracheal mite *Locustacarus buchneri* Stammer (Acari: Podapolipidae), are far more abundant in commercial than wild bumble bees (Fig. 1). Only the protozoan *Apicystis bombi* Liu, MacFarlane and Pengelly (Neogregarinida: Ophrocystidae), which infects the fat-bodies of bumble bees, does not seem to occur more commonly in commercial than wild bees (M.C. Otterstatter, unpublished). All of these parasites infect a range of bumble bee species and spread extensively within host colonies, thereby reducing colony survival and reproduction and (or) the foraging efficiency of individual workers (*C. bombi*: Brown et al., 2003; Otterstatter et al., 2005; *N. bombi*: Fisher and Pomeroy, 1989; *L. buchneri*: Husband and Sinha, 1970; *A. bombi*: MacFarlane et al., 1995). Furthermore, at least for intestinal protozoa, shared flower use by infected and susceptible bumble bees is sufficient for extensive spread of pathogens between individuals (Durrer and Schmid-Hempel, 1994) and colonies (Imhoof and Schmid-Hempel, 1999).

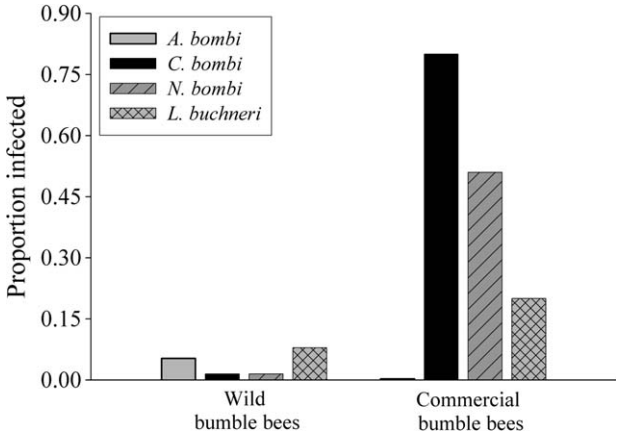

Fig. 1 – The prevalence of four parasites in wild-caught and commercial bumble bees. Note that each bar represents data from a separate study, and that all studies were on Canadian populations (wild bees: MacFarlane et al., 1995 [*A. bombi*, *n* = 2977 queens); Liu, 1973 [*C. bombi*, *N. bombi*, *n* = 133 queens]; Otterstatter and Whidden, 2004 [*L. buchneri*, *n* = 1016 queens, workers, males]; commercial bees: Otterstatter M.C., unpublished [*A. bombi*, *C. bombi*, *n* = 20 colonies]; Whittington and Winston, 2003 [*N. bombi*, *n* = 49 colonies]) with the exception of the study on *L. buchneri* in commercial bees (*n* = 367 colonies, Goka et al., 2000), which considered European populations.

Frequent interaction at flowers between pathogen-infected commercial bees and wild conspecifics and congeners provides the necessary components for pathogen spillover from greenhouse to wild bumble bee populations. Such spillover could harm native bumble bee populations and thus warrants further investigation. In this study, we assess the likelihood of pathogen spillover from greenhouse to wild bumble bees by comparing the prevalence of four pathogens (*C. bombi*, *N. bombi*, *L. buchneri*, and *A. bombi*) among bumble bees foraging in close proximity to large-scale commercial greenhouses to their prevalence among bumble bees foraging in areas without commercial greenhouse operations.

2. Materials and methods

2.1. *Sampling methods*

Using sweep nets, we collected foraging bumble bees from six sites in southwestern Ontario, Canada during the summers of 2004 and 2005 (Fig. 2). Collection sites were chosen based on the presence or absence of large-scale commercial greenhouse operations and previous surveys of pathogens in wild bumble bees. For our first 'greenhouse site', we sampled bees from three different areas near the city of Leamington, which has the largest concentration of commercial greenhouses in North America (Agriculture and Agri-Food Canada, 2004). These three areas included roadside ditches and old fields and were within 500 m of at least one commercial greenhouse, which is well within the maximum distance that bumble bees travel from the colony to forage (Goulson and Stout, 2001; Darvill et al., 2004). Our second greenhouse site, hereafter referred to as the Exeter site, was approximately 40 km north of the city of London. As in Leamington, we collected bees along roadside ditches within 500 m of a large-scale (~40 acre) greenhouse operation. Our 'non-greenhouse sites' were over 50 km from any commercial greenhouse operations but had comparable habitats and bumble bee species. For our first and second non-greenhouse sites, we sampled bees along a 1 km section of the Eramosa River in the city of Guelph and along roadside ditches within 3 km of the northwest side of the town of Belwood; these same two areas were surveyed for bumble bee pathogens by Liu (1973) and MacFarlane (1974). For our third non-greenhouse site, we sampled bees from three areas located near the St. George campus of the University of Toronto (hereafter referred to as the Toronto site). For our final non-greenhouse site, we collected bees along roadside ditches within 1 km of the Thames River on the northwest and southwest limits of the city of London. We sampled bees in early (June–July) and late (August–September) summer during 2004 (Leamington, Guelph, Toronto) and 2005 (Exeter, London, Belwood). Each greenhouse site was sampled within, at most, one week of a non-greenhouse site to minimize the effects of seasonal changes in pathogen prevalence. At all sites, we collected foraging bees during the morning and afternoon by walking various trajectories (e.g., along the length of a roadside ditch) for multiple hours and collecting all visible bumble bees. Additionally, we recorded the number of bees caught per hour as an estimate of collection effort. Bees were placed individually in plastic vials for transport to the laboratory, and then transferred from vials into microcentrifuge tubes and frozen at −20 °C for later dissection.

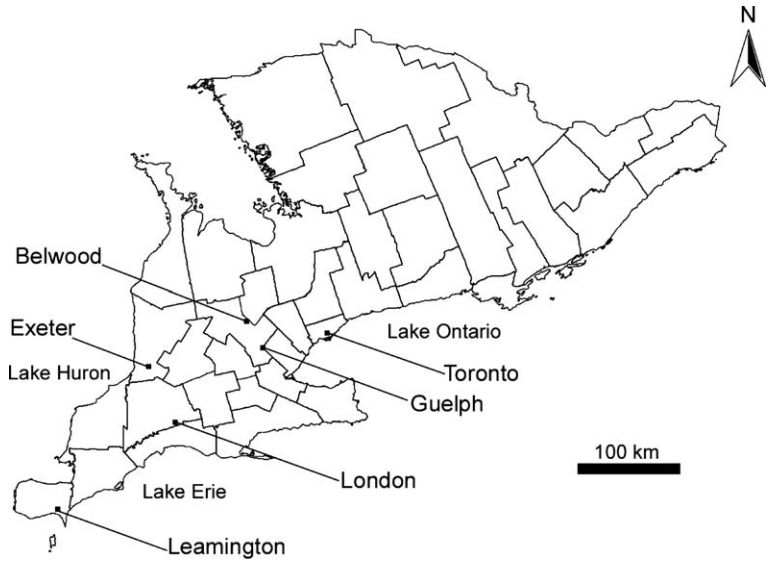

Fig. 2 – Map of southern Ontario, Canada, showing the six sites that were sampled for pathogens of bumble bees during the summers of 2004 and 2005.

2.2. *Identification and dissection*

We dissected and examined bees for three common pathogens of commercially-produced colonies, *C. bombi*, *N. bombi* and *L. buchneri*. Additionally, we tested all bees for *A. bombi*, which infects wild bumble bees in southern Ontario (MacFarlane et al., 1995), but has not been reported in commercial colonies. Bees were examined blind to the location of collection.

Before dissection, we identified each bee to species (Laverty and Harder, 1988), noted whether or not it had collected discernable pollen loads, and then removed the right forewing for our measures of body size (radial cell length: Gerloff et al., 2003) and bee age (wing wear: Cartar, 1992). For each bee, we removed the entire gut tract posterior to the junction of the mid- and fore-gut regions (the posterior gut has highest concentration of *C. bombi* and *N. bombi* in infected bees) and fat bodies lying along the inner abdominal wall (which have the highest concentration of *A. bombi*) and crushed each separately on a clean slide in a drop of distilled water. These samples were examined at 160× magnification for the protozoa *C. bombi*, *N. bombi*, and *A. bombi*. Lastly, we examined the air sacs of each bee under a dissecting microscope for the presence of *L. buchneri*. These four pathogens are well-described (MacFarlane et al., 1995) and easily distinguishable following our protocol. We scored bees as infected or uninfected for each of the four pathogens (we considered a bee infected if a single protozoan or single mite was observed) and ranked the intensity of infection for each protozoan species (1 = light infection, ≈10–100 cells observed, to 3 = heavy infection, ≈1000 or more cells observed), and counted the number of gravid females mites per bee.

2.3. *Statistical analysis*

G-tests of independence (Sokal and Rohlf, 1995) were used for comparing the frequency of infection by each pathogen species between collecting locations, worker and male bees, and between sampling dates at a location. We also used *G*-tests to compare the proportion of infected (*N. bombi* and *C. bombi*) and uninfected workers from the same location which were collecting pollen at the time of capture. We compared the wing wear (correlate of bee age) of infected and uninfected bees from the same location, and the intensity of infection by each parasite between locations, using Wilcoxon two-sample tests (*Z* statistic) when comparing two samples, and Kruskal–Wallis tests (χ^2 statistic) when comparing more than two samples (SAS Institute, 1999). We compared the radial cell length (correlate of bee size) of infected (*C. bombi* or *N. bombi*) and uninfected bees using ANOVA and included collecting date (early or late) and bee species (only *B. bimaculatus*, *B. griseocollis*, and *B. impatiens* were collected in sufficient numbers for this analysis) as explanatory variables.

3. Results

We collected and screened for pathogens 500 worker and 128 male bumble bees belonging to 12 species during the summers of 2004 and 2005. Overall, bumble bees were similarly abundant at all sites (approximately 25 collected per hour, on average), except Guelph (8 per hour), and the relative abundance of each bumble bee species was comparable between sites. Across all sites, *A. bombi* infected 1.8% of bees, *C. bombi* 7.0%, *N. bombi* 5.1%, and *Locustacarus buchneri* 3.5%. At all six study locations, and for all pathogen species, the frequency of infection did not differ significantly between worker and male bees (*G* tests, $P > 0.11$ in all cases) or among sampling days ($P > 0.17$ in all cases).

Near commercial greenhouse operations, the intestinal pathogen *C. bombi* infected 27% (Exeter) and 15% (Leamington) of bees, but was entirely absent at our four sites lacking commercial greenhouses. We also found a second intestinal pathogen, *N. bombi*, infecting 15% of bees near greenhouses in Leamington, but occurring in less than 4% of bumble bees elsewhere. Two other pathogens, the protozoan *A. bombi* and

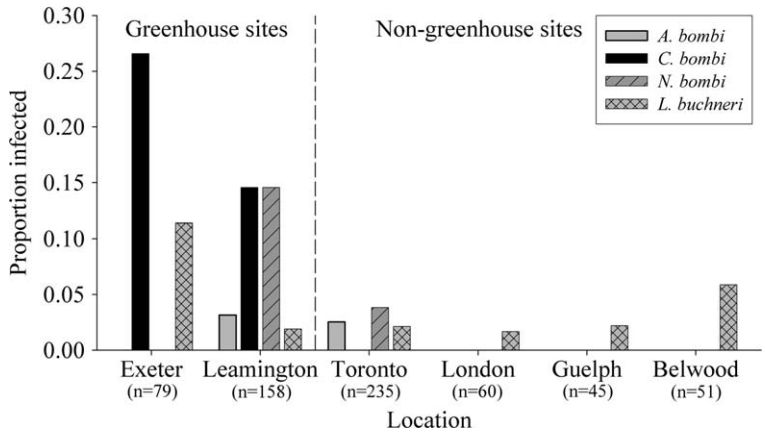

Fig. 3 – Proportion of bumble bees (all species pooled) infected by four parasites in southwestern Ontario during the summers of 2004 and 2005. The pathogen *C. bombi* infected a greater proportion of bees at our Leamington greenhouse site than at all of the non-greenhouse sites (Belwood, Guelph, London, and Toronto) combined ($G = 59.8$, $P < 0.001$) and was even more prevalent at our Exeter greenhouse site than at Leamington ($G = 4.8$, $P = 0.03$). Our two greenhouse sites also had a greater proportion of bees infected by the pathogens *N. bombi* (Leamington, $G = 45.4$, $P < 0.001$) and *L. buchneri* (Exeter, $G = 13.9$, $P < 0.05$) than elsewhere. In contrast, the prevalence of *A. bombi* did not differ across sites ($G = 10.6$, $P > 0.05$).

the tracheal mite *L. buchneri*, were similarly rare (6% of bees or less) across all sites except Exeter, where tracheal mites were slightly more common (11% of bees) than elsewhere (Fig. 3). The same pattern appeared when we considered only late-summer sampling dates, which suggests that differences in pathogen prevalence between sites were not a reflection of seasonal trends. In addition to being infected more often, bees foraging near commercial greenhouses in Leamington tended to have more intense infections of *N. bombi* than bees foraging away from greenhouses (Toronto) ($Z = -2.39$, $P = 0.017$). We could not test whether this pattern was consistent across all sites because *N. bombi* was absent at our other sampling locations. We did not find that infection by *A. bombi* (Leamington vs. Toronto, $Z = -1.01$, $P = 0.31$) or *L. buchneri* (all sites, $\chi^2 = 4.51$, $P = 0.48$) varied in intensity among locations, nor did the intensity of infection by *C. bombi* differ between our two greenhouse sites (Exeter vs. Leamington, $Z = 0.93$, $P = 0.35$). Note that we could not compare *C. bombi* intensity between greenhouse and non-greenhouse sites as bees foraging distant to greenhouses did not harbor this pathogen.

The prevalence of *C. bombi* was substantially higher near commercial greenhouses for all of the five most abundant bumble bee species that we collected (Table 1). The same pattern was apparent in less abundant bumble bee species ('Other' species category, Table 1). The prevalence of *N. bombi* was also greater near commercial greenhouses for all bumble bee species, except *B. rufocinctus*. Although our goal was to determine pathogen prevalence among wild bumble bees, it is possible that some of the *B. impatiens* we collected at Exeter and Leamington were commercially-reared bees which escaped from nearby greenhouses. Indeed, *B. impatiens* were more abundant at our greenhouse sites (proportion of catch: 41%) than elsewhere (30%) ($G = 7.7$, $P = 0.006$). However, excluding this species from the analyses did not alter the overall frequency of infection by *C. bombi* or *N. bombi* at Leamington (*G* tests, $P = 0.45$ and $P = 0.86$, respectively) or the frequency of infection by *C. bombi* at Exeter ($G = 0.17$, $P = 0.83$).

We did not find that infected and uninfected bees differed consistently in age, size, or propensity to collect pollen. Overall, wing wear (a correlate of bee age) did not differ between locations ($P > 0.05$ for all bumble bee species), and was similar among bees from the same location that were infected or uninfected with intestinal protozoa (*C. bombi* or *N. bombi*) (Leamington: $\chi^2 = 2.61$, $P = 0.27$; Toronto: $Z = 1.15$, $P = 0.25$).

Table 1 – Prevalence of intestinal protozoa *C. bombi* and *N. bombi* among the most common bumble bee species collected in southwestern Ontario during the summers of 2004 and 2005 (data pooled)

Bee species (*n*)	Greenhouse sites		Non-greenhouse sites	
	C. bombi	*N. bombi*	*C. bombi*	*N. bombi*
B. bimaculatus (130)	8.7%	6.3%	0.0%	3.7%
B. fervidus (50)	5.3%	18.4%	0.0%	0.0%
B. griseocollis (98)	10.2%	10.2%	0.0%	7.7%
B. impatiens (212)	25.0%	9.4%	0.0%	0.0%
B. rufocinctus (55)	75.0%	0.0%	0.0%	4.7%
Other (83)	11.1%	0.0%	0.0%	0.0%

Bumble bee species that were absent or poorly represented at one or more of our collecting sites are pooled into the 'Other' category (*B. borealis*, *B. pennsylvanicus*, *B. perplexus*, *B. sandersoni*, *B. terricola*, *B. vagans*).

Compared to uninfected workers, the proportion of workers collecting pollen at the time of capture did not differ for bees infected by *C. bombi* (Leamington and Exeter pooled, uninfected: 59%, $n = 107$; infected: 67%, $n = 31$; $G = 1.1$, $P = 0.29$) or *N. bombi* (Leamington only, uninfected: 80%, $n = 266$; infected: 65%, $n = 20$; $G = 0.81$, $P = 0.37$). At our Toronto location, bees infected with *N. bombi* were larger on average than uninfected bees (LSmean ± SE radial cell length, 2.95 ± 0.10 mm vs. 2.61 ± 0.03 mm; $F_{1,136} = 10.15$, $P = 0.002$) after accounting for significant differences in body size between species and collecting dates. However, this pattern did not hold for other locations or for bees infected or uninfected by *C. bombi* ($P > 0.05$ in all cases).

4. Discussion

Pathogens are common among bumble bees that are reared commercially for large-scale greenhouse pollination (Goka et al., 2000; Houbaert et al., 1986; Kwon et al., 2003; MacFarlane et al., 1995; Niwa et al., 2004; Otterstatter et al., 2005; Whittington and Winston, 2003). However, a large number of foragers from commercial colonies escape from greenhouses and potentially share flowers with wild bees (Whittington et al., 2004). We predicted that such circumstances could lead to the transmission of disease, so called 'pathogen spillover' (Power and Mitchell, 2004), from commercial to wild bumble bees. Indeed, we found that the intestinal pathogen *Crithidia bombi* infected 27% (Exeter site) and 15% (Leamington site) of wild bumble bees foraging near commercial greenhouses, but was entirely absent in bumble bees collected elsewhere. Further, a second intestinal pathogen, *Nosema bombi*, was three times more prevalent among bumble bees near greenhouses at our Leamington site than elsewhere. Both *C. bombi* and *N. bombi* are common in commercial bumble bees (Otterstatter et al., 2005; Whittington and Winston, 2003) and capable of spreading to wild bees at flowers (Durrer and Schmid-Hempel, 1994; Schmid-Hempel, 1998). In contrast, the pathogen *Apicystis bombi*, which is not known to exist in commercial bumble bees, occurred at similar levels across all sites. The differences in infection rates are not attributable to differences in bee-species composition, bee abundance, or bee age between sites. Prior to the use of commercial bumble bees in Canada, MacFarlane (1974) and Liu (1973) found that *C. bombi* and *N. bombi* infected a very small proportion of wild bumble bees in southern Ontario (<2% of bees on average). After re-visiting the same sites sampled by MacFarlane and Liu (Belwood and Guelph), and several others, we conclude that intestinal pathogens have remained rare (currently, <4% of bees infected on average) only at sites distant to commercial greenhouses. Spillover of pathogens from commercial to wild bumble bees near greenhouses is the most likely cause of these patterns.

Pathogen spillover can occur when a heavily infected 'reservoir' host population interacts with a closely-related 'non-reservoir' population, thereby allowing disease to spread (Daszak et al., 2000; Power and Mitchell, 2004). Often, feral animals that escape from infected, domestic populations act as a conduit through which pathogens spillover into wild populations (Dobson and Foufopoulos, 2001). Although we could not directly witness pathogen spread between commercial and wild bees (which are indistinguishable in the field), it almost certainly occurs because (1) commercial bumble bees often escape from greenhouses at our Leamington (Morandin et al., 2001) and Exeter (M.C. Otterstatter, personal observation) sites, (2) escaped commercial bumble bees visit a variety of plant species outside the greenhouse (Whittington et al., 2004), and (3) shared flower use is sufficient for the transmission of intestinal protozoa between individual bumble bees (Durrer and Schmid-Hempel, 1994) and colonies (Imhoof and Schmid-Hempel, 1999). Furthermore, surveys conducted in our lab during 2003–2005 have revealed a high prevalence of pathogens, particularly *C. bombi*, in commercial bumble bees, including stock from a producer that supplies greenhouses in Leamington and Exeter (M.C. Otterstatter, unpublished data). Nevertheless, in order to fully assess the risk that commercial bumble bee pose towards their wild counterparts, further studies are needed which explicitly compare pathogen prevalence between commercial and wild populations. The abundance of intestinal pathogens that we observed among all bumble bee species at Leamington and Exeter suggests that disease may be spreading across bumble bee species, as is known to occur when infected, domestic animals aggregate with wildlife at common food sources (Dobson and Foufopoulos, 2001). It is also possible that pathogens could spread from commercial to wild bumble bee colonies if infected workers enter colonies other than their own (a process know as 'drifting', Schmid-Hempel, 1998). This may explain why the tracheal mite *L. buchneri*, which is common in commercial bumble bees (Goka et al., 2000) but typically spreads from adult workers to brood within colonies (van den Eijnde and de Ruijter, 1998), was more common at our Exeter greenhouse site than at sites lacking greenhouses. However, further study is needed to determine whether pathogens spread between bumble bee colonies via drifting workers.

In recent decades, wild bumble bee populations have declined in North America and Europe (Williams et al., 2005; Goulson, 2003). Although habitat loss (Osborne and Corbet, 1994; Williams, 1986) and increased competition from introduced bees (Goulson, 2003; Thomson, 2004) are thought to contribute to these declines, in many cases the causes remain elusive (Goulson et al., 2005). Remarkably, the role of disease has received little attention, despite evidence that pathogens, particularly intestinal protozoa, harm bumble bee colonies and their workers. For example, *C. bombi* can reduce the reproductive output of bumble bee colonies by 40% (Brown et al., 2003), and reduce the survival of individual workers by 50% when food is scarce (Brown et al., 2000). In the absence of effects on host survival, *C. bombi* can still reduce the foraging efficiency of workers, which likely diminishes the growth of infected colonies (Otterstatter et al., 2005). The elevated prevalence and intensity of *N. bombi* infections that we observed among wild bees near Leamington greenhouses is also of concern because this pathogen is thought to have contributed to the recent collapse of commercial *B. occidentalis* populations in North America (Whittington and Winston, 2004). Interestingly, *N. bombi* occurred more often in large-bodied bees at our Toronto location, which may suggest that, in the absence of unnatural sources of pathogens like commercial greenhouses, the tendency of

large bees to spend more time foraging (Goulson et al., 2002) exposes them to greater risks of infection. Because commercial bumble bees are used in, and escape from, large-scale greenhouses year round, the spillover of pathogens likely occurs continuously throughout the spring and summer months when wild bees are active. Although our study focused only on infection of worker and male bees during the summer, wild bumble bee populations might suffer most from the damaging effects of *C. bombi* and *N. bombi* on the colony founding success of spring queens (Brown et al., 2003; Fisher and Pomeroy, 1989). Currently, commercial bumble bees are used for greenhouse pollination in Australia, Israel, Japan, and in parts of North America and Europe (Asada and Ono, 2002; Dag and Kammer, 2001; de Ruijter, 1997; Hingston, 2005), and have also been used in outdoor orchards in the United States, Canada (Stubbs and Drummond, 2001; Whidden, 1996) and New Zealand (Griffin et al., 1991). Our study is the first to investigate pathogen spillover from commercial to wild bumble bees; clearly, this phenomenon deserves immediate attention in other areas where commercial bees are being used. Future work should examine the rate at which infected, commercial bumble bees visit plants (and possibly other bumble bee nests) outside the greenhouse, the distance that pathogens spread from commercial greenhouses, and the extent to which these pathogens have become established in wild bee populations. Because many pathogens can certainly move from one *Bombus* host species to another, it will be particularly important to study transmission, mortality, and morbidity in a range of host species.

Acknowledgements

We thank Nora Saona, Kieran Samuk, and Sarah Tanzini for assistance with bee collecting. Funding was provided by the Natural Science and Engineering Research Council.

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