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Pathogen prevalence in commercially reared bumble bees and evidence of spillover in conspecific populations

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ABSTRACT

Worldwide, wild bumble bees (*Bombus* spp.) are experiencing marked declines, with potentially up to 11% of species currently under threat. Recent studies from North America suggest that disease transmission from commercially reared bumble bees to wild populations has led to marked range contractions in some species. In Europe, data on the prevalence of pathogen spillover from commercial to wild bumble bee populations is lacking, despite the widespread production and transport of hives within the EU since the early 1980s. We determined the permeability of cropping systems to commercial bumble bees, and quantified the prevalence of four pathogens in commercial *Bombus terrestris* hives and adjacent conspecific populations at increasing distances from greenhouses in Ireland. Commercial bumble bees collected from 31% to 97% of non-crop pollen, depending on the cropping system, and hives had markedly higher frequencies of two gut parasites, *Crithidia* spp. and *Nosema bombi*, compared to adjacent populations, but were free of tracheal mites. The highest prevalence of *Crithida* was observed within 2 km of greenhouses and the probability of infection declined in a host sex- and pathogen-specific manner up to 10 km. We suggest implementing measures that prevent the interaction of commercially reared and wild bumble bees by integrating the enforcement of national best management practices for users of commercial pollinators with international legislation that regulates the sanitation of commercial hives in production facilities.

1. Introduction

Human-mediated changes in landscapes and shifts in wildlife populations are the primary drivers of pathogen outbreaks worldwide (Daszak et al., 2000; Jones et al., 2008). In the past two decades, the emergence of infectious diseases in humans, such as Ebola, severe acute respiratory syndrome and avian influenza A viruses, have served to increase scientific and public awareness of the causal links between wildlife, domestic animals and human populations in driving outbreaks (Jones et al., 2008). The transmission of infectious agents from domesticated populations to sympatric wild populations, known as pathogen spillover (Daszak et al., 2000), has infamously led to marked declines in wild vertebrate populations, such as occurred in Kenya when 90% of wild African buffalos succumbed to rinderpest virus that originated from imported Asian cattle (Mack, 1970). In contrast to vertebrate studies, cases of pathogen spillover in insects are rare and the aetiology of insect diseases are largely unknown, despite accumulating evidence of human-mediated population decline and extinction (Samways, 2005; Dunn, 2005).

Insect pollinators play an essential functional role in most terrestrial ecosystems, maintaining the reproduction and genetic diversity of wild flora and productivity in agricultural systems (Klein et al., 2007; Potts et al., 2010). There is growing concern that pollinators may be declining at a global scale, particularly as the cultivation of pollinator dependent crops has steadily increased in the last 45 years (Aizen and Harder, 2009). The best evidence for regional declines of entire bee communities comes from Europe, where citizen science data from Britain and The Netherlands indicate that 52% and 67%, respectively, of areas surveyed experienced a decline in bee species richness since 1980 (Biesmeijer et al., 2006). Currently, across Europe 37–67% of bee species are on lists of conservation concern (Patiny et al., 2009). Habitat loss and fragmentation is considered the most important factor driving bee declines (Winfree et al., 2009), but the role of disease in

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pollinator declines has recently been the focus of intense research, primarily due to dramatic declines of the major commercial pollinator, the honeybee *Apis mellifera* (Neumann and Carreck, 2010). However, wild bumble bees (*Bombus*) have also experienced marked declines with potentially 11% of all species worldwide listed as "near threatened" or above using the IUCN Red List criteria (Williams and Osborne, 2009; Winfree, 2010). For some North American species, pathogen spillover from commercial to wild bumble bee populations is hypothesized to have contributed to rapid range contractions (up to 87% in the past 30 years; Thorp, 2003; Cameron et al., 2011; Szabo et al., 2012).

Since the development of breeding techniques in the late 1980s allowing year-round production, bumble bee hives are now mass produced for the pollination of over 20 different crops (Velthuis and van Doorn, 2006). Currently, commercially reared hives are imported by over 50 countries across the globe with an estimated annual value of €55 million (Velthuis and van Doorn, 2006; Ings, 2007). However, the trade in bumble bees frequently involves introductions of non-native species, e.g. *Bombus terrestris* has been imported by over 57 countries, 16 are outside its native range (Ings, 2007; Ings et al., 2010). *B. terrestris* has now become established in the wild in Japan (Inari et al., 2005), Chile (Ruz, 2002) and Argentina (Torretta et al., 2006), and was intentionally introduced into New Zealand in 1885 and spread to Tasmania in 1992 (Schmid-Hempel et al., 2007). In parallel with establishment, empirical data now supports other negative impacts associated with the introduction of non-native bumblebees such as modification of native plant community structures (Kenta et al., 2007), displacement of native bumble bee species (Inoue et al., 2008), hybridization between closely related taxa (Kanbe et al., 2008; Kraus et al., 2011) and pathogen spillover from non-native commercial to native wild populations (Goka et al., 2006).

Commercial bumble bee rearing facilities provide ideal conditions for the development of pathogens and parasites due to the high density of hosts facilitating disease transmission, and the provision of ad libitum food that increases the likelihood of host survival and reproduction, despite potentially high pathogen loads (e.g. Brown et al., 2000). Elevated parasite loads have been reported in commercially reared species: tracheal mites (*Locustacarus buchneri*) in European hives of *B. terrestris* in Japan (Goka et al., 2000); microsporidian infection in North American *B. occidentalis* (Whittington and Winston, 2003), and intestinal protozoa in North American *B. impatiens* (Otterstatter and Thomson, 2008). Transmission to wild bees is facilitated by the lack of preventative measures taken to reduce the escape of commercial bumble bees from the target crop, e.g. up to 73% of pollen collected by foragers in tomato greenhouses originated from plants outside the greenhouse (Whittington et al., 2004). The potential spread of pathogens from commercial to wild *Bombus* populations was first documented in studies of the tracheal mite *L. buchneri* in Japan, where mitochondrial DNA sequences indicated that haplotypes found in commercially reared *B. ignitus* originated in European rearing facilities (Goka et al., 2001) but are now present in native populations due to spillover (Goka et al., 2006). Colla et al. (2006) found elevated levels of *Crithidia* and *Nosema* parasites in two Canadian bumble bee populations close to greenhouses actively using commercial *B. impatiens* for pollination, compared to four populations with no commercial bumble bees. Furthermore, the spatial pattern of infection within the immediate area of greenhouses agreed with predictions from spillover models of initial primary infection with *Crithidia* (Otterstatter and Thomson, 2008).

Despite the lack of evidence directly linking spillover and decreased abundance in wild populations (reviewed in Meeus et al., 2011), major bumble bee parasites have been shown to impose significant morbidity on laboratory populations of *Bombus*. *Crithida bombi* and the recently identified *C. expoeki* (Schmid-Hempel and Tognazzo, 2010) infect multiple *Bombus* species in Europe and North America and are known to reduce lifetime reproductive output of *B. terrestris* queens by 40% when infected prior to hibernation (Brown et al., 2003), and increase worker mortality rate by 50% in conjunction with starvation (Brown et al., 2000). The virulence of another generalist gut parasite, the microsporidian *N. bombi*, varies across species (Rutrecht and Brown, 2009), but can deform wings, decrease the survival of workers and males, prevent queens from mating (Otti and Schmid-Hempel, 2007, 2008). Rapidly declining North American bumble bee species have a higher prevalence of *N. bombi* compared to stable species (Cameron et al., 2011). The neogregarine *Apicystis bombi* infects the adipose tissue of bumble bees and has been correlated with high mortality in post-hibernation *B. pratorum* queens (Rutrecht and Brown, 2008). Although apparently absent from native species in Argentina, *A. bombi* has been found in 4–12% of invasive *B. terrestris* (Plischuk et al., 2009, 2011) and has now spread to honey bees (*A. mellifera*) in the same region (Plischuk et al., 2011). Finally, high levels of tracheal mite *L. buchneri* infestation have been associated with lethargy and the cessation of foraging in workers (Husband and Shina, 1970), and reduced lifespan in Canadian *B. occidentalis* (Otterstatter and Whidden, 2004), but it now appears to have been successfully controlled by commercial breeders after spillover to wild populations was confirmed (Goka et al., 2006).

The increasing global demand for commercial bumble bees coupled with the potential elevated incidence and prevalence of disease in commercial hives (compared to wild colonies) and lack of measures preventing the escape of bees from target cropping systems may perpetuate chronic pathogen spillover from commercial to wild *Bombus* populations. Furthermore, laboratory studies suggest that pathogen transmission is greatest among closely related bumble bees (Durrer and Schmid-Hempel, 1995; Schmid-Hempel and Loosli, 1998) and allopatric infections result in higher mortality (Imhoof and Schmid-Hempel, 1998); thus, pathogens escaping from commercial hives would most likely spread and have the greatest negative impacts on wild conspecifics. Therefore, we aim to: (i) quantify the permeability of different strawberry cropping systems to commercial bumble bees; (ii) assess the potential of commercially reared *B. terrestris* colonies to act as pathogen reservoirs by comparing the prevalence of four pathogens (*A. bombi*, *Crithidia* spp., *N. bombi* and *L. buchneri*) among commercially reared hives and allopatric conspecific populations; and (iii) determine the likelihood of pathogen spillover from commercial bees to allopatric conspecifics by dissecting and recording parasitic infections present among bumble bees foraging in areas of decreasing proximity to horticultural activities where commercially reared bumble bees were employed.

2. Materials and methods

2.1. Study system

To determine the prevalence and potential spread of disease from imported bumble bees, native species of the subgenus *Bombus sensu stricto* were collected using sweep nets from six locations of intensive strawberry production (henceforth 'sites') in eastern Ireland between 4 May and 21 August 2008 (Fig. 1). Sites were chosen based on the presence of large-scale commercial strawberry farms that had imported bumble bees for a minimum of 10 years and that utilized three cropping systems: greenhouses, plastic tunnels and field-grown crops; thereby ensuring that bumble bees are imported throughout the strawberry growing season.