



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



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



Tomás E. Murray<sup>a,b,\*</sup>, Mary F. Coffey<sup>a,c</sup>, Eamonn Kehoe<sup>d</sup>, Finbarr G. Horgan<sup>a,e</sup>

<sup>a</sup> Teagasc, Oak Park Research Centre, Oak Park, Carlow, Co. Carlow, Ireland

<sup>b</sup> Martin-Luther University Halle-Wittenberg, Institute for Biology, Department of Zoology, D-06120 Halle (Saale), Germany

<sup>c</sup> University of Limerick, Department of Life Sciences, Limerick, Ireland

<sup>d</sup> Teagasc, Advisory Office, Johnstown Castle Estate, Co. Wexford, Ireland

<sup>e</sup> International Rice Research Institute, Crop and Environmental Sciences Division, DAPO Box 7777, Metro Manila, Philippines

## ARTICLE INFO

### Article history:

Received 2 April 2012

Received in revised form 21 August 2012

Accepted 22 October 2012

Available online 21 January 2013

### Keywords:

Apicystis

Bombus

Crithidia

Locustacarus

Nosema

Parasite

## 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 *Crithidia* 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.

© 2012 Elsevier Ltd. All rights reserved.

## 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 (Winfrey et al., 2009), but the role of disease in

\* Corresponding author at: Martin-Luther University Halle-Wittenberg, Institute for Biology, Department of Zoology, Hoher Weg 8, D-06120 Halle (Saale), Germany. Tel.: +49 345 5526502; fax: +49 345 5527428.

E-mail address: [tomas.murray@zoologie.uni-halle.de](mailto:tomas.murray@zoologie.uni-halle.de) (T.E. Murray).

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

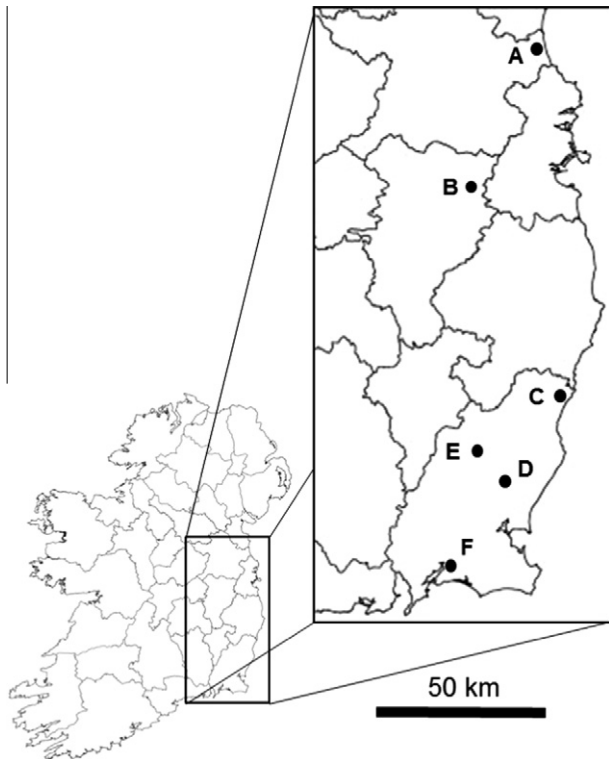
impose significant morbidity on laboratory populations of *Bombus*. *Crithidia bombi* and the recently identified *C. expoeki* (Schmid-Hempel and Tognazzo, 2010) infect multiple *Bombus* species in Europe and North America and are known to reduce lifetime reproductive output of *B. terrestris* queens 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.



**Fig. 1.** Map of eastern Ireland indicating the six sites sampled for pathogens of *B. terrestris* during the summer of 2008.

## 2.2. Pollen sampling and identification

To determine the permeability of strawberry cropping systems, pollen samples were collected from workers returning to commercial hives within each cropping system across sites. Within each cropping system, 10 pollen samples were collected per day over three days, resulting in a total of 540 pollen samples. Workers returning to commercial hives were caught with a sweep net, the pollen load removed from both corbicula, then placed in a 1.5 ml Eppendorf tube and stored at  $-20^{\circ}\text{C}$  within 8 h. Samples were then homogenized in 200  $\mu\text{l}$  distilled water by vortexing for 30 s and 100  $\mu\text{l}$  of the homogenate smeared on a glass microscope slide, dried at  $25^{\circ}\text{C}$  for 4 h, mounted on fuchsin gel and examined using light microscopy at  $400\times$  magnification. 250 pollen grains were counted, identified to species where possible and verified against reference slides prepared from known local flora (Sawyer, 1981).

## 2.3. Bumble bee sampling and identification

At each site, a minimum of 50 *Bombus* s.s. were collected at four radial distances from the site centroid: 250 m, 500 m, 1 km, 2 km; all within the estimated flights distances of worker (1.5–4.0 km; Osborne et al., 2008) and male (2.6–9.9 km; Kraus et al., 2009) *B. terrestris*. Additionally, we sampled bumble bees within a 500 m radius at a paired ‘control’ site located 10 km away in a random direction from each strawberry site; a distance beyond the normal bumble bee flight distance from the commercial site, but representing a comparable ecology and bumble bee community in the absence of commercial strawberry production and bumble bee importation. All sites, inclusive of the control sites, were a minimum of 15 km apart and each site was considered an independent sample. To minimize bias associated with seasonal changes in pathogen prevalence, all radial distances per site and control site were sampled within a 2-week period. Cryptic species of the

subgenus *Bombus* s.s. were identified using polymerase chain reaction restriction length polymorphism (PCR-RFLP) as in Murray et al. (2008). Individuals positively identified as *B. terrestris* were then dissected and examined for the presence of four common bumble bee pathogens: *A. bombi*, *Crithidia*, *N. bombi*, and *L. buchneri* (MacFarlane et al., 1995).

To assess the pathogen prevalence in commercially imported *B. terrestris* hives, five workers were randomly sampled from each of 68 commercial hives imported by the growers involved in this study. Bees were sampled directly from hives supplied by Koppert B.V. (Berkel en Rodenrijs, The Netherlands) and Syngenta Bioline Bees B.V. (Weert, The Netherlands) upon arrival at each strawberry site and before they were opened and exposed to native pathogens. All field-caught and commercial bees were immediately stored in individual microcentrifuge tubes at  $4^{\circ}\text{C}$  and then transferred to a  $-20^{\circ}\text{C}$  freezer within 8 h for later genetic identification and dissection.

## 2.4. *B. terrestris* pathogen screening

The abdomen of each bee was dissected and a sample of the fat bodies coating the inner abdominal wall (which have the highest concentration of *A. bombi*, and *N. bombi* in heavily infected bees), the Malpighian tubules associated with the mid-gut (primary site of *N. bombi* infection), and the contents of the hind-gut (highest concentration of *Crithidia*) were each crushed separately on a clean glass slide in a drop of distilled water. 20 visual fields of these slides were then examined at a magnification of  $400\times$  for the protozoa *A. bombi*, *Crithidia* and *N. bombi*. Finally, the air sacs of each bee were examined for the presence of *L. buchneri* under a dissecting microscope using  $20\times$  magnification. Bees were scored as infected or uninfected for each of the four pathogens. Prevalence, rather than intensity of infection, was recorded due to multiple confounding factors determining individual infection intensities and prevalence at the colony or population-level (e.g. Baer and Schmid-Hempel, 2003; Rutrecht and Brown, 2009). Bee size was quantified by measuring the intertegular span using a digital calipers and bee age estimated by qualifying the extent of wing wear using a four-point scale (modified from Michener (1974)).

## 2.5. Statistical analyses

Nonparametric Kruskal–Wallis and pairwise Mann–Whitney tests were conducted on the pollen data collected across cropping systems at each site. To determine the likelihood of pathogen spillover, conservative site-level analyses were first implemented using each site as a replicate and randomization tests to assess differences in the prevalence of infection across sites, distances, ages and sexes. Spatial autocorrelation of disease prevalence was assessed by Mantel tests of geographic distance versus the proportion of individuals infected across sites. All randomization tests and post-hoc Tukey’s honest significant difference (HSD) tests were two-tailed and significance evaluated via 5000 randomizations without replacement as implemented in Poptools (Hood, 2010). Randomizations were conducted within, rather than across, sites to retain the observed level of inter-site variance. Associations between the proportion of bees infected, size and sampling date were assessed using Spearman’s rank correlation coefficients in R v2.13.0 (R Core Team, 2011).

Individual-based analyses using binomial generalized linear mixed models in the R package lme4 v0.999375-39 (Bates et al., 2010) were used to investigate the determinants of parasite presence–absence. For each parasite, distance, date, host sex, size, age, and the presence–absence of accompanying parasites were included as fixed effects and site included as a random effect, and evaluation of diagnostic plots indicated that model assumptions

were not violated (Crawley, 2007). The analyses were conducted twice using different distance factors: (i) using a subset of the data whereby all bees collected within 500 m of greenhouses were compared to those collected within 500 m of control sites; and (ii) using data from all distances and log-transformed distance as continuous variable. For commercial bee data, company was included as a fixed effect and colony as a random effect. All two-way interactions were investigated and models simplified according to Akaike Information Criterion. Post-hoc tests were conducted using Tukey's HSD test in the multcomp package v1.2-6 (Hothorn et al., 2008).

### 3. Results

#### 3.1. Permeability of strawberry cropping systems

Overall, 63.7% of the pollen collected by returning foragers ( $n = 540$ ) to commercial hives was not strawberry pollen (Table S1). We observed marked differences in the proportion of strawberry pollen collected ( $H = 11.825$ ,  $df = 2$ ,  $p = 0.003$ ) and the number of non-crop taxa represented in pollen loads ( $H = 10.784$ ,  $df = 2$ ,  $p = 0.005$ ) across cropping systems (Fig. 2). The proportion of strawberry pollen returning to commercial hives in greenhouses was markedly higher (median = 0.695, range = 0.374–1.000), than

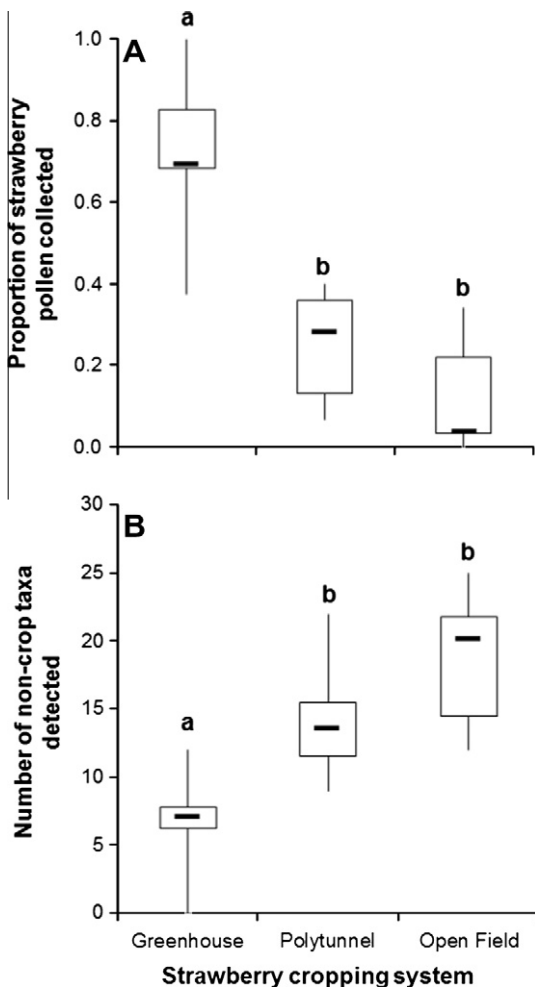


Fig. 2. Boxplot of the proportion of strawberry pollen and number of non-crop taxa represented in pollen loads collected by workers returning to commercial hives in each cropping system ( $n = 540$ ). Letters above bars indicate statistically homogeneous groups using pairwise Mann–Whitney U tests.

in polytunnel (0.281, 0.068–0.399,  $U = 2.000$ ,  $p = 0.010$ ) or open field systems (0.034, 0.000–0.340,  $U < 0.001$ ,  $p = 0.004$ ), with similar proportions collected in both between polytunnel and open field systems ( $U = 7.000$ ,  $p = 0.078$ ). In parallel, fewer non-crop plant taxa were observed in pollen loads returning to hives in greenhouses (7.0, 0.0–12.0), compared to polytunnels (13.5, 9.0–22.0,  $U = 2.000$ ,  $p = 0.010$ ) or open field systems (20.0, 12.0–25.0,  $U = 0.500$ ,  $p = 0.005$ ), with no statistical difference between polytunnel or open field systems ( $U = 10.000$ ,  $p = 0.199$ ).

#### 3.2. Pathogen prevalence in commercial hives

We screened 340 workers from 68 commercial hives (39 Koppert; 29 Syngenta) of *B. terrestris*. The tracheal mite *L. buchneri* and larvae of a conopid fly *Physocephala* sp. were not detected in commercial hives (Fig. 3). There was no statistical difference in the frequency of *Crithidia* (Koppert, 36.8%; Syngenta, 34.5%;  $z = -2.431$ ,  $df = 1$ ,  $p = 0.323$ ) or *N. bombi* (Koppert, 56.4%; Syngenta, 65.5%;  $z = 0.548$ ,  $df = 1$ ,  $p = 0.583$ ) infections between commercial suppliers. Overall, 73.5% of hives were infected; a single *A. bombi* infection was recorded (1.5% of hives screened), compared to 35.3% *Crithidia* spp. and 61.8% *N. bombi*, with 25.0% of hives being co-infected with both *Crithidia* spp. and *N. bombi* (Fig. 3).

#### 3.3. Trends across sites in pathogen prevalence

We collected and screened 847 bumble bees (205 males; 642 workers) of *B. terrestris*. Overall, five species of parasite were detected, of which two, the tracheal mite *L. buchneri* and larvae of a conopid fly *Physocephala* sp., were detected at very low prevalence (*L. buchneri*, 4 sites, 7 infected bees; *Physocephala* sp., 6 sites, 14 infected bees). Therefore, no further analyses were conducted for these parasites. There was no spatial autocorrelation across sites in the proportion of *B. terrestris* infected with *A. bombi* ( $r = -0.113$ ,  $p = 0.596$ ), *Crithidia* ( $r = 0.339$ ,  $p = 0.198$ ) or *N. bombi* ( $r = 0.289$ ,  $p = 0.133$ ) and the frequency distribution of bee age ( $G = 16.919$ ,  $df = 15$ ,  $p = 0.324$ ) or size (Kruskal–Wallis  $\chi^2 = 5.743$ ,  $df = 5$ ,  $p = 0.332$ ) did not differ across sites. For each pathogen, there was no correlation between the proportions of bees infected and sampling date or bee size (Table 1).

#### 3.4. Trends across individuals in probability of infection

Controlling for variability across sites, the presence–absence of parasites infecting individual bees was used to investigate factors

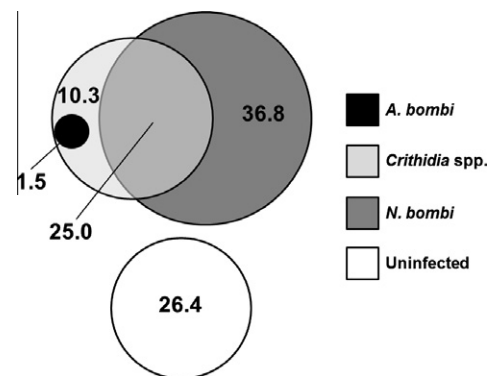


Fig. 3. Diagram illustrating the percentage of uninfected and infected commercial hives ( $n = 68$ ), and the percentage of single and multiple infections with *A. bombi*, *Crithidia* spp. and *N. bombi*. No hive with all three pathogens was observed in this study. *A. bombi* was recorded in a single hive as a multiple infection with *Crithidia* spp.

**Table 1**

Summary of randomization tests and Spearman's rank correlations conducted on the prevalence of *A. bombi*, *Crithidia* spp. and *N. bombi* in field-caught populations of *B. terrestris*. Randomization test two-tailed statistical significance was evaluated using 5000 randomizations without replacement. Significant results are highlighted in bold.

Variable	n	<i>A. bombi</i>			<i>Crithidia</i> spp.			<i>N. bombi</i>		
		Test statistic	df	p	Test statistic	df	p	Test statistic	df	p
<i>Randomization test</i>										
Distance	6	F = 1.905	4, 29	0.145	F = 3.594	4, 29	<b>0.037</b>	F = 0.197	4, 29	0.920
Sex	6	D <sup>a</sup> = 0.010	–	0.634	D = 0.266	–	<b>0.016</b>	D = 0.047	–	0.195
Age	6	F = 0.065	3, 23	0.979	F = 4.603	3, 23	<b>0.033</b>	F = 0.457	3, 23	0.565
<i>Spearman's rank correlation</i>										
Date	12	$\rho = -0.272$	10	0.389	$\rho = -0.371$	10	0.236	$\rho = -0.147$	10	0.648
Size	209	$\rho = 0.040$	207	0.562	$\rho = -0.039$	207	0.589	$\rho = -0.125$	207	0.072

<sup>a</sup> D, difference in mean proportion of infected bees between sexes (males – workers).

**Table 2**

Output of the binomial generalized linear mixed model for the probability of *Crithidia* infection in *B. terrestris*. df = 1 for all variables and significant results are highlighted in bold.

Variable	Worker (n = 642)				Male (n = 205)			
	CE <sup>a</sup>	SE	z	p	CE	SE	z	p
Distance	–0.498	0.227	–2.194	<b>0.028</b>	0.240	0.338	0.711	0.477
Age	–0.074	0.126	–0.584	0.560	0.189	0.170	1.113	0.266
Date	–0.010	0.004	–1.904	0.057	0.013	0.006	1.826	0.068
Size	0.125	0.168	0.745	0.456	–0.359	0.476	–0.754	0.451
<i>A. bombi</i>	–0.276	0.401	–0.688	0.491	–1.978	0.779	–2.540	<b>0.011</b>
<i>N. bombi</i>	–0.228	0.329	–0.694	0.488	–0.431	0.375	–1.149	0.251

<sup>a</sup> Coefficient estimate.

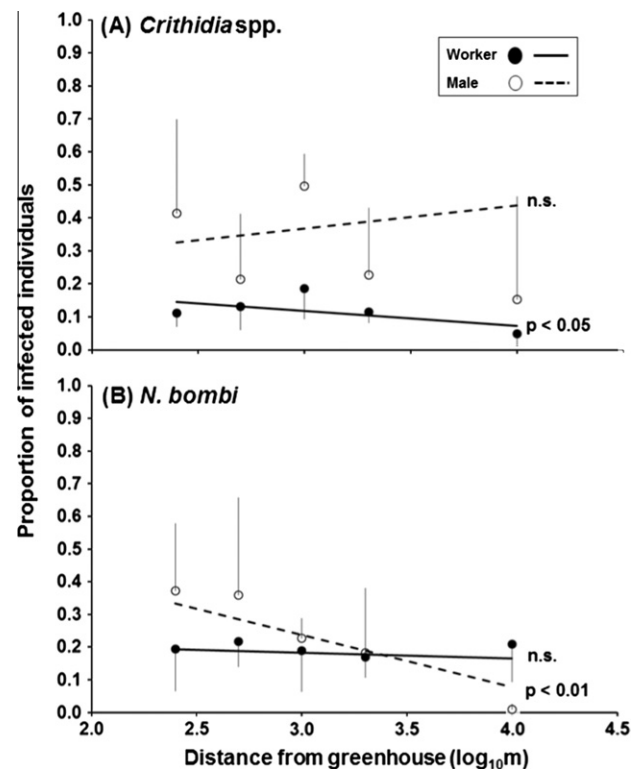
affected the probability of infection. The GLMM analyses using distance as either a categorical (prevalence within 500 m of greenhouses vs. control sites) or continuous variable were qualitatively identical (Tables S2–S5, Fig. S1), therefore only the latter analyses is presented below.

There was a sex-specific interaction with distance in regards to the probability of infection with *Crithidia* ( $z = -1.063$ ,  $p = 0.038$ ) and *N. bombi* ( $z = 2.376$ ,  $p = 0.018$ ; Table S6). Analyzing the sexes separately, the correlation between infection with *Crithidia* and distance remained significant for workers ( $z = -2.194$ ,  $p = 0.028$ ) but not for males ( $z = 0.240$ ,  $p = 0.477$ ; Table 2); the probability of infection in workers decreasing with distance with  $p_{250m} = 0.149$ , compared to  $p_{10km} = 0.076$  (Fig. 4A). Males had higher probabilities of *Crithidia* infection (Fig. 4A), but contrary to workers, the probability of infection was not associated with distance ( $z = 0.240$ ,  $p = 0.477$ ), but declined in the presence of *A. bombi* co-infection ( $z = -2.540$ ,  $p = 0.011$ ; Table 2).

The probability of infection with *N. bombi* decreased with distance from greenhouses in both sexes, but after separate analyses remained significant for males ( $z = -2.748$ ,  $p = 0.006$ , Table 3;  $p_{250m} = 0.352$ ,  $p_{10km} = 0.092$ , Fig. 4B) and not for workers ( $z = -0.832$ ,  $p = 0.405$ ; Table 3). The negative correlation between the probability of *A. bombi* infection and distance was not significant ( $z = -0.957$ ,  $p = 0.339$ ; Table S6). However, individuals infected with *A. bombi* had a lower probability of co-infection with either *Crithidia* ( $z = -1.964$ ,  $p = 0.050$ ) or *N. bombi* ( $z = -1.984$ ,  $p = 0.047$ ; Table S6). In contrast, the probabilities of co-infection with *Crithidia* (workers,  $z = -0.694$ ,  $p = 0.488$ ; males,  $z = -1.149$ ,  $p = 0.251$ ; Table 2) and *N. bombi* (workers,  $z = -0.781$ ,  $p = 0.435$ ; males,  $z = -1.150$ ,  $p = 0.250$ ; Table 3) were independent.

#### 4. Discussion

Numerous parasite species are present in commercial bumble bee hives resulting in elevated pathogen loads in adjacent conspecific populations. We found that the intestinal pathogens *N. bombi* and *Crithidia* infected 60% and 35%, respectively, of commercially-



**Fig. 4.** Sex-specific differences in the proportion of infected individuals (workers,  $n = 642$ ; males  $n = 205$ ) with distance from greenhouses for (A) *Crithidia* and (B) *N. bombi*. Circles indicate observed values  $\pm 95\%$  C.I. and regression lines are from the binomial generalized linear mixed models (Tables S1 and S2) with associated statistical significance (n.s., not significant).

reared *B. terrestris* hives imported into Ireland, that these parasites can be found at elevated levels in conspecifics up to 2 km from greenhouses and that the dynamics of pathogen spillover are

**Table 3**  
Output of the binomial generalized linear mixed model for the probability of *N. bombi* infection in *B. terrestris*. df = 1 for all variables and significant results are highlighted in bold.

Variable	Worker (n = 642)				Male (n = 205)			
	CE <sup>a</sup>	SE	z	p	CE	SE	z	p
Distance	-0.146	0.175	-0.832	0.405	-1.127	0.410	-2.748	<b>0.006</b>
Age	-0.024	0.102	-0.232	0.816	-0.016	0.184	-0.085	0.932
Date	-0.006	0.004	-1.529	0.126	0.009	0.006	1.472	0.141
Size	-0.068	0.139	-0.491	0.623	0.835	0.514	1.625	0.104
<i>A. bombi</i>	-0.524	0.362	-1.450	0.147	-0.863	0.675	-1.278	0.201
<i>Crithidia</i>	-0.259	0.331	-0.781	0.435	-0.428	0.372	-1.150	0.250

<sup>a</sup> Coefficient estimate.

sex-specific. Furthermore, this is the first study to detect the neogregarine *A. bombi* in commercial bumble bee hives, a pathogen associated with serious physical and behavioral effects in bumble bees. However, our data is in agreement with Goka et al. (2006) whereby the tracheal mite *Locustacarus buchneri* appears to have been eliminated from commercial colonies of *B. terrestris*. Clearly, despite the continued efforts of commercial producers to maintain parasite-free bombicultural facilities, the high density of hosts in commercial settings and the high permeability of cropping systems (Fig. 2) provides ideal conditions for the proliferation and transmission of pathogens and parasites (Goka et al., 2000, 2006; Whittington et al., 2004; Colla et al., 2006; Otterstatter and Thomson, 2008).

#### 4.1. Pathogen spillover in *Bombus*

Pathogen spillover typically occurs when infectious agents are transmitted from a heavily infected reservoir population to a sympatric wild population, and is the major driver of emerging infectious diseases in vertebrates (Daszak et al., 2000). Across six sites in eastern Ireland and sampling at five distances from greenhouses, we found that the prevalence of *Crithidia* within adjacent populations of *B. terrestris* was highest at 11–18% within 2 km of greenhouses compared to 5% at 10 km. We also observed a decline in the probability of *Crithidia* infection in workers (25–11%) and of *N. bombi* infection in males (49–12%) from 250 m to 10 km from greenhouses that used commercial hives (Fig. 4). These data support previous preliminary Canadian surveys of bumble bee parasites whereby 15–27% and 15% of bees within 500 m of two greenhouse sites containing commercial bumble bees (*B. impatiens*) were infected with *C. bombi* and *N. bombi*, respectively, in contrast to 0% and <4% detectable infection at four sites without commercial hives (Colla et al., 2006). Similarly, in a follow-up study by Otterstatter and Thomson (2008), *C. bombi* infections from commercial to Canadian wild bumble bees agreed with predictions for spillover from spatially explicit models where prevalence was greatest (10–30%) within 500 m and declined to below 5% between 1.5 and 4.5 km from greenhouses. Given the disparity between the Irish (*B. terrestris*-strawberry production) and Canadian (*B. impatiens*-tomato/bell pepper production) systems, the congruence in results strongly supports the generality of increased infection rate in wild bumble bees due to pathogen spillover near agricultural areas that use commercial bumble bees.

The threat posed by pathogen spillover to wild bumble bees is twofold: (i) the introduction of novel or commercially adapted strains of parasites; (ii) commercial species may act as reservoirs of parasites leading to increased prevalences in sympatric wild populations (Stout and Morales, 2009; Meeus et al., 2011). However, there is a serious lack of empirical data regarding the epidemiology of most pathogens associated with bumble bees, particularly wild populations (Salathé and Schmid-Hempel, 2011; Ruiz-González et al., 2012). We found the spread of disease up to 10 km from greenhouses importing commercial colonies of

*B. terrestris* to be highly sex- and parasite-specific, where a decrease in *Crithidia* infection was detected in workers with increasing distance from greenhouses, but not in males, and vice versa for *N. bombi* infection. As horizontal transmission is facilitated by commercial bees foraging outside of greenhouses (Fig. 2) and both males and workers are produced in abundance by commercial *B. terrestris* hives in agricultural habitats (Goulson et al., 2002), our data may reflect the interaction between sex-specific flight-range and pathogen-specific modes of transmission. Workers of *B. terrestris* do not forage in the immediate vicinity of their nest but, depending on the landscape structure, typically forage between 0.2 km and 1.5 km (Osborne et al., 2008; Wolf and Moritz, 2008). In contrast, males typically fly between 2.6 and 9.9 km and, therefore, may be important long-range vectors of disease (Kraus et al., 2009). With regards to the parasites, *C. bombi* transmission is thought to be rapid and extensive as new hosts are quickly infected through the faeces in the nest (Otterstatter and Thomson, 2008) and through shared use of flowers by foragers (Durrer and Schmid-Hempel, 1995). In comparison, *N. bombi* is believed to spread slowly through novel populations as transmission primarily occurs via contaminated pollen or nectar fed to the larvae (Rutrecht et al., 2007) with subsequent inter-colony infections through drift of infected adults into non-natal colonies. Therefore, relative to workers, the long flight range of males and rapid transmission of *Crithidia* may have obscured any spatial trend in this sex over the distance sampled in this study. However, this does not explain how the relatively slow rate of transmission of *N. bombi* may have contributed to the weak decline in infection observed in near-flying workers compared to the marked decline in far-flying males. An alternative explanation may be that workers infected with *N. bombi* are less active and less likely to leave the nest (Shykoff and Schmid-Hempel, 1991), whereas males permanently leave the nest within a few days of eclosion (Goulson, 2003), thus infected workers are under-represented in field-caught samples and spatial trends in *N. bombi* infection are detected solely in males.

#### 4.2. Within-host multiparasitic interactions

In natural populations, host-parasite interactions typically involve multi-parasitized hosts and multi-host parasites (reviewed in Rigaud et al., 2010). Numerous theoretical and empirical studies of multiparasitic interactions within a host indicate that the two most likely outcomes are: exclusion by the most effective competitor or ongoing competition between parasites. For *A. bombi*, our data suggests the latter, where the probability of infection with *A. bombi* was 0.11, but in the presence of either *Crithidia* or *N. bombi* co-infection, this dropped to 0.06 for both parasites. Currently, little is known about its mode of transmission or epidemiology, but queens of *B. pratorum* rapidly die if infected with *A. bombi* in the spring (Rutrecht and Brown, 2008). Additionally, *A. bombi* did not overlap with other parasitic species in *B. pratorum* queens, but co-infections with the tracheal mite *L. buchneri* were

observed in workers (2.2%, Rutrecht and Brown, 2008). Together, this suggests that *Crithidia* and *N. bombi* may competitively exclude *A. bombi*, which may subsequently benefit host populations by depressing the prevalence of this highly virulent parasite, particularly given the high mortality rate of queens at a critical time in the hosts' lifecycle. Given our confirmation of the presence of *A. bombi* in commercial European colonies of *B. terrestris* and its recent discovery in honeybees and invasive *B. terrestris* in North Patagonia (Plischuk et al., 2011), the complex dynamics of this parasite should be a priority for future research.

#### 4.3. Study limitations

As this study was conducted within the native range of *B. terrestris*, commercial workers and males are indistinguishable in the field from native *B. terrestris audax*. Therefore, the observed pattern of infection within 2 km of greenhouses may be a product of the majority of bees collected being infected commercial *B. terrestris*, rather than pathogen spillover *per se*. However, although it not possible to directly observe pathogen spillover in the field, we do not believe this was the case. Firstly, unlike a previous study where the commercial and native bee were also indistinguishable in the field (Colla et al., 2006; Otterstatter and Thomson, 2008), we found no evidence of higher abundances of *B. terrestris* closer to greenhouses. With equal sampling effort, no statistical difference was found in the number of bees collected within 250 m of greenhouses versus 10 km (T.E.M., unpubl. data). Secondly, native *B. terrestris audax* queens are morphologically distinct from commercial *B. terrestris*, of which we collected 23 of the former and 11 of the latter near to greenhouses during the course of this study. Finally, the prevalence of *A. bombi* in commercial workers (0.3%) was quite different to the field-caught sample (12.3%; Fig. S2). Although the number and identity of colonies represented in the field-caught sample is currently unknown, we believe that the pattern of infection observed was not strongly biased by oversampling infected commercial bees within 2 km of greenhouses. At minimum, our data confirm the high permeability of strawberry cropping systems to commercial bees, the elevated level of pathogens in commercial hives and the presence of infected 'reservoir' populations adjacent to greenhouses with a high potential for spillover to wild bees.

#### 4.4. Recommendations for commercial bumble bee management and policy

For many agricultural systems the benefits of commercial bumble bee crop pollination are clear (Velthuis and van Doorn, 2006), but along with these quantifiable benefits should be an equally intensive appraisal of the risks associated with commercial bumble bee importation. Many countries are signatories to international phyto- and zoo-sanitary agreements that are meant to prevent the spread of pests and parasites, but in almost all cases, health certification is based on parasites of direct threat to honey bees (*A. mellifera*), not bumble bees. Some countries (e.g. Canada, China, Israel, Mexico, Turkey, and the United States) have prohibited the importation of non-native bumble bees in favor of native congeners or conspecifics, but this still does not resolve the issue of elevated levels of parasites in commercial production facilities. Clearly, as in the case of the tracheal mite *L. buchneri*, bumble bee producers are committed to eliminating parasites in their commercial populations. However, even with the recent development of molecular tools (Meeus et al., 2010a, 2010b), extensive screening of commercial populations and queens harvested from the wild may prove too costly and treatments for most pathogens are currently unavailable (Meeus et al., 2011).

Analogous to the use of agri-chemicals, increasing the awareness of end-users and local enforcement of best management

practices may ultimately be the most cost-effective way of mitigating the risks associated with commercial bumble bees. Given the high proportion of non-crop pollen collected by commercial bumblebees in all cropping systems recorded in this study (median of 30.5% greenhouses, 71.9% polytunnels, 96.6% open field; Fig. 2), interventions preventing the interaction of commercial and wild populations, such as the successfully established method of netting protected crop systems (greenhouses and plastic tunnels; Goka, 2010), negate the dangers of pathogen spillover and introgression (Kraus et al., 2011). However, commercial bumble bees are extensively used in open crop systems, despite a significant lack of data regarding the efficacy of commercial bumble bees in these systems (Lye et al., 2011). Furthermore, a posteriori, we found no relationship between the area of each cropping system per site and pathogen prevalence (Table S7), primarily due to the dominance of highly permeable open field systems found on all sites. Therefore, in combination with improved management practices, further legislative action is necessary to: (i) improve the health certification of commercial bumble bees before export or distribution; (ii) integrate with existing quarantine and inspection services upon importation; (iii) include environmental risk assessment and proof of pollination limitation as preconditions to importation. In combination with ongoing efforts from scientists, conservation bodies and commercial companies, encouraging the responsible use of commercial bumble bees could minimize or even eradicate pathogen spillover.

#### Acknowledgements

The authors wish to thank P. Martin and M. Nuñez Lopez for assistance in the field, A. Brennan and C. Bryne for help in the laboratory, and the members of the Irish Soft Fruit Growers Association for access to their facilities. We also thank Falk Huettmann and two anonymous referees for many helpful comments on the manuscript. This project was funded through a Research Stimulus Grant (06 348) from the Irish Department of Agriculture, Fisheries and Food.

#### Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.biocon.2012.10.021>.

#### References

- Aizen, M.A., Harder, L.D., 2009. The global stock of domesticated honey bees is growing slower than agricultural demand for pollination. *Curr. Biol.* 19, 915–918.
- Baer, B., Schmid-Hempel, P., 2003. Bumblebee workers from different sire groups vary in susceptibility to parasite infection. *Ecol. Lett.* 6, 106–110.
- Bates, D., Maechler, M., Bolker, B., 2010. lme4: linear mixed-effects models using Eigen and R Package Version 0.999375-39.
- Biesmeijer, J.C., Roberts, S.P.M., Reemer, M., Ohlemüller, R., Edwards, M., Peeters, T., Schaffers, A.P., Potts, S.G., Kleukers, R., Thomas, C.D., Settele, J., Kunin, W.E., 2006. Parallel declines in pollinators and insect-pollinated plants in Britain and The Netherlands. *Science* 313, 351–354.
- Brown, M.J.F., Loosli, R., Schmid-Hempel, P., 2000. Condition-dependent expression of virulence in a trypanosome infecting bumblebees. *Oikos* 91, 421–427.
- Brown, M.J.F., Schmid-Hempel, R., Schmid-Hempel, P., 2003. Strong context-dependent virulence in a host-parasite system: reconciling genetic evidence with theory. *J. Anim. Ecol.* 72, 994–1002.
- Cameron, S.A., Lozier, J.D., Strange, J.P., Koch, J.B., Cordes, N., Solter, L.F., Griswold, T.L., 2011. Patterns of widespread decline in North American bumble bees. *Proc. Nat. Acad. Sci.* 108, 662–667.
- Colla, S.R., Otterstatter, M.C., Gegeer, R.J., Thomson, J.D., 2006. Plight of the bumble bee: pathogen spillover from commercial to wild populations. *Biol. Conserv.* 129, 461–467.
- Crawley, M.J., 2007. *The R Book*. John Wiley & Sons, Chichester, UK.
- Daszak, P., Cunningham, A.A., Hyatt, A.D., 2000. Emerging infectious diseases of wildlife – threats to biodiversity and human health. *Science* 287, 443–449.



- Dunn, R.R., 2005. Modern insect extinctions, the neglected majority. *Conserv. Biol.* 19, 1030–1036.
- Durrer, S., Schmid-Hempel, P., 1995. Parasites and the regional distribution of bumblebee species. *Ecography* 18, 114–122.
- Goka, K., 2010. Introduction to the special feature for ecological risk assessment of introduced bumblebees: status of the European bumblebee, *Bombus terrestris*, in Japan as a beneficial pollinator and an invasive alien species. *Appl. Entomol. Zool.* 45, 1–6.
- Goka, K., Okabe, K., Niwa, S., Yoneda, M., 2000. Parasitic mite infestation in introduced colonies of European bumblebees, *Bombus terrestris*. *Jpn. J. Appl. Entomol. Zool.* 44, 47–50.
- Goka, K., Okabe, K., Yoneda, M., 2006. Worldwide migration of parasitic mites as a result of bumblebee commercialization. *Popul. Ecol.* 48, 285–291.
- Goka, K., Okabe, K., Yoneda, M., Niwa, S., 2001. Bumblebee commercialization will cause worldwide migration of parasitic mites. *Mol. Ecol.* 10, 2095–2099.
- Goulson, D., 2003. *Bumblebees: Their Behaviour and Ecology*. Oxford University Press, Oxford, UK.
- Goulson, D., Hughes, W.O.H., Derwent, L.C., Stout, J.C., 2002. Colony growth of the bumblebee, *Bombus terrestris*, in improved and conventional agricultural and suburban habitats. *Oecologia* 130, 267–273.
- Hood, G.M., 2010. Poptools Version 3.2.3. <<http://www.poptools.org/>>.
- Hothorn, T., Bretz, F., Westfall, P., Heiberger, R.M., 2008. Multcomp: simultaneous inference in general parametric models. R Package Version 1.0-0.
- Husband, R.W., Shina, R.N., 1970. A review of the genus *Locustacarus* with a key to the genera of the family Podapolipidae (Acarina). *Ann. Entomol. Soc. Am.* 63, 1152–1162.
- Imhoof, B., Schmid-Hempel, P., 1998. Patterns of local adaptation of a protozoan parasite to its bumblebee host. *Oikos* 82, 59–65.
- Inari, N., Nagamitsu, T., Kenta, T., Goka, K., Hiura, T., 2005. Spatial and temporal pattern of introduced *Bombus terrestris* abundance in Hokkaido, Japan, and its potential impact on native bumblebees. *Popul. Ecol.* 47, 77–82.
- Ings, T.C., 2007. *Bombus terrestris*, humble pollinator or assiduous invader? PhD, University of London.
- Ings, T.C., Ings, N.L., Chittka, L., Rasmont, P., 2010. A failed invasion? Commercially introduced pollinators in Southern France. *Apidologie* 41, 1–13.
- Inoue, M., Yokoyama, J., Washitani, I., 2008. Displacement of Japanese native bumblebees by the recently introduced *Bombus terrestris* (L.) (Hymenoptera: Apidae). *J. Insect Conserv.* <http://dx.doi.org/10.1007/s10841-007-9071-z>.
- Jones, K.E., Patel, N.G., Levy, M.A., Storeygard, A., Balk, D., Gittleman, J.L., Daszak, P., 2008. Global trends in emerging infectious diseases. *Nature* 451, 990–993.
- Kanbe, Y., Okada, I., Yoneda, M., Goka, K., Tsuchida, K., 2008. Interspecific mating of the introduced bumblebee *Bombus terrestris* and the native Japanese bumblebee *Bombus hypocrita sapporoensis* results in inviable hybrids. *Naturwissenschaften* 95, 1003–1008.
- Kenta, T., Inari, N., Nagamitsu, T., Goka, K., Hiura, T., 2007. Commercialized European bumblebee can cause pollination disturbance. An experiment on seven native plant species in Japan. *Biol. Conserv.* 134, 298–309.
- Klein, A.-M., Vaissière, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C., Tscharntke, T., 2007. Importance of pollinators in changing landscapes for world crops. *Proc. R. Soc. Biol. Sci. Ser. B* 274, 303–313.
- Kraus, F.B., Wolf, S., Moritz, R.F.A., 2009. Male flight distance and population substructure in the bumblebee *Bombus terrestris*. *J. Anim. Ecol.* 78, 247–252.
- Kraus, F., Szentgyörgyi, H., Rozej, E., Rhode, M., Morón, D., Woyciechowski, M., Moritz, R., 2011. Greenhouse bumblebees (*Bombus terrestris*) spread their genes into the wild. *Conserv. Genet.* 12, 187–192.
- Lye, G.C., Jennings, S.N., Osborne, J.L., Goulson, D., 2011. Impacts of the use of nonnative commercial bumble bees for pollinator supplementation in raspberry. *J. Econ. Entomol.* 104, 107–114.
- MacFarlane, R.P., Lipa, J.J., Liu, H.J., 1995. Bumble bee pathogens and internal enemies. *Bee World* 76, 130–148.
- Mack, R., 1970. The great African cattle plague epidemic of the 1890s. *Trop. Anim. Health Prod.* 2, 210–219.
- Meeus, I., De Graaf, D.C., Jans, K., Smagghe, G., 2010a. Multiplex PCR detection of slowly-evolving trypanosomatids and neogregarines in bumblebees using broad-range primers. *J. Appl. Microbiol.* 109, 107–115.
- Meeus, I., Smagghe, G., Siede, R., Jans, K., de Graaf, D.C., 2010b. Multiplex RT-PCR with broad-range primers and an exogenous internal amplification control for the detection of honeybee viruses in bumblebees. *J. Invertebr. Pathol.* 105, 200–203.
- Meeus, I., Brown, M.J.F., De Graaf, D.C., Smagghe, G., 2011. Effects of invasive parasites on bumble bee declines. *Conserv. Biol.* 25, 662–671.
- Michener, C.D., 1974. *The Social Behavior of the Bees*. Harvard University Press.
- Murray, T.E., Fitzpatrick, U., Brown, M.J.F., Paxton, R.J., 2008. Cryptic species diversity in a widespread bumble bee complex revealed using mitochondrial DNA RFLPs. *Conserv. Genet.* 9, 653–666.
- Neumann, P., Carreck, N.L., 2010. Honey bee colony losses. *J. Apic. Res.* 49, 1–6.
- Osborne, J.L., Martin, A.P., Carreck, N.L., Swain, J.L., Knight, M.E., Goulson, D., Hale, R.J., Sanderson, R.A., 2008. Bumblebee flight distances in relation to the forage landscape. *J. Anim. Ecol.* 77, 406–415.
- Otterstatter, M.C., Thomson, J.D., 2008. Does pathogen spillover from commercially reared bumble bees threaten wild pollinators? *PLoS ONE* 3, e2771.
- Otterstatter, M., Whidden, T.L., 2004. Patterns of parasitism by tracheal mites (*Locustacarus buchneri*) in natural bumble bee populations. *Apidologie* 35, 351–357.
- Otti, O., Schmid-Hempel, P., 2007. *Nosema bombi*: a pollinator parasite with detrimental fitness effects. *J. Invertebr. Pathol.* 96, 118–124.
- Otti, O., Schmid-Hempel, P., 2008. A field experiment on the effect of *Nosema bombi* in colonies of the bumblebee *Bombus terrestris*. *Ecol. Entomol.* 33, 577–582.
- Patiny, S., Rasmont, P., Michez, D., 2009. A survey and review of the status of wild bees in the West-Palaearctic region. *Apidologie* 40, 313–331.
- Plischuk, S., Martín-Hernández, R., Prieto, L., Lucía, M., Botías, C., Meana, A., Abrahamovich, A.H., Lange, C., Higes, M., 2009. South American native bumblebees (Hymenoptera: Apidae) infected by *Nosema ceranae* (Microsporidia), an emerging pathogen of honeybees (*Apis mellifera*). *Environ. Microbiol. Rep.* 1, 131–135.
- Plischuk, S., Meeus, I., Smagghe, G., Lange, C.E., 2011. *Apicystis bombi* (Apicomplexa: Neogregarinorida) parasitizing *Apis mellifera* and *Bombus terrestris* (Hymenoptera: Apidae) in Argentina. *Environ. Microbiol. Rep.* 3, 565–568.
- Potts, S.G., Biesmeijer, J.C., Kremen, C., Neumann, P., Schweiger, O., Kunin, W.E., 2010. Global pollinator declines: trends, impacts and drivers. *Trends Ecol. Evol.* 25, 345–353.
- R Core Team, 2011. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <<http://www.R-project.org/>>.
- Rigaud, T., Perrot-Minnot, M.-J., Brown, M.J.F., 2010. Parasite and host assemblages: embracing the reality will improve our knowledge of parasite transmission and virulence. *Proc. R. Soc. B: Biol. Sci.* 277, 3693–3702.
- Ruiz-González, M.X., Bryden, J., Moret, Y., Reber-Funk, C., Schmid-Hempel, P., Brown, M.J.F., 2012. Dynamic transmission, host quality, and population structure in a multihost parasite of bumblebees. *Evolution*. <http://dx.doi.org/10.1111/j.1558-5646.2012.01655.x>.
- Rutrecht, S.T., Brown, M.J.F., 2008. The life-history impact and implications of multiple parasites for bumble bee queens. *Int. J. Parasitol.* 38, 799–808.
- Rutrecht, S.T., Brown, M.J.F., 2009. Differential virulence in a multiple-host parasite of bumble bees: resolving the paradox of parasite survival? *Oikos* 118, 941–949.
- Rutrecht, S.T., Klee, J., Brown, M.J.F., 2007. Horizontal transmission success of *Nosema bombi* to its adult bumble bee hosts: effects of dosage, spore source and host age. *Parasitology* 134, 1719–1726.
- Ruz, L., 2002. Bee pollinators introduced into Chile: a review. In: Kevan, P., Imperatriz-Fonseca, V.L. (Eds.), *The Conservation Link between Agriculture and Nature*. Ministry of Environment, Brasília.
- Salathé, R.M., Schmid-Hempel, P., 2011. The genotypic structure of a multi-host bumblebee parasite suggests a role for ecological niche overlap. *PLoS ONE* 6, e22054.
- Samways, M.J., 2005. *Insect Diversity Conservation*. Cambridge University Press, Cambridge, UK.
- Sawyer, R., 1981. *Pollen Identification for Beekeepers*. University College Press, Cardiff.
- Schmid-Hempel, P., Loosli, R., 1998. A contribution to the knowledge of *Nosema* infections in bumble bees, *Bombus* spp. *Apidologie* 29, 525–535.
- Schmid-Hempel, R., Tognazzo, M., 2010. Molecular divergence defines two distinct lineages of *Crithidia bombi* (Trypanosomatidae), parasites of bumblebees. *J. Eukaryot. Microbiol.* 57, 337–345.
- Schmid-Hempel, P., Schmid-Hempel, R., Brunner, P.C., Seeman, O.D., Allen, G.R., 2007. Invasion success of the bumblebee, *Bombus terrestris*, despite a drastic genetic bottleneck. *Heredity* 99, 414–422.
- Shykoff, J.A., Schmid-Hempel, P., 1991. Incidence and effects of four parasites in natural populations of bumble bees in Switzerland. *Apidologie* 22, 117–125.
- Stout, J.C., Morales, C.L., 2009. Ecological impacts of invasive alien species on bees. *Apidologie* 40, 388–409.
- Szabo, N.D., Colla, S.R., Wagner, D.L., Gall, L.F., Kerr, J.T., 2012. Do pathogen spillover, pesticide use, or habitat loss explain recent North American bumblebee declines? *Conserv. Lett.* <http://dx.doi.org/10.1111/j.1755-263X.2012.00234.x>.
- Thorp, R.W., 2003. Bumble bees (Hymenoptera: Apidae): commercial use and environmental concerns. In: Strickler, K., Cane, J.H. (Eds.), *For Non-Native Crops, Whence Pollinators of the Future?* Thomas Say Publications in Entomology, Proceedings of the Entomological Society of America, Lanham, MD, pp. 21–40.
- Torretta, J.P., Medan, D., Abrahamovich, A., 2006. First record of the invasive bumblebee *Bombus terrestris* (L.) (Hymenoptera, Apidae) in Argentina. *Trans. Am. Entomol. Soc.* 131, 285–289.
- Velthuis, H.H.W., van Doorn, A., 2006. A century of advances in bumblebee domestication and the economic and environmental aspects of its commercialization for pollination. *Apidologie* 37, 421–451.
- Whittington, R., Winston, M.L., 2003. Effects of *Nosema bombi* and its treatment fumagillin on bumble bee (*Bombus occidentalis*) colonies. *J. Invertebr. Pathol.* 84, 54–58.
- Whittington, R., Winston, M.L., Tucker, C., Parachnowitsch, A.L., 2004. Plant-species identity of pollen collected by bumblebees placed in greenhouses for tomato pollination. *Can. J. Plant Sci.* 84, 599–602.
- Williams, P.H., Osborne, J.L., 2009. Bumblebee vulnerability and conservation world-wide. *Apidologie* 40, 367–387.
- Winfree, R., 2010. The conservation and restoration of wild bees. *Ann. N. Y. Acad. Sci.* 1195, 169–197.
- Winfree, R., Aguilar, R., Vázquez, D.P., LeBuhn, G., Aizen, M.A., 2009. A meta-analysis of bees' responses to anthropogenic disturbance. *Ecology* 90, 2068–2076.
- Wolf, S., Moritz, R.F.A., 2008. Foraging distance in *Bombus terrestris* L. (Hymenoptera: Apidae). *Apidologie* 39, 419–427.