

Factors affecting parasite prevalence among wild bumblebees

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Abstract. 1. Bumblebees are important pollinators in North America and are attacked by a range of parasites that impact their fitness; however, few studies have investigated the extent or causes of parasitism in North America.

2. This study used a 2-year multi-site survey of bumblebee parasitism to ask: (i) how common are parasitoid conopid flies and the internal parasites *Crithidia bombi* and *Nosema bombi* in Massachusetts; and (ii) what factors are correlated with parasitism?

3. Infection rates by all three parasites were higher in this study than previously documented in North America. Overall, conopids infected 0–73% of bees in each sample, *C. bombi* infected 0–82% of bees, and *N. bombi* infected 0–32%.

4. Conopid flies infected female bees more than males and intermediate-sized bees more than large or small bees. *Crithidia bombi* infection rates were higher in certain bee species and sites, and exhibited a unimodal pattern of prevalence over time. *Nosema bombi* parasitism was higher in male than female bees.

5. Infection by *N. bombi* in two rare bumblebee species was higher than expected based on parasitism rates of common bee species but *C. bombi* infection was lower.

If high prevalence of *N. bombi* in these bumblebee species is common, parasitism may be a potential cause of their decline.

6. Given the documented effects of these parasites, the high levels of infection may affect bee populations in Massachusetts and threaten the stability of their valuable ecosystem services.

Key words. *Bombus*, bumblebee, Conopidae, *Crithidia bombi*, *Nosema bombi*, parasitism.

Introduction

In the past decade there has been a worldwide decline in the abundance of many different pollinator taxa, including bees, butterflies, and hummingbirds (Allen-Wardell *et al.*, 1998; Kearns *et al.*, 1998; Biesmeijer *et al.*, 2006; Berenbaum *et al.*, 2007). Wild pollinators play a critical ecological role in most ecosystems, and are increasingly important to crops as pollination service by honeybees is threatened by colony collapse disorder (Biesmeijer *et al.*, 2006; Vanengelsdorp *et al.*, 2007; Winfree *et al.*, 2007). There are many hypotheses regarding the causes of pollinator decline, including loss of habitat and the effects of invasive species, but in many cases we lack basic information about what factors regulate wild pollinator populations or could cause species decline (Ghazoul,

2005). Given that wild pollinators are important conservation priorities and have increasing economic importance in agriculture, it is imperative to improve our understanding of factors that impact their populations.

Disease and parasites caused feral honeybee disappearance in the late 1900s, and have also been implicated in the recent dramatic losses of managed honeybee colonies due to colony collapse disorder (Southwick & Southwick, 1992; Allen-Wardell *et al.*, 1998; Cox-Foster *et al.*, 2007; Oldroyd, 2007). These examples from honey bees demonstrate the dramatic impacts that parasites and diseases of pollinators can have on pollinator populations, but we know much less about the extent of disease and parasitism in wild pollinator populations. In order to make informed decisions about pollinator management and conservation priorities, it is essential to document the extent of and variation in parasite loads, and to elucidate factors that may contribute to parasitism.

Bumblebees (*Bombus* spp., Hymenoptera: Apidae) pollinate a wide range of wild plants and agricultural crops in

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North America (Kearns & Thomson, 2001). There is mounting evidence that certain species are in decline, or have disappeared entirely from much of their original range (Colla & Packer, 2008). For instance, on the west coast of North America, commercial rearing of *Bombus occidentalis* collapsed due to an outbreak of *Nosema bombi* (Microsporidia: Nosematidae), possibly due to a foreign strain of this pathogen, and concurrently, wild populations of *B. occidentalis* suffered precipitous declines (Winter *et al.*, 2006). On the east coast, *Bombus affinis* was once a widespread, common bumblebee, but is now locally extinct throughout most of its former range, and several other species, such as *B. pensylvanicus* and *B. fervidus* have declined in abundance (Colla & Packer, 2008). Although there is no direct evidence that pathogens were involved in the declines of *B. occidentalis*, *B. affinis* and several other closely related species, there remains concern that exposure to a non-native pathogen might have played a role (Osborne *et al.*, 2008). Given these patterns, further data are needed on the impacts of parasites on this important pollinator clade.

Bumblebees are attacked by a range of parasites, all of which can reduce colony fitness. Several species of parasitoid conopid flies (Diptera: Conopidae) attack foraging bumblebees on the wing, and lay their eggs inside the bee's abdomen. The infectious protozoan *Crithidia bombi* (Zoomastigophora: Trypanosomatidae; *Crithidia* hereafter) is a gut parasite that can be contracted at flowers via faecal transmission (Durrer & Schmid-Hempel, 1994; Schmid-Hempel *et al.*, 1990). The microsporidium *Nosema bombi* (*Nosema* hereafter) is another parasite of bumblebees that is transmitted through faeces (Fisher, 1989). Conopids and *Crithidia* can affect colony reproduction and worker foraging behaviour (Schmid-Hempel & Durrer, 1991; Shykoff & Schmid-Hempel, 1991a; Gegear *et al.*, 2005, 2006). *Nosema* may reduce colony fitness and worker survival (Otti & Schmid-Hempel, 2007). Thus, there is evidence that these parasites could affect local abundance of bumblebee populations, and some, such as *Crithidia* and *Nosema*, have been implicated in the overall decline of bumblebees (Berenbaum *et al.*, 2007; Osborne *et al.*, 2008). However, few studies of bumblebee parasitism in North America have taken place in Canada (Macfarlane & Pengelly, 1974; Otterstatter *et al.*, 2002; Otterstatter, 2004; Colla *et al.*, 2006), and therefore, the extent of parasitism is unknown over a large part of bumblebees' range in North America.

There are many factors that could influence parasitism in bumblebee populations, including geographic and temporal variation in a number of environmental variables such as temperature, bee physiology, and bee behaviour. For example, in Europe the prevalence of *Crithidia* was between 50% and 80% in lowland sites, but only 10% in alpine sites (Shykoff & Schmid-Hempel, 1991b; Korner & Schmid-Hempel, 2005). Additionally, prevalence of *Crithidia*, *Nosema*, and conopid flies all increased over the course of the spring and summer both in Europe and Canada (Korner & Schmid-Hempel, 2005). In North America, high infection rates of *Crithidia* and *Nosema* in wild bumblebees have only been documented near greenhouse operations using cultivated *Bombus impatiens* for pollination (Colla *et al.*, 2006). These managed colonies often have high levels of infection by *Crithidia* and *Nosema* (Gegear

et al., 2005), thereby potentially increasing infection rates for wild bumblebee individuals foraging nearby. Other aspects of bee biology may also affect the likelihood of being attacked or infected. For example, conopid parasitism is higher in female hosts of intermediate size in Canada, and in larger hosts in Europe (Muller *et al.*, 1996; Otterstatter, 2004). Furthermore, there is evidence that members of the subgenus *Bombus sensu stricto* suffer higher levels of attack by tracheal mites (Otterstatter & Whidden, 2004). Aspects of bee physiology and behaviour that could increase risk of parasitism should be investigated in order to understand how parasites may impact different bumblebee species or populations.

Given the importance of pollination in plant reproduction in wild ecosystems and the increasing economic value of wild pollinators as honeybees decline, it is imperative to document parasite prevalence in wild bees and determine factors associated with increased risk of attack or infection. This 2-year study documented the prevalence of parasites in 13 populations of bumblebees in western Massachusetts, addressing two questions:

- 1 How commonly are bumblebees parasitised by conopid flies, *Crithidia bombi*, and *Nosema bombi*?
- 2 Is the probability of parasitism for an individual bee related to bee species, sex, body size, date of collection, and collection site?

Methods

Field samples

Bees were collected from old-field meadows throughout western Massachusetts during the summers of 2006 and 2007. Meadows were primarily conservation or wildlife management areas that were mowed once a year (Table 1). Typical of eastern North American old fields, the dominant floral resources for pollinators were milkweed (*Asclepias* sp., Asclepiaceae), goldenrod (*Solidago* spp., Asteraceae), and various members of the pea family, including clovers and vetches (*Trifolium* spp., *Vicia* sp., and others, Fabaceae).

During 2006 bees were collected from 10 old-field meadows (Table 1, Fig. 1). Each meadow was sampled once, collecting from 4 to 35 bees depending on abundance. Bees were collected with a sweep-net and placed in individual vials. Bees were collected, regardless of species, throughout the habitat from a variety of flowering plants, resulting in a representative sample of the bee species present. These methods are similar to those used in other studies and an effort was made to collect every bee seen to avoid biased sampling of species that are easier to catch (Otterstatter, 2004). Initially, bumblebee workers were primarily collected, in order to consistently sample one guild of bees, but there were not enough workers at all sites on all dates for adequate sampling. At 8 out of 10 meadows, when sufficient workers could not be collected, males were collected as well (Table 1).

During 2007 bees were again collected in 13 old-field meadows (Table 1, Fig. 1), encompassing a greater geographic

Table 1. Site description with date and number of bees collected at each site. A dash (—) indicates a site that was not sampled during that year.

ID	Site description	Latitude, longitude	Bees collected in 2006			2007: Sample 1			2007: Sample 2		
			Date	Females	Males	Date	Females	Males	Date	Females	Males
AC	Amherst College conservation area	42°21'39.43"N, 72°30'18.46"W	24/7	34	5	13/8	28	1	20/8	28	2
CI	University of Massachusetts campus	42°23'47.14"N, 72°31'1.63"W	31/7	3	5	—	—	—	—	—	—
SW	Southampton Wildlife Management Area, Massachusetts Department of Fish and Wildlife	42°13'2.16"N, 72°40'51.92"W	8/8	6	4	—	—	—	—	—	—
GF	Graves Farm, Massachusetts Audubon Society conservation area	42°24'7.32"N, 72°42'15.05"W	—	—	—	21/7	9	20	17/8	25	5
HC	Herman Covey Wildlife Management Area, Massachusetts Department of Fish and Wildlife	42°15'5.54"N, 72°20'39.84"W	—	—	—	11/7	29	1	8/8	19	12
HM	Haskin's Meadow. Amherst municipal conservation area	42°25'48.83"N, 72°29'26.32"W	12/8	25	5	7/8	25	3	14/8	24	5
LW	Leyden Wildlife Management Area, Massachusetts Department of Fish and Wildlife	42°39'17.48"N, 72°35'55.00"W	—	—	—	14/7	22	11	10/8	24	5
NH	Acadia conservation area, Easthampton, Massachusetts Audubon society	42°17'45.70"N, 72°38'39.37"W	—	—	—	1/8	25	5	24/8	25	5
Q17	Quabbin – gate 17. Massachusetts Department of Conservation and Recreation	42°26'49.22"N, 72°22'19.84"W	9/8	47	2	7/25	30	0	22/8	28	2
Q20	Quabbin – gate 20. Massachusetts Department of Conservation and Recreation	42°26'32.38"N, 72°20'42.51"W	11/8	40	1	27/7	28	1	29/8	30	0
RE	Road's End, Massachusetts Audubon Society conservation area	42°25'24.79"N, 72°55'45.84"W	—	—	—	13/7	29	1	15/8	29	1
SA	Sweet Alice, Amherst municipal conservation area	42°19'6.10"N, 72°31'18.68"W	16/8	23	8	3/8	19	10	18/8	28	1
TF	Turner's Falls high school	42°35'1.89"N, 72°32'30.06"W	—	—	—	18/7	23	5	21/8	22	7
WF	Wentworth farms, Amherst municipal conservation area	42°21'42.17"N, 72°29'30.35"W	17/8	32	5	26/7	28	2	31/8	23	7
WW	Wildwood, Amherst municipal conservation area	42°23'34.51"N, 72°30'24.22"W	14/8	30	9	15/7	10	19	28/8	10	20

area than in 2006. Each meadow was sampled twice over the course of July and August. Due to logistical constraints, all sites were not sampled concurrently (Table 1). Sampling methods were as in 2006, with 30 bees collected per site. For all but two collections, sufficient females could not be found and some males were collected.

Assessing parasitism

Bees were brought to the laboratory and provided with sugar water *ad libitum* until either they died from parasites or several weeks had passed. Prior to dissection, bees were identified to species using an online key to the *Bombus* (<http://www.discoverlife.org/mp/20q>) and the length of the

radial cell (base of median plate to distal end of radial cell) was measured as a crude indicator of bee size. While this measure is best used within a single bee species and sex (Harder, 1985), rearing bees for conopid larvae prevented us from obtaining bee dry weight, and so we rely on radial cell length as an approximate indicator of bee body size. Bees were dissected within 24 h of death and inspected for the presence of conopid parasitoids. The gut was then removed, ground in a drop of water on a slide, and inspected at 100× magnification for *Crithidia* and *Nosema*. In some cases conopid larvae had consumed the entire gut and screening for microorganisms was not possible, leading to different sample sizes for different parasites. In 2006, this represented 101 out of 338 bees collected, in 2007, 78 out of 779 bees. Bees that contained conopid larvae were returned to the growth chamber, so that

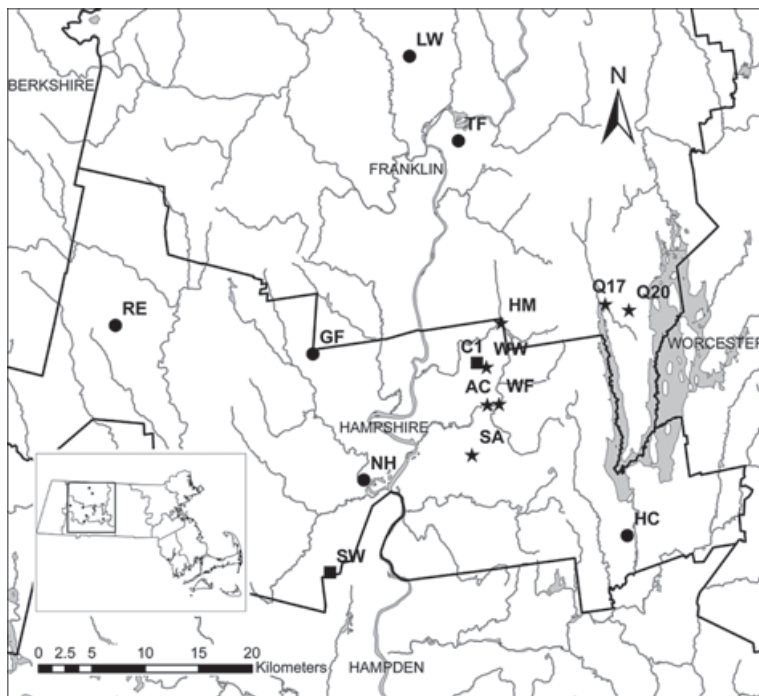


Fig. 1. Map showing field site locations in Western Massachusetts. Stars (★) are sites sampled in both 2006 and 2007, squares (■) are sites sampled only in 2006, and circles (●) are sites sampled only in 2007. Site labels are two-letter identifiers for each site. Other labels are county names. For site details see Table 1.

the larvae could pupate. Pupae were exposed to 2 months at 8 °C, then returned to room temperature and allowed to emerge. Those that emerged as adults were killed, pinned, and keyed to genus (Smith & Peterson, 1987). All reared conopids were from the genus *Physocephala*.

Analysis

Bee parasitism was quantified by calculating the proportion of bees attacked by each parasite overall and on each sample date for both 2006 and 2007. All further statistical analyses were conducted on the 2007 data, which had sufficient replication to statistically examine factors associated with parasitism. To examine effects of parasitism on survival in the laboratory, each bee's residual lifespan was calculated as the number of days a bee survived after being captured in the field. For each parasite we then compared survival curves for infected and uninfected bees (Proc Lifetest, SAS Institute, 2007).

To examine what factors might affect parasitism, separate logistic regressions were conducted for each parasite (Proc LOGIST, SAS Institute, 2007), with bee sex, species, and site as factors, and date of collection and wing size as continuous variables. There was insufficient replication to test for interaction terms because only two bee species occurred at all sites, thus this analysis is limited to main effects. For categorical effects that were significant, χ^2 tests were used to determine which individual levels of each factor differed from the random expectation based on population level infection. The Dunn–Sidak method was used to account for multiple tests by adjusting the alpha level based on the number of χ^2 tests for each parasite (Sokal & Rohlf, 1995).

Restriction of the analysis to main effects limits interpretation of certain patterns. For example, males increase over the season and certain species are abundant earlier, or later during the summer. Normally interactions between such phenological effects and sampling date would be detected by a significant time by species or time by sex interaction; the logistic analysis of main effects cannot distinguish a significant change in parasitism over time from the effects of bee phenology. In order to examine temporal patterns in more detail, when there was a significant effect of date on parasitism least squares means (LS means hereafter) were calculated for each date by site combination, accounting for the effects of species and sex. These analyses were conducted using Proc GENMOD, including species and sex as explanatory variables (LS means cannot be calculated in Proc LOGIST). In this analysis, LS means estimate parasitism on each sampling date for each site independent of the abundance of bumblebee species or changes in the sex ratio over time. These independent parasitism rates were then regressed on sampling date to determine whether a significant relationship between sampling date and parasitism remained after other effects were accounted for. The shape of the relationship was tested by adding the squared date term to the model and testing its significance. If it was not significant, it was removed.

A large proportion of the bees sampled were *B. impatiens*, the species that is used in commercial hives (Table 2). Given that it could not be determined whether individuals from this species were wild or managed, data were re-analysed without *B. impatiens*. This did not qualitatively change the results of any statistical analysis of parasitism (results not shown), and data are presented with *B. impatiens* included.

Because *B. pensylvanicus* and *B. fervidus* are of particular conservation interest but were too rare to include in the main analyses, two-way tables and Fisher's exact test were used to examine whether infection by each parasite was different in each rare species than in the rest of the species pooled together.

Results

Bumblebee species abundance

Two hundred and eighty-nine female workers and 49 males from eight species were collected in 2006 and 628 female workers and 151 males from nine species were collected in 2007 (Table 2). *Bombus impatiens* Cresson 1863, *B. griseocollis* Degeer 1773, *B. vagans* Say 1837, and *B. bimaculatus* Cresson 1863 were the most widespread, common species (Table 2). *Bombus fervidus* (Fabricius, 1798) and *Bombus pensylvanicus* (DeGeer, 1773) were the rarest bee species (Table 2). We also caught a large number of males, and one queen, from the parasitic species, *Bombus citrinus* (subgenus *Psithyrus*; Table 2).

Conopid parasitism

Conopids infected 60% of all bees in 2006 ($n = 297$ bees, range 0–73% by site) and 22% of all bees in 2007 ($n = 779$ bees, range 10–32%). Despite the non-significant overall

model, there was a significant association between sex and conopid parasitism, and a marginally significant effect of body size. Females were attacked more than males (Table 3, 22% of females infected versus 13% of males), and intermediate sized bees tended to be more frequently attacked than large or small bees (squared term: $P = 0.063$). Conopid parasitism was not significantly different between common bees and *B. pensylvanicus* or *B. fervidus* (*B. pensylvanicus*: $n = 15$, Fisher's exact $P = 0.36$; *B. fervidus*: $n = 7$, Fisher's exact $P = 0.22$).

Crithidia parasitism

Crithidia infected 24% of bees in 2006 ($n = 197$ bees, range 0–53% by site) and 51% in 2007 ($n = 670$ bees, range 4–82%). *Crithidia* parasitism varied with bee species, collection date and site (Table 3). Sex and body size were unrelated to parasitism (Table 3). *Bombus griseocollis* and *B. ternarius* had lower than average infection rates (*B. griseocollis*: $n = 105$, $\chi^2 = 20.07$, $P < 0.0001$; *B. ternarius*: $n = 41$, $\chi^2 = 21.54$, $P < 0.0001$ respectively) while *B. impatiens* had higher than average infection rates ($n = 278$, $\chi^2 = 7.64$, $P = 0.0057$; Fig. 2a). For sites, Haskin's Meadow (HM) had higher than average infection rates ($\chi^2 = 11.63$, $P = 0.0006$) while Road's End (RE), Turner's Falls high school (TF) and Wildwood (WW) all had lower infection rates (RE, $\chi^2 > 9.0$, $P < 0.002$ for all; Fig. 2b). *Crithidia* exhibited a unimodal

Table 2. Summary of *Bombus* species collected.

<i>Bombus</i> species	Seasonal start of activity	2006			2007			Number of sites where found
		Total	Male	Female	Total	Male	Female	
<i>B. bimaculatus</i> Cresson, 1863	Early	15	11	4	69	47	22	12
<i>B. citrinus</i> (Smith, 1854)	—	8	8	0	37	36	1*	9
<i>B. fervidus</i> (Fabricius, 1798)	Late	0	0	0	16	3	13	6
<i>B. griseocollis</i> (DeGeer, 1773)	Early	15	2	12	121	34	87	13
<i>B. impatiens</i> Cresson, 1863	Late	271	19	252	303	1	302	13
<i>B. pensylvanicus</i> (DeGeer, 1773)	Early	0	0	0	7	1	6	4
<i>B. perplexus</i> Cresson, 1863	—	13	8	5	28	21	7	7
<i>B. ternarius</i> Say, 1837	Early	6	1	5	51	6	45	5
<i>B. vagans</i> Say, 1837	Late	11	0	11	147	2	145	12
Total		338	49	289	779	151	628	

*This was a queen.

Table 3. Results of three separate logistic regressions examining association between various factors and parasitism by conopids, *Crithidia* or *Nosema*.

	d.f.	Conopids		<i>Crithidia bombi</i>		<i>Nosema bombi</i>	
		Wald's χ^2	<i>P</i> -value	Wald's χ^2	<i>P</i> -value	Wald's χ^2	<i>P</i> -value
Overall model	21	23.1807	0.3344	81.100	<0.0001	37.8344	0.0135
Species	6	4.6923	0.5838	20.317	0.0024	10.6054	0.1014
Sex	1	5.9388	0.0148	0.3162	0.5739	10.2703	0.0014
Site	12	10.699	0.5549	38.246	0.0001	11.0916	0.5211
Collection date	1	0.4212	0.5163	6.9723	0.0083	3.4986	0.0614
Wing size	1	2.9934	0.0836	1.9049	0.1675	1.4888	0.2224

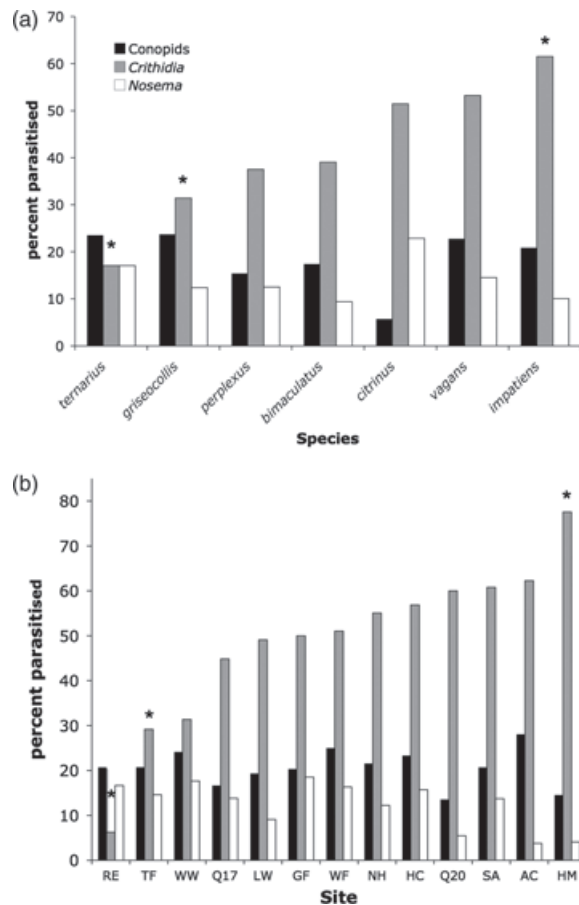


Fig. 2. (a) Per cent of each bumblebee species infected with conopids, *Crithidia bombi* and *Nosema bombi* in 2007. (b) Proportion of bumblebees collected at each site infected with conopids, *Crithidia bombi* and *Nosema bombi* in 2007. Asterisks over bars represent species or sites with parasitism rates significantly different from the average infection rate within that parasite only.

distribution of infection over the summer (in logistic regression, squared term $P < 0.001$, Fig. 3). This pattern remained when LS means accounting for the effects of species and sex were calculated for each sampling date and regressed on date (regression model: d.f. = 2, $F = 6.66$, $P = 0.005$; date squared: d.f. = 1, $P = 0.008$). Thus, for simplicity of interpretation, proportion infection rates instead of LS means are presented in Fig. 3. Both *B. pennsylvanicus* and *B. fervidus* had significantly lower *Crithidia* infection than common bee species (*B. pennsylvanicus*: Fisher's exact $P = 0.015$; *B. fervidus*: Fisher's exact $P = 0.006$). While infection of common bees was approximately 50%, only 13% of *B. fervidus* and zero *B. pennsylvanicus* were infected by *Crithidia*.

Nosema parasitism

Nosema infected 13% of bees captured in 2006 ($n = 195$ bees, range 3–38% by site) and 11% of bees in 2007 ($n = 670$ bees, range 0–32%). *Nosema* parasitism was

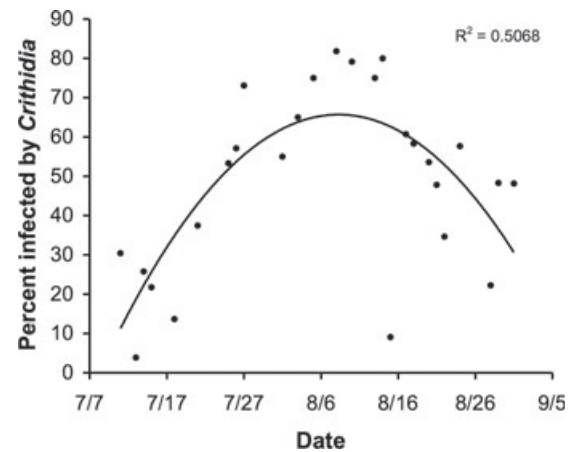


Fig. 3. Percent of bumblebees collected on each sampling date in 2007 infected with *Crithidia bombi*.

significantly associated with sex and marginally significantly associated with collection date. Species, wing length, and collection site were not related to parasitism (Table 3). Twenty-three per cent of males were infected versus 13% of females, and *Nosema* infection declined over the season. However, the pattern of *Nosema* decline over the season disappeared when prevalence was examined with LS means accounting for the effects of sex. This indicates that the pattern of decline is likely due to changing abundance of males over time, rather than an overall decrease in *Nosema* prevalence. *Nosema* infection was significantly higher in both *B. pennsylvanicus* and *B. fervidus* compared with common bee species (*B. pennsylvanicus*: Fisher's exact $P = 0.015$; *B. fervidus*: Fisher's exact $P = 0.015$). Whereas infection of common bees was approximately 12%, 33% of *B. fervidus* and 42% of *B. pennsylvanicus* were infected by *Nosema*.

Residual lifespan

Residual lifespan differed between bees due to conopid parasitism (log-rank test: $\chi^2 = 18.52$, d.f. = 1, $P < 0.0001$). On average, conopid-parasitised bees survived fewer days in the laboratory than healthy bees (parasitised: $x = 5.56 \pm 0.29$ days, unparasitised: $x = 7.63 \pm 0.28$; Fig. 4b). Residual lifespan did not differ due to *Crithidia* infection (Log-rank test: $\chi^2 = 0.49$, d.f. = 1, $P = 0.49$; Fig. 4b). Residual lifespan also differed due to *Nosema* infection (log-rank test: $\chi^2 = 11.9$, d.f. = 1, $P = 0.0006$); on average, *Nosema*-infected bees survived more days in the laboratory than healthy bees (infected: $x = 10.2 \pm 0.76$ days, uninfected: $x = 6.9 \pm 0.27$; Fig. 4c).

Discussion

Parasite infection levels

Infection rates by all three parasites were higher in this study than previously documented in North America. *Crithidia* infected 24% of bees in 2006, and a remarkable 51% in

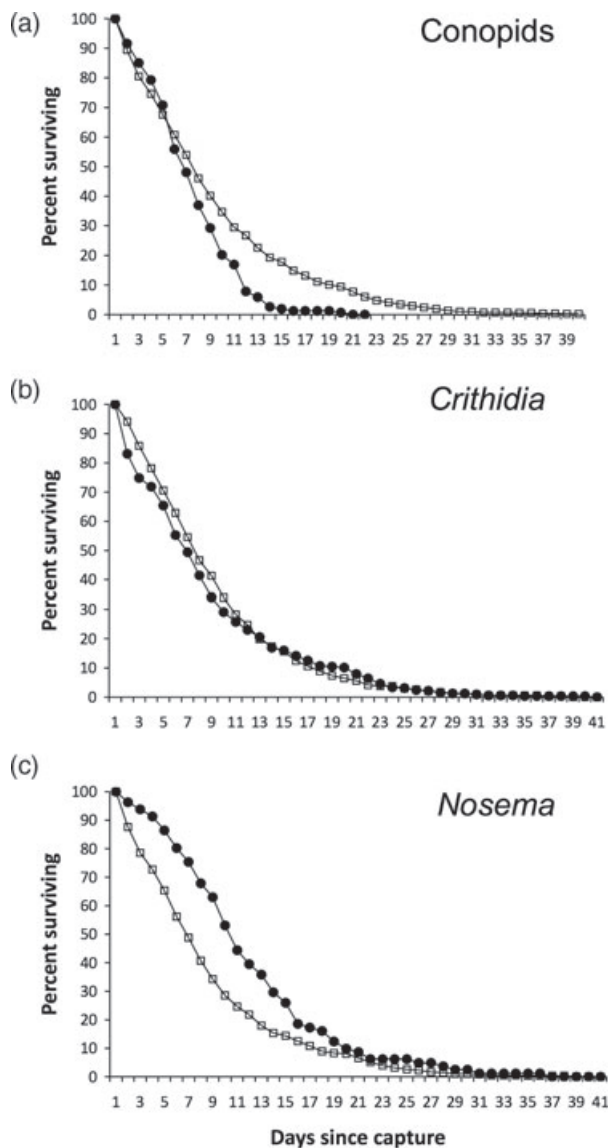


Fig. 4. Survival curves of the residual lifespans of bees infected and uninfected by: (a) conopids, (b) *Crithidia bombi*, and (c) *Nosema bombi*. Black circles represent infected bees and open squares are uninfected bees.

2007, with up to 80% of bees infected at a single site. Conopids parasitised 60% of bees in 2006 and 22% in 2007. *Nosema* infected 11–13% of bees each year. Both *Crithidia* and conopids varied in infection rates between years, but it cannot be determined whether this is due to year-to-year variation or to sampling different sites in 2006 and 2007. Infection by all parasites was similar to rates documented in Europe, and higher than previously found in wild bees in North America. While *Crithidia* infection rates of up to 80% have been documented in Europe (Shykoff & Schmid-Hempel, 1991b), studies in Canada have only found *Crithidia* in wild bumblebees collected near commercial greenhouses (Colla *et al.*, 2006). Similarly, *Nosema* has been found at similar

levels in Europe (0–10%, Shykoff & Schmid-Hempel, 1991b; Korner & Schmid-Hempel, 2005), whereas in Canada *Nosema* was found in only about 5% of wild bees (Colla *et al.*, 2006). Conopid parasitism mimicked this pattern; prevalences in western Massachusetts were comparable to levels in Europe (20–62% parasitism), but higher than has been documented in North America (about 10%; Schmid-Hempel & Durrer, 1991; Otterstatter, 2004). The high infection rates found here suggest the need for more studies of bumblebee parasitism in North America, since this may be the first study documenting the abundance of these natural enemies in the U.S.A.

The only place in North America that *Crithidia* and *Nosema* have so far been documented at numbers comparable to those found here has been in proximity to greenhouses that use managed *Bombus impatiens* for pollination of tomato crops. *Crithidia* infected on average 25% of wild bees at greenhouse sites (but up to 75% of one species), versus none in isolated populations (Colla *et al.*, 2006). *Nosema* followed a similar pattern, although infection was overall lower than *Crithidia* (Colla *et al.*, 2006). It has been proposed that bees from highly infected managed colonies escape the greenhouse to forage at common flowers with wild bees, where pathogens are transmitted (Durrer & Schmid-Hempel, 1994; Colla *et al.*, 2006). Greenhouse tomato crops are not common in Massachusetts (USDA, 2007); however, bumblebees are used for pollination in blueberry and apple orchards (Stubbs & Drummond, 2001; Thomson & Goodell, 2001). If managed bumblebees are used near sites surveyed in this study, spillover from those colonies could produce the high infection levels documented here. However, spillover may not be the only cause of high parasitism in the current study. Relatively isolated sites, separated by over 3 km of open water and forest from any orchard that may use commercial hives of bumblebees (Q17 and Q20), still had high levels of infection by *Crithidia* (~45% and 60%) and *Nosema* (~14% and 5%). While the maximum documented flight range of bumblebees (up to 1.5 km, Osborne *et al.*, 2008) would allow transmission across this distance, the depauperate nature of the intervening landscape suggests that this unlikely. Examining patterns of parasitism at sites near farms with known use of managed bumblebees and comparing them to isolated sites would be the best way to determine whether commercial bumblebee hives could be responsible for the high parasitism levels observed in this study.

Compared to *Crithidia* and *Nosema*, little work has examined factors determining local conopid abundance. In North America, conopids have previously been studied in Alberta, Canada, where infection rates averaged 10% (range: 0–30% depending on sampling date and site), and in Ontario, Canada, where conopids infected at most 10% of bees sampled (Macfarlane & Pengelly, 1974; Otterstatter, 2004). These numbers are low compared with the 20–60% infection rates in the current study. Given that conopids were only identified to genus in this study, the high levels of parasitism documented here could be due to the presence of different conopid species. Further taxonomic work on samples collected here will help clarify this. Alternately, colder winter temperatures in Alberta may lead to lower overwintering survival of pupating conopids, and overall lower abundances of the

parasitoid (Otterstatter *et al.*, 2002; Otterstatter, 2004). This hypothesis is supported by studies in Europe, where conopids at lower elevations were at higher abundances compared with alpine sites (Schmid-Hempel *et al.*, 1990; Korner & Schmid-Hempel, 2005). The study in Ontario examined conopid parasitism around Guelph, which is in a similar climatic zone to western Massachusetts, yet had lower levels of conopid parasitism than documented here (Macfarlane & Pengelly, 1974; USDA, 1990). Conducting field surveys of conopid parasitism in different climates, such as the southern U.S.A., and laboratory experiments on overwintering survival of conopid pupae would clarify whether winter temperatures regulate conopid parasitism.

Parasitism of *B. fervidus* and *B. pensylvanicus*, two species that have declined in the past 20 years (Colla & Packer, 2008), was examined to determine if these rare species were attacked at different rates than more common species. Rare species were less infected by *Crithidia* but more infected by *Nosema* compared with common bumblebee species. Conopid parasitism was not different between the rarer and more common bumblebee species. It is important to note that these results are based on low sample sizes of the rare species. However, the differences in infection by *Crithidia* and *Nosema* may represent interesting patterns for further study. Research in Europe has documented higher parasite infection rates, and parasite diversity in widespread bumblebee species relative to rare species (Durrer & Schmid-Hempel, 1995). It has been suggested that exposure and subsequent resistance to a range of parasites gives common species a competitive advantage over rare species (Price *et al.*, 1986). *Crithidia* infection may follow this pattern for North American bumblebees; however, high *Nosema* infection in rare species indicates that it is a potentially important exception. Further examination of these patterns may reveal potential causes for the declines of these species. High infection by *Nosema* may be reducing populations of *B. fervidus* and *B. pensylvanicus*, or these species may be limited competitively by lack of exposure and resistance to other parasite species.

Residual lifespan

Each parasite had a unique effect on residual lifespan of bumblebees in the laboratory. Parasitism by conopids was associated with shorter lifespans in the laboratory, whereas infection by *Nosema* was associated with longer survival, and *Crithidia* had no effect (Fig. 4). These differences in residual laboratory survival could be due to the direct effects of parasites on laboratory survival, or differences in the ages of parasitised and unparasitised bees at capture. For instance, bees infected with conopids survived about 2 fewer days in the laboratory than healthy bees. This may be because conopids are parasitoids, which kill their hosts in a few weeks, are likely to shorten laboratory survival (Schmid-Hempel & Schmid-Hempel, 1996). Previous work on conopids in Europe found overall longer residual bee lifespans than those found here (7.8 days for infected bees and 15.7 days for uninfected bees). This may be due to differences in laboratory conditions; parasitoids in bees kept at higher temperatures may develop

faster, and even healthy bees may expend more energy and die faster.

Crithidia had no effect on bee survival in the laboratory (Fig. 4b). Given that *Crithidia* is considered to be a relatively benign parasite that only exhibits lethal effects under stressful conditions (Brown *et al.*, 2000), it may not be expected to affect laboratory survival when food is abundant. Surprisingly, *Nosema* increased bee residual lifespan by nearly 50% (Fig. 4c). Given that *Nosema* is considered a relatively lethal parasite, these results were unexpected (Otti & Schmid-Hempel, 2007). However, previous studies found that the lethal effects of *Nosema* varied between bumblebee species (Rutrecht & Brown, 2009). Furthermore, worker bees infected with *N. apis* tend to reduce their activity levels and stay within the colony (Showers *et al.*, 1967). If *N. bombi* has a similar effect, then *Nosema*-infected bees in the laboratory may exert less energy trying to escape and thus survive longer.

Factors associated with parasitism

In addition to documenting parasitism levels in bumblebees in western Massachusetts, this study also examined factors that could explain variation in parasitism levels. These included spatial and temporal differences in collection of samples, as well as individual variation including species, sex, and body size.

Conopid flies parasitised female bees more than males, and medium-sized bees tended to be more frequently attacked, although this pattern was not statistically significant. Both these findings correspond with Otterstatter (2004), suggesting that a larger sample size may have enabled us to detect patterns at a statistically significant level. Females may be attacked more because their foraging behaviour brings them into contact with conopids more often than males, who spend much of their time patrolling for females rather than foraging at flowers (Otterstatter, 2004). There may also be a host size trade-off for conopid females such that larger hosts provide more resources for conopid offspring but are better able to escape oviposition attempts, leading to highest parasitism in mid-sized bees (Otterstatter, 2004). However, studies in Europe found a linear increase in conopid parasitism with host size (Muller *et al.*, 1996); this pattern needs further investigation.

Bee species differed in *Crithidia* infection rates. *Bombus impatiens* had higher than average levels of infection by *Crithidia*, while *B. griseocollis* and *B. ternarius* had lower than average infection rates (Fig. 2a). High infection of *B. impatiens* may be due to its use in commercial hives, which have high levels of infection of both *Crithidia* and *Nosema*. High levels of infection in *B. impatiens* have been found close to greenhouses using managed colonies; however, as in previous studies, it cannot be determined whether the *B. impatiens* individuals collected in this study were wild bees or from nearby managed colonies (Colla *et al.*, 2006). It is also interesting to note that *B. impatiens* is a late-season bee, whereas *B. griseocollis* and *B. ternarius*, which both experienced lower than average infections, are early season bees (Table 2, Fig. 2a; Kearns & Thomson, 2001). *Bombus bimaculatus*, another

early season bee, also had lower than average infection rates, although this was not significant at the adjusted P -value ($\chi^2 = 5.17$, $P = 0.02$; Fig. 2a). Low infection of early season bees could be explained in two ways, given that *Crithidia* exhibited a unimodal pattern of prevalence over the summer (Fig. 3). First, by starting their colony cycles early in the summer, early season bees may avoid exposure to the highest levels of *Crithidia* later in the season. Alternately, the temporal increase in *Crithidia* over the early part of summer may be due to the increased abundance of more susceptible host species. However, when temporal patterns of *Crithidia* parasitism were examined independent of the effects of species, the unimodal pattern was maintained, indicating that this pattern is likely not due to the effects of bee phenology. This distinction has important implications for conservation. In a broad survey of factors associated with risk of decline in bumblebee species, a late start to the foraging season was positively associated with declining populations (Williams *et al.*, 2009). The current study suggests that in addition to time limitation on colony development, late season bees in Massachusetts may also be disproportionately impacted by parasites.

Crithidia infection also varied significantly between sites. Geographic variation in bumblebee species presence may be responsible for variation in the prevalence of *Crithidia*. For instance, Road's End (RE) had a far lower than average rate of *Crithidia* infection (Fig. 2b), and the bumblebee species most frequently caught there was *B. ternarius*. *Bombus ternarius* also experienced lower than average rates of infection, so that low infection at RE could be due to the presence of mostly *B. ternarius*. Because sites had different bee communities, it is not possible to disentangle variation due to bee species from geographic variation. Further research focusing on the extent and mechanisms of species-level resistance to infection could shed light on whether differences in bee communities or geography alone underlie variation in parasitism across sites and over time.

Higher *Nosema* infection rates in males compared with females is consistent with previous research in Europe (Shykoff & Schmid-Hempel, 1991b). It has been observed that workers infected with *Nosema* are less active and less likely to leave the nest (Shykoff & Schmid-Hempel, 1991b). Male bees do not have this option, as they are all forced to leave the nest. Thus we may see higher levels of infection in field-sampled males than in workers because diseased workers are disproportionately in the nest (Shykoff & Schmid-Hempel, 1991b). Alternately, males of many invertebrates suffer higher levels of parasite infection than females, and invest less in immune defence (Zuk & McKean, 1996; Zuk & Stoehr, 2002). This difference has been explained by life-history differences in the sexes, where males compete heavily for mating opportunities, possibly at the expense of their own immune function (McKean & Nunney, 2001; Schmid-Hempel, 2005).

Overall, it appears that by virtue of phenology, location or biology, certain bees are at greater risk of attack by the three parasites studied here. These results also suggest that different parasites will impact pollinator populations in different ways, for example by affecting males in the case of *Nosema*, or by causing female forager mortality in the case of conopids

(Table 3). Bee species that are active later in the season may be exposed to higher levels of *Crithidia* but lower levels of *Nosema*, making the interaction between bee phenology and the effects of parasitism complex. Given the difficulty of acquiring data on rare bees, it is difficult to ascertain whether parasites are a reasonable explanation for declines in certain species of bumblebees in eastern North America. However, this study did find higher than average prevalence of *Nosema* in two relatively rare bumblebee species: *B. pennsylvanicus* and *B. fervidus*, indicating that these species may suffer higher infection by this parasite than more common species.

Conclusion

This study is the first to document the prevalence of bumblebee parasitism in the U.S.A. Infection levels by three different parasites in western Massachusetts were higher than has previously been found in North America. While pathogen spillover from managed colonies is a potential explanation for the high abundances of *Crithidia* and *Nosema* in wild bees, it does not explain the presence of both of these parasites at high levels in geographically isolated sites, nor why conopids are also common. Given that this is the first study of this type in the U.S.A., sampling at more southern latitudes will clarify the role that climate plays in conopid abundance. This study also demonstrates that parasitism rates may be related to several aspects of the bee's biology, although important factors vary between parasites. The potential causes of variation in parasitism rates have important implications for conservation, as particular bee species may suffer higher incidence of infection, making their populations more susceptible to decline or extinction. The high levels of parasite infection shown in this study combined with their documented impacts on colony reproduction suggest that *C. bombi*, *N. bombi*, and parasitoid conopids are likely to affect bee populations in Massachusetts. This may threaten the stability of bumblebee pollination of crops, as well as conservation of rare bumblebee species.

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