Journal of Invertebrate Pathology 107 (2011) 220–224

Contents lists available at ScienceDirect

Journal of Invertebrate Pathology

journal homepage: www.elsevier.com/locate/jip

Short Communication

Survey of bumble bee (*Bombus*) pathogens and parasites in Illinois and selected areas of northern California and southern Oregon

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ARTICLE INFO

Article history:
Received 9 March 2011
Accepted 20 April 2011
Available online 27 April 2011

Keywords:
Microsporidia
Protozoans
Tracheal mites
Parasitoids
Phoretic mites
Nosema bombi
Crithidia bombi
Locustacarus buchneri
Conopidae

ABSTRACT

Pathogens have been implicated as potential factors in the recent decline of some North American bumble bee (*Bombus*) species, but little information has been reported about the natural enemy complex of bumble bees in the United States. We targeted bumble bee populations in a state-wide survey in Illinois and several sites in California and Oregon where declines have been reported to determine presence and prevalence of natural enemies. Based on our observations, most parasites and pathogens appear to be widespread generalists among bumble bee species, but susceptibility to some natural enemies appeared to vary.

1. Introduction

Bumble bees (*Bombus* spp.) are vitally important native pollinators of natural and agricultural ecosystems (Kremen et al., 2002; Velthuis and van Doorn, 2006) and appear to be suffering severe range reductions in Europe (Goulson et al., 2005; Williams, 2005), Asia (Yang, 1999; Xie et al., 2008) and North America (Thorp, 2005; Colla and Packer, 2008; Grixti et al., 2009; Cameron et al., 2011). In Europe, factors reported to cause range shifts and decreasing abundance include agricultural intensification, climate change, and habitat fragmentation with diminished floral resources (Biesmeijer et al., 2006; Goulson et al., 2008; Williams and Osborne, 2009). In North America, *Bombus* species range reduction and declining relative abundance have been reported to be associated with pathogens (Thorp, 2005; Colla et al., 2006; Otterstatter and Thomson, 2008; Cameron et al., 2011). Thorp (2005) proposed that a microsporidian pathogen, *Nosema bombi* (Nosematidae), known to infect European *Bombus* species, may have invaded North American populations and become an important agent of decline, and Cameron et al. (2011) found significantly higher prevalence of *N. bombi* in declining populations of *Bombus s. s.* (*Bombus occidentalis*) and *Thoracobombus* (*Bombus pensylvanicus*) compared to populations of stable species. The geographic origin of *N. bombi* strains in the declining species has not yet been determined, however, and the potential role of other parasites or pathogens in the decline of North American species is also unknown. The pathogens *Crithidia bombi* (Trypanosomatidae) and *N. bombi*, the metazoan parasite *Locustacarus buchneri* (Podapolipidae) and conopid flies (Conopidae) are all capable of reducing longevity (Otterstatter and Whidden, 2004), colony fitness, (Schmid-Hempel and Durrer, 1991; Schmid-Hempel, 2001; Otterstatter and Whidden, 2004; Gegear et al., 2006; Colla et al., 2006; Otti and Schmid-Hempel, 2007; van der Steen, 2008) and learning among foragers (Gegear et al., 2005, 2006).

We surveyed existing populations of *Bombus* species in their native Illinois range, and conducted a preliminary survey of bumble bee species in northern California and southern Oregon to address the lack of data on diseases and parasitoids in the US. We identified an array of different pathogens and parasites found in declining and stable species and obtained prevalence data for *C. bombi*, *L. buchneri* and, collectively, various dipteran and hymenopteran parasitoids.

2. Materials and methods

Bumble bee populations were sampled in or near 20 established natural areas across the state of Illinois in 2006–2007, eight sites in

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0022-2011/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.jip.2011.04.008

California (2006) and five sites in Oregon (2006) (Table 1). Sites included natural areas, roadsides and open pastures. Sites in Illinois were sampled multiple times within and across years. Worker and male bees were collected with an aerial net; queens were collected occasionally if workers and males were absent. Collected bees were transported on ice to the laboratory where they were dissected immediately or stored at −80 °C for later dissection. A total of 1351 individuals from Illinois and 307 individuals from California and Oregon were identified to species using published keys (La Berge and Webb, 1962; Thorp et al., 1983), cuticle was examined for phoretic mites, and tissues were microscopically examined for pathogens and parasitoids.

Nosema bombi ribosomal DNA (rDNA) sequences were obtained from infected tissues of host individuals using standard techniques for microsporidia (Table 2). Sequences were aligned unambiguously in BioEdit 7.0.0 (Hall, 1999) and compared to European isolates of *Nosema bombi* and other microsporidia using the GenBank BLAST search program.

Differences in prevalence of parasite and pathogen infection (Illinois samples only) were evaluated using logistic regression. Explanatory variables, including two and three-way interactions, were year, month, region, site, species, and caste. The model, including variables and their interactions, was tested initially, and any insignificant terms ($p > 0.05$) were removed in a backward stepwise fashion, until all terms were significant ($p < 0.05$). All statistical analyses were implemented in SAS v. 9.1 (SAS Institute).

3. Results and discussion

Our surveys of *Bombus* spp. in Illinois, northern California and southern Oregon suggest that pathogens and parasites (with the exception of viruses which we did not evaluate) are widespread generalists in the host genus, as was observed for different species of European bumble bees (Shykoff and Schmid-Hempel, 1991; Tay et al., 2005). The prevalence of different natural enemies varied among host species, however, with some consistently at high or low levels, or not present in a host species, suggesting that the level of host susceptibility to several of the parasites and pathogens may be species specific (Table 3) (Gillespie, 2010). Because collection numbers of some *Bombus* species in Illinois, California and Oregon were low, conclusions about occurrence and host specificity of pathogens and parasites for these species cannot be made. Although overall prevalence of parasitoids was high for some species, we did not observe high pathogen prevalence as reported for sites in Massachusetts (Gillespie, 2010).

Table 1
Prevalence of four pathogens and parasites recovered from *Bombus* species at Illinois sites (2006 and 2007), and California and Oregon sites in 2006.

Collection site	GPS coordinates	*N*	Tracheal mites[a]	Parasitoids[a]	*Crithida*[a]	*Nosema*[a]
North Region						
Iroquois Co.	40°59.658′N:087°35.612′W	38	7.89	31.58	2.63	0.00
Henderson Co.	41°01.252′N:090°55.607′N	55	3.64	23.64	3.64	0.00
Peoria county	40°48.981′N:089°48.735′W	68	2.94	13.24	0.00	0.00
LaSalle county	41°16.769′N:089°01.192′W	72	12.50	33.33	0.00	0.00
Lee county	41°38.367′N:089°31.060′W	101	17.82	35.64	0.00	0.99
Combined sites		334	10.17	28.14	0.90	0.30
Central Region						
Macoupin county 1	39°12.750′N:089°58.661′W	30	0.00	0.00	3.33	0.00
Macoupin county 2	39°14.444′N:089°55.602′W	59	3.39	25.42	3.39	1.69
Logan county	40°07.082′N:089°23.838′W	61	1.64	14.75	9.84	0.00
Schuyler county	40°14.014′N:090°53.609′W	65	9.23	10.77	1.54	0.00
Mason county	40°23.454′N:089°51.836′W	77	0.00	3.90	18.18	0.00
De Witt County	40°07.079′N:088°55.479′W	78	3.85	23.08	2.56	0.00
Champaign County	40°07.876′N:088°08.345′W	79	49.37	21.52	1.27	0.00
Vermillion County	40°03.592′N:087°33.904′W	81	19.75	32.10	13.58	0.00
Combined sites		530	12.64	17.92	7.17	0.19
South Region						
Hamilton county	38°14.117′N:088°43.295′W	23	0.00	30.43	0.00	0.00
Jersey county	38°58.692′N:090°32.012′W	37	13.51	18.92	8.11	0.00
Jasper county	38°53.571′N:088°18.503′W	41	0.00	19.51	0.00	0.00
Washington county	38°16.272′N:089°21.179′W	42	7.14	19.05	9.52	2.38
Pope county	37°22.880′N:088°39.323′W	53	0.00	47.17	24.53	0.00
Jackson county	37°46.622′N:089°22.248′W	58	3.45	44.83	22.41	0.00
Lawrence county	38°43.324′N:087°50.337′W	86	2.33	12.79	18.60	2.32
Combined sites		340	3.53	27.06	14.41	0.88
California						
Garberville 1	40°06.949′N:123°48.469′W	1	0.00	0.00	0.00	0.00
Willits	39°34.978′N:123°26.640′W	1	0.00	0.00	0.00	0.00
Bodega Bay	38°19 N: 122.02′W	2	0.00	0.00	50.00	0.00
Humbolt, Arcata	40°53.689′N:124°04.697′W	6	16.67	0.00	33.33	50.00
Humbolt 2	40°47.860′N:124°02.224′W	11	18.18	0.00	0.00	0.00
Colfax	39°05.198′N:120°57.310′W	10	0.00	10.00	10.00	0.00
Nevada City	39°16.231′N:121°03.565′W	10	0.00	10.00	20.00	0.00
Montague	41°43.397′N:122°31.865′W	24	0.00	29.00	0.00	4.17
Oregon						
Grizzly Peak	42°17.772′N:122°36.778′W	3	0.00	0.00	33.33	0.00
Ashland 1	42°13.650′N:122°35.730′W	26	0.00	7.69	15.38	0.00
Ashland 2	42°10.899′N:122°40.197′W	13	0.00	23.00	0.00	0.00
Gold Hill	42°27.676′N:123°01.000′W	16	0.00	18.75	62.50	0.00
Mt. Ashland	42°04.645′N:122°42.608′W	53	0.00	11.32	3.77	0.00

Regions: North = north of 40°25′N; Central = between 39°00′N and 40°25′N; South = south of 39°00′ N.
N = total number of individual bees collected at each site.
[a] Prevalence.

Table 2
Primer pairs and annealing temperatures for *Nosema bombi*.

Primer	Sequence	Region	Annealing temperature
ss18f	CACCAGGTTGATTCTGCC		
ss1492r	GGTTACCTTGTTACGACTT	Approx. 1200 bp; SSUrDNA	51
ss530f	GTGCCAGC(C/A)GCCGCG	Approx. 470 bp; 360 bp from 5′	
ss1047r	AACGGCCATGCACCA	end SSUrDNA	53
ss1061f	GGTGGTGCATGGCCG	Approx. 600 bp; ITS region,	
ls228r[a]	GTTAGTTTCTTTTCCTCC	partial SSUrDNA and LSUrDNA	50

[a] Vossbrinck et al. (1993); all other primers from Weiss and Vossbrinck (1999).

Table 3
Overall occurrence of four parasites in *Bombus* species in Illinois in 2006 and 2007, and California and Oregon in 2006. *Bombus* species reported to occur in these ranges but not recovered in this study have been added for reference.

Bombus Species	N	Tracheal mites[a]	Parasitoids[b]	*Crithidia*[b]	*Nosema*[b]
Illinois					
affinis	1	0.00	0.00	0.00	0.00
ashtoni	0	–	–	–	–
auricomus	37	0.00	13.51	0.00	0.00
bimaculatus	235	43.40	30.64	5.53	0.43
citrinus	2	0.00	0.00	0.00	50.00
fervidus	8	0.00	50.00	0.00	0.00
fraternus	0	–	–	–	–
griseocollis	429	0.00	28.67	3.96	0.23
impatiens	427	2.58	14.52	14.05	0.00
pensylvanicus	28	0.00	21.43	0.00	3.57
rufocinctus	0	–	–	–	–
vagans	37	0.00	21.62	0.00	2.70
variabilis	0	–	–	–	–
California and Oregon					
appositus	11	0.00	0.00	0.00	0.00
bifarius	11	0.00	36.36	0.00	0.00
californicus	9	0.00	11.11	0.00	0.00
caliginosus	4	0.00	0.00	0.00	25.00
fernaldae	0	–	–	–	–
fervidus	6	0.00	33.33	0.00	16.67
flavifrons	11	0.00	9.09	9.09	0.00
franklini	0	–	–	–	–
griseocollis	3	0.00	33.33	0.00	0.00
insularis	0	–	–	–	–
melanopygus	14	0.00	0.00	28.57	7.14
mixtus	11	18.18	9.09	27.27	0.00
morrisoni	0	–	–	–	–
nevadensis	1	0.00	100.00	0.00	0.00
occidentalis	0	–	–	–	–
rufocinctus	6	0.00	0.00	0.00	0.00
sitkensis	2	50.00	0.00	0.00	50.00
suckleyi	0	–	–	–	–
vandykei	30	0.00	3.33	3.33	0.00
vosnesenskii	57	0.00	19.30	24.56	0.00

N = number of individuals of each *Bombus* species collected.
[a] Tr. Mites = tracheal mites (*L. buchneri*).
[b] Prevalence (%).

3.1. Parasitoids

Conopid flies and hymenopteran parasitoids (undetermined to species) were found parasitizing all *Bombus* species and all castes for which more than three individuals were collected (Table 3), and at 19 of 20 sites (Table 1), corroborating the findings of a broad *Bombus* host range in Canada (Otterstatter et al., 2002). Typically, one parasitoid larva per host was observed (primarily conopid flies), but multiple hymenopteran parasitoids were dissected from individuals collected at more than half of the sites and in most hosts (Tables 1 and 3).

3.2. Mites

Tracheal mites, *L. buchneri*, were recovered from the metasomal air sacs of collected bees. The parasite was strongly host-specific, preferring *Bombus bimaculatus* (Goldblatt and Fell, 1984) but occasionally occurring in *Bombus impatiens* (Table 3) in the same sites where infected *B. bimaculatus* were recovered. Too few individuals of *Bombus vagans*, another reported host (Goldblatt and Fell, 1984), were collected for evaluation. *L. buchneri* was recovered from 15 of the 20 sites sampled (Table 1), and in every site where more than two *B. bimaculatus* individuals were collected. Prevalence in *B. bimaculatus* ranged from 9.1 to 100% and averaged 43.4%. There was a sharp increase in *L. buchneri* prevalence in *B. bimaculatus* from June to July in 2006 (20.9% to 56.0% overall) and in 2007 (15.0–59.0% overall). Prevalence in *B. impatiens* remained low throughout the summer months (1.9–2.7%). The three reported midwestern hosts (Goldblatt and Fell, 1984; this study) are in the same subgenus, *Pyrobombus*, as two western species, *Bombus. mixtus* and *Bombus. sitkensis*, from which we recovered the mite. No infested bees were found in the Oregon sites. Tracheal mites are reported to affect *Bombus* behavior and reduce longevity (Otterstatter and Whidden, 2004), which may stress *Bombus* spp. colonies that are struggling due to other factors.

Of three phoretic mite species recovered from *Bombus*, *Kuzinia* sp. (Acaridae) was the most common and had the broadest host range. It was recovered from *Bombus auricomus*, *B. bimaculatus*, *Bombus griseocollis*, *B. impatiens*, and *B. vagans* in Illinois. Prevalence was 12.2% overall in 2006 and 7.8% in 2007; infestations were higher in *B. bimaculatus* than in all other Illinois species combined in both 2006 and 2007. In California and Oregon, *Kuzinia* was recovered from *Bombus flavifrons*, *Bombus melanopygus*, *B. mixtus*, *Bombus vandykei*, and *Bombus vosnesenskii*. Scutacarid mites (Scutacaridae) occurred in lower numbers, on fewer *Bombus* species and at fewer sites than the acarid. Mites in this family were recovered from the propodia of *B. auricomus*, *B. griseocollis*, *B. bimaculatus* and *B. impatiens* workers and males in Illinois. The overall prevalence in six sites spanning the north, central and south regions of Illinois was 1.2% in 2006 and 2.1% 2007. Parasitid mites (Parasitidae) were nearly as common as the Acaridae and also appeared to have a broad host range within the host genus. Parasitid mites were recovered from the propodia of *B. auricomus*, *B. bimaculatus*, *B. fervidus*, *B. griseocollis*, *B. impatiens* and *B. pensylvanicus* workers and males at most sites in Illinois. The overall prevalence was 10.1% in 2006 and 2.0% in 2007.

3.3. Protozoa

Crithidia bombi was recovered from three *Bombus* species in Illinois, *B. impatiens*, *B. bimaculatus* and *B. griseocollis*, and from five species collected in eight sites in the western United States. In the West, prevalence was relatively high in three host species, *B.*

melanopygus, *B. mixtus*, and *B. vosnesenskii*. *C. bombi* has aslo been reported infecting *B. fervidis* and *B. rufocinctus* in North America (Otterstatter and Thomson, 2008). In Illinois, *C. bombi* was recovered from most sites and was observed at highest prevalence, more than 14%, in *B. impatiens*. Prevalence levels were lower for *B. bimaculatus* and *B. griseocollis* (Table 1). Overall prevalence of *C. bombi* among infected species in Illinois was 1.0% in 2006 and 5.7% in 2007 (Table 3); combining data from the 2 years, the overall prevalence was highest in June and lowest in August. *C. bombi* was more abundant in the southern half of Illinois (Table 1); prevalence decreased with increasing latitude. *C. bombi* inhibits colony founding, reduces host longevity and colony fitness, and adversely affects worker behavior (Brown et al., 2000, 2003; Schmid-Hempel, 2001; Gegear et al., 2006), which can potentially add stress to individual colonies as well as contribute to the declines of susceptible species.

Apicystis bombi (Neogregarinida) was recovered from bumble bees in three central Illinois sites, and three sites in Oregon. The neogregarine pathogen was recovered from one *B. bimaculatus* specimen in each of four Illinois sites and was also observed in one individual *B. griseocollis* and one *B. impatiens.* In Oregon, *A. bombi* was recovered from one *B. vosnesenskii*, one *B. vandykei*, and in three *B. mixtus* individuals. The overall prevalence and the prevalence in each host species were low in both Illinois and Oregon, which is probably typical of this pathogen (Lipa and Triggiani, 1996). *A. bombi* was recovered only from species collected in the largest numbers, therefore, the complete host range was probably not represented in our study.

3.4. Microsporidia and other fungi

Although microsporidia were rarely observed in Illinois *Bombus* spp., individuals of five different species, belonging to four different subgenera (*B. bimaculatus*, *B. griseocollis*, *B. vagans*, *B. citrinus* and *B. pensylvanicus;* Table 1), were infected with *N. bombi.* The microsporidium was also recovered from four different species, *B. fervidus*, *B. melanopygus*, *B. sitkensis* and *B. caliginosus*, in two subgenera in California. The 16S rDNA and ITS regions were sequenced or partially sequenced from Illinois samples of *B. citrinus* and *B. vagans*, and *B. griseocollis* and from *B. fervidus* in the West. These sequences were identical to those of European isolates of *Nosema bombi* obtained from GenBank. *N. bombi* appears to have broad host range in North American *Bombus* spp., as it does in European *Bombus* spp. (Tay et al., 2005), and is not specific to a particular subgenus. Our data do not suggest that this pathogen is other than naturally occurring in Illinois or that a different *N. bombi* strain has been introduced. Too few western bees were evaluated to make an assessment.

In addition to the microsporidia, other fungi represented by four different morphotypes were recovered from alimentary tissues of 44 live *B. griseocolis*, *B. impatiens*, and *B. bimaculatus* in Illinois, and in 1 *B. flavifrons* in Oregon. Hyphae were present within the tissues, which were degraded. The fungi were not identified because the samples lacked conidia, and their effects on *Bombus* spp. are unknown.

Pathogens and parasites that have evolved with the host are unlikely to cause widespread decline of a host species, although severe local declines or even extinctions are possible when virulent natural enemies reach high prevalence levels and environmental (resting) stages are persistent (Anderson and May, 1981; Richards et al., 1999). Natural enemies do, however, decrease colony success of bumble bees (Schmid-Hempel, 2001; Otti and Schmid-Hempel, 2007; Otterstatter et al., 2002) and may compound the effects of other stress factors such as climate change, crop monoculture, pesticide use and habitat loss caused by human activities (Kremen et al., 2002; Kearns et al., 1998; Thompson, 2001), thus contributing to range reduction and species declines. A recent nationwide study in the United States determined presence and prevalence of *N. bombi* (Cameron et al., 2011) and *C. bombi* (Cordes et al., unpublished data). This study contributes additional baseline data on these two pathogens as well as new survey information on additional pathogens and parasites of North American *Bombus* species, providing much needed comparative data for future evaluations of bumble bee health.

Acknowledgments

We thank C. Rasmussen for rDNA sequencing J. Grixti for assistance with the 2007 field collections and S. Buck, University of Illinois Natural Areas Program. This research was funded in part by the United States Fish & Wildlife Service, Portland, Oregon, Cooperative Agreement Order Nos. 34205M090 and 101816M577, US Department of Agriculture CSREES-NRI, (Grant # 2007-02274), USDA Cooperative State Research, Education and Extension Service, Hatch Project number #AD-421 No. ILLU-875-302-0205249, and the University of Illinois.

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