

Survey for *Nosema bombi*: a Potential Causative Agent for Decline of *Bombus franklini* and *Bombus occidentalis*

Final Report: U.S. Fish & Wildlife Purchase Order #101816M577
Progress Report U.S. Fish & Wildlife Purchase Order #101817M496

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Introduction: Several species of bumble bees (*Bombus* spp.) are apparently declining in different regions of North America. Among them, *B. franklini* has disappeared from the Pacific Northwest, northern California and southern Oregon, and *B. occidentalis*, a formerly widespread western native, has been reported to be in decline in California, Oregon (Thorp, 2005) and Colorado (J. Thomson, personal communication). In the Midwest, the range of *B. pensylvanicus* has contracted (Grixti et al., 2009), and *B. affinis* is exceedingly rare where it was once common in the eastern U.S. (Colla and Packer, 2008). While there are a considerable number of factors that may be involved in the decline of bumble bee species, increased pressure on bee populations due to inadvertent use of commercial bees infected with the microsporidium *Nosema bombi* or other pathogens, and subsequent escape from greenhouses has been suggested as a potential factor (Flanders et al. 2003; Colla et al., 2006). Three issues complicate this supposition. First, no comprehensive studies have evaluated natural enemies of bumble bees in the U.S., so there are little or no extant baseline data to use as a reference. Second, it is not known whether *N. bombi* or other microsporidia occur naturally in U.S. bumble bee populations or may have been introduced from Europe or elsewhere. Finally, disappearing populations of *B. occidentalis* and other bumble bee species in California and other areas of the U.S. may be difficult to analyze due to low population densities and lack of information about locations of the ‘receding edge’ of the currently utilized habitat.

Purpose of Research: To investigate the role of pathogens, particularly microsporidia, as a factor in the decline of *Bombus franklini*, *B. occidentalis*, and *B. pensylvanicus*. This project, US Fish & Wildlife Purchase Order #101816M577, provided a second season of collections (following project PO #134205M090) of *Bombus* spp. in the midwestern and northwestern U.S. We continue to gather information leading to understanding the potential role of pathogens in the decline of *Bombus* species in the western and Midwestern United States.

Study objectives:

1. *Determine the extent of N. bombi invasion in the western and midwestern U.S. by surveying populations of B. occidentalis, B. franklini if feasible, B. pensylvanicus, B. impatiens and other potentially affected species; determine whether other microsporidian species are present.*
2. *Develop protocols to determine the relative susceptibility to N. bombi of declining Bombus spp. compared to nondeclining species (e.g. B. impatiens)*
3. *Susceptibility testing*

Research Report: In the summer of 2005 with funding from US Fish & Wildlife Service (Project No. PO 134205M090), we initiated pilot studies to evaluate the parasites and pathogens of bumble bee populations in the Pacific Northwest and Midwest (Illinois) with the goal of addressing the issues identified above and to begin efforts to resolve the question about release of

N. bombi from bees reared in Europe and sent to California. The results of these studies were covered in the final report for the project.

In the second two seasons funded by the project addressed in this report, bumble bees sent to the Illinois Natural History Survey from Oregon and Northern California were evaluated for presence of parasites and pathogens, including *Nosema bombi*. This was a relatively small collection and prevalence could not be established for the natural enemies. The most frequently observed parasites and pathogens were tracheal mites, conopid (Diptera) parasites, *Crithidia* sp. (Protozoa) and *Nosema bombi* (Table 1) Bees were also collected from 20 sites in Illinois in the 2006 and 2007 seasons where no known commercial bee releases have taken place (Table 2).

Table 1. Overall occurrence of four parasites in *Bombus* species in northern California and southern Oregon in 2006.

<i>Bombus</i> species	N	Tracheal Mites ^a	Conopid Parasitoids ^a	<i>Crithidia</i> ^a	<i>Nosema</i> ^a
<i>appositus</i>	11	-	-	-	-
<i>bifarius</i>	11	-	yes	-	-
<i>californicus</i>	9	-	yes	-	-
<i>caliginosus</i>	4	-	-	-	yes
<i>fernaldae</i>	0	-	-	-	-
<i>fervidus</i>	6	-	yes	-	yes
<i>flavifrons</i>	11	-	yes	yes	-
<i>franklini</i>	0	-	-	-	-
<i>griseocollis</i>	3	-	yes	-	-
<i>insularis</i>	0	-	-	-	-
<i>melanopygus</i>	14	-	-	yes	yes
<i>mixtus</i>	11	yes	yes	yes	-
<i>morrisoni</i>	0	-	-	-	-
<i>nevadensis</i>	1	-	yes	-	-
<i>occidentalis</i>	0	-	-	-	-
<i>rufocinctus</i>	6	-	-	-	-
<i>sitkensis</i>	2	yes	-	-	yes
<i>suckleyi</i>	0	-	-	-	-
<i>vandykei</i>	30	-	-	yes	-
<i>vosnesenskii</i>	57	-	yes	yes	-

N = total number of individuals of each *Bombus* species collected;

Table 2. Presence of four parasites in *Bombus* species in Illinois in 2006 and 2007

<i>Bombus</i> Species	N	Tracheal Mites ^a	Conopid Parasitoids ^a	Multiple Parasitism ^b	<i>Crithidia</i> ^a	<i>Nosema</i> ^a
<i>affinis</i>	1	-	-	-	-	-
<i>ashtoni</i>	0	-	-	-	-	-
<i>auricomus</i>	37	-	yes	yes	-	-
<i>bimaculatus</i>	235	yes	yes	yes	yes	yes
<i>citrinus</i>	2	-	-	-	-	yes
<i>fervidus</i>	8	-	yes	yes	-	-
<i>fraternus</i>	0	-	-	-	-	-
<i>griseocollis</i>	429	-	yes	yes	yes	yes
<i>impatiens</i>	487	yes	yes	yes	yes	-
<i>pensylvanicus</i>	28	-	yes	yes	-	yes
<i>rufocinctus</i>	0	-	-	-	-	-

<i>vagans</i>	37	-	yes	-	-	yes
<i>variabilis</i>	0	-	-	-	-	-

N = number of individuals of each *Bombus* species collected; Tr. Mite = prevalence of tracheal mites (*L. buchneri*);

The low numbers of individuals of each species collected and sites represented in the western U.S. (data not shown) do not allow predictions about susceptibility of the remaining *Bombus* spp. to these natural enemies. This was considered a pilot study and the data provided the basis for more robust collections in 2007. The Illinois collections (Table 2) were more comprehensive and were conducted in the same sites (N=20; Figure 1) for two full seasons. Three species, *B. griseocollis*, *B. bimaculatus* and *B. impatiens* were collected in sufficient numbers to gain insights on prevalence of pathogens and parasites. We were unable to obtain collections from Canada for evaluation.

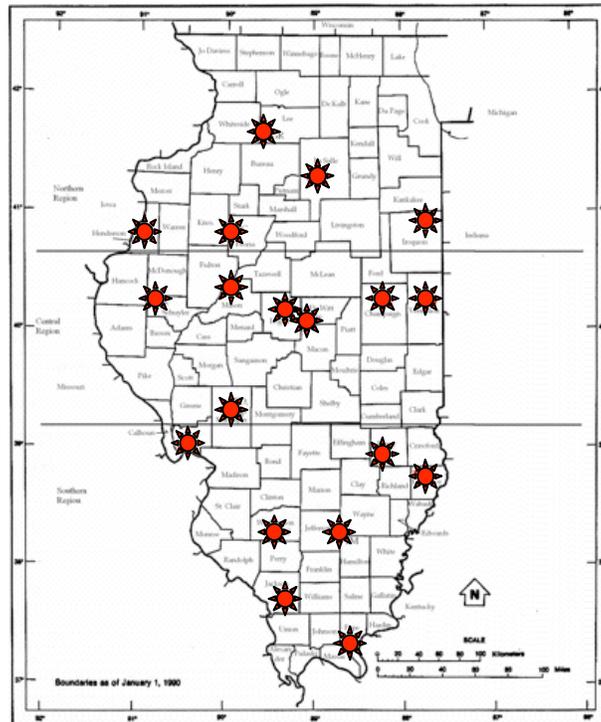


Figure 1. *Bombus* species collection sites in Illinois, 2005-2007.

Of the microsporidia isolated from collected bumble bees and sequenced, the sequences or partial sequences of the small subunit rDNA were identical to sequences of *Nosema bombi*, a naturally-occurring pathogen of European *Bombus* species. The rDNA gene is, however, known to be highly conserved in the microsporidia and we are working to identify additional genes or molecular techniques to evaluate potential differences between European and North American isolates.

Based on the pilot data collected in the summers of 2005-2006, we began more comprehensive and targeted regional surveys in collaboration with Drs. James Strange and Terry Griswold at the USDA-ARS Pollinating Insect Research Unit, Logan, Utah. In 2007, the western sites were chosen to delineate the 'receding edge' of *B. occidentalis* populations.

Bumble bees collected from California, Nevada, Oregon and Utah in the summer of 2007 with Dr. Strange have been processed in the laboratory; microsporidia were recovered from six *Bombus* species: *B. occidentalis*, a species of concern, *B. mixtus*, *B. vosnesenskii*, *B. californicus*, *B. huntii* and *B. bifarius*. Ribosomal DNA sequence data from these bees also appears to be a match with the European isolate of *N. bombi* and, as reported for the European *Bombus* species, the microsporidium appears to have a broad host range within the genus. *Bombus* populations were relatively low during the collection period and we cannot make predictions at this time regarding susceptibility or resistance of all collected *Bombus* spp. to *N. bombi*. We are currently evaluating the 2008 collection and will provide an additional report at the end of 2009 with the termination of PO #101817M496.

Outcomes, Products and Continuing Studies:

We developed a new collaboration with Drs. James Strange and Terry Griswold at the USDA-ARS Pollinating Insect Research Unit, Logan, Utah to assess the current status of bumble bee populations, study the potential role of pathogens/parasites in decline of *Bombus* spp., and assess the population structure and diversity of target *Bombus* species. We were awarded a USDA CSREES NRI grant (Aug. 2007-Aug. 2010). Pilot studies (reported here) were completed and a manuscript detailing the survey work is in progress. These studies also comprised the thesis research for Ms. Christina North, who earned her M.S. degree from the Department of Entomology, University of Illinois in 2008. We continue our studies supported in part by funding from the third cooperative agreement, U.S. Fish & Wildlife PO#101817M496. This third round of funding provided for a second collection in Oregon in the summer of 2008 (Solter) with Dr. Strange, and will provide 2009 summer salary for a new graduate student, Mr. Nils Cordes. Mr. Cordes will continue studies on *Nosema bombi*. He is currently completing dissections for collections made in the summer of 2008 to identify *Nosema*-infected populations, and is developing infection protocols to produce the microsporidium in the laboratory. He has also made plans to conduct comparative studies of susceptibility of a declining species, *B. occidentalis*, and a stable species, *B. impatiens*. This work will be conducted at the University of Illinois/INHS laboratory and at the USDA laboratory in Logan, Utah. *Bombus* collections and evaluation will continue in the summer of 2009.

References:

- Colla, S. R., M. C. Otterstatter, R. J. Gegear, and J. D. Thomson. 2006. Plight of the bumble bee: pathogen spillover from commercial to wild populations. *Biological Conservation* 129: 461-467.
- Colla, S. and L. Packer. 2008. Evidence for decline in eastern North American bumblebees (Hymenoptera: Apidae), with special focus on *Bombus affinis* Cresson. *Biodiversity and Conservation* 17, 1379-1391.
- Grixti, J.C., L.T. Wong, L.T., S.A. Cameron, C. Favret. 2009. Decline of bumble bees in the American Midwest. *Biological Conservation* 142, 75-84.
- Flanders, R. V., W. F. Wehling, and A. L. Craghead. 2003. Laws and regulations on the import, movement and release of bees in the United States. In K. Strickler and J. H. Cane (eds.). *For nonnative crops, whence pollinators of the future?* Thos. Say Publ. in Entomol., Proc. Entomol. Soc. Am., Lanham, MD. pp. 99-111.
- Thorp, R. W. 2005. Species Profile: *Bombus franklini*. In Shepherd, M. D., D. M. Vaughan, and S. H. Black (Eds). *Red List of Pollinator Insects of North America*. CD-ROM Version 1 (May 2005). Portland, OR: The Xerces Society for Invertebrate Conservation.