

Surveying Midwestern Ant (Hymenoptera: Formicidae) Communities to Facilitate Successful Karner Blue (*Lycaeides melissa samuelis* Nabokov) Introductions

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Abstract: Mutualisms between ants and butterfly larvae, particularly those in the family Lycaenidae, can greatly enhance the survival of both groups. The Karner blue butterfly (*Lycaeides melissa samuelis* Nabokov; KBB) is an endangered species that has experienced significant range reduction, including extirpation from Minnesota. Current KBB conservation efforts include a plan to introduce KBB to a novel location in Minnesota. Since ant-tending is known to decrease KBB mortality, ants may play a crucial role in successful introduction efforts. This study sought to assess potential ant-KBB interactions by sampling ant communities at three sites: those with a current KBB population, those with a history of KBB presence, and those proposed for KBB introduction. We found the presence of KBB, both historic and present, to impact the diversity of both total ants and the diversity of ants known to tend KBB larvae. The site for the proposed KBB introduction had lower ant diversity in terms of overall ant composition and for those species known to tend to KBB larvae.

Introduction

Mutualism is a type of symbiotic relationship in which both species benefit, though the nature and degree of benefits can vary. Unlike parasitism, where one species benefits at the expense of another, or commensalism, where one species benefits while the other is unaffected, mutualism provides advantages to both partners. These benefits can include shelter [1], protection [2], and nutrient supplementation [3], providing access to otherwise unavailable resources. Ants

(Hymenoptera: Formicidae) are one of the most well recorded groups of insects to participate in these mutualistic relationships. Several ant species are known to form facultative mutualistic interactions with various other insects [4]. One such interaction is that of ants and Karner blue butterfly (*Lycaeides melissa samuelis* Nabokov) larvae [5]. Ants protect these larvae from predation and parasitoids, and in return, they collect honeydew from special glands located on the abdomen of KBB larvae [5]. Several studies have previously explored

KBB-ant community relationships [6-10], however little is known about how this relationship could influence the species composition of ants, as KBB-tending ants receive nutrient supplementation that non-tending ants do not. Less is known about Minnesotan ant populations or which ant species participate in KBB tending behavior.

KBB was historically found across the northeastern United States, primarily found in oak savannas containing its obligate host plant, *Lupinus perennis* L. (wild blue lupine) (Fabales: Fabaceae) [11]. While previously abundant, KBB has lost much of its home range due to anthropogenic habitat conversion [12] and was listed as federally endangered in 1992. With the loss of Minnesota's oak savannas, KBB has disappeared from the state. Recently a plan has been proposed to introduce KBB to a new site in Minnesota where it has not been previously recorded [13]. This location contains one of the most diverse restored prairies in the state with newly planted populations of wild lupine [14]. However, the ant populations in this area have not been considered a part of this novel introduction proposal.

The goal of this research was to assess ant populations at locations of known KBB occurrence, previous KBB occurrence, and proposed KBB introduction. We hypothesize that the presence of KBB will impact ant population composition due to the inherent nutritional benefits of tending behavior. We further sought to investigate whether there were legacy effects, or long-term influence, of KBB's former presence on existing ant populations.

Methods

Sites

Ant populations were assessed at a site known to hold a reproducing KBB population, at a site of previous KBB occurrence, and at a site that has been proposed for KBB introduction (Figure 1). KBB has been recorded at Fish Lake Wildlife Area in Wisconsin (FL) and was confirmed to be present at the time of ant sampling. The presence of KBB was previously known at the Whitewater Wildlife Management Area in Minnesota (WW) but has not been detected at this site since 2009. The cause of decline at Whitewater Wildlife Management Area is unknown. This site was sampled to assess the potential legacy effects of KBB-ant interactions. KBB is not known from Crow-Hassan Park Reserve in Minnesota, nor is this site in the known range of the species.

Sampling

Sampling protocols followed the methods of Agosti et al. [15]. Two 100 meter transects were established at each site. Ten standard pitfall traps were placed along each transect, evenly spaced every 10 meters. Pitfall traps consisted of a 120 mL specimen cup placed into the ground so that the lip of the cup was even with the ground. A Styrofoam plate was suspended above each trap by 3 nails, leaving a roughly 20 cm gap between the ground and the plate. Each trap was filled halfway with polyethylene glycol mixed with a drop of dish soap to break the surface tension. Once activated, pitfall traps were in place for 7 days. On collection, traps were removed from the ground, sealed, and returned to the lab. Pitfall traps were activated across two time periods,

one corresponding to the first emergence of KBB and the other to its second emergence of KBB (FL:6/02/22-6/09/22, 7/19-7/26/2022; WW: 5/30-6/07/2022, 7/20-7/27/2022; CH: 6/01-/08/2022, 7/22-7/29/2022).

All ant specimens were sorted from the other specimens collected into traps and transferred into 70% ethanol. Specimens were identified morphologically to species level using published keys [16-17]. All ant species were categorized as either i) known to tend to KBB immatures, or ii) not known to tend to KBB immatures. Caterpillar tending was determined based on published records of KBB-ant tending interactions [6-10].

Statistical analyses

Collected ants at each site were characterized based on richness (genus, species) and diversity. We examined the number of species shared between each of the three sites. Diversity was evaluated using Simpson's diversity index [18] to minimize bias from rare species and sampling effort, while comparing sites with different species compositions. Simpson's diversity was calculated for each site based on i) all collected ants, and ii) only ants known to actively tend KBB. Analyses were conducted in R using the vegan 2.6-8 package [19].

Results

A total of 2,174 ant specimens were collected from all sites and sampling periods (Table 1). Genus and species richness was greatest at FL (12 genera, 22 spp.), followed by WW (12 genera, 18 spp.), and CH (8 genera, 15 spp.) Diversity was similar across all sites (CH: 0.611, FL: 0.732, WW: 0.661) however when

only tending ant species were considered, both FL (0.615) and WW (0.594) appeared more diverse than CH (0.316). Despite WW containing a higher proportion of tending species than FL, WW had a lower overall specimen count than FL (Figure 2 & Table 1). This discrepancy led to FL having higher diversity for overall ant species as well as tending ant species.

Discussion

Our results suggest a diverse ant fauna across the three study sites. Overlap in species composition was moderate, with each site maintaining many unique species. Sites with a known KBB history were more similar to each other in overall species composition and known tending species compared to the proposed introduction site. These results suggest that the presence of KBB may have an impact on ant diversity. The ant composition at WW further suggests that KBB presence could have a possible legacy effect on ant populations.

Environmental filtering often acts as a determining factor in structuring ant communities, particularly at broad spatial scales [20]. This process is especially influential when species must share key functional traits to persist under similar conditions. In this study, we expected the ant community at CH to differ from WW and FL due to the absence of KBB. If KBB-associated honeydew acts as a selective resource, it could drive environmental filtering by favoring an increase in KBB-tending species. Our results align with this expectation, as we observed a greater diversity of KBB-tending species in FL and WW, sites with a historical KBB presence.

The role of ant diversity in caterpillar survival, including KBB larvae, remains complex. The ecological dominance of ants can shape biodiversity across trophic levels [21]. However, not all ant species contribute equally to Lycaenid survival, as tending behavior can be highly species-specific [22]. It is uncertain whether KBB introduction would result in a substantial change in ant community composition at this site. Ant community composition is a result of environmental filtering and resource-driven shifts. Traits such as thermal tolerance and competitive ability strongly influence ant fitness under varying environmental conditions [23]. Our study did not assess the full complement of factors responsible for ant community composition

across the three sites. Further research is needed to determine the impact of factors beyond KBB in shaping these communities.

The factors influencing species composition at a site include abiotic factors, biotic factors, and the ability of the species to find favorable habitats. While our study examined a limited number of sites, our results suggest spatial variation in ant communities is present. In particular, the presence, or past presence, of KBB was found to be associated with higher ant diversity. This was particularly true for those species known to tend KBB. As such, our results suggest the survival of KBB in Minnesota may be benefited by introduction into habitats with a known KBB history.

Supplements



Figure 1. Map of Minnesota and Wisconsin, showing the sites surveyed for ants in red. The diamond indicates CH, Crow-Hassan. The point FL indicates Fish-Lake. The point WW indicates Whitewater.

Subfamily	Genus	Specific epithet	Specimens	Ref. for tending
Dolichoderinae	<i>Dolichoderus</i>	<i>pustulatus</i>	7	Herms (1996)
Dolichoderinae	<i>Dolichoderus</i>	<i>sp.</i>	1	
Dolichoderinae	<i>Tapinoma</i>	<i>sessile</i>	34	Bleser (1992), Lane (1999)
Formicinae	<i>Camponotus</i>	<i>pennsylvanicus</i>	16	Packer (1991)
Formicinae	<i>Camponotus</i>	<i>sp.</i>	2	
Formicinae	<i>Formica</i>	<i>fossiceps</i>	2	
Formicinae	<i>Formica</i>	<i>incerta</i>	15	Savignano (1994), Lane (1999)
Formicinae	<i>Formica</i>	<i>laeviceps</i>	1	
Formicinae	<i>Formica</i>	<i>pallidefulva</i>	3	Savignano (1994), Herms (1996)
Formicinae	<i>Formica</i>	<i>pergandei</i>	2	
Formicinae	<i>Formica</i>	<i>sanguinea</i>	1	
Formicinae	<i>Formica</i>	<i>dolosa</i>	35	
Formicinae	<i>Formica</i>	<i>fusca</i>	31	Bleser (1992)
Formicinae	<i>Formica</i>	<i>obscuriventris</i>	109	
Formicinae	<i>Lasius</i>	<i>pallitarsis</i>	1	
Formicinae	<i>Lasius</i>	<i>sp.</i>	7	
Formicinae	<i>Lasius</i>	<i>neoniger</i>	1120	Savignano (1994), Lane (1999)
Formicinae	<i>Nylanderia</i>	<i>parvula</i>	128	
Formicinae	<i>Nylanderia</i>	<i>sp.</i>	4	
Formicinae	<i>Prenolepis</i>	<i>imparis</i>	1	Lane (1999)
Myrmicinae	<i>Aphaenogaster</i>	<i>mariae</i>	1	
Myrmicinae	<i>Aphaenogaster</i>	<i>rudis</i>	7	Packer (1991)
Myrmicinae	<i>Aphaenogaster</i>	<i>treatae</i>	37	
Myrmicinae	<i>Crematogaster</i>	<i>cerasi</i>	6	Savignano (1994)
Myrmicinae	<i>Crematogaster</i>	<i>lineolata</i>	105	Herms, 1996
Myrmicinae	<i>Crematogaster</i>	<i>sp.</i>	5	
Myrmicinae	<i>Monomorium</i>	<i>minimum</i>	18	
Myrmicinae	<i>Myrmica</i>	<i>americana</i>	50	Herms (1996), Lane (1999)
Myrmicinae	<i>Myrmica</i>	<i>latifrons</i>	404	Lane (1999)
Myrmicinae	<i>Myrmica</i>	<i>punctiventris</i>	9	Packer (1991)
Myrmicinae	<i>Solenopsis</i>	<i>molesta</i>	73	
Myrmicinae	<i>Temnothorax</i>	<i>ambigus</i>	1	
Myrmicinae	<i>Temnothorax</i>	<i>sp.</i>	5	
Myrmicinae	<i>Tetramorium</i>	<i>caespitum</i>	3	Bleser (1992)
Myrmicinae	<i>Tetramorium</i>	<i>immigrans</i>	1	
Ponerinae	<i>Ponera</i>	<i>pennsylvanica</i>	8	

Table 1. Ant species collected during sampling in 2022. References are given for evidence of tending of ant species by KBB.

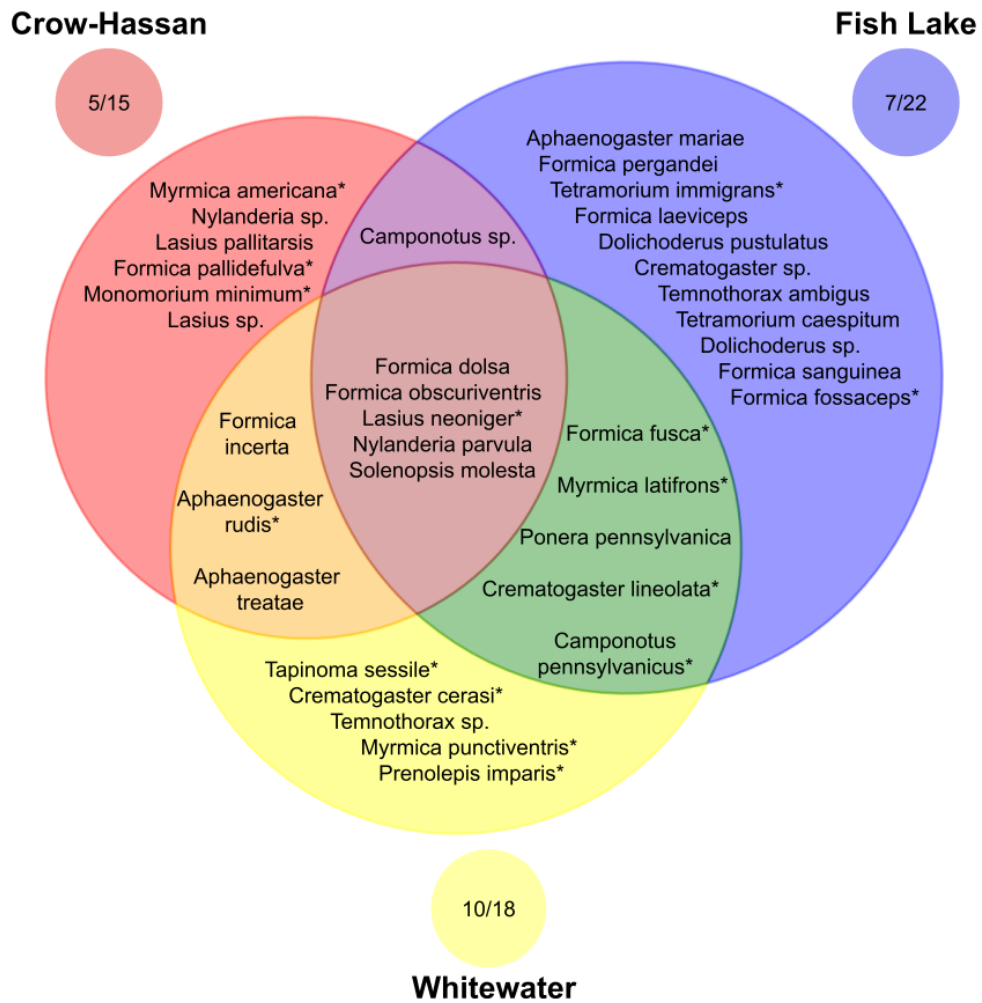


Figure 2. Shared species found at each site. Small outer circles show the number of tending species/total species at each site, while larger circles show shared species between the three sites (*Tending species).

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