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humulus* (Lepidoptera: Lycaenidae)**

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**FACULTATIVE MYRMECOPHILY (HYMENOPTERA:
FORMICIDAE) IN THE HOPS BLUE BUTTERFLY,
CELASTRINA HUMULUS (LEPIDOPTERA:
LYCAENIDAE)¹**

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ABSTRACT: The hops blue butterfly (*Celastrina humulus*) is a rare North American butterfly, found in the Rocky Mountain region of the United States in areas where its host plant, wild hops (*Humulus lupulus*), grows in abundance. As a member of the Lycaenidae we considered it likely that myrmecophiles may exist with ants found in *C. humulus* habitats. To better understand the ecology of this species and guide conservation efforts, we investigated and documented myrmecophily with multiple ant species. We found seven ant species with close associations with *C. humulus* larvae.

KEY WORDS: *Camponotus*, *Celastrina humulus*, conservation, *Formica*, hops blue butterfly, myrmecophily, *Myrmica*, *Pogonomyrmex*, *Tapinoma*

The Lycaenidae are a diverse family of approximately 6,000 species worldwide and include coppers, hairstreaks, and blues (Eliot, 1973; Ackery et al., 1999). Many of these butterflies are known to have evolved relationships with ants, called myrmecophilies (Pierce et al., 2002). The underlying associations between the ants and butterflies can vary from parasitic, usually associated with specific ant species, to mutualistic or commensal myrmecophilies, usually less specific relationships with several different ant species (Pierce et al., 2002; Fiedler, 2012). These latter relationships typically provide a benefit to the butterfly larvae without incurring costs to the ants (Fiedler, 1999). Commonly, lycaenid larvae provide tending ants with beneficial sugar or amino acid-rich nectar rewards in exchange for defense against predation and parasitoid attack (Fiedler and Maschwitz, 1988; Fiedler and Maschwitz, 1989). The lycaenid larvae receive a survival and competitive advantage over other lepidopteran herbivores that cannot feed in areas that are patrolled by predatory ants (Fiedler, 1991; Fiedler and Saam, 1995).

The hops blue butterfly (*Celastrina humulus* Scott and Wright 1998) is a locally distributed western Nearctic lycaenid butterfly, occurring along the Colorado Front Range, and possibly as far north as Montana (Scott and Wright, 1998; Fisher, 2009). *Celastrina humulus* feed on wild hops (*Humulus lupulus* L.), which grow along riparian drainages and gulch bottoms with plentiful solar exposure (Scott and Wright, 1998; Punttenney and Schorr, 2016). Populations of *C. humulus* can be locally abundant, but limited to areas where wild hops grow, because females lay a majority of their eggs on the male flower buds of the wild

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hops plant (Scott, 1992). In the southern part of their range, occupancy rates of *C. humulus* increase with the size of the patch of wild hops (Puntenney and Schorr, 2016).

As with many lycaenid butterflies, myrmecophilies likely exist for *C. humulus* (Scott and Wright, 1998). Microscopic secretory structures commonly associated with the release of sedentary volatiles that aid in the mitigation of ant aggression and the provision of honeydew rewards are found on *C. humulus* (Fiedler and Maschwitz, 1989; Pierce et al., 2002; J. A. Scott, personal communication). Additionally, occupancy rates of *C. humulus* increase at wild hops patches where ant colonies are present (Schorr, unpublished data). Because of the spatial association with ant colonies, the presence of possible myrmecophilous organs, and the frequency of myrmecophilies in the Lycaenidae, we initiated a study to identify and describe the ecological nature of myrmecophily in the *C. humulus* populations in the southern part of the species' range.

METHODS

Using data from *C. humulus* occupancy studies (Puntenney and Schorr, 2016), 15 sites were selected along a 22.5-km (14-mile) stretch of Monument Creek located on the grounds of the United States Air Force Academy (Academy), El Paso County, Colorado (Puntenney and Schorr, 2016). At study sites, we (TDK) would walk within the riparian, shrub-dominated habitats adjacent to Monument Creek looking for patches of hops. Once patches were found we would locate and follow female *C. humulus* (Fig. 1A) to the locations where they were ovipositing (Fig. 1B). Surveys were conducted during the expected adult flight period, starting on June 6th and continued until July 27, 2016 (Scott, 1992). Each site where egg-laying was observed was flagged for recognition on each subsequent visit. After egg-laying we (TDK) began revisiting each location every second or third day to locate larvae. During subsequent observations, we (TDK) would spend two hours searching male and female hops flowers looking for larvae.

Surveys were conducted from 7:00 AM to 12:00 PM and 5:00 PM to 7:00 PM daily (Scott and Wright, 1998). Ant behaviors, such as active antennation, walking back and forth over larvae, alarm behavior, and aggression were noted (Ballmer and Pratt, 1991). Photographic and videographic data were recorded using a Samsung Galaxy S4 mobile telephone (Samsung Electronics Co. Ltd, Seoul, Korea). Voucher specimens were collected using sweep nets, aspiration, and hand capture. All specimens were preserved and donated to the C. P. Gillette Museum of Arthropod Diversity (Colorado State University, Fort Collins, Colorado).

RESULTS AND DISCUSSION

All 15 survey locations had adult *C. humulus* in flight. We found egg-laying occurring at all sites, and we found larvae at all sites. A total of 72 egg-laying events were observed and a total of 128 larvae were detected (Figs. 2A, 2B).



Fig. 1. Hops blue butterfly (*Celastrina humulus*) adult females. A. Dorsal view of an adult female with wings open; and B. An adult female ovipositing on male wild hops (*Humulus lupulus*) flowers. Photographs by Michael Menefee, Colorado Natural Heritage Program.

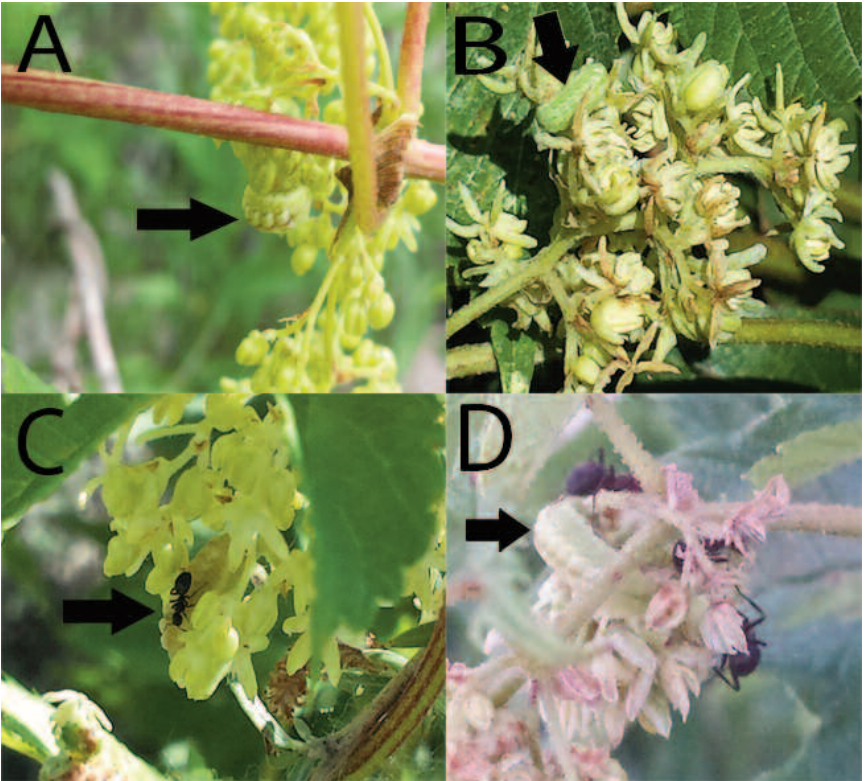


Fig. 2. Hops blue butterfly (*Celastrina humulus*) larvae and tending ants. Black arrows identify the location of larva. A. *C. humulus* larva on male hops (*Humulus lupulus*) flowers; B. *C. humulus* larva on male hops flowers; C. *C. humulus* larva being tended by *Tapinoma sessile*; and D. *C. humulus* larva tended by *Formica* sp.

Myrmecophily was observed at 10 of the 15 study sites. The ants observed tending *C. humulus* larvae included *Tapinoma sessile* (Say) (Fig. 2C), three species from the genus *Formica* (Fig. 2D), *Camponotus herculeanus* L., *Pogonomyrmex occidentalis* (Cresson), and *Myrmica rugosa* (Mayr). Though attendant ants in the genus *Formica* could not be identified down to species, there were consistent differences in physical characters, i.e. body size, coloration, and nest structure, that regularly correlated with locality leading to the assumption that there were three different species of *Formica* tending larvae despite the absence of verified species identifications. Although larvae of each instar were available, ants were not observed tending larvae between first and third instars, but were only found tending fourth instar lycaenid larvae. When not tending larvae, ants were observed collecting pollen and nectar from male flower buds.

Upon the initial discovery of fourth instar larvae, formicid workers would display aggressive behaviors and the *C. humulus* larvae would cease feeding to withdraw their heads into their thickened hood of the prothoracic shield (Malicky, 1970). Early ant aggression included mandibular probing and acidopore, and recruitment of fellow nestmates in attempts to harvest the discovered prey. The aggression typically lasted less than five seconds after larval discovery, but sometimes subsided only after the aggressor had recruited a number of nestmates. Aggressive behaviors were followed by antennal behaviors exploring the entire larva. Antennation would cease when the ant reached the posterior segment of the larva where an antennation occurred similar to that seen when tending their own ant brood (Pierce et al., 2002). Once posterior antennation had occurred, the *C. humulus* larvae resumed feeding and a honeydew droplet was excreted and imbibed by the tending ant. This behavior was observed amongst all observed species of ants. Nearly 30% (4) of the sites hosted more than one of the documented myrmecophilous ant species.

The presence of particular ant taxa frequently appeared to depend upon the surrounding habitats. *Camponotus herculeanus* only occurred in areas where fallen pine and deciduous trees were readily available (Harmon et al., 1986). Similarly, the wetland-associated *M. rugosa* was only in areas of periodic flooding of riparian edges (Gregg, 1963). In the study sites located in arid high plains grasslands, *P. occidentalis* was observed tending *C. humulus* larvae (Johnson, 2000).

Myrmecophily of *C. humulus* showed a size bias for interactions. When multiple species of ants were observed in an area, only the larger ant taxa were observed tending larvae. For example, when the smallest *Formica* species was found foraging in conjunction with *C. herculeanus*, the latter was found tending *C. humulus* larvae in all observed instances of myrmecophily. Similarly, when *T. sessile* was observed foraging in the presence of a larger myrmecophilous *Formica* species, the smaller *T. sessile* were never observed tending the caterpillars. In areas where myrmecophilous *Formica* spp. and *M. rugosa* were observed foraging together, the larger *Formica* species was the only species observed tend-

ing *C. humulus* fourth instars. Finally, in arid areas where *Formica* spp. and *P. occidentalis* species foraged together, only the larger *P. occidentalis* was found tending *C. humulus* larvae.

The largest myrmecophilous ant species (*Formica* spp., *C. herculeanus*, and *P. occidentalis*) tended *C. humulus* larvae in groups of three to five workers. Each group included at least one soldier, and they defended the caterpillars from sphecids and pompilid attacks on several occasions. Furthermore, when ants perceived the researcher's presence as a threat, the larger taxa readily stood their ground and attacked the researcher's probes and camera. The smaller myrmecophilous ant species (*Formica* sp., *M. rugosa*, and *T. sessile*), tended *C. humulus* larvae in groups no larger than two.

Individuals of the smaller ant taxa regularly fled from larger predatory insects. In one instance, individuals abandoned a *C. humulus* larva to a predaceous pentatomid (*Podisus* sp.), which successfully preyed upon the abandoned larva. These species would regularly flee when the vegetation was disturbed. *Tapinoma sessile* was only observed tending *C. humulus* larvae when nearby nesting sites were releasing alates. This was observed on three occasions at one site. At no other time was *T. sessile* observed tending *C. humulus* larvae.

These facultative myrmecophilies between *C. humulus* and ants that use the same riparian floodplains confirms some of the myrmecophilies suspected in the northern parts of Colorado (Scott and Wright, 1998). This butterfly is of special concern because of its limited range and host-specificity (Puntenney and Schorr, 2016), and understanding the nature of the myrmecophilies may be important to *C. humulus* conservation (New, 1993). The *C. humulus* myrmecophilies likely provide a tangible benefit, such as increased larval growth and success (Fiedler and Saam, 1995; Fraser et al., 2001), but we did not monitor larval development and survival to assess comparative changes. Also, it is likely that various ant species provide some level of defense against predators or parasites (Pierce et al., 2002), despite ants' inability to deter a pentatomid predator. Larvae of *C. humulus* feed on male hops flower buds that provide an abundant source of protein, and it is likely that this diet provides the caterpillars with excess proteins, which may be used to provide ants with proteins and essential amino acids that are otherwise difficult for the ants to acquire (Pierce, 1985; Fiedler and Saam, 1995).

The size bias seen in tending ant species may indicate larvae-mediated rewards for select species or competition among the ant species for rewards. Namely, the presence of any of the four larger ant species (*Formica* spp., *C. herculeanus*, and *P. occidentalis*) provided defense against some predators including pentatomids, sphecids, and pompilids, while smaller ant species may be less effective at predator defense (Fraser et al., 2001). The four large ant taxa tended in groups of 3-5 workers, often with a soldier caste present, suggesting that these ant taxa may benefit from investing larger numbers of workers in caterpillar defense. The three small ant taxa tended only in groups of 1-3 workers, often without a soldier caste present, suggesting that these ant taxa may benefit less from investing

smaller numbers of workers in caterpillar defense. This tendency for fewer individuals of the smaller ant species to be present whenever tending larvae, suggests the larvae may actively try to recruit more of the larger ant species to provide better defense (Hölldobler and Wilson, 1990). Also, these larger ant species were seen displacing smaller ant species, and it is possible the larger species were competing for ant-mediated rewards, or the rewards were more appealing to these larger ant species. It is possible that *C. humulus* larvae are capable of recruiting multiple tending ants by sequestering and offering secretions of particular nutritive value or of particular volume in exchange for a level of defense (Fiedler and Maschwitz, 1988; Fielder and Maschwitz, 1989). Alternately, some ants may rely on larvae rewards only during particular events. *Tapinoma sessile* may extend its foraging to atypical sources during energy intensive events like fighting and release of reproductive alates (Hölldobler and Wilson, 1990). The infrequency of tending by *T. sessile* suggests that it is likely an opportunistic feeding strategy rather than a myrmecophily.

The nature of *C. humulus* relationships to individual ant species is unclear. Based on the diversity of ant taxa tending the larvae, the myrmecophily does not appear to be species-specific, but rather a more generalist mutualism or commensalism (Pierce et al., 2002). The initial predatory behaviors seen in some attendant ants is not convincing evidence of obligate associations, because initial aggression could result in successful predation of *C. humulus* larvae by non-mutualistic partners. Although species-general obligate myrmecophilies have been observed, they are uncommon (Pierce et al., 2002).

The formicid attendance of fourth instar larvae suggest that the dorsal nectary organs and other miscellaneous ant-related secretory structures do not develop in *C. humulus* larvae until the fourth instar, where they persist possibly into the pupa (Pierce et al., 2002). The rearing temperatures, humidity, and light availability documented for *C. humulus* are similar to those recorded for certain ant species' subterranean winter nesting conditions (Hölldobler and Wilson, 1990; Scott, 1992). It could be possible that *C. humulus* takes advantage of ant provisioned shelter during pupation as such behaviors are not uncommon in other lycaenids (Pierce et al., 2002). Further investigations into pupation of *C. humulus* in the wild would help in explaining the nature of the butterfly's myrmecophily.

Although there may be a higher likelihood of a patch of wild hops being occupied by *C. humulus* adults when ant mounds are present, the abundance of *C. humulus* adults was more dependent upon the abundance of host plants rather than the abundance of myrmecophilous ants (Schorr, unpublished data). Oviposition did not appear to be dependent upon the presence of myrmecophilous ants because there were multiple records of oviposition and larvae at hops plants without tending ants. All of this suggests that *C. humulus* adults do not rely heavily upon ants as oviposition cues, as has been documented among other lycaenid myrmecophilies (Pierce and Elgar, 1985).

Given the conservation status of *C. humulus* in a region where its habitat has been reduced (Kuby et al., 2007), it is vital to understand as much as possible about its natural history and any constraints that might limit populations or distribution. This study expanded the list of myrmecophilies for this species, but it is important to further study the cost and benefits of these relationships for *C. humulus* larvae (Fiedler, 2001). It is also important to know the ants' role in *C. humulus*' larval development and survivorship. Such investigations may clarify the importance of ant taxa for invertebrate conservation, and aid in understanding the value of healthy ecosystems, such as the riparian systems at the Academy, for maintaining greater diversity of ants, butterflies, and other species (Philpott et al., 2010).

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Table 1. *Celastrina humulus* adults and larvae seen and ant-larvae interactions at 15 locations along Monument Creek, U.S. Air Force Academy, El Paso County, Colorado, June - July 2016.

Site	<i>C. humulus</i> adult	larvae	Number of individuals that palpatated larvae					Total
			<i>Formica</i> <i>spp.</i>	<i>Pogonomyrmex</i> <i>occidentalis</i>	<i>Myrmica</i> <i>rugosa</i>	<i>Camponotus</i> <i>herculeanus</i>	<i>Tapinoma</i> <i>sessile</i>	
1	7	18	2	0	0	0	0	2
2	3	6	5	0	0	0	2	7
3	5	12	0	0	0	0	0	0
4	2	6	0	0	0	0	0	0
5	2	7	0	0	0	11	0	11
6	16	23	8	0	6	0	0	14
7	3	9	0	0	0	0	0	0
8	2	3	0	0	0	0	0	0
9	4	11	7	0	0	0	0	7
10	3	5	11	0	0	0	0	11
11	4	3	0	0	0	0	0	0
12	1	6	0	6	0	0	0	6
13	2	3	5	0	0	0	0	5
14	1	4	6	0	0	0	0	6
15	4	12	6	0	7	0	0	13