Facultative ant mutualism in the rare hops azure butterfly, *Celastrina humulus* (Lycaenidae)

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Introduction to the Study System

Conservation of rare species relies on understanding how biotic and abiotic factors shape abundance [1]. The hops azure butterfly (*Celastrina humulus*, Family Lycaenidae) is ranked as a G2 Imperiled species by NatureServe [2]. Butterflies lay eggs on male flowers of native hops, *Humulus lupulus* var. *neomexicanus* (Fig. 1A). Larvae develop quickly feeding on the pollen-rich male inflorescences (Fig. 1B). Like other lycaenids, hops azure larvae can form associations with several species of ants [3], which walk over the caterpillars and drink nectar from specialized organs (Fig. 1C). Kubik and Schorr (2018) found hops azure larvae tended by ants in 10 of 15 sites along Monument Creek near Colorado Springs, CO [3]. Given the well documented benefits of mutualism with ants [4], it is unclear why many larvae do not form associations with ants. **Our objective was to assess which biotic and abiotic factors were associated with ant tending.**

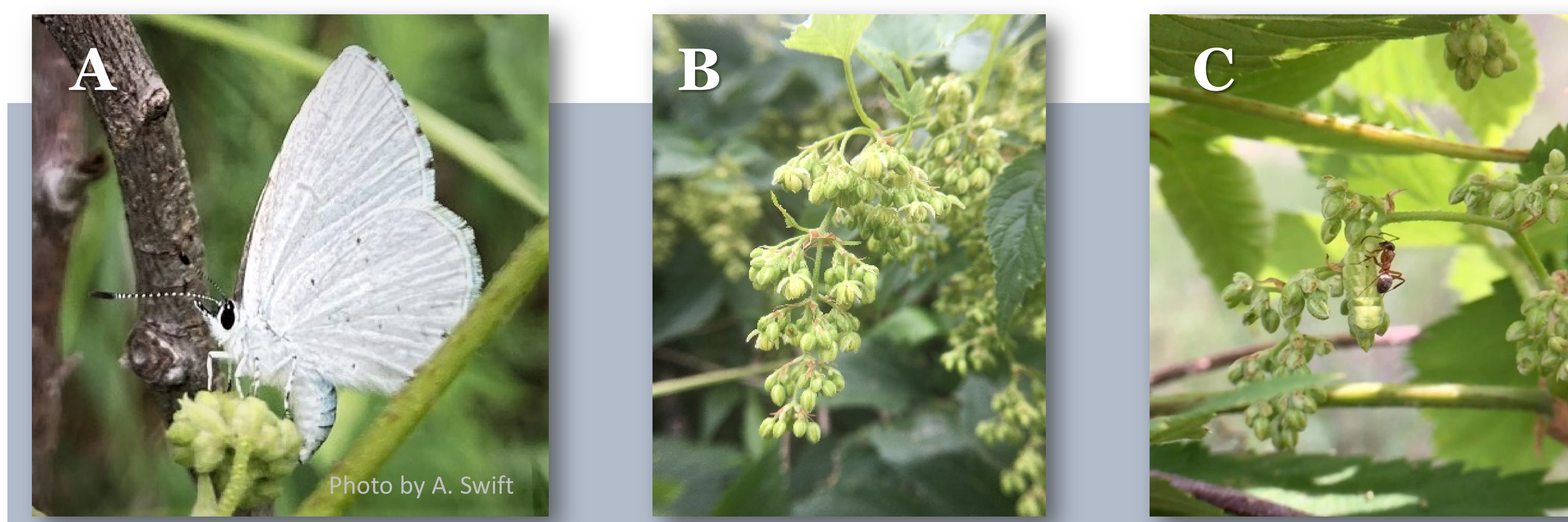
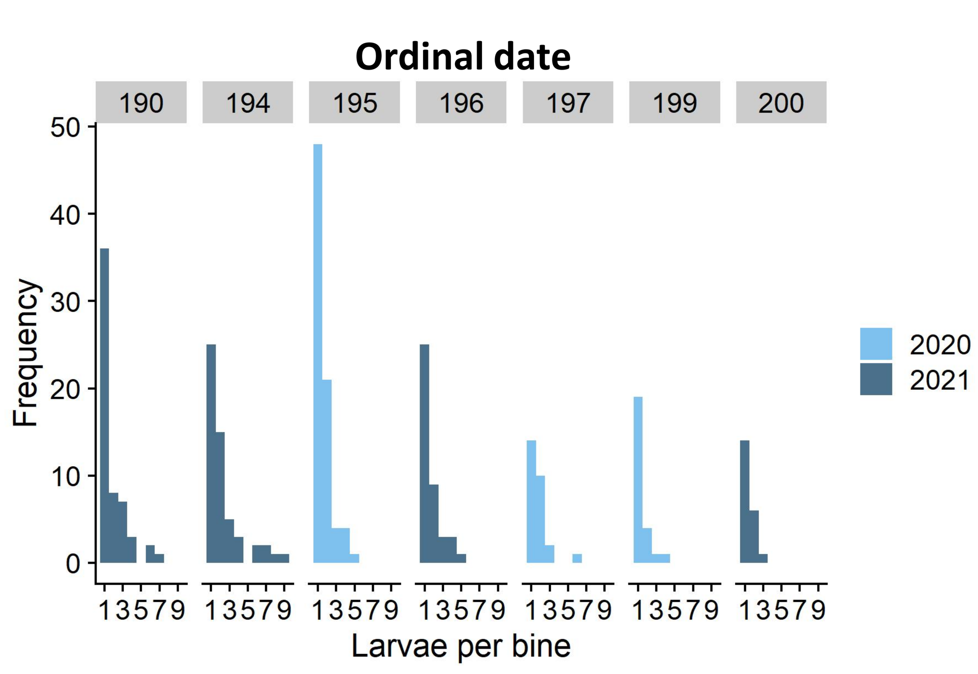


Figure 1 Female hops azure laying eggs on an early-stage male inflorescence (A), panicle of male flowers of hops (B), and an ant tending a larvae while it feeds on male flowers (C); in panel C, flowers show characteristic herbivory of pollen inside sepals.

Methods In the Field

We surveyed larvae on **134 unique bines** (flowering vines) along a 1.5 km section of Monument Creek in Colorado Springs, CO. Once larvae were found, we used replicate surveys to record the number of larvae on the bine, larval developmental stage, ant attendance, ant species, and floral phenology (Fig. 2). We ranked phenology of male flowers using a numerical score (Fig 3). To account for variation among host plants and their environments, we measured light (PAR) above each bine using a meter and patch area. **We surveyed bines within the same host plant population in 2020 and 2021**.



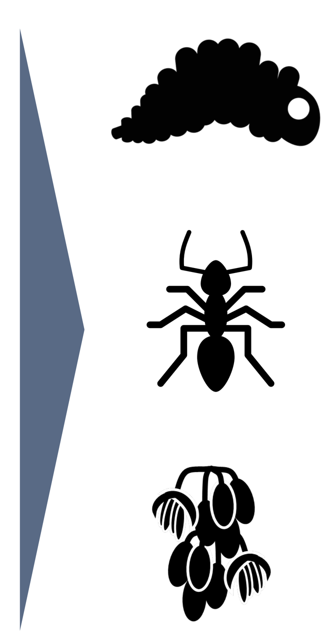


Figure 3 Male hops inflorescences at varying phenological stages; at stage 1, buds have elongated into oval, but anthers remain inside of sepals; by stage 6, anthers have begun to dehisce.

Model Selection and Results

We used a model selection approach to determine which of the factors we measured best explained variation in likelihood of ant tending (Y/N). We fit mixed effects models with binomially distributed errors using the 'glmer()' in the package *lmerTest* [5]. The model set included univariate, additive, and 2-way interactive models (Table 1). After initial analysis failed to support models that included an effect of larval stage, we did not include this factor in the final model set. We obtained AICc and ΔAIC scores from the *AICcmodavg* package [6]. All analyses were performed in R version 4.1.1 [7].

Table 1 Models and fit statistics

Model	Weight	ΔAICc
Ants? ~ Larvae + Flowerstage + Area + (1 | Year)	0.567	0
Ants? ~ Larvae + Flowerstage + Area+ Light + (1 | Year)	0.752	0.590
Ants? ~ Larvae + Area + Light + (1 | Year)	0.884	1.644
Ants? ~ Larvae + Flowerstage * Light + Area + (1 | Year)	0.946	2.705
Ants? ~ Larvae + Flowerstage * Area + (1 | Year)	0.987	3.949
Ants? ~ Larvae + Flowerstage * Area + Light + (1 | Year)	0.998	3.965
Ants? ~ Larvae + Area * Light + (1 | Year)	0.999	4.439
Ants? ~ Larvae + Flowerstage + (1 | Year)	1	4.923
Ants? ~ Larvae + (1 | Year)	1	8.849

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What's Associated with Ant Tending?

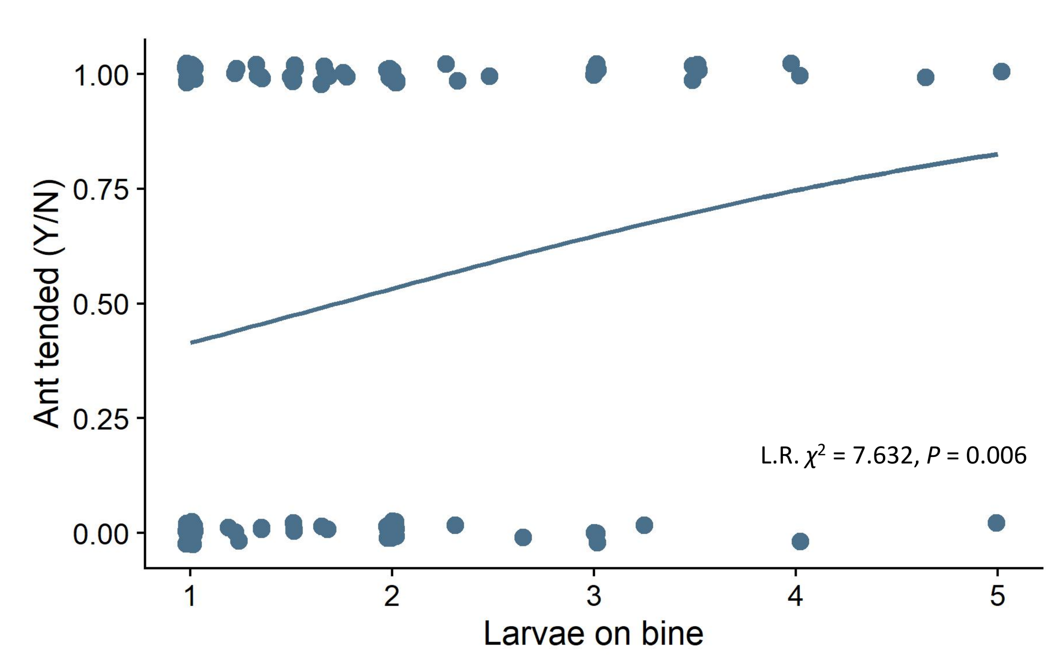

Figure 4 Greater densities of larvae per bine was associated with increased likelihood of ant tending. **More larvae may simply attract more ants.** Such density dependence can be common in ant mutualisms [6]

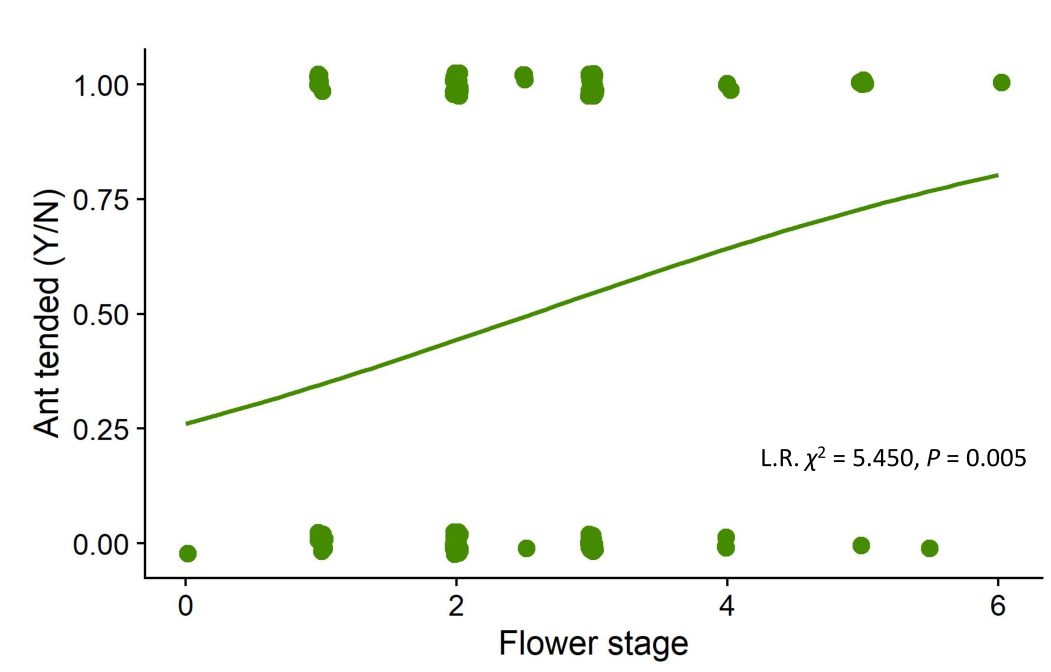

Figure 5 Larvae on bines with later stage flowers were more likely to be ant tended. Flowering phenology can mediate associations between herbivores and ants [9]. **Nectar from larvae feeding on later stage flowers may be more attractive to ants** [10].

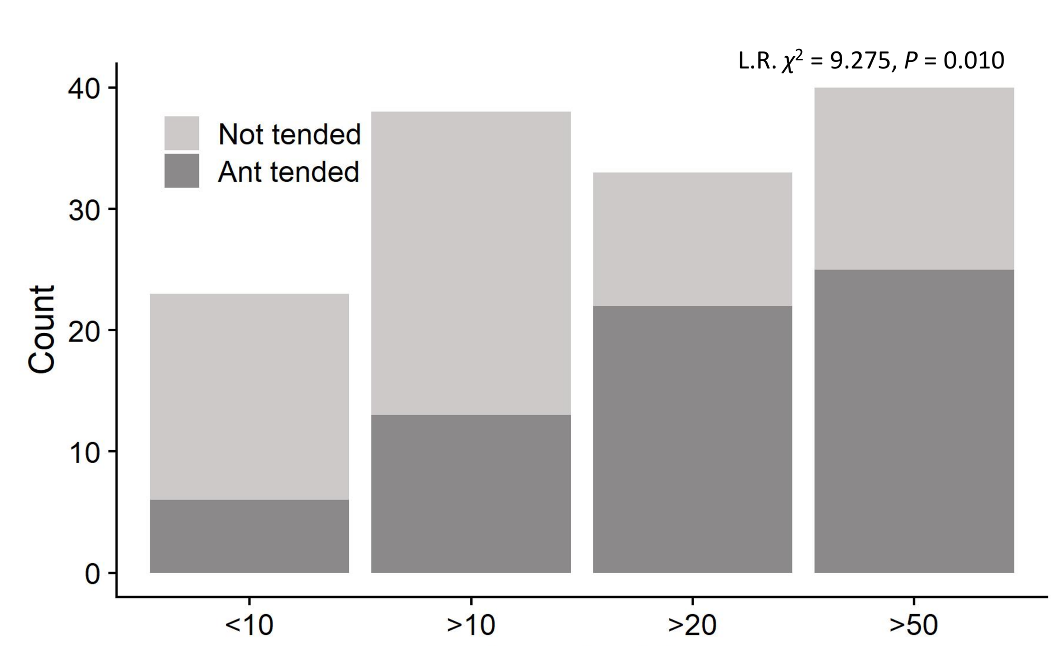

Figure 6 Larvae on bines in larger patches had increased likelihood of ant tending. **Patch area can be a signal of site quality perhaps due to greater nitrogen**, a key factor in these mutualisms [11].

Acknowledgements

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