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Title: Variation of wasp behavior patterns and pollination behavior on *Ligusticum porteri*

Introduction:

Wasps (order *Hymenoptera* except *Symphyta*, *Anthophila*, and *Formicidae*) are important predators, pollinators, and parasitoids which interact with a large number of plant systems. Adult wasps are typically unable to eat solid food and will frequently visit the nectaries of flowers while pursuing prey or hosts for their young (Fei et al 2023, Brock et al 2021). Most wasp species are parasitoids, with several species present on *Ligusticum porteri*, a perennial herb in the *Apiaceae* family (Noyes 2019, Mooney et al 2021).

Ligusticum porteri (*Apiaceae*) is a major component of high elevation meadows and the understory of aspen stands (Langenheim 1962). *L. porteri* has several distinct phases of growth on its flowering stalks and may or may not grow flowering stalks during any given season. The growth rate of flowering stalks depends on the rate of snow melt during the spring and early summer (Ingersoll 1991), which allows for study on the phenological overlap between *L. porteri* and various arthropod visitors (Mooney et al 2023). Many adult insects feed on the nectar of *L. porteri*, with wasps, true bugs, and flies being especially common visitors (Mooney et al 2016). A major driver of insect abundance in the system is the high quantity of nectar produced by the flowers in floral nectaries, which bring in substantial numbers of small flying insects (Terrell et al. 2009). The pursuit of prey, nectar, and hosts may lead to substantial pollination for the plant, which is not visited frequently by bees (Pyke et al 2012). This mode of pollination is vastly understudied compared to pollination by bees and other insect groups (Brock et al 2021). Aphids, sessile phloem feeding insects in order *Hemiptera*, are common herbivores on umbellifers. The flowering stalks of *L. porteri* are typically colonized by aphid alates, winged forms of the otherwise wingless insects, which then reproduce wingless offspring clonally. This form of reproduction can cause explosive population growth allowing populations to double in only a few days (Guldemand 1998). Due to their high sugar diet, aphids secrete high amounts of sugar rich honeydew, which is fed upon by so called “dairying” ants, which protect the aphids from predators and parasitoids in exchange. Parasitoid wasps in subfamily *Aphidiinae* and family *Aphelinidae* parasitize and function as a top-down control for the aphids found on *L. porteri* (Mooney et al, unpublished data).

Methods

Long-term Monitoring Study

The *Ligusticum* project has been operating at RMBL under Dr. Emily Mooney for over a decade. This project involves the random selection of plants at sites with various elevations, 300 meters overall, and various levels of shade, snowmelt timing, temperature, and other abiotic and abiotic factors. Plants of *L. porteri* consist of leaves emergent from an underground rhizome plus one to several flowering stalks, each with clusters of flowers known as umbels (Terrell et al 2009). The phenology of flowering stalks on *L. porteri* are scored and tracked via guidelines for their terminal and primary umbels (Robinson et al. 2017). Additionally, arthropod presence is tracked on the flowering stalks of each selected plant, with more specific identification given to ants. Within aphid colonized plants, the team tracks several types of natural enemies, including counts of the number of parasitized aphids known as ‘mummies’. Specimens are collected in late June every year through sweep netting, preserved, then sorted to order level and counted throughout the year at the University of Colorado Colorado Springs (UCCS) campus. Every individual is retained, allowing for follow-up projects related to the study system or new questions that may emerge. Data has also been collected on aphid parasitoids on the plant specifically, with Aphelinidae and Aphidiinae sorted out of the general Hymenoptera category for abundance research. Temperature and snow melt data is collected for each site over the season via HOBO temperature sensors.

Wasp Behavioral Observations

Behavioral observations were conducted at 8 sites from the project’s 20 existing sites twice per week, with the time of day of each visit varied. 10 plants were randomly selected from individuals not used for the census at the beginning of each observation, with all plants being observed at sites with 10 or fewer plants. 20-minute focal observations were conducted at each site, with each plant observed for an equal amount of time. When wasps were present on a focal plant, their behaviors were cataloged using an ethogram (Table 1), allowing for the development of a time budget for wasp behaviors on *L. porteri*. The ethogram used was based on a published ethogram for on-flower behavior of wasps (Bernauer et al 2022). The behaviors of wasps were recorded at 15 -second intervals until they left the host plant or 5 minutes had passed. As large wasps are prone to being startled by human presence, half of the allotted time for each plant was spent observing it from at least one meter away, with the remaining time being spent right next to plant to track smaller individuals. If a wasp left and returned to the focal host plant, it was recorded as a new visitation event. Due to the small size and high diversity of wasps, species-level identification is not feasible in the field setting (Bernauer et al 2022). Instead, a list of morphotypes, groupings of individuals based on body shape, was used to categorize wasps. In addition to behavior and morphotype, data were collected for time of day, plant

phenology, if the wasp left to another *L. porteri* plant or a different plant, site elevation, and temperature.

Specimen Collection and Pollen Analysis

44 wasps of various morphotypes were collected from flowering stalks of *L. porteri* using a butterfly net and an aspirator. They were then frozen for transport to UCCS, where they will be identified to lowest taxonomic level and swabbed for pollen using fuschin gel. The pollen will then be analyzed via light microscopy and matched to a pollen sample collected from *L. porteri* in the field. Additionally, the *L. porteri* sample slide will be digitized and uploaded to the Global Pollen Project database.

Results:

Morphotype frequency

Far more observations were made of the aphid parasitoids than any other morphotype, making up 50.49 percent of all observations (Table 2). Other apocrita and large ichneumonids made up most of the remaining observations, making up 20.91 percent and 14.79 percent of the observations respectively. Additionally, all observations of large ichneumonids were made on plants with a total phenology score of 5.75 or lower, while other groups had observations at both early and late stages. Despite their presence in preliminary research, sawflies were entirely absent in the data set.

Behavior frequency

Wasps spent most of their time on *L. porteri* resting, locomoting, or foraging, with little interaction with other arthropods (Figure 1). Only 10 aggressive, predatory, or parasitic interactions (biting, divebombing, or ovipositing) with other individuals were observed. Foraging of nectar was also far more common than foraging of pollen or grabbing the anthers as a mode of contacting pollen across all morphotypes except sphecoid wasps. Walking was the primary mode of transportation for aphid parasitoids and other apocrita, but flying was more common for large ichneumonids.

Variation in behavior based on time of day

NOTE: Add chi squared values

Temperature is expected to be a major predictor of pollination behavior rates. Time of day is used as a proxy for temperature, as temperature data has not yet been collected

from the HOBO data sensors present at each site. Foraging, including grappling with anthers, was more common during the midday time block than in the morning or afternoon (Figure 2, Test statistic = , $p=0.013$), with no significant difference in pollen contacting behavior rates between morning and afternoon (Test statistic = , $p=0.612$). Morphotype was another major predictor of pollen contacting behavior rates (Test statistic = , $p<0.001$), with large ichneumonids engaging in pollen-contacting behavior far more often than any other group (Figure 4). The aphid parasitoid and small ichneumonid groups had similar pollen-contacting behavior rates (Test statistic = , $p=0.860$) at roughly 25 percent.

Variation in behavior based on plant phenology

Pollen-contacting behavior also varied significantly between phenological stages (Test statistic = , $p<0.01$). Predictably, post-hoc testing revealed that the significance in variation was driven by differences between fruiting stage plants and both early and late flowering stages (Figure 3). Due to the plant's difference in phenology between terminal and primary umbels, some pollination behavior was observed on fruiting stage stalks, but at less than half of the rate on flowering and late-flowering stage stalks.

Discussion:

Variation in Behavior Based on Morphotype

The data suggest that any pollination of *L. porteri* by wasps is primary driven by the nectar foraging behavior of large ichneumonid wasps. In a system which does not typically attract bees (Pyke et al 2012), large wasps may fill a similar role. More research would be needed to understand selection of plants by large ichneumonids, but all individuals in this study which left during the observation period left to either the same or an unknown plant.

Aphid parasitoids spend the vast majority of their time resting or walking, typically on plants with aphid colonies but on separate umbels from any dairying ants. The low frequency of interaction between aphid parasitoids and their hosts suggests that the wasps may have a high success rate when parasitizing aphids. This is supported by literature, which shows an oviposition success rate of at least 80 percent in ideal conditions (Fink et al. 1995). The interaction between aphid parasitoids and dairying ants is also likely to be an important factor in oviposition success rates, as fewer aphid mummies are found in populations with more aphids (Mooney et al, unpublished data). While this suggests a top-down control of aphids by their parasitoids, it may also be caused in part by the presence of ants on larger colonies.

Variation in Behavior Based on Plant Phenology

The low rate of pollination behavior on fruiting stalks suggests that wasps are unlikely to be major pollinators of late-stage *L. porteri* primary umbels. While this may simply be caused by the low relative number of flowering and late-flowering stage stalks compared to those with lower phenology scores, *L. porteri* flowering stalks typically have more primary umbels than terminal umbels. Other candidates for pollination of primary umbels include beetles, true bugs, and true flies. The major known visitors of *L. porteri* are flies (Kearns 1992), but very little else is known about the system's insect floral visitation. In other Apiaceae, Hymenopterans are known to make up the vast majority of visitors at 84 percent (Koul et al. 1993), so the prevalence of flies on *L. porteri* is unusual. While substantial data is available for insects living on the plant, more research is needed to better understand the system's insect visitation and dynamics between visitors.

Variation in Behavior Based on Plant Phenology

Far more pollen-contacting behavior was observed during the midday than either other grouping of times, suggesting that wasps have a temperature optimum at which they conduct foraging behavior. While this is a new finding for this study system, behavior variations based on heat and time of day have been noted on other plants in the same family, with peak foraging occurring between early morning and 2 PM (Koul et al. 1993, Tomlinson 2012). Small wasps in particular show strong variation in activity in response to heat, with nectar providing a potentially important water source for thermoregulation (Polidori et al. 2020).

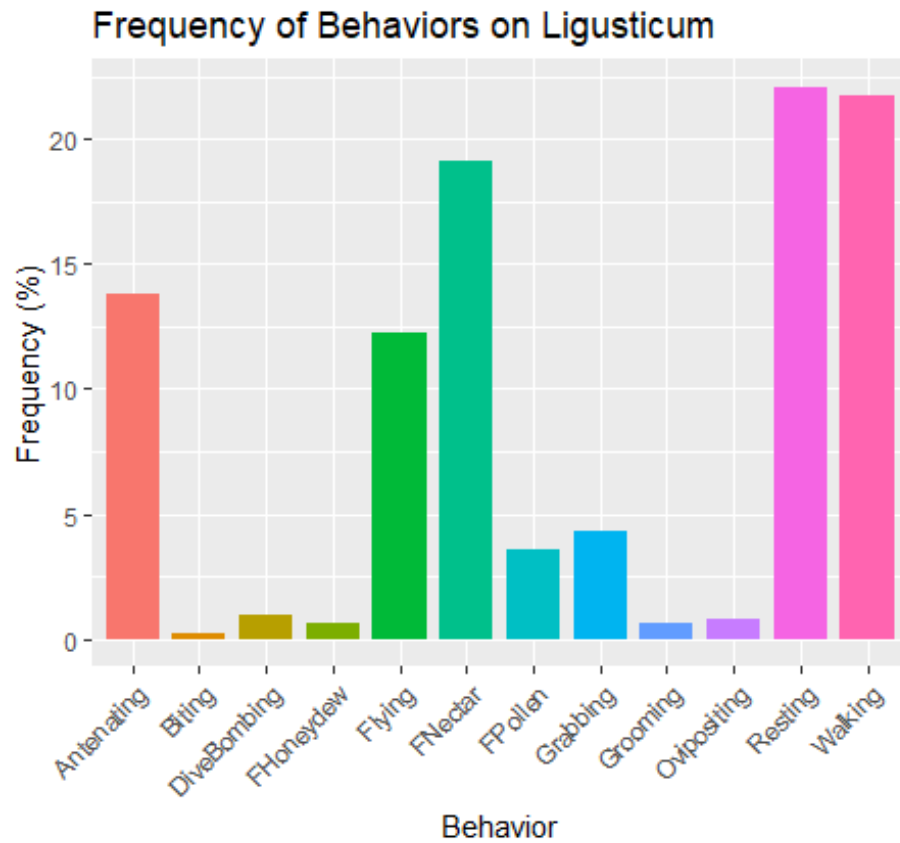


Figure 1: Behavior frequencies
on *Ligusticum porteri*.

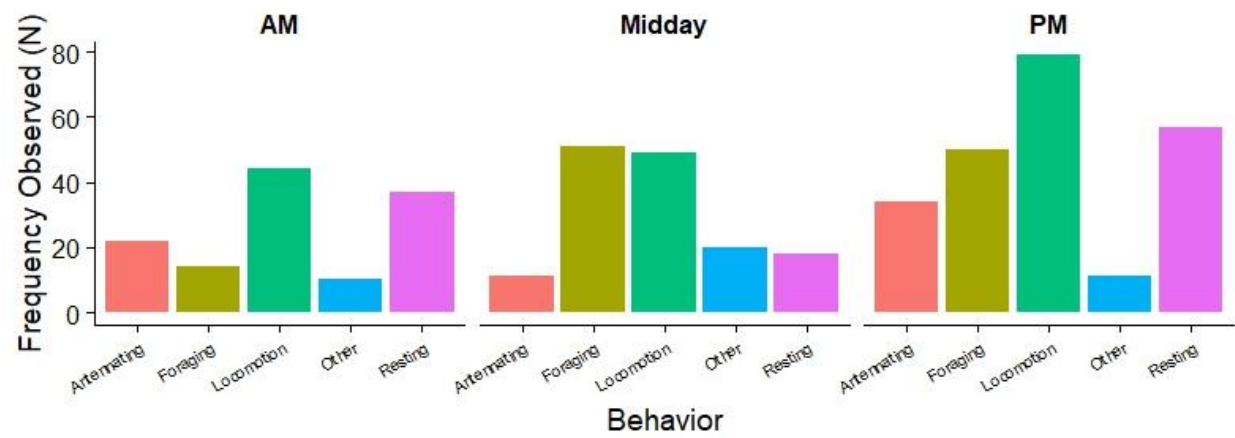


Figure 2: Behavior group frequencies by time of day. (LR $\chi^2 = 8.6233$, $P = 0.01341$)

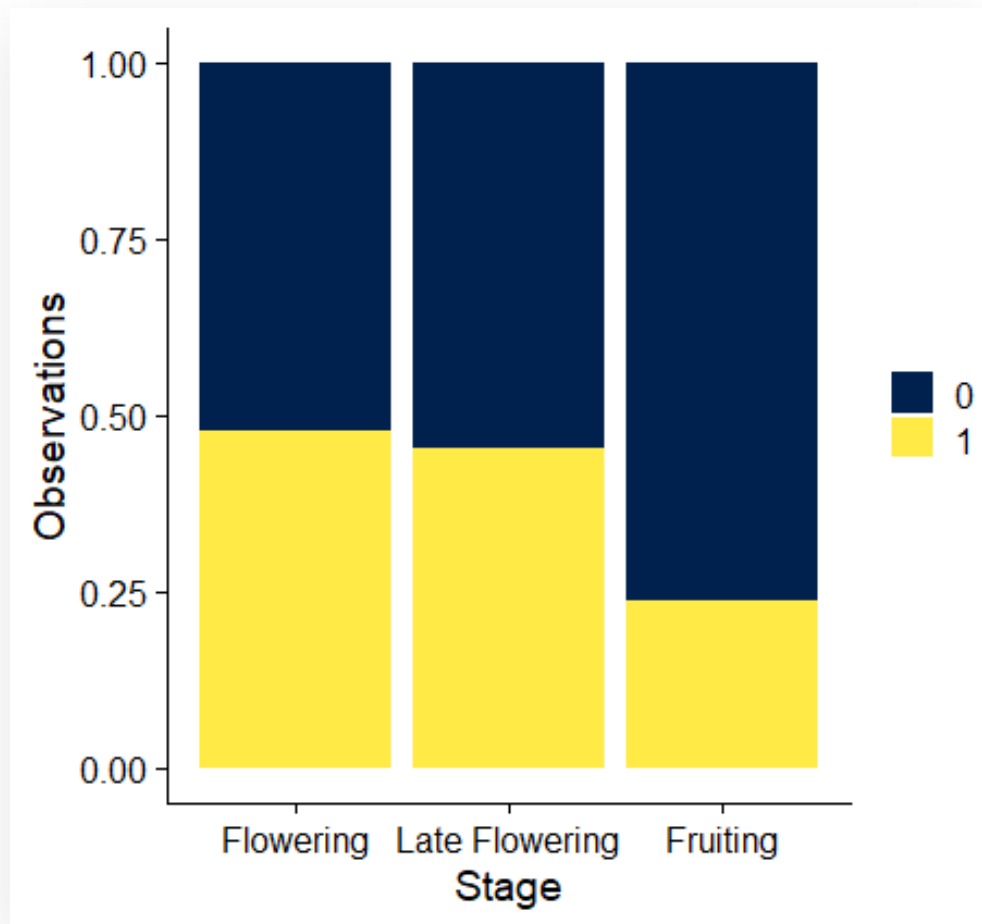


Figure 3: Pollen-contacting behavior by overall plant phenology score. A score of 1 denotes pollen-contacting behavior while a score of 0 denotes all other behavior.

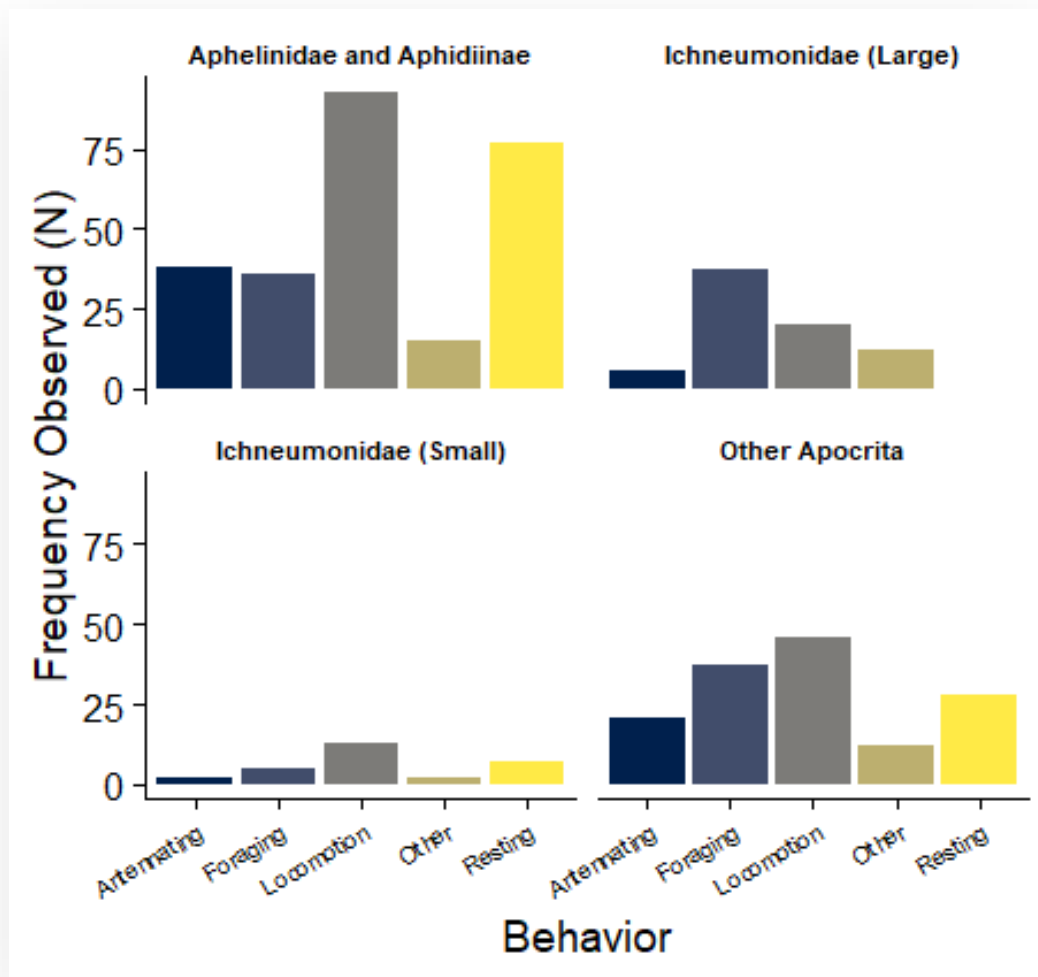


Figure 4: Behavior group frequencies by morphotype group.

Category	Behavior	Description
Foraging	Pollen forage	Visitor forages for pollen on anthers using mouthparts, legs or bodily vibrations
	Nectar forage	Visitor forages for nectar from the top of the flower, through the anthers and stigmas, or from the side at the base of the anthers
Locomotion	Walking	Visitor moves about the flower on foot
	Flying	Visitor moves in the air with use of wings
Resting	Resting	Visitor sits on flower without moving or performing any other behavior
Other	Antennating	Visitor uses antennae to sense the flower, a conspecific or heterospecific visitor
	Grooming	Visitor grooms self with mouthparts or legs
	Biting	Visitor bites or is bitten by another conspecific or heterospecific visitor
	Chasing	Visitor quickly follows a conspecific or heterospecific on foot, moving them away from or off the flower.
	Dive bombing	Visitor either flies directly into another conspecific or heterospecific and displaces the visitor while remaining airborne or is displaced by an airborne conspecific or heterospecific.
	Grabbing	Visitor grabs or grapples with the anthers using front legs, not foraging for pollen; mouthparts not interacting with anthers
	Oviposition	Visitor injects eggs into a host plant or animal by bending its abdomen forward and piercing it with its ovipositor.

Table 1: Ethogram with behavior descriptions for wasp visitation of flowering stalks
(Bernauer et al. 2022)

Morphotype	Count of observations
Aphid Parasitoid	259
Other Apocrita	106
Large Ichneumonoid	75
Small Ichneumonoid	29
Sphecoid	20
Pimpla	11
Vespidae	7
Total	507

Table 2: Count of wasp observations by morphotype.

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