

Effects of Temperature on Voltinism in Subalpine Potter Wasps (Vespidae: Eumeninae)

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ABSTRACT

Voltinism, the number of generations produced per year, is under both genetic and environmental control in insects. The rapid temperature increases associated with climate change could distort the information insects are obtaining from environmental cues and possibly alter the number of generations produced per year. This summer I investigated the effects of temperature on voltinism in potter wasps (Vespidae: Eumeninae). Previous observations suggest that some eumenines around RMBL are bivoltine, while most are univoltine. I focused on whether variables such as temperature, time of year at which an egg was laid, source population, or taxon may be responsible for this variation. I observed individual wasps building nests at multiple study sites varying in elevation, collected completed nests from said sites, isolated eggs or larvae from these nests and subjected them to one of two temperature conditions (boxes exposed to warm or cool temperatures) to observe the effects on development and time until an individual began cocooning. Warm boxes experienced average maximum daily temperatures 4-18°C warmer than cooler boxes. A generalized linear mixed model showed that while site of origin, date laid and box pair had no effect on rates of cocooning, final age, temperature treatment, and source nest did. These results demonstrate how environmental factors influence wasp development. While genetic effects may factor in as well, more work must be done to conclude that the effect of source nest isn't merely a result of additional environmental factors such as those experienced in early life (before the start of experimental treatments) or maternal effects. These results add to our understanding of the broader consequences of climatic warming for insect life-history strategies and potential changes in eumenine wasp populations.

INTRODUCTION

In areas with large annual variability in temperature, survival is dependent on development and reproduction occurring during favorable seasons. Adaptations to synchronize diapause, or dormancy, with unfavorable seasons are critical for survival in many species, including many arthropods (Stearns 1992). At the same time, reproducing as early in the season as environmental conditions permit, which can allow for multiple generations in a growing season, can result in rapid population increase and, potentially, more rapid adaptation to environmental conditions. However, attempting multiple generations in a single growing season can be risky since lengths of times when temperatures are high enough and food is plentiful can vary across years.

Voltinism, the number of generations produced per year, is under both genetic and environmental control in insects (Scoble 1995). Depending on the species and environmental conditions, insects can be univoltine (having one generation per year), bivoltine (two generations per year), multivoltine (more than two generations per year) or even semivoltine (one generation per two years). Different types of voltinism can be observed in the same species and even among siblings. Partial voltinism, or parsivoltinism, is defined as the presence of different levels of voltinism in the same population, such as a population with both uni- and bivoltine individuals (Tochio & Tepedino, 1982). This variation could be due to genetic variation and/or lack of consistent environmental conditions to produce the same life-history in all individuals. While not entirely independent of climate, elevational changes have also been shown to contribute to voltinism strategy. For example, dark bush-crickets were found to have shorter life cycles at higher elevations, even under the same environmental conditions. These results have been attributed to local adaptation to harsher climates, particularly in mountainous regions (Černecká et al., 2020).

In widespread species that experience variable climates, using environmental variables to cue which life history strategy (e.g., univoltine vs multivoltine) to employ could be advantageous. However, how climate change will alter the effectiveness of these reproductive strategies is unknown. The rapidity of temperature changes could distort the information insects are obtaining from environmental cues (Van Dyck et al. 2015). For example, if higher temperatures cue early emergence, but temperature drops steeply at the end of the season, insects may not reach the proper developmental stage to overwinter. Nevertheless, studies of numerous species, including bees, moths and butterflies, have demonstrated that development at higher temperatures, such as those associated with climatic warming, tends to increase the number of generations produced per year (Altermatt 2009). Forrest et al. (2019) also showed that voltinism was impacted by the date an individual bee (*O. iridis*) was laid, with later laid individuals being less likely to emerge in the first rather than second year. Since season length had no apparent effect on development it was suggested that maternal cues may indicate how much of the season remains. This cue could increase survival for those who may need to diapause quickly at the end of the season or those who have more than enough time to emerge and build nests again before

the summer ends. In fact, maternal cues controlling timing of diapause in their offspring have been demonstrated in other insects (Scharf et al., 2010).

This summer I investigated the effects of temperature on voltinism in several species of potter wasps (Vespidae: Eumeninae) in the Rocky Mountains of Colorado. Previous observations in the study area suggested that some Eumeninae around RMBL are bivoltine, while most are univoltine (J. Forrest, unpublished data). I focused on the effects of temperature, while also investigating how time of year at which an egg was laid, source population, or species may contribute to variations in development rates or voltinism. To answer this question I observed individual wasps building nests at multiple study sites varying in elevation, collected the nests from said sites, isolated eggs from these nests and subjected them to one of two temperature conditions to observe their effects on development.

I hypothesized the following: (1) under high-temperature conditions, rates of direct development (i.e. development to adulthood during the first growing season, rather than diapause development to overwinter as larva/prepupa before maturing to adulthood the following growing season), will be significantly higher than under low-temperature conditions (demonstrating environmental control). (2) Under similar temperature conditions, certain nests/species will have higher rates of early emergence/bivoltinism (suggesting genetic control). (3) Finally, earlier laid wasps will be more likely to directly develop (as shown previously in high-elevation solitary bees; Forrest et al. 2019).

SPECIFIC METHODOLOGIES

Experimental Set-up & Study Organism:

Nests and field data were collected over a 6-week period in six subalpine sites located in the Rocky Mountains of Colorado, USA (Figure 1). Sites ranged in elevation from 2,730 to 3,440 meters and were located at a minimum distance of 1.5 km from one another to prevent oversampling from any individual population. Sites were established in 2013-14, when “trap-nests” were set up for studies on solitary bees. Wasps at these sites were not collected or subject to any experimentation between 2015 and 2022. The trap nests consist of wooden blocks with holes drilled 14 cm deep and paper straws inserted to allow for nest removal and inspection.

Potter wasps (Vespidae: Eumeninae) are a cosmopolitan group making up about 3,579 species worldwide, five of which have been identified from trap nests around RMBL: two species of *Symmorphus* and three *Ancistrocerus* spp. (Picket & Carpenter 2010). These wasps normally nest in borings found in dead trees. Following emergence and mating in early summer, females will attempt to find a hole to build a nest in. Once a suitable hole is found, she will begin to build a series of “brood cells” inside the tube. In each cell she will lay one egg (fertilized with stored sperm if female) suspended by a flexible thread from the roof of the cell. She then collects food for her offspring, typically consisting of caterpillars or beetle larvae (though *Symmorphus cristatus* feeds particularly on Coleoptera larvae). The prey will be stung and paralyzed to preserve it until the egg hatches and wasp larvae consume it. Female wasps tend to be laid first in

larger cells (due to intersexual differences in wasp size). Brood cells are separated from one another by walls made of mud, and the nest is closed with an additional mud plug at the outer end of the boring to prevent parasites from entering into the nest. The mud walls and outer plug are chewed through during emergence of adult offspring (Krombein 1967).

These wasps exhibit complete metamorphosis with distinct egg, larva, pupa, and adult/imago stages. They tend to be bi- or uni-voltine with some strains having evidence of univoltine development or mixes depending on the location (Fye 1965). Among univoltine individuals, overwinter diapause occurs at the end of larval stage/prepupa and pupation occurs the following spring before emergence as adults to continue the cycle (Krombein 1967).

Data Collection:

Nesting:

At most sites, I checked nesting blocks at least once every 4 days from late June to late July. The highest-elevation site (Virginia Basin) was only visited once per week due to the longer travel time. Females seen building a nest were captured and marked with non-toxic paint on their thorax to allow for tracking of individuals and their future nests. These individuals were also filmed to aid in later taxon identification using an identification atlas of northeastern Vespidae (Buck et al., 2008). (However, these data were not used in analysis due to the low proportion of nests constructed by known and identified wasps.)

At every site visit, wasp nests (contained within their paper straws) were marked with the current date on the side of each straw to determine the approximate date when each egg was laid and discern if the nest had been abandoned. Nests were removed as soon as they were completed (had final outer mud plug) and brought back to the lab. All nests were collected on the first day of observed completion to ensure that offspring were at an early-enough stage of development. Only eggs and larvae (up to 1 cm, though 86% were ≤ 0.5 cm) were used in the experiment since it was assumed that later stages would be less likely to respond to temperature changes while developing. About 60% of nests were also left in the field and observed for signs of emergence at each visit until August 6th, 2022 (Table 1). If emergence was seen, nests were collected and dissected to verify.

Table 1: Total number of eumenine nests at each site, which were collected or remained in the field, as well as the number of nests from which wasps emerged in 2022, of those nests left in the field. (Abandoned nests were not included.)

Site	Number of Nests	Collected	Remaining	Emerged
Brush Creek	53	16	37	2
Rosy Point	49	24	25	0
Kebler Clearing	7	2	5	0
401 Trail	50	16	34	2
Kebler Pass	27	10	16	0
Virginia Basin	15	10	5	0
Total	201	78	123	4

Test groups:

Once nests were back at the lab I opened each brood cell, noting the number and kind of prey present in the cell (ex. gray beetle, red-striped caterpillar). Prey type was not used in the final analysis but served to confirm the genus, since only *Symmorphus* are known to primarily collect beetle larvae. I also noted the number of cells in each nest, date the nest was completed and collected, and any additional notes about contents (presence of nest parasites, dried prey, etc). Cell contents were carefully transferred into gelatine capsules (size 00) so as to not damage the egg or prey. Each viable cell (which had a live egg or wasp larva) was put in one of two temperature treatments (warm or cool) alternating such that siblings next to one another in the nest (and therefore closest in age) were placed in opposite treatments. Gelatine capsules were put in petri dishes for extra weather protection (since capsules are water soluble) and subsequently placed into wooden boxes secured with mesh and tied to prevent accidental opening or escape of emerging wasps. I started with over 250 viable cells that had live wasp eggs or larvae inside, allowing for about 25 individuals in each of 10 boxes (5 per treatment).

Temperature Experimentation:

I used 10 total boxes (5 boxes for each of the two temperature treatments), which I deployed in a paired design. The warm-treatment boxes were left in areas of direct sun (south sides of buildings), while boxes in the cool-temperature treatment were left in areas of shade (north sides of buildings). Boxes were labeled with letters A-J, with pairs of boxes (e.g., A and B, C and D) being placed around the same building and filled with siblings from the same nest.

Each box was equipped with a HOBO temperature logger to verify that there were temperature differences between the treatments. The loggers recorded temperatures once every 30 minutes as well as daily maximum and minimum temperatures. The first wasps entered the temperature treatments on July 1st and the last started on July 13th. After July 21st, 2022, parafilm was also used to cover petri dishes to prevent mortality due to desiccation. Once an individual had experienced 2 weeks in its outdoor temperature treatment, it was removed and placed in an incubator programmed to a 10-25°C daily cycle. All individuals were treated for the same amount of time.

Developmental Tracking:

After placement of individual wasps in boxes, I observed developmental stages every other day, keeping track of the date when each developmental stage was reached (e.g., egg, larva, initiation of cocoon spinning, pupa) as well as recording survival. Developmental data was recorded from the day of nest dissection until August 13th. Cocoon spinning was used as an indication of the individual reaching the prepupal stage. Extremely opaque cocoons were assumed to not have pupated (though this was only relevant in about two cases). Individuals that didn't survive were removed and the date of death recorded, as well as the stage they died at. (Mortality and date of death were not used in the final analysis.)

Due to the limited number of pupating individuals (only one, from a warm treatment), models were based on rates of cocooning. While initiation of cocoon construction is not a direct measurement of voltinism, faster development allows for more time for emergence and increases the likelihood of success for bivoltine individuals.

Data Analysis:

To determine effects of treatment (warm/cool) and other factors on probability of cocoon construction, I ran a generalized linear mixed model with binomial error distribution using the package lme4 in R (Bates et al., 2015; R Core Team, 2022; RStudio Team, 2020). Fixed effects were age at time of final observation and treatment. The response variable examined was whether the individual had started a cocoon or not (indication of prepupal stage). Random effects were the site an individual was collected from, source nest, and box pair. Plots were made using ggplot2 (Wickham, 2016).

RESULTS

Field Surveys of Nests:

Across the 6 sites I visited, I was able to film and identify a total of 51 individual wasps (Table 1). Some videos were taken of unmarked wasps. All unknowns were confirmed to be different individuals so as to avoid repeat counting. From the videos I was able to distinguish genera; both *Ancistrocerus* and *Symmorphus* were present. Some wasps were identifiable to species: *Ancistrocerus albophaleratus*, *A. antilope*, *A. albolacteus*, *Symmorphus cristatus*, and *S. albomarginatus*. However, since species identities could not be confirmed I limited my categorization to genus (Table 2).

Table 2: Numbers of individually marked nesting females of each genus at all 6 sites. Low numbers at Kebler Clearing and Virginia Basin were due to scarcity of eumenines at these sites.

Site	Elevation (m)	<i>Ancistrocerus</i>	<i>Symmorphus</i>	Total
Brush Creek	2,730	6	6	12
Rosy Point	2,875	4	9	13
Kebler Clearing	2,956	2	0	2
401 Trail	3,020	13	0	13
Kebler Pass	3,040	11	0	11
Virginia Basin	3,440	0	0	0
Total	-	36	15	51

Even though most (43 of 78) nests were collected from known/identified individuals, we also collected nests for which the mother's identity was unknown. There was an average of 1.3 nests per marked individual, though it is important to note that these do not account for nests made outside of our nesting blocks, or ones that were completed between site visits (i.e. in under 4 days). In total from 78 nests I collected 120 brood cells at the egg stage and 139 at the larval stage to put into treatments (an average of about three live individuals per nest). Across both

treatments, a total of 66 individuals survived until the end of the experiment (a survival rate of 25%). Of those that survived, 62 had reached the cocooning stage.

Temperature Treatments:

All warm-treatment boxes showed higher average temperatures than their cool-treatment counterparts, but to varying degrees. For example, boxes A and B both show cyclical temperature changes throughout the day (Figure 3), and while minimum daily temperatures were not correlated with treatment type (Figure 4a), box A (warm) was consistently warmer than box B. All other box pairs also showed higher maximum daily temperatures in those boxes on south sides of buildings (“warm treatments”; Figure 4c). When comparing the mean of maximum daily temperatures (across all days during which the treatments were in place), warm-treatment boxes were 4-18°C hotter (Figure 4b).

Developmental Tracking:

Due to the limited number of pupating individuals, models were based on rates of cocooning. Though there weren’t enough data points to analyze, the one individual that pupated had been placed in a warm treatment. It was estimated to have been laid on July 2nd and took 4.5 weeks to develop into a pupa.

Rates of cocooning were significantly higher in the warm treatment, indicating that warmer temperatures positively affect developmental rate ($z = 2.3$, $p = 0.024$, Figure 5). Of 56 individuals that reached the cocoon stage, 32 were in the warm treatment and 24 were in the cool treatment. The probability of reaching cocoon stage by the end of the experiment increased with wasp age, as expected ($z = 2.5$, $p = 0.011$, $n = 247$ wasps).

Random effects such as the site a nest was collected from and block pair did not contribute to intercept variance. Additionally, while there was a measurable amount of variance attributable to source nest, accounting for nest ID didn’t significantly improve model fit (likelihood-ratio test, $\chi^2 = 0.15$, $p = 0.69$). Finally, there was no detectable effect of date laid on likelihood of an individual cocooning ($z = 1.6$, $p = 0.11$).

DISCUSSION

Wasps subjected to the warmer treatment were more likely to construct cocoons prior to the end of the study and therefore developed faster. This indicates that the probability of direct development is influenced by environmental factors—specifically temperature. It is important to note that the temperature treatments imposed here did not expose wasps to unnaturally high or low temperatures. They were still insulated by petri dishes and wooden boxes, and like natural nests were either in direct sunlight or shade. The temperature difference between warm and cool treatments should therefore be similar to what nests built in shade vs. direct sunlight would experience. It would be interesting to test whether wasps have preferences (given sufficient nest availability) between south- and north-facing holes. Other cavity nesters have been shown to

choose holes based upon orientation, which predicts temperature and often subsequent survival (Wilson et al., 2020; Hranitz et al., 2009). Rising temperatures caused by climate change would likely increase developmental rates more than the difference between nests in sun vs. shade. However, more work will need to be done to better understand how climate change will influence each population.

While wasp emergence rates were low in the experimental treatments, emergence in the field was not much higher. Out of the 124 nests remaining in the field, only 4 had signs of emergence by August 6th. Of those that emerged, all were completed in June or early July (two on June 16th, one on June 29th and one on July 12th). Among lab-raised individuals, of the 56 that survived to cocoon, only one of the warm treated individuals (estimated laid date on July 2nd) pupated by August 6th. Thus, most of the individuals that pupated were laid relatively early in the season, which lends support to the hypothesis that date laid would negatively affect rates of pupation. However, among experimental individuals, I detected no significant effect of date laid on likelihood of an individual cocooning, perhaps due to the limited date range (by August 5th only 10 surviving wasps were estimated to have been laid before July 2nd, while most emerged individuals were laid earlier). Additionally, relatively small sample size could be a factor, since rates of early emergence in the field were only 3%, indicating low rates of bivoltinism in the study population, at least during the year of this study.

Compared to other populations such as those studied in Northern Ontario by Fye (1965), estimated rates of bivoltinism are much lower at RMBL. Research on *Ancistrocerus* (including *A. antilope* and *A. albophaleratus*) in 1961-2 showed significantly more bivoltine individuals, with only one univoltine nest recorded out of 43. Additionally, rates of mid-summer emergence were estimated at 81% (Fye 1965). The discrepancy between the two locations is likely due to genetic and environmental factors. Since work on these insects has not been done previously at RMBL, repeated data collection over multiple years would be required to determine typical proportions of bivoltine individuals year to year and the relative effects of genetics and environmental conditions.

The site at which a nest originated did not affect developmental rate. However, the source nest, though not significant, explained some variation in the likelihood of reaching cocooning stage. Effects of source nest on development rate could indicate that direct development is influenced by genetic factors; however, maternal effects or shared early-life environmental conditions could also be responsible.

Cocoon formation was observed in individuals from both genera, with no obvious difference between genera in rates of cocoon formation (Figure 5). Due to the large number of unidentified individuals, these data could not be analyzed. However, there was a difference between the two genera in terms of elevational range. While at lower sites the genera were nearly equally abundant, higher elevation sites showed fewer to no *Symmorphus* (Figure 2). This indicates that the range of *Symmorphus* species could be limited by elevation, as seen previously in a multitude of species including other eumenines (Ribeiro et al., 2019). However, more work

would need to be done to determine exactly what factors are limiting their range that aren't affecting *Ancistrocerus*.

In conclusion, this summer I found that environmental factors contributed to rates of development of potter wasps in the RMBL area. Under warmer (but still natural) temperatures, rates of development were faster. This more rapid development could allow for multiple generations in a summer, and if survival rates weren't too impacted, lead to higher rates of population growth and fitness increases through faster adaptation. However, higher temperatures without longer seasons could prevent individuals from reaching critical development stages, leaving them trapped and causing low survival. Alternatively, populations that present less plasticity in voltinism may experience a loss of fitness as seasonal conditions change. More research looking at extreme temperatures associated with climate change would be needed to give insight on how climatic warming will affect these populations. It will be important to look at the effects of warming on survival as well as voltinism. More work will need to be done to better understand effects of climate change on these populations of predatory wasps, and how changes in their populations may impact the communities in which they live.

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FIGURES

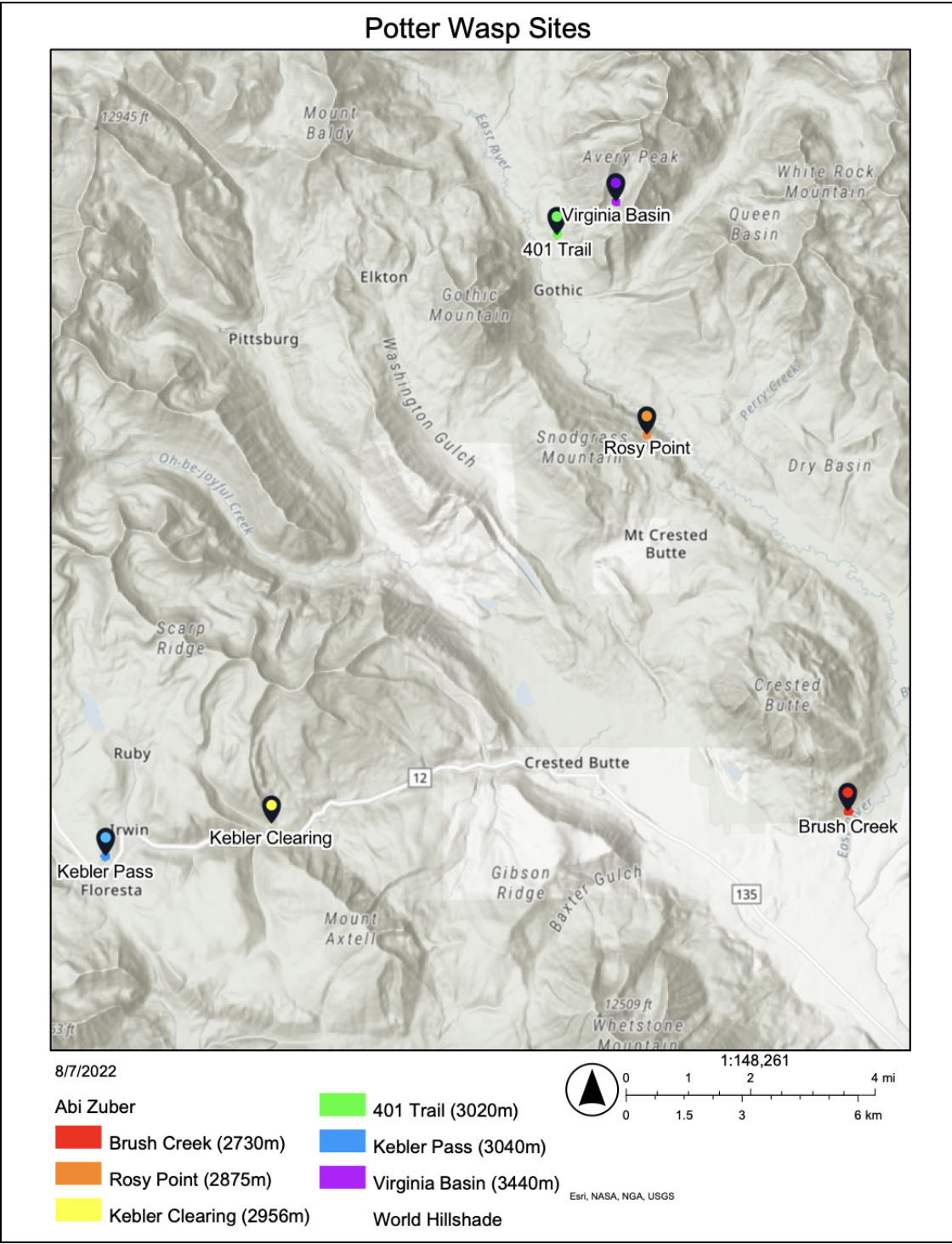


Figure 1: The six field sites of collection and their elevations, Gunnison County CO, USA.



Figure 2: Number of caught wasps of each genus at each site. Sites are in ascending order of elevation.

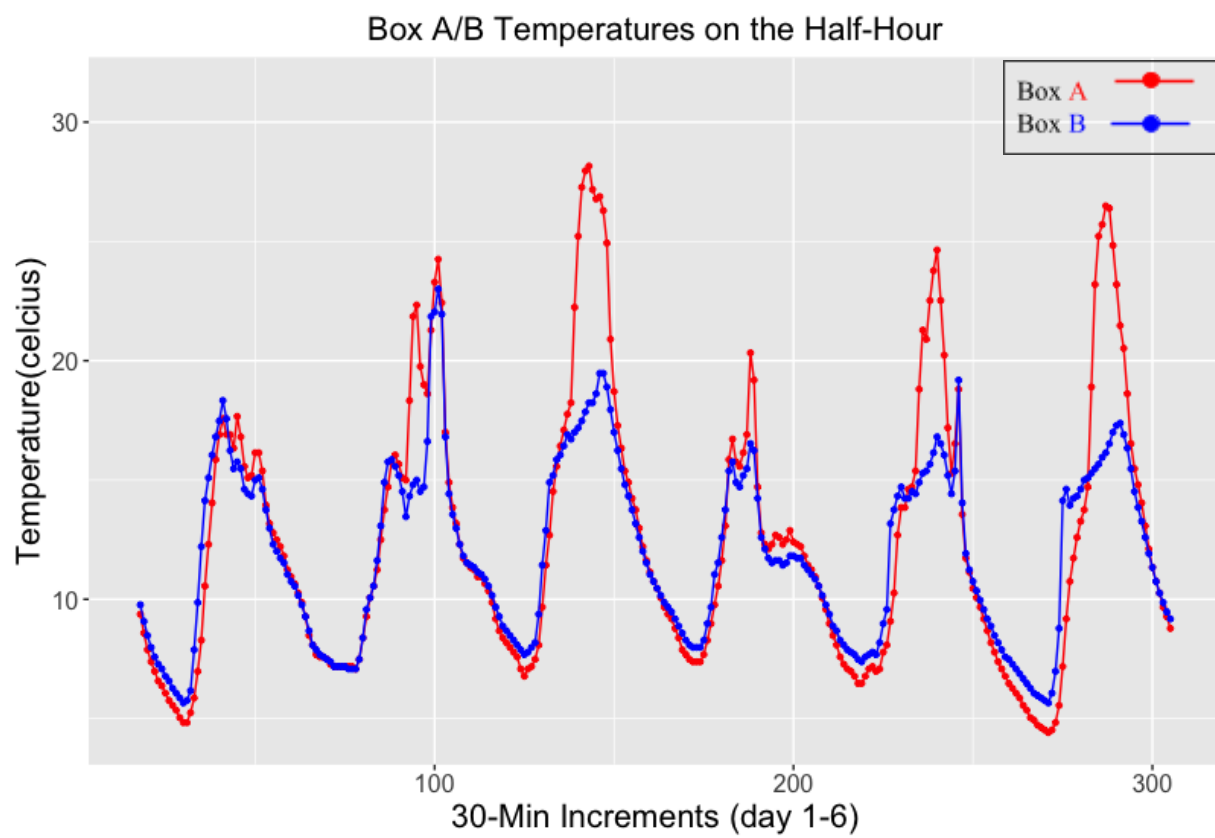


Figure 3: Temperatures of Boxes A and B during the first 6 days of treatment (July 1st- July 6th).

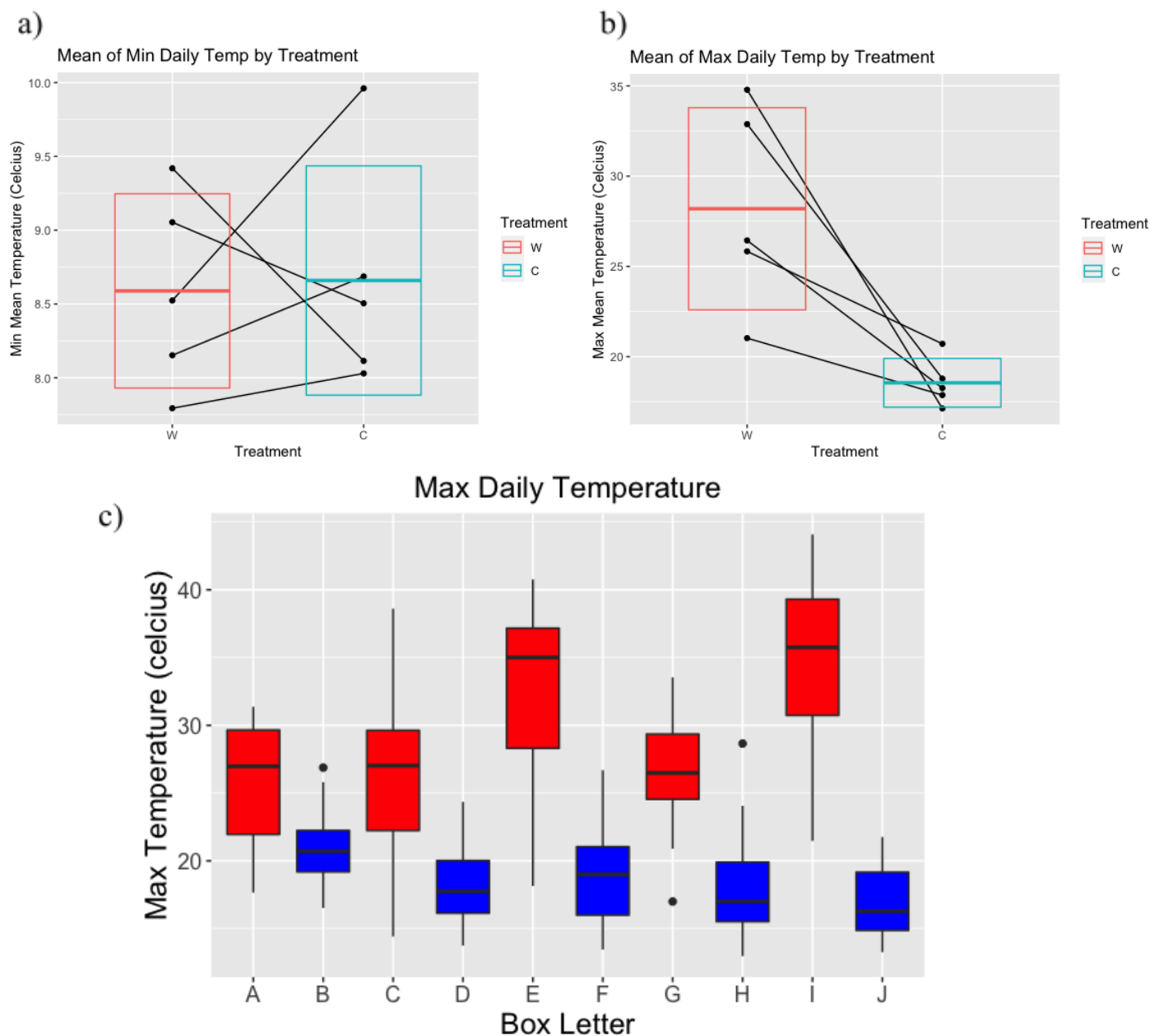


Figure 4: Minimum and maximum daily temperatures of each box pair by treatment. a) Mean of minimum daily temperature by treatment (w=warm, c=cool), showing no association between mean minimum and treatment. (Lines connect paired boxes.) b) Mean of maximum daily temperature by treatment (w=warm, c=cool), showing consistently higher values in warm treatment (Lines connect paired boxes.). c) Box plots of each box's maximum daily temperature of the recordings taken throughout the experiment with an average sample size of 28 days of recorded temperature/treatment (min=24, max=34). Red color denotes warm treated boxes while blue color denotes cool treated boxes (South or North side of building respectively.)

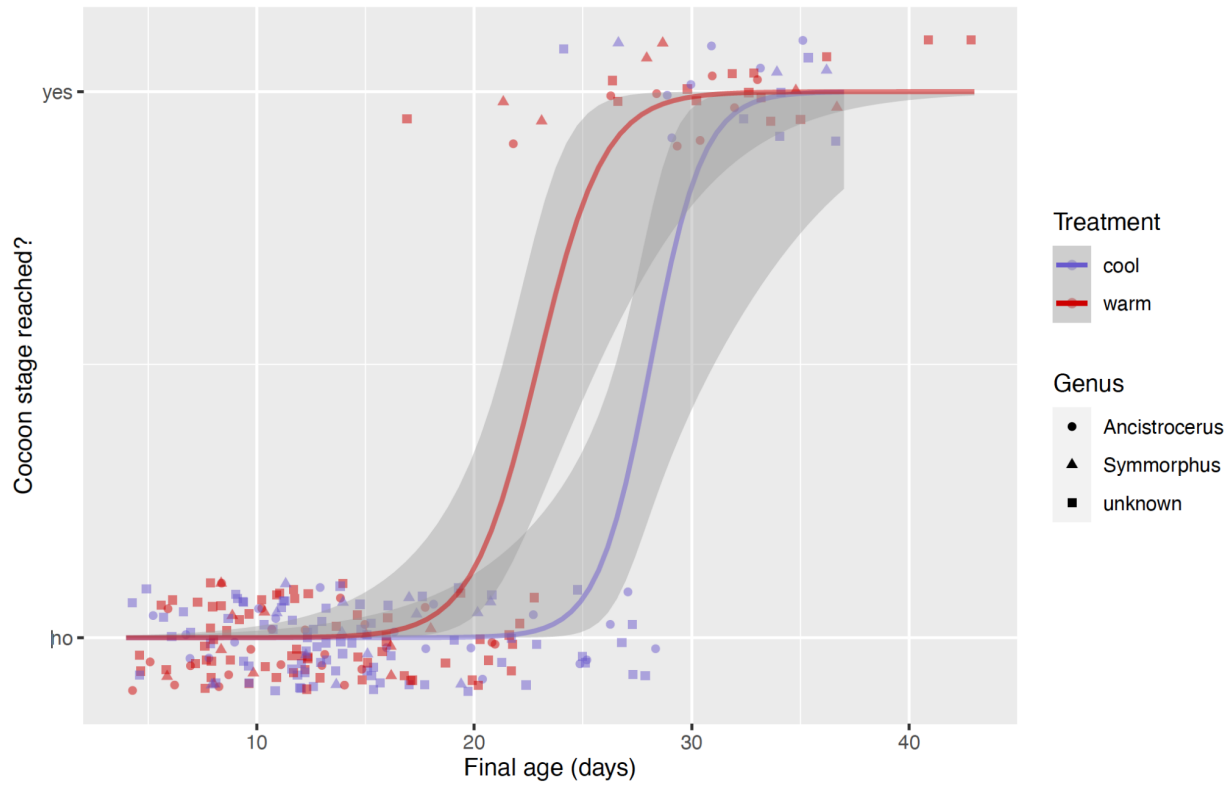


Figure 5: Rates of cocooning based on age at last observation and treatment. Points are colored and shaped to indicate treatment and taxon. Logistic regression is plotted for each treatment. Those oldest individuals that have still not begun cocooning are in the cool treatment, while the youngest individuals to begin cocooning were in the warm treatment. Points were jittered vertically for better visualization.