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Variations in Thermoregulation Strategies of Northern and Southern Populations of Mischocyttarus mexicanus

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Variations in Thermoregulation Strategies of Northern and Southern Populations of *Mischocyttarus mexicanus*

by

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ABSTRACT

Mischocyttarus mexicanus is a species of eusocial paper wasp that is found across much of the Southeastern coastal region of the United States. Contrasting wintering strategies have been seen in northern versus southern populations. Anecdotally, northern populations overwinter by clustering on top of palmetto fronds, their preferred nesting substrate, in a sort of "taco" shape, while southern populations stand on their nests that hang on the underside of the palmetto frond throughout the year. I tested three hypotheses related to the adaptive value of northern over-frond clustering behavior: 1) Over-frond clustering is thermoregulation related, meaning it would be temperature driven. 2) Clustering on top of fronds provides protection from nests falling more often in the winter. 3) Clustering on top of fronds prevents wasps from losing grip on the underside nesting substrates due to cold immobility. To test these hypotheses, I placed iButton temperature probes on the top and underside of *M. mexicanus* nesting fronds in Louisiana and Mississippi during the winter (the southern range limit for overwintering clustering behavior). I also performed nest falling surveys across seasons. Lastly, I compared ambient temperatures to the temperature at which this species loses motor control (CTmin). Results suggested that this behavior was not thermoregulatory. I did, however, find that nests had a higher probability of falling in the winter than warmer ones, which supports the hypothesis of protection from nest falling. Lastly, ambient temperatures in the winter dropped below CTmin, suggesting that wasps cluster on top of fronds during cold winters to prevent falling off of nests due to cold coma. This could allow us an explanation of the change in behavior of the northern populations, as temperatures more often reach cold enough values to hinder mobility significantly. Compared to other

eusocial insects, even being native to Mississippi, *Mischocyttarus mexicanus* has not been heavily researched in the past, which our research findings hope to help rectify. Also, by understanding climate impacts on certain species, we can understand the connection between animal physiology and changing climate.

Keywords: climate change, cold physiology, eusocial, thermoregulation

DEDICATION

To my father, who fostered a sense of curiosity within me from childhood and supported me during every step.

ACKNOWLEDGMENTS

I would like to acknowledge my mentor, Dr. Kaitlin Baudier, without whom I would not have had the encouragement and guidance to complete this thesis. I also would like to acknowledge Alycia Johnson and Kristin Robinson for accompanying me on data collection as well as assisted with experimental design aspects. I would like to thank Jean Lafitte National Park and City Park for providing access to the field sites surveyed in this study. I also would like to acknowledge the University of Southern Mississippi Honors College, as without the resources provided I would not have been able to participate in research in this capacity.

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CT_{min}	Minimum critical temperature
USM	The University of Southern Mississippi

CHAPTER I: INTRODUCTION

Paper Wasp Biology

Paper wasps in the subfamily Polistinae are typically classified as eusocial organisms because of the social structures and organization these species employ (Gunnels, 2007). Eusocial insects, such as paper wasps, serve as useful model organisms to study how social strategies differ among populations (Miller et al., 2022). There is also a high level of diversity within the subfamily Polistinae, which includes some of the most species rich genera of all eusocial insects (Miller et al., 2019). There are two genera of paper wasps that inhabit the southeastern United States: *Polistes* and *Mischocyttarus*. The species of focus for this study was *Mischocyttarus mexicanus*. Previous studies have found some paper wasp species to overwinter together, such as in the North American species *Polistes metricus* and *Polistes fuscatus* (Erram & Judd, 2017). However, only anecdotes exist describing similar overwintering behavior in *M, mexicanus* (Pers. Comm., Kaitlin Baudier & Floria Mora Kepfer-Uy). Based on these anecdotal observations, this clustering behavior appears to be absent in southern Florida populations but present elsewhere in this species' range. Northern populations, such as those in North Carolina, overwinter by clustering on top of palmetto fronds, their preferred nesting substrate, in a sort of "taco" shape, while southern populations, such as those in Mississippi and Louisiana, stand on their nests that hang on the underside of the palmetto frond throughout the year (Pers. Comm., Kaitlin Baudier & Floria Mora Kepfer-Uy). In this study, I present a test of three hypothesized adaptive functions of this stereotyped wintertime behavior.

Nest Building & Microclimates

Paper wasps are so named because of the nests they construct, typically of a paper-like material made of collected plant fibers mixed with water and saliva (Miller et al., 2022). Nesting site selection is important in *M. mexicanus*, with nest-founding females taking multiple factors into account when selecting where new nest initiations will take place (Gunnels et al., 2008). Recolonization of abandoned nests has not been observed in paper wasps where this behavioral trait has been studied well, such as in *Polistes carolina* (Seppa et al., 2012) and *Ropalidia plebeiana* (Oberprieler & Spradberry, 2009).

Temperature does play an important role in the nests of these insects, as once nests are initiated temperature impacts the larval development and emergence (Hocherl et al., 2016). It makes sense, then, for there to be capabilities of nest thermoregulation present (Hocherl et al., 2016). I therefore hypothesized that *M. mexicanus* wasps cluster on the top of palmetto fronds to gain access to warmer, more favorable microclimates during the winter. In the winter, tops of leaves may receive more midday sun, which could benefit survival of the overwintering wasps. Similar fluctuating thermal regimes have been found to increase winter survival in leafcutting bees (Rinehart et al., 2013).

Seasonality, Nest Integrity, and Falling

Mischocyttarus mexicanus follow seasonal patterns in nest building, with new nests started in the late winter or early spring (Gunnels, 2007). The spring season is an important one, as once nests are founded in the spring it typically is one solitary foundress (a fertile female forming the colony), or a small number of foundresses, that begin these nests and become reproductively active (Leadbeater et al., 2010). A study by Gunnel (2007) also found a significant decrease in maintenance and upkeep of the nests before early spring, so mainly in the months of December and January. For this reason, I hypothesized that an adaptive value of clustering on the tops of palmetto fronds in the winter could be protection against nest falling. Alternatively, I hypothesized that the wasps may also cluster on the tops of the fronds in the winter because wintertime temperatures in these regions drop low enough to send them into total cold coma, in which case, being atop the frond prevents them from falling to the ground where they could be vulnerable to predation.

CHAPTER II: METHODS

Testing the Nest Falling Hypothesis

I conducted surveys of nest falling across seasons to find out whether nest falls are indeed more common in the late winter period. My study site was the Couturie Forest in City Park, New Orleans, LA. Three surveys were conducted on July 3, 2022 on December 3, 2022, and on March 5, 2023. These dates coincided roughly with weather changes, the first freeze occurring in late November and warm temperatures mostly returning late March. As such, the first period captured about 5 months of warm weather and the second captured about 3 months of cold weather. In the July survey, I marked the first 35 active *M. mexicanus* nests I came across in the area when searching the undersides of palmetto fronds. For each nest I came across, I made note of the GPS coordinates and I marked the nest using a red sharpie to write on the underside of the palmetto frond. Only nests between ground level and seven feet above ground level were included. I also took pictures of some of the nests that were more difficult to locate on the palmetto plant, to attempt to eliminate trouble locating the same nests in the return surveys and to ensure as much confidence as possible in future survey comparisons. In December I returned to the same nest sites and counted how many of them were absent before locating enough new nests to replace those that had fallen, so that N = 35 nests for both cold and warm periods. For the final survey in March, I counted how many nests of the 35 from December had fallen. I then ran a Fisher's Exact Test in R (v4.1.2; R Core Team 2021) to compare the proportion of falling and non-falling nests over the warm months versus cold months.

Testing the Thermoregulation Hypothesis

To assess if being on the top of the frond in the winter was a thermoregulation strategy, I compared above-frond and below-frond temperatures. For each replicate palmetto, iButton probes (Maxim Integrated™, San Jose, CA, USA) were deployed on the top of the palmetto frond, the bottom of the palmetto frond, and 1 m away at the same height above ground (between 70 cm and 102 cm above ground). I did this for four palmettos in New Orleans, LA and Hattiesburg, MS where active nests of *M. mexicanus* had been observed the summer before. These locations included the Walker Science Building garden at the University of Southern Mississippi (USM) (MS), in the Couturie Forest of City Park in New Orleans (LA), in the Jean Lafitte Barataria Preserve (LA), as well as on a palmetto in Uptown New Orleans (LA). The probes were deployed from February 10 to 13, 2022 and were all collected on March 6, 2022. The probes in Hattiesburg were deployed for a total of twenty-four days. Probes at the three locations in Louisiana recorded for twenty-one days. Upon collection, I took the probes to the lab and I downloaded the data points from all twelve probes. The twelve files were in excel form and separated by location and microclimate and the next step was to compile all twelve files into one masterfile and then convert that file into CSV form for further analysis through the programming software R. When I created the masterlist, I also omitted the first and last data point of recorded temperature for all microclimates and all locations to account for any misleading data upon deployment and retrieval.

Using R (v4.1.2; R Core Team 2021) I compared daily temperatures across microclimates using a linear mixed-effect analysis. I used the lmer function in the lme4 package for this analysis. This was done to test the hypothesis, as it would give the best statistical analysis of if the temperature ranges were significantly greater in one microclimate versus another. Assumptions of evenness of variance and normal distribution were met. In three separate analyses, I compared daily mean temperature, daily maximum temperature, and daily minimum temperature above the frond, below the frond and 1 m away while including location (Uptown, City Park, Jean Lafitte, or USM) as a random variable.

Testing the Cold-Coma Hypothesis

To test the hypothesis that the adaptive value of over-frond clustering is to prevent wasps from falling due to low-temperature induced cold coma, I compared the minimum critical temperatures (CT_{min}) of overwintered *M. mexicanus* to recorded ambient temperatures used to test the previous hypothesis. CT_{min} is the temperature below which an ectothermic animal loses mobility (Hazel et al., 2011). Values for CT_{min} in overwintering wasps were collected by a collaborator for another in-lab project (Unpublished data) using 38 wasps from City Park and Uptown colonies assayed in February 2022. Wasps were allowed to acclimate for four days at 26° C with a 13:11 light:dark cycle and humidity ranging from 76% to 98%. Wasps from these populations were put into conical tubes placed into an EchoTherm™ IC20 cooling bath (Torrey Pines Scientific, Carlsbad, California, USA). They were then ramped down in temperature at a rate of 1 ˚C every 10 minutes. The coldest temperature at which they retained mobility (assessed as any movement response to tapping) was considered their CT_{min}. I compared these values to gathered ambient temperatures using a histogram.

CHAPTER III: RESULTS

Nest Falling Results

A Fisher's exact test was used to find the proportionality between the number of nests that stayed versus nests that fell and how those numbers varied with changing seasonality (Table 1). The Fisher's exact test showed that nests were more likely to fall in the three cold months from December to March than during the five sampled hot months from July to December ($p = 9.02 \times 10^{-6}$). Although the number of days between each survey differed, the shorter winter period still saw more nests falling than in the summer, but rate per day was not recorded to keep it seasonally focused.

Microclimate Results

Results of the linear mixed-effect analysis suggest that there was no significant difference in daily max temperature above, below, or away from palmetto fronds ($X^2 = 1.16$, df = 2, p = 0.559; Figures 1 & 2). Daily minimum temperatures across microclimates were also not significantly different ($X^2 = 0.02$, df = 2, p = 0.990; Figures 1 & 2). Daily mean temperature was the same across the sampled microclimates as well ($X^2 = 0.05$, df = 2, p = 0.972; Figures 1 & 2).

Cold Coma Results

The average temperature at which *M. mexicanus* lost all mobility (CT_{min}) was 2.97˚C ± 0.68 SD. When comparing overwintering CT_{min} values and ambient temperatures, it appears that temperatures did drop below the temperature at which *M.*

mexicanus would lose all mobility during the three weeks of winter temperature recordings (Figure 3).

CHAPTER IV: DISCUSSION

Striking similarity in temperature above and below palmetto fronds caused me to reject the hypothesis that the wasps are aggregating on the top of the palmetto frond because it is warmer there (Figures 1 & 2). This is of note, considering the level at which temperature relates to physiological output in this and similar species (Gunnels, 2007). Results showed that it actually is not warmer on upper sides of the palmetto fronds when compared to the temperatures of the undersides of the fronds or as compared to ambient air temperature away from the palmetto. It should be noted though, that probes were placed on the surface of the frond, with direct contact, to ensure that when these results were gathered they could be as accurate in rejecting or accepting the hypothesis as possible.

There were temperatures recorded that made these wasps lose entire body mobility. It is plausible that aggregating on top of palmetto fronds may be of adaptive value because when these wasps lose all body mobility, they will not fall to the ground. These recorded temperatures and associated values of critical temperatures of the species (Figure 3) support this thought for the behavior exhibited. Other behaviors, rather than aggregating, have also been noted in the related species, such as cohabitation for overwintering (Erram & Judd, 2017).

Similarly, I found evidence that the nest itself is more likely to fall in cold months. One reason the nest might be more likely to fall in winter is because the wasps themselves are rendered immobile. The wasps being rendered immobile means they would be unable to upkeep the maintenance necessary to keep the nests from falling or losing structural integrity. A previous study also found a significant decrease in maintenance and upkeep of the nests before early spring, so mainly in the months of December and January (Gunnels, 2007). This higher likelihood of nests falling in these months could explain why overwintering wasps aggregate on top of the frond and not just on top of the nest itself. I believe it would be beneficial for future studies to dive deeper into this aspect of overwintering behavior, perhaps by placing cameras in nesting areas and observing the rate of upkeep in various months and how that level of upkeep changes with the seasons. I believe it would be interesting to determine if the temperature can truly be correlated to nest upkeep and maintenance efforts.

Limitations and Caveats

There were a few notable limitations and unexpected roadblocks that came about during this study. The first was with the way in which the monitored nests were marked with sharpie on the palmetto plants. I had made numbered marks with red sharpie on each underside of the palmettos, large enough to be visible and identifiable. Upon return in December and March, the goal was to find these marks and be able to identify each specific nest, but by the first return survey many of the sharpie marks were no longer present. Thankfully, photos were taken of a select number of the nests, so photo matching could be done on nests where the sharpie marks had washed away or been worn off. Also, the possession of the exact GPS coordinates from the initial survey helped to improve confidence in my assessment of nests that were still present when I returned. However, it is of note that the absence of the markings diminished full confidence that it was the same nest being observed in each returning survey. If repeated future improvements to this methodology could include using a small amount of tagging tape on the stem of the frond with the active nests, or even placing a small notch in a frond that has a nest being studied. The issue with the tagging tape or other methods suggested is the worry about general public interference. City Park is a high traffic area and the nests being observed for our research are typically right on the walking paths, so any obvious markings may cause curiosity from people and possible harm to the nests or other disruptions in general.

Another limitation of this study was the seasonal replicates being different lengths than expected. The original protocol I planned had one replicate spanning between July and October and one spanning between October and April, each with 114 days between an ideal “spring/summer season” and “October/winter season”. The seasonal replicates that ended up being used were from July 3, 2022 to December 3, 2022 and then December 3, 2022 to March 5, 2022. This means the warm weather replicate was 153 days and the cooler replicate was 92 days in length. More warm months were recorded then, because of this, but even with a longer warm replicate, more nests were recorded that fell in the shorter winter replicate.

Importance

The results I present in this study are important to science in a few ways. First, learning about how animals are adapted to current and previous climates helps us understand how they will fair in a changing climate. The direct impacts of climate change on this abundant native pollinator are understudied, but it is important to include physiological and behavioral information in such assessments. This research also helps to fill natural history and biogeographic knowledge gaps for *M. mexicanus*. The species is anecdotally undergoing a range expansion throughout coastal regions of eastern North America but with a changing range expansion, it would help us to gain a better understanding of this species' ecology. These findings about the thermal physiology and behavioral ecology of this species broaden our understanding of winter survival tactics in other eusocial insects.

Tables and Figures

Table 1: Comparison of nests that had fallen versus nests that remained based on seasonality.

	Fallen	Remained
July to December (warm)	10	25
December to March (cool)	29	6

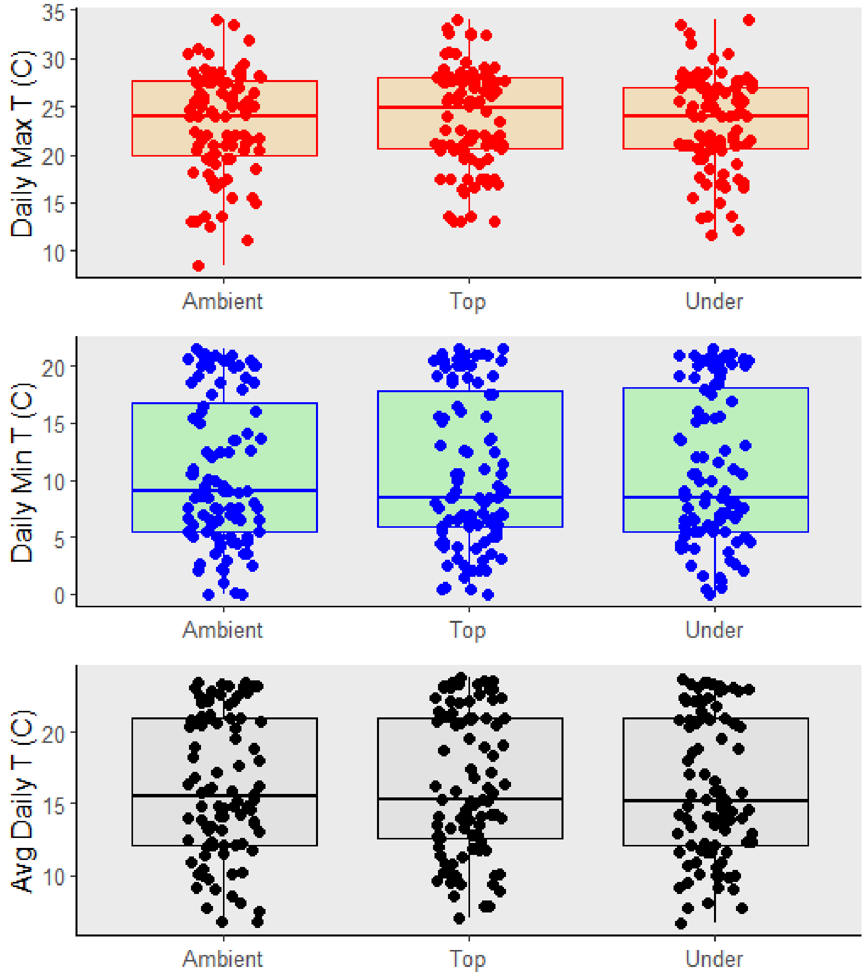

Figure 1. A comparison of the daily maximum, minimum, and mean temperatures found for each of the microclimates. "Ambient" probes were placed 1 meter away from the palmetto. "Top" probes were placed on the top side of the palmetto frond. "Under" probes were placed on the underside of the palmetto frond.

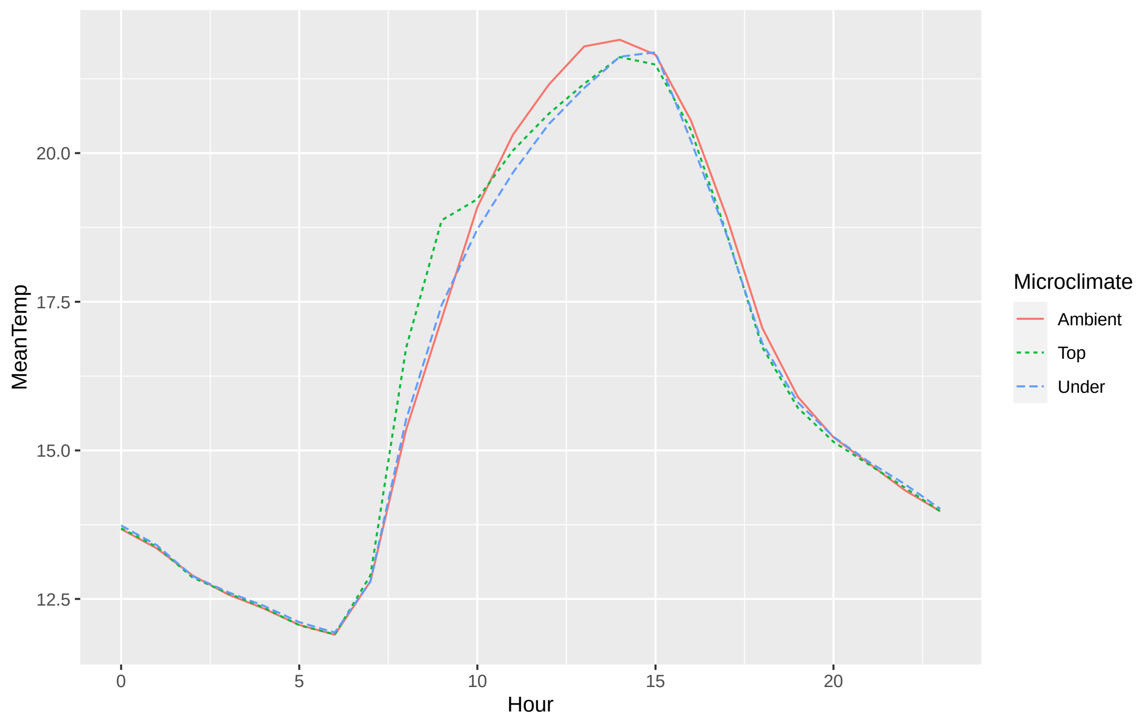

Figure 2. Temperature by hour at the three different microclimates sampled. Lines show averages across days and across four replicate palmettos.

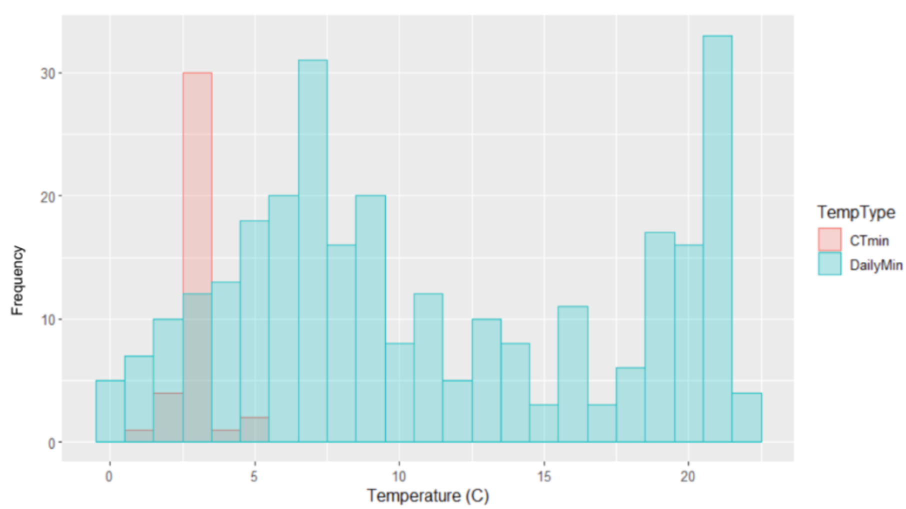

Figure 3. Histogram showing overlap of lower critical temperatures (CTmin) and daily low temperatures from February 10 to March 6, 2022, suggesting that overwintering wasps experienced total mobility loss on multiple nights.

APPENDIX A: IRB APPROVAL LETTER

Honors Scholar and Thesis Advisor IRB and IACUC Statement

I, Taylor Guild ______________________ w10006007
(Student Name) **(Student ID)**

have read the IACUC and IRB resource sheets and consulted my advisor

Dr. Kailin Baudier
(Advisor Name)

about the need for approval from one of these committees.

We have determined that my planned study:

Does not [✓] **Does** [ ] **require IRB approval**

I have submitted/will submit my protocol to IRB on ____________ **(date)**

I already have IRB approval ____________ **(date obtained)** **Protocol #** _________

Does not [✓] **Does** [ ] **require IACUC approval**

I have submitted/will submit my protocol to IACUC on __________ **(date)**

I already have IACUC approval ____________ **(date obtained)** **Protocol #** _________

I understand that, if IRB or IACUC approval is needed, data collection or experimentation may not begin until my protocol has been approved.

Student Signature: ______________________ Date: 04/07/2022

Advisor Signature: Kaitlin Baudier ______________________ Date: ______________

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