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### Context-Dependent Behavior, Reproduction and Brain Structure in Newly-Established Colonies of the Primitively Eusocial Wasp, *Mischocyttarus mexicanus*

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UNIVERSITY OF MIAMI

CONTEXT-DEPENDENT BEHAVIOR, REPRODUCTION AND BRAIN  
STRUCTURE IN NEWLY-ESTABLISHED COLONIES OF THE PRIMITIVELY  
EUSOCIAL WASP *MISCHOCYTTARUS MEXICANUS*

By

Floria Mora Kepfer

A DISSERTATION

Submitted to the Faculty  
of the University of Miami  
in partial fulfillment of the requirements for  
the degree of Doctor of Philosophy

Coral Gables, Florida

May 2011

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Context-Dependent Behavior, Reproduction and Brain  
Structure in Newly-Established Colonies of the  
Primitively Eusocial Wasp, *Mischocyttarus mexicanus*

(May 2011)

Abstract of a dissertation at the University of Miami.

Dissertation supervised by Professor Keith D. Waddington.

No. of pages in text. (92)

Reproductive division of labor is the most distinctive characteristic of the social Hymenoptera; some individuals reproduce and others forego their own reproduction to raise non-descendant offspring. In species where females are reproductively totipotent and lack morphologically distinct castes, there is potential for reproductive conflict because more than one female in a colony may attempt direct reproduction.

I focused my dissertation research on a subtropical population of the primitively eusocial paper wasp, *Mischocyttarus mexicanus*, to investigate the initiation, establishment, and development of the colony before the emergence of adult offspring. Female *M. mexicanus* exhibit variation in behavior and task performance, and switch between reproductive and non-reproductive roles. These changes in behavior and reproduction may be influenced by social context.

In three studies, I investigated the role of social context on reproduction, behavior, and brain structure. In the first study, I tested the role of body size, reproductive potential, and immediate egg-laying potential on the reproductive tactic employed by females. I found that large females either became solitary foundresses or became part of a group-initiated colony. In contrast, small females left their natal colony and pursued joining other colonies. This joiner tactic is unique to this population and has not been

observed in temperate zone populations. I also found that subordinate females had the potential to lay eggs if given the opportunity. This suggests an incentive to remain in a colony for future opportunities of direct reproduction

In the second study, I investigated the effect of three variables on non-nestmate acceptance: non-nestmate age, stage of colony development, and non-nestmate aggressive behavior. I demonstrated that non-nestmate acceptance was context-dependent. Both non-nestmate age and stage of colony development had an effect on the proportion of accepted non-nestmates. Although, non-nestmate aggressive behavior did not affect non-nestmate acceptance, it did trigger an aggressive response from colony nestmates.

In the third study, I assessed the relationship of Mushroom Bodies (MB) volume, the brain neuropils associated with learning and memory, to environmental conditions and social interactions. I compared MB volume of newly-established colonies initiated by solitary foundresses to groups of foundresses. In addition, I performed laboratory experiments to differentiate between the effect of environmental conditions and social interactions. I found a positive relationship between MB volume and environmental conditions including light intensity and foraging experience. In contrast to previous studies, I found no association between MB volume and social interactions. Ovary development was positively correlated with MB development. This result suggests that although reproductive dominance is established in newly-initiated colonies, social dominance may not yet be established.

In summary, my studies found an effect of social context on behavior, adoption of reproductive tactics and brain structure in colonies of *M. mexicanus* during the offspring pre-emergence phase.

## **Dedication**

My parents and my sister Diana encouraged my love for science and gave me their unconditional love and advice throughout this journey.

My partner Chris helped me in every step of the way. His love, support, and faith in me became my inspiration to finish this dissertation.



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My family encouraged me and supported my decision to move away from home to pursue a doctorate. Their love has overcome distance. I will always be grateful for the sacrifices my parents made so I could have a good education and learn English at an early age. I also thank them for letting me keep bugs in the fridge. My sister has been the best friend and greatest listener towards my passion for science. My brother Roberto, has given me support and encouragement as we have built a relationship through the distance. My better half, Chris, has helped me in every possible way with this project but mostly, he has believed in my capacity as a scientist even at times of doubt. I thank you for your unconditional love and support.

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## **Chapter 1**

### **Introduction**

#### **Background**

In social Hymenopteran colonies, females of one or more generations live together in an interplay of cooperation and conflict (Michener 1974, Savoyard and Gamboa 1999, Heinze 2010). These colonies are characterized by reproductive division of labor: only one or a few females reproduce, and the remaining females forego direct reproduction to raise non-descendent offspring (Wilson 1971). Although cooperation is fundamental to maintaining a stable reproductive unit, there is potential for reproductive conflict in species where females lack morphological castes and are reproductively totipotent (Röseler 1991, Ratnieks and Reeve 1992). Conflicts can involve direct reproduction attempts of several females in a colony and manipulation of egg-laying and rearing the brood of other colony members (Ratnieks et al. 2006).

Such is the case in primitively eusocial wasps, where females switch between reproductive and non-reproductive roles and change task performance depending on the social context of the colony (Röseler 1991, O'Donnell 1996, Tibbets 2007, Gunnels 2007, Gunnels et al. 2008). In contrast to highly eusocial species with determined castes, division of labor in primitively eusocial wasps is strongly influenced by social interactions. The most aggressive female becomes the primary reproductive in the colony

and the remaining females become auxiliaries (Reeve 1991, Röseler and Röseler 1989, Röseler 1991, O'Donnell 1998b). The primary reproductive female often avoids performing behaviors that are risky or require energy expenditure, such as foraging (West-Eberhard, 1981, O'Donnell, 1998a, 1998b).

Although the primary reproductive is the female with greatest ovary development, cofoundresses and offspring in group-initiated colonies can show variation in reproductive physiology (Markiewicz and O'Donnell 2001). Thus, more than one female may compete for reproductive dominance in a colony (Röseler 1991). After a female emerges as an adult, she either can remain as part of her natal colony or leave. If she remains on her natal colony, she can pursue direct reproduction or become an auxiliary. If she leaves, she can initiate nest construction as a solitary foundress and perform all colony tasks or initiate nest construction as part of a group of foundresses who share task performance (Reeve 1991, Röseler 1991, Reeve et al. 1998).

Additionally, instead of initiating a new colony, females can usurp a foreign nest to become the primary reproductive or adopt an orphaned nest (Klahn 1988, Starks 1998). In both cases, the usurpers and adopters destroy the eggs and early instar larvae and replace them with their own (Klahn 1988, Starks 2001). Adult females also frequently switch nests (Sumner et al. 2007). Previous studies have extensively investigated these different reproductive tactics. However, these studies did not explore colony initiation and subsequent development, and the establishment of reproductive and social dominance.

After colony establishment, the females maintain group integrity by using chemical cues to distinguish between nestmates and non-nestmates as potential usurpers

(Reeve 1989; Sherman et al. 1997). These chemical cues are hydrocarbons found on the nest and on the cuticle of colony members (reviewed in Dani 2006). Females learn these identifying chemical cues soon after they emerge as adults and compare this template to the cues of other wasps (Gadagkar 1985). Each colony develops a specific chemical profile during establishment, and newly-emerged females acquire the chemical profile gradually (Arathi et al. 1997, Panek et al. 2001). However, in some primitively eusocial wasps and bees, approximately 25% of the females in a colony are not related (Queller et al. 2000, Kûdo et al. 2007, Zanette and Field 2008, Soro et al. 2009). These results further suggest nest-switching and also that some females were originally non-nestmates subsequently accepted into colonies other than their natal one.

Prior studies show a higher acceptance rate of non-nestmates immediately after the emergence of the first adult offspring compared to colonies with several generations of adults (Gamboa et al. 1991b, Gamboa 1996). These studies suggest that selection favors changes in the acceptance threshold of non-nestmates under different social contexts throughout a colony's development (Reeve 1989, Starks et al. 1998). Nevertheless, it is unknown whether acceptance of non-nestmates is context-dependent in the offspring pre-emergence stage. During this stage of colony development, either a single foundress or a small group of foundresses perform all the tasks in a newly-established colony without the work of adult offspring.

Another unexplored factor in newly-established colonies is the relationship between the development of brain structures and division of labor and social dominance. Previous studies in honeybees, ants, and wasps have investigated the Mushroom Bodies (MB), the brain neuropils associated with learning and memory. These studies provide

evidence for structural changes in MB associated with foraging experience (Gronenberg et al. 1996, Fahrbach et al. 1998, O'Donnell et al. 2004). In established colonies of primitively eusocial wasps, social and reproductive dominance determined by aggressive behavior are associated with MB development (O'Donnell et al. 1998b). Dominant females that spent more time in the nest than foragers had increased MB development (O'Donnell et al. 2007, Molina and O'Donnell 2008a). Also, social dominance and ovary development are positively correlated with MB volume (Molina and O'Donnell 2007). Therefore, in established colonies after emergence of the adult offspring, changes in MB volume are more strongly associated with ovary development and social dominance, than with foraging. To date, no study has investigated changes in MB while social and reproductive dominance are being established during colony initiation.

### **Study species**

I used colonies of the primitively eusocial wasp, *Mischocyttarus mexicanus*, to determine the effect of the social context on reproductive tactics, non-nestmate acceptance and associated behavioral interactions, and changes in brain structure. *M. mexicanus* was originally found in the Bahamas, Puerto Rico, Cuba, and the southeastern U.S.A. and has recently expanded to Texas (Carpenter et al. 2009). I focused on newly-established colonies in a subtropical population located in Kendall Indian Hammocks Park, Florida (25°69'N, 80°38'W). The population does not face strong seasonal changes and colony cycles are asynchronous, with active colonies in different stages throughout the year. Females are highly variable in reproduction and behavior in the offspring pre-emergence

phase (Mora-Kepfer pers. obs). After colony establishment, one female becomes the primary reproductive and the other females become auxiliaries (Röseler 1991). Nest switching is frequent and females switch between solitary and group founding strategies, and more than one female within a colony can be reproductively viable (Litte 1977, Hermann et al. 1985, Clouse 1995, 2001, Gunnels 2007, Gunnels et al. 2008).

### **Objectives**

The main goal of my dissertation was to investigate several aspects of behavior, reproduction, and brain structure during colony establishment of *M. mexicanus*. Specifically, I focused on the effect of social context on: 1) the reproductive tactics used to establish colonies, 2) the acceptance of non-nestmates in recently-established colonies, and 3) brain development and its relationship with foraging and social interactions.

In Chapter 2, I performed a detailed analysis of the reproductive tactics in this population. I marked aggregations of females and solitary females and observed the construction of the first cell in each nest. During the initiation of nest construction and colony establishment, I determined which reproductive tactics were present and tested for the effects of body size, reproductive potential, and immediate egg-laying potential on adoption of each tactic. I also performed experimental manipulations to simulate joining behavior of females that left their natal nest to join a foreign one. I determined the proportion of accepted large and small joiners by both solitary foundresses and groups of foundresses.

In Chapter 3, I examined the effects on the acceptance of non-nestmates of three variables: 1) non-nestmate age, 2) stage of colony development within the offspring pre-emergence phase, and 3) non-nestmate aggressive behavior when joining a foreign colony. I additionally tested for possible interactions among these three variables. Finally, I investigated whether colony size had an effect on non-nestmate acceptance.

In Chapter 4, I tested for an association between MB volume and environmental conditions and also social interactions in newly-established colonies. I assessed differences between MB volume in solitary foundresses and groups of foundresses in field colonies, and between primary reproductives and auxiliaries. To discriminate between the effects of environmental conditions and social interactions, I performed experimental manipulations in laboratory colonies. Finally, I used both body size and ovary development as proxies for reproductive dominance and determined their relationship with MB volume.

My dissertation research provides critical knowledge on the effects of social context on colony initiation and establishment in primitively eusocial wasps. I provide novel information on initiation of nest construction and the reproductive tactics employed by females to establish colonies. My results provide the first evidence for the effect of the social context on non-nestmate acceptance as colonies are initiated and develop before the emergence of adult offspring. Finally, my findings show a positive relationship of MB volume with ovary development and foraging, but no association with social interactions in newly-established colonies.

## Chapter 2

### **Reproductive tactics are associated with body size, reproductive potential, and immediate egg-laying potential in *Mischocyttarus mexicanus* females (Hymenoptera: Polistinae)**

#### **Summary**

In primitively eusocial wasps, adult females use alternative reproductive tactics to maximize reproductive success under different social and environmental circumstances. Previous research shows that primitively eusocial wasps are relatively plastic in their behavior, they lack morphologically distinct castes, and that more than one female may attempt direct reproduction in a colony. Their behavioral and reproductive plasticity make them an excellent model system to test whether the choice of reproductive tactic is related to reproductive potential and opportunities for direct reproduction of each female in a colony. Here, I test the effect of body size, reproductive potential, and immediate egg-laying potential on adoption of specific reproductive tactics in a subtropical population of the wasp *Mischocyttarus mexicanus*. My results indicate that large females became solitary foundresses or initiated a colony as a group of foundresses. In contrast, small females left their natal nest and pursued joining other colonies, which has not been observed in temperate populations of *M. mexicanus*. Small joiners were accepted more frequently by solitary foundresses than by groups of foundresses. Joiners also exhibited low reproductive potential and low immediate egg-laying potential compared to large females. Even though the reproductively dominant female in a colony

had the highest reproductive potential, other females were capable of laying eggs, regardless of their subordinate role. My findings show that reproductive tactics are associated with body size, reproductive capability, social environment, and opportunities for direct reproduction.

## **Background**

In animal societies, different behaviors have evolved to maximize the reproductive success of individuals under a wide range of conditions (Tarborsky et al. 2008, Tarborsky and Brockman 2010). Such is the case in the social Hymenoptera, in which females employ different reproductive tactics (Ratnieks et al. 2006, Foitzik et al. 2010), and the choice of tactic may be influenced by genetic, physiological, social and/or ecological factors (Trivers and Hare 1976, Röseler 1991, Keller and Reeve 1994, Field et al. 1998). The evolution of alternative reproductive tactics might be explained by differences in the reproductive capability of females in a colony (Tibbetts 2007). Some females might be subfertile or reproductively constrained while others are not (West-Eberhard 1975, Sumner et al. 2010). Additionally, other factors such as the high cost of solitary nesting (Clouse 1995) and low availability of nesting sites (Gunnels 2007) may influence the reproductive tactic employed. Similarly, indirect fitness benefits from raising the offspring of kin may influence a female's choice (Hamilton 1964), as might the benefits of remaining on the natal nest for inheritance of the nest (Queller et al. 2000), or availability of other nesting sites (Field et al. 2006). Different reproductive tactics allow females to respond adaptively to both environmental and social conditions (Gross 1996).



Here, I investigate the factors that influence the choice of reproductive tactics during the initiation of nest construction and colony establishment in the primitively eusocial wasp, *Mischocyttarus mexicanus*.

Primitively eusocial wasps lack morphologically distinct castes and show a degree of reproductive plasticity, as many females in the colony can reproduce directly (West 1967, Litte 1977, O'Donnell 1996, Tibbetts 2007). During colony establishment, females compete for reproductive dominance of the colony and one female becomes the primary reproductive while the remaining females become auxiliaries (Chandrashekara and Gadagkar 1991, reviewed in Röseler 1991, Sinha et al. 1993). Females pursue different choices in nest initiation and behavior during colony establishment (Reeve 1991, Röseler 1991, Yamane 1996). They can initiate nests as a solitary foundress, or instead, as a group of foundresses (Pardi 1948, Litte 1977, Reeve 1991; Nonacs and Reeve 1995). When a female emerges as an adult, she can either remain in her natal nest or leave (Appendix I). If the female decides to stay in her natal nest, she can pursue two alternative reproductive tactics: compete with the other females to become the primary reproductive or instead remain in the colony as an auxiliary (Röseler 1991). If the female decides to leave, she can initiate construction of a new nest as a solitary foundress, join a foreign colony (Starks 1998, Sumner et al. 2007, Mora-Kepfer in prep), adopt an orphaned nest (Starks 2001), or usurp another colony to become the primary reproductive (Clouse 1995, Nonacs and Reeve 1995).

The choice of reproductive tactic may be influenced by different factors, including body size, reproductive potential, and immediate egg-laying potential (Reeve 1991, Röseler 1991). Previous studies suggest an association between body size and the

rank of a female in the hierarchy of a colony (West-Eberhard 1975, reviewed in Röseler 1991, Smith et al. 2008). Additionally, body size has been associated with fighting ability (Turillazi and Pardi 1977), thus large females may have an advantage in acquiring reproductive dominance in a colony over small females. Although there is limited evidence, body size is positively correlated with reproductive potential (Lin and Michener 1972, Reeve 1991, Cervo et al. 2008). There is also mixed evidence that ovary development (as a measurement of reproductive potential) is associated with aggression and social dominance in paper wasps (Röseler 1991, Markiewicz and O'Donnell 2001, Molina and O'Donnell 2008b, but see Gunnels 2007 and Izzo et al. 2010). In colonies initiated by a group of foundresses, the primary reproductive female may retain dominance by inhibiting to some degree the reproduction of the auxiliary females (West-Eberhard 1978, Smith et al. 2009). However, if auxiliaries remain in group-initiated colonies because of future reproductive incentives, they should be able to lay viable eggs regardless of their subordinate role. For example, an auxiliary may have low reproductive potential compared to the primary reproductive, but not necessarily have low immediate egg-laying potential if given the opportunity to lay an egg.

Reproductive tactics are also affected by seasonality in temperate zones (Gunnels 2007, Starks 1998, Starks 2001). A study of a temperate population of *Mischocyttarus mexicanus* revealed that females received different payoffs using alternative reproductive tactics during different seasons Gunnels (2007). In addition, the exclusive effect of body size of a female on the adoption of a reproductive tactic may be difficult to determine as age and size can co-vary in environments with defined seasons (Smith et al. 2008). Therefore, exploring the effect of body size in an environment without defined seasons

would clarify its potential association with the reproductive tactic adopted by each female in a colony.

In this study, I performed a detailed analysis of the reproductive tactics in a subtropical field population of the paper wasp *Mischocyttarus mexicanus*. This population is asynchronous, with active colonies in different stages of the life cycle throughout the year, and does not face strong seasonal constraints (Mora-Kepfer in prep). I determined which reproductive tactics were present in this population compared it the reproductive tactics of a population in a temperate zone. I tested the effects of: 1) body size, 2) reproductive potential, and 3) immediate egg-laying potential of the females adopting each tactic. I predicted that if body size is related with reproductive dominance within a colony, small females should adopt auxiliary roles in group-initiated nests, and large females should become primary reproductives on a group-initiated nest or a solitary foundress. Second, I predicted that primary reproductives in group-initiated colonies should have greater ovary development than solitary foundresses, and subordinate females should have less ovary development. Third, I predicted that in group-initiated colonies, primary reproductives should have higher immediate egg-laying potential than auxiliaries, but auxiliaries should be able to lay viable eggs regardless of their subordinate role.

Finally, I experimentally manipulated females to simulate the reproductive tactic of joining a colony other than its natal one. These non-natal colonies could be those initiated by a group of foundresses or initiated by a solitary foundress. I performed two joiner treatments. In treatment 1, I simulated a female of large body size approaching a colony and exhibiting joining behavior. I expected joiners to be more frequently accepted

by solitary foundresses, compared to groups, because solitary foundresses may benefit from having nestmates aid in care of offspring and nest construction. In treatment 2, I simulated the same joining behavior using a second set of joiners which consisted of small females. I predicted that small joiners might not be perceived as a high usurpation threat and consequently would be accepted more frequently than large joiners.

## **Methods**

### *Study site and data collection*

I conducted my study in Kendall Indian Hammocks Park, Florida (25°69'N, 80°38'W) between May 2009 and June 2010. I selected 33 colonies in an asynchronous subtropical population of the paper wasp *M. mexicanus* and observed them exclusively during the period of colony initiation. I marked females with Testors® enamel paint as they rested on potential nesting sites before initiation of nest construction. Each marked wasp was monitored daily for two hours; I categorized the type of colony initiation as solitary initiation or group initiation (Appendix II). I documented the construction of the first cell and the total number of females involved in cell construction (Appendix III). Solitary foundresses initiated nest construction individually, but were later approached by other females that attempted to join them (Appendix IV). If the foundress allowed these joiner females to remain 5-10 cm in proximity to the newly initiated nest, they subsequently became accepted as colony members. When an accepted joiner started performing colony tasks, I marked her with paint for unique recognition. Group foundresses initiated nest

construction together. I observed each colony for two hours during three consecutive days after nest initiation to detect potential joiners.

The reproductive behavior of each wasp in a colony was categorized by using an established ethogram for *M. mexicanus* (Gunnels 2007, Appendix V). After the 3-day observation period, I collected the colonies at night to ensure that all females were present. I stored each marked female individually in the aldehyde-based fixative Prefer (Anatech, LDT) for future dissection to determine their reproductive role in the colony.

#### *Morphological measurements and dissections*

I photographed the dorsal view of the head and thorax of each wasp from the 33 collected colonies with an Axiocam MRm camera (Zeiss). I used the image analysis software Axio Vision Rel. 4.8 software (2009) to measure three morphological traits to estimate body size: the minimum intereye distance of the head (Min IE), mesonotum length (MSL), and mesonotum width (MSW) of the thorax (Fig 2.1a). I performed a principal component analysis and used the first principal component to estimate size using these three body size measurements for each wasp. I used the loadings for each wasp to create a new variable called PC1 “body size” that combined all three measurements and met the assumptions of normality (Gunnels 2007).

I dissected the metasoma (‘abdomen’) of each female and photographed the ovaries. I estimated the reproductive potential as the mean area of all the oocytes in the ovary. I measured the maximum length and width of each oocyte using the image analysis software described above (Fig 2.1b). Since oocytes are elliptically shaped, I

calculated the area of each individual oocyte as  $\pi \times [1/2] \text{ width} \times [1/2] \text{ length}$  (Molina and O'Donnell, 2007, 2008a).

Because the reproductive tactics in this subtropical population of *M. mexicanus* were unknown, I determined which tactics were present based on previous categorical descriptions of Polistinae wasps (Litte 1977, Reeve 1991, O'Donnell 1996, O'Donnell 1998b, Starks 1998, Starks 2001, Gunnels 2007). For colonies initiated by a group of foundresses, I determined two reproductive tactics: 1) the female with the highest reproductive potential was considered the primary reproductive and 2) other females in the colony were considered auxiliaries. I also determined two reproductive tactics for the colonies initiated by a solitary foundress and subsequently joined by other females: 1) the female who initiated nest construction alone was categorized as the solitary foundress, and 2) the females that were subsequently accepted by the solitary foundress and became subordinate, were categorized as joiners. Other potential tactics employed were: 1) usurpation when a female left its natal nest and instead of becoming a subordinate joiner pursued reproductive dominance in another nest, and 2) adoption of an orphaned nest. To avoid pseudoreplication, I determined the ovary area of all auxiliaries in each colony, and used the mean ovary area as the single measurement of reproductive potential of the auxiliaries per colony. I performed the same calculations to obtain a single measurement per colony for the joiners.

I measured the length of the largest oocyte as a conservative representation of immediate egg-laying potential for each female (Fig 2.1c). Developed oocytes in *M. mexicanus* are on average 1.2 mm long (Gunnels 2007). Therefore, I used oocyte length to determine whether a female had the ability to lay a viable egg if an opportunity arose,

independently of its reproductive tactic. I calculated the mean oocyte length for all auxiliaries and the mean oocyte length of all joiners as an estimate of their immediate egg-laying potential. Additionally, I collected one newly laid egg from 22 colonies. I determined the length of each laid egg as a control measurement and compared these with the length of the most developed oocyte per ovary.

### *Joiner simulation experiment*

#### Treatment 1

During the nest initiation phase, I simulated the approach of a joiner in 19 colonies initiated by a group and 14 colonies initiated by a solitary female. I selected joiners by isolating one female wasp from each foreign nest. I measured the three previously described body measurements for each wasp: minimum intereye distance (Min IE), mesonotum length (MSL), and mesonotum width (MSW) to create a new composite variable named PC1 body size (Fig 2.1). I selected as large joiners, the females that measured at least the average of primary reproductives previously collected.

Each joiner was cooled on ice, and marked distinctively. When the joiner became active, I put the end of a thin wooden stick in front of the wasp and it climbed onto the stick. The potential joiner was placed approximately 5 cm away from the colony to simulate previously observed behavior of approaching joiners (Mora-Kepfer unpublished). I excluded any events in which the introduced joiner did not walk freely towards the nest after being introduced. Each test colony and simulated joiner was used one time.

To prevent a possible effect of philopatry resulting in high joiner acceptance (reviewed in Röseler 1991), I selected joiners randomly from nests located 1.5 km away from my focal colonies. I videotaped interactions between colony residents and the simulated joiner and categorized the outcome. An outcome was considered as “accepted” if the joiner was allowed to stay close to the colony and “rejected” if the joiner was forced off the nest and not permitted to remain in proximity (5-10 cm) to the nest.

I used established ethograms for *Mischocyttarus* to categorize the behavior of the colony members as aggressive or non-aggressive (Itô 1993, Gunnels 2007). An interaction was scored “aggressive” if the colony residents physically attacked a joiner by grappling or biting it. An interaction was scored “not aggressive” if the colony residents did not physically attack the joiner. Using the videotaped interactions, I determined aggression levels from 1 to 4 based on the aggression scores established by Stuart and Herbers (2000). I assigned a score of zero if the interactions consisted of grooming and/or antennation but no aggression. A score of one indicated mild aggression such as pushing. A score of two indicated increased aggression such as chasing and biting. A score of three indicated high aggression with physical dragging and forcing the joiner off the nest. A score of four was assigned when a colony resident tried to kill a joiner by stinging, grappling, and biting while forcing it off the nest. I assigned the aggression scores blindly to the code of each taped interaction.

### Treatment 2

I selected only small joiners and introduced them in proximity to 19 group colonies and 17 solitary foundresses. I chose the small joiners by collecting females from foreign nests



and measured three previously described morphological traits to calculate. PC1 size variable as previously described. Small joiners were selected as females with the average size of joiners previously collected. I also videotaped each trial and assigned an aggression score from 0-4 for each interaction to establish the outcome as accepted or rejected. I used each simulated joiner and test colony only once.

### *Statistical analyses*

I used a Mann–Whitney *U* test to determine if the number of nestmates differed between group foundress colonies and colonies initiated by a foundress and joiners. I performed one-way ANOVAS followed by Tukey HSD post hoc tests to compare body size, reproductive potential, and immediate egg-laying potential among the four reproductive tactics. Data that did not meet normality assumptions were log-transformed. To assess the relationship between size and both reproductive potential and immediate egg-laying potential for each of the four reproductive tactics, I used Spearman correlations.

I compared the acceptance rate of joiners in group initiated colonies and colonies initiated by a solitary foundress with a *G*-test. In Treatments 1 and 2, I used Mann–Whitney *U* tests to test for aggression levels in solitary and group founding colonies towards joiners in each introduction treatment. I tested for differences in aggression levels towards joiners by the females grouped in each of the four reproductive tactics using Kruskal-Wallis tests followed by pairwise comparisons similar to a post hoc Tukey test. I used SPSS 17 to perform all the analyses (SPSS, Inc. 2008).

## Results

### *Behavioral field observations and morphological measurements*

In this population of *M. mexicanus*, I detected four reproductive tactics: two tactics in group initiated colonies and two other tactics in colonies initiated by a solitary foundress (Appendix II). In group initiated colonies, the “primary reproductive” was the female with the highest reproductive potential and “auxiliaries” were the remaining females in the colony. In colonies initiated by a single female, the “solitary foundress” was the female who initiated nest construction alone and “joiners” were the approaching females that were allowed to remain on the nest (Appendix IV). The joiner tactic is exclusive to this subtropical population and has not been reported in temperate populations of *M. mexicanus*. I did not observe any usurpation attempts or adoption of orphaned colonies.

Of the 33 observed and collected colonies, 19 were initiated by a group of foundresses and 14 by a solitary foundress. Every colony initiated by a solitary foundress was approached by females, and at least one female was accepted and allowed to remain on the nest as a joiner after the second or third day of nest construction. Group initiated colonies within an average of 3 days after initial nest construction had a higher number of nestmates (median = 4) than colonies initiated by a solitary foundress and subsequently joined by other females (median = 2) (Mann–Whitney  $U = 63$ ,  $df = 1$ ,  $P = 0.042$ ,  $N_{\text{colonies}} = 30$ ).

Size varied significantly among individuals employing the four reproductive tactics ( $F_{3,56} = 4.36$ ,  $P = 0.01$ , Fig. 2.2a). Joiners were significantly smaller than auxiliaries, primary reproductives, and solitary foundresses (Tukey HSD,  $P = 0.002$ ). Reproductive potential also differed among reproductive tactics. ( $F_{3,56} = 26.57$ ,  $P <$

0.001, Fig. 2.2b). Primary reproductives in group-initiated colonies had greater reproductive potential than solitary foundresses (Tukey HSD,  $P = 0.04$ ). However, solitary foundresses had significantly higher reproductive potential than auxiliaries (Tukey HSD,  $P = 0.001$ ). Immediate egg-laying potential was also significantly different among reproductive tactics ( $F_{3,56} = 11.4$ ,  $P < 0.001$ , Fig. 2.2c). Primary reproductives and solitary foundresses had the highest immediate egg-laying potential in comparison to auxiliaries (Tukey HSD,  $P = 0.001$ ) and joiners (Tukey HSD,  $P = 0.001$ ). However, auxiliaries had a significantly higher immediate egg-laying potential than joiners (Tukey HSD,  $P = 0.01$ ). These results reveal that joiners had the smallest body size, lowest reproductive potential, lowest immediate egg-laying potential, and consequently, the greatest disadvantage in direct reproduction.

When all reproductive tactics were grouped together, I found significant positive relationships between size and reproductive potential (Spearman rank correlation  $r = 0.46$ ,  $N = 58$ ,  $P = 0.01$ ) and size and immediate egg-laying potential (Spearman rank correlation  $r = 0.48$ ,  $N = 59$ ,  $P = 0.01$ ). In contrast, when I analyzed the relationship between body size and reproductive potential in each of the four reproductive tactics, I found strong positive relationships between size and both reproductive potential and immediate egg-laying potential only in the joiner tactic. A larger body size was positively correlated with higher reproductive potential and egg-laying potential only in joiners, the smallest wasps. In contrast, I found no correlation of size and both reproductive potential and immediate egg-laying potential within the three other reproductive tactics (Table 2.1)

### *Joiner simulation experiment*

#### Treatment 1

The proportion of accepted large joiners was similar for both group and solitary initiated colonies ( $G = 1.14$ ,  $df = 1$ ,  $P = 0.28$ ,  $N = 33$ , Fig. 2.3a). Both group-initiated colonies and colonies initiated by a solitary female were more aggressive in interactions that resulted in joiner rejection than in interactions where the joiner was accepted (Mann–Whitney  $U = 20$ ,  $df = 1$ ,  $P = 0.001$ ,  $N = 33$ , Fig 2.3c.). In both group and solitary-initiated colonies, residents exhibited similar aggression levels towards joiners (Mann–Whitney  $U = 137$ ,  $df = 1$ ,  $P = 0.88$ ,  $N = 33$ ).

#### Treatment 2

Small joiners were accepted more frequently by solitary foundresses than by females in group-initiated colonies ( $G = 5.95$ ,  $df = 1$ ,  $P = 0.01$ ,  $N = 36$ , Fig. 2.3b). Colony residents in both group and solitary-initiated colonies were also more aggressive in interactions where small joiners were rejected and than in interactions where the small joiner was accepted (Mann–Whitney  $U = 1.2$ ,  $df = 1$ ,  $P = 0.001$ ,  $N = 36$ , Fig 2.3d). Similarly to Treatment 1, residents in both group and solitary-initiated colonies did not differ in aggression levels towards joiners (Mann–Whitney  $U = 210$ ,  $df = 1$ ,  $P = 0.11$ ,  $N = 36$ ).

### **Discussion**

My study reveals novel information on the initiation of nest construction in solitary and group-initiated colonies. I followed marked aggregations of females and solitary females

and observed the construction of the first cell. I found four reproductive tactics. In group-initiated colonies, I found two tactics: a primary reproductive foundress and one or more auxiliaries. In colonies initiated by one female I found two tactics. The initial solitary foundress who began nest construction was subsequently pursued by other females after nest initiation who were subsequently accepted as joiners. Large females became either: primary reproductives, auxiliaries, or solitary foundresses. In contrast, most joiners were small females.

This subtropical population of *M. mexicanus* reveals differences in nest initiation and reproductive tactics from a temperate population (Gunnels 2007, 2008). In temperate areas, the choice of reproductive tactics was influenced by the season: large females nested as solitary foundresses in the spring, and instead as group foundresses during the summer. Low availability of nesting sites resulted in a high frequency of group-initiated colonies in those temperate populations (Gunnels et al. 2008). In contrast, in this subtropical population, I found both solitary and group initiated-nests throughout the year, but colonies initiated by solitary females were less frequent than group-initiated ones. Group-initiated colonies may be more prevalent because they are more successful in resisting predation, cannibalism of brood, and usurpation attacks (Litte 1977; Clouse 1995; Klahn 1988, Shakarad and Gadakgar 1995).

The joiner tactic is exclusive to this subtropical population and has not been observed in temperate populations. My findings reveal that females that pursued the joiner auxiliary tactic were always small and joined nests initiated by a solitary foundress. The joiner simulation treatments provide evidence that small joiners are accepted more frequently by solitary foundresses than by groups of foundresses. This result suggests that

solitary foundresses may benefit from accepting small females as joiners. However, small and large joiners were accepted in similar proportions by solitary foundresses. This result does not support the prediction that large joiners may be perceived as a usurpation threat (Clouse 1995), and consequently be rejected more frequently. Large females may be difficult to reject by a solitary foundress in contrast to a group of foundresses defending the nest together. Litte (1977) showed that solitary foundresses frequently abandon newly-established nests instead of incurring the high cost of defending them. Additionally, aggressive responses by colony residents may be more costly than abandoning the nest. My data show that high levels of aggression by colony residents resulted in rejection of the potential joiner.

These results bring into question why a small female would join a colony initiated by a single foundress instead of remaining as an auxiliary in its natal nest. My data demonstrates that group-initiated nests always had more females compared with colonies initiated by a solitary foundress with subsequent joiners. Thus, a female may attempt to join a solitary foundress to have future opportunities to become the reproductive dominant (Queller et al. 2000, Cant and Field 2001, Field et al. 2006), because it would be part of a much smaller colony than if she stayed in her natal one. Small females also have a disadvantage in fighting ability when in contact with a large female (Turillazi and Pardi 1977), leaving the small female at a disadvantage to fight for reproductive dominance in their natal colony.

I found that the different reproductive tactics are associated with plasticity in reproductive potential and immediate egg-laying potential. Joiners had the lowest reproductive potential in contrast to the other three tactics. None of the accepted joiners

became the reproductive dominant female; instead they became subordinates to the solitary foundresses. Although joiners experienced reduced direct reproduction, this does not necessarily mean that they are subfertile (West-Eberhard 1975, Craig 1983). Previous studies of paper wasps and sweat bees demonstrated that if the reproductive dominant female is experimentally removed, an auxiliary can develop ovaries and lay eggs (Hughes et al. 1987, Strassmann et al. 2004, Smith et al. 2009), regardless of their role or body size. Additionally, body size is not associated with fecundity, ovary development, or dominance (Markiewicz and O'Donnell 2001, Smith et al. 2008). Therefore, my results suggest that ovary development in joiners is constrained by the presence of the reproductively dominant female (Röseler 1991), but joiners can potentially lay viable eggs.

Although primary reproductives had the most developed oocytes in group-initiated colonies, the ovaries of auxiliaries had both developed and underdeveloped oocytes. Auxiliaries also had higher immediate egg-laying potential than joiners. Thus, the results support my prediction that auxiliaries should be able to lay eggs regardless of their subordinate role. Prior studies found that eggs laid by auxiliaries are eaten by other auxiliaries and the primary reproductive (Ratnieks and Wenseleers 2005, Ratnieks et al. 2006, Alaux et al. 2007). However, not all eggs laid by auxiliaries may be detected, resulting in direct reproduction (Strassmann 1983). In addition, the colonies used in the present study were newly-initiated and the reproductive dominance recently established, so primary reproductives may not have had enough contact with auxiliaries to inhibit their ovary development (Röseler 1991).

Female *M. mexicanus* have the potential to switch between non-reproductive and reproductive roles (Gunnels 2007), which may influence large females to remain as auxiliaries in group-initiated nests. Since body size is associated with an individual's fighting ability (Turillazi and Pardi 1977), auxiliaries have higher chances of direct reproduction when competing with a similarly sized primary reproductive (Reeve et al. 1998). Another possibility is that auxiliaries similarly sized to the primary reproductive, may remain as 'hopeful reproductives' for a future chance at direct reproduction (West-Eberhard 1978). It is not known whether accepted joiners are more likely to be the kin of the colony than rejected intruders..

In conclusion, I provide evidence for an association of reproductive potential and immediate egg-laying potential with the employed reproductive tactic. I also show an effect of body size and lack of seasonality, as small females pursued the joining tactic in this subtropical population, which has not been observed in temperate populations of *M. mexicanus*. Future genetic studies in this population would clarify if joiner acceptance is influenced by relatedness among females. In addition, mechanisms of nestmate recognition and the effect of the social context on joiner acceptance should be explored in this species where individual females exhibit reproductive and behavioral plasticity.



Table 2.1. Spearman correlation coefficients between PC1 size and ovary area (mm<sup>2</sup>) and between PC1 size and oocyte length (mm) in wasps of the four reproductive tactics. Bold script indicate positive significant relationships ( $P < 0.001$ )

Colony initiation tactic	PC 1 size per tactic	Ovary Area	Oocyte length
Group	Primary reproductive	0.176	0.432
Group	Auxiliary	0.269	0.406
Foundress + joiners	Solitary foundress	0.075	0.496
Foundress + joiners	Joiner auxiliary	<b>0.829</b>	<b>0.769</b>

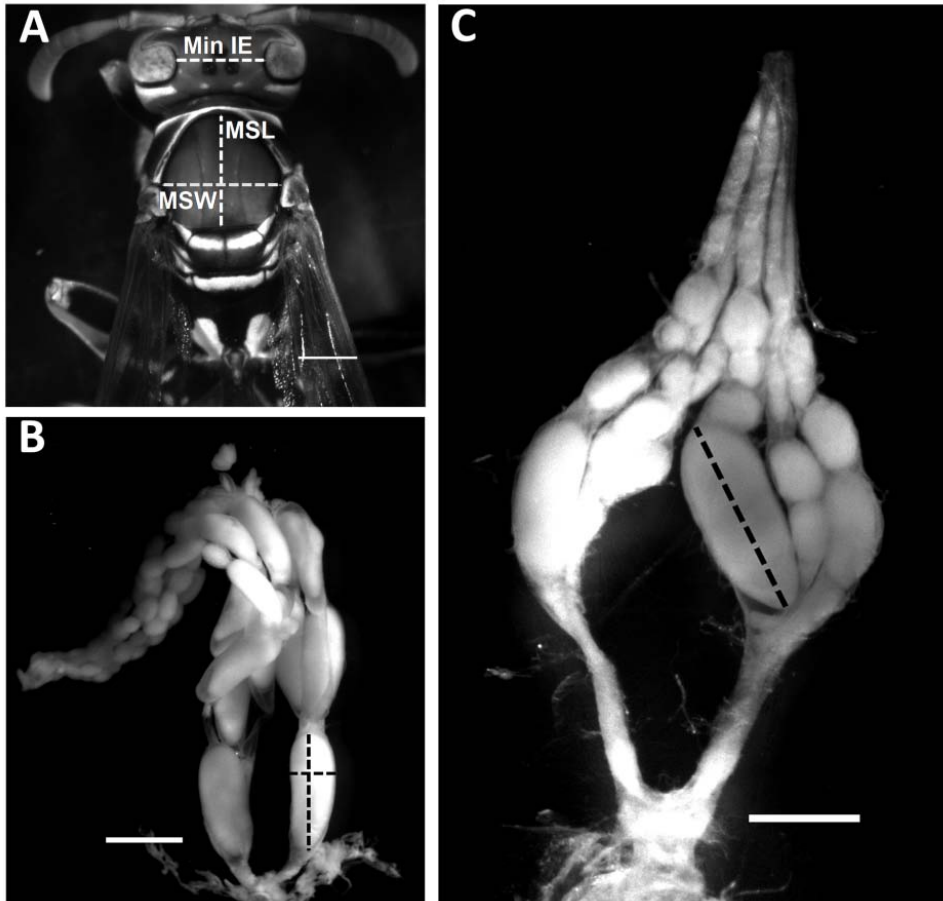
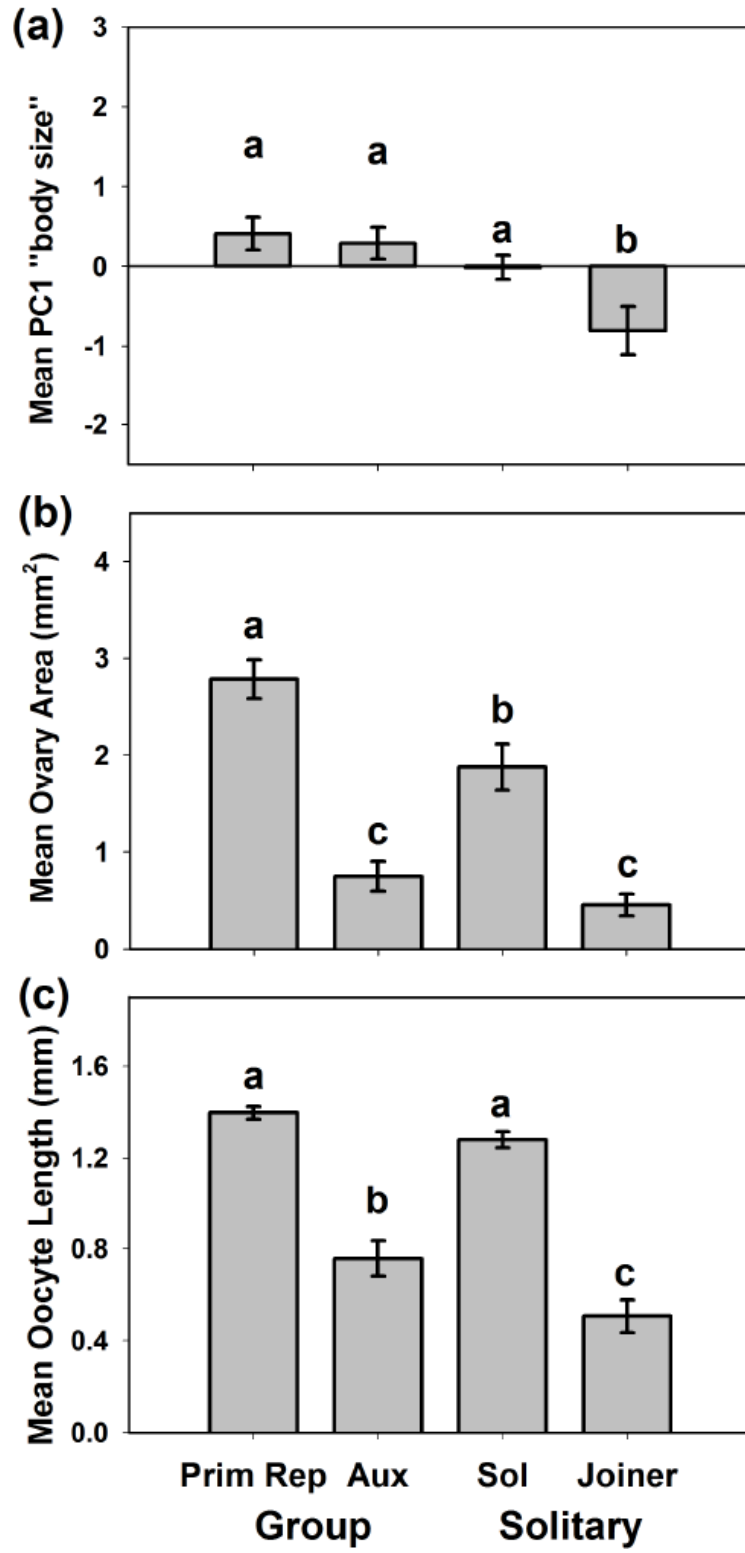


Figure 2.1 Measurements of morphological variables in *Mischocyttarus mexicanus*. a) Dorsal view of the head and thorax. Minimum intereye distance was measured as the shortest distance between eye margins (Min IE), mesonotum length as the distance between the posterior and anterior mesonotum margins (MSL), and mesonotum width as the distance between the left and right mesonotum margins (MSW), scale bar = 1 mm; b) developed oocytes of an ovary, the length and width of each oocyte was measured, scale bar = 1 mm; c) ovary with both developed and underdeveloped oocytes of an auxiliary, with a large oocyte ready to be laid as an egg. The length of the largest oocyte was measured, scale bar = 0.5 mm.

Figure 2.2 *Next page*. Comparisons among the four reproductive tactics of *M. mexicanus* females in a) mean PC1 “body size” (obtained as an amalgamated variable from combining the measurements of MIN IE, MSL, and MSW): negative numbers indicate small individuals and positive numbers indicate large individuals, b) mean reproductive potential, ovary area (mm<sup>2</sup>), and c) mean immediate egg-laying potential, oocyte length (mm). Labels of tactic categories: primary reproductives (Prim Rep), auxiliaries (Aux), Solitary Foundresses (Sol) and Joiners (Joiner). Each bar shows the mean and error bars show the standard error. Bars topped with the same letter do not differ significantly at  $P < 0.001$ , one way ANOVA tests with a subsequent Tukey test.



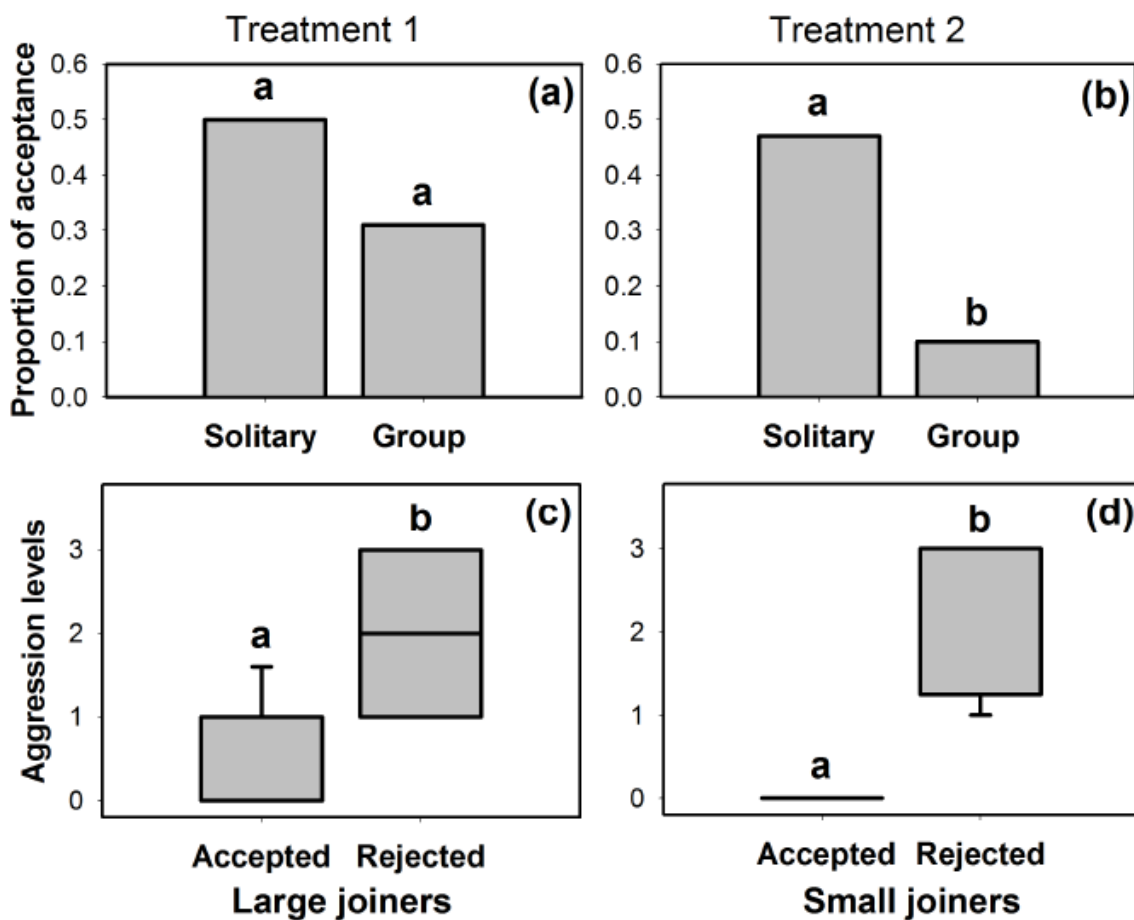


Figure 2.3 a) Proportion of acceptance in solitary and group-initiated foundress colonies in Treatment 1, large body size joiners (large joiners) and b) Treatment 2, small sized joiners (small joiners). Bars topped by the same letter do not differ significantly at  $P < 0.01$ , G tests. c) Aggression levels of the colony residents towards accepted and rejected large joiners in Treatment 1, and d) towards accepted and rejected small joiners in Treatment 2. Aggression levels ranged from 1 to 4; 0 being a score of no aggression, 1 as mild aggression (pushing), 2 as increased aggression (chasing and biting), and 3 as high aggression (physical dragging, and the joiner being forced off the nest). Box and whisker plots of aggressive categories show the median, 25<sup>th</sup> and 75<sup>th</sup> percentile (box) and 5<sup>th</sup> and 95<sup>th</sup> percentile (whiskers). Bars topped by the same letter do not differ significantly at  $P < 0.001$ , Mann–Whitney  $U$  tests.

## Chapter 3

### **Context-dependent acceptance of non-nestmates in a primitively eusocial paper wasp**

#### **Summary**

In primitively eusocial wasps, colonies maintain integrity by recognizing and rejecting approaching non-nestmates. However, in certain social contexts, non-nestmates are accepted instead. The optimal acceptance threshold model posits that the acceptance threshold of non-nestmates shifts depending on the context. In colonies of the wasp *Mischocyttarus mexicanus* that had not yet produced offspring, I tested for shifts in the acceptance threshold. I compared non-nestmate acceptance in early versus late colonies, acceptance of young and old non-nestmates, and aggressive interactions between nestmates and a joining non-nestmate. My results show that acceptance of non-nestmates was context-dependent with an effect of both non-nestmate age and stage of colony development. Young non-nestmates were more frequently accepted in early colonies than in other contexts. In late colonies in comparison to early colonies, a high rejection of both young and old non-nestmates suggests a fitness payoff to avoiding potential colony usurpers. Non-nestmate aggressive behavior did not have a direct effect on non-nestmate acceptance, but it triggered an aggressive response from colony nestmates. These findings reveal variation in the acceptance threshold in the offspring pre-emergence phase and suggest both the effect of lack of recognition and the social context on non-nestmate acceptance.

## **Background**

In the eusocial insects, the ability to discriminate nestmates from non-nestmates by using chemical cues is a key component to maintain group integrity (Wilson 1971, Gadagkar 1985, Waldman 1987, Sherman et al. 1997, Reeve 1989, Vásquez and Silverman 2008, Penn and Frommen 2010). However, discrimination may be difficult when there is overlap in these chemical cues between colony nestmates and non-nestmates (Lacy and Sherman 1983). This overlap in recognition cues might increase the possibility of recognition errors such as rejecting colony nestmates (rejection error) and accepting non-nestmates (acceptance error) (Sherman et al. 1997). Alternatively, recognition of non-nestmates may not always cause their rejection (Reeve 1989), and in certain social contexts, it may be beneficial for nestmates to accept non-nestmates into their colony (Kûdo et al. 2007).

Primitively eusocial wasps use durable chemical cues learned after adult emergence that consist of a cuticular hydrocarbon profile specific to each colony (Singer and Espelie 1992, Lorenzi et al. 1996). Nestmates compare the learned templates from their colony to the hydrocarbon cues on other wasps (Gadagkar 1985, Gamboa et al. 1986a, Gamboa et al. 1986b, Mateo 2004). Previous studies have demonstrated that each colony acquires its specific hydrocarbon profile gradually as it is being established (Arathi et al. 1997, Panek et al. 2001). Similarly, after an adult wasp ecloses and ages, it acquires the specific profile of its colony and is recognized by its nestmates.

The optimal acceptance threshold model posits that in fluctuating conditions, recognition systems should not be fixed, but instead shift depending on the context (Reeve 1989). This model assumes variability in recognition cues within individuals in a

population, and higher similarity in the cues among nestmates when compared to non-nestmates. This model also posits that the optimal acceptance threshold depends on the fitness consequences of accepting and rejecting nestmates and non-nestmates. As the fitness cost increases, the acceptance threshold increases and non-nestmates are more frequently rejected. Previous studies in primitively eusocial wasps show that the acceptance threshold shifts during different stages of the offspring post-emergence phase (Gamboa 1991a, Starks et al. 1998). Non-nestmate acceptance may also be influenced by colony size and aggressive interactions between a non-nestmate and the colony nestmates (Clouse 2001, Buczkowski and Silverman 2005). Because of these conflicting pressures, variation in non-nestmate acceptance might be adaptative and a given non-nestmate could be accepted in a specific social context and rejected in others (Reeve 1989, Gamboa et al. 1991b). However, little is known about how the social context may influence non-nestmate acceptance in newly-established colonies before the emergence of adult offspring.

During colony establishment there is competition among foundresses in a colony and females exhibit reproductive and behavioral variation (O'Donnell 1996, Gunnels 2007, Gunnels et al. 2008). Nest-switching is also frequent as individual females may leave their natal nest and pursue joining other colonies (Sumner et al. 2007). After colony establishment, the original foundress or foundresses in a colony must decide to accept or reject approaching non-nestmates. In this early phase when there are not yet adult offspring, all the work is performed by foundresses. Therefore, it might favor the colony to accept non-nestmates as subordinate workers when there are no offspring helpers. Additionally, colonies initiated by a group of foundresses have higher survival rates when



exposed to predators and parasitoids than colonies established by a solitary foundress (Klahn 1988). However, foreign wasps from other colonies frequently attempt to usurp the reproductively dominant role, commit brood theft, or cannibalize the offspring in other colonies (Klahn 1988, Clouse 1995).

This study tests the hypothesis that non-nestmate acceptance is context-dependent in a primitively eusocial paper wasp, *Mischocyttarus mexicanus*, during the offspring pre-emergence phase. In this species, colonies can be initiated either by a solitary foundress or a group of foundresses (Litte 1977, Hermann et al. 1985, Clouse 2001). Additionally, active colonies in different stages of the life cycle are observed all year long in subtropical populations (Mora-Kepfer unpublished). In active colonies, some wasps leave natal nests to become part of other colonies (Litte 1977). Several females in a colony can be reproductively viable, and they can switch between solitary and group founding strategies (Gunnels 2007, Gunnels et al. 2008).

I tested the effect of three variables on acceptance of non-nestmates: non-nestmate age, stage of colony development, and non-nestmate aggressive behavior when joining a foreign colony. I also examined possible interactions among these three variables, and whether colony size had an effect on non-nestmate acceptance. First, I predicted that colonies in early stages of development might recognize old non-nestmates with a distinct chemical profile, but accept them to benefit from their help as subordinate workers. Second, young non-nestmates that are acquiring their chemical profile may not be recognized as foreign due to the acceptance error, and be accepted more frequently. Third, aggressive non-nestmates may be perceived as a usurpation threat, elicit an aggressive response by the colony nestmates, and be less frequently accepted.

## Methods

### *Non-nestmate introduction trials*

I conducted this study from July until December 2007 in Kendall Indian Hammocks, a Miami-Dade County park in Kendall, Florida (25°69'N, 80°38'W). For the experimental colonies, I exclusively selected colonies that had not yet produced adult offspring. The average duration of this offspring pre-emergence phase is 55 days in *M. mexicanus* (Litte 1977). I divided the experimental colonies into two categories: “early colonies” and “late colonies” (Appendix VI). Early colonies were within the first week of being established and consisted of a foundress or small group of foundresses, eggs and first instar larvae (Appendix VI). Late colonies were approximately five weeks old with eggs, larvae, and pupae close to emerging as adults, but no adult offspring (Appendix VI).

I selected adults from marked nests to serve as non-nestmates and introduced them into experimental early and late colonies. To avoid the possible effects of philopatry (reviewed in Röseler 1991) and higher non-nestmate acceptance, I collected non-nestmates 1.5 km away from the experimental colonies they were introduced into. I divided the non-nestmates into two categories: 1) young non-nestmates and 2) old non-nestmates. Young non-nestmates were specifically introduced on their first day as adults, because wasps less than 24 hours old have not yet acquired the specific chemical profile of their colony (Panek et al. 2001; Lorenzi et al. 2004). I selected the old non-nestmates by marking newly emerged females and 7 days later introduced them to the experimental nests to make sure they had acquired their colony's chemical profile.

A trial consisted of one behavioral interaction between experimental colony nestmates and a non-nestmate. I isolated each non-nestmate individually and placed it in

a cooler. When the wasp became inactive, I used the tip of an insect pin to paint its thorax with Testors® enamel paint for individual identification. When the wasp became active again, I introduced a thin wooden stick to the container and the wasp grasped the stick. I placed the non-nestmate in contact with an experimental colony and waited until it walked without prodding onto the experimental nest. This method avoided manipulation of the wasps with forceps that might affect their behavior. I excluded events when the non-nestmate wasp did not walk in proximity of the foreign nest.

I examined the effect of both age of non-nestmates (young and old) and stage of the experimental colony (early and late) on the acceptance of non-nestmates. My experiment consisted of four treatments: 1) introduction of a young non-nestmate into an early colony, 2) introduction of a young non-nestmate into a late colony, 3) introduction of an old non-nestmate into an early colony and, 4) introduction of an old non-nestmate into a late colony. To ensure that each interaction outcome was an independent event, I used each non-nestmate and each experimental colony only one time, for a total of 84 interactions. As a control, I removed one resident wasp from its natal colony by using the same methodology previously described and introduced the resident back into its own colony. I repeated this control procedure for 11 young residents and 11 old residents during the same months I performed the experimental introductions.

#### *Aggressive interaction scoring*

I videotaped each interaction between a non-nestmate and the experimental colony nestmates (N= 84), and timed the duration of each interaction. The outcome of each interaction was categorized as acceptance if the non-nestmate was allowed to remain in

the experimental nest or rejection if the non-nestmate was physically forced off the nest. Additionally, I recorded from videotape the number of nestmates interacting with the non-nestmate. I performed night censuses when all colony nestmates are present in their colony.

For each interaction, I categorized the behavior of experimental colony nestmates and the introduced non-nestmate as non-aggressive or aggressive by using established ethograms (Itô 1993; Gunnels 2007). If the interacting wasps antennated or did not have any physical contact, the interaction was classified as non-aggressive behavior. If the interaction consisted of physical attacks such as biting, grappling or physically forcing the non-nestmate off the nest, the behavior was classified as aggressive.

I assigned the aggression level of each interaction using the aggression scores established by Stuart and Herbers (2000). I observed the videotaped interactions and assigned a level of aggression from zero to four to both the non-nestmate and colony nestmates. A score of zero was assigned if interactions included only grooming and/or antennation and the non-nestmate was accepted. A score of one was assigned when wasps exhibited mild aggression such as pushing and the non-nestmate in some cases might still be accepted by the colony. A score of two was given if a wasp exhibited aggression such as biting and chasing and there was rejection. A score of three was assigned in interactions where there was physical dragging and a wasp was rejected and forced off the nest. Finally, a score of four was assigned to interactions in which a wasp tried to kill a non-nestmate by biting, stinging, and grappling, even in cases when the non-nestmate had been rejected and left the nest. The assignment of the scores for the aggression levels was done blind to the introduction treatment of each videotaped interaction.

*Statistical analyses*

To detect an effect of non-nestmate age, stage of colony development and non-nestmate aggression in the acceptance of a non-nestmate, I performed Generalized Linear Models (GLM) with a binomial error structure. For all GLM, I tested for the main effect of each variable and the interactive effects among variables on acceptance of non-nestmates. The Akaike Information Criteria (AIC) was used to determine the most parsimonious model in the candidate set (Akaike 1973). The AIC value for each model  $i$  was calculated and was rescaled to  $\Delta AIC$  ( $AIC_i - AIC_{\min}$ ) to choose the best fit model and avoid the effect of sample size and arbitrary constraints (Caswell 2001, Burnham and Anderson 2004). In a candidate set, the models with  $\Delta AIC \leq 2$  are well supported and the simplest model is the best fitted. Full details on selecting the best fit model are described in Burnham and Anderson (2004). GLM analyses were performed using the software R (R Development Core Team 2009).

After detecting an effect of non-nestmate age, I used a Chi-Square test to compare non-nestmate acceptance between young and old non-nestmates. After detecting an effect of colony stage, I used the same test to compare non-nestmate acceptance between early and late colonies. I tested the effect of both non-nestmate age and colony stage on differences in the acceptance threshold by comparing the proportion of accepted non-nestmates among the four treatments using replicated G-tests of goodness-of-fit (Sokal and Rohlf 1997). Subsequently, I performed pair-wise comparisons to determine differences between introduction treatments. To test for the effect of the total number of colony nestmates and the number of nestmates present during introductions on non-nestmate acceptance, I performed Nominal Logistic Regressions.

Chi-square tests were used to determine the effect of non-nestmate age on presence of aggressive behavior by both resident wasps and non-nestmates. Using Mann-Whitney  $U$  tests, I determined differences in aggression levels: towards old and young non-nestmates and between nestmates in early and late colonies. Kruskal-Wallis tests were performed to determine differences in aggression levels among 1) nestmates and 2) non-nestmates in the four introduction treatments with subsequent pair-wise comparisons. A Logistic Regression was used to test the effect of non-nestmate aggressive behavior on the aggressive response from nestmates. SPSS 17 was used to analyze behavioral interactions (SPSS, Inc. 2008).

## **Results**

### *Non-nestmate acceptance*

From the nine candidate GLM, Model 8 was the best fit, supported by a value of  $\Delta AIC = 0$  (Table 3.1). This model reveals significant main effects of non-nestmate age and colony stage on non-nestmate acceptance (Table 3.2). The model did not detect any significant interactive effects between these two variables. Non-nestmate aggression did not have an effect on non-nestmate acceptance (Table 3.2).

Of 84 non-nestmate introductions, twenty four percent were accepted into a foreign nest. The majority of the young non-nestmates (93%) were accepted into early nests in comparison with only 7% of old non-nestmates being accepted ( $\chi^2 = 13.89$ , d. f. = 1,  $N = 82$ ,  $P = 0.002$ ). I also found that nestmates of early colonies accepted more non-

nestmates (33%) than did those of late colonies (14 %) ( $\chi^2 = 5.27$ ,  $df = 1$ ,  $N = 84$ ,  $P = 0.02$ ).

I found a significant overall difference in non-nestmate acceptance among the four introduction treatments and the control ( $G = 82.26$ ,  $df = 4$ ,  $P < 0.001$ ,  $N=106$ , Fig 3.1). Subsequent pair-wise comparisons revealed significant differences in non-nestmate acceptance between young non-nestmates accepted into early nests in comparison to the other three treatments and the control. The majority of young non-nestmates introduced to early colonies were accepted (62%). The other three treatments resulted in significantly lower acceptance: 9% of old non-nestmates were accepted by late colonies, 18% of young nestmates were accepted by late colonies and 5% of old non-nestmates were accepted by early colonies. This result indicates a higher acceptance proportion of young non-nestmates exclusively in early colonies and a lower acceptance proportion of non-nestmates in the other three treatments. In the control procedure, all reintroduced nestmates were accepted back into their own nest by their colony nestmates.

The number of nestmates present at the time of the introduction did not have an effect on non-nestmate acceptance ( $\chi^2 = 0.812$ ,  $P = 0.84$ ,  $R^2 = 0.01$ ,  $N_{\text{colonies}} = 71$ ). Likewise, total colony size including nestmates not present at the nest at the time of the introduction did not have an effect on non-nestmate acceptance ( $\chi^2 = 3.51$ ,  $P = 0.78$ ,  $R^2 = 0.003$ ,  $N_{\text{colonies}} = 81$ ).

#### *Aggressive interactions*

Few young non-nestmates, (14%) in comparison to older non-nestmates (45%), exhibited aggressive behavior towards colony nestmates ( $\chi^2 = 8.33$ ,  $df = 1$ ,  $N = 69$ ,  $P = 0.003$ ). A

similar trend was found in the aggressive behavior of colony nestmates towards non-nestmates. Fifty percent of colony nestmates reacted aggressively towards young non-nestmates and ninety two percent towards old non-nestmates ( $\chi^2 = 16.88$ ,  $df = 1$ ,  $N = 77$ ,  $P = 0.0001$ ).

Old non-nestmates were more aggressive towards colony nestmates than were young non-nestmates (Mann–Whitney  $U = 1067.5$ ,  $df = 1$ ,  $P = 0.04$ ,  $N = 84$ ). Non-nestmates exhibited only mild aggression and did not differ in aggression levels toward colony nestmates in early and late colonies (Mann–Whitney  $U = 748$ ,  $df = 1$ ,  $P = 0.14$ ,  $N = 84$ ). Colony nestmates exhibited higher levels of aggression towards old non-nestmates than young non-nestmates (Mann–Whitney  $U = 1144.5$ ,  $df = 1$ ,  $P = 0.028$ ,  $N = 85$ ). Nestmates in late colonies were significantly more aggressive towards non-nestmates than in early colonies (Mann–Whitney  $U = 533$ ,  $df = 1$ ,  $P = 0.01$ ,  $N = 85$ ). Nestmates responded more aggressively when encountering aggressive non-nestmates than non-aggressive non-nestmates ( $\chi^2 = 9.76$ ,  $P = 0.001$ ,  $R^2 = 0.14$ ,  $N_{\text{colonies}} = 84$ ).

The joint effects of non-nestmate age and colony stage on aggression levels differed among treatments. Non-nestmates exhibited signs of mild aggression towards colony nestmates in three of the four introduction treatments. However, in comparison to higher levels of aggression by nestmates, non-nestmates exhibited low levels of aggression (Kruskal-Wallis test,  $\chi^2 = 7.35$ ,  $df = 3$ ,  $P = 0.061$ ,  $N = 86$ , Fig 3.2a). Nestmates of early colonies exhibited mild aggression towards young non-nestmates in contrast to a significantly higher aggression in the three other treatments (Kruskal-Wallis test,  $\chi^2 = 18.70$ ,  $df = 3$ ,  $P < 0.001$ ,  $N = 85$ , Fig 3.2b).



## Discussion

This is the first study to provide evidence for context-dependent acceptance of non-nestmates by wasp colonies in the offspring pre-emergence phase. Although the non-nestmates were not kin, they were accepted by other colonies in certain contexts. The acceptance threshold shifted according to both colony stage and non-nestmate age, and non-nestmates were more frequently accepted in early colonies than in late colonies. Young non-nestmates also were accepted more frequently than old non-nestmates, coinciding with studies of the offspring post-emergence phase (Arathi et al. 1997, Panek et al. 2001). This variation in the acceptance of non-nestmates suggests that the response of acceptance or rejection of non-nestmates depends on both recognition of the non-nestmates and the specific needs of a colony.

High acceptance of young non-nestmates in early colonies may occur because young non-nestmates are difficult to recognize because they are in the process of acquiring an established chemical profile (Lorenzi et al. 1996, Panek et al. 2001, Lorenzi et al. 2004). Additionally, in recently established early colonies, nestmates are still acquiring and learning the chemical profile of their own colony (Singer and Espelie 1992, reviewed in Gamboa 2004). Thus, it may be difficult to differentiate between nestmates and non-nestmates during this learning period. In contrast, late colonies have an established colony profile and as they recognize non-nestmates, the acceptance threshold is raised and the proportion of accepted non-nestmates is lowered. Young non-nestmates may be accepted as subordinate workers because young *Mischocyttarus* females are relatively inactive and show limited ovary development until their second week of adult

life (Molina and O'Donnell 2008b). Arathi et al. (1997) showed that young non-nestmates are inexperienced and non-aggressive, and are easier to mold into subordinate workers. Additionally, accepting young non-nestmates may be beneficial because groups of females are better defended against predators, parasitoids, and usurpation attempts than a single foundress (Litte 1977, Clouse 1995, Klahn 1988).

My results support the findings of previous studies of the offspring post-emergence phase where non-nestmates were accepted more frequently after the emergence of the first adult offspring compared to more developed colonies with several generations of adults (Gamboa et al 1991b; Gamboa 1996). They also are consistent with the predictions of the optimal acceptance threshold model which posits that selection favors shifts of the acceptance threshold within the same colonies in different contexts (Reeve 1989). This model predicts that variation in non-nestmate acceptance depends on the trade-off between the fitness costs of accepting non-nestmates (acceptance error) versus those of rejecting its own nestmates (rejection error). Therefore, there might be a high pay-off to context-dependent acceptance of non-nestmates resulting in high offspring survival and consequently, high fitness (Starks et al. 1998).

The acceptance of non-nestmates in some contexts and rejection in others may also be related to the needs of the colony in different stages of development. In the pre-emergence phase, the fate of the colony depends exclusively on the foundress or foundresses (West 1967; Clouse 2001). Foundresses in early colonies of *M. mexicanus* have a low investment in offspring and are known to abandon early nests when threatened by usurpers instead of incurring a higher cost of defending the brood (Litte 1977). To accept non-nestmates as subordinate workers in early colonies that have

invested little in offspring might represent a high benefit towards nest construction, foraging, and defense. In contrast, late colonies have invested in brood rearing and pupae are about to eclose resulting in the valuable first set of adult workers. Judd (1998) reported high nestmate aggression in late stages of the colony cycle as there was a high brood investment that represented direct reproductive investment. When a late colony is highly invested in offspring, the risk of potential usurpation and brood theft (Clouse 1995; Khlan 1988) might influence a decrease in non-nestmate acceptance.

The interactions between nestmates and the approaching non-nestmates also revealed context-dependent differences in aggression. Although non-nestmate aggressive behavior did not have an effect on non-nestmate acceptance, aggressiveness in non-nestmates did trigger an aggressive response from nestmates. An individual's prior experience may also influence aggressive behavior (Hsu et al. 2006). In the Argentinean ant, *Linepithema humile*, aggressive behavior towards non-nestmates increases with age and experience of the colony nestmates (Van Wilgenburg et al. 2010). Previous interactions of colony nestmates with potential usurpers may lead to elevated aggression in later encounters with non-nestmates. As a result, further encounters with aggressive non-nestmates may be perceived as a usurpation threat to the colony.

My findings raise interesting questions about the mechanisms that underlie acceptance of non-nestmates. In other bee and wasp species, nest switching is also frequent (reviewed in Reeve 1991; Sumner 2007), and approximately 25% of the colony members are not related to their nestmates (Queller et al. 2000; Soro et al. 2009, Zanette and Field 2008). Those studies suggest that unrelated subordinates may join foreign colony because of future inheritance of a territory (Queller et al. 2000) or opportunities to

enhance direct fitness by becoming a reproductive (Cant and Field 2001; Field et al. 2006; Leadbeater et al. 2010), coinciding with the results of this present study.

Relatedness among nestmates in the *M. mexicanus* population in this study has yet to be determined. A low relatedness among colony members might influence more frequent acceptance of non-nestmates. Another unexplored question is the potential effect of changes in the chemical profile of individuals throughout colony stages on the acceptance of non-nestmates. The chemical profile is known to change within colonies over time (Vander Meer et al. 1989), so recognition cues also may change throughout the different stages of the colony cycle. Future studies of these cues, and their experimental manipulation, would provide valuable insights into the mechanisms that influence context-dependent acceptance of non-nestmates.

Table 3.1 Comparison of the effect of Non-nestmate age, A, Non-nestmate aggression, N, and colony stage, S, on non-nestmate acceptance in a foreign colony by generalized linear models with a binomial error structure. The main effect between variables (+), the interaction between variables (x), and both main effect and interactions between variables were tested. The Akaike Information Criteria (AIC) was calculated for each model i. The most parsimonious model was selected by using the lowest  $\Delta AIC$  ( $AIC_i - AIC_{\min}$ ). Models 2-9 were obtained by simplifying Model 1 step by step. Variables were subtracted on each consecutive model.

Model i	Variables	AIC	$\Delta AIC$
M1	A+ N + S + AxN + NxS + AxS + AxNxS	86.19	0.3
M2	A+ N + S + AxN + NxS + AxS	87.96	2.07
M3	A+ N + S + AxN + NxS	88.6	2.71
M4	A+ N + S + AxN + AxS	85.99	0.1
M5	A+ N + S + NxS + AxS	87.73	1.84
M6	A+ N + S + AxN	86.7	0.81
M7	A+ N + S + NxS	87.41	1.52
M8	A+ N + S + AxS	85.88	0
M9	A+ N + S	85.92	0.03

Table 3.2 Effect of non-nestmate age, colony stage, non-nestmate aggression, and interactions between non-nestmate age and colony stage on non-nestmate acceptance for the selected best fit model (M8). N= 84 interactions between a non-nestmate and colony nestmates. Bold highlights reflect a significant effect of the tested variable.

Variables	Model 8			<i>P</i>
	DF	Residuals	DF residuals	
Non-nestmate age	1	10.69	81	<b>0.001</b>
Colony stage	1	5.15	80	<b>0.02</b>
Non-nestmate aggression	1	0.69	82	0.4
Interaction age and stage	1	2.03	79	0.15

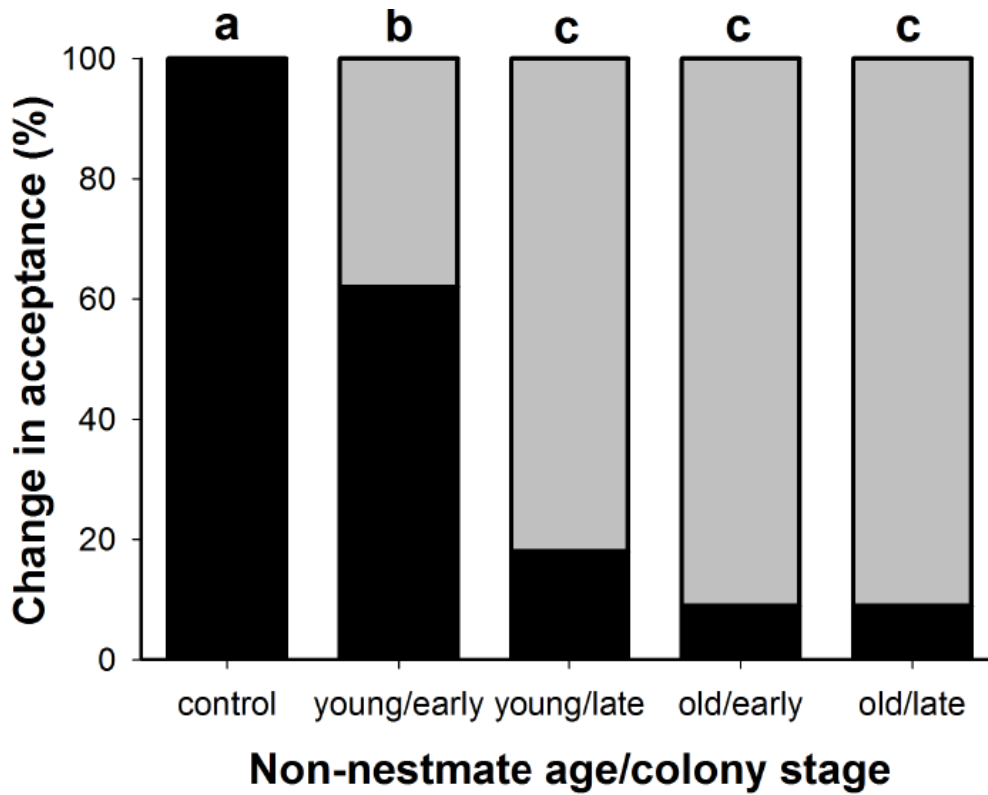


Figure 3.1 Percentage of acceptance for control and four introduction treatments: 1) young non-nestmates introduced into early colonies, 2) young non-nestmates into late colonies, 3) old non-nestmates into early colonies and 4) old non-nestmates into late colonies. The control treatment consisted of re-introducing a wasp into its own nest. Black bars indicate acceptance and grey bars indicate rejection. Bars topped by the same letter do not differ significantly at  $P < 0.005$ , Replicated  $G$ -tests of goodness of-fit.

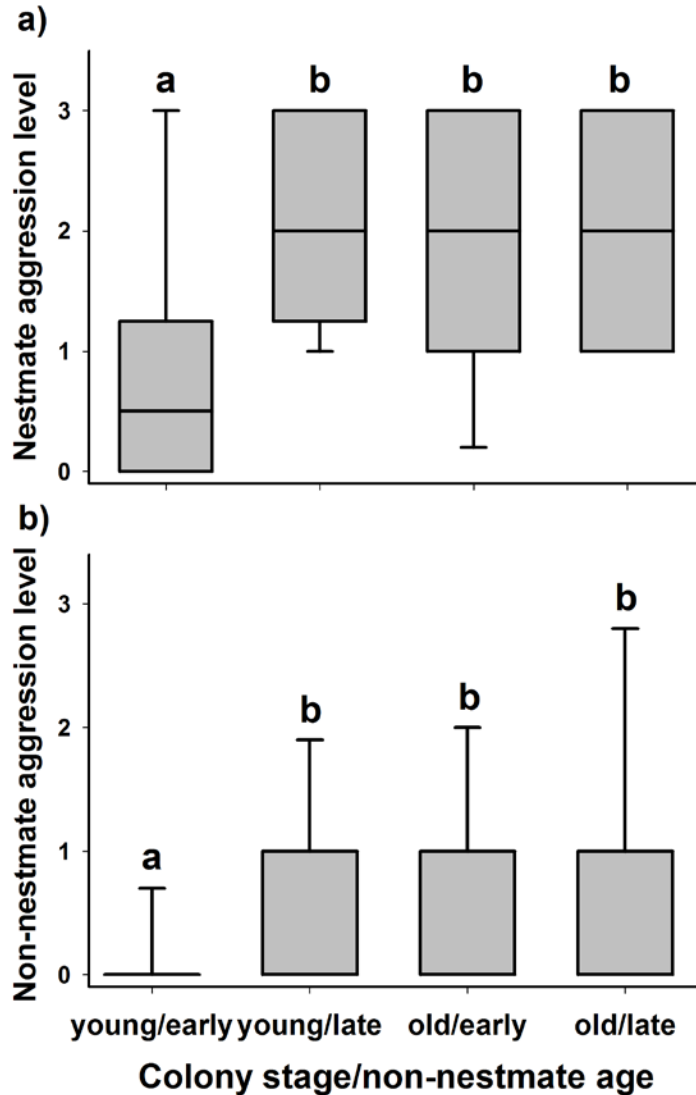


Figure 3.2 Aggression levels between the introduced non-nestmate and colony nestmates in the four experimental treatments: a) aggressive response of colony nestmates toward the introduced non-nestmate and b) aggressive response of the non-nestmate toward colony nestmates. Box and whisker plots of aggressive categories show the median, 25<sup>th</sup> and 75<sup>th</sup> percentile (box) and 5<sup>th</sup> and 95<sup>th</sup> percentile (whiskers). Bars topped by the same letter do not differ significantly at  $P < 0.001$ , Kruskal-Wallis tests with subsequent pairwise comparisons.



## Chapter 4

### **Mushroom body volume is associated with environmental conditions but not with social interactions in newly-established colonies of the primitively eusocial wasp, *Mischocyttarus mexicanus***

#### **Summary**

The brain structure of primitively eusocial wasps varies, particularly in the calyx of the Mushroom Bodies (MB), the neuropils associated with learning and memory. Previous studies have demonstrated that foraging experience and social dominance by aggression are associated with changes in MB calyx substructures during the offspring post-emergence phase. Here, I extend those studies by examining possible associations between MB development and environmental conditions and social interactions, in solitary foundresses and groups of foundresses in newly-established field colonies of *Mischocyttarus mexicanus*. I also used experimental treatments in the laboratory designed to discriminate between the environmental conditions and social interactions in differences in MB volume. A change in collar + basal ring volume, the calyx substructure that receives optical input, was associated with environmental conditions such as light intensity and foraging experience. In contrast to previous studies, I found no association between access to social interactions and volume of the lip, the calyx substructure that receives information from the antennal lobes. In newly-established colonies, reproductive dominance was established, with one primary reproductive female per colony. In these colonies, ovary development was positively correlated with calyx volume. Group foundresses and solitary foundresses exhibited similar calyx volume, which may be

related to variation in behavior, ovary development, and frequent nest-switching in this species. My results suggest that social dominance may be established as the colony develops and structural changes in the calyx may be associated with social dominance after the colony transitions from newly-established to later stages of the colony cycle.

## **Background**

Insect brains exhibit structural variation that is associated with cognitive and information-processing demands (Erber et al. 1980, Heisenberg et al. 1995, Gronenberg et al. 1996, Gronenberg 2001). In particular, the paired neuropils called Mushroom Bodies (MB) have been extensively studied because of their function as brain centers for learning and memory (Heisenberg 1998, Mizunami et al. 1998, Farris 2005, reviewed in Fahrbach 2006). MB are associated with responses to cognitive demands (Withers et al. 1993, Fahrbach et al. 2003, Farris et al. 2001), spatial memory (Mizunami et al. 1993, Zars 2000), and individual behavior (Molina and O'Donnell 2007, 2008a). The relationship between MB structure and social behavior has been of long-standing interest, as previous studies suggest that an increase in MB development is associated with the evolution of sociality in insects (reviewed in Gronenberg and Riveros 2009).

In eusocial Hymenoptera, females within a colony perform different tasks and these may demand specific cognitive abilities and differences in brain architecture (Molina et al. 2009). For example, MB development varies with task performance in honeybee and ant workers (Erber et al. 1980, Withers et al. 1993, Gronenberg et al. 1996, Farris et al. 2001). This variation has been detected within distinct anatomical regions of

the MB: the calyx and the Kenyon cell bodies (Fahrbach 2006). The Kenyon cell bodies are clusters of intrinsic neurons whose dendrites arborize into the cup-shaped calyx (Ehmer and Hoy 2000, Gronenberg 2001). The calyx, which receives input from other neural regions, is divided into the lip, the collar, and the basal ring. The lip receives input from the antennal lobes, the collar receives visual information from the optic lobes, and the basal ring receives information from both the antennal and optical lobes (Gronenberg 2001).

Light intensity is an environmental condition that may affect the development of neural regions that process visual information (Gronenberg and Liebig 1999, Julian and Gronenberg 2002). Species with open-comb nests are exposed to ambient light and may rely on visual cues more than species with enclosed nests where brood and on-nest workers are exposed to reduced light levels (Jeanne 1975, Molina and O'Donnell 2009). Previous studies found that the calyx volume undergoes a drastic change at the onset of foraging in species that perform different tasks associated with age, known as temporal polyethism (Withers et al 1993, 1995, Durst et al. 1994, Gronenberg et al. 1996). In honeybees and highly eusocial wasps, there is a significant increase in the calyx/kenyon cell body ratio as workers transition from tasks inside the enclosed nest to foraging outside the nest in complex visual environments (Withers et al. 1993, Fahrbach et al. 1998, O'Donnell et al. 2004). Foraging workers have larger calyx volume than similarly-aged honeybee and ant workers placed in cages and prevented from foraging (Gronenberg et al. 1996, Fahrbach et al. 1998, Farris et al. 2001, Fahrbach 2006, Kühn-Bühlmann and Wehner 2006). Nevertheless, other Hymenopteran species without temporal polyethism also exhibit an increase in brain volume associated with foraging

experience. In workers of the bumblebee *Bombus occidentalis*, brain volume was positively associated with foraging experience and body size but not with age (Riveros and Gronenberg 2009). In the solitary bee, *Osmia lignaria*, females start foraging immediately after adult emergence. MB volume increased with foraging experience in this solitary bee, when compared to females that were prevented from foraging (Withers et al. 2007). Therefore, development of the collar and basal ring, the visual processing subregions of the MB, may be associated with the onset of foraging and changes in light intensity in both solitary and social Hymenoptera.

The evolution of sociality and behavioral interactions among colony members has also been proposed to affect brain development (Dujardin 1850, Howse 1975, reviewed in Gronenberg and Riveros 2009). There is mixed evidence that social species have enlarged MB compared to related solitary species (Straussfeld et al. 1998, reviewed in Farhbach 2006, but see Farris and Shulmeister 2011). Smith et al. (2010) found an association between brain development and the transition from solitary to social behavior in a facultatively eusocial bee. Their study proposes that the task of maintaining social dominance affects higher MB development. In some primitively eusocial wasps with weak temporal polyethism, dominance is maintained predominantly by aggressive interactions (O'Donnell 1998b, O'Donnell et al. 2007, Molina and O'Donnell 2007, 2008a, 2008b). In these species, changes in MB structure are associated with social interactions. Socially dominant females of the primitively eusocial *Mischocyttarus mastigophorus* had more developed MB compared to the Kenyon cell body (O'Donnell et al. 2007), and the calyx volume increased with age (Molina and O'Donnell 2008a). In *Polistes instabilis*, MB calyx volume also was associated with social dominance and

ovary development (Molina and O'Donnell 2007). Thus, in these species the most aggressive female is both the social and reproductive dominant female during the offspring post-emergence phase. To date, it is unknown if this positive relationship between MB and social dominance is similar in recently established colonies before the emergence of adult offspring.

I focus on newly-established colonies of the primitively eusocial wasp, *Mischocyttarus mexicanus*. Females of this species exhibit high reproductive and behavioral variation in the offspring pre-emergence phase (Mora-Kepfer in prep). After colony establishment, one female becomes the primary reproductive and the other females become auxiliaries (Röseler 1991). In active colonies, nest switching is frequent as females leave natal nests to become part of other colonies (Litte 1977). Many females in a colony can be reproductively viable and they can switch between solitary and group founding strategies (Litte 1977, Hermann et al. 1985, Clouse 1995, 2001, Gunnels 2007, Gunnels et al. 2008).

The goal of my study was to test for an association between MB volume and environmental conditions and social interactions. I tested for differences between MB volume in solitary foundresses and females that initiated field colonies as a group. I predicted that because *M. mexicanus* females are plastic in behavior and reproductive potential, and frequent nest-switching occurs, there would be no differences in MB volume between group and solitary foundresses. I also assessed MB volume in primary reproductives and auxiliaries. If dominance is established by aggressive interactions in newly-established colonies, primary reproductives should have greater MB volume than auxiliaries (O'Donnell et al. 2007, Molina and O'Donnell 2007, Molina et al. 2009).

Alternatively, if dominance by aggression is established as the colony develops, MB volume should not differ between primary reproductives and auxiliaries in newly-established colonies. In addition, I discriminated between the effects of environmental conditions and social interactions in MB volume in the laboratory. I predicted that in this species that builds nests with open combs, light intensity and foraging experience are linked to the development of large visual processing regions. Therefore, the absence of foraging and diminished light intensity would result in less development of the collar + basal ring that receives visual information, compared to the same structure of foragers. As the lip is the calyx substructure that receives neural input from the antennal lobes, I predicted an increase in lip volume in interacting groups of females compared to solitary females. Finally, I investigated the relationship between MB volume and two proxies for reproductive dominance: body size and ovary development.

## **Methods**

### *Study site and data collection*

I observed nine group-initiated colonies and eight solitary nest foundresses of *Mischocyttarus mexicanus* between February 2009 and October 2010 in Kendall Indian Hammocks Park, Florida (25°69'N, 80°38'N). The colonies were located during construction of the first nest cell and each female was made individually recognizable with marks of Testors® enamel paint. Each colony was videotaped for thirty minutes daily during five consecutive days. The tapes were decoded to determine the primary reproductive female and the auxiliaries on each colony. On day five of observations, I

collected each nest and wasps after dark to ensure the presence of all the wasps. Each marked female was stored individually in an aldehyde-based fixative (Prefer, Anatech, Ltd.). In October of 2010, I collected twenty-four additional nests with live pupae and no adults for transport to the laboratory for two experimental treatments.

### *Laboratory treatments*

I placed nests with live pupae in the laboratory at 26°C with 11 hours of artificial lighting to simulate field conditions in subtropical Florida. I glued each of the collected nests to a small palm fragment. The palm fragment was glued to the lid of a transparent cylindrical cage that measured 13cm diameter by 10 cm tall. Females had enough room to fly briefly inside the cage but not enough volume to forage.

Each cage contained: one nest with live pupae, a sugar cube, water, silkworms as prey *ad libitum*, and construction paper as a source of fiber for cell construction. The caged colonies were divided randomly into two experimental treatments. In treatment one, 14 colonies initiated by a group of foundresses were exposed to social interactions within their cage, but could not forage. As adults emerged, I kept the females and removed males, because male field adults disperse from the natal nest soon after emergence. On average, there were 3 females in each cage, all emerging during 1 or 2 days. In treatment two, 10 solitary foundresses were in complete isolation, with no access to foraging and no social interactions. Before the adults emerged, I removed all but one pupa per nest. When the female adult emerged, it had access to food, water, and paper fiber for construction but never had visual contact with other wasps. For both treatments,

each cage was isolated by a cardboard divider so wasps could not see individuals in other cages. Colonies were maintained for 30 days, collected, and stored in Prefer fixative.

#### *Morphological measurements and dissections*

Females from the 17 field colonies and 24 laboratory-reared colonies were photographed with an Axiocam MRm camera (Zeiss). I measured three morphological traits to estimate body size using the image analysis software Axio Vision Rel. 4.8 software (2009): the minimum intereye distance (Min IE), mesonotum length (MSL), and mesonotum width (MSW) of the thorax. I created a new variable that combined these three measurements by performing a principal component analysis and used the first principal component to estimate body size. The values for each wasp were used to calculate a composite new variable called PC1 body size that met the assumptions of normality (Gunnels 2007).

To estimate the reproductive potential of each female in a colony, I dissected the metasoma ('abdomen') and photographed the ovaries. I measured the maximum length and width of each oocyte in the ovary using Axio Vision Rel. 4.8. Because oocytes are roughly elliptical, the area of each oocyte was calculated as  $\pi \times [1/2] \text{ width} \times [1/2] \text{ length}$  (Molina and O'Donnell 2007, 2008a, 2008b). I calculated the mean area of the oocytes in the ovaries of solitary females and of groups of foundresses. For group-initiated colonies, I determined the reproductively dominant female as the female with the most developed ovaries, and considered the remaining females as subordinate auxiliaries. To avoid pseudoreplication, I selected the primary reproductive and one randomly selected auxiliary in the group-initiated field and laboratory-reared colonies. The head capsules of



all females from field and laboratory colonies were stored individually in Prefer fixative for subsequent histology.

### *Histology*

Head capsules were prepared as follows (total n = 41: 9 females of field group-initiated colonies, 8 field solitary foundresses, 14 females of group-initiated colonies reared in the laboratory and 10 solitary foundresses in the laboratory). I removed the antennas and mandibles from each head capsule to improve resin infiltration. I dehydrated each head capsule through a series of ascending ethanol concentrations and acetone: resin concentrations. The samples were placed in a tissue rocker and alternated with placement under vacuum to improve infiltration. I placed the embedded head capsules in plastic molds filled with resin in a 60°C oven to polymerize for 72 hours. I sectioned each embedded head capsule using a rotary microtome and disposable stainless steel blades; the section thickness was 17µm. Individual sections were placed in a drop of 20% acetone and each section of a specimen was placed in consecutive order on gelatin-coated slides. Slides were dried on a slide warmer and then stained using Toluidine blue (Nissl stain).

I photographed every other section by using an AxioCam MRm camera connected to an Axiovert 200 inverted microscope (Zeiss). I divided the MB calyx structures into two distinct regions: lip and collar + basal ring (Fig 4.1). Collar and basal ring were grouped because the boundaries between these two structures were not clear (Molina and O'Donnell 2008a). I used the image analysis software Axio Vision Rel. 4.8 software (2009) to outline the areas of the lip, collar + basal ring, and Kenyon cell body region and

count the pixels within the outlined area (Fig. 4.1). To calculate the volume of each structure, I summed up all the individual products of the structure area and the distance between section planes (34  $\mu\text{m}$ ). Quantification of sections was done on one hemisphere of the brain and blind to the code of each specimen

### *Statistical Analyses*

I used volumes ratios instead of absolute volumes to avoid effects of body size on volume of each MB substructure (Mares et al. 2005, Wehner et al. 2007). I determined the volume ratios of the MB substructures and the kenyon cell body for each specimen: calyx/kenyon cell body, lip/kenyon cell body, (collar + basal ring)/kenyon cell body, and lip/(collar + basal ring). To test for differences in the volume ratios of MB substructures of females in solitary and group-initiated colonies in the field and laboratory, I performed one-way analyses of variance (ANOVA) followed by Tukey HSD post hoc tests. I performed Spearman correlations to assess the relationship between body size and both calyx volume and kenyon cell body volume. I also used Spearman correlations to examine the relationship between ovary development and both calyx volume and kenyon cell body volume, and to explore the relationship between body size and ovary area. Finally, I used a Chi-square test to compare the presence of laid eggs in solitary versus group-initiated colonies reared in the laboratory. Data that did not meet normality assumptions were log-transformed or square-root transformed. All analyses were performed with SPSS 17 (SPSS, Inc. 2008).

## Results

### *Body size, ovary development, and brain volume*

Body size was not significantly correlated with either calyx volume ( $r = 0.075$ ,  $P = 0.54$ ,  $N = 40$ ) or kenyon cell body volume ( $r = 0.15$ ,  $P = 0.34$ ,  $N = 40$ ). Body size was significantly different among groups (one-way ANOVA,  $F_{3,37} = 4.13$ ,  $P = 0.013$ ).

Laboratory solitary females were significantly smaller than field solitary foundresses (Tukey HSD,  $P = 0.01$ ), females in field group-initiated colonies (Tukey HSD,  $P = 0.04$ ), and laboratory group colonies (Tukey HSD,  $P = 0.04$ ). Body size was not correlated with ovary development ( $r = 0.284$ ,  $P = 0.075$ ,  $N = 40$ ).

Ovary development was significantly correlated with calyx volume ( $r = 0.42$ ,  $P = 0.006$ ,  $N = 40$ ) (Fig. 4.2). In contrast, ovary development was not correlated with kenyon cell body volume ( $r = 0.17$ ,  $P = 0.283$ ,  $N = 40$ ). There were no significant differences in mean ovary development among field group foundresses, field solitary foundresses, laboratory group foundresses, and laboratory solitary foundresses (one-way ANOVA,  $F_{3,37} = 2.50$ ,  $P = 0.43$ ). Ovary development differed significantly among groups (one-way ANOVA,  $F_{3,19} = 8.94$ ,  $P = 0.001$ ). Primary reproductives in both the field (Tukey HSD,  $P = 0.02$ ) and the laboratory (Tukey HSD,  $P = 0.02$ ) had significantly greater ovary development than laboratory auxiliaries. A *post hoc* Tukey test did not detect differences between field primary reproductives and laboratory reproductives ( $P = 0.93$ ), and between field auxiliaries and laboratory auxiliaries ( $P = 0.39$ ). Laboratory group foundresses laid eggs in 93% of the colonies, compared to only 25% of solitary females (G test = 10.80,  $P = 0.001$ ,  $N_{\text{colonies}} = 22$ ). These eggs were not fertilized because females in the laboratory were not exposed to males.

### *Changes in MB development*

Both field solitary and group foundresses had higher calyx/kenyon cell body ratio than laboratory solitary and group foundresses (one way ANOVA,  $F_{3,37} = 13.74$ ,  $P < 0.001$ , Fig 4.3a, Tukey HSD *post hoc* pairwise comparisons, field solitary foundress versus laboratory group foundresses  $P < 0.001$ ; field solitary foundress versus laboratory solitary foundress  $P = 0.001$ ; field group foundresses versus laboratory solitary foundress  $P = 0.005$ ; field group foundresses versus laboratory group foundresses  $P < 0.001$  ).

Calyx/kenyon cell body ratio did not differ between group and solitary females in both field (Tukey HSD  $P = 0.88$ ) and laboratory colonies (Tukey HSD  $P = 0.84$ ). When examining separately the ratios of each calyx substructure with kenyon cell body region, I found different patterns. Lip/kenyon cell body ratio did not differ among the four categories (one way ANOVA,  $F_{3,37} = 2.23$ ,  $P = 0.096$ , Fig 4.3b). Group and solitary foundresses in field colonies had higher (collar + basal ring)/kenyon cell body ratio than group colonies and laboratory solitary females (one way ANOVA,  $F_{3,37} = 17.36$ ,  $P < 0.001$ , Fig 4.3c, Appendix VII, Tukey HSD *post hoc* pairwise comparisons, field solitary foundress versus laboratory group foundresses; field solitary foundress versus laboratory solitary foundress; field group foundresses versus laboratory solitary foundress; field group foundresses versus laboratory group foundresses,  $P < 0.001$  for all ). Both field solitary foundresses and group foundresses had significantly lower lip/(collar + basal ring) ratio than laboratory solitary foundresses and group foundresses (one way ANOVA,  $F_{3,37} = 4.95$ ,  $P = 0.005$ , Fig 4.3d, Tukey HSD *post hoc* pairwise comparisons, field solitary foundress versus laboratory group foundresses; field solitary foundress versus

laboratory solitary foundress; field group foundresses versus laboratory solitary foundress; field group foundresses versus laboratory group foundresses  $P < 0.01$  for all).

Field primary reproductives and auxiliaries did not differ in calyx/kenyon cell body ratio, but had higher calyx/kenyon cell body ratio than laboratory primary reproductives and auxiliaries (one way ANOVA,  $F_{3,29} = 8.73$ ,  $P < 0.001$ , Fig 4.4a, Tukey HSD *post hoc* pairwise comparisons, field primary reproductives versus laboratory primary reproductives  $P < 0.001$ ; field primary reproductives versus laboratory auxiliaries  $P = 0.001$ ; field auxiliaries versus laboratory primary reproductives  $P = 0.04$ ; field auxiliaries versus laboratory auxiliaries  $P = 0.02$ ). In contrast, lip/kenyon cell body ratio did not differ between primary reproductives and auxiliaries in both field and laboratory colonies (one way ANOVA,  $F_{3,29} = 1.34$ ,  $P = 0.299$ , Fig 4.4b). In field primary reproductives and auxiliaries, the (collar + basal ring)/kenyon cell body ratio were similar but were significantly higher than laboratory primary reproductives and auxiliaries (one way ANOVA,  $F_{3,29} = 14.33$ ,  $P < 0.001$ , Fig 4.4c, Tukey HSD *post hoc* pairwise comparisons, field primary reproductives versus laboratory primary reproductives  $P < 0.001$ ; field primary reproductives versus laboratory auxiliaries  $P < 0.001$ ; field auxiliaries versus laboratory primary reproductives  $P = 0.01$ ; field auxiliaries versus laboratory auxiliaries  $P = 0.006$ ). Primary reproductives and auxiliaries in field group colonies did not differ in lip/(collar + basal ring) ratio but had significantly lower lip/(collar + basal ring) ratio than primary reproductives and auxiliaries in laboratory group colonies (one way ANOVA,  $F_{3,29} = 7.92$ ,  $P = 0.001$ , Fig 4.4d, Tukey HSD *post hoc* pairwise comparisons, field primary reproductives versus laboratory primary reproductives  $P = 0.03$ ; field primary reproductives versus laboratory auxiliaries  $P <$

0.04; field auxiliaries versus laboratory primary reproductives  $P = 0.005$ ; field auxiliaries versus laboratory auxiliaries  $P = 0.03$ ).

## **Discussion**

I found an association between environmental conditions and changes in MB volume in newly established colonies of *M. mexicanus*. Females from field colonies had higher calyx/kenyon cell body ratios compared to laboratory females that were prevented from foraging were exposed to diminished ambient light intensity. Previous studies of honeybees, ants, and highly eusocial paper wasps also had this pattern (Fahrbach et al. 1998, Gronenberg et al. 1996, O'Donnell et al. 2004, Kühn-Bülmann and Wehner 2006). Those earlier studies of species with temporal polyethism indicated that the onset of foraging is positively correlated with calyx development. For example, honeybees that were spatially, socially, and visually deprived had enlarged calyx volume, suggesting a programmed expansion of the MB neuropil in the first days of adult life (Fahrbach et al. 1998).

*M. mexicanus* and other primitively eusocial wasps, do not have temporal polyethism (Gunnels 2007, O'Donnell et al. 2007). Factors such as exposure to complex environments, spatial memory, and visual stimuli may affect changes in calyx volume. Molina et al. (2009) found that species that build open comb nests with constant exposure to light had increased collar and basal ring volume, the MB substructures that receive visual input. Similarly, laboratory colonies used in the present study that were exposed to diminished light intensity also had low collar and basal ring volume compared to females

from field colonies. However, the onset of flight and foraging may also explain collar and basal volume (Molina and O'Donnell 2007). In honeybee queens and males, flight initiation was positively correlated to MB volume (Fahrbach et al. 1995, 1997). In my laboratory treatments, females could not forage and could only fly very briefly inside their cages. Therefore, lack of foraging may also affect development of the MB areas that receive visual input, supported by the lower collar and basal ring volume of laboratory females compared to field females.

I found that change in volume of the MB substructures was not associated with social interactions. This result suggests that groups of foundresses in newly-established nests may have not interacted enough for social dominance to be established. In contrast, previous studies found that social dominance does affect changes in MB structure in the offspring post-emergence phase in *M. mastigophorus* (O'Donnell et al. 2007, Molina and O'Donnell 2008a). Social dominance may be established gradually as newly-initiated colonies develop and transition into later stages of the colony cycle. Consequently, after social dominance is established in *M. mexicanus*, MB volume may differ between dominant and subordinate females. One possibility is that socially dominant females have enlarged MB compared to auxiliaries in the offspring post-emergence stage but not in earlier colony phases. Previous studies reported this pattern in other primitively eusocial wasps. Dominant females of *M. mastigophorus* had better developed MB calyces than subordinate females that spent more time foraging during the offspring post-emergence phase (O'Donnell et al. 2007, Molina and O'Donnell 2008a). Their results indicate that dominance, determined by aggressive behavior, was associated with MB development.

Similarly, in *Polistes instabilis*, MB calycal volume and social dominance were positively correlated in colonies with adult offspring (Molina and O'Donnell 2007).

Calyx development of primary reproductives and auxiliaries did not differ. This result suggests that differential MB development is associated with aggressive interactions as the colony develops and nestmates age. A study of *M. basimacula* and *M. angulatus* reported low aggression levels among colony nestmates in the offspring pre-emergence phase and high aggression in the offspring post-emergence stages of the colony cycle (Ito, 1985). In *M. mexicanus*, aggressive interactions also increase within the offspring pre-emergence phase as the colony shifts from newly-established to the imminent emergence of the adult offspring (Mora-Kepfer in prep).

Although social dominance may not be yet established, my results indicate that reproductive dominance is determined during colony establishment in *M. mexicanus*, in agreement with previous studies (reviewed in Röseler 1991). Immediately after the first cells of the nest are built, one female becomes the primary reproductive with higher ovary development compared to the other females in the colony (Mora-Kepfer unpublished data). I discovered a positive relationship between ovary development and calyx volume, in agreement with the results reported for *P. instabilis* and *M. mastigophorus* in the offspring post-emergence phase (Molina and O'Donnell 2007, Molina and O'Donnell 2008a).

The calyx/kenyon cell body ratio did not differ between solitary foundresses and group foundresses, in both field and laboratory colonies. My results support the prediction that MB volume does not differ significantly among group and solitary foundresses because females in *M. mexicanus* have high variation in behavior and



reproduction. For example, females frequently switch nests and alternate between being part of a group of foundresses and solitary females (Gunnels 2007, Mora-Kepfer and Gunnels in prep). My data differ from a study of *Polistes dominulus*. Females in group-initiated colonies had larger collar volume than solitary foundresses in colonies in the offspring pre-emergence phase (Ehmer et al. 2001). Primary reproductives also had larger collar volumes than auxiliaries in groups of foundresses. They also found greater antennal lobe volume in group foundresses than in solitary foundresses, which I did not explore in my study.

My data did not support the prediction of a positive relationship between social interactions and the lip that receives input from the antennal lobes. The lip/kenyon cell body ratios were similar in laboratory solitary females with no exposure to social interactions and laboratory females that interacted in group-initiated colonies. This pattern may be affected by the age of females kept in the laboratory treatments. Laboratory solitary females and group foundresses were only 30 days old and then sectioned. This may not have been enough time for females to interact and for brain structures to develop differentially. Molina and O'Donnell (2008a) found a positive correlation between age and calyx volume in colonies of *Mischocyttarus mastigophorus* in the offspring post-emergence phase.

In conclusion, there was a positive association between foraging experience/light intensity and MB regions that receive visual input, but no association was found between MB regions that receive olfactory input and social interactions. This result strongly suggests an effect of the phase of colony development on change in brain volume. Nevertheless, social dominance may not yet be determined in newly-established colonies

compared to developed colonies. Future studies should address the association between social dominance and MB development at different stages in the colony cycle. Finally, the potential effect of age on MB structural development should be assessed and differentiated from the role of social experience and aggressive dominance.

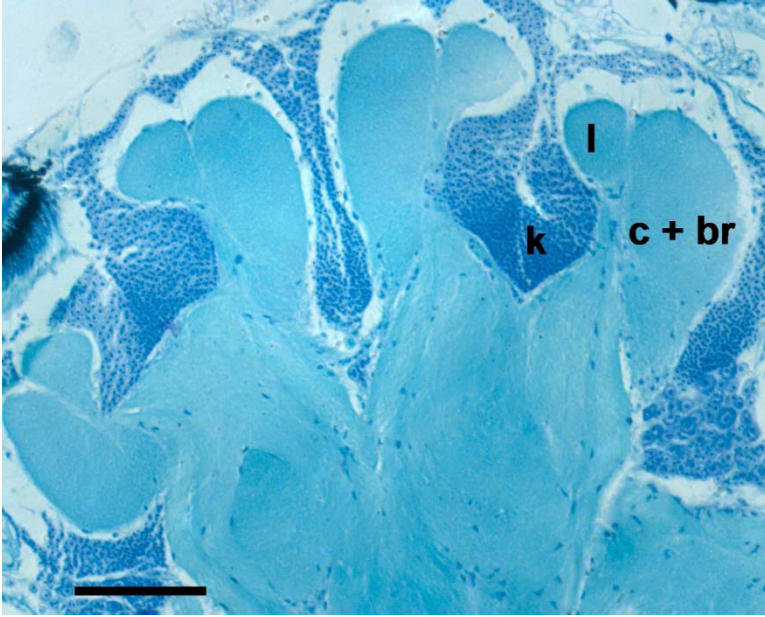


Figure 4.1 Frontal section of the Mushroom Body (MB) of a female *Mischocyttarus mexicanus*. Labels of structures: Kenyon cell bodies (k) and the calyx substructures lip (l) and collar + basal ring (c + br). Scale bar = 0.1 mm

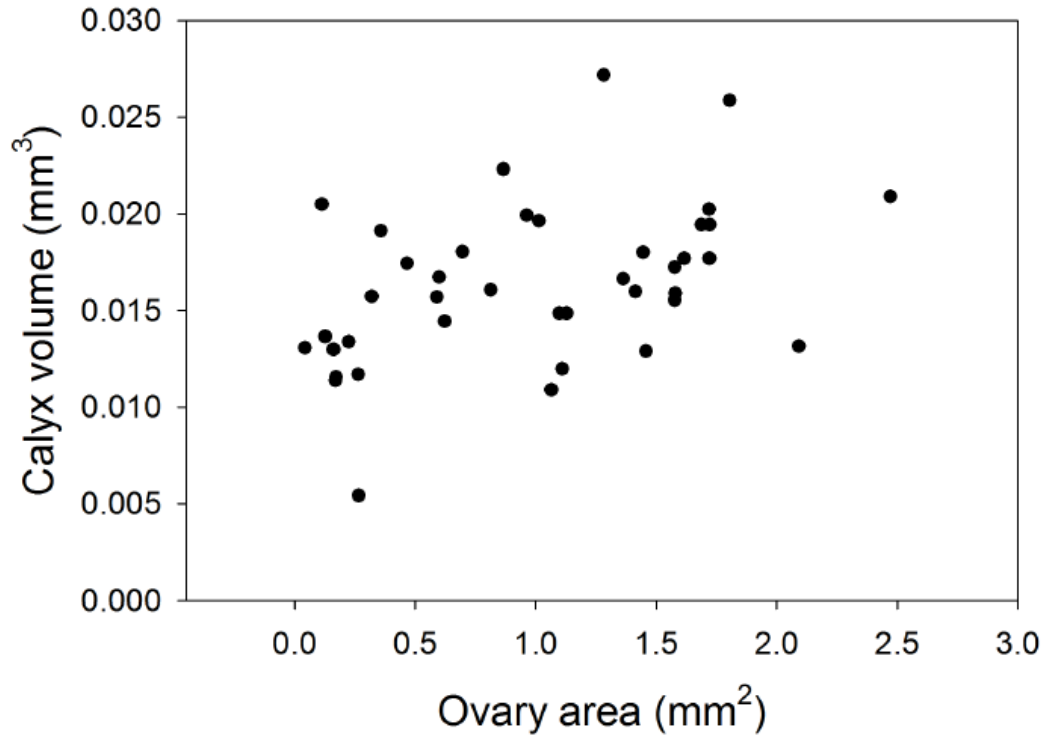


Figure 4.2 Scatterplot showing the positive relationship between ovary area (mm<sup>2</sup>) as an indicator of ovary development, and calyx volume (mm<sup>3</sup>). Spearman correlation,  $r = 0.448$ ,  $P = 0.006$ ,  $N = 40$ .

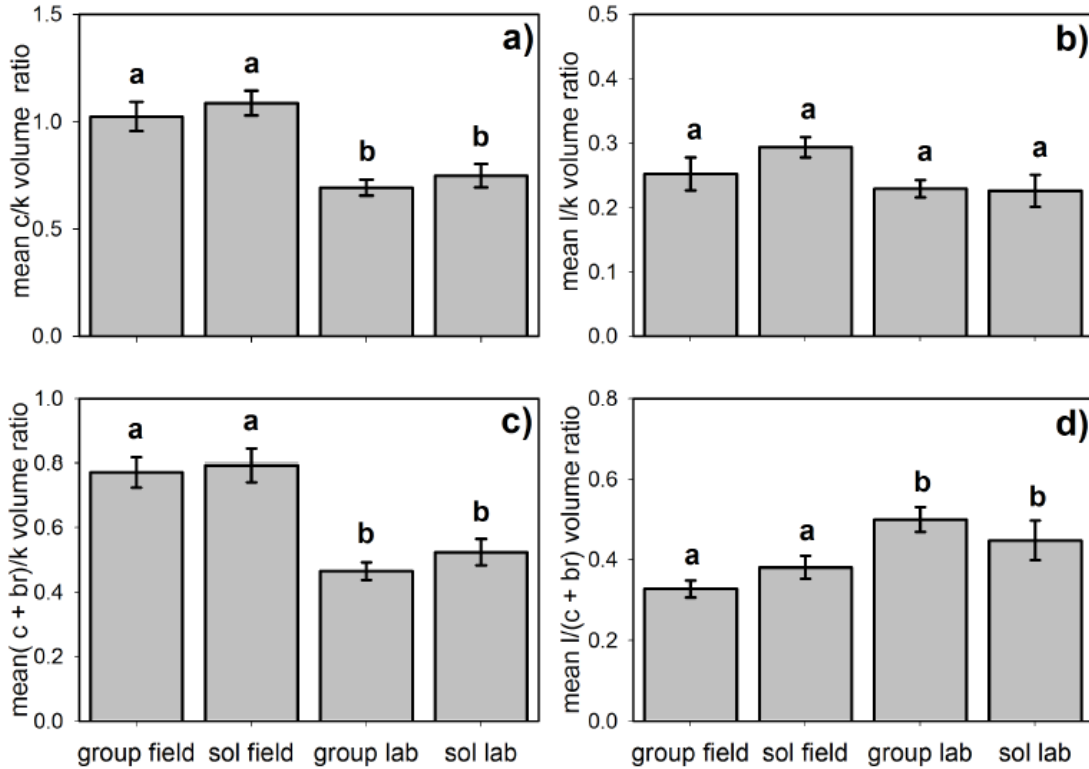


Figure 4.3 Comparison among group foundresses from field colonies who foraged (group field), field solitary foundresses who foraged (sol field), group foundresses in the laboratory who did not forage but had social interactions (group lab), and solitary females in the laboratory who did not forage and did not have access to social interactions (sol lab). a) Mean calyx/ kenyon cell bodies (c/k) volume ratio, b) mean lip/ kenyon cell bodies (l/k) volume ratio, c) mean collar + basal ring/ kenyon cell body [(c + br)/k] volume ratio, and d) lip/collar + basal ring/ kenyon cell bodies [l/(c + br)] volume ratio. Each bar shows the mean and error bars show the standard error. Note differences in Y axis range in each figure. Bars topped with the same letter do not differ significantly at  $P < 0.001$ , one way ANOVA with a subsequent Tukey test.

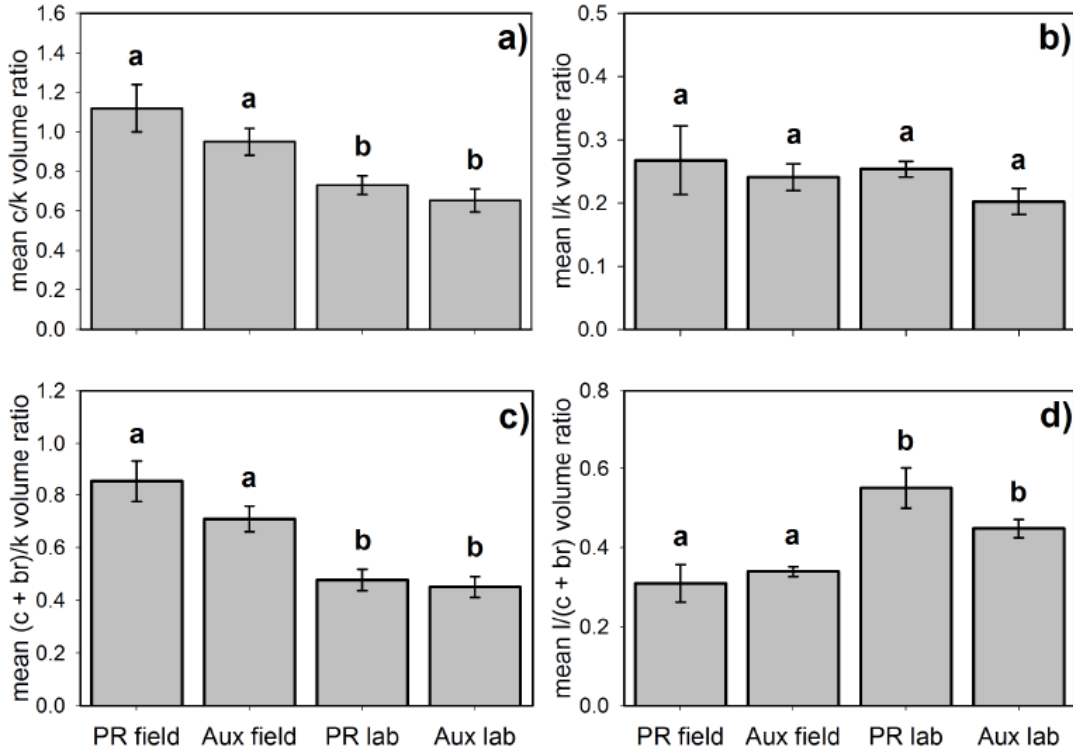


Figure 4.4 Comparison of the mean calyx/kenyon cell bodies (c/k) volume ratio in the reproductively dominant female as primary reproductive female in field colonies (PR field), auxiliaries in field colonies (Aux field), the reproductively dominant female as primary reproductive female in laboratory colonies (PR lab), and auxiliaries in laboratory colonies (Aux lab). a) Mean calyx/kenyon cell bodies (c/k) volume ratio, b) mean lip/kenyon cell bodies (l/k) volume ratio, c) mean collar + basal ring/kenyon cell body [(c + br)/k] volume ratio, and d) lip/collar + basal ring/kenyon cell bodies [l/(c + br)] volume ratio. Each bar indicates the mean and error bars indicate the standard error. Bars topped with the same letter do not differ significantly at  $P < 0.001$ , one way ANOVAs with a subsequent Tukey test.

## Chapter 5

### Conclusion

I investigated reproductive tactics, non-nestmate acceptance and associated behavioral interactions, and changes in brain volume in colonies during the offspring pre-emergence phase of *Mischocyttarus mexicanus*. Although my study focused on one species, my results provide critical insights into the dynamics of colony initiation and establishment in primitively eusocial wasps.

This is the first study to document the initiation of nest construction and the reproductive tactics employed by females to establish colonies. I examined the role of body size, reproductive potential, and immediate egg-laying potential on reproductive tactics used by individual females. I discovered that large females became primary reproductives or auxiliaries in group-initiated colonies, or solitary foundresses. Small females became joiners in nests initiated by solitary foundresses.

Although primary reproductives had the highest ovary development, auxiliaries had the potential to lay viable eggs, suggesting that the auxiliary tactic is adopted with possibility of future opportunities for direct reproduction. Small females had the lowest reproductive potential and immediate egg-laying potential and therefore were the least likely to reproduce directly. My findings suggest that a small female may join a solitary female and become part of a smaller colony and have higher opportunities of direct reproduction rather than competing with large females in their natal nests. Finally, the

joiner tactic adopted by small females in this subtropical population has not been observed in temperate populations of this species.

This was also the first study to provide evidence for the effect of social context on the acceptance of non-nestmates in early and late colonies before the emergence of adult offspring. Both non-nestmate age and colony stage had an effect on the acceptance threshold of non-nestmates. However, aggressive behavior of non-nestmates did not affect non-nestmate acceptance.

Young non-nestmates were accepted more frequently than old non-nestmates, which coincides with previous studies of colonies after the emergence of offspring. The acceptance rate of young non-nestmates into early colonies was significantly higher than in other contexts. My findings suggest that young non-nestmates may be more difficult to recognize because they have not yet acquired the specific chemical profile of their colony. Similarly, early colonies may not have an established chemical profile making it difficult for females to distinguish their nestmates from non-nestmates.

My results also are consistent with the predictions of the optimal acceptance threshold model (Reeve 1989). This model posits that selection favors a shifting non-nestmate acceptance threshold within the same colony in different contexts. For example, my data show a high rejection of both young and old non-nestmates in late colonies. These late colonies are highly invested in offspring pupae that will become the valuable first set of adult workers, suggesting a fitness payoff to rejection of non-nestmates as potential colony usurpers. Therefore, context-dependent acceptance of non-nestmates results in offspring survival and consequently, high fitness.



Finally, my study provides novel evidence on the association between foraging and changes in Mushroom Body structure in newly-established colonies. Results from my laboratory experiments, in which I prevented adult females from foraging and colonies were exposed to diminished light intensity, support findings of previous studies of other social Hymenoptera in the offspring post-emergence phase. In field colonies, females had higher calyx/Kenyon cell body ratio compared to females in light-deprived conditions that did not forage in the laboratory. In particular, the collar and basal ring, the calyx substructures associated with visual input, were less developed.

However, I found no relationship between social interactions and MB development. MB volume was similar in solitary females and foundresses in group-initiated colonies. In newly-initiated colonies, reproductive dominance was established and ovary development showed a positive relationship with MB volume. On the contrary, MB volume showed no association with social dominance. My findings suggest that social dominance is established as a colony develops, and structural changes in the calyx may be associated with social dominance after the colony transitions from newly-established to later stages of the colony cycle.

In conclusion, my research provides the first broad and comprehensive study of the behavior, reproductive tactics, and brain structure in newly-established colonies of a primitively eusocial wasp. During colony initiation and subsequent establishment, females showed variation in behavioral interactions, reproductive tactics, and changes in brain structure. Of particular interest, my findings suggest an important effect of the social context throughout the offspring pre-emergence phase of colony development,

which makes *M. mexicanus* an ideal model system for further studies of the mechanisms and evolution of sociality.

### **Future studies**

I intend to extend my research in directions that were not addressed in my dissertation. First, it is crucial to investigate the mechanisms associated with nestmate recognition. The composition of the chemical profile in this species is yet to be determined and it is unknown whether chemical cues change throughout a colony's development. The role of visual cues in nestmate recognition in this species is also unknown. Studies of these cues and their experimental manipulation would provide essential information on the mechanisms that affect nestmate recognition and non-nestmate acceptance. Second, genetic studies are needed to establish the relatedness among colony nestmates to clarify the role of kin recognition in nest-switching, joining attempts and non-nestmate acceptance. Third, it is necessary to determine the moment when social dominance is established after colony initiation to understand how dominance and aggressive behavior are related to changes in brain structure. Finally, the relationship between MB development and social dominance at different stages of the colony cycle should be explored and differentiated from the effect of age.

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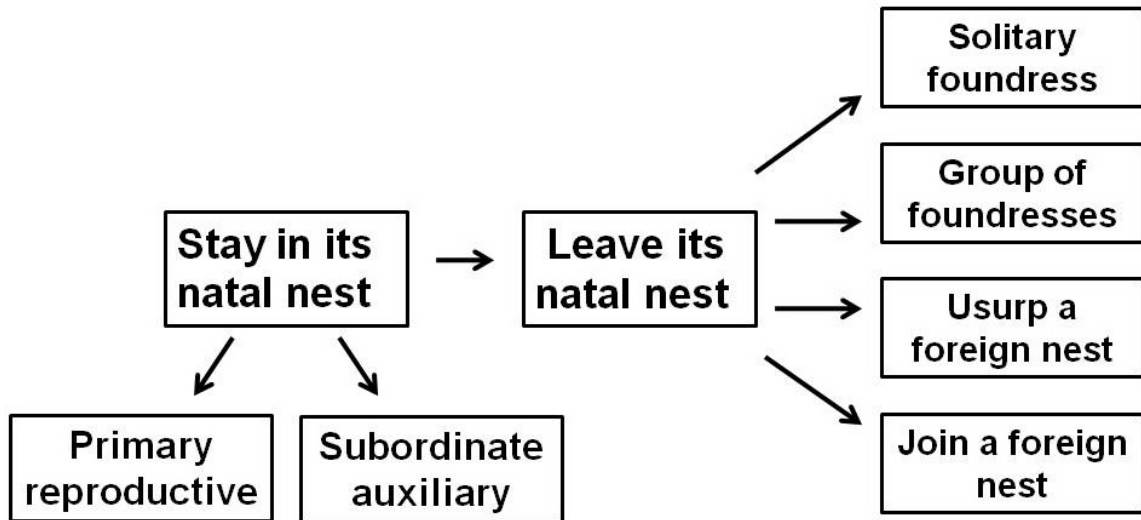
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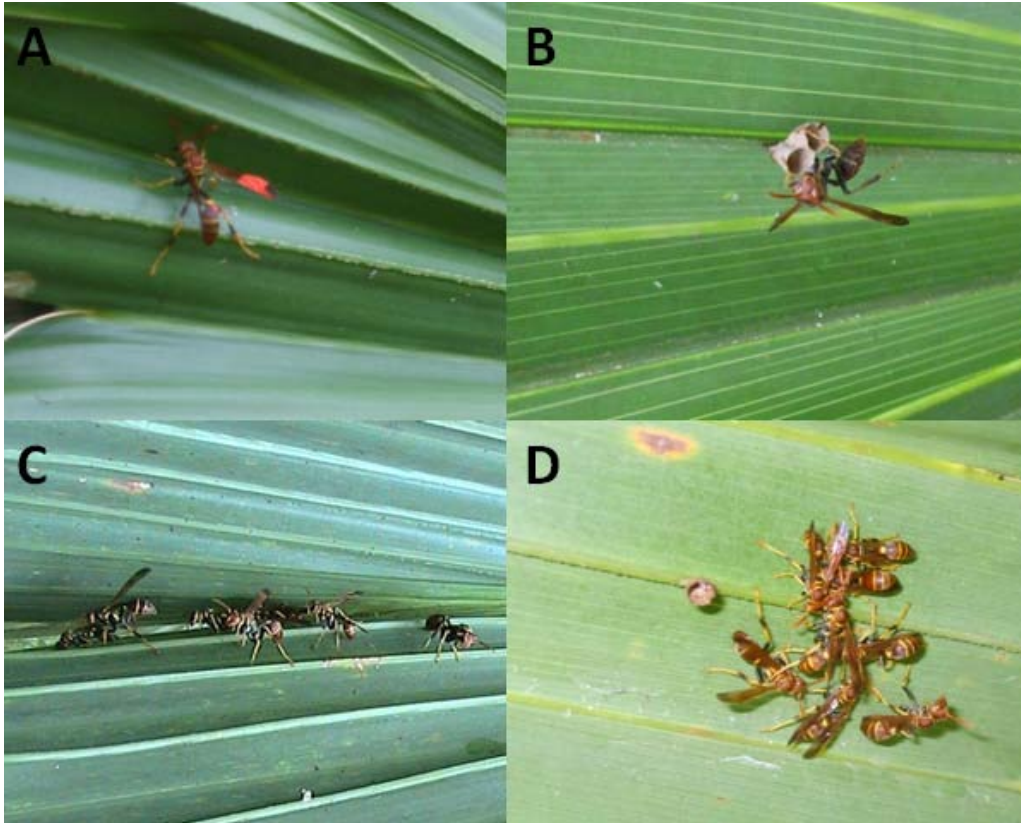
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## Appendices

Appendix I. Decision tree of the alternative reproductive tactics described for primitively eusocial paper wasps.



Appendix II. Colony initiation tactics of *Mischocyttarus mexicanus* females. A) Solitary female resting on palm. This female initiated nest construction two days later. B) Solitary foundress on a newly-initiated nest. C) Aggregation of females resting on palm. These females initiated nest construction one day later. D) Newly-initiated nest constructed by a group of foundresses.



Appendix III. Solitary foundress of *Mischocyttarus mexicanus* initiates nest construction by building the nest pedicel.





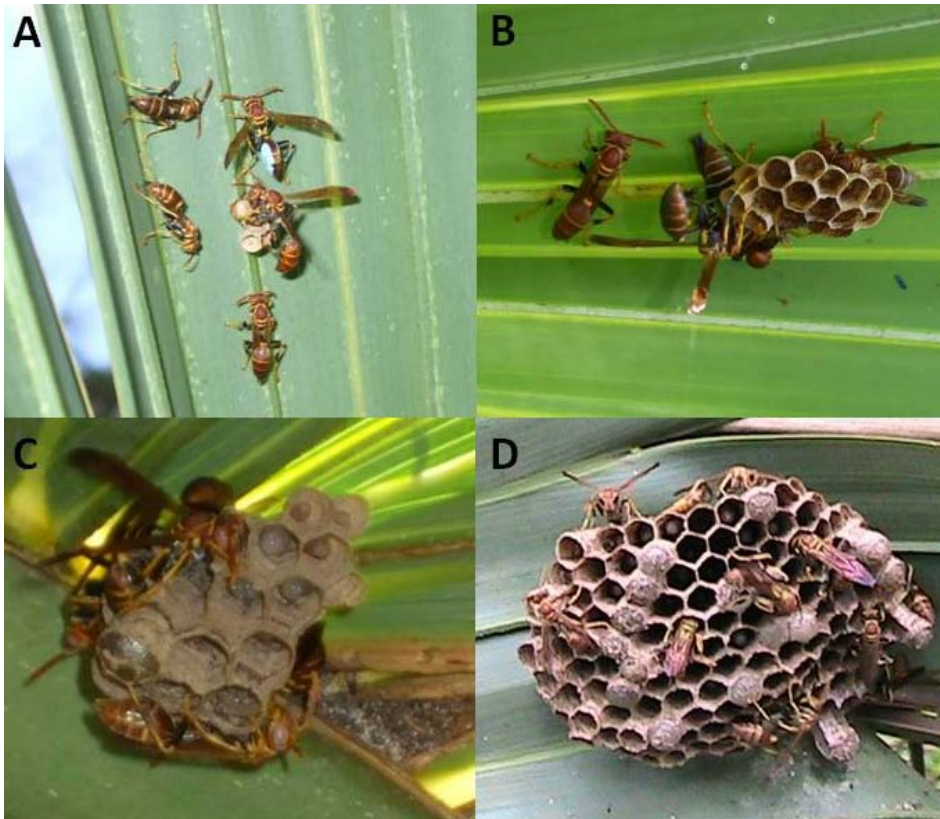
Appendix IV. Joiner tactic in *Mischocyttarus mexicanus*. A small female leaves her natal nest and pursues joining a solitary foundress on a recently constructed nest.



Appendix V. Primary reproductive of a group-initiated colony of *Mischocyttarus mexicanus* lays an egg inside a cell. An auxiliary female remains close to the nest.



Appendix VI. Stages of colony development in nests constructed by a group of foundresses of *Mischocyttarus mexicanus*. A) Newly-established colony with foundresses. B) Early colony with foundresses, eggs and young instar larvae. C) Late colony with the foundresses, eggs, larvae, and pupae about to emerge as the first generation of adult offspring. D) Developed colony with several generations of adult females in the offspring post-emergence phase



Appendix VII. Frontal section of the Mushroom Body (MB) of *Mischocyttarus mexicanus* females. A) Foundress from a group-initiated field colony. B) Foundress from a group-initiated colony in the laboratory that was prevented from foraging. Labels of structures: Kenyon cell bodies (k) and the calyx substructures lip (l) and collar + basal ring (c + br). Scale bar = 0.1 mm

