Social interactions as an ecological constraint in a eusocial insect

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The ecological constraints model (ECM) was formulated to predict how different ecological factors affect cooperative-breeding vertebrates. Within these groups some individuals help raise nondescendent young because of constraints that limit their own reproduction (e.g. limited foods, reproductive opportunities and nesting sites). Despite similarities between cooperative-breeding vertebrates and facultatively social insects, few studies have explicitly and experimentally determined how the ECM applies to social insects. In this study, we examined the potential effects of predation, density and nest site limitation as well as differences in nest site quality on the alternative nesting tactics of the paper wasp *Mischocyttarus mexicanus*. Accurate field estimates of density were possible because multiple nests are initiated in isolated *Sabal palmetto* trees. Results from a 17-month census showed more solitary nesting when density was low and nest sites were plentiful. In addition, females appeared to prefer nests initiated on unoccupied east-facing fronds. We then examined the potential effect of density and nest site limitation in a field experiment by adding or removing fronds from *S. palmetto* and forcing all females to renest. The percentage of haplo-metrotic females decreased when fronds were removed (increased density treatment), increased when fronds were added (decreased density treatment), and showed a mixed response when frond number remained unchanged (control density treatment). Nest site limitation did not appear to affect renesting females: the percentage of available unoccupied fronds was not affected by treatment. *Mischocyttarus mexicanus* females responded primarily to their social environment within palm groves and to variation in nest site quality, instead of constraints that typically affect cooperative-breeding vertebrates.

Keywords: alternative tactic; facultatively social; kin selection; *Mischocyttarus mexicanus*; nesting; nest site limitation; paper wasp; reproductive plasticity

Evidence suggesting that some social animals forgo reproduction to help others remained an evolutionary paradox until the realization that individuals could gain indirect fitness (Hamilton 1964). Reproductive altruism can now be explained if animals help relatives raise extra offspring (i.e. kin selection). Although this theory appears to offer a single, unifying explanation for reproductive altruism, subsequent literature has tended to focus on different elements of kin selection. Studies on social insects have stressed the importance of genetic relatedness among individuals within reproductive groups (e.g. Sayama & Takahashi 2005; Troniti et al. 2005; Wenseleers et al. 2005). In contrast, much of the vertebrate literature has identified ecological factors that limit a potential helper’s opportunity to reproduce independently, the ecological constraints model (ECM) (Emlen 1982, 1995). The ECM predicts that individuals will help others raise nondescendant young cooperatively when ecological factors, including inadequate foods, breeding territories and nest sites, limit their own reproduction. While the ECM is well established in the vertebrate literature (e.g. Bergmüller et al. 2005; Dickinson & McGowan 2005; Randall et al. 2005), the importance of ecological constraints to the evolution of insect sociality remains comparatively untested despite the fact that the form of sociality observed in social vertebrates and invertebrates often appears to involve similar costs and benefits (Brockmann 1997).

Ecological constraints certainly affect social insects. For example, queen number within polygynous ant colonies (i.e. a colony with multiple mated queens) can
be influenced by nest site and food limitation: queen number increases within colonies when critical resources are in short supply (e.g. Herbers 1986; Adams & Tschinkel 2001; Cahan 2001; Ingram 2002). Despite clear examples among polygynous ants, it is difficult to generalize between highly eusocial insects and cooperatively breeding vertebrates because of striking differences in their biology. However, comparisons can be made between cooperatively breeding vertebrates and primitively eusocial insects that are facultatively social during nest initiation because of their similar biology: individuals can establish nests with or without the aid of helpers and many individuals are totipotent (i.e. have the ability to develop from a non-reproductive to a reproductive state as adults; Brockmann 1997). As with polygynous ants, ecological constraints appear to affect group formation among primitively eusocial insects (Strassmann & Queller 1989; Richards & Packer 1996; Brockmann 1997). For example, Field et al. (1998) showed that helper Liestenogaster flavolineata (Vespidae, Stenogastrinae) preferentially aid high-quality nests where they can expect to queue for future reproductive opportunities after the host queen dies (Field et al. 2000). This situation is analogous to a cooperatively breeding vertebrate helper that differentially associates with a high-quality territory because it expects to inherit or ‘bud’ off from that territory in the future (Komdeur & Edelaar 2001a, b; Buston 2004).

Despite similarities, there are differences between cooperatively breeding vertebrates and primitively eusocial insects that may affect how each responds to ecological constraints (Brockmann 1997). Adult eusocial insects are short-lived compared to the developmental time of their offspring, while adult cooperative-breeding vertebrates are relatively long-lived. Additionally, most primitively eusocial insects are not territorial beyond the immediate borders of their nest. By comparison, many cooperative-breeding vertebrates defend territories that stretch vast distances away from their nest. A detailed examination of facultatively social insect species will help us to identify the common and unique ecological constraints that affect reproductive decisions in cooperatively breeding vertebrates and social insects.

In this study, we evaluated ecological constraints predicted to affect the nesting tactics of female Mischocyttarus mexicanus, a eusocial paper wasp common in the southeastern U.S.A. (Vespidae, Polistinae). During the nest initiation phase of colony development, M. mexicanus is also facultatively social: females initiate solitary (lone foundress) and group (multiple foundresses) nests within the same population (Litte 1977; Hermann et al. 1985; Clouse 1997, 2001). Group nests are semisocial during this phase, although all nests, regardless of whether they are initiated by one foundress or multiple foundresses, become eusocial after workers eclose. In addition, nearly all adult females of any generation are reproductively viable (Gunnels 2006). Finally, M. mexicanus nesting tactics are plastic: females can switch between haplometrotic (i.e. solitary) and pleometrotic (i.e. group) nest initiation as adults (Gunnels 2006).

In the observational portion of this study (see Observational Census below), the effects of predation, density and nest site limitation were considered. Nest orientation, position and exposure angle (see Methods) were also evaluated because each could reflect differences in nest site quality. In an associated experiment (see Field Experiment below), the effects of density and nest site limitation were explicitly examined. In this experiment, every individual within the population was forced to respond to increases or decreases in potentially limited resources. This allowed us to overcome a limitation inherent to most ecological constraints studies in which populations are either supplemented or deprived of a limited resource and only a segment of the population responds. In both sections of this study, we determined whether the frequency of pleometrotic females changed with each assessed factor. If ecological constraints affect M. mexicanus nesting tactics, then fewer females should pursue haplometrotic nesting with increased density and predation, decreased nest site availability and increased nest site quality.

**METHODS**

**Study Species**

We examined wild females as they initiated new nests in Gainesville, Florida, U.S.A. In this population, females initiate and develop nests for 10 months each year (C. W. Gunnels & T. Kimbrell, unpublished data). During this period, colony growth can be separated into two biologically defined seasons: an early season (February–April), which consists exclusively of newly initiated nests, and a late season (May–November), where newly initiated and postemergence nests coexist (C. W. Gunnels & T. Kimbrell, unpublished data).

We took advantage of the nesting behaviour of M. mexicanus to determine whether foundresses respond to ecological constraints in the field. Multiple, noninteracting nests are initiated in the same Subal palmetto Walter tree (Hermann et al. 1985). Coexisting nests in each tree represent discrete subpopulations within the total population of Gainesville. This nesting behaviour allowed us to consider ecological constraints that might affect individuals at the level of the tree (e.g. density) as well as the nest (e.g. predation).

**Observational Census**

We conducted a biweekly census of M. mexicanus over 17 months (April 2002–September 2003) to determine whether potential ecological constraints (i.e. density, predation, nest site limitation, nest site location and nest orientation) correlated with the frequency of helping in an unmanipulated environment. We found nests by visually inspecting every frond that could be reached from a standing position in a S. palmetto grove, which was defined as every tree that was in physical contact with a neighbouring S. palmetto (range 1–3 trees; N = 29 groves). Fronds positioned above a standing position were not included in the study.

During the day of a census, we recorded the number of newly initiated and previously established nests as well as the number of sampled fronds. Newly initiated nests
(i.e. nests containing only eggs) were individually labelled by marking the frond with a nest specific code (i.e. tree identification and nest number) adjacent to the peduncle of the nest. Density was estimated based on the number of females (female density) or nests (nest density) found relative to the number of sampled fronds at each S. palmetto grove. Our density estimates included both newly arriving as well as previously established residents. We chose these density estimates because this allowed us to include all early season observations when every female was initiating new nests. For each newly initiated nest, we also recorded the compass direction of the nest (i.e. nest orientation) and nest position on the frond. Nest orientation was the direction of the nest from the trunk of the S. palmetto tree. Nest position was classified as inside curl or outside curl relative to the frond costa (Fig. 1; modified from Hermann et al. 1985). For nests positioned on the outside curl, we recorded nest exposure angle as the space created between opposing leaflets (Fig. 1). We measured nest position and exposure angle because each factor could reflect a colony’s risk of predation and/or parasitism. If this were the case, then we predicted that nests on the inside curl and nests with larger exposure angles should experience greater risks. We measured nest orientation because it could reflect a colony’s response to abiotic conditions, such as the amount of light that each nest received.

Later that same night, we recorded the number of females at every nest (both newly initiated and previously established) because M. mexicanus are diurnal and all females return to and remain on their nest at night. In addition to providing us with a density estimate, this information was also used to determine the nesting tactic of newly initiated females: haplometrotic nests (Hap) consisted of a solitary female and pleometrotic nests (Pleo) contained two or more females. When possible we recorded the source of mortality for each lost nest (Table 1). We did not collect this information for nests if there was any confusion categorizing the mortality source.

Field Experiment

We conducted a field experiment to examine the effects of density and nest site limitation on M. mexicanus during March 2004 (Fig. 2). During an initial census, the number of females and nests were recorded from every sampled frond in each S. palmetto grove (N = 21). Nests were located and marked during the day; female number and nesting tactics were determined later that same night. Sabal palmetto groves were then blocked into seven groups of three groves each according to their initial density of females: the three groves with the highest initial density of females were grouped into one block, followed by the next three groves, and so on until the three groves with the smallest initial density of females were grouped into the last block. The groves within each block were then randomly assigned to one of three treatments: increased, decreased, or control density treatment. To create the increased density treatment, 50% of sampled fronds were removed from a grove. The decreased density treatment was created by increasing the number of fronds by 50%. This was accomplished by taking fronds cleaned of wasps and nests and attaching them to the trunk of trees in the decreased density treatment. Finally, the number of palm fronds was left unchanged in the control treatment. After altering frond number, all nests were removed from the trees. This action forced every female in the experiment to reinitiate a new nest. A second, final census was conducted 14 days later, in which we recorded the number of females (and their nesting tactic) and the number

![Figure 1. Mischocyttarus mexicanus nest locations on Sabal palmetto fronds observed in Gainesville, Florida, U.S.A. (modified from Hermann et al. 1985). For nests positioned on the outside curl of live fronds, exposure angle of nests was recorded as the space created between opposing leaflets.](image-url)
Table 1. Mortality sources of *Mischocyttarus mexicanus* nests*

<table>
<thead>
<tr>
<th>Mortality source</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abandon</td>
<td>No adults found at the nest. Pupae, larvae and/or eggs as well as the nest were still present. No evidence of nest damage.</td>
</tr>
<tr>
<td>Ant</td>
<td>No adults found at the nest. Pupae, larvae and/eggs were also missing, although the nest was present. Nest frequently showed signs of damage, including holes at the base of cells.</td>
</tr>
<tr>
<td>Large vertebrate</td>
<td>Nests, adults and offspring were missing. The nest peduncle and palm frond were still present. Palm trees were not physically damaged, as might be expected if nests were lost to extreme weather or humans, see below.</td>
</tr>
<tr>
<td>Human</td>
<td>Nests, adults and offspring were missing. Either the frond or the entire <em>Sabal palmetto</em> tree was missing: humans frequently cut off lower fronds of <em>S. palmetto</em> trees for aesthetic reasons. Hurricanes and other large storms were not observed during the study.</td>
</tr>
</tbody>
</table>

*Diagnostic characters used to categorize mortality source of *Mischocyttarus mexicanus* nests in Gainesville, Florida, U.S.A. Descriptions of mortality sources modified from Litte (1977) and Hermann & Chao (1984).*

of nests on each sampled frond. We chose to conduct the field experiment during the early season because all nests within the population were newly initiated at the same developmental stage, pre-emergence (C. W. Gunnels & T. Kimbrell, unpublished data).

**Analyses**

For the observational census, we evaluated the effects of female density, nest density, nest position, percentage of unoccupied fronds and season, as well as all two-way interactions on nesting tactics of foundress females with a forward stepwise logistic regression. Season (i.e. early versus late season nest initiation) was included in the analysis because reproductive decisions of females can change depending on the season (Gunnels 2007). For nests positioned on the inside curl, a second logistic regression was used to determine whether exposure angle, female density, nest density, season, and all their two-way interactions predicted nesting tactic. If female or nest density appeared to affect nesting tactic, then we ran a Mann—Whitney *U* test to determine whether density of previously established residents affected nesting tactic of initiating females. We used a binomial test to determine whether females showed a preference for unoccupied palm fronds by comparing the probability of initiating the only nest on a frond relative to the proportion of available unoccupied fronds. We also used a binomial test to determine whether females established more nests along the inside or outside curl of fronds against an expected probability of 0.5. We used a Rayleigh test to determine whether nests were oriented in a particular direction. If nest orientation was nonrandom, the orientation of nesting tactics and seasons were compared separately with a Mardia—Watson—Wheeler test (Zar 1996). We evaluated the field experiment with ANOVAs to examine effects of density treatment, experimental manipulation (i.e. initial versus final census) and the interaction between density treatment and experimental manipulation. We also examined the effects of the block design in these ANOVAs to determine whether pairing palms according to their initial female density influenced nesting tactic. Analyses were completed with SPSS 13.0 (2004, SPSS, Chicago, IL, U.S.A.), except for circular statistics, which were evaluated with Oriana 2.0 (2004, Kovach Computing Services, Anglesey, U.K.). Unless stated otherwise, central tendencies are shown as mean ± 95% CI.

**RESULTS**

**Observational Census**

Of the 244 newly initiated nests found during the observational portion of the study, the majority of nests were haplometrotic (63.64%; *Hap* = 153 nests, *Pleo* = 91 nests). However, only a minority of *M. mexicanus* females actually set up these solitary nests (28.2%; 153 of 541 females). The remaining 382 females established pleometrotic nests (mean ± 95% CI = 4.3 ± 3.74—4.86; range 2—12 females).

Most nests were initiated on trees that contained other *M. mexicanus* colonies (6.5 ± 5.9—7.1; range 1—17): only 9 of 244 nests were initiated in an otherwise unoccupied tree. All nests were initiated on live fronds. In addition, most nests were initiated on previously unoccupied fronds (93.03%), which was more frequent than predicted by the percentage of available unoccupied fronds (i.e. fronds lacking a *M. mexicanus* nest; binomial test: *Z* = 7.17, *N* = 244, *P* < 0.001; median ± 95% CI = 67.47 ± 65.19—69.75% unoccupied fronds/sampled fronds). Females initiated more nests on the outside curl (80.7%) than on the inside curl (19.3%) of palm fronds (binomial test: *Z* = 6.7, *N* = 244, *P* < 0.001). Females initiated nests on trees that contained available unoccupied fronds (99.6%) in all but one case.

Based on the forward stepwise selection criterion for all initiated nests, the interactions between female density and percentage of unoccupied fronds as well as the interaction between nest position and percentage of unoccupied fronds were the only factors included in a model predicting *M. mexicanus* nesting tactic (Table 2; Nagelkerke *R*² = 0.276). Pleometrosis increased at a *S. palmetto* grove when female density was high and unoccupied fronds were few (Fig. 3a). Females were more likely to initiate pleometrotic nests on the outside curl of fronds when unoccupied fronds were rare and to initiate haplometrotic nests on the outside curl when unoccupied fronds were common (Fig. 3b). The positive relationship between female density and pleometrosis was maintained when the density estimate was limited to previously established residents (Mann—Whitney *U* test: *U* = 6257.5, *N*ₜₜ = 117,
Pleometrotic foundresses established nests in exposed positions and haplometrotic females set up their nests in closed positions as female density increased (Fig. 4). Nest orientation was nonrandom: on average, nests were initiated on easterly facing palm fronds (Rayleigh test: $Z = 6.03$, $N = 174$, $P = 0.002$; $65.72 \pm 34.5^\circ$–$98.65^\circ$). In addition, north–south orientation differed between newly established haplometrotic and pleometrotic nests (Mardia–Watson–Wheeler test: $W = 7.37$, $P = 0.025$; Fig. 5): haplometrotic nests faced northeast ($41.95 \pm 14.22^\circ$–$69.68^\circ$, $N = 111$) and pleometrotic nests faced southeast ($132.74 \pm 86.56^\circ$–$178.93^\circ$, $N = 63$). Orientation of newly initiated early and late season nests did not differ (Mardia–Watson–Wheeler test: $W = 4.46$, $P = 0.108$; early season: $42.87 \pm 358.62^\circ$–$87.12^\circ$, $N = 110$; late season: $92.77 \pm 56.11^\circ$–$129.43^\circ$, $N = 64$).

Haplometrotic and pleometrotic nests were affected by different sources of mortality (chi-square test: $\chi^2 = 9$, $P = 0.029$, $N = 153$). A greater proportion of haplometrotic nests were lost to abandonment (Hap = 0.177 versus Pleo = 0.1) and ants (Hap = 0.46 versus Pleo = 0.325). Pleometrotic nests were at a slightly greater risk of human caused loss (Hap = 0.097 versus Pleo = 0.275). There was no appreciable difference in the proportion of haplometrotic and pleometrotic nests that were lost to large vertebrates (Hap = 0.265 versus Pleo = 0.3).

Field Experiment

The percentage of solitary females was affected by the interaction between density treatment and experimental manipulation (ANOVA: $F_{2,30} = 4.142$, $P = 0.026$). The percentage of haplometrotic females decreased in all blocks by an average of 44.9% after density was increased (initial census = $55.83 \pm 29.22^\circ$–$82.44^\circ$; final census = $10.9 \pm 0^\circ$–$24.54^\circ$, $N = 7$; Fig. 6a). The percentage of haplometrotic females increased in all but one block by an average
of 11.4% when density was decreased (initial census = \(34.84 \pm 13.43-56.25\%\); final census = \(46.27 \pm 11.19-81.35\%\), \(N = 7\); Fig. 6b). There was a mixed response in the control treatment. The percentage of haplometrotic females increased in three of seven blocks by an average of 5.8% (initial census = \(37.15 \pm 15.41-58.9\%\); final census = \(42.97 \pm 14.67-71.28\%\), \(N = 7\); Fig. 6c). The percentage of haplometrotic females was not affected by the treatment, experimental manipulation or block (ANOVA: density: \(F_{2,30} = 0.277, P = 0.76\); manipulation: \(F_{1,30} = 1.097, P = 0.303\); block: \(F_{6,30} = 0.537, P = 0.776\)).

The number of females found at each grove appeared to decline after females were forced to renest (manipulation: \(F_{1,30} = 2.905, P = 0.084\); initial census = \(22.86 \pm 11.97-33.74\) females/grove; final census = \(16.62 \pm 7.82-25.42\) females/grove), although the difference was not significant. Female number was not affected by density treatment, the interaction between treatment and experimental manipulation, or block (density: \(F_{2,30} = 1.627, P = 0.213\); density × manipulation: \(F_{2,30} = 0.126, P = 0.882\); block: \(F_{6,30} = 1.563, P = 0.192\)). Although the number of fronds within treatments changed, the percentage of unoccupied fronds was not affected by density treatment, block, or the interaction between density treatment and experimental manipulation (density: \(F_{2,30} = 0.345, P = 0.69\); block: \(F_{6,30} = 0.436, P = 0.849\); density × manipulation: \(F_{2,30} = 0.246, P = 0.783\)). Unoccupied fronds were also available in every palm grove after the manipulation. In addition, the percentage of unoccupied fronds appeared to increase after the experimental manipulation (density: \(F_{1,30} = 3.88, P = 0.058\); initial census = \(61.54 \pm 52.79-70.3\%\); final census = \(72.91 \pm 64.73-81.09\%\)).

**DISCUSSION**

Female *M. mexicanus* clearly reacted to ecological constraints when deciding between haplometrotic and pleometrotic nesting. Foundresses responded to the density of conspecifics within the local population at palm groves and to variation in quality of different nest sites. In the observational census, females established pleometrotic nests in palm groves with many conspecifics and few unoccupied fronds, and they established haplometrotic nests in groves with fewer conspecifics and numerous unoccupied fronds. The relationship between female density and nesting tactic was also supported by results of the field experiment. The percentage of females establishing haplometrotic nests decreased dramatically after density was increased. Haplometrotic nesting also increased after female density was decreased, although this change was less extreme.

Results of the field experiment may reflect a nonlinear response by *M. mexicanus* to changes in density. Nonlinear relationships that affect behaviour have been observed in other eusocial insects. In ants, pheromone trail strength and distance between colonies can have disproportionate effects on the foraging behaviour of workers (Sumpter & Beekman 2003; Sumpter & Pratt 2003; Weeks et al. 2004). If *M. mexicanus* do show a nonlinear response to density, then females in our study appeared to receive a relatively low population density, because they responded less strongly to the decreased density treatment than they did to the increased density treatment. However, it seems more likely that females in our study responded differently to alternative treatments because some of the added fronds used to create the decreased density treatment died during the experiment. In the census, we determined that females only initiate nests on live fronds. Consequently, females may have perceived a more dense population in the decreased density treatment than we intended because they avoided added fronds that died during the experiment. However, we did not collect data on the number of fronds that died during the experiment, so we cannot discriminate between these hypotheses.

*Mischocyttarus mexicanus* females also responded to variation in nest orientation and position, suggesting that they do perceive differences in nest site quality. Females

**Table 2.** Results of forward stepwise regression identifying variables associated with the nesting tactics of *Mischocyttarus mexicanus* females from all initiated nests (\(N = 242\)) and from nests initiated along the outside curl of palm fronds (\(N = 197\)).

<table>
<thead>
<tr>
<th>Included variables</th>
<th>Coefficient</th>
<th>SE</th>
<th>Wald</th>
<th>(P)</th>
<th>Odds ratio*</th>
<th>95% CI odds ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All nests†</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>-0.77</td>
<td>0.366</td>
<td>4.721</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female density × unoccupied fronds</td>
<td>0.014</td>
<td>0.003</td>
<td>28.797</td>
<td>&lt;0.001</td>
<td>1.014</td>
<td>1.009-1.019</td>
</tr>
<tr>
<td>Nest position × unoccupied fronds</td>
<td>-0.016</td>
<td>0.005</td>
<td>10.478</td>
<td>0.001</td>
<td>0.985</td>
<td>0.975-0.994</td>
</tr>
<tr>
<td><strong>Nests along outside curl‡</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>-1.771</td>
<td>0.3</td>
<td>34.895</td>
<td>&lt;0.001</td>
<td>1.015</td>
<td>1.008-1.021</td>
</tr>
<tr>
<td>Female density × exposure angle</td>
<td>0.015</td>
<td>0.003</td>
<td>20.229</td>
<td>&lt;0.001</td>
<td>1.015</td>
<td>1.008-1.021</td>
</tr>
</tbody>
</table>

*Odds ratio describes the increased probability of pleometrotic nesting for each increase of one unit in the independent variable. Two nests were removed from the analysis because we did not have a density estimate.
†Effects of season, female density, nest density, nest position, unoccupied fronds, female density × season, nest density × season, nest position × season, unoccupied fronds × season, female density × nest density, female density × nest position, nest density × nest position and nest density × unoccupied fronds were not included in the final model.
‡Effects of season, female density, nest density, unoccupied fronds, exposure angle, female density × season, nest density × season, unoccupied fronds × season, exposure angle × season, female density × nest density, female density × unoccupied fronds, nest density × exposure angle and unoccupied fronds × exposure angle were not included in the final model.
biased nest initiation towards the east-facing fronds. By biasing the location of their nest, females should experience increased activity during morning hours because of higher temperatures and increased luminance (Stabentheiner et al. 2003; Spiewok & Schmolz 2006; Weissel et al. 2006). Females were also more likely to initiate nests along the outside curl of fronds. Nesting on the outside curl may help individuals hide. These locations are comparatively concealed because of the restricted space created by opposing leaflets of the frond.

Differences between nest sites also appeared to affect the nesting tactic of *M. mexicanus*. Pleometrotic nests tended to face the south while haplometrotic nests primarily faced the north. In Florida where the sun passes across the southern horizon, south-facing nests would be both brighter and warmer than north-facing nests. This orientation may create a higher-quality nest site that promotes group nesting on south-facing fronds because of the increased temperature and solar exposure. In addition, larvae on south-facing fronds may develop more rapidly because of higher temperatures (Porter 1988).

The response of *M. mexicanus* females to their social environment within palm groves and variation in nest site quality could reflect unidentified benefits or costs associated with group living. Females may prefer pleometrotic nesting because of benefits associated with grouping that outweigh lost opportunities for independent reproduction. Among primitively eusocial insects, pleometrotic nests typically survive longer, grow faster and reinitiate more rapidly after nest loss than haplometrotic nests (Strassmann & Queller 1989; Reeve 1991). Multifoundress *M. mexicanus* nests similarly experience higher rates of survival and developmental rates as well as faster reinitiation than solitary nests (Litte 1977; Clouse 2001). In this case, the increased frequency of pleometrotic nesting in dense environments may reflect the increased likelihood of finding a cooperative partner, regardless of whether floating wasps attempt to join nests (Shreeves et al. 2003) or residents bid for their help (Reeve & Keller 2001). Alternatively, a female's response to her social environment may be a consequence of costs resulting from conspecifics, including cannibalism, usurpation and, potentially, brood parasitism (Nonacs & Reeve 1995; Shakarad & Gadagkar 1995). Nesting *M. mexicanus* do experience both intercolony cannibalism and nest usurpation (Clouse 1995). There is also the possibility that cannibalistic females engage in intercolony brood parasitism when an intruder lays an egg in the empty cell of the cannibalized egg or larva. In each case, nesting *M. mexicanus* would be at greater risk as female density increases. In dense environments, females may choose to group on pleometrotic nests to minimize these risks. Protection is presumably accomplished by continuous guarding of the developing brood, and at least one female, typically the primary reproductive, is always on a pleometrotic nest (Gunnels 2007). Consequently, there is always a foundress that could ward off intruders. By comparison, a haplometrotic nest
is exposed every time the female leaves to forage for food or pulp. Haplometrotic females in dense populations could minimize these costs by establishing nests in fronds with a small nest exposure angle, a finding observed in this study. The possibility that conspecifics could represent a cost that promotes pleometrosis is further supported by the observation of rare, low-intensity aggression towards nestmates (Gunnels 2007), limited defence against predators (Litte 1977; Clouse 2001) and immediate, high-intensity aggression towards non-nestmates (Clouse 1995). Nesting *M. mexicanus* appear to react intensely only to those intruders that could cannibalize, usurp and potentially parasitize their nest.

Our results could be explained as a response to nest site limitation. Most nests were initiated on unoccupied fronds. Furthermore, females observed during the census were more likely to establish pleometrotic nests when available nest sites (i.e. percentage of unoccupied fronds) were rare. Finally, females showed the strongest response in the field experiment to frond reduction. Nest site limitation is frequently noted for its effects on the nesting decision of many facultatively social ants (e.g. Herbers 1986; D’Ettorre et al. 2005; Feldhaar et al. 2005). These ants appear to be affected by nest site limitation because they establish their colonies in organic substrates, such as acorns, seeds and plant nodules. Organic nest sites can become limited if they decay simultaneously, as observed in temperate populations (Herbers 1986). Consequently, queens will establish pleometrotic colonies as the stock of nest sites disappears.

Despite some supporting evidence, nest site limitation does not appear to strongly influence the nesting tactics of *M. mexicanus*. In both the observational census and field experiment, females established pleometrotic nests even though there were available nest sites (unoccupied fronds) in all but one observed *S. palmetto*. We would expect *M. mexicanus* foundresses to avoid pleometrosis and establish solitary nests on these unoccupied fronds if they were nest site limited (sensu stricto). In addition, the percentage of unoccupied fronds did not differ before and after the manipulation in any treatment. If nest site limitation had a strong effect on female nesting tactic, then the percentage of unoccupied fronds should have decreased when fronds were removed, increased when fronds were added, and remained unchanged in the control after the experimental manipulation. Finally, our data suggesting the importance of nest site limitation could be explained because nest site limitation and female density are probably confounded (e.g. unoccupied nest sites are rare when female density are high), which could create a false positive result for either factor.

Similarly, Queller & Strassmann (1988) rejected nest site limitation as an explanation for group size in a cliff-dwelling population of *Polistes annularis*. Upon first examination, nest site limitation appeared to affect group size of newly initiated *P. annularis* nests: the available substrate (i.e. cliff overhangs) was finite, multifoundress nests were common, and females frequently renested either on or next to their natal nests (Strassmann 1988). Despite some support for nest site limitation, Queller & Strassmann (1988) rejected the hypothesis because the number of multifoundresses in pleometrotic nests did not change in response to fluctuation in the number of nests and females within the population. Had nest site limitation been critical then group size should have decreased when population size was small.

Responses of primitively eusocial insects, including *M. mexicanus*, to ecological constraints are similar to those of cooperatively breeding vertebrates. Individuals reproduce with helpers when constraints are severe and they reproduce without helpers when conditions are more benign. In social insects, this is described by differences in the frequency of pleometrotic and haplometrotic nesting. Despite similarities, there are striking differences in the biology of primitively eusocial insects and cooperatively breeding vertebrates that may affect how each group responds to constraints. In the end, these differences may be so extreme that they may limit our ability to develop specific predictions that can be generalized among taxa (Pen & Weissing 2000). For example,
cooperatively breeding vertebrates are relatively long-lived and tend to defend expansive territories beyond their nests. Consequently, cooperatively breeding vertebrates are frequently nest site limited. Helpers assist because they hope to eventually inherit or bud from the reproductive territory (Legge & Cockburn 2000; Komdeur & Edelaar 2001a, b). By comparison, short-lived, nonterritorial primitively eusocial insects do not appear to be nest site limited, although the cost of initiating a new nest is comparatively high (Queller & Strassmann 1988; Strassmann 1988; Field et al. 1998). At this point, it is still unclear how these differences will affect our ability to generalize among cooperatively breeding vertebrates and social insects.

Detailed examination of ecological constraints should also help clarify some of the recent controversy that surrounds kin selection theory (Wilson & Hölldobler 2005). Among their many concerns, Wilson & Hölldobler (2005) felt that kin selection made genetic relatedness the dominant force resulting in social evolution. While high genetic relatedness certainly promotes altruism, kin selection does not require high relatedness between individuals, only a net advantage to the altruist. For example, altruism exists in reproductive groups of distantly related individuals (e.g. Strassmann et al. 1989; Keller & Reeve 1994; Strassmann et al. 1995; Wiernasz et al. 2004; Stiver et al. 2005), although low relatedness would seem to discourage this behaviour. Despite its prevalence in the literature (e.g. Beeckman & Oldroyd 2005; Trontti et al. 2005; McLeish et al. 2006), relatedness is only one component that a potential altruist considers according to kin selection theory (Foster et al. 2006). In addition to relatedness, the presence of altruism (e.g. pleometrotic nest initiation) is affected by the benefit to the recipient and the cost suffered by the altruist. Potential altruists will be influenced by intrinsic and/or extrinsic factors, such as relative body size and ecological constraints, because these factors affect benefits and costs. For example, an individual with few reproductive opportunities (e.g. high ecological constraints) would be more likely to help than an individual with many reproductive opportunities (e.g. low ecological constraints). While relatedness is clearly important, individuals must weigh a variety of factors when deciding whether to help (Foster et al. 2006). In the end, Wilson & Hölldobler’s (2005) criticisms have successfully focused attention on neglected aspects of kin selection theory, including the role of ecological constraints.

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