

Response from Editor

Dear Terry,

I have now received the reports from the two referees I had contacted to evaluate your manuscript IS1194, and copy these below. The two referees agree about the potential interest of this manuscript, but also put forward several comments and questions. I also attach a file of the manuscript in which I indicated as track changes a number of technical editorial corrections that are to be made (this has altered the line numbers - the line numbers mentioned in the referee reports correspond with those in the submitted version). I evidently leave it up to you whether you are prepared to revise and re-submit the manuscript along these comments raised by the referees, or prefer to try another journal. When sending a revised version, please clarify in a cover letter how you have dealt with each of the referee comments.

In order to follow the editorial procedure, I would like to make clear the deadline for receiving a revised version (as a *.doc file) is 6 months, which for this manuscript corresponds with a deadline of 14 February 2010 (in case I have not received a revised manuscript before this date, this contribution will be considered as a new submission).

Best regards,
xxxxx

Reviewer 1

This is a very interesting paper that examines the role of two ecological factors (food availability and nest site availability) controlling the level of polygyny in ant nests. It is within the scope of the journal and should be of interest to many social insect researchers. I have some concerns about the design of the study/experiments and analysis of the data that should be addressed, but they are not fatal to the scientific content of the conclusions. Also the introduction and discussion need to be revised so that explanations (especially for the predictions and conclusions about the competition hypothesis) are more clear. I am especially concerned about the author's conclusion that competition (which is vaguely defined) is more important than competition for food in controlling queen number. I have suggested that the author incorporate some additional literature references in the paper, especially a series of papers published by Herbers and her co-workers that have explored the role of food and nest supplementation on sociometric measures in ant nests (including polygyny) in temperate forest ants. While the system they researched is different from the system McGlynn is using it would be interesting to know if tropical and temperate forest ants behave the same way to similar manipulations.

Specific comments are provided in a separate document. [the subsequent 6 pages]

Review of IS1194

T. McGlynn

Adaptative queen number in thief ants: competition, food resources or nest limitation?

Reviewer: xxxxx

Concerns about the study/experimental design.

For both the study and the experiment: Are there slave-makers that affect litter *Solenopsis* spp.? Foitzik et al. (2004), the full citation is at the end of this review, found that the presence of slave-makers strongly affects how ant nests will respond to food and nest site competition in temperate forest ants. Indeed plots with high slave-maker pressure and low slave-maker pressure responded differently to the same food and nest supplementation regimes. If slave-makers are present than their possible effect should be addressed.

The plots excavated are only 1m². In the temperate forest plots of this size are too small to adequately sample the range of ant nests even in the richest habitats. Figure 2A shows that nest density per 10 m² ranges from approximately 1-20 (although it is not clear that it is the density of thief-ants only or all ants species found). Indeed in the discussion the author's suggests that in the clumped food supplementation treatment ants moved out of the plot and thus any effect of clumped food supplementation may have been confounded by low sample size. [Note: Based on information in the methods the sample size for the clumped treatment was 48 plots and the largest sample size was 55 plots which is not a huge difference. Also, the author suggests that this treatment showed greater variation than the other treatments in queen number; this can be tested by comparing the coefficient of variation for the 5 treatments.] A table or figure showing the density of litter *Solenopsis* spp. nests and all ant species nests for each treatment might help show that the plot size is adequate.

If the nest density was collected in 1m² plots why is figure 2A showing density per 10m² plot? This needs to be explained.

The author needs to justify the use of logistic regression to determine the extent to which queen number is explained by colony size. Correlation, rather than regression, should be done; regression assumes queen number is a function of worker number however, if multiple queens are laying fertilized eggs then worker number might be a function of queen number too. Also, he states in his paragraph on statistical analysis that he did those regressions separately for each experiment and presents separate regression equations in the results section, but combines the data from experiment 1 and 2 in figure 1. This is confusing and there's no justification for this. Also, he should make it clear if only the control nests were used for this analysis in the Food and Litter Manipulation experiment. Using the other treatments will confound that regression.

In the Environmental Gradient study:

The author chose the sampling plots to encompass the soil-fertility gradient. Why was soil-fertility gradient the chosen and does this ensure that the entire range of thief ant habitats are sampled? Foitzik et al. (2004) found that sampling blocks of cavity dwelling ants react very differently to food and nest supplementation manipulations so that plot choice for these types of studies is critical. The author should justify his use of soil-fertility gradient as the deciding factor in where to place his plots.

The author states that the diet of litter *Solenopsis* spp. is not known then uses density of Collembola as one measure of natural variation in food levels (in addition to all non-ant litter arthropods). Why were Collembola chosen for this? These data should be deleted unless there is a compelling reason to include them.

In the Food and Litter Manipulation experiment:

Would trampling the litter affect food availability or foraging ability? Compacted litter may make harder for foragers to move about and find food. (Alternatively, if they forage on the litter surface it may make it easier for them to find food.) Also, it may squash the available arthropods and thus reduce food availability. Indeed the author states that trampling does limit access to resources (page 7 line 131). This needs to be addressed as a potential confounding variable.

How did the author estimate new worker production? This is not explained and it could be critical especially if it was done by counting worker pupae (as I infer from the legend to table 3). What is the lag time between egg laying and pupation for workers? Given that the duration of each treatment ranged from 1 week to 4 months there may not have been enough time for the nest to make changes in their allocation strategies that could be measured by counts of worker pupae. In addition, nests may change allocation in response to food by changing packaging decisions (Backus 1993; Herbers and Banschbach 1998) and these would not be apparent by counts of pupae. The author needs to expand on how these data were collected and whether he is confident that he could have detected changes in worker production given the time frame of the experiment.

Concerns about the competition hypothesis and the conclusion from the natural population study that competition is a factor in controlling queen number.

I am confused about the competition explanation for queen number throughout this paper. If the author means inter-specific interference competition (ISIC) by the word competition then the data shows that this is occurring; as the density of ant nests increases then queen number increases. There is an alternative explanation for this result however; an increase in the density of nests may be due to an increase in the availability of food (which is probably distributed patchily) so that the changes in nest density are driven by food competition. Also, bigger nests (more workers and more queens) may be able to be supported by the food availability. Thus, the level of inter-specific interference

competition is confounded by food competition and the conclusion that competition is driving changes in queen number may not be correct.

This is recognized, but not commented on by the author, in Table 1 where the predictions for the Food-Nest Manipulation experiment for the influence of food and competition are the same: clumped food supplementation leads to increased queen number.

The food manipulation experiment can help to untangle this. The author states that a clumped food supplementation manipulation can result in an increase in inter-specific interference competition (if that is what he means by that word). Presumably the diffuse food manipulation doesn't do this. Thus, if there is a difference in the response of queen number to the two manipulations then the relative effects of ISIC and food competition can be untangled. The data seem to show that the clumped food treatment did increase ISIC as expected on the basis of prior work and as shown by the decrease in the density of nests in those treatments. (This is why data on nest density should be included.) Yet, there was no effect of clumped feeding on ISIC. There was an effect of diffuse feeding on queen number. Thus, it is reasonable to conclude that competition for food is more important than ISIC. This matches the published data on temperate forest ants that show changes in food supply can change a whole suite of sociometric measures (Herbers and Banschbach 1999; DeHeer et al. 2001 and Foitzik et al. 2004).

The author needs to be more specific about what he means by competition in this paper and how he will detect it, especially if he wants to support his assertion that "It is unlikely that food itself enhanced polygyny..." made on Page 10¶3.

Specific comments on the text/figures and tables

Introduction

Page 3 ¶1. The word Tschinkel (1991) coined was sociometry, not socioecology.

Page 3 ¶1. There is a contradiction between sentences 2 and 3 that needs to be resolved. The last sentence of the paragraph needs to be re-written as it implies that only polygyny can be studied this way.

Page 4 ¶1. I'm unclear what the author means by competition here. Later in the paper it seems that he's using the word competition as a short form for "interspecific interference competition". If this is what he means he needs to define it in the paragraph. Also, the two rationales he provided for why competition could influence queen number also apply to the other two factors he discusses. Rationale 1 is really competition for nest sites whereas rationale 2 is related to food limitation. This paragraph needs to be re-written so that the author's meaning is more clear.

Page 4 ¶2 and 3. Herbers and her co-workers used food and nest site supplementation

experiments to address the relative importance of these conditions on temperate forest ants. These papers might be used to put the current studies into context. Full references to these papers are at the end of the paper.

Page 4 ¶4. The author should refer to and explain table 1 in this paragraph to justify the first sentence. Methods

Page 5. ¶2. Re-write sentence 2 for narrative flow.

Page 5. ¶3. Re-write sentences 1, 2 and 3 for narrative flow. This information describes a study, not an experiment.

Page 7 ¶3. Delete this paragraph and put the relevant information in results where needed. Results Throughout. Be consistent with the number of decimals that data and P values are recorded to.

Page 9 ¶1. In this paragraph prediction A refers to density of ant nests and prediction B refers to density of ants. In table 1 prediction A refers to density of ants and prediction B refers to density of ant nests. These need to be reconciled so they match.

Make clear whether density of nests and density of ants presented in figure 2 is all species found or only the 16 litter dwelling *Solenopsis used in the analysis*.

Page 9 ¶2. Rewrite sentence 2 for narrative flow.

Discussion

Page 10 ¶1. Change first sentence so that results match predictions, not predictions match results. Add that queen number increases with food availability to this summary of the findings.

Page 10 ¶2. The last sentence (We found that diffuse food...) is a non sequitar. That is addressed in the next paragraph so either delete that sentence or combine paragraphs.

Page 10 ¶3. The conclusion that food supplementation did not increase the production of new workers can not be made if the time lag between the beginning of treatments and nest collection is shorter than the time lag to produce pupae. Also, as mention before nests can respond by changing packaging and not numbers so the manipulations may affect growth but not numbers. Finally, it is possible that the nests are already growing (producing workers) at an optimum level so that additional resources are moved into reproduction (Backus 1995) and not growth.

Page 10 ¶4. The second sentence is a non sequitar.

Page 11¶1. The conclusion that colonies with higher queen numbers will have more genetic diversity depends on whether all queens are able to lay fertilized eggs. If the ovaries on the extra queens are suppressed then there is no increase in genetic variation in multiple queen nests over single queen nests.

Page 11¶2. If nest limitation affects polygyny why was there no effect of litter removal? I have no doubts that nest limitation does affect polygyny; that conclusion was also found for some temperate forest ants by Herbers and her co-workers but the lack of an effect of nest removal needs to be addressed.

I suggest the author consider adding a paragraph to make integrate his data on tropical ants with what is known from similar experiments on temperate forest ants. It would be interesting to know how these two groups of ants compare changes in the same resources to determine if the patterns are universal or location specific.

References

I have not verified that the format and punctuation for each reference is correct but there are numerous inconsistencies that need to be corrected.

There are two papers cited in the text that are not listed in the references section: Clark and Clark 2000; McGlynn and Kirksey 2000.

The Herbers 1982 reference is part of the Herbers 1993 entry and needs to be moved to the correct place in the list.

Figure 1

Why combine data from the study and the experiment in the figure when the regressions are done separately? Either do the correlations (the suggested correct analysis) on the lumped data or present two figures.

Figure 2

Both A and B: make clear that the density is per 10m² in the axis label. Better yet use 1m². Panel B: Put regression equation and r² value in figure legend not on the figure.

Both figures: Make sure the font is correct size and style for the journal.

Table 1

The predictions listed here need to be better justified. (Especially the ones regarding the competition hypothesis.) This can be done by making the table entries longer and more clear or in the text. Expected direction of relationship should be more explicit, EG. Queen

number decreases with a decreased arthropod density.

There is a typo in the heading for the 3rd column (net instead of nest).

There is no explicit prediction for the litter removal manipulation (which would be prediction L).

It would be easier for the reader to refer to the table if the prediction letter came first in the cell EG. Prediction C: Queen number decreases with a decreased arthropod density.

Table 2 and 3

Center entries in columns.

Make sure the number of decimal places is consistent with the results text.

In table 2 the ANOVA results are in the heading but in table 3 they are at the bottom. Be consistent with placement.

Papers I have referred to in the review

Backus, V.L. 1993 Packaging of offspring by nests of the ant, *Leptothorax longispinosus*: parent-offspring conflict and queen-worker conflict. *Oecologia* 95:283-289.

Backus, V.L. 1995 Rules for allocation in a temperate forest ant: Demography, natural selection, and queen-worker conflict. *American Naturalist* 145:775-796.

DeHeer, C.J., V.L. Backus, and J.M. Herbers. 2001 Sociogenetic responses to ecological variation in the ant *Myrmica punctiventris* are context dependent. *Behavioural Ecology and Sociobiology* 49:375-386

Foitzik, S. V.L. Backus, A. Trindl and J.M. Herbers. 2004 Ecology of *Leptothorax* ants: Impact of Food, Nest Sites and Social Parasites. *Behavioural Ecology and Sociobiology*. 55:484-493

Herbers, J.M. and V.S. Banschbach. 1998 Food supply and reproductive allocation in forest ants: repeated experiments give different results. *Oikos* 83:145-151.

Herbers J.M. and V.S. Banschbach. 1999 Plasticity of social organization in a forest ant species. *Behav. Ecol. Sociobiol.* 45:451-465.

Reviewer 2

General comments

This is a small study on the impact of environmental cues on queen number in tropical thief ants. It appears to be an re-analysis of a data set, which was originally accumulated for other purposes and, as such, some procedures are a little questionable. For example, to study nest site limitation, study plots were trampled, but the direct effect of this physical manipulation was not evaluated. However, some of the findings in this study are rather interesting. A number of hypotheses and their predictions are reported in Table 1, but

they should also be introduced more carefully in the introduction. In general, the link between these hypotheses, their predictions and the experimental approach becomes not really clear. Also the conclusions in the discussion are often a little too simple and additional studies should be cited. The manuscript should be carefully revised.

Specific points

Title: It would be more informative to rephrase the title in such a way, that the results of the study become apparent e.g. Nest site limitation and competition influence queen number in thief ants

Abstract

Line 24 The first sentence gives the impression that queen number is still completely unexplained in ants. This is certainly not true. Both theoretical and empirical studies addressed this question and there is evidence for a number of environmental parameters, which affect queen number in ants. Please rephrase this sentence

Line 28 It remains unclear what was tested in the first experiment. Please rephrase so that it becomes apparent how the competition hypothesis was tested with this experiment

Line 32 Please clarify the results by writing: i.e. Queen number increased under nest site limitation and competition....

Introduction

Line 42 The author cites here a nearly two decade's old paper to support the notion that the socioecology of ants is still poorly understood. However, in the last 18 years advances were made. Please cite some more recent papers.

Line 62-76 The first two hypotheses can be combined into one. In general, re-adoption of daughter queens (secondary polygyny) is expected under difficult environmental conditions (Bourke & Heinze 1994) These can be extreme temperatures, nest site limitation or competition. A second hypothesis would state that polygyny increases genetic diversity, which could be beneficial for a colony due to enhanced parasite resistance or because it enhances foraging efficiency or division of labor.

Line 83-88 Please report more directly here how these different hypothesis are tested with the experiments. This remains unclear at the end of the introduction.

Methods

Line 95 Can an observation be an experiment? Instead of experiment, I would term it Approach 1

Line 99-110 I remains unclear what was tested against what here. Did you study how queen number per ant nest varies with food availability?

Line 128 Did you check which proportion of nest sites were crushed? Did you also crush uninhabited nest sites? Which percentage of nest sites remained untouched?

Line 132 I did not understand this sentence: How did you destructively sample over four months? Did you resample each plot several times?

Line 143 Because I do not know this sampling protocol, I would like to ask whether it ensured that ant colonies and their queens stayed together? Did you know afterwards how many ant nests were found per plot? How likely is it that you found each queen, which was present in a plot?

Results

Line 189 Albeit only less than 1% of the colonies where queenless or workerless, I do not understand, why queen less nests were removed from the data base. Queenlessness can arise through either death of the queen or polydomy and both are natural phenomena in ants.

Line 195 Prediction 1 should also be explained in the text. Table 1 should be much more integrated with the introduction. These predictions in the table come out of the blue.

Discussion

Line 199-202 The start sentence is awkward. Why not simply stating that the experiment and the observation give support to the nest site limitation and competition hypothesis. Also it is unnecessary to point out that the results are discussed on the basis of related research. This is what is expected from a scientific paper. Rather remove this sentence and discuss the findings right away!

Line 206 Change to: “ In addition, we found that experimental diffuse food...”

Line 214 It is unnecessary to name these hypotheses with letters. Please explain in short which hypothesis you refer to. Otherwise, the reader has to have table 1 at side, to understand the discussion.

Line 223 There are also studies which showed a negative effect of polygyny and lower intracolony relatedness on colony productivity (e.g. Linksvayer 2008)

Line 253 Is it really clear that thief ants actively adjust their queen number? Is it not also possible that competing ants profit from the manipulation and force the tiny thief ants to few nests site, ie. Interspecific competition affects polydomy?

Table 1 Please explain the hypothesis and their diverse predictions carefully in the introduction! What evidence from other ants is there for these predictions?

Cover letter to editor with revision

Dr. xxxxxx:

I am pleased to submit to *Insectes Sociaux* a revision of the manuscript renamed, “Polygyny in thief ants responds to competition and nest limitation but not food resources.” I have made many changes to improve the manuscript in accordance with the recommendations of both referees.

Referee 2 prescribed one major change. In accordance, I have altered the introduction and Table 1 so that the predictions of each of the three hypotheses and their predictions are presented in the introduction in each paragraph describing the hypotheses.

I have made many other alterations to satisfy the remaining concerns of Referee 2. A partial list of these many small changes include: an explanation of destructive sampling without replacement; more detail in the sampling protocol to explain that nests were collected in their entirety; citations indicating more information about the consequences

of relatedness and polygyny for efficiency; and more explicit explanations about how experimental manipulations altered the variables of interest.

The one suggestion that I did not adopt was to recast the original three hypotheses as two hypotheses regarding secondary polygyny and genetic relatedness. I did not make this change because the original hypotheses center around external ecological influences on colonies, and because in nearly all cases it is highly likely that secondary polygyny prevails in this system (even if the function of polygyny is to increase workforce genetic diversity). I briefly address this matter in the introduction of the revised manuscript.

Because both referees took exception to the label “experiment” to describe hypothesis testing using observational data, I have renamed the two parts of the study as “components.”

I have accounted for the itemized recommendations of Referee 1 by making many changes to the manuscript. A partial list includes: an increase in references to work on polygyny in temperate ants, including papers by the Herbers group as suggested; a clarification in the figure captions on the scales of samples per plot (1m^2) and per site in the gradient component (10m^2); a clarification on the effects of trampling relative to controls; a clarification on the measure of new worker production and time scale of treatments; more explicit wording that competition is measured using the density of heterospecific ants, in terms of workers and nests; I provided a reference featuring the term “socioecology,”; and deletion of the data on springtail abundance.

There were a few remaining remarks of Referee 1 that did not prescribe changes in the manuscript, but instead involved concerns about experimental design or interpretation. I address these matters here in the covering letter in a fashion that I believe will satisfy these concerns. Most of these items are applicable to temperate forests, but have less bearing on studies on litter ants in the tropics. One major concern was that spatial heterogeneity in slavemaking ants would be driving polygyny in this system. This cannot be the case, as there are no dulotic species at this site to my knowledge. (If slave-makers in do occur, then they are at such a low density to have a null effect, as they had to have escaped detection in an exhaustive 16-year sampling regime conducted by Jack Longino, and I have found no suggestion of dulosis in nests containing *Solenopsis* workers). The referee was also concerned that the timeframe of the experiment was not adequate to observe changes in the ontogeny of colonies, however in tropical rain forests, I and others have shown (and cited to this effect in the present manuscript), the time elapsed in this study is more than adequate.

Another concern of Referee 1 was the sampling scale of excavations at the 1m^2 scale. I did not excavate soil, but rather collect litter as this is the nesting environment of thief ants. This spatial scale has long been a standard size for samples and manipulations of tropical leaf litter environments, in prior papers by my myself, Kaspari, Vasconcelos, and others and is prescribed as a standardized method in the Agosti et al (2000) book as cited, as well as the forthcoming Ant Ecology book (Lach et al 2010). xxxxx’s concern was that I was not sampling the range of ant nests - I assume this means foraging range;

this concern is moot as I was only sampling nests and measuring density. As for adequately representing each site, I replicated samples at an appropriate scale to acquire an adequate sample size such that samples were independent of one another. Referee 2 requested information about density of *Solenopsis* and other species at the spatial scale at which I sampled; these data are available in the paper, in which I report the number of 1m² plots sampled and the number of nests of all species and of *Solenopsis* encountered in these plots.

Referee 1 was concerned that have confounded “competition” with “interspecific interference competition.” I have not made such an error, though I have made modest changes to emphasize that competition is evaluated in terms of density, rather than behavior. This concern will be ameliorated with the following two points. First, I use the term competition as it is defined in ecology textbooks, as an interaction among species with overlapping niches. In this case, a competitor is a nest or worker that potentially consumes or inhabits the same resources as *Solenopsis*. To the best of our knowledge, nearly all litter-nesting ants in this site broadly compete with one another among many resource axes. Second, nearly all competitive interactions among tropical litter-nesting ants are interspecific as a consequence of high species richness. The probability that two *Solenopsis* colonies of the same species may interact with one another so low that it may be disregarded.

Referee 1 was concerned that I conducted a logistic regression to evaluate the relationship between colony size and queen number, when correlation would be the appropriate procedure. I changed the methods section to accurately reflect that I conducted both correlation and ordinal logistic regression. Referee 1 recommended that I create two panels in Figure 1; because the relationship was equivalent in both components, I feel would be distracting to the reader to create two equivalent panels and make the figure redundant with the text. She was concerned that I designated a dependent variable to conduct the analysis; the model at hand in this manuscript is to account for factors predictive of queen number. I have avoided the inference of causation where in this case there is only a very weak correlation.

One final remark of Referee 1 was that the litter removal was redundant with the nest destruction treatment, because potential nests would be taken away with the litter removal treatment. This concern may be satisfied by a statement in the methods (which was present in the original submission) that the litter removal was constrained to leaves, and that all colonies and twigs and other coarse debris (which is favored nesting material) were retained in the treatment.

If you have any questions about these revisions, or if further amendments to the manuscript are required, please let me know.

Sincerely,
[submitted electronically]
Terry McGlynn