Dear Dr. Alisha DSouza

EMID: 82c35267b56053f8

JoVE59044 “Evaluating the productivity of social wasp colonies (Vespinae) located, collect and reared following traditional Japanese techniques”

I am returning herewith the above-mentioned manuscript revised according to your e-mail of September 28. I appreciate the editor’s and reviewers' careful and precise comments on my manuscript. I considered that the all the comments/questions were appropriate. I carefully revised protocol section according to the instructions of the editor. Furthermore, following the reviewers’ advices, I believe that the method I proposed has been clarified further by this revision.

The one-by-one replies to the editor’s and reviewer’s comments (BLACK) were replied in “Response to Reviewers” in BLUE letters (also see PDF). The changes made in the original text were indicated in RED.

I would like to thank the editor, the associate editor, and the reviewers for their helpful comments and hope that the revised manuscript is now suitable for publication in Journal of Visualized Experiments.

Yours sincerely,

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**Editorial comments:**  
  
1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

Thank you very much for inviting me to submit a revised draft. I carefully proofread the manuscript.

2. Please revise the title to be clear and concise.

I changed the title ”Evaluating the productivity of social wasp colonies (Vespinae) located, collect and reared following traditional Japanese techniques

” to “A method of evaluating the productivity of social wasp colonies (Vespinae) and an introduction to the traditional *Vespula* wasp hunting” (Line 2-3). I hope that you agree.

3. Line 101: Please update the reference format to a superscripted number.

I updated the reference format to a superscripted number (Line 112).

4. Please revise the protocol text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).

I ensured to avoid using of any personal pronouns in protocol.

5. Please revise the protocol to contain only action items that direct the reader to do something (e.g., “Do this,” “Ensure that,” etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as “could be,” “should be,” and “would be” throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a “Note.” Please include all safety procedures and use of hoods, etc. However, notes should be used sparingly and actions should be described in the imperative tense wherever possible. Please move the discussion about the protocol to the Discussion.

I revised most of the protocol as you indicated (Line 118-259). I have rewritten action items in the imperative tense. I also added the notes in protocol.

6. Please add more details to your protocol steps. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol. Please ensure you answer the “how” question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. See examples below.

I have added some sentences for clarifying the protocol steps, especially first step (Evaluating colony productivity) (Line 118-127).

7. 1.1: It is unclear how to obtain and prepare the cells for counting. Please add such details.

I have added more details of protocol steps in 1.1. (Line 120-121).

8. Lines 115-116: Please describe how to calculate the average area of worker and queen cells. Please add more specific details (e.g. button clicks for software actions, numerical values for settings, etc.)

I added sentences that I calculated the area from the ratio of the actual scale length to the number of pixels (Line 122-127).

9. Line 147: What is used to cut?

I use box cutter to cut a plastic bag for making flags (Line 152-153).

10. Line 148: Please specify the type of meat.

I use chicken heart or cuttlefish as flagged bait meat (Line 154-156).

11. Line 161: How many baits are used for a specific area?

I use 50-100 baits which attract wasps for a specific area (Line 145-146).

12. The Protocol should be made up almost entirely of discrete steps without large paragraphs of text between sections. Please simplify the Protocol so that individual steps contain only 2-3 actions per step and a maximum of 4 sentences per step. Use sub-steps as necessary. Please move the discussion about the protocol to the Discussion.

I have simplified the protocol so that each step contains only 2-3actions (Line 118-259). I moved the explanation for understanding the result to representative results section (Line 296-298). I also moved the discussion about the protocol to Figure legend (L348).

13. Please include single-line spaces between all paragraphs, headings, steps, etc.

I revised the manuscript as include single-line spaces between all paragraphs, headings, steps, etc.

14. After you have made all the recommended changes to your protocol (listed above), please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol.

I highlighted 2.75 pages for identifying the essential steps of the protocol for the video.

15. Please highlight complete sentences (not parts of sentences). Please ensure that the highlighted part of the step includes at least one action that is written in imperative tense.

I ensured that the highlighted part of step includes some actions.

16. Please include all relevant details that are required to perform the step in the highlighting. For example: If step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be highlighted.

I highlighted some steps according to the above instructions.

17. Figure 5: Please change the x-axis label “sample size(N)” to “Sample size (N)”.

I have revised the x-axis label.

18. Please upload each Figure individually to your Editorial Manager account as a .png, .tiff, .pdf, .svg, .eps, .psd, or .ai file.

I uploaded each Figure individually as an EPS file as you indivated.

19. Please upload each Table individually to your Editorial Manager account as an .xls or .xlsx file.

I uploaded Table 1 as an .xlsx file.

20. Please remove the titles and Figure Legends from the uploaded figures. The information provided in the Figure Legends after the Representative Results is sufficient.

Yes. I removed the title and Figure legends from the figures.

21. Discussion: As we are a methods journal, please also discuss critical steps within the protocol, any modifications and troubleshooting of the technique, and any limitations of the technique.

I have added some sentences that showed some limitation of the technique (Line 374-375, 394-397).

Again, thank you for giving me the opportunity to strengthen my manuscript with your valuable comments and queries.

**Reviewers' comments:**  
  
Reviewer #1:  
  
Manuscript Summary:  
In this nice paper, the author in effect describes three useful techniques to improve the study of vespine wasps. The first is the use of meconia counts, rather than cell counts, to estimate colony productivity. While the former has been used before, the latter is much more common, so a paper demonstrating its superiority and effectiveness is welcome. The most valuable contribution I can see is the second method described (the proposed subject of the video): the techniques used to locate wild vespula colonies. I have used similar methods but find this approach attractive, and hope to try it soon. Finding colonies in the wild is perhaps the most limiting step in studying these fascinating but often difficult animals. As these insects cannot be reared artificially, nests must be collected from the wild for study. Thus, this technique addresses an important problem in wasp biology methodology. The fact that it has been developed as the result of a cultural practice in Gifu of collecting these wasps for use by the wider community is interesting as well. Finally, the paper also describes how the authors transplant and keep these wasps in the lab. The details of this will be interesting to anyone who has tried to do this. It is not an easy task, and the methodology presented here is an improvement on my previous attempts. Publications like these are a service to the community, where otherwise such methodological advances are either lost, or spread very slowly and imperfectly via word of mouth.

I appreciate your precise and valuable comments.

Major Concerns:  
I have none.  
  
Minor Concerns:  
I find the paper generally well written and clear. I suggest light copyediting by the journal editors to ensure proper grammar, though mistakes are few. Another minor suggestion would be to group the sections 2.4 (The structure of the carrying box) and 2.5 (excavating the nest) with the third section on rearing Vespula, rather than the end of the second. To me, this would be a more logical arrangement, since these methods pertain directly to transplanting the nests. Some studies will not require transplanting, and instead study colonies where they are found. Thus, I think these fit better with the third section than the second.

I agree with your suggestion that some studies will not require the transplanting (the section 2.4. and 2.5.). Therefore, I moved the section 2.4. and 2.5. from section 2 “Finding *Vespula* nest”. On the other hand, I think that transplanting and rearing a colony are different topics. Therefore, I made new section “Transferring the nest” which included “The structure of the carrying box” and “excavating the nest” as sub-steps. I believe that this revision is more appropriate.

Reviewer #2:  
  
Manuscript Summary:  
General comments:  
The ideas of counting meconia and flag-tracking adults are not noevl to wasp researchers. However, illustrating these methods visually in a well-done videa could be a valuable contribution.

Thank you for giving me the opportunity to strengthen my manuscript with your valuable comments and queries.

Major Concerns:  
None  
  
Minor Concerns:  
Lines 40-46: Careful when talking about fitness. Workers in social insect colonies do not directly count toward fitness- they are more like investment in body growth. Only the gynes (new queens) and males count toward fitness.

I have incorporated this comment in Line 51-57.

Line 57 correct: number of larval cells will could overestimate

I corrected it as you indicated (Line 66).

Line 122: Sufficient by what criterion? Explain…

That criterion is that standard error of the number of meconial per cell is within 0.05. I have added it (Line 145-146).

Line 143: This requires marking the wasps…  
I agree with your comment. I added a sentence about marking wasps to identify individuals before an action required marking the wasps (Line 179-180).

In Figure 5, add a horizontal dashed line to indicate the target SE of 0.05.

I have incorporated this comment and added the horizontal dashed line in Figure 5.

Reviewer #3:  
  
This is an interesting method that could be useful for a number of different wasp species, including many species of Polistes and Vespula. My main concern with the study is how do we know that this method is accurate. The author suggests that is a useful and accurate method, but doesn't provide data to assure us of this statement. I am, however, not sure how you'd test the method. You'd need to estimate the total number of workers produced over the lifespan of a nest and correlate that with the meconia count estimates. However, I don't think that is a problem for the publication of the method, but perhaps this issue should be acknowledged.

I understood the concern about accuracy of estimation the number of adults by counting meconia by reviewer3. Therefore, I have added a sentence to inform readers about it (Line376-377). I think this change now better.

Overall, I think this is an interesting method worthy of publication. The manuscript could use a degree of revision as outlined below. A key point might be to try and generalise the manuscript: at the moment it is focussed on the Japanese Vespula species. However, methods used for this species will not work for all Vespula.

Thank you for your suggestion. I have incorporated your comments in discussion (Line 396-399). I mentioned that this tracking method cannot be used for all vespula.

Key points:  
  
-- On page 4 there is a single square bracket "[" that should be removed. There are also no units on the average wet weight of the Vespula worker. I'm not actually sure why the worker weight is needed?

I removed “[” as you indicated. I described the worker weight to readers to understand the relationship between the weight of an adult wasp and the flag.  
  
-- In section 2.1 on Baiting, I don't think that the exact species of fish are needed here. Japanese dace aren't frequently available in countries like New Zealand. I think just a description of the fish and approximate weight of fish is needed here. The suggested flag size must also be dictated by the size of the wasp and what it is able to carry.

I cancelled to describe the exact species of fish. Approximate weight of the pieces of fish is about 10mg.

-- On lines 195- 197 the author suggests stamping on the ground to have workers stay in the nest. For many species including those that I work with, stamping your foot on the ground would result in a flood of wasps coming out of the nest to attack.

Although the stamping on the ground is useful for collecting *V. shidai*, *V. flaviceps* and *V. vulgaris* nests in Japan. I understood that the stamping on the ground makes it hard to get nests in other species. Therefore, I have added a note that the stamping method may be not suitable for collecting nests in other species (Line 236-238).  
  
-- Section 3 talks about "Rearing Vespula". The methods suggested are brief and may need more details. I'd also note that the nest box size and dimensions are very small for many Vespula. Too small for species like V. germanica or V. vulgaris.

I added a step (Line 275). I also added a note that the size of the nest box should be made according to each species (Line 264-265).

-- Lines 257-258. Why not look at the coefficient of variation here rather than the SE?

The number of individuals produced is calculated by multiplying the sample mean by total number of cells. Therefore, I believe that the standard error which represents the magnitude of error of the sample mean would be more appropriate than the coefficient of variation because easy to know the error of the estimation.

-- On lines 307- 309 you state that "our study shows that the estimate of the number of meconial provides a better estimate of the overall number of individuals produced and of the number of queen wasps produced (i.e., a better indicator of colony fitness". As noted above, how do you know that? What data do you have to make that comparative statement? The number of queens produced is really the key estimator of fitness, but there should only ever be one cohort of queens, so that there should only ever be one meconial deposit left in each queen cell. Also, the author switches between "I" and "we" and "our" in the paper. I suggest standardizing.

I have reflected this comment to the discussion (Line 369-371). I understood that I can not discuss about estimation of fitness from my data by your indication. I canceled to claim that the number of new queens produced (a part of the fitness) can be known by the number of meconia in queen combs as you indicated. I hope that you agree.

Sincerely,

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