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Operationalizing transitions in individuality

Yohay Carmel and Ayelet Shavit

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Review timeline

Original submission:	9 March 2019
1st revised submission:	2 December 2019
2nd revised submission:	15 January 2020
Final acceptance:	15 January 2020

Note: Reports are unedited and appear as submitted by the referee. The review history appears in chronological order.

Review History

RSPB-2019-0576.R0 (Original submission)

Review form: Reviewer 1

Recommendation
Major revision is needed (please make suggestions in comments)

Comments to the Author
This is a very worthwhile paper on the topic, not the least because it understands what was written previously (not so common....). The four criteria in Table 1 and their use are the core of the paper.

Some suggested changes, though:
1. Include the term "fraternal" in the title. The paper is good enough without overstating its case.
2. I consider a statement on p. 10 somewhat inaccurate:
"inseparability plays an important causal role in driving a METI from a collection of individuals into a new collective level of individuality, and that the importance of inseparability has been largely overlooked by most major evolutionary transition models."
Well, in the 1995 book and the accompanying review article in nature JMS and Szathmary DID highlight this aspect. In fact, in the review aricle one reads:
"There are common features that recur in many of the transitions: (1) Entities that were capable of independent replication before the transition can only replicate as parts of a larger unit after it" -- so this feature could not possibly have a more prominent role than listed as item 1.

3. The paper is nice, but largely phenomenological. Mention that no causal analysis is given about how and why the different features interlink.
4. It is not only cheating that is a problem, more generally it is the lack of replicative synchrony. Think of tranposons, meiotic drivers, parthenogenetic variants, cancer, egg-laying worker bees, etc. The fact that cancer cells ususally (with the exception of a few transmissive cancers) cannot survive on their own does not mean that they do not, as a result of a peculiar microevolutionary process, undermine the integrity of the organism.
5. Analyse how your fatures might be mapped onto the phasis of social group formation, maintenance and transformation as given by Bourke in his book Principles of Social Evolution.
After due revision this paper will be very good.

Incidentally, citation of Ref. 46 is incorrect. It should be:
Maynard Smith J. (1988a). Evolutionary progress and the levels of selection. In: Nitecki M.H. (eds). Evolutionary Progress. Chicago, University of Chicago Press.

Review form: Reviewer 2

Recommendation
Reject – article is scientifically unsound

Scientific importance: Is the manuscript an original and important contribution to its field?
Poor

General interest: Is the paper of sufficient general interest?
Good

Quality of the paper: Is the overall quality of the paper suitable?
Poor

Is the length of the paper justified?
No

Should the paper be seen by a specialist statistical reviewer?
No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
No

It is a condition of publication that authors make their supporting data, code and materials available - either as supplementary material or hosted in an external repository. Please rate, if applicable, the supporting data on the following criteria.

> **Is it accessible?**
> N/A
>
> **Is it clear?**
> N/A
>
> **Is it adequate?**
> N/A

Do you have any ethical concerns with this paper?
No

Comments to the Author
This paper is an attempt to 'operationalize' the conception of individuality as it transitions between hierarchical levels of the Tree of Life, eg. cells, multicellular organisms, eusocial societies. The general approach is a comparison between multicellular systems, focusing on the volvocine green algae and select metazoan lineages, and eusocial societies. I think the approach, explicitly linking the origin of multicellularity and the origin of eusociality, is highly valuable and desperately needed. I commend the authors for their attempt at this difficult problem. Unfortunately, while careful comparison of these transitions could be highly valuable and informative, this paper falls too short in its attempt.

I think the paper suffers a few major weaknesses:
1. There is a general lack of thoroughness in regards to the literature search. For example- the authors say this is the first quantitative comparison (abstract) but don't appropriate credit Queller & Strassman 2009 (PTRSB) for their quantitative comparison of individuality. Indeed, the 2009 paper is more quantitative than the 4 categorical variables explored here. Additionally, the authors lament the paucity of comparisons between multicellularity and eusociality (first paragraph, page 3). While I agree, the authors haven't discussed the seminal Santelices 1999 (TREE) or Folse & Roughgarden 2010 (Quarterly Review of Biology) papers. On page 4, many papers are highly relevant but not discussed- Bonner 2004 (Evolution), Bell & Mooers 1997 (Bio J of Linnean Society). Solari et al. 2006 (AmNat) is also highly relevant to the order of reproductive/non-reproductive differentiation.
2. Hanschen et al 2017 (Philosophy Theory Practice in Biology) builds a similar table to table 1, exploring different volvocine algae and definitions of individuality in depth. That previous work includes far more detail and accuracy. It is hard to justify publication of this paper given that it doesn't build upon the work of that 2017 paper, rather has several inaccuracies (Gonium does disassociate, Gonium could be argued to have nonreproductive specialization based on center/periphery gradients, Volvox has nonreproductive specialization; see Kianianmomeni 2014 Current Genetics). While the current attempt to extend the work of Hanschen et al 2017 to apply to eusocial societies is necessary, such an attempt must improve upon existing frameworks in terms of detail and accuracy.
3. Much of the paper is based on 3 criteria (number of units, connectivity, number of different types of units). However, these measures have relevant to the field long before the cited McShea & Brandon and Herrara-Paz. A thorough review of these concepts would be valuable. This is especially true if they are choosing to make non-canonical decisions regarding the definition of individuality. For example, requiring non-reproductive differentiation is both unusual and not sufficiently explained or justified. Why is this trait important from a first principles argument? Given most of the field of the evolution of individuality emphasizes reproductive specialization, why is non-reproductive specialization the hallmark of individuality?
4. General lessons of order of traits arising along METIs, the required cell numbers, etc, arises from only 16 comparisons, which are simplified from some thousands of species. The selection of these 16 taxa is critical but unjustified. The reader is not convinced that these 16 is appropriate (eg, is there no need to discuss Pandorina or Eudorina in the volvocine section?) or meaningfully representative of the diversity present in these clades. Similarly, is it meaningful to compare slime molds to mammals given a 1.4 billion year divergence? Shouldn't comparisons be between as-similar-as-possible-ecological-niches? This approach applies well to the volvocine algae, but comparing sponges to Dictyostelium without justification is spurious. Similarly, termites and ants represent thousands of species, the diversity of which is completely lacking in this paper. Given the diversity of ecological niches and social organization across these insect clades, appropriately exploring this diversity is critical to the conclusions of such a study. Similarly, social spiders would be very informative to this avenue. Additionally, as above, some of the values in table 1 are controversial- slime molds can't reproduce with the multicellular body, suggesting inseparability. Slime molds are de facto reproductive specialization, in the sense that some cells reproduce and others don't. What else is required for reproductive specialization? The authors

suggest because all Dictyo cells are totipotent, there isn't reproductive specialization, but programmatic differentiation in humans occurs much the same way.
5. There seems to be little justification or conclusive value to the paper. While the concept superficially is obviously valuable, and I think it ultimately is, the paper's framework provides no lessons learned or novel insights this framework allows. What is this conceptual framework used for? What have we learned?

Minor comments:
1. The connection of METIs and complexity, while briefly touched upon is vague and confusing.
2. The authors suggest a lack of vocabulary prevents progress (page 3), but this is stated without examples or sufficiently making a case. Worse still, the authors then use "inseparability" to replace the common term "indivisibility".
3. The authors assume that "intermediates" exist as a meaningful concept (page 5, first paragraph of "Application of these scheme to two types of METI). Herron et al 2009 PNAS argued against the concept of intermediates based on time calibrated phylogenetics. Volvocine lineages have existed in (statistically predicted) similar form for hundreds of millions of years. These are stable evolutionary strategies in and of themselves, not intermediates to Volvox.
4. There is some confusing logic regarding inseparability (page 9)- how does inseparability play a causal role in collective levels of individuality? Once inseparability is achieved, isn't heritability and variation automatically transferred to the higher level and selection at the lower level disappears? Wouldn't that mean individuality is at the higher level then? In the same paragraph, what is "partial inseparability"? At the end of this paragraph, the inseparability is suggested to be a causal facilitator of reproductive specialization, but given the lack of explanation or discussion, this appears to be a post hoc ergo propter hoc fallacy- just because inseparability comes first, doesn't mean it causes the later reproductive specialization.
5. Page 10- reproductive specialization occurs before non-reproductive specialization simply because if any 'non-reproductive' specialization occurred first, it would inevitably affect the cell growth and division rates, immediately converting it to reproductive specialization (even if not total reproductive specialization).
6. Page 10- spelling: "posses"
7. Page 10- in the conclusion, wouldn't inseparability be irreversible by definition?

Review form: Reviewer 3

Recommendation
Accept with minor revision (please list in comments)

Scientific importance: Is the manuscript an original and important contribution to its field?
Excellent

General interest: Is the paper of sufficient general interest?
Excellent

Quality of the paper: Is the overall quality of the paper suitable?
Good

Is the length of the paper justified?
Yes

Should the paper be seen by a specialist statistical reviewer?
No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
No

It is a condition of publication that authors make their supporting data, code and materials available - either as supplementary material or hosted in an external repository. Please rate, if applicable, the supporting data on the following criteria.

Is it accessible?
Yes

Is it clear?
Yes

Is it adequate?
Yes

Do you have any ethical concerns with this paper?
No

Comments to the Author
This is an excellent paper, eminently worthy of publication. The framework for understanding individuation is new, it is operational, and it is applicable to a wide range of organisms, as the authors demonstrate by applying it to everything from colonial algae to social insects. Best of all, they use the framework to make what appear to be some new discoveries about transitions in individuality. (At least, new to me.) Most interesting is the discovery that mutual dependence among lower-level units precedes division of reproductive labor.

As you will see in my marginal notes on the manuscript, I have some complaints, mostly about the absence of certain conceptual connections and citations. These include connections to my papers, and while it makes me uncomfortable to promote my own work in context of the review process, I really think the authors need to at least see and consider that work. We are clearly up to the same thing: operationalizing individuality. And while there is no reason for them to adopt my approach – I'm a fan of multiple approaches in this area – they really ought to be aware of it. Also, they should be aware of and certainly cite some of the considerable work on individuality in the literature on colonial marine invertebrates from the 1970s and 1980s, notable the work Boardman, Cheetham, McKinney, Mackie, and others. (See marginal remarks in the manuscript for some specific citations.)

Decision letter (RSPB-2019-0576.R0)

28-May-2019

Dear Dr Carmel:

I am writing to inform you that your manuscript RSPB-2019-0576 entitled "OPERATIONALIZING MAJOR EVOLUTIONARY TRANSITIONS" has, in its current form, been rejected for publication in Proceedings B.

This action has been taken on the advice of referees, who have recommended that substantial revisions are necessary. With this in mind we would be happy to consider a resubmission, provided the comments of the referees are fully addressed. However please note that this is not a provisional acceptance.

The resubmission will be treated as a new manuscript. However, we will approach the same reviewers if they are available and it is deemed appropriate to do so by the Editor. Please note that resubmissions must be submitted within six months of the date of this email. In exceptional circumstances, extensions may be possible if agreed with the Editorial Office. Manuscripts submitted after this date will be automatically rejected.

Please find below the comments made by the referees, not including confidential reports to the Editor, which I hope you will find useful. If you do choose to resubmit your manuscript, please upload the following:

1) A ‘response to referees’ document including details of how you have responded to the comments, and the adjustments you have made.
2) A clean copy of the manuscript and one with 'tracked changes' indicating your 'response to referees' comments document.
3) Line numbers in your main document.

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Sincerely,
Proceedings B
mailto: proceedingsb@royalsociety.org

Associate Editor
Board Member: 1
Comments to Author:
This paper is submitted as 'research' but possibly it is better classified as 'review', and I think that the author should consider this in developing a revised version. The paper seeks to clarify and operationalise the concept of a major evolutionary transition, an important topic in evolutionary biology. The analysis highlights the importance of 'inseparability' as a threshold, finds that system size is strongly related to complexity, and examines the order of appearance of complexity indicators in different evolutionary transitions (finding some similarities and also some differences across groups). The reviewers came to quite divergent conclusions, although all see a high value in the attempted synthesis - Even reviewer 2, who is the most critical, says the approach “is highly valuable and desperately needed”. The issues do seem to be resolvable overall, but are serious and will require some serious consideration and rewriting. First, the literature review needs to be more thorough and criteria for Major Transitions justified, as detailed by Reviewer 2 (points 1 - 3) and Reviewer 1 (point 2), and Reviewer 3 (comments on the PDF, e.g. see see: McShea, D.W. 2015 Evolutionary Biology 43:531–542.), whose recommendations should be followed. Second, the selection of the 16 taxa and the broad comparisons made need to be more throroughy justified (Reviewer 2, point 4). Third, the conclusions need to be more strongly developed (Reviewer 2, point 5) in general and in specific relation to how the different features interlink (Reviewer 1, point 3)

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s)
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Some suggested changes, though:
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Referee: 2

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Referee: 3

Comments to the Author(s)
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Author's Response to Decision Letter for (RSPB-2019-0576.R0)

See Appendix A.

RSPB-2019-2805.R0

Review form: Reviewer 1

Recommendation
Accept as is

Comments to the Author
Lovely paper, duly revsied.

Review form: Reviewer 2

Recommendation
Accept with minor revision (please list in comments)

Scientific importance: Is the manuscript an original and important contribution to its field?
Acceptable

General interest: Is the paper of sufficient general interest?
Acceptable

Quality of the paper: Is the overall quality of the paper suitable?
Acceptable

Is the length of the paper justified?
Yes

Should the paper be seen by a specialist statistical reviewer?
No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
No

It is a condition of publication that authors make their supporting data, code and materials available - either as supplementary material or hosted in an external repository. Please rate, if applicable, the supporting data on the following criteria.

Is it accessible?
N/A

Is it clear?
N/A

Is it adequate?
N/A

Do you have any ethical concerns with this paper?
No

Comments to the Author
The authors have done an excellent job incorporating my previous suggestions and I commend them for the willingness to include such revisions. I think the paper is much improved and deserves to be published. I include two minor comments:

1. On page 13 third paragraph, they mention Gonium has inseparability but Table 1a has been updated based on my previous comments that Gonium does not have inseparability.
2. The material in the appendices is interesting and provides valuable context. The authors should consider including this material in the main text of the manuscript.

Decision letter (RSPB-2019-2805.R0)

08-Jan-2020

Dear Dr Carmel

I am pleased to inform you that your manuscript RSPB-2019-2805 entitled "OPERATIONALIZING FRATERNAL TRANSITIONS IN INDIVIDUALITY" has been accepted for publication in Proceedings B.

The referee(s) have recommended publication, but also suggest some minor revisions to your manuscript. Therefore, I invite you to respond to the referee(s)' comments and revise your manuscript. Because the schedule for publication is very tight, it is a condition of publication that you submit the revised version of your manuscript within 7 days. If you do not think you will be able to meet this date please let us know.

To revise your manuscript, log into https://mc.manuscriptcentral.com/prsb and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions," click on "Create a Revision." Your manuscript number has been appended to denote a revision. You will be unable to make your revisions on the originally submitted version of the manuscript. Instead, revise your manuscript and upload a new version through your Author Centre.

When submitting your revised manuscript, you will be able to respond to the comments made by the referee(s) and upload a file "Response to Referees". You can use this to document any changes you make to the original manuscript. We require a copy of the manuscript with revisions made since the previous version marked as ‘tracked changes’ to be included in the ‘response to referees’ document.

Before uploading your revised files please make sure that you have:

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2) A separate electronic file of each figure (tiff, EPS or print-quality PDF preferred). The format should be produced directly from original creation package, or original software format. PowerPoint files are not accepted.

3) Electronic supplementary material: this should be contained in a separate file and where possible, all ESM should be combined into a single file. All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI.

Online supplementary material will also carry the title and description provided during submission, so please ensure these are accurate and informative. Note that the Royal Society will not edit or typeset supplementary material and it will be hosted as provided. Please ensure that the supplementary material includes the paper details (authors, title, journal name, article DOI). Your article DOI will be 10.1098/rspb.[paper ID in form xxxx.xxxx e.g. 10.1098/rspb.2016.0049].

4) A media summary: a short non-technical summary (up to 100 words) of the key findings/importance of your manuscript.

5) Data accessibility section and data citation
It is a condition of publication that data supporting your paper are made available either in the electronic supplementary material or through an appropriate repository.

In order to ensure effective and robust dissemination and appropriate credit to authors the dataset(s) used should be fully cited. To ensure archived data are available to readers, authors should include a 'data accessibility' section immediately after the acknowledgements section. This should list the database and accession number for all data from the article that has been made publicly available, for instance:
• DNA sequences: Genbank accessions F234391-F234402
• Phylogenetic data: TreeBASE accession number S9123
• Final DNA sequence assembly uploaded as online supplemental material
• Climate data and MaxEnt input files: Dryad doi:10.5521/dryad.12311
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If you wish to submit your data to Dryad (http://datadryad.org/) and have not already done so you can submit your data via this link http://datadryad.org/submit?journalID=RSPB&manu=(Document not available) which will take you to your unique entry in the Dryad repository. If you have already submitted your data to dryad you can make any necessary revisions to your dataset by following the above link. Please see https://royalsociety.org/journals/ethics-policies/data-sharing-mining/ for more details.

6) For more information on our Licence to Publish, Open Access, Cover images and Media summaries, please visit https://royalsociety.org/journals/authors/author-guidelines/.

Once again, thank you for submitting your manuscript to Proceedings B and I look forward to receiving your revision. If you have any questions at all, please do not hesitate to get in touch.

Sincerely,
Professor Gary Carvalho
mailto: proceedingsb@royalsociety.org

Associate Editor
Comments to Author:
The authors have carefully and thoroughly revised their paper in line with the recommendations of the reviewers, and the reviewers are happy with the outcome - only two minor changes are required by Reviewer 2. They have also considered my query over whether it should be more appropriately categorised as review rather than research, and I am happy with their reasoning for keeping it as research.

Reviewer(s)' Comments to Author:
Referee: 1
Comments to the Author(s).
Lovely paper, duly revsied.

Referee: 2
Comments to the Author(s).
The authors have done an excellent job incorporating my previous suggestions and I commend them for the willingness to include such revisions. I think the paper is much improved and deserves to be published. I include two minor comments:

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Author's Response to Decision Letter for (RSPB-2019-2805.R0)

See Appendix B.

Decision letter (RSPB-2019-2805.R1)

15-Jan-2020

Dear Dr Carmel

I am pleased to inform you that your manuscript entitled "OPERATIONALIZING TRANSITIONS IN INDIVIDUALITY" has been accepted for publication in Proceedings B.

You can expect to receive a proof of your article from our Production office in due course, please check your spam filter if you do not receive it. PLEASE NOTE: you will be given the exact page length of your paper which may be different from the estimation from Editorial and you may be asked to reduce your paper if it goes over the 10 page limit.

If you are likely to be away from e-mail contact please let us know. Due to rapid publication and an extremely tight schedule, if comments are not received, we may publish the paper as it stands.

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Thank you for your fine contribution. On behalf of the Editors of the Proceedings B, we look forward to your continued contributions to the Journal.

Sincerely,
Editor, Proceedings B
mailto: proceedingsb@royalsociety.org

Appendix A

28-May-2019

Dear Dr Carmel:
I am writing to inform you that your manuscript RSPB-2019-0576 entitled "OPERATIONALIZING MAJOR EVOLUTIONARY TRANSITIONS" has, in its current form, been rejected for publication in Proceedings B.
This action has been taken on the advice of referees, who have recommended that substantial revisions are necessary. With this in mind we would be happy to consider a resubmission, provided the comments of the referees are fully addressed. However please note that this is not a provisional acceptance.
The resubmission will be treated as a new manuscript. However, we will approach the same reviewers if they are available and it is deemed appropriate to do so by the Editor. Please note that resubmissions must be submitted within six months of the date of this email. In exceptional circumstances, extensions may be possible if agreed with the Editorial Office. Manuscripts submitted after this date will be automatically rejected.
Please find below the comments made by the referees, not including confidential reports to the Editor, which I hope you will find useful. If you do choose to resubmit your manuscript, please upload the following:
1) A ‘response to referees’ document including details of how you have responded to the comments, and the adjustments you have made.
2) A clean copy of the manuscript and one with ‘tracked changes‘ indicating your ‘response to referees‘ comments document.
3) Line numbers in your main document.

Sincerely,
Proceedings B

Dear Editor,
We are very grateful for the many constructive comments made by the Board Member and three referees. All three referees are doubtless experts in this field, and their bibliographic recommendations were very helpful. We spent much time reading the many books and articles they suggested, hence our late response. We thus added many relevant references to the manuscript. In order to get additional feedback on our analysis, particularly regarding Table 1, we have then sent the revised manuscript to Prof. Tamar Keasar, a zoologist specializing in invertebrates and in particular in bees. She pointed out few important omissions from our table, notably nematodes and tardigrades. We revised table 1 accordingly. Considering the special properties of these organisms lead us to reconsider the section 'ETI progression scheme as a predictive model', and we decided to delete it, together with Table 2. We believe that our current manuscript is much better rooted in the wider context of previous research.

The line numbers below refer to the 'track changes' version. Changes in Table 1, are not marked (for clarity), but are documented below.

Board Member: 1
Comments to Author:

This paper is submitted as 'research' but possibly it is better classified as 'review', and I think that the author should consider this in developing a revised version.
We understand why the paper may be perceived as a review, and therefore we clarified its research aspects in the text. In a nutshell, a review is a summary of existing literature, while a research paper concerns hypothesis testing. Here, our suggested operationalization scheme leads to a clear hypothesis that we test (**lines 109-111**), using data analysis (**Table 1**), and therefore we conceive this manuscript a research paper rather than a review.

The paper seeks to clarify and operationalize the concept of a major evolutionary transition, an important topic in evolutionary biology. The analysis highlights the importance of 'inseparability' as a threshold, finds that system size is strongly related to complexity, and examines the order of appearance of complexity indicators in different evolutionary transitions (finding some similarities and also some differences across groups). The reviewers came to quite divergent conclusions, although all see a high value in the attempted synthesis - Even reviewer 2, who is the most critical, says the approach “is highly valuable and desperately needed”. The issues do seem to be resolvable overall, but are serious and will require some serious consideration and rewriting. First, the literature review needs to be more thorough and criteria for Major Transitions justified, as detailed by Reviewer 2 (points 1 - 3) and Reviewer 1 (point 2), and Reviewer 3 (comments on the PDF, e.g. see: McShea, D.W. 2015 Evolutionary Biology 43:531–542.), whose recommendations should be followed. Second, the selection of the 16 taxa and the broad comparisons made need to be more throroughy justified (Reviewer 2, point 4). Third, the conclusions need to be more strongly developed (Reviewer 2, point 5) in general and in specific relation to how the different features interlink (Reviewer 1, point 3)
We thank the associate editor, as well as the three reviewers for their specific comments. Our reviewers have no doubt dedicated much time and effort to their task, and their comprehensive and clear critical comments were helpful, and substantially changed and improved the paper.
We worked hard to address all these issues in the revised version, as detailed below. In the rare cases that we disagreed with a reviewer, we offered an explicit justification. Below we explain and document the changes we made in the text; the line numbers refer to the 'track changes' version.

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s)
This is a very worthwhile paper on the topic, not the least because it understands what was written previously (not so common....). The four criteria in Table 1 and their use are the core of the paper. Thank you.

Some suggested changes, though:
1. Include the term "fraternal" in the title. The paper is good enough without overstating its case.

Corrected accordingly. We agree with the reviewer, that this manuscript studies specifically fraternal transitions. We have revised the title accordingly, and we now mention it also in the first paragraph of the introduction (**lines 39-41**) and in the conclusion (**line 321**). We also decided to omit the word *'Major'* from the term *'Major Evolutionary Transitions in Individuality'*, and we now use the shorter term *Evolutionary Transitions in Individuality* (hereafter, ETI).

2. I consider a statement on p. 10 somewhat inaccurate:
"Inseparability plays an important causal role in driving a METI from a collection of individuals into a new collective level of individuality, and that the importance of inseparability has been largely overlooked by most major evolutionary transition models."
Well, in the 1995 book and the accompanying review article in nature JMS and Szathmary DID highlight this aspect. In fact, in the review aricle one reads:
"There are common features that recur in many of the transitions: (1) Entities that were capable of independent replication before the transition can only replicate as parts of a larger unit after it" -- so this feature could not possibly have a more prominent role than listed as item 1.
We agree with the reviewer and this omission was corrected accordingly. Indeed, JMS and Szathmary have identified inseparability as the common feature of all major transitions, and we acknowledge it in the **Discussion, lines 275-277**.

3. The paper is nice, but largely phenomenological. Mention that no causal analysis is given about how and why the different features interlink.
We agree with the reviewer and corrected accordingly. This paper is indeed largely phenomenological (although we provide some inferences based on our preliminary study, see for example the **discussion, lines 285-290**). We now mention explicitly that no actual causal mechanism is provided (**introduction, 3rd paragraph, lines 62-64**). Also, in view of this comment, we now briefly cover some of the literature on alternative possible mechanisms (**introduction, starting in 54, and starting in 85**) with references to prominent literature on these issues.

4. It is not only cheating that is a problem, more generally it is the lack of replicative synchrony. Think of tranposons, meiotic drivers, parthenogenetic variants, cancer, egg-laying worker bees, etc. The fact that cancer cells usually (with the exception of a few transmissive cancers) cannot survive on their own does not mean that they do not, as a result of a peculiar microevolutionary process, undermine the integrity of the organism.
We fully agree and corrected accordingly. We added this notion to the respective paragraph (**discussion, lines 293-298**). Thank you.

5. Analyse how your fatures might be mapped onto the phasis of social group formation, maintenance and transformation as given by Bourke in his book Principles of Social Evolution.
We thank the reviewer for this advice which we have applied. Some ideas and much of the specific examples on the transition to eusociality are taken from Bourke's book. His

contribution is referred to in nine separate places in our article, including the last paragraph of methods section, appendices, Table 1, etc.

After due revision this paper will be very good.
Thanks. We are glad to hear this and we hope the revision matches this expectation.

Incidentally, citation of Ref. 46 is incorrect. It should be:
Maynard Smith J. (1988a). Evolutionary progress and the levels of selection. In: Nitecki M.H. (eds). Evolutionary Progress. Chicago, University of Chicago Press.
Corrected accordingly. Thank you.

Referee: 2
Comments to the Author(s)

This paper is an attempt to 'operationalize' the conception of individuality as it transitions between hierarchical levels of the Tree of Life, eg. cells, multicellular organisms, eusocial societies. The general approach is a comparison between multicellular systems, focusing on the volvocine green algae and select metazoan lineages, and eusocial societies. I think the approach, explicitly linking the origin of multicellularity and the origin of eusociality, is highly valuable and desperately needed. I commend the authors for their attempt at this difficult problem. Unfortunately, while careful comparison of these transitions could be highly valuable and informative, this paper falls too short in its attempt.
We thank the reviewer for noticing the importance of attempting such a highly generalized operationalization. We are aware that it may suffer some simplicity, which may be inevitable given our attempt to construct a very general scheme (see Richard Levins' 1966 famous paper on the necessary tradeoff between generalization, precision and accuracy in model building).

I think the paper suffers a few major weaknesses:
1. There is a general lack of thoroughness in regards to the literature search. For example- the authors say this is the first quantitative comparison (abstract) but don't appropriate credit Queller & Strassman 2009 (PTRSB) for their quantitative comparison of individuality. Indeed, the 2009 paper is more quantitative than the 4 categorical variables explored here. Additionally, the authors lament the paucity of comparisons between multicellularity and eusociality (first paragraph, page 3). While I agree, the authors haven't discussed the seminal Santelices 1999 (TREE) or Folse & Roughgarden 2010 (Quarterly Review of Biology) papers. On page 4, many papers are highly relevant but not discussed- Bonner 2004 (Evolution), Bell & Mooers 1997 (Bio J of Linnean Society). Solari et al. 2006 (AmNat) is also highly relevant to the order of reproductive/non-reproductive differentiation.
We thank the reviewer for listing these important publications. The current version is corrected accordingly. We revised the relevant text, accounting for all the papers listed above. We added a new section titled 'operationalization schemes for transitions in individuality', with a detailed account of those schemes (**introduction, lines 85-115**). This

section describes the quantitative approach of Queller and Strassman (2009), as well as two other approaches (Mcshea, Hanschen et al). In addition, Queller and Strassman's general approach [2009] is now described in **appendix 1**. The contributions of Santelices, and Folse and Roughgarden, are added to **Appendix 1**; The studies of Bonner, Bell & Moorse, and Solari et al, are now described in the **1st paragraph of the 'Results and Discussion' section**.

2. Hanschen et al 2017 (Philosophy Theory Practice in Biology) builds a similar table to table 1, exploring different volvocine algae and definitions of individuality in depth. That previous work includes far more detail and accuracy. It is hard to justify publication of this paper given that it doesn't build upon the work of that 2017 paper, rather has several inaccuracies (Gonium does disassociate, Gonium could be argued to have nonreproductive specialization based on center/periphery gradients, Volvox has nonreproductive specialization; see Kianianmomeni 2014 Current Genetics). While the current attempt to extend the work of Hanschen et al 2017 to apply to eusocial societies is necessary, such an attempt must improve upon existing frameworks in terms of detail and accuracy.

We thank the reviewer for this important input; we corrected accordingly. Indeed, Hanschen et al (2017) presents a complete and valuable approach to operationalizing the transition to multicellularity, and it is one of the first attempts to systematically operationalize a major transition. We now describe this paper, and compare between their scheme and ours (**lines 93-104**).

We also complied with the reviewer's proposition to enhance Table 1. We modified Table 1 accordingly (Gonium does dissociate, we thank the reviewer for this important correction). The reviewer also suggests that Volvox and possibly Gonium have nonreproductive specializations. In our manuscript, nonreproductive specializations is inferred if cells specialize exclusively and irreversibly in different nonreproductive tasks. According to Kianianmomeni (2014), the article mentioned by the reviewer, the only differentiation in Volvox and Gonium is between somatic and reproductive cell types.

The reviewer also asks that we enhance the list of specific criteria within the operationalization scheme, following Hanschen et al 2017. There are six such criteria that change during ETIs, of which two criteria appear in our scheme as well (we now acknowledge this in the **Introduction, lines 85-104**). Two additional criteria suggested by Hanschen et al (2017) (physiological unity, and spatial / temporal boundaries) may not be applicable to transitions where lower-level units are motile (such as ants). The two other criteria (group-level adaptation and multi-level selection) are indeed very important and general criteria that could be valuably applied in comparison between ETIs. However the concepts of 'group-level adaptation'and 'multi-level selection' are also highly theoretical and abstract (see the different meanings of these terms in Lloyd, 2017 and their different general schemas by Okasha 2007 and Birch 2017). Hot debates over group adaptation and multi-level section also have a long history (e.g. Williams (1966) and Maynard Smith (1987) versus D.S. Willson (1975) and Sober (1987). These theoretical criteria of group-level adaptation and multi-level selection are relevant for better understanding ETIs, but because we aim for an agreed general set of directly measurable parameters, we chose not to include them in our scheme. All these considerations now appear in the **Introduction** (lines 85-104).

3. Much of the paper is based on 3 criteria (number of units, connectivity, number of different types of units). However, these measures have relevant to the field long before the cited McShea & Brandon and Herrara-Paz. A thorough review of these concepts would be valuable. This is especially true if they are choosing to make non-canonical decisions regarding the definition of individuality. For example, requiring non-reproductive differentiation is both unusual and not sufficiently explained or justified. Why is this trait important from a first principles argument? Given most of the field of the evolution of individuality emphasizes reproductive specialization, why is non-reproductive specialization the hallmark of individuality?

We agree that an expanded justification of the set of complexity-based parameters to evaluate ETIs is in place. This point was corrected accordingly. We now elaborate on this issue in the **introduction (lines 105-115), and in the Methods (117-144)**. We included a justification for the principles and rationale of our approach, as well as the criteria for selecting operationalization parameters.

The reviewer refers to our choice of criteria as follows: "…*they are choosing to make non-canonical decisions regarding the definition of individuality*." We regrettably disagree on this point, since even after reading the additional literature suggested by the reviewer we are not aware of any clear canonical decision regarding the definition of individuality. More importantly, we do not seek to quantify the process of individuation, nor the exact point in the transition where individuality switches from lower level to higher level. Our aim here is to quantify the multi-facet concept of complexity (rather than individuality) during these transitions (see 1st paragraph of the **Discussion** section). Having said that, we follow Michod (2007), in suggesting that inseparability marks the switch to individuality (this point appeared already in the original version, see the **Discussion, lines 268-270**).

The reviewer is concerned with the inclusion of nonreproductive specialization in our scheme. Nonreproductive specialization (also termed 'the variation between lower-level units', and often quantified as the number of cell types) was used as an important criterion in this context in seminal studies which we humbly follow, including Bonner's '*The evolution of complexity by means of natural selection*', Bourke's '*Principles of Social Evolution*', Mcshea's '*Three trends in the history of life*', and others. Nonreproductive specialization allows us to distinguish between initial- and advanced phases of a transition.

4. General lessons of order of traits arising along METIs, the required cell numbers, etc, arises from only 16 comparisons, which are simplified from some thousands of species. The selection of these 16 taxa is critical but unjustified. The reader is not convinced that these 16 is appropriate (eg, is there no need to discuss Pandorina or Eudorina in the volvocine section?) or meaningfully representative of the diversity present in these clades.
---Corrected accordingly. Thanks for noting this omission of Pandorina and Eudorina, both were added to **Table 1**. We agree that 16 comparisons may be insuffient for a comprehensive understanding of ETIs. However, we believe it is sufficient for our modest goal, which is to develop an operationalization scheme and conduct a preliminary evaluation. Third, we agree that a justification for selecting these certain taxa rather than others was missing in the previous version. The species choices for Table 1 are now explicitly

and generally explained in the methods (**178-181**): "*A taxon was added to the table if it satisfied two conditions: (1) it differs in at least one feature from all other records in its group already existing in the table. The rationale for this condition is to avoid inflating the table with records that are identical for all columns except the taxon name. (2) Enough data exists to reliably classify that taxon in all criteria.*"

Similarly, is it meaningful to compare slime molds to mammals given a 1.4 billion year divergence? Shouldn't comparisons be between as-similar-as-possible-ecological-niches? This approach applies well to the volvocine algae, but comparing sponges to Dictyostelium without justification is spurious.
Corrected accordingly. We agree that slime molds are different from all other organisms in that general group, since (presumably) they have not originated from the same transition to multicellularity (although see Dickinson 2012 [1]). We have now removed slime molds from that general group, and renamed the group 'metazoa' (animals). All organisms in this group developed from the same transition to multicellularity. We added an additional organism, Choanoflagellates, and grouped it with slime molds (Table 1b). This new group is called 'partial multi-cellularity'.

Similarly, termites and ants represent thousands of species, the diversity of which is completely lacking in this paper. Given the diversity of ecological niches and social organization across these insect clades, appropriately exploring this diversity is critical to the conclusions of such a study.
Corrected accordingly. Termites and ants are indeed a diverse and variable groups, and we revised Table 1d to briefly account for this diversity as revealed in the context of our scheme. We also added the following text to the discussion (**lines 255-259**): *'Ants and termites are two groups of numerous species, with colony sizes from dozens to millions (termites) or even billions (ants). Corroborating results of this study, strong relations were found between colony size, and inseparability, reproductive specialization, and nonreproductive specialization* [2–4]. *A full account of the variability in these two groups is beyond the scope of this article'.*

Similarly, social spiders would be very informative to this avenue.
We are thankful for this comment. We added social spiders to ***Table 1***d.

Additionally, as above, some of the values in table 1 are controversial- slime molds can't reproduce with the multicellular body, suggesting inseparability. Slime molds are de facto reproductive specialization, in the sense that some cells reproduce and others don't. What else is required for reproductive specialization? The authors suggest because all Dictyo cells are totipotent, there isn't reproductive specialization, but programmatic differentiation in humans occurs much the same way.
Indeed, in Slime Molds some cells reproduce while others do not. However, to the best of our knowledge, each Slime Mold cell could in principle commit to either reproduction or a somatic function, and there is no telling which cell would commit to either function (see for example Rainey 2015 [5]). Our view of reproductive specialization requires, at a minimum, that some cells have higher propensity to reproduce than others. Nonetheless, the definition

of reproductive specialization used here is more stringent, namely that some units are entirely and irreversibly incapable of reproduction (Slime Molds do not satisfy either condition). This latter definition allows one to empirically determine presence/absence of reproductive specialization. We now clarify this in the caption of Table 1b.

5. There seems to be little justification or conclusive value to the paper. While the concept superficially is obviously valuable, and I think it ultimately is, the paper's framework provides no lessons learned or novel insights this framework allows. What is this conceptual framework used for? What have we learned?
Regrettably, we disagree on this point. We believe that there is value in our attempt to operationalize ETIs in the context of complexity, especially given that this matter is the subject of hot scientific debates. Fortunately, the two other reviewers clearly agree with our view. The value of this approach is indicated in the first and last comment of reviewer #1, and the first comment of reviewer #3. Anyhow, in view of this comment, we now explicate the value of operationalizing ETIs in the **Conclusion (lines 320-331).**

Minor comments:

1. The connection of ETIs and complexity, while briefly touched upon is vague and confusing.
---We agree with the reviewer and corrected accordingly. In view of this comment, we now elaborate on the inherent connection between ETIs and complexity, in the **last paragraph of the Introduction** section (**lines 105-115**).

2. The authors suggest a lack of vocabulary prevents progress (page 3), but this is stated without examples or sufficiently making a case. Worse still, the authors then use "inseparability" to replace the common term "indivisibility".
---We agree. We deleted the sentence regarding ''lack of common vocabulary'.

Regarding the terms 'inseparability / indivisibility', we gave much thought to this comment. It is our opinion that the term 'inseparability' better describes the situation in which an individual lower-level unit cannot survive and reproduce on its own. In contrast, the term 'indivisibility' is ambiguous; it refers also to the breakdown of a modular organism into smaller units. For example, some plants may be divisible into multiple clones, but any single lower-level unit is not separable. That said, we agree with the reviewer that the term 'indivisibility' is widely used in the context of ETIs, while 'inseparability' is less commonly used. In this version, we conserved the term 'inseparability'; we are willing to change it to 'indivisibility' upon an editorial advice.

3. The authors assume that "intermediates" exist as a meaningful concept (page 5, first paragraph of "Application of these scheme to two types of METI). Herron et al 2009 PNAS argued against the concept of intermediates based on time calibrated phylogenetics. Volvocine lineages have existed in (statistically predicted) similar form for hundreds of millions of years. These are stable evolutionary strategies in and of themselves, not intermediates to Volvox.

We thank the reviewer and have clarified this point in the text. We view this as a semantic issue. In our view, the concept of *intermediate* does not presume a relatively short time spent by the taxon at a certain evolutionary phase, nor does it presume an inevitable change of a taxon to another phase. For example, an amoeba is an organism that has not gone through the transition to multicellularity, while a dolphin represents organisms that have completed this transition. In between these two extremes, there could conceivably be organisms that have gone through part of the transition but did not complete it. We term such organisms 'intermediates', because they possess some- but not all characters of the transition, but we do not view them as precursors of other organisms. We now clarify this point in **lines 175-177**.

4. There is some confusing logic regarding inseparability (page 9) -- how does inseparability play a causal role in collective levels of individuality? Once inseparability is achieved, isn't heritability and variation automatically transferred to the higher level and selection at the lower level disappears? Wouldn't that mean individuality is at the higher level then? In the same paragraph, what is "partial inseparability"? At the end of this paragraph, the inseparability is suggested to be a causal facilitator of reproductive specialization, but given the lack of explanation or discussion, this appears to be a post hoc ergo propter hoc fallacy- just because inseparability comes first, doesn't mean it causes the later reproductive specialization.

---Corrected accordingly. We changed these sentences in the text in an attempt to clarify our reason. The reviewer provides here a better explanation for the role of inseparability than ours, and we have included the reviewer's notion in the revised version: *"Once inseparability is achieved, … heritability and variation [are] automatically transferred to the higher level, and selection at the lower level disappears*." We adopted this notion (**Lines 270-273**). We are grateful for the reviewer's contribution to making our point clearer.

The reviewer asks *what is 'partial inseparability?'.* We now changed this term to 'incomplete inseparability', and explain in the text that in some cases inseparability is incomplete; in such cases, with a very low probability, a lower level unit may regain its independence (for example, a worker may become a queen).

The reviewer writes "*just because inseparability comes first, doesn't mean it causes the later reproductive specialization*". We agree that temporal order alone does not entail causality. To avoid confusion, in view of this comment we changed the text in this part, but in view of another comment by reviewer #3 on the same item: ''*Interesting. New!!'',* we decided not to omit this point altogether.

5. Page 10- reproductive specialization occurs before non-reproductive specialization simply because if any 'non-reproductive' specialization occurred first, it would inevitably affect the cell growth and division rates, immediately converting it to reproductive specialization (even if not total reproductive specialization).
Corrected. We thank the reviewer for this important note. We adopted this idea and added it into the **discussion (lines 299-302).**

6. Page 10- spelling: "posses"

Corrected.

7. Page 10- in the conclusion, wouldn't inseparability be irreversible by definition?
Thank you. We deleted those words from the text.

Referee: 3

Comments to the Author(s)
This is an excellent paper, eminently worthy of publication. The framework for understanding individuation is new, it is operational, and it is applicable to a wide range of organisms, as the authors demonstrate by applying it to everything from colonial algae to social insects. Best of all, they use the framework to make what appear to be some new discoveries about transitions in individuality. (At least, new to me.) Most interesting is the discovery that mutual dependence among lower-level units precedes division of reproductive labor.
We thank the reviewer for this comment, and for the many helpful comments below.

As you will see in my marginal notes on the manuscript, I have some complaints, mostly about the absence of certain conceptual connections and citations. These include connections to my papers, and while it makes me uncomfortable to promote my own work in context of the review process, I really think the authors need to at least see and consider that work. We are clearly up to the same thing: operationalizing individuality. And while there is no reason for them to adopt my approach – I'm a fan of multiple approaches in this area – they really ought to be aware of it. Also, they should be aware of and certainly cite some of the considerable work on individuality in the literature on colonial marine invertebrates from the 1970s and 1980s, notable the work Boardman, Cheetham, McKinney, Mackie, and others. (See marginal remarks in the manuscript for some specific citations.)
Corrected. We have carefully read these books and chapters, and have related to them in the revised version, as detailed in our responses to the specific remarks made directly on the PDF. Those PDF remarks and our responses appear following the major comments below.

<u>Introduction, 1st paragraph</u>. Should cite *Pettersson, M. 1996. Complexity and evolution. Cambridge University, Press, Cambridge*. Also, I would like to make the authors aware of my own papers documenting the trend. *McShea, D.W. 2001. The hierarchical structure of organisms: a scale and documentation of a trend in the maximum. Paleobiology 27:405-423.* And, *McShea, D.W. and M.A. Changizi. 2003. Three puzzles in hierarchical evolution. Integrative and Comparative Biology 43:74-81.*
Thanks. The text was corrected accordingly. Indeed, we were not aware of these relevant studies and are grateful to the reviewer. All of these studies are now cited in the first three paragraphs of the **Introduction** (**lines 28-65**) and some of them are cited in other places as well. Moreover, we now provide a brief account of existing approaches to describe progress along ETIs (in the new subsection '**Operationalization schemes for transitions in individuality'**). These studies include papers mentioned by the reviewer, as well as studies mentioned by reviewer #2, and others.

Introduction, 1st paragraph. There were transitions to what is effectively the eusocial level (although not called that) among the marine invertebrates, notably the bryozoans, much earlier than in the insects (McShea and Changizi 2003).
Corrected accordingly. Thanks for pointing out this important omission. The case of colonial marine invertebrates is now described in the Introduction (**lines 43-52)**. In particular, bryozoans, corals, syphonophores, and tunicates, include organisms representing different stages according to our scheme; they make excellent candidates for a future examination, as well as for independent tests of our scheme. We considered including these groups within the detailed analysis here (Table 1); we then realized that their extreme diversity in this context would shift our paper from a focus on a conceptual approach to a focus on a zoological survey. This is explained in the **Methods (204-206)**. We cannot fully address this group within the limits of this current paper, thus we plan to apply our operationalization scheme to colonial marine invertebrates in a separate project.

Introduction, 2nd paragraph. Let me strongly encourage the authors to take a look at, and ultimately cite, some of the considerable literature on individuation in colonial marine invertebrates, most of it in the 1970's and 1980's, notably:

Boardman, R. S., A. H. Cheetham, and W. A. Oliver Jr., eds. 1973. Animal colonies: development and function through time. Dowden, Hutchinson, and Ross, Stroudsburg, Penn.

Larwood, G., and B. R. Rosen, eds. 1979. Biology and systematics of colonial organisms. Systematics Association Special Volume 11. Academic Press, London.

McKinney, E K. 1984. Feeding currents of gymnolaemate bryozoans: better organization with higher colonial integration. Bulletin of Marine Sciences 34:315-319.

Mackie, G. 0. 1986. From aggregates to integrates: physiological aspects of modularity in colonial animals. Philosophical Transactions of the Royal Society of London B 313:175-196.

Corrected accordingly. We thank the reviewer for this meticulous bibliographic advice, taking us back to the origin of many current ideas. We have read the literature, in order to write a full paragraph on colonial invertebrates (**Introduction** chapter**, 2nd paragraph**).

Introduction, 2nd paragraph. I'm sure this comes across as self-promotion, and I try to avoid that, but in this case, I really can't help myself. See: McShea, D.W. **2015**. Three trends in the history of life: An evolutionary syndrome. *Evolutionary Biology* 43:531–542.
Corrected accordingly. Thanks for pointing us to this paper. It is indeed relevant and we now refer to it in several places in the manuscript. BTW, it was actually published in 2016.

Introduction, 2nd paragraph. See McShea 2001: general and consistent terminology developed.
We thank the reviewer and corrected accordingly. We deleted this sentence, and added two paragraphs on existing approaches to characterize ETIs **(lines 85-115)**.

Introduction, 2nd paragraph. Again, I wouldn't be mentioning these here if they were not directly relevant to the statement in the text in which the citations appear:
McShea, D.W. 2001. The "minor transitions" in hierarchical evolution and the question of directional bias. *Journal of Evolutionary Biology* 14:502-518. Marcot, J. and D.W. McShea.

2007. Increasing hierarchical complexity throughout the history of life: phylogenetic tests of trend mechanisms. *Paleobiology* 33:182-200.
Thanks for pointing us to these studies, which we have read with interest, and cited two of them in the new subsection '**Operationalization schemes for transitions in individuality**'.

Introduction, 2nd paragraph.*'…there is no general schematic framework for evaluating progression along METIs'*. -- But there is ... see my papers above. ... I should add that the fact that I have developed just such a consistent, schematic framework, takes nothing away from the authors' framework here. We need multiple approaches, and the one offered here looks like an excellent one. (See next marginal note.).
We thank the reviewer for this pluralistic view. We deleted that erroneous claim. We added a new section that discusses previous frameworks.

Introduction, 2nd paragraph. Again, see McShea 2001 and McShea and Changizi 2003. The authors should say something about the differences between the approach developed there and their own operationalization.
We agree with the referee and ccorrected accordingly. Indeed, we were unaware of these studies, and indeed they proposed a complete scheme for operationalizing ETIs. The respective sentences were deleted. These studies are now mentioned in a new subsection ''**Operationalization schemes for transitions in individuality', starting in line 85.**

Methods, 1st paragraph. *'To date, there is no standard and widely accepted method to quantify complexity in living systems'*. Not true. A standard method -- complexity as number of part types or degree of differentiation among parts -- was formally proposed in McShea 1993, 1996 (although it has a considerable history before that -- see Bonner's book on complexity: Bonner, J. T. 1988. *The Evolution of Complexity by Means of Natural Selection*. Princeton University Press, Princeton). And it has been used since then by Valentine et al. (1994), who used number of cell types to document a trend in complexity in metazoans. It is now also a standard usage in molecular biology, applied to describe changes in numbers of genes or numbers of protein types involved in various molecular mechanisms (e.g., Doolittle 2012, Finnegan et al. 2012). Really the whole recent literature on constructive neutral evolution (CNE) adopts this view of complexity as well.
We agree with the reviewer and the text was corrected accordingly. We now discuss the number of cell types as an indicator of complexity, referring to the above-mentioned literature (**Methods, lines 138-144**). In our scheme we only use a binary variable to denote the presence or absence of non-reproductive specialization. That said, future work in the line suggested by the reviewer can indeed enhance our method by using the number of unit-types (e.g., cell types in a multi-cellular organism) as a continuous variable, documenting advanced stages of individuation. Such a project, although relevant and important, is beyond the scope of our present study, since we are concerned mainly with changes during the transition itself.

Methods, 1st paragraph. A useful addition to the discussion. The relevance of connectivity is underappreciated, in my view. Still, I think the authors should keep in mind that all four of these notions are conceptually independent. That is, they are capturing different and incommensurate concepts, not different aspects of a single concept. If all of them are going

to be called complexity, then I think it should be made clear that they are complexity in different senses.
Corrected accordingly. We agree that these parameters represent independent concepts, and we now clarify the point in a new paragraph (**Introduction, lines 105-115, and again in Methods, lines 122-125**). That said, we believe that at the same time, these parameters taken together capture, at least partially, the elusive and multi-facet notion of complexity in biological systems, and we clarify this point in the text as well (**same place**).

Methods, 1st paragraph. Complexity as number of levels is now called "vertical complexity," while complexity as number of part types (differentiation) is called "horizontal complexity" (Sterelny 1999). The two are conceptually independent of each other.
We thank the referee and corrected accordingly (**lines 85-91)**.

Methods, 2nd paragraph. This is fine, but the authors should take a look at a contrasting approach to connectivity in the context of METIs: chapter 1 in Ed Venit's thesis: Evolutionary Trends in the Individuation and Polymorphism of Colonial Marine Invertebrates, 2007, Duke University.
We have read this thesis with interest. Although potentially very useful, we are not sure how can we measure connectivity in other transitions, for example, in social insects. We view the extension of our scheme into a scheme of continuous variables, a topic for future projects, and we hope to cooperate with the reviewer on such a project.

Methods, 2nd paragraph. Excellent Framework!
Thanks.

Page 5, line 15. *'We separated these two types of specialization because they can appear at very different stages during a transition and thus could be indicative of different degrees of complexity along the METI continuum'.* **--Excellent framework**.
Thanks. Much appreciated.

Page 5, line 22. *System size is a good variable to use in this context.* John Bonner talks quite a bit about its relevance to METIs in his book on complexity. See also Anderson, C., and D.W. McShea. 2001. Individual versus social complexity, with particular reference to ant colonies. Biological Reviews (of the Cambridge Philosophical Society) 76: 211-237.
---Thanks, we agree about the importance of size and corrected the text accordingly (1st paragraph of the **Discussion**, lines 208-215).

Page 6, line 4. This scheme is the heart of this paper. It makes sense, it is operational, and at least the combination of variables in new.
--- Thanks.

Page 6, line 4. Citations needed to the large literature on caste differentiation dating back to Oster and Wilson's book?
We thank the reviewer and corrected accordingly. We now mention caste differentiation with relevant citations, although in a different location than proposed by the reviewer (**Introduction, 2nd paragraph**, lines 49-52).

Page 6, line11. Am puzzled: there are enormous numbers of taxa -- e.g., among the algae -- that are multicellular but poorly individuated, by the authors' own definition of that term. In claiming that nearly all are fully-fledged individuals they seem to be limiting their claim to taxa nested within the three major multicellular clades, the animals, plants, and fungi.
Our claim was indeed erroneous. These two sentences have been deleted.

Page 6, line13. These three are exceptions, yes, but are they the ONLY ones? Hmmm.
This sentence was deleted.

Page 7 line 2. *Without exception, once a specific parameter (inseparability, reproductive specialization, or nonreproductive specialization) appears in an organism, it also appears in all larger organisms within the same general group (Table 1)* – **Interesting**.
Thank you.

Page 7 line 3. I see what the authors' are trying to say with Table 1, and the point is a good one, but I'm not sure this is the right way to say it. In the top half of the table, if one were to change the order in which the columns are listed (say inseparability, then nonreprod spec, and then reprod spec), wouldn't the pluses and minuses look pretty random? I think what they mean is that as body size goes up, the total number of pluses goes up.
Corrected accordingly. We thank the reviewer for pointing to that unclear explanation. We meant to say that in each column by itself, the vertical sequence of + / - signs is perfectly ordered; had it been random, one would imply no correlation between size and other parameters. We have clarified this explanation, **Discussion lines 219-226**.

Results and Discussion, 2nd paragraph. *In volvocine algae, inseparability appears before reproductive specialization, while in the two other groups they appear together. In contrast, nonreproductive specialization appears much later (in systems > 10^6 units). This pattern is repeated in the two types of METI, suggesting that even this preliminary operationalization yields insights into the general causal processes that produced major evolutionary transitions.* **Yes! Interesting discovery!**
Thank you.

Table 1, slime molds. I thought slime molds had reproductive specialization…
Indeed, a situation like in Slime Molds, where some cells reproduce while others do not – could be considered as reproductive specialization. However, to the best of our knowledge (e.g., Rainey 2015), each Slime Mold cell could in principle commit to either reproduction or a somatic function, and there is no telling which cell would commit to either function. Thus, all cells have the same probability to reproduce. In our opinion, a minimum requirement for reproductive specialization is that some cells have higher propensity to reproduce than others. This condition is not met by Slime Molds.
Table 1, footnote 'f'. But the fact they eventually commit to a certain phenotype, isn't that enough to give them reprod div of labor?
This is an interesting point. Apparently, the concept of reproductive specialization could be defined in more than one way. For the purpose of the present study, we define it as follows (modified from previous version): '*Reproductive specialization is marked as present when*

some units are capable of reproduction, while other units are entirely and irreversibly incapable of reproduction'.

Page 9 line 19. '*Therefore, we suggest that inseparability is better understood as a causal facilitator of reproductive specialization rather than its byproduct'.* Interesting. New!!
Thanks.

Table 2, stage 1. Inseparability, no specialization, social colonies. *'Not Applicable'*. Why not applicable?
Corrected accordingly. In social insects, this situation may indeed not be applicable; it is hard to conceive motile units that are inseparable without any specialization. We now realize that this combination may be common among marine colonial invertebrates, and we corrected Table 2 accordingly. We plan to devote a future project to analyzing invertebrate colonies using the scheme presented here. Thank you for making this important comment.

Appendix 1, line 10 from bottom. There is also the view of an individual as simply as object, one with certain structural properties -- like boundedness, connectedness, etc. -- irrespective of function and of evolution. It starts with a non-biological view of individuals and layers the biology on top of that. (If interested, see my paper.)
We read the 2001 article (thanks), and used it elsewhere (**Methods, 2nd paragraph**). In order not to repeat ourselves we did not refer to it in **Appendix 1.**

Appendix 1, bottom line. Sounds right.

Thank you.

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2. Bourke AFG. 1999 Colony size, social complexity and reproductive conflict in social insects. *J. Evol. Biol.* **12**, 245–257.
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4. Burchill AT, Moreau CS. 2016 Colony size evolution in ants: macroevolutionary trends. *Insectes Soc.* **63**, 291–298.
5. Rainey PB. 2015 Precarious development: the uncertain social life of cellular slime molds. *Proc. Natl. Acad. Sci.* **112**, 2639–40.

Appendix B

08-Jan-2020

Dear Dr Carmel

I am pleased to inform you that your manuscript RSPB-2019-2805 entitled "OPERATIONALIZING FRATERNAL TRANSITIONS IN INDIVIDUALITY" has been accepted for publication in Proceedings B.

The referee(s) have recommended publication, but also suggest some minor revisions to your manuscript. Therefore, I invite you to respond to the referee(s)' comments and revise your manuscript. Because the schedule for publication is very tight, it is a condition of publication that you submit the revised version of your manuscript within 7 days. If you do not think you will be able to meet this date please let us know.
… …
Once again, thank you for submitting your manuscript to Proceedings B and I look forward to receiving your revision. If you have any questions at all, please do not hesitate to get in touch.

Sincerely,
Professor Gary Carvalho
Thanks. Much appreciated.

Associate Editor
Comments to Author:
The authors have carefully and thoroughly revised their paper in line with the recommendations of the reviewers, and the reviewers are happy with the outcome - only two minor changes are required by Reviewer 2. They have also considered my query over whether it should be more appropriately categorised as review rather than research, and I am happy with their reasoning for keeping it as research.
We are happy with this positive outcome.

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s) .
Lovely paper, duly revsied .
Thanks.
Referee: 2

Comments to the Author(s) .
The authors have done an excellent job incorporating my previous suggestions and I commend them for the willingness to include such revisions. I think the paper is much improved and deserves to be published. I include two minor comments:

1 .On page 13 third paragraph, they mention Gonium has inseparability but Table 1a has been updated based on my previous comments that Gonium does not have inseparability.
---Corrected accordingly. We have now revised the text accordingly, on p. 14 and on p. 15.

2. The material in the appendices is interesting and provides valuable context. The authors should consider including this material in the main text of the manuscript.
---Corrected accordingly. Both appendices are now included within the main text.

In addition, we wish to omit the term 'fraternal' in the title of the article. The fact that we refer to fraternal transitions only is mentioned three times in this article (introduction, discussion, conclusions). We feel that its mention in the title is unnecessary. Moreover, being a specific jargon, unknown to many biologists – it may actually alienate readers.