Review of "Meta-population structure and the evolutionary transition to multicellularity"

General comments

Rose *et al* attempt to explain how multicellular individuals could evolve through the evolution of different types of cells, cooperating in a nascent multicellular group.

Firstly, there are a few experimental points that I think need clarifying. I should note here that I do not feel I have the expertise to comment in detail about the experimental methods, but perhaps these observations could lead to more clarity in the manuscript.

- A key question for me is whether the non-sticky 'germ' cells have to arise de novo every generation? (For it to be considered a true germline, these cells would need to arise through a developmental program, and not through random mutation and loss of the 'sticky' gene). Could the authors please clarify.
- Another key point is whether the cells in these life cycles can survive on their own. A key feature of obligate multicellular organisms is that they are unable to survive and reproduce outside of the multicellular body – however it seems to me that these cells (of both types) can function outside of this life cycle. Could the authors please explain this more.
- I would like to highlight that it seems unlikely that this specific experimental setup reflects 'real-life' scenarios that could have occurred at the beginning of the evolution of multicellularity. I wonder if they could give some more examples of where these types of life cycles could be found in extant species?

Secondly, I would like to raise several general theoretical points in relation to possible misunderstandings of social evolution theory.

- It has been shown that obligate multicellularity (= individuality) has only arisen when multicellular groups are formed through cells sticking together after division, thus guaranteeing that relatedness between them = 1 (Fisher *et al*. 2013). This is relevant, because (as far as I understand it) the experiments in this manuscript essentially produce genetically identical (non-mixed ecology) and non-genetically identical (mixed ecology) treatments, where group formation is being experimentally manipulated. Therefore, from what I can see, the experiments support the idea that clonal relatedness is important – which has been shown many times in a very strong collection of papers (most crucially the work of Ashleigh Griffin & Stuart West). This literature is notably absent from the bibliography.
- The authors imply that group selection is "often dismissed as a rare occurrence" (line 508). This is a misunderstanding of social evolution theory, which I think it is extremely important to clarify. From West *et al.* 2015, Fig 4 legend: "Natural selection will lead to the evolutionarily stable strategy (ESS), which will be the strategy that maximizes inclusive fitness, irrespective of the consequences at the group level. We would expect natural selection to lead to maximization of group fitness, and thus think of the group as a fitness-maximizing individual, only in extreme cases where there is no within-group conflict". This is NOT saying that group selection doesn't happen in nature, it is in fact saying that it is always individual level inclusive fitness maximization, which appears as/is equal to group-level selection BUT ONLY WHEN the individuals within the group are without conflict (and this is most likely when relatedness = 1). In other words, inclusive fitness is always

maximized, and sometimes (when r = 1) this coincides with maximizing group fitness aswell, and this is when we see group adaptation.

Detailed comments

Line 33-35 – sentence doesn't read well and needs editing

Line 55 -56 – this implies that the lower-level units didn't (or don't) 'participate' in evolution by natural selection. Both the lower-level units and the higher-level units are subject to natural selection, and I think that needs to be made clearer here.

Line 58 – 59 – I disagree with this statement. Some of the explanations for the evolution of multicellular life centre on the origins of group reproduction, but not all of them by a long way. Some of the explanations centre of suppression of cheating, some on the mode of group formation, others on the emergence of complexity and division of labour.

Line 60 – 61 – I don't quite understand this statement, but I think it is saying that the somatic part of the life cycle is the part which experiences selection and the germline does not? Needs to be made clearer in order to be understood.

Line 66 - 67 – The fundamental requirements for the evolution of multicellularity are not a bottleneck and reproductive specialisation. Multicellularity is a very broad term, encompassing many facultative forms that only transiently exist as multicellular groups (e.g. *Dictyostelium,* many ciliates and algal species) that do not go through strong bottlenecks or have lifetime reproductive specialization. Even if we are talking about the requirements for obligate, complex multicellularity, I would argue that the fundamental requirement is clonality between the cells (where relatedness = 1), and in fact these are the only lineages where we ever see the evolution of obligate multicellularity. Reproductive specialization is an effect, not a cause, of some multicellular groups.

Line 71 – 72 – I would argue that this question is posed incorrectly. The higher-level doesn't have to 'constrain' the lower-level in true multicellular individuals – as they are formed from clonal cells. Therefore this question is only relevant to non-clonal multicellular groups, where relatedness is normally lower and therefore conflict is present between the different cells. ETIs have never occurred with non-clonal multicellular groups, so this question is misleading.

Line 119 – 122 – Again, I disagree that 'decoupling of fitness' is necessary for transitions to higher levels of individuality. In fact, much evidence suggests that it is in fact the alignment of fitness interests between the cell-level and the group-level (resulting from r = 1) that allows the transition to occur.

Line 352 – 358 – I would argue that these life cycles are a consequence, rather than a cause of the transition to obligate multicellularity organisms.