Shape, form, function and Leishmania pathogenicity: from textbook descriptions to biological understanding

Jack Sunter and Keith Gull

Article citation details
Open Biol. 7: 170165.
http://dx.doi.org/10.1098/rsob.170165

Review timeline
Original submission: 5 July 2017
Revised submission: 4 August 2017
Final acceptance: 4 August 2017

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

Review History

RSOB-17-0165.R0 (Original submission)

Review form: Reviewer 1

Recommendation
Accept with minor revision (please list in comments)

Are each of the following suitable for general readers?

a) Title  
Yes

b) Summary  
Yes

c) Introduction  
Yes

Is the length of the paper justified?
Yes

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Comments to the Author

Shape, form, function and Leishmania pathogenicity: from textbook descriptions to biological understanding by Jack Sunter and Keith Gull.

This is a narrative review of "form and function" in Leishmaniasis coming from the foremost lab in this area. It is very well written - nice clear descriptions and justifications of some aspects of their current thinking which is likely to be read and well received by those interested in this area. It's strength lies in the pitching of new ideas but it's weakness is that it is primarily descriptive, containing quite a lot of unsubtended speculation (e.g. flagellar sensing of macrophage health) and is fairly uncritical of the literature base that it summarizes using it for inspiration rather than relying on its accuracy. It does not attempt to undertake or report any specific novel data syntheses.

The title speaks directly to both morphology and pathogenicity and I was hoping that they would use the opportunity to explore in detail the evidence that leishmanial morphology has any role in pathogenesis. Clearly the considerable variation in leishmanial pathology - from apathogenic through a spectrum of tropism and severity to lethal and disfiguring diseases amongst apparently morphologically indistinguishable lineages suggests that it may actually be that no such link exists. Equally, however, infective stages have different morphologies and motility to non-infective ones which argues that that form is important to niche adaptation. Many genes associated with motility and morphogenesis have now been identified (and could be usefully tabulated in a review like this) and for all of these it is fairly easy to evaluate whether they are evolving in the leishmania genus (and sub genuses?) under selective pressure and correlate this with host and vector etc. At any rate, some sort of synthesis of the genomic and functional genomic data relating to morphogenesis with what is contained in this review already would in my opinion take this from being a very interesting review to being an exceptional and tremendously useful review, so this is my main suggestion.

Note
I found figure 2 a bit inaccessible when considering the sources of the data reported. The legends (unnecessarily I think) cite methods on cell shrinkage during processing and not the source of the populations which is much more germane to their interpretation. There is a lot of info in quite a complex composite that one would need to go back to source to interpret them and I think the figure might be usefully modified to illustrate the key narrative points and ensure that the review self contained. Also this composite, if simplified may be a good opportunity to showcase actual images of parasites stages if available.

Minor notes I think protozoal is misused where protozoan is meant in most cases - (assuming we continue to employ protozoa as a useful classification at all). i.e.The protozoal disease - leishmaniasis (or protozoal infection - leishmanosis)The protozoan parasite - Leishmania major
In line 98, "the traverse through the sandfly digestive tract" suggests to me it is remaining in the lumen rather than penetrating tissue which is not the authors intent I think. Ins 100-103 add little and could be cut.

Review form: Reviewer 2

Recommendation
Accept with minor revision (please list in comments)

Are each of the following suitable for general readers?

a) Title
   Yes

b) Summary
   Yes

c) Introduction
   Yes

Is the length of the paper justified?
Yes

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

Is it clear how to make all supporting data available?
Not Applicable

Is the supplementary material necessary; and if so is it adequate and clear?
Not Applicable

Do you have any ethical concerns with this paper?
No

Comments to the Author
Manuscript (RSOB-17-0165)
Shape, form, function and Leishmania pathogenicity: from textbook descriptions to biological understanding
General comments
This is generally a well written review on the shape, form, function and Leishmania pathogenicity. It was particularly useful to highlight the issue of using morphological parameters to determine cell types and the need for cell markers. I also felt this manuscript would be of more value to a general audience. However, it was somewhat difficult to read in its current form as the reference numbers in the text did not match the reference numbers in the bibliography suggesting this was not thoroughly checked before submission.
Amendments
1. Leishmania shape and form:
   a. Lines 37-39, ‘As seen with other parasites such as Plasmodium and trypanosomes some of these developmental forms are proliferative whereas others are quiescent and pre-adapted for transmission to the next host [1].’ Ref 1 I could not get full access to this reference to check it was appropriate but I would like other references to be added here and include references that support the statement about Plasmodium and trypanosomes.
b. Line 43 remove ‘a’.
c. References 4, 5, 6 and 7 are T. brucei references to include these you need to be clear these are not Leishmania references.

2. Defining diversity: different species, different diseases, different cells in the vector and host:
b. Line 92- add basal body to Figure 1A
c. Line 95- ref 13 & 14 in the text I think should be ref 12 & 13
d. Line 98- ref 15 in the text should be ref 14

3. Promastigote to amastigote transition:
a. Ref 16-18 in text should 15-17, ref 19 should be 18, ref 20-22 should be 19-21, this is all the way through the manuscript so please correct references
b. Line 176-182- be clear you are talking about African trypanosomes ref 4.
c. Line 230- see ref Doehl et al 2017 Nat Comm 8 (1) 57

4. Figure legends:
a. Figure 1B- I would like more explanation in the legend

5. References:
a. As stated above these all need to be checked and corrected so ref number in text matches the actual reference

Decision letter (RSOB-17-0165)

25-Jul-2017
Dear Dr Sunter,

We are pleased to inform you that your manuscript RSOB-17-0165 entitled "Shape, form, function and Leishmania pathogenicity: from textbook descriptions to biological understanding" has been accepted by the Editor for publication in Open Biology. The reviewer(s) have recommended publication, but also suggest some minor revisions to your manuscript. Therefore, we invite you to respond to the reviewer(s)' comments and revise your manuscript.

Please submit the revised version of your manuscript within 14 days. If you do not think you will be able to meet this date please let us know immediately and we can extend this deadline for you.

To revise your manuscript, log into https://mc.manuscriptcentral.com/rsob 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, please 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" in "Section 6 - File Upload". You can use this to document any changes you make to the original manuscript. In order to expedite the processing of the revised manuscript, please be as specific as possible in your response to the referee(s).

Please see our detailed instructions for revision requirements https://royalsociety.org/journals/authors/author-guidelines/.
Before uploading your revised files please make sure that you have:

1) A text file of the manuscript (doc, txt, rtf or tex), including the references, tables (including captions) and figure captions. Please remove any tracked changes from the text before submission. PDF files are not an accepted format for the “Main Document”.

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. Please note that PowerPoint files are not accepted.

3) Electronic supplementary material: this should be contained in a separate file from the main text and meet our ESM criteria (see http://royalsocietypublishing.org/instructions-authors#question5). 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/rsob.2016[last 4 digits of e.g. 10.1098/rsob.20160049].

4) A media summary: a short non-technical summary (up to 100 words) of the key findings/importance of your manuscript. Please try to write in simple English, avoid jargon, explain the importance of the topic, outline the main implications and describe why this topic is newsworthy.

Images
We require suitable relevant images to appear alongside published articles. Do you have an image we could use? Images should have a resolution of at least 300 dpi, if possible.

Data-Sharing
It is a condition of publication that data supporting your paper are made available. Data should be made available either in the electronic supplementary material or through an appropriate repository. Details of how to access data should be included in your paper. Please see http://royalsocietypublishing.org/site/authors/policy.xhtml#question6 for more details.

Data accessibility section
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

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

Sincerely,

The Open Biology Team
mailto:openbiology@royalsociety.org
Author's Response to Decision Letter for (RSOB-170165)

See Appendix A.

Decision letter (RSOB-17-0165.R1)

04-Aug-2017

Dear Dr Sunter

We are pleased to inform you that your manuscript entitled "Shape, form, function and Leishmania pathogenicity: from textbook descriptions to biological understanding" has been accepted by the Editor for publication in Open Biology.

You can expect to receive a proof of your article from our Production office within approx. 5 working days. Please let us know if you are likely to be away from e-mail contact during this period. Due to rapid publication and an extremely tight schedule, if comments are not received, we may publish the paper as it stands.

Article processing charge
Please note that the article processing charge is immediately payable. A separate email will be sent out shortly to confirm the charge due. The preferred payment method is by credit card; however, other payment options are available.

Thank you for your fine contribution. On behalf of the Editors of Open Biology, we look forward to your continued contributions to the journal.

Sincerely,

The Open Biology Team
mailto: openbiology@royalsociety.org
We were impressed by the speed and clarity of the review of our manuscript and were happy with the positive comments that both reviewers had. We found the comments they had very useful and have used them to extend and improve the manuscript.

Please find attached the revised manuscript and figures and our response to the reviewers. We have addressed each point raised by the reviewers and provided our responses to the reviewers in the blue text. Given the positive response from the reviewers and the changes we have made to the manuscript we hope that this improved manuscript is now acceptable for publication.
Shape, form, function and Leishmania pathogenicity: from textbook descriptions to biological understanding by Jack Sunter and Keith Gull.

This is a narrative review of "form and function" in Leishmaniasis coming from the foremost lab in this area. It is very well written - nice clear descriptions and justifications of some aspects of their current thinking which is likely to be read and well received by those interested in this area. Its strength lies in the pitching of new ideas but it's weakness is that it is primarily descriptive, containing quite a lot of unsubtended speculation (e.g. flagellar sensing of macrophage health) and is fairly uncritical of the literature base that it summaries using it for inspiration rather than relying on its accuracy. It does not attempt to undertake or report any specific novel data syntheses.

We thank the reviewer for their comments on the manuscript and have found their input useful in improving the manuscript. We have added extra detail at key points throughout the manuscript to take this work beyond the descriptive and highlight important areas that have not been addressed to date.

The title speaks directly to both morphology and pathogenicity and I was hoping that they would use the opportunity to explore in detail the evidence that leishmanial morphology has any role in pathogenesis. Clearly the considerable variation in leishmanial pathology - from apathogenic through a spectrum of tropism and severity to lethal and disfiguring diseases amongst apparently morphologically indistinguishable lineages suggests that it may actually be that no such link exists. Equally, however, infective stages have different morphologies and motility to non-infective ones which argues that that form is important to niche adaptation. Many genes associated with motility and morphogenesis have now been identified (and could be usefully tabulated in a review like this) and for all of these it is fairly easy to evaluate whether they are evolving in the leishmania genus (and sub genuses?) under selective pressure and correlate this with host and vector etc. At any rate, some sort of synthesis of the genomic and functional genomic data relating to morphogenesis with what is contained in this review already would in my opinion take this from being a very interesting review to being an exceptional and tremendously useful review, so this is my main suggestion.

We thank the reviewer for these useful comments, which highlight a key observation that these morphologically similar amastigotes are able to cause a spectrum of disease pathology. We have now expanded on this point and in doing so have improved the manuscript.

1) We have added further details and discussion about the morphology of amastigotes forms, highlighting work that shows there are differences in size between amastigotes of different species and that amastigotes of some species have a structural modification called a posterior invagination. However, there appears not to be a simple link between these morphological differences and the disease pathology.

2) On the reviewer's suggestion we have generated a summary table of the various morphological/motility mutants in Leishmania and have added this to the manuscript. This table shows that there a number of morphological/motility mutants found; however, the mutants are generally knockouts of well conserved proteins and these tend to have dramatic effect on the
morphology/motility of the cell. It therefore may be the case that the subtle modulations of protein expression, which are not apparent to the eye, have more influence or absence of certain proteins.

note

I found figure 2 a bit inaccessible when considering the sources of the data reported. The legends (unnecessarily I think) cite methods on cell shrinkage during processing and not the source of the populations which is much more germane to their interpretation. There is a lot of info in quite a complex composite that one would need to go back to source to interpret them and I think the figure might be usefully modified to illustrate the key narrative points and ensure that the review self contained. Also this composite, if simplified may be a good opportunity to showcase actual images of parasites stages if available.

We have removed the citations from the legend as the reviewer suggested and have introduced illustrations of the different promastigote morphologies to help the reader understand our arguments. The figure legend has been expanded to include more information about the cells used for the analysis in 2F/G and how this analysis was performed so that this review is now standalone. We decided to retain 2F/G as we believe there are powerful illustrations of the problem of trying to define cell types on morphology alone and will now be more accessible given the clearer and more extensive legend.

minor notes

I think protozoal is misused where protozoan is meant in most cases - (assuming we continue to employ protozoa as a useful classification at all). i.e.

The protozoal disease - leishmaniasis (or protozoal infection - leishmanosis)

The protozoan parasite - Leishmania major

We thank the reviewer for spotting this and have changed the text accordingly.

Ins 98 "the traverse through the sandfly digestive tract" suggests to me it is remaining in the lumen rather than penetrating tissue which is not the authors intent I think.

This phrasing was intentional as current evidence shows that Leishmania parasites remain in the digestive tract and do not penetrate through tissues as is found for Trypanosoma brucei in the tsetse fly.

Ins 100-103 add little and could be cut.

We thank the reviewer for this comment and have removed this paragraph.
Referee: 2

Comments to the Author(s)

Manuscript (RSOB-17-0165)

Shape, form, function and Leishmania pathogenicity: from textbook descriptions to biological understanding

General comments

This is generally a well written review on the shape, form, function and Leishmania pathogenicity. It was particularly useful to highlight the issue of using morphological parameters to determine cell types and the need for cell markers. I also felt this manuscript would be of more value to a general audience. However, it was somewhat difficult to read in its current form as the reference numbers in the text did not match the reference numbers in the bibliography suggesting this was not thoroughly checked before submission.

We thank the reviewer for their positive comments about the manuscript and for also spotting the referencing problem. We have now been through the manuscript after making the required changes and have ensured that the references are now correct.

Amendments

1. Leishmania shape and form:
   a. Lines 37-39- ‘As seen with other parasites such as Plasmodium and trypanosomes some of these developmental forms are proliferative whereas others are quiescent and pre-adapted for transmission to the next host [1].’ Ref 1 I could not get full access to this reference to check it was appropriate but I would like other references to be added here and include references that support the statement about Plasmodium and trypanosomes.

      We have added extra references here to refer Leishmania, trypanosomes and Plasmodium.

   b. Line 43 remove ‘a’.

      We have removed ‘a’ from this sentence.

   c. References 4, 5, 6 and 7 are T. brucei references to include these you need to be clear these are not Leishmania references.

      We have added an extra section into the introduction that acknowledges much of our basic information of Leishmania cell form and function is derived from trypanosomes and have added a caveat about the potential pitfalls this may cause.

2. Defining diversity: different species, different diseases, different cells in the vector and host:

      We thank the reviewer for spotting this error and have double checked and corrected all references.
b. Line 92- add basal body to Figure 1A

We have added the basal body to the promastigote and amastigote cartoons in figure 1A.

c. Line 95- ref 13 & 14 in the text I think should be ref 12 & 13

We thank the reviewer for spotting this error and have double checked and corrected all references.

d. Line 98- ref 15 in the text should be ref 14

We thank the reviewer for spotting this error and have double checked and corrected all references.

3. Promastigote to amastigote transition:

a. Ref 16-18 in text should 15-17, ref 19 should be 18, ref 20-22 should be 19-21, this is all the way through the manuscript so please correct references

We thank the reviewer for spotting this error and have double checked and corrected all references.

b. Line 176-182- be clear you are talking about African trypanosomes ref 4.

We thank the reviewer for spotting this error and again this sentence has fallen victim to the referencing issue and is actual referring to the work done by Wheeler and Sunter on ‘Flagellar pocket restructuring through the Leishmania life cycle involves a discrete flagellum attachment zone’. We have now corrected this referencing error.

c. Line 230- see ref Doehl et al 2017 Nat Comm 8 (1) 57

We thank the reviewer from bringing this recent paper to our attention and have added the reference to the manuscript and modified the text accordingly.

4. Figure legends:

a. Figure 1B- I would like more explanation in the legend

We have added more detail to the figure legend for 1B to clearly explain the life cycle.

5. References:

a. As stated above these all need to be checked and corrected so ref number in text matches the actual reference

We thank the reviewer for spotting this error and have double checked and corrected all references.