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Shear-sensitive adhesion enables size-independent adhesive performance in stick insects

David Labonte, Marie-Yon Struecker, Aleksandra Birn-Jeffery and Walter Federle

Article citation details
Proc. R. Soc. B **286**: 20191327.
http://dx.doi.org/10.1098/rspb.2019.1327

Review timeline

Original submission:	6 June 2019
Revised submission:	8 August 2019
Final acceptance:	26 September 2019

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

Review History

RSPB-2019-1327.R0 (Original submission)

Review form: Reviewer 1 (Christofer J. Clemente)

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?
Excellent

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 paper explores the interaction between shear forces and adhesive forces in insects as body size increases. The authors use a series of clever experiments to illustrate the mechanism by which shear stresses may change across body sizes, explaining previous findings and in turn describing a novel mechanism through which animals may alleviate the size dependent limitations of adhesion in insects.

Overall the paper is excellent, the writing is clear and well-constructed, in a language that is accessible to a broad scientific audience, and will therefore make this paper of interest to a broad group of readers. I had very few criticisms of the paper, and I found myself following along with the logical flow presented by the authors.

There were however a few sections which I thought required clarification.
Lines 166-173 describes an experiment where the pad is pulled along the surface proximally towards the body, and then 'pulled' off at an angle of 150 deg relative to the surface. From reading this I understood that the pull off would also be proximal (i.e. to measure adhesive force) yet figure 3B appears to show an arrow indicating a push off, in the distal direction? Perhaps I misunderstand the figure, though the cartoon pad appears to show the distal portion orientated to the left? Perhaps an inset of the cartoon indicating which direction is proximal vs distal might clarify the orientation.

Lines 192: "under natural conditions pads will frequently be sheared passively…" As I understand it, this condition would only be true if the insect was hanging upside down from a smooth surface, or would only apply to the legs above the BCOM on a vertical surface? Otherwise we might expect different loading regimes, for example during level walking? Might be worth clarifying?

The one nagging doubt I had while reading this paper, which is actually addressed by the authors in the last paragraph of the discussion, is the reference to the size-independence adhesive performance. The authors certainly show that within the size range tested adhesive performance

is not compromised, but they also show that this is due to a shift in the mechanism of adhesion. They even show the body size at which this mechanism changes on line 205 (wow!!). However we know from previous studies that adhesion is not size independent (e.g. Labonte et al. 2016), and there is a size limitation above which we no longer see animals with adhesive pads. The most incredible part of this paper is that the authors give a likely mechanism for why this would be the case (e.g. Line 270-272): the maximum distance which a pad can slide before its orientation becomes unfavourable for attachment. To me this is the most important conclusion of the paper and it is hidden at the end of the discussion! My suggestion would be to include this earlier on, perhaps the last sentence in the abstract? Though perhaps this is just a matter of style.
C.

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?
Good

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

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

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Is it accessible?
No

Is it clear?
Yes

Is it adequate?
No

Do you have any ethical concerns with this paper?
No

Comments to the Author

Summary

This study investigates the scaling of stick insect adhesion across a broad range of size, focusing on normal, pull-off adhesion and shear. The authors suggest that there is a shear component of adhesion that is able to explain size independent performance measures that have been reported previously. The results of this work have important implications for the field of bio-adhesion science, most specifically they help explain what more "efficient" pads may actually be.

I believe that this work is of great interest and was well done. However, the lack of an introduction made it very difficult for me, someone who studies biological adhesive systems, to follow. I am concerned about how this will read to those outside the field. The difficulty stems our field itself. For instance, terms such as adhesion, shear, whole body measurement, whole body-force, efficiency, friction, shear force, static shear force, shear stress, static shear stress, adhesive stress, sliding, and several others were all used in this text. Many were not defined and were thus hard to follow for non-experts and for experts, since we all seem to have different definitions. A glossary could rectify this, as well as very careful word choice that stays consistent, but the lack of introduction also limits our understanding of the implications of this work to the field. Specifically the authors note several key implications in the text, but without an introduction it is difficult to understand why they are important and the background from which they came. This includes the difference between normal pull-off adhesion and shear (or friction), the undefined and interesting term "efficiency" in biological adhesion studies that we all wonder about, and the root of this paper, the issue with scaling adhesive systems (among others). I do not think an introduction needs to be long, but a couple paragraphs would really help.

In addition to my suggestion for an introduction, I have a few other comments to consider:

1. Is the morphology (even adhesive pad chemistry?) the same among all instars?
2. How do you know your results scale with area or with length? i.e., what is the fundamental theory behind this?(The introduction could clarify this)
3. Your results noting the difference between friction and adhesion are not all that novel, we know that adhesion is less than friction in many systems, what is interesting is your control of shear. In an introduction you could give a couple examples of what has been done on adhesion/friction and argue that the control of shear in a biological adhesive system has not been investigated. In synthetic adhesives this has of course been done.
4. It would be nice to see an example of the linear relationship between shear force and adhesion. Could go in the introduction.
5. Figure 2, the stick figures of the stick insects are not obvious at first and make the graph look a bit messy. I don't think they are needed either, it is clear there is a range of body sizes tested by looking at the x-axis.
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8. I'm getting caught up in the terms, what is the difference between shear force and pad sliding (line 173)
9. What are the implications of this work for hairy pads?
10. line 248 - again, the terms ,I got it, but I had to read this line multiple times to understand it.
11. This manuscript and the title itself is a bit too far reaching. You cannot conclude that what you have found can be translated to all climbing animals. Please clarify in the title. In the text it would be good to remind the readers that this is one species of insect. There are three main types of adhesive systems (for terrestrial organisms), you only tested one. It is also possible that not all organisms scale in this way, you only tested one species with growth instars, but not multiple species, or within a single species from different environments or with variable roles etc. Finally,

there could be other solutions to our question of "efficiency", such as material or chemical variation that has not been addressed well. This contribution is the best I have seen as an attempt to explain this, but it is important to remind the readers that there could still be other components of importance.

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?
Good

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

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

Is the length of the paper justified?
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Should the paper be seen by a specialist statistical reviewer?
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Is it accessible?
N/A

Is it clear?
N/A

Is it adequate?
N/A

Do you have any ethical concerns with this paper?
Yes

Comments to the Author
The authors present the scaling data for shear adhesion (instars) as a function of mass and find that it scales with pad area. In contrast the adhesion (normal) of single pads scale as a function of length. If the single pads were sheared before the normal pull-off experiments, the scaling is much more consistent with area instead of length. The authors propose that the larger insects use

shear as a way to enhance adhesion. The shearing results in loss of contact-mediating fluid and the authors propose that this results in increase in normal (or shear) adhesion. They suggest that this could be the mechanism for increasing adhesion rather than increasing the pad area more than the isometric scaling of pad area with mass.
This study is well designed and I recommend publication.

However, I encourage the authors to add an introduction. This will make it easier to read and provide context. Would like the authors to discuss the reason behind the secretion of this contact mediating fluid and the information on the chemistry of this liquid. Also it will be helpful if the authors discuss any available information on pad morphology. This could be part of the introduction.

Decision letter (RSPB-2019-1327.R0)

22-Jul-2019

Dear Dr Labonte:

Your manuscript has now been peer reviewed and the reviews have been assessed by an Associate Editor. The reviewers' comments (not including confidential comments to the Editor) and the comments from the Associate Editor are included at the end of this email for your reference. As you will see, the reviewers and the Editors have raised some concerns with your manuscript and we would like to invite you to revise your manuscript to address them.

We do not allow multiple rounds of revision so we urge you to make every effort to fully address all of the comments at this stage. If deemed necessary by the Associate Editor, your manuscript will be sent back to one or more of the original reviewers for assessment. If the original reviewers are not available we may invite new reviewers. Please note that we cannot guarantee eventual acceptance of your manuscript at this stage.

To submit your revision please log into http://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.

When submitting your revision please upload a file under "Response to Referees" - in the "File Upload" section. This should document, point by point, how you have responded to the reviewers' and Editors' comments, and the adjustments you have made to the 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.

Your main manuscript should be submitted as a text file (doc, txt, rtf or tex), not a PDF. Your figures should be submitted as separate files and not included within the main manuscript file.

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Use of animals and field studies:
If your study uses animals please include details in the methods section of any approval and licences given to carry out the study and include full details of how animal welfare standards were ensured. Field studies should be conducted in accordance with local legislation; please include details of the appropriate permission and licences that you obtained to carry out the field work.

Data accessibility and data citation:
It is a condition of publication that you make available the data and research materials supporting the results in the article. Datasets should be deposited in an appropriate publicly available repository and details of the associated accession number, link or DOI to the datasets must be included in the Data Accessibility section of the article (https://royalsociety.org/journals/ethics-policies/data-sharing-mining/). Reference(s) to datasets should also be included in the reference list of the article with DOIs (where available).

In order to ensure effective and robust dissemination and appropriate credit to authors the dataset(s) used should also be fully cited and listed in the references.

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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.

For more information please see our open data policy http://royalsocietypublishing.org/data-sharing.

Electronic supplementary material:
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. Please try to submit all supplementary material as a single file.

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].

Please submit a copy of your revised paper within three weeks. If we do not hear from you within this time your manuscript will be rejected. If you are unable to meet this deadline please let us know as soon as possible, as we may be able to grant a short extension.

Thank you for submitting your manuscript to Proceedings B; we look forward to receiving your revision. If you have any questions at all, please do not hesitate to get in touch.

Best wishes,
Victoria Braithwaite

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Professor V A Braithwaite
mailto: proceedingsb@royalsociety.org

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Associate Editor, Comments to Author:
Dear Dr. Labonte,
Thank you for submitting your manuscript entitled “Shear-sensitive adhesion enables size-independent adhesive performance in climbing animals” to the Proceedings of the Royal Society. I have received three peer reviews that are overall supportive of the manuscript but also raise some important concerns.

I appreciate that your manuscript describes the scaling of adhesion forces with body size in insects that use hairy pads to climb on surfaces. The main finding of the study is that shear sensitivity of the adhesive strength ensures that adhesive performance does not change over a considerable size range in stick insects, whose body mass spans more than two orders of magnitude from first instar to adult.

All three reviewers are overall positive about the manuscript and its findings, but two reviewers lament the weak exposition due to the lack of an introduction. They strongly encourage the authors to provide a stronger conceptual framework and to define core concepts, some of which, such as efficiency, have no agreed-upon definition and therefore really need to be formally defined whenever they are used. I have to agree with the reviewers that this manuscript needs a stronger exposition and needs to define core terminology. Reviewer 2 also cautions against overgeneralisation and I would encourage the authors to provide more nuanced or stronger arguments to support their conclusions, particularly that this study’s findings extend beyond this species and this form of adhesion.

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Reviewers' Comments to Author:

Referee: 1

This paper explores the interaction between shear forces and adhesive forces in insects as body size increases. The authors use a series of clever experiments to illustrate the mechanism by which shear stresses may change across body sizes, explaining previous findings and in turn describing a novel mechanism through which animals may alleviate the size dependent limitations of adhesion in insects.

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There were however a few sections which I thought required clarification.
Lines 166-173 describes an experiment where the pad is pulled along the surface proximally towards the body, and then ‘pulled’ off at an angle of 150 deg relative to the surface. From reading this I understood that the pull off would also be proximal (i.e. to measure adhesive force)

yet figure 3B appears to show an arrow indicating a push off, in the distal direction? Perhaps I misunderstand the figure, though the cartoon pad appears to show the distal portion orientated to the left? Perhaps an inset of the cartoon indicating which direction is proximal vs distal might clarify the orientation.

Lines 192: “under natural conditions pads will frequently be sheared passively…” As I understand it, this condition would only be true if the insect was hanging upside down from a smooth surface, or would only apply to the legs above the BCOM on a vertical surface? Otherwise we might expect different loading regimes, for example during level walking? Might be worth clarifying?

The one nagging doubt I had while reading this paper, which is actually addressed by the authors in the last paragraph of the discussion, is the reference to the size-independence adhesive performance. The authors certainly show that within the size range tested adhesive performance is not compromised, but they also show that this is due to a shift in the mechanism of adhesion. They even show the body size at which this mechanism changes on line 205 (wow!!). However we know from previous studies that adhesion is not size independent (e.g. Labonte et al. 2016), and there is a size limitation above which we no longer see animals with adhesive pads. The most incredible part of this paper is that the authors give a likely mechanism for why this would be the case (e.g. Line 270-272): the maximum distance which a pad can slide before its orientation becomes unfavourable for attachment. To me this is the most important conclusion of the paper and it is hidden at the end of the discussion! My suggestion would be to include this earlier on, perhaps the last sentence in the abstract? Though perhaps this is just a matter of style.
C.

===

Referee: 2

Summary
This study investigates the scaling of stick insect adhesion across a broad range of size, focusing on normal, pull-off adhesion and shear. The authors suggest that there is a shear component of adhesion that is able to explain size independent performance measures that have been reported previously. The results of this work have important implications for the field of bio-adhesion science, most specifically they help explain what more "efficient" pads may actually be.

I believe that this work is of great interest and was well done. However, the lack of an introduction made it very difficult for me, someone who studies biological adhesive systems, to follow. I am concerned about how this will read to those outside the field. The difficulty stems our field itself. For instance, terms such as adhesion, shear, whole body measurement, whole body-force, efficiency, friction, shear force, static shear force, shear stress, static shear stress, adhesive stress, sliding, and several others were all used in this text. Many were not defined and were thus hard to follow for non-experts and for experts, since we all seem to have different definitions. A glossary could rectify this, as well as very careful word choice that stays consistent, but the lack of introduction also limits our understanding of the implications of this work to the field. Specifically the authors note several key implications in the text, but without an introduction it is difficult to understand why they are important and the background from which they came. This includes the difference between normal pull-off adhesion and shear (or friction), the undefined and interesting term "efficiency" in biological adhesion studies that we all wonder about, and the root of this paper, the issue with scaling adhesive systems (among others). I do not think an introduction needs to be long, but a couple paragraphs would really help.

In addition to my suggestion for an introduction, I have a few other comments to consider:
1. Is the morphology (even adhesive pad chemistry?) the same among all instars?
2. How do you know your results scale with area or with length? i.e., what is the fundamental theory behind this?(The introduction could clarify this)
3. Your results noting the difference between friction and adhesion are not all that novel, we know that adhesion is less than friction in many systems, what is interesting is your control of shear. In an introduction you could give a couple examples of what has been done on adhesion/friction and argue that the control of shear in a biological adhesive system has not been investigated. In synthetic adhesives this has of course been done.
4. It would be nice to see an example of the linear relationship between shear force and adhesion. Could go in the introduction.
5. Figure 2, the stick figures of the stick insects are not obvious at first and make the graph look a bit messy. I don't think they are needed either, it is clear there is a range of body sizes tested by looking at the x-axis.
6. Figure 2a, why are there lines extending beyond the graph? I don't understand what they mean to show.
7. Figure 2b, the figure makes sense, but the caption for it does not. Isn't this just showing adhesive stress vs. body weight is linear?
8. I'm getting caught up in the terms, what is the difference between shear force and pad sliding (line 173)
9. What are the implications of this work for hairy pads?
10. line 248 - again, the terms ,I got it, but I had to read this line multiple times to understand it.
11. This manuscript and the title itself is a bit too far reaching. You cannot conclude that what you have found can be translated to all climbing animals. Please clarify in the title. In the text it would be good to remind the readers that this is one species of insect. There are three main types of adhesive systems (for terrestrial organisms), you only tested one. It is also possible that not all organisms scale in this way, you only tested one species with growth instars, but not multiple species, or within a single species from different environments or with variable roles etc. Finally, there could be other solutions to our question of "efficiency", such as material or chemical variation that has not been addressed well. This contribution is the best I have seen as an attempt to explain this, but it is important to remind the readers that there could still be other components of importance.

===
Referee: 3

The authors present the scaling data for shear adhesion (instars) as a function of mass and find that it scales with pad area. In contrast the adhesion (normal) of single pads scale as a function of length. If the single pads were sheared before the normal pull-off experiments, the scaling is much more consistent with area instead of length. The authors propose that the larger insects use shear as a way to enhance adhesion. The shearing results in loss of contact-mediating fluid and the authors propose that this results in increase in normal (or shear) adhesion. They suggest that this could be the mechanism for increasing adhesion rather than increasing the pad area more than the isometric scaling of pad area with mass.
This study is well designed and I recommend publication.

However, I encourage the authors to add an introduction. This will make it easier to read and provide context. Would like the authors to discuss the reason behind the secretion of this contact mediating fluid and the information on the chemistry of this liquid. Also it will be helpful if the authors discuss any available information on pad morphology. This could be part of the introduction.

Author's Response to Decision Letter for (RSPB-2019-1327.R0)

See Appendix A.

RSPB-2019-1327.R1 (Revision)

Review form: Reviewer 1 (Christofer J. Clemente)

Recommendation
Accept as is

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

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

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

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
The authors have done a great job of revising the manuscript and have addressed all of my concerns. I think the addition of an introduction is also advantageous as it makes the paper more accessible to a broader audience. I have no further suggestions for this manuscript.

Review form: Reviewer 2

Recommendation
Accept as is

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

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

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

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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
Thank you for making the suggested changes. This manuscript is much improved. I have no further comments.

Review form: Reviewer 3

Recommendation
Accept as is

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

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

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?
Yes

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.
Yes

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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
No additional comments.

Decision letter (RSPB-2019-1327.R1)

26-Sep-2019

Dear Dr Labonte

I am pleased to inform you that your manuscript entitled "Shear-sensitive adhesion enables size-

independent adhesive performance in stick insects" 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.

If you have any queries regarding the production of your final article or the publication date please contact procb_proofs@royalsociety.org

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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.

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,
Victoria Braithwaite

Professor V A Braithwaite
Editor, Proceedings B
mailto: proceedingsb@royalsociety.org

Appendix A

Reply to referees

We sincerely thank all referees for the time they invested, and for the critical yet constructive comments on our manuscript. We reply to all comments in detail below.

Referee: 1

This paper explores the interaction between shear forces and adhesive forces in insects as body size increases. The authors use a series of clever experiments to illustrate the mechanism by which shear stresses may change across body sizes, explaining previous findings and in turn describing a novel mechanism through which animals may alleviate the size dependent limitations of adhesion in insects.

Overall the paper is excellent, the writing is clear and well-constructed, in a language that is accessible to a broad scientific audience, and will therefore make this paper of interest to a broad group of readers. I had very few criticisms of the paper, and I found myself following along with the logical flow presented by the authors.

There were however a few sections which I thought required clarification.
Lines 166-173 describes an experiment where the pad is pulled along the surface proximally towards the body, and then 'pulled' off at an angle of 150 deg relative to the surface. From reading this I understood that the pull off would also be proximal (i.e. to measure adhesive force) yet figure 3B appears to show an arrow indicating a push off, in the distal direction? Perhaps I misunderstand the figure, though the cartoon pad appears to show the distal portion orientated to the left? Perhaps an inset of the cartoon indicating which direction is proximal vs distal might clarify the orientation.

We thank the referee for pointing out this inaccuracy in our language. To avoid this misunderstanding (which arose because we used "pull" in two different ways), we now write that pads were "detached" at an angle of 150 deg; this indeed corresponds to a (partial) pushing motion, as indicated in the cartoon inset.

Lines 192: "under natural conditions pads will frequently be sheared passively…" As I understand it, this condition would only be true if the insect was hanging upside down from a smooth surface, or would only apply to the legs above the BCOM on a vertical surface? Otherwise we might expect different loading regimes, for example during level walking? Might be worth clarifying?

We entirely agree with the referee, and have changed the wording to:

"Under natural conditions, pads will be sheared passively whenever adhesive forces are required..."

We note that our initial statement was correct even in the situations hinted at by the referee: legs below the BCOM during vertical climbing (and all legs during upright locomotion) are still "sheared", albeit in a direction corresponding to a push.

The one nagging doubt I had while reading this paper, which is actually addressed by the authors in the last paragraph of the discussion, is the reference to the size-independence adhesive performance. The authors certainly show that within the size range tested adhesive performance is not compromised, but they also show that this is due to a shift in the mechanism of adhesion. They even show the body size at which this mechanism changes on line 205 (wow!!). However we know from previous studies that adhesion is not size independent (e.g. Labonte et al. 2016), and there is a size limitation above which we no longer see animals with adhesive pads. The most incredible part of this paper is that the authors give a likely mechanism for why this would be the case (e.g. Line 270-

272): the maximum distance which a pad can slide before its orientation becomes unfavourable for attachment. To me this is the most important conclusion of the paper and it is hidden at the end of the discussion! My suggestion would be to include this earlier on, perhaps the last sentence in the abstract? Though perhaps this is just a matter of style.

We thank the referee for the helpful suggestion. We have considered moving this conclusion into the abstract, but are afraid this won't be possible without significant additional explanation, which would reduce the number of words available to render the abstract understandable to a more generalist audience. In addition, this conclusion remains somewhat of a conjecture, which needs to be corroborated with further experiments (for example on the scaling of static shear stress). In order to give this point more weight, we added a sentence to the introduction referring to possible constraints of the two strategies to increase adhesion in larger animals.

Referee: 2

This study investigates the scaling of stick insect adhesion across a broad range of size, focusing on normal, pull-off adhesion and shear. The authors suggest that there is a shear component of adhesion that is able to explain size independent performance measures that have been reported previously. The results of this work have important implications for the field of bio-adhesion science, most specifically they help explain what more "efficient" pads may actually be.

I believe that this work is of great interest and was well done. However, the lack of an introduction made it very difficult for me, someone who studies biological adhesive systems, to follow. I am concerned about how this will read to those outside the field. The difficulty stems our field itself.

We now added an introduction to our manuscript.

For instance, terms such as adhesion, shear, whole body measurement, whole body-force, efficiency, friction, shear force, static shear force, shear stress, static shear stress, adhesive stress, sliding, and several others were all used in this text. Many were not defined and were thus hard to follow for non-experts and for experts, since we all seem to have different definitions. A glossary could rectify this, as well as very careful word choice that stays consistent, but the lack of introduction also limits our understanding of the implications of this work to the field.

We agree with the referee that the number of terms can be confusing, and that, unfortunately, not all terms are used correctly in the literature. We have added a brief note on terminology at the beginning of the methods section:

Throughout this manuscript, we use `shear force' to refer to forces applied parallel to the surface (they are counteracted by `friction forces'). `Adhesive force', in turn, refers to the normal component of the force resisting detachment for whole animals or individual adhesive pads. `Shear stress' and `adhesive stress' refer to these forces when normalised by contact area. We use 'pad efficiency' for the maximum adhesive stress a pad can produce. `Static shear stress' is the maximum stress which can be applied parallel to the surface without causing the pads to slide, i. e. to move relative to the surface.

Specifically the authors note several key implications in the text, but without an introduction it is difficult to understand why they are important and the background from which they came. This includes the difference between normal pull-off adhesion and shear (or friction), the undefined and interesting term "efficiency" in biological adhesion studies that we all wonder about, and the root of this paper, the issue with scaling adhesive systems (among others). I do not think an introduction needs to be long, but a couple paragraphs would really help.

We now added an introduction to our manuscript, in which we address these points. The difference between normal pull-off and shear is now addressed in the methods section, where we define how we use these terms in the manuscript.

In addition to my suggestion for an introduction, I have a few other comments to consider:

1. Is the morphology (even adhesive pad chemistry?) the same among all instars?

There is no evidence for changes either in morphology (adhesive pad area follows isometry, see Tab.1) or in chemistry, though to the best of our knowledge, no one has looked systematically. However, we believe that the results of our study are consistent with the absence of such changes: In the absence of a shear force, adhesive force scales with a length scale, as is expected for spherical or tape-like contacts. We present a plausible hypothesis for why this changes during whole body measurements, and corroborate this hypothesis with detailed experiments.

2. How do you know your results scale with area or with length? i.e., what is the fundamental theory behind this?(The introduction could clarify this)

This point is now addressed in the introduction.

3. Your results noting the difference between friction and adhesion are not all that novel, we know that adhesion is less than friction in many systems, what is interesting is your control of shear. In an introduction you could give a couple examples of what has been done on adhesion/friction and argue that the control of shear in a biological adhesive system has not been investigated. In synthetic adhesives this has of course been done.

We are not entirely sure what exactly the referee is referring to, and would be grateful for further clarification. Adhesion and friction are fundamentally different (see for example Israelachvili's classic text book), though adhesion may influence friction (e.g. via hysteresis) and vice versa (via frictional dissipation, see ref 9). We are unaware of a single biological system in which adhesive forces exceed friction forces, and also make no statement to this or a related end in our manuscript. Instead, we show that the well-established link between applied shear force and resulting adhesive force provides animals with a mechanism to alter the `efficiency' of their pads. We explain this reasoning in more detail in the newly added introduction.

4. It would be nice to see an example of the linear relationship between shear force and adhesion. Could go in the introduction.

Examples for this relationship can be found in refs 9, 10 and 12, as cited in the manuscript; in particular ref 12 has detailed examples for hairy, smooth, wet and dry adhesive pads. These examples include extensive and detailed data on this relationship for the species studied here (ref 9). Due to space constraints, we decided not to include an additional figure to visualise this relationship in this manuscript.

5. Figure 2, the stick figures of the stick insects are not obvious at first and make the graph look a bit messy. I don't think they are needed either, it is clear there is a range of body sizes tested by looking at the x-axis.

We removed the illustrations of the stick insects from the figure.

6. Figure 2a, why are there lines extending beyond the graph? I don't understand what they mean to show.

This is an error that must have happened in the conversion of the figure to the final pdf – we apologise. These lines are short in our version of the figure, and illustrate the three slopes 1, 2/3 and 1/3, corresponding to mass, area and length-scaling, respectively.

7. Figure 2b, the figure makes sense, but the caption for it does not. Isn't this just showing adhesive stress vs. body weight is linear?

The data is plotted on a double logarithmic scale, so the relationship is not linear (we have now added this to the caption, to avoid this misunderstanding). Instead, the adhesive stress increases with body mass in a way sufficient to achieve size-independent adhesion, even if pads only grew isometrically (see also the newly added introduction). The cause of this change in stress is that the shear forces applied during detachment grew faster than pad area; they were scaled with body mass, as seems reasonable for freely climbing insects, and as is outlined in the caption.
For clarification, we changed the wording of the caption to

“Because of the linear relationship between applied shear force and measured adhesion, applying a shear force corresponding to the insect's body weight increases the scaling coefficient of adhesion, leading to an apparent increase in pad efficiency.”

8. I'm getting caught up in the terms, what is the difference between shear force and pad sliding (line 173)

We have added a note on terminology in the methods section (see above).

9. What are the implications of this work for hairy pads?

We believe that our experiments constitute strong support for the hypothesis that the coupling between adhesive force and applied shear force provides a mechanism which allows large climbing animals to maintain size-independent performance. As this coupling appears to be independent of pad morphology and alleged adhesive mechanism (see refs 9, 10, and 12), we believe that the conclusions of our manuscripts should also hold for hairy pads, though this of course will need to be tested experimentally.

10. line 248 - again, the terms ,I got it, but I had to read this line multiple times to understand it.

We have added a note on terminology in the methods section to clarify the multiple terms used in the manuscript (see above).

11. This manuscript and the title itself is a bit too far reaching. You cannot conclude that what you have found can be translated to all climbing animals. Please clarify in the title. In the text it would be good to remind the readers that this is one species of insect. There are three main types of adhesive systems (for terrestrial organisms), you only tested one. It is also possible that not all organisms scale in this way, you only tested one species with growth instars, but not multiple species, or within a single species from different environments or with variable roles etc. Finally, there could be other solutions to our question of "efficiency", such as material or chemical variation that has not been addressed well. This contribution is the best I have seen as an attempt to explain this, but it is important to remind the readers that there could still be other components of importance.

We completely agree with the words of caution by the referee. However, there is no reason to assume that the mechanism we describe is limited to our study species, or even to a particular type

of adhesive pad. The effect arises as a direct consequence of the linear relationship between shear force and adhesion. As this relationship holds for small, large, hairy, smooth, dry and wet pads (see Federle and Labonte, in press, now cited in the manuscript, ref 12), so should the knock-on effect on scaling. At present, this does admittedly remain conjecture, however likely it may be, and so we changed the title to: “Shear-sensitive adhesion enables size-independent adhesive performance in stick insects”.

Referee: 3

The authors present the scaling data for shear adhesion (instars) as a function of mass and find that it scales with pad area. In contrast the adhesion (normal) of single pads scale as a function of length. If the single pads were sheared before the normal pull-off experiments, the scaling is much more consistent with area instead of length. The authors propose that the larger insects use shear as a way to enhance adhesion. The shearing results in loss of contact-mediating fluid and the authors propose that this results in increase in normal (or shear) adhesion. They suggest that this could be the mechanism for increasing adhesion rather than increasing the pad area more than the isometric scaling of pad area with mass.
This study is well designed and I recommend publication.

However, I encourage the authors to add an introduction. This will make it easier to read and provide context.

We have now added an introduction to the manuscript.

Would like the authors to discuss the reason behind the secretion of this contact mediating fluid and the information on the chemistry of this liquid. Also it will be helpful if the authors discuss any available information on pad morphology. This could be part of the introduction.

Our study focuses on the scaling of adhesive forces, and the loss of pad secretion appears to be involved in changes in `pad efficiency’; our results therefore corroborate previous hypotheses, and add another potential function to the list (We briefly address some potential functions of the pad secretion in L 228-237). More generally, the exact function(s) of the pad secretion remain unclear, but have been subject of a recent study from our group (ref 8), which discusses several hypotheses in more detail. We have added this and another reference in L 230, to address the comment of the referee:

Second, sliding results in the depletion of the contact-mediating liquid secreted by the adhesive pads [see Fig. 3 A. 9, 27, 28. For more details on the function and chemical composition of the secretion, see refs. [8, 29]].

Regarding morphology, we now cite information on pad allometry across and within taxa of climbing animals in the Introduction.