Photoreflectance/scattering measurements of spider silks informed by standard optics

Sean J. Blamires, Douglas J. Little, Thomas E. White and Deb M. Kane

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Review timeline
Original submission: 13 December 2019
Revised submission: 26 March 2020
Final acceptance: 30 March 2020

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

Note: This manuscript was transferred from another Royal Society journal with peer review.

Review History
RSOS-192174.R0 (Original submission)

Review form: Reviewer 1

Is the manuscript scientifically sound in its present form?
Yes

Are the interpretations and conclusions justified by the results?
Yes

Is the language acceptable?
Yes

Do you have any ethical concerns with this paper?
No

Have you any concerns about statistical analyses in this paper?
No

Recommendation?
Accept as is
Comments to the Author(s)
I have reviewed a prior submission of this manuscript. As stated before, I find a study that uses a standard optical measurement technique to characterize the visibility of spider silk interesting and important, as spider silk plays a major role in spider evolution and diversification, while its visual properties are understudied.

Despite a number of considerations from myself and other reviewers, in my opinion, none of these were of the nature that would undermine the strength of this study. The authors addressed them carefully and I think the manuscript is now in a publishable form.

Review form: Reviewer 2

Is the manuscript scientifically sound in its present form?
Yes

Are the interpretations and conclusions justified by the results?
Yes

Is the language acceptable?
Yes

Do you have any ethical concerns with this paper?
No

Have you any concerns about statistical analyses in this paper?
Yes

Recommendation?
Accept with minor revision (please list in comments)

Comments to the Author(s)
This version of the manuscript is much improved over the first version. I think that the data and methodology are worth publishing and it seems appropriate for this journal. I have a number of minor comments.

- Some, but not all of the reference to "ancient vs. modern" spiders has been changed. Again, it would be more appropriate to refer to more and less recently derived groups.
- Through most of the manuscript the authors refer to the silk they are studying as "dragline or frame/radial" then later they start referring to this silk as major ampullate or MA silk, then not until page 7 ln 24 is the abbreviation actually defined. Readers familiar with the spider silk literature will know what is going on here, but it would be better to add an explanation in the introduction that explicitly states that spiders use secretions from the major ampullate (MA) glands to produce dragline, frame, and radials.
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Decision letter (RSOS-192174.R0)

16-Mar-2020

Dear Miss Kane

On behalf of the Editors, I am pleased to inform you that your Manuscript RSOS-192174 entitled "Photoreflectance/Scattering Measurements of Spider Silks Informed by Standard Optics" has been accepted for publication in Royal Society Open Science subject to minor revision in accordance with the referee suggestions. Please find the referees’ comments at the end of this email.

The reviewers and handling editors have recommended publication, but also suggest some minor revisions to your manuscript. Therefore, I invite you to respond to the comments and revise your manuscript.

• Ethics statement
If your study uses humans or animals please include details of the ethical approval received, including the name of the committee that granted approval. For human studies please also detail whether informed consent was obtained. For field studies on animals please include details of all permissions, licences and/or approvals granted to carry out the fieldwork.

• Data accessibility
It is a condition of publication that all supporting data are made available either as supplementary information or preferably in a suitable permanent repository. The data accessibility section should state where the article's supporting data can be accessed. This section should also include details, where possible of where to access other relevant research materials such as statistical tools, protocols, software etc can be accessed. If the data has been deposited in an external repository this section should list the database, accession number and link to the DOI for all data from the article that has been made publicly available. Data sets that have been deposited in an external repository and have a DOI should also be appropriately cited in the manuscript and included in the reference list.

If you wish to submit your supporting data or code to Dryad (http://datadryad.org/), or modify your current submission to dryad, please use the following link: http://datadryad.org/submit?journalID=RSOS&manu=RSOS-192174

• Competing interests
Please declare any financial or non-financial competing interests, or state that you have no competing interests.

• Authors’ contributions
All submissions, other than those with a single author, must include an Authors’ Contributions section which individually lists the specific contribution of each author. The list of Authors should meet all of the following criteria; 1) substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data; 2) drafting the article or revising it critically for important intellectual content; and 3) final approval of the version to be published.

All contributors who do not meet all of these criteria should be included in the acknowledgements.
We suggest the following format:
AB carried out the molecular lab work, participated in data analysis, carried out sequence alignments, participated in the design of the study and drafted the manuscript; CD carried out the statistical analyses; EF collected field data; GH conceived of the study, designed the study, coordinated the study and helped draft the manuscript. All authors gave final approval for publication.

• Acknowledgements
Please acknowledge anyone who contributed to the study but did not meet the authorship criteria.

• Funding statement
Please list the source of funding for each author.

Please ensure you have prepared your revision in accordance with the guidance at https://royalsociety.org/journals/authors/author-guidelines/ -- please note that we cannot publish your manuscript without the end statements. We have included a screenshot example of the end statements for reference. If you feel that a given heading is not relevant to your paper, please nevertheless include the heading and explicitly state that it is not relevant to your work.

Because the schedule for publication is very tight, it is a condition of publication that you submit the revised version of your manuscript before 25-Mar-2020. Please note that the revision deadline will expire at 00.00am on this date. If you do not think you will be able to meet this date please let me know immediately.

To revise your manuscript, log into https://mc.manuscriptcentral.com/rsos and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with Decisions". Under "Actions," click on "Create 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 referees 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 referees. We strongly recommend uploading two versions of your revised manuscript:

1) Identifying all the changes that have been made (for instance, in coloured highlight, in bold text, or tracked changes);
2) A 'clean' version of the new manuscript that incorporates the changes made, but does not highlight them.

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

1) A text file of the manuscript (tex, txt, rtf, docx or doc), references, tables (including captions) and figure captions. Do not upload a PDF as your "Main Document";
2) A separate electronic file of each figure (EPS or print-quality PDF preferred (either format should be produced directly from original creation package), or original software format); 3) Included a 100 word media summary of your paper when requested at submission. Please ensure you have entered correct contact details (email, institution and telephone) in your user account;
4) Included the raw data to support the claims made in your paper. You can either include your data as electronic supplementary material or upload to a repository and include the relevant doi within your manuscript. Make sure it is clear in your data accessibility statement how the data can be accessed;
5) All supplementary materials accompanying an accepted article will be treated as in their final form. Note that the Royal Society will neither edit nor typeset supplementary material and it will be hosted as provided. Please ensure that the supplementary material includes the paper details where possible (authors, article title, journal name).

Supplementary files will be published alongside the paper on the journal website and posted on the online figshare repository (https://rs.figshare.com/). The heading and legend provided for each supplementary file during the submission process will be used to create the figshare page, so please ensure these are accurate and informative so that your files can be found in searches. 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.

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

Kind regards,
Anita Kristiansen
Editorial Coordinator
Royal Society Open Science
openscience@royalsociety.org

on behalf of Miles Padgett (Subject Editor)
openscience@royalsociety.org

Associate Editor Comments to Author:
Comments to the Author:
Thank you for taking such care in the revision of this transferred manuscript - the reviewers are broadly satisfied that you have taken the requested changes into consideration; however, a number of tweaks remain necessary to get the paper over the line. Please take the same degree of care with these outstanding changes, and we'll look forward to receiving the final version of the paper in the near future.

Reviewer comments to Author:
Reviewer: 1

Comments to the Author(s)
I have reviewed a prior submission of this manuscript. As stated before, I find a study that uses a standard optical measurement technique to characterize the visibility of spider silk interesting and important, as spider silk plays a major role in spider evolution and diversification, while its visual properties are understudied.
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Reviewer: 2

Comments to the Author(s)
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- p6, In 49-56 could use some references for the optical explanations.

Author's Response to Decision Letter for (RSOS-192174.R0)

See Appendix A.

Decision letter (RSOS-192174.R1)

30-Mar-2020

Dear Miss Kane,

It is a pleasure to accept your manuscript entitled "Photoreflectance/Scattering Measurements of Spider Silks Informed by Standard Optics" in its current form for publication in Royal Society Open Science. The comments of the reviewer(s) who reviewed your manuscript are included at the foot of this letter.

You can expect to receive a proof of your article in the near future. Please contact the editorial office (openscience_proofs@royalsociety.org) and the production office
(openscience@royalsociety.org) to let us know if you are likely to be away from e-mail contact -- if you are going to be away, please nominate a co-author (if available) to manage the proofing process, and ensure they are copied into your email to the journal.

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Thank you for your fine contribution. On behalf of the Editors of Royal Society Open Science, we look forward to your continued contributions to the Journal.

Kind regards,
Andrew Dunn
Royal Society Open Science Editorial Office
Royal Society Open Science
openscience@royalsociety.org

on behalf of Prof Miles Padgett (Subject Editor)
openscience@royalsociety.org

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Appendix A

Anita Kristiansen
Editorial Coordinator

Royal Society Open Science
openscience@royalsociety.org

on behalf of Miles Padgett (Subject Editor)
openscience@royalsociety.org Response to Reviewers

25 March 2020

Dear Anita and Prof Padgett,

Thank you for the notification that, subject to completing minor revisions requested by one of the reviewers, our manuscript will be acceptable for publication in RSOS. Please see a detailed list of Reviewer 2's comment and our response below.

We look forward to the next stages of the publication process.

Yours sincerely,

(Electronic signature)

Deb Kane (on behalf of all authors)

Response to reviewers

Reviewer 1 did not request any revisions.

Reviewer 2

- Some, but not all of the reference to "ancient vs. modern" spiders has been changed. Again, it would be more appropriate to refer to more and less recently derived groups.

There are two uses of "modern" and one use of the term “ancient” in the revised manuscript:

“A prior study of the reflectance of spider silks from 18 species reported results for three species of modern orb weaving spiders (Nephila clavipes, Argiope argentata and Micrathena Schreibersi) as having reduced reflectance in the UV range. (Modern in the context used here means more recently derived). The reduced UV reflectance was interpreted as an adaptive advantage in making the silks less visible to insects. Herein a standard, experimental technique for measuring the reflectance spectrum of diffuse surfaces, using commercially available equipment, has been applied to samples of the silks of four modern species of orb weaving spiders: Phonognatha graeffei; Eriophora transmarina; Nephila plumipes; and Argiope keyserlingi.”
“Nonetheless if silk visibility by day is the selective driving silk reflectance, we might expect *E. transmarina* silk to be slightly more reflective in the UV than that of *A. keyserlingi*. *Phonognatha graefei*, or leaf curling spider, is reported as a nocturnally active web building spider of the family Araneidae (but formerly classed with the more ancient family Tetragnethidae).”

In these 3 instances the word is chosen to link with published work being referred to and/or to reflect common usage description that will be clear to most readers. On balance we think the word choice, as it currently is, will be more readily understood by more readers. We nevertheless added that “modern in the context used here means more recently derived” to show that we use it in the same evolutionary context that the reviewer has interpreted it.

- Through most of the manuscript the authors refer to the silk they are studying as "dragline or frame/radial" then later they start referring to this silk as major ampullate or MA silk, then not until page 7 ln 24 is the abbreviation actually defined. Readers familiar with the spider silk literature will know what is going on here, but it would be better to add an explanation in the introduction that explicitly states that spiders use secretions from the major ampullate (MA) glands to produce dragline, frame, and radials.

The first use of the term major ampullate silks was on page 2 of the submitted manuscript. In response to the reviewers comment we have changed page 2 line 7 to include major ampullate and introduced (MA) in page 2 paragraph 4 line 1.

The species *Argiope keyserlingi*, and *Plebs eburnus* have been studied and shown to have high optical quality dragline/radial (i.e. major ampullate) silks [11-17].

“In this study we measured the photoreflectance/scattering of major ampullate (MA) silks from four species of spider:”

p3, ln14 "of" should be added between "silks" and "some"

Corrected “The study examined the silks of some derived ecribellate species”

p3, para1 could use some more references

Without some more specific suggestion of what referencing is missing from this paragraph we are unsure of what the reviewer thinks is missing. This paragraph deals with the actual previous measurements of the optical properties of silks that are relevant to this paper. There is a literature on measuring birefringence of spider silks, which we have mentioned and have included references to, but that was done for completeness. That research is not directly relevant here. We don’t think we have missed any references that should be included.

p3, In 9 "," should be added between "measurements" and "but"

Corrected
p3, ln 23 you say "conspecific", but I think you mean "congeneric" unless these are really the same species?

The term we should have used is ‘congener’. We have thus changed the text in question to:

“N. plumipes is a congener of N. clavipes and A. keyserlingi is a congener of A. argentata”

p3, ln 58 "AR" is not defined

Corrected “Such a layer could act as an antireflection (AR) coating with a centre wavelength determined by the thickness of the layer. The centre wavelength could be in the UV. The aqueous layer on a capture silk, could potentially form an AR coating, for example.”

p5, ln 7 I don’t know what "between" is referring to

The paragraph has been changed to make the meaning clear.

“We collected six females (identified visually) of each of the four species of spider (N. plumipes, E. transmarina, A. keyserlingi, and P. graeffei, totalling 24 spiders) from locations in suburban Sydney, and taken to the laboratory at the University of New South Wales in eastern Sydney for silk collection. The six were a combination of non-gravid adult and sub-adult spiders.

Section 3.2 my understanding is that you are using a single strand, from a single spinneret, although as you state, the silk functions as paired strands. Do you have an expectation for how this might affect results? Or an explanation for why you made this choice?

The experiment uses a single thread from the spinnerets so the silk sample used is a double cylinder thread. We have in separate studies undertaken theoretical modelling of scattering from a double cylinder and compared it to a single cylinder. Models of scattering from a double cylinder (both ours and the one referenced [18]) involve some approximation and are not exact. The scattering from a double cylinder changes the detail of the maxima and minima in the scattering pattern with angle but it does not make a significant difference to the angular spread of the main lobe of the scattering pattern (both forward and back scattered). Hence, we have not obfuscated the main narrative here with, what in the context of the paper, is unnecessary detail, by discussing scattering from a double cylinder in any more detail.

p5, ln 60 is this pattern because it is a big spider and therefore produces big silk?

Yes, it is primarily because the silks have the largest diameter. We have added a sentence to make this explicit.

“It is noted that it is the silks of this nocturnal species that has the largest photoreflectance signal per silk in this study. This is primarily because of their larger diameter.”

p6, ln 49-56 could use some references for the optical explanations.

The eight lines of text associated with this comment are:
“The light that is collected as the “reflected” signal has been scattered by the shaped silks, and this includes light that has propagated through the “largely transparent” silk and ultimately been back scattered to the collection fibre (fig. 2(c) and fig. 1). Thus, two physical mechanisms contribute to a reduced signal at shorter wavelengths. (i) Scattering – it is known that shorter wavelength light is scattered more than longer wavelengths in Rayleigh scattering within a dielectric, and, the form of the silks as a scatterer can lead to a differentiated collection of scattered light at shorter wavelengths compared to longer wavelengths within the numerical aperture of the collection optics (Fig. 1) [28, 29]. (ii) Wavelength dependent absorption of some of the light by the silk could be occurring. Thus, the reduced signal is considered real but it is attributed to wavelength dependent absorption and/or wavelength dependent scattering effects, and not reflection.”

We have added references to this section as requested.