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Modelling the effect of birth and feeding modes on the development of human gut microbiota

Xiyan Xiong, Sara L. Loo, Li Zhang and Mark M. Tanaka

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1st revised submission:	24 November 2020
2nd revised submission:	8 December 2020
Final acceptance:	9 December 2020

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

Review History

RSPB-2020-1810.R0 (Original submission)

Review form: Reviewer 1 (Allen Rodrigo)

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?
Yes

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

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

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

Is it accessible?
Yes

Is it clear?
Yes

Is it adequate?
Yes

Do you have any ethical concerns with this paper?
No

Comments to the Author
This is an interesting paper, accounting for variation in microbiome composition as a consequence of feeding and birth practice.

The paper is relatively easy to follow, for a mathematically literate biologist. The methods are clearly explained, and I have not particular comments on the results or the discussion. I do have one small question about the assumptions in the extended model, specifically, the immune clearance rates of Bifidobacteria and commensals, mu_b and mu_c, respectively. Why is it assumed that mu_b<mu_c? If anything, I would have thought that, biologically, the immune response generated by Bifidobacteria would mean that mu_b>mu_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?
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

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
See attached file. (See Appendix A)

Decision letter (RSPB-2020-1810.R0)

03-Nov-2020

Dear Ms XIONG:

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 Associate Editor 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.

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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.

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

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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,
Professor Hans Heesterbeek
mailto: proceedingsb@royalsociety.org

Associate Editor
Board Member: 1
Comments to Author:
It was difficult to find reviewers at the moment so the review process took longer than usual. The reviewers agree that this is a good piece of modelling work but ask some questions about the modelling assumptions that should be (briefly) addressed in a revised version - likely by giving a little extra discussion of how the model was built.

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s)
This is an interesting paper, accounting for variation in microbiome composition as a consequence of feeding and birth practice.

The paper is relatively easy to follow, for a mathematically literate biologist. The methods are clearly explained, and I have not particular comments on the results or the discussion. I do have one small question about the assumptions in the extended model, specifically, the immune clearance rates of Bifidobacteria and commensals, mu_b and mu_c, respectively. Why is it assumed that mu_bmu_c.

Referee: 2
Comments to the Author(s)
See attached file

Author's Response to Decision Letter for (RSPB-2020-1810.R0)

See Appendix B.

Decision letter (RSPB-2020-1810.R1)

07-Dec-2020

Dear Ms XIONG

I am pleased to inform you that your manuscript RSPB-2020-1810.R1 entitled "Modelling the effect of birth and feeding modes on the development of human gut microbiota" has been accepted for publication in Proceedings B.

The Associate Editor has recommended publication, but also remarks on the referencing in your manuscript. Indeed, all references in the text are '?', indicating that perhaps you forgot to run Bibtex, or did not run Latex twice, before uploading the revised manuscript. Please check what

has happened and revise accordingly. Because the schedule for publication is very tight, it is a condition of publication that you submit the revised version of your manuscript within 7 days. If you do not think you will be able to meet this date please let us know.

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

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

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

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

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It is a condition of publication that data supporting your paper are made available either in the electronic supplementary material or through an appropriate repository.

In order to ensure effective and robust dissemination and appropriate credit to authors the dataset(s) used should be fully cited. To ensure archived data are available to readers, authors should include a 'data accessibility' section immediately after the acknowledgements section. This should list the database and accession number for all data from the article that has been made publicly available, for instance:
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- Phylogenetic data: TreeBASE accession number S9123
- Final DNA sequence assembly uploaded as online supplemental material
- Climate data and MaxEnt input files: Dryad doi:10.5521/dryad.12311

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http://datadryad.org/submit?journalID=RSPB&manu=(Document not available) which will take you to your unique entry in the Dryad repository. If you have already submitted your data to dryad you can make any necessary revisions to your dataset by following the above link. Please see https://royalsociety.org/journals/ethics-policies/data-sharing-mining/ for more details.

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

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

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

Decision letter (RSPB-2020-1810.R2)

09-Dec-2020

Dear Ms XIONG

I am pleased to inform you that your manuscript entitled "Modelling the effect of birth and feeding modes on the development of human gut microbiota" 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.

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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.

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

Sincerely,
Proceedings B
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Appendix A

Report on Modelling the effect of birth and feeding modes on the development of human gut microbiota

Scientists have learned a great deal about the effects of our gut micro-flora on human health in recent decades. Yet much is unknown. For example, how does it initialize and what are the effects of a C-section verses a natural birth and what are the effects of formula feeding rather than breast feeding. The medical literature appears to be contradictory on these issues. The authors construct very simple mathematical models of Lotka-Volterra type to explore these issues. The simplest model involves competition among two strains of Bifidobacteria (here labeled B1 and B2) and a generic commensal bacteria, labeled C. B1 is fed with supplied breast milk the amount of which declines exponentially and therefore B1 goes extinct; before doing so, its digestion of breast milk augments the growth rate of B2 via cross-feeding. An extended model takes account of the effects of an immune response against the bacteria stimulated by B1.

A strength of the paper is that some of the contradictory results reported in the literature can be mimicked in the observed dynamics of the model under appropriate parameter regimes.

While I have some reservations about some modeling assumptions and parameter choices, to be described below, I believe the authors have made an interesting and possibly useful contribution and therefore I recommend publication of a suitably revised version.

My main complaint is that very little motivation is given for some modeling assumptions, the choice of parameters, and initial data for the models. For such crude models, this may be expected. However, very little attempt is made to explore the dynamical consequences of alternative choices for parameters and initial data. See below for details.

Technical Issues:

For the competition model, what biological motivation can be given for the assumptions $\alpha_c > 1$ and $f_3 \gg f_2$; why not maintain equality in the latter unless there is evidence supporting it. The main issue with the competition model is whether the competition between B and C is stronger or weaker ($\alpha > 1$ or $\alpha < 1$) than intra-specific competition. This choice dictates which of the outcomes of Figure 3 prevail. The authors need to address this point from a biological perspective head on. The outcomes displayed in Figure 2 depend on the choice $\alpha = 2$; it would be reasonable to provide a similar plot in the case that $\alpha < 1$.

The extended model involving the immune component. It seems strange that the authors assume that the immune response rate is a multiple of the milk-digesting B1 alone instead of some linear combination of B1,B2 and C, while simultaneously assuming that the dominant effect of immune response is to depress commensals C ($\mu_c > \mu_b$). Is there some biological evidence for these two separate modeling decisions?

It is clear that B1 tends to zero exponentially fast and this implies that M is uniformly bounded for positive time. Therefore, the effect of immune response on bacteria is relatively muted. If instead, the immune response rate were some linear combination of the bacterial types then M would grow linearly with time and then it is easy to see that all variables in the extended model would go extinct. Thus, I see a mathematical reason why the authors chose to assume that the immune response rate is a multiple of the milk-digesting B only. Many modelers assume that the immune response is dampened in the absence of stimulation. An alternative model might have the form $\dot{M} = a_1 B_1 + a_2 B_2 + a_3 B_3 - \mu M$ although this introduces another parameter μ.

Appendix B

Xiyan Xiong
Sara Loo
Li Zhang
Mark Tanaka
UNSW SYDNEY
Sydney NSW 2052
Australia
✉ *xiyan.xiong@student.unsw.edu.au*

The Editors
Proceedings of the Royal Society B

November 24, 2020

Dear editor,

Below we respond to all of the reviewers' comments and provide details of the revisions we have made to address these comments. We thank the reviewers and the editor for their suggestions, which have led to improvements in our manuscript.

Referee: 1

1. *The paper is relatively easy to follow, for a mathematically literate biologist. The methods are clearly explained, and I have not particular comments on the results or the discussion. I do have one small question about the assumptions in the extended model, specifically, the immune clearance rates of Bifidobacteria and commensals, μ_b and μ_c, respectively. Why is it assumed that $\mu_b < \mu_c$.*

   Studies in the literature show that IgA stimulated by Bifidobacteria restricts pathogens and potentially harmful commensal bacteria from attaching to the epithlelium [3, 6]. For this reason, we assume that the immune system favours the establishment of mutualists by making them more resistant to the immune response than commensals. Since we include a much wider range of species in our commensal population, we assume that they are on average more susceptible to this immune clearance, and thus choose $\mu_b < \mu_c$. We had previously included some introduction to the relationship between the immune compartment and Bifidobacteria, which we now emphasise in Section 2.2, lines 185-189. The stimulation of IgA by Bifidobacteria was also outlined in the introduction.

Referee: 2

1. *Scientists have learned a great deal about the effects of our gut micro-flora on human health in recent decades. Yet much is unknown. For example, how does it initialize and what are the effects of a C-section verses a natural birth and what are the effects of formula feeding rather than breast feeding. The medical literature appears to be contradictory on these issues. The authors construct very simple mathematical models of Lotka-Volterra type to explore these issues. The simplest model involves competition among two strains of Bifidobacteria (here labeled B1 and B2) and a generic commensal bacteria, labeled C. B1 is fed with supplied breast milk the amount of which declines exponentially and therefore B1 goes extinct; before doing so, its digestion of breast milk augments the growth rate of B2 via cross-feeding. An extended model takes account of*

the effects of an immune response against the bacteria stimulated by B1. A strength of the paper is that some of the contradictory results reported in the literature can be mimicked in the observed dynamics of the model under appropriate parameter regimes. While I have some reservations about some modeling assumptions and parameter choices, to be described below, I believe the authors have made an interesting and possibly useful contribution and therefore I recommend publication of a suitably revised version. My main complaint is that very little motivation is given for some modeling assumptions, the choice of parameters, and initial data for the models. For such crude models, this may be expected. However, very little attempt is made to explore the dynamical consequences of alternative choices for parameters and initial data. See below for details.

We thank the reviewer for their comments. We have addressed their concern by clarifying our choices of parameters and undertaking some additional sensitivity analysis following their suggestion. We address these in the specific points below.

2. *For the competition model, what biological motivation can be given for the assumptions $\alpha_c > 1$ and $f_3 > f_2$; why not maintain equality in the latter unless there is evidence supporting it.*

   In our models, we focus on the mutualistic interaction between the infant and fibre-consuming Bifidobacteria populations, instead of competition between them. This cross-feeding effect helps the $B2$ Bifidobacteria population survive through infancy even though only $B1$ is able to metabolise breast milk. This is described in the literature [1, 4, 5]. This cross-feeding action is described in the introduction in lines 54-57. Thus, we choose $\alpha_c > 1$ as cross-feeding is beneficial to the $B2$ population. We clarify this point in lines 151-156.

   We note, however, that in our model, the level of cross-feeding does not ultimately affect the abundance of Bifidobacteria and commensals at steady state; the cross-feeding coefficient α_c is linked to HMO-consuming Bifidobacteria $B1$, which is eliminated when solid foods replace milk. Therefore, while we appreciate the point made here, our conclusions will not be greatly altered by exploring alternative values of α_c. We indicate this in lines 155-156.

   For the reviewer's second point regarding our choice of $f_3 > f_2$ we note that the commensal population in our model includes a wider range of species than the Bifidobacteria population. Therefore, we assume that the supply of commensals from the environment f_3 is higher than that of Bifidobacteria f_2. We describe this in additional detail in lines 144-150 of the manuscript. Furthermore, as we presented in our Supplementary Material, Figure S9 explores the effect of varying the environmental supply of the bacterial populations (f_2 and f_3) on equilibria. This figure also shows outcomes when $f_2 = f_3$. As the supply of commensals (f_3) increases, the relative abundance of commensal ($B3$) at equilibria increases. Higher f_3 values enables the dominance of $B3$ at low breast milk level. We described these effects in lines 314-324 of the manuscript.

3. *The main issue with the competition model is whether the competition between B and C is stronger or weaker ($\alpha > 1$ or $\alpha < 1$) than intra-specific competition. This choice dictates which of the outcomes of Figure 3 prevail. The authors need to address this point from a biological perspective head on. The outcomes displayed in Figure 2 depend on the choice $\alpha = 2$; it would be reasonable to provide a similar plot in the case that $\alpha < 1$.*

   In our initial submission, Figure 3 demonstrated the effect of $\alpha < 1$ and $\alpha > 1$ on steady-states of our quasi-steady-state approximation. We have now also included the temporal dynamics of the competition model where $\alpha = 0.7$ in the Supplementary Material Figure S2. This is the equivalent plot to the temporal dynamics (for $\alpha = 2$) given in Figure 2, but with $\alpha = 0.7$. The equilibria for this parameter setting is independent of birth mode; this is consistent with the trajectory shown in the quasi-steady-state nullclines plot, which we had previously included. We refer to this plot in lines 208-210 and 234-239, and clarify the biological significance of this threshold effect in lines 239-243.

4. *The extended model involving the immune component. It seems strange that the authors assume that the immune response rate is a multiple of the milk-digesting B1 alone instead of some linear combination of B1, B2 and C, while simultaneously assuming that the dominant effect of immune response is to depress commensals C ($\mu_c > \mu_b$). Is there some biological evidence for these two separate modeling decisions? It is clear that B1 tends to zero exponentially fast and this implies that M is uniformly bounded for positive time. Therefore, the effect of immune response on bacteria is relatively muted. If instead, the immune response rate were some linear combination of the bacterial types then M would grow linearly with time and then it is easy to see that all variables in the extended model would go extinct. Thus, I see a mathematical reason why the authors chose to assume that the immune response rate is a multiple of the milk-digesting B only. Many modelers assume that the immune response is dampened in the absence of stimulation. An alternative model might have the form $\dot{M} = a_1B_1 + a_2B_2 + a_3B_3 - \mu M$ although this introduces another parameter μ.*

   We thank the reviewer for this insight and suggestion. Bifidobacteria stimulate the production of IgA in the infant's gut as part of the neonatal immune development, as described in the literature [7, 2]. We assume that the immune system (which is stimulated by Bifidobacteria) favours the establishment of mutualists. That is, we assume that these Bifidobacteria have adapted to humans in such a way as to efficiently stimulate the immune system while being relatively resistant to its effects. Exploring the effect of this immune response together with breast milk is the main motivation of our extended model. We describe this in additional detail in lines 185-192 of the manuscript.

   While these considerations led us initially to focus on the effect of infant Bifidobacteria only, we take the point made by the reviewer. Therefore, we now include a generalisation in which the immune response is governed by $\dot{M} = \gamma(B_1 + B_2) - \mu M$. We choose not to include a B_3 component for the immune compartment, as we are

interested in the IgA-stimulating effect of Bifidobacteria rather than commensals, particularly in relation to breast feeding. We have included a brief description of this new version in Section 2.2 lines 194-197, with additional detail provided in the Supplementary Material. In the Supplementary Material, Figure S10 shows that the model is insensitive to birth modes as the immune clearance dominates over changes in initial condition. Further, Figure S11 explores the effect of varying γ and μ on the relative abundance of $B2$ and $B3$. The growth of immune response γ favours Bifidobacteria $B2$ while the damping factor μ encourages the commensal $B3$.

We have also made small changes to repair typos and other minor issues. All changes can be seen in the attached version of the manuscript below.

We are grateful to the reviewers and the editor for their suggestions which have led to improvements in our manuscript. We look forward to your response.

Sincerely,

Xiyan Xiong

Publications

[1] M. Egan, M. O. Motherway, M. Kilcoyne, M. Kane, L. Joshi, M. Ventura, and D. van Sinderen. Cross-feeding by bifidobacterium breve ucc2003 during co-cultivation with *Bifidobacterium bifidum* PRL2010 in a mucin-based medium. *BMC Microbiol*, 14(1): 282, 2014.

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[3] N. J. Mantis and S. J. Forbes. Secretory IgA: arresting microbial pathogens at epithelial borders. *Immunol Invest*, 39(4-5):383–406, 2010.

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[5] F. Turroni, C. Milani, S. Duranti, L. Mancabelli, M. Mangifesta, A. Viappiani, G. A. Lugli, C. Ferrario, L. Gioiosa, A. Ferrarini, et al. Deciphering bifidobacterial-mediated metabolic interactions and their impact on gut microbiota by a multi-omics approach. *ISME J*, 10(7):1656, 2016.

[6] B. J. Underdown and J. M. Schiff. Immunoglobulin A: strategic defense initiative at the mucosal surface. *Annu Rev Immunol*, 4(1):389–417, 1986.

[7] H. Yasui, N. Nagaoka, A. Mike, K. Hayakawa, and M. Ohwaki. Detection of bifidobacterium strains that induce large quantities of IgA. *Microb Ecol Health Dis*, 5 (3):155–162, 1992.