Dear Editor,

We have revised the manuscript according to the detailed comments from you and from the reviewers. We think the manuscript has benefited substantially from the careful scrutiny of the presentation and from the additional work included. Our detailed responses to the comments are presented below. We hope that you find the current version satisfactory.

On behalf of the authors,
Petteri Karisto

Dear authors,

The three exports who reviewed your paper during the first round have looked into your revised version of the manuscript and have provided me with comments. This time, I also read and reviewed your paper in detail (see my comments, written in Markdown, below), and found many opportunities for improvement. I therefore ask that you revise the manuscript to take into account my own and the other reviewer's comments. Please also provide a detailed point-by-point response to all comments. After this, I will be able to further consider the preprint for recommendation.

I look forward to receiving your revised manuscript.

Best regards,

Jorge Peña

Review of "Positive fitness effects help explain the broad range of Wolbachia prevalences in natural populations"

Major comments

1. One of the main merits of this paper is that it revisits previous models of cytoplasmatic incompatibility in the literature, and fills the gaps in some theoretical understanding of this phenomenon. In this respect, it is unfortunate that you are not able to prove analytically the stability of the equilibria of the haplodiploid models, and instead are forced to run a numerical analysis. I encourage you to devote some time to see if you can make some analytical progress. Perhaps necessary and sufficient conditions are difficult to obtain, but either necessary or sufficient conditions can be obtained more easily? Or perhaps a different strategy might prove useful? For instance, you seem to rely quite a bit on the manipulation of the explicit solutions of the equilibria. Perhaps working directly with implicit expressions leads to some progress (see, e.g., the next comment)?

> We appreciate the desire to make the final product as complete as possible, when it comes to proving the essential results. We found the suggestions to prove the generality of the ^p_F > ^p_M results very helpful; the stability properties, however, were not quite as easy, and we hope that the editor sees the value of this MS even if not all results come with a full proof. We have throughout strived to write for ecologists, not mathematical biologists, and this makes us believe that our

contribution will be ultimately judged based on how clearly it manages to explain why the results make biological sense, not on the completeness of the proofs. That said, we do find that the extra progress between round 2 and 3 has been absolutely worthwhile – see our next reply below.

2. In line 314, you say: "In all cases that we explored in the haplodiploid models, the infection frequency was higher in females than in males". I think that this statement is actually easy to prove for the female-killing effect. To see this, note that, at equilibrium, $\Delta p_M = 0$ and hence, by setting the second line of Eq. 4 to zero and solving for $\hat{p}_M$, $\hat{p}_M = \frac{\hat{p}_F ft}{\hat{p}_F f + 1-\hat{p}_F}$. It follows from this equation that the ratio $\hat{p}_F/\hat{p}_M$ is equal to $\frac{\hat{p}_F f + 1-\hat{p}_F}{\hat{p}_F f t} = \frac{1}{t}+\frac{1}{\hat{p}_F ft} - \frac{1}{ft}$, which can be rewritten as $\frac{1}{t} \left[1+\frac{1}{f} \left(\frac{1}{\hat{p}_F}-1\right)\right]$. From this, it is then easy to see that, for any interior equilibrium $\hat{p}_F \in (0,1)$, the term inside round brackets is positive, and hence that the term within square brackets is greater than one. As $t \in (0,1]$, it follows that the whole ratio $\hat{p}_F/\hat{p}_M$ must be greater than one and hence that $\hat{p}_F > \hat{p}_M$ holds, finishing the proof. Could you double check that this is a valid proof and, if so, include it in the paper? I wonder also if a similar reasoning allows for a similar proof for the case of the masculinization effect (I did not check).

Thank you for encouraging us to work on this more thoroughly. We did not adopt the above calculation as is, as the ratio $\hat{p}_F/\hat{p}_M$ above appears to have an extra p_F in the denominator. Correcting that part breaks the reasoning that followed this step of the proof.

However, we found a way to prove this claim for both models (we had a partial proof in an earlier version and thanks to this encouragement decided to pursue it further). We present the proofs in the new Appendix C, and we thank the editor for making us work on this a little harder than we had before, it is a very worthwhile addition in our mind.

3. Figures. The authors should pay more attention to the figures and their captions, as I think that they can still be much improved.

3.1. In particular, for Fig. 1 I suggest (i) that the x range is always [0,1] (currently panels b, d, and f have smaller ranges), and

The axes have been adjusted to improve visibility of the interesting parts of the plots within limited panel size.

(ii) that the label of the y axis in panels a and b do not have the additional $\times 10^2$ or $\times 10^3$ (instead, show a rescaled y axis).

We changed the labelling.

Also in this Fig., a better visualization strategy seems to be not to impose limits on the x and y ranges, but rather to let matplotlib decide on this.

The axes have been adjusted to improve visibility of the interesting parts of the plots within limited panel size.

3.2. Figures 4 and 6 should have a legend describing what the different shades of the areas refer to (this is currently done in the caption).

> We prefer keeping the explanation in the caption, since there is no short unambiguous explanation for the colours and hence the legend would be undesirably large, essentially a text element within the figure.

3.3. The image quality of Figure 7 should be improved (it currently looks pixelated).

> Thanks for noting this, now fixed.

3.4. All mathematical symbols in the figures should be rendered with LaTeX, e.g., the f=0.8 on top of Fig. 7A should be rendered as $f=0.8$, the t on the y axis should be rendered as t, etc.

> Fixed these.

3.5. I urge you to write the names of the variables in the labels of the axis of all figures. For example, the label of the x axes of the panels of Fig. 2 should read "infected proportion, p" (or similar), the y axis labels should read "change in infected proportion, Δp" (or similar), etc.

> Done.

1.6. The y label of panel a of Fig. 5 should be p_M, not p_F. Here, as per the comment above, these labels should be enhanced by providing the name of the variable the symbols refer to.

> Corrected.

4. As per the last comment in the first page of the review of reviewer 1, mathematical formulas are parts of a sentence, and punctuation and grammar rules apply. Please revise carefully your manuscript in light of this comment.

> Done.

5. In your response to reviews, you reply to all (but for the first, about a figure that you introduced) the general comments by reviewer 2 by saying that they are interesting questions but that they are beyond the scope of the present paper. At the very least (if you indeed found the comments interesting) you should devote some lines of the Discussion to these possible extensions and potential future work.

> Done.

6. Appendices. I think that it would be worthwhile reorganizing the appendices in the following way: Appendix A: Diplodiploid model (both equilibria and linear stability analysis). Appendix B: Haplodiploid models. B.1 Female-killing, B.2 Masculinization.

Minor comments

1. l. 31: "without" -> "without a"

> Done.

2. l. 49: "frequency-dependence" -> "frequency dependence"

Done.

3. l. 65: "on-going" -> ongoing

Done.

4. l. 74: "CI inducing symbiont" -> "CI-inducing symbiont"

Done.

5. l. 79: "lower" -> "a lower"

Done.

6. l. 86: "(0.5-1)" -> "between one half and one"

Done.

7. l. 88: "be close" -> "be too close"

Done.

8. l. 90: "in wide" -> "in a wide"

Done.

9. l. 91: "without the invasion barrier" -> "without an invasion barrier"

Done.

10. l. 92: "to different" -> "to the results of different"

Done.

11. l. 95: "in diplodiploid" -> "in a diplodiploid"

Done.

12. l. 118: denominator of the fraction appearing in the last line of eq. 1: This can be also written as the expression appearing in the denominator of Eq. 1 of Engelstadter 2009. Unless you have a good reason to prefer writing the expression as you do (if so, explain) I suggest you stick to the expression reported in Engelstadter 2009, so that it is more clear that the model is the same.

We originally retained the structure from above separating the terms for uninfected and infected females focusing on the biological meaning, but the suggestion from the editor will likely help readers to see the connection to earlier work (and the biological structure is already given in the previous line). Changed to the form of Engelstädter & Telschow 2009.

13. l. 123: $B=-1+f-L$ -> $B=f-1-L$

Done.

14. l. 132: $r=-B/2A$ -> $r=-B/(2A)$

Done.

15. l. 134: "with $0 < t, L \leq 1$": From this, it seems you are assuming $t \in (0,1]$, $L \in (0,1]$. But then, p=1 can be an equilibrium for $t=1$, and this is not reported below. Also, it would be clearer if you write $0 < t \leq 1$, $0 < L \leq 1$.

Yes, we indeed assume that the parameters t and L can be one. If both of them were 1, and f was also 1, then p2=1. That is not an issue for the consideration below where we look at whether p2 can be below one half. Do you refer to something else that we are missing?

16. l. 138: "shows this" -> "shows that this"

Done.

17. l. 144: "range (0.5-1)" "interval $(1/2,1)$"

We changed the wording to “close to one half”.

18. l. 145: "based on" -> "given"

Done.

19. l. 146: $r>0.5$ -> "$r>1/2$"

Done.

20. l. 148: "The conclusion arises that" -> "Hence,"

Done.

21. l. 162: "poor spread" -> "selection against"

22. l. 162: "better success" -> "selection for"

We associate “selection” with evolutive force, which is not really the case here when we speak about population dynamics at different frequencies of infected individuals. We would prefer to keep this and the previous wording as they are.

23. l. 166: "we get $\lim_{p \to 0} \Delta p = pf t − p = (f t − 1)p$": $\lim_{p \to 0}$ is missing at the beginning of the final expression. The limit $\lim_{p \to 0} \Delta p$ is zero, not $(f t − 1)p$.

Thanks for noticing this. Our argument is better phrased by using the language of Taylor expansions, and we now made our point that way.

24. l. 175: "$f t$ is clearly below 1" -> "$f t$ is below 1"

Done.

25. l. 189: "$t \in]0,1]$": Rather use $($ for open interval, as it is more standard (see one of the comments of reviewer 3).

Changed (this and other occurrences).

26. l. 205: "compare Fig. 2c,e" -> "compare Fig. 2c and/to Fig. 2e"

Done.

27. l. 215: "(; also shown in Fig. S1": remove ;

Done.

28. l. 217: "Figure 2b shows examples" -> "Figure 2c shows examples"

Done.

29. l. 221: "Higher values of L lead to equilibria with a higher prevalence of Wolbachia" (and the rest of the paragraph): Is there a proof of this statement? If yes, refer to it. If not, prove and show the proof in the appendix.

This is description/explanation of the figure. We added a reference to the figure to clarify this.

30. l. 230: "instead, higher f increases the frequency". Prove.

Here we again refer to a figure. We don't think proving this mathematically would add to the manuscript aimed to biologically oriented audience.

31. l. 234: "curves in 2e" -> "curves in Fig. 2e"

Done.

32. l. 248: "Effect [...] are shown" -> "The effect [...] is shown"

Done.

33. caption of Fig. 4:
"non-trivial stable equilibrium $\hat{p}_2 > 1/2$" -> "non-trivial stable equilibrium with invasion threshold $\hat{p}_2 > 1/2$";
"and stable equilibrium $\hat{p}_2 < 1/2$" -> "and non-trivial stable equilibrium $\hat{p}_2 < 1/2$"

Added these and improved the caption further. The first one (light grey) contains both with and without invasion threshold, which is now specified in the caption.

34. l. 269: in "Leptopilina type", write the quotation marks as ``'' instead of "" so that they are nicely rendered in LaTeX. Apply these changes also below and in other parts of the manuscript.

Done, thanks.

35. l. 297: "that this applies for" -> "that this applies to"

Done.

36. l. 298: "but only for" -> "but only to"

Done.

37. l. 301: "(examples of equilibria: Fig. 5)" -> "(for examples of equilibria, see Fig. 5)"

Done.

38. l. 329: "they can occur only if f > 1." Try to avoid as much as possible writing formulas in the Discussion. In this case, you can simply state the inequality in words.

Done.

39. l. 341: "is unstable. See also" -> "is unstable; see also"

Done.

40. l. 343: "e ciency of transmission and relative fecundity" -> "e ciency of transmission, and relative fecundity"

Done.

41. l. 356: "when studying" -> "when studying the"

Done.

42. l. 358: ""if the proportion infected daughters" -> ""if the proportion [of] infected daughters"

Good point, thanks.

43. l. 359: "than the proportion daughters" -> "than the proportion [of] daughters"

Done.

44. p. 25: "it is simple to see that" -> "it is easy to see that"

Done.

45. p. 26: "(i.e. the square brackets)" -> "(i.e. the expression within square brackets)"

Done.

46. p. 26 after eq. A2: "f_{lim}" -> "f_{lim}"

Done.

47. l. 454: "egg into haploid that" -> "egg into a haploid that"

Done.

48. p. 27: "i.e. we show with contradiction" -> "i.e. we show

by contradiction"

Done.

49. p. 27: "Thus, $r_F >= 1/2$" -> $r_F \geq 1/2$"

Done.

50. p. 27: You need to write something to introduce the last equation.

Done.

51. p. 28: "the slope of that function $−2fLt(1 + f (t − 1)) < 0$ and the root" -> "the slope of that function is negative, and the root satisfies"

Done.

52. l. 461: "to equal condition" -> "to the equivalent condition"

Done.

53. l. 466: "and the root $\hat{k} \geq 1$" -> "and $k \geq 1$ holds"

Here we think the original would be fine, considering “\geq” is a verb, but changed anyway as the alternative is ok too. :)

54. l. 468: "As A4 is equivalent with" -> "As (A4) is equivalent to the condition"

Fixed.

55. p. 29. There's a missing period at the end.

Added.

56. l. 517: "Since $ft > 0$ always" -> "Since $ft > 0$ always holds"

Done.

57. right after eq. B6: "multiply with" -> "multiply both sides of the inequality by"

Done.

58. l. 540: "The left-hand side of this equation is called the Jacobian matrix." There are several problems with this statement. First, this is not an equation, this is an inequality. Second, the left-hand side is not a Jacobian matrix, but (as I understand from the context) the absolute value of the (real) dominant eigenvalue of the Jacobian matrix. Here, the notation you use for this is more distracting than helpful. I'd go for something like $|\lambda| < 1$ (or $|\zeta| < 1$, since you are already using this notation) and then would explain in words what this λ (or ζ) is, having described the Jacobian matrix M as you do below.

Good points, we have reworked the writing of the entire section to improve clarity.

59. l. 542: "We will denote the dominant eigenvalues by ζ." You denoted it below instead by λ_1.

Fixed

60. p. 34, last line: "have the following general shape": Write down the g and h functions.

We now present the functions g and h in their respective sections and state that in this part.

61. p. 36 "We can rewrite the term under the square roots in equilibria above as follows": Couldn't you already write the expressions for the equilibrium in this form from the beginning? It would save some space.

> True, good point. We rewrote this part and moved the note about existence of the invasion threshold (using the suggested simplification) to the first occurrence of the formula in Appendix A, as it fits there more logically.

62. l. 556: "$h(\hat{p}_F) = \hat{p}_M/\hat{p}_F$". You defined h in p. 34 as a function of two variables, but only one variable is given here.

> Done.

63. Equations between l. 556 and l. 558: The left hand side is not evaluated at equilibrium, but the right hand side is. Correct.

> Done.

64. l. 560: Multiply by minus one and rearrange the term in square brackets on the right hand so that the expression looks similar to eq. B9 already at this point.

> Done.

65. l. 572: "I" -> "we"

> Done.

66. p. 38: "the term before square root": It is unclear which term this refers to. Rewrite.

> Done.

67. l. 604: "whenever they occurred in" -> "whenever they occurred within a"

> Done.

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R1

The authors have adequately revised the manuscript according to my suggestions. Even though the formal analysis of the haplodiploid case is not complete, the numerical results seem to suggest the generality of the presented results. Related to this numerical analysis, I have a question that had escaped me during my first review:

page 38, line 580: it is not clear to me for which equilibrium (pˆ1 or pˆ2) the condition $0 < pˆM , pˆF \leq 1$ applies. I guess, it is meant to apply to both? But in that case, the parameter regime is naturally restricted to ft < 1 because otherwise pˆM (and I believe also pˆF) of pˆ1 is negative. Hence, the condition $0 < pˆM , pˆF \leq 1$ should only apply to pˆ2 to fully explore the stated parameter space. Could the authors clarify what they did? Maybe they could even visualize the locations of pˆ1 and/or pˆ2? (based on my small-scale numerical analysis the stability claims in the Appendix seem valid)

Thanks for noting this potential point of confusion. We analysed entire parameter space but only assessed the stability of an equilibrium when it appeared in the biologically reasonable range. We clarified the writing.

Overall, this manuscript is a nice theoretical study that complements the existing theory of *Wolbachia*-induced cytoplasmic incompatibilities. I do not have any remaining objections (besides the one point stated above that requires some clarification).

Thanks for this favourable evaluation.

I have spotted a couple of typos, inconsistencies and inaccuracies (mostly in the appendix) that I mention here:

page 10, line 215: additional semicolon in the brackets

Removed.

inconsistent references to figures: The authors sometimes use Fig., sometimes F/figure and sometimes just state the figure panels (e.g. line 223). I suggest to use one notation and to stay consistent (my preference is Fig. XY).

We checked the references and unified to "Fig. xx", or to "Figure xx" when it was placed in the beginning of a sentence.

page 11, line 223: graph → graphs

Fixed.

legend Figure 2: "Δp as a function of p" is not a proper sentence

Fixed the sentence structure.

Figure 5, panel a: wrong y-axis label

Done.

Figure 7: Maybe this figure can be moved into the appendix. My impression is that the figure does not provide much additional information at the (comparably high) cost of being a completely new figure type compared to the previous figures.

We prefer keeping it here, since it is important for the discussion about infection frequencies across parameter space.

page 21, line 341: additional dot after 'unstable'

Fixed.

page 21, line 348: analysis → analyses

Done.

page 24, line 419: eg. → e.g.

Done.

Appendix, formulas: Mathematical formulas are typically considered as part of a sen- tence. Therefore, punctuation rules apply to them. E.g., if an equation is at the end of a sentence a dot should be added (e.g. Eq. (A.2)) or if the sentence continues after the equation, often a comma should close the expression (e.g. Eq. (A.1)). Similarly, a sequence of equations should be separated by commas (e.g. Eq. (A.1)), but if calculations are done over multiple lines, these should not be separated by commas (e.g. equation after Eq. (B.4)).

Done.

Inconsistent reference to equations: Sometimes the authors refer to equations by Eq., sometimes by E/equation and sometimes just by number (with or without braces). Similar to the figures inconsistency, I suggest to use a single format and to stay consistent (my preference is Eq. (XY)).

Checked the references and unified to "Eq. (xx)", or to "Equation (xx)" when it was placed in the beginning of a sentence.

page 27, above last formula: r_F >= 1/2 → $r_F \geq ½$

Fixed.

page 27, last line before formula: additional dot at end of sentence

Removed.

page 28, sentence after first formula: missing verb(s)

The mathematics in the sentence includes the verbs, "When f _is_ smaller or equal to 1, the slope…"

page 31, line 504: I suggest to add that pˆ1 is below 0 when ft > 1. This helped me to understand why (and when) the dynamics change in one dimension.

Added.

page 33, line 529: "ineq. (B6)" – this is wrong reference to an equation. The equation (B6) is an inequality, but one can not refer to it as an 'inequation'. I suggest to write: 'left hand side of Eq. (B6)'.

Fixed.

page 34, line 543: eigenvalues → eigenvalue (there is only one dominant eigenvalue)

Yes.

page 37, line 572: I → we

Done.

page 38, line 577: I suggest to write '$\{0.01 \leq L, t \leq 1\} \times \{0.01 \leq f \leq 3\}$' to avoid confusion about the range of the parameters (I accidentally read 1 times 0.01 and got confused for a bit about the notation). (applies as well to the masculinization section)

Fixed at both places.

page 38, line 578: delete ‘limits included’ (this is clear from using ≤ instead of <)

Done, thanks.

page 38, line 578/579: I suggest to write 1, 000, 000 instead of 1 000 000, to avoid the line split at undesired places

Fixed with non-breaking spaces.

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R2

The authors have now taken into account most of my comments, thanks! I guess the remaining minor comments I have are more a question of style than anything else, so feel free to ignore, and no need for an answer!

Thank you for the helpful comments!

1. There is still an occurrence of “pleiotropic” in the abstract

   We wanted to keep it once to retain the possibility for creating the mental connection to pleiotropic effects, while hopefully not disrupting most readers too much.

2. L217: figure 2b --> 2c

   Fixed.

3. L234: 2e --> 2c compared to 2e

   Done.

4. The zoom could be more pronounced in the new panels of figure 1. It is still hard to spot the smallest equilibrium.

   We zoomed in.

5. L332: you could remove the word “currently”

   Done.

6. L309: “two different ways in which males can be produced”: here also, you are talking about the masculinization case. While you have now clarified this for the following sentence, it should also apply to that one.

   The part after colon explains that this is speaking about masculinization.

7. L349: Unless I missed something, the sentence “As the previous analysis usually assumed f<=1, they also observed stable equilibria above one half” is redundant and could be removed.

We want to keep this, although it is a bit redundant, to highlight the difference.

8. L358: “the proportion” --> “the proportion OF” (two occurrences)

Done.

9. L 458+4: “>=” has not been written properly in tex

Fixed.

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R3

As before, I think the paper is well written, clear, and correct, and so I see little need for substantial changes. I think the authors have done a good job of addressing the minor points that I previously had. I only have a few minor typographical queries.

Thank you.

Line 74: ‘effects of CI inducing symbionts’ rather than ‘effect of Ci inducing symbiont’?

Done.

Line 189: First square bracket round the wrong way?

Changed to more a common notation for open interval (0, 1].

Line 215: Semi-colon seems to be floating here?

Fixed.

Line 217: Do you mean Figure 2B here?

Actually, 2c and d in the current version, thanks.

Figure 2: I was a little confused with panels c,d,e,f. As far as I understand it, the difference between c and d, and e and f, is the value of L. L=0.85 for c and e, and L=0.35 for d and f? I am guessing this based panels a and b, so I think could be cleared up a little.

The main difference is that $ft>1$ in c,d and $ft<1$ in e,f. This is noted in the titles of the panels.