Population dynamics of Wolbachia symbionts playing Dr. Jekyll and Mr. Hyde

Jorge Peña *based on reviews by 3 anonymous reviewers*

A recommendation of:

Scripts used to obtain or analyze results
- *https://doi.org/10.5281/zenodo.6443870*

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Recommendation

"Good and evil are so close as to be chained together in the soul"
Robert Louis Stevenson, Dr. Jekyll and Mr. Hyde

Maternally inherited symbionts—microorganisms that pass from a female host to her progeny—have two main ways of increasing their own fitness. First, they can increase the fecundity or viability of infected females. This "positive fitness effects" strategy is the one commonly used by mutualistic symbionts, such as *Buchnera aphidicola*—the bacterial endosymbiont of the pea aphid, *Acyrthosiphon pisum* [4]. Second, maternally inherited symbionts can manipulate the reproduction of infected females in a way that enhances symbiont transmission at the expense of host fitness. A famous example of this "reproductive parasitism" strategy is the cytoplasmic incompatibility (CI) [3] induced by bacteria of the genus *Wolbachia* in their arthropod and nematode hosts. CI works as a toxin-antidote system, whereby the sperm of infected males is modified in a lethal way (toxin) that can only be reverted if the egg is also infected (antidote) [1]. As a result, CI imposes a kind of conditional sterility on their hosts: while infected females are compatible with both infected and uninfected males, uninfected females experience high offspring mortality if (and only if) they mate with infected males [7].

These two symbiont strategies (positive fitness effects versus reproductive parasitism) have been traditionally studied separately, both empirically and

theoretically. However, it has become clear that the two strategies are not mutually exclusive, and that a reproductive parasite can simultaneously act as a mutualist—an infection type that has been dubbed "Jekyll and Hyde" [6], after the famous novella by Robert Louis Stevenson about kind scientist Dr. Jekyll and his evil alter ego, Mr. Hyde. In important previous work, Zug and Hammerstein [7] analyzed the consequences of positive fitness effects on the dynamics of different kind of infections, including "Jekyll and Hyde" infections characterized by CI and other reproductive parasitism strategies. Building on this and related modeling framework, Karisto et al. [2] re-investigate and expand on the interplay between positive fitness effects and reproductive parasitism in *Wolbachia* infections by focusing on CI in both diplodiploid and haplodiploid populations, and by paying particular attention to the mathematical assumption structure underlying their results.

Karisto et al. begin by reviewing classic models of *Wolbachia* infections in diplodiploid populations that assume a "negative fitness effect" (modeled as a fertility penalty on infected females), characteristic of a pure strategy of reproductive parasitism. Together with the positive frequency-dependent effects due to CI (whereby the fitness benefits to symbionts infecting females increase with the proportion of infected males in the population) this results in population dynamics characterized by two stable equilibria (the *Wolbachia*-free state and an interior equilibrium with a high frequency of *Wolbachia*-carrying hosts) separated by an unstable interior equilibrium. *Wolbachia* can then spread once the initial frequency is above a threshold or an invasion barrier, but is prevented from fixing by a proportion of infections failing to be passed on to offspring. Karisto et al. show that, given the assumption of negative fitness effects, the stable interior equilibrium can never feature a *Wolbachia* prevalence below one-half. Moreover, they convincingly argue that a prevalence greater than but close to one-half is difficult to maintain in the presence of stochastic fluctuations, as in these cases the high-prevalence stable equilibrium would be too close to the unstable equilibrium signposting the invasion barrier.

Karisto et al. then relax the assumption of negative fitness effects and allow for positive fitness effects (modeled as a fertility premium on infected females) in a diplodiploid population. They show that positive fitness effects may result in situations where the original invasion threshold is now absent, the bistable coexistence dynamics are transformed into purely co-existence dynamics, and *Wolbachia* symbionts can now invade when rare. Karisto et al. conclude that positive fitness effects provide a plausible and potentially testable explanation for the low frequencies of symbiont-carrying hosts that are sometimes observed in nature, which are difficult to reconcile with the assumption of negative fitness effects.

Finally, Karisto et al. extend their analysis to haplodiploid host populations (where all fertilized eggs develop as females). Here, they investigate two types of cytoplasmic incompatibility: a female-killing effect, similar to the CI effect studied in diplodiploid populations (the "Leptopilina type" of Vavre et al. [5]) and a masculinization effect, where CI leads to the loss of paternal chromosomes and to the development of the offspring as a male (the "Nasonia type" of Vavre et al. [5]). The models are now two-sex, which precludes a complete analytical treatment, in particular regarding the stability of fixed points. Karisto et al. compensate by conducting large numerical analyses that support their claims. Importantly, all main conclusions regarding the interplay between positive fitness effects and reproductive parasitism continue to hold under haplodiploidy.

All in all, the analysis and results by Karisto et al. suggest that it is not necessary to resort to classical (but depending on the situation, unlikely) mechanisms, such as ongoing invasion or source-sink dynamics, to explain arthropod populations featuring low-prevalent *Wolbachia* infections. Instead, low-frequency equilibria might be simply due to reproductive parasites conferring beneficial fitness effects, or Wolbachia symbionts playing Dr. Jekyll (positive fitness effects) and Mr. Hyde (cytoplasmatic incompatibility).

References

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Conflict of interest:

The recommender in charge of the evaluation of the article and the reviewers declared that they have no conflict of interest (as defined in the code of conduct of PCI) with the authors or with the content of the article.

Reviews
Toggle reviews

Evaluation round #2
DOI or URL of the preprint: *https://doi.org/10.1101/2022.04.11.487824*

Version of the preprint: 3

Author's Reply, 31 Oct 2022

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Decision by *Jorge Peña*, posted 19 Sep 2022

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)?

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).

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 (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). 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.

    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).

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

    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.

    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.

    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.

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.

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.

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"
2. l. 49: "frequency-dependence" -> "frequency dependence"
3. l. 65: "on-going" -> ongoing
4. l. 74: "CI inducing symbiont" -> "CI-inducing symbiont"
5. l. 79: "lower" -> "a lower"
6. l. 86: "(0.5-1)" -> "between one half and one"
7. l. 88: "be close" -> "be too close"
8. l. 90: "in wide" -> "in a wide"
9. l. 91: "without the invasion barrier" -> "without an invasion barrier"
10. l. 92: "to different" -> "to the results of different"
11. l. 95: "in diplodiploid" -> "in a diplodiploid"
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.
13. l. 123: $B=-1+f-L$ -> $B=f-1-L$
14. l. 132: $r=-B/2A$ -> $r=-B/(2A)$
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 $0 < t \leq 1$, $0 < L \leq 1$.
16. l. 138: "shows this" -> "shows that this"
17. l. 144: "range (0.5-1)" "interval $(1/2,1)$"
18. l. 145: "based on" -> "given"
19. l. 146: $r>0.5$ -> "$r>1/2$"
20. l. 148: "The conclusion arises that" -> "Hence,"
21. l. 162: "poor spread" -> "selection against"
22. l. 162: "better success" -> "selection for"
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 $(f t − 1)p$.
24. l. 175: "$f t$ is clearly below 1" -> "$f t$ is below 1"
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).
26. l. 205: "compare Fig. 2c,e" -> "compare Fig. 2c and/to Fig. 2e"
27. l. 215: "(; also shown in Fig. S1": remove ;
28. l. 217: "Figure 2b shows examples" -> "Figure 2c shows examples"
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.
30. l. 230: "instead, higher f increases the frequency". Prove.
31. l. 234: "curves in 2e" -> "curves in Fig. 2e"
32. l. 248: "Effect [...] are shown" -> "The effect [...] is shown"
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 $\hat{p}_2 < 1/2$"
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.
35. l. 297: "that this applies for" -> "that this applies to"
36. l. 298: "but only for" -> "but only to"

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

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.

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

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

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

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

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

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

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

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

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

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

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

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

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"

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

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

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

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

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

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

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.

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

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

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.

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.

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

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.

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

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

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

Reviewed by anonymous reviewer, 06 Sep 2022

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Reviewed by anonymous reviewer, 01 Aug 2022

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!

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

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

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

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

5.   L332: you could remove the word "currently"

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.

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.

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

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

Reviewed by anonymous reviewer, 12 Aug 2022

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.

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

Line 189: First square bracket round the wrong way?

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

Line 217: Do you mean Figure 2B here?

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.

Evaluation round #1
DOI or URL of the preprint: *https://doi.org/10.1101/2022.04.11.487824*

Version of the preprint: 2

Author's Reply, 28 Jul 2022

Download author's reply

Decision by Jorge Peña, posted 22 Jun 2022

Dear authors,

I have now received three expert reviews on your preprint. As you will see below, all reviewers agree that your manuscript addresses an interesting question, that the paper is well written, and that the analysis seems to be correct. However, they also raise several questions and comments that suggest different ways of improving the analysis and presentation of your results. Having read your paper, I agree with the reviewers' assessments, and see many opportunities for improvement.

In particular, and in line with some of the reviewers' comments, I would like you to pay particular attention to (i) the possibility of adding a figure illustrating cytoplasmic incompatibility and the workings of the model (as suggested by reviewers 2 and 3), (ii) being more concise in pages 7-10 (as suggested by reviewer 2), and (iii) the comment on the (unnecessarily complicated?) use of matrix calculus (suggested by reviewer 1).

I therefore ask that you revise the manuscript to take into account these and 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.

In addition to the reviewers' specific comments, I have some of my own. First, I would like you to pay attention to a typographical detail. You often write 0.5 for either "1/2" or "one half". The form "1/2" looks nicer in math mode or in equations; the form "one half" looks nicer when writing sentences. For instance, in line 29 you could write "can produce low frequency ($<1/2$)" and in line 31 you could write "any stable equilibrium close to one half". Second, I found some of the choices for naming variables a bit strange. In the kind of population dynamics models that you study, time is usually denoted by (lower case) t, not (upper case) T, as you do in the manuscript. This choice might be due to the fact that you already use t for one of the parameters of the model. My suggestion would be that you stick to the standard norm of using t for time but use an alternative symbol for the transmission parameter (that you now represent by t). Third, you write down equations (1) and (2) in the form $p_{T+1}=g(p_T)$ for a given function g, but then in Fig. 1 you illustrate the dynamics by plotting Δp_T as a function of p_T. I would stick to one or the other way of writing these recursions and would try to be consistent, i.e., I would either stick to the way equations (1) and (2) are written now, but then change Fig. 1 to show p_{T+1} as a function of p_T, or I would keep Fig. 1 as it is but then rewrite equations (1) and (2) in difference form. When making one change or the other, please also try to be consistent with the way you write down the equations for the haplodiploid cases. Fourth, many equations in the Appendix seem to be copy-pasted directly from (I guess) Mathematica, leading to cumbersome expressions that are difficult to parse. As an example, the factor $f(-1+(-1+k)L(-1+t)t)$ appearing in p. 39 could be rewritten as $f[L(k-1)t(t-1)-1]$ (note also the use of square brackets, consider using e.g., $\left($ and $\right)$ instead of simply $($ and $)$), which is shorter and easier to parse. I encourage you to make these changes throughout the manuscript. Finally, although all reviewers agree that the manuscript is well written, I found some grammatical mistakes and typos---please revise the writing carefully and edit the manuscript accordingly.

I look forward to receiving your revised manuscript.

Best regards,

Jorge Peña

Reviewed by anonymous reviewer, 12 Jun 2022

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Reviewed by anonymous reviewer, 04 May 2022

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Summary

Cytoplasmic incompatibility is a reproductive strategy used by certain strains of Wolbachia (and other matrilineally transmitted bacteria), whereby infected males only produce viable offspring with infected females. This means of reproductive manipulation has frequency dependent effects, as benefits to infected females increase with the number of infected males in the population.

This paper studies the ecological dynamics of Wolbachia spread and – in particular - considers the consequences of the Wolbachia providing direct fitness benefits to the female host, which have previously been ignored. Contrary to previous models, they find that stable, low frequency equilibria of Wolbachia infections can be maintained provided such fitness effects are positive. Moreover, when there are direct fitness benefits, then there needn't be an infection threshold that initially must be crossed. They suggest that this might provide a parsimonious explanation of the low Wolbachia frequencies seen in some natural populations. In addition to an investigation of diplodiploids, they also consider this effect under haplodiploidy, considering two distinct types of cytoplasmic incompatibility, a direct male-killing strategy, and a masculinising male strategy.

General comments

Overall, I thought this paper was excellent, and would be of great interest to the readers of PCI Ecology. I thought both the empirical and theoretical motivation for the question was clearly laid out, and the authors choice of approach appeared appropriate. The models were well explained, notation was clear, and I was able to recreate the key results from the descriptions provided. In addition, the authors did a thorough job outlining not only the results of their models, but much of the intuition behind these results too. This personally gave me a much richer understanding of the population dynamics, which I really appreciated. Moreover, the additional investigation of the two forms of cytoplasmic incompatibility in haplodiploids was incredibly interesting, and I think lends itself to some neat empirical tests. Generally, I think the paper is well written, clear, and correct, and so I see little need for substantial changes. The suggestions I do make, I hope the authors find useful.

Specific comments

Description of cytoplasmic incompatibility.

Whilst many of the readers of this paper may already be well acquainted with the workings of cytoplasmic incompatibility, nonetheless I feel like a small diagram would be of great help to succinctly explain the phenomena. This is particularly true for the two haplodiploid cases. Perhaps punnet square like crosses showing viable/non viable offspring (e.g. similar to Engelstädter & Telschow (2009), Figure 1)?

Fertilisation rate k

I thought that the parameter k (the fertilisation rate) could just be explained a little more. As far as I understood, this is just the proportion of eggs that a female allows to be fertilised? i.e. in a Wolbachia free population this would be the primary sex ratio? With the assumption that k might be much higher in an infected population as females attempt to counteract the sex-ratio effect?

Masculinising vs female-killing CI

I thought that the differences between these two mechanisms of CI was very interesting, and thought that the results the authors presented might lend themselves to some clear empirical patterns, and so maybe these could be expanded upon a little more. Two points jumped out to me.

Firstly, from Figure 4, it appeared that the female-killing effect had a lower invasion threshold, and higher equilibrium frequency, than the masculinising strategy. This to me intuitively made sense, as by 'masculinising' the offspring from non-infected females rather than simply killing them, the infected males are diluted in the population, reducing their effects. Thus, one might expect those populations with female-killing CI to have higher Wolbachia loads than those with masculinising CI,

although from Figure 5 it was difficult to see whether this was true. If this is correct, and the authors were able to expand upon this a little more – either in the discussion or in the haplodiploid section - then I think that would be worthwhile.

Secondly, the sex-differences in the Wolbachia infection rate under haplodiploid CI seems a very interesting and clear empirical prediction, and a clear point of comparison to be made with the diplodiploid CI. Especially as the qualitative pattern of higher infection rates in females appears to hold pretty much under any of the specified parameters. I wondered if this point had previously been made, if not then I think is worthy of being made a bit more explicit, and worth noting any empirical work that had been done on this. This pattern might also provide a nice test of hypotheses to other explanations for low frequencies of Wolbachia, i.e. if low frequencies are maintained by gene flow with sex-biased dispersal.

Sex-specific positive effects

As far as I can understand the positive (and negative) fitness effects investigated in this paper (and previous analyses) are exclusively restricted to females. I wondered whether this was worth the authors commenting on, and potentially speculating (or indeed modelling) the potential effects of positive and negative fitness effects falling upon males instead. Whilst I suspect that this would have relatively little consequence to the qualitative patterns previously shown (due to matrilineal nature of Wolbachia inheritance, and so is in effect a little bit more like just scaling L), I think it nonetheless might be worthwhile, particularly as some of the potential sources of positive fitness effects may extend to both sexes, but nonetheless it is only the positive (or negative) effects that have any substantial bearing upon the ecological dynamics.

Figures

On the whole I think the figures are clear and understandable. One comment would be that maybe Figure 5 could be explicitly split up into two figures (maybe restrict the male to the Appendix?), as I found it initially a bit of work to understand the male frequencies on the plot, and comes out relatively poorly in Grayscale.