Color code:
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to do/thoughts/questions between coauthors (temporary)
authors' response

Line numbers (L#) in authors' comments refer to those in the revised PDF.

See diff.pdf for highlighted differences between the original submission and the revised version.

1 Reviewer 1

The turnover within a species from one genetic means of sex determination to another is surprisingly common, and it poses a challenging puzzle for evolutionary geneticists. Existing theory on the topic suggests that there must be either sexually-antagonistic selection or selection variants that bring the sex ratio closer to 1:1. This paper builds explicit models of the situation and asks about the role of selection in the haploid stage (i.e. meiotic drive and gametic selection), and finds that such effects can also serve as the primary driver of turnover of sex determining mechanisms. While this is a rather focused topic, it is a striking evolutionary puzzle, and the paper presents a very satisfying solution that is much more general than we had previously thought. It also overturns the primacy of Fisherian sex ratio evolution as the driver of the dynamics of genes of this sort.

The authors devise a satisfyingly simple genetic scenario, with one ancestral sex-determining locus, one locus under selection, and a third locus for the nascent sex determining locus. They manage to collapse some messy algebra into terms like the "haplotypic growth rate" that allow the reader to intuit many of the results. I only have a few suggestions:

1.1

The selected locus allows for three modes of selection: gametic selection (weighting the frequencies of gamete genotypes), the usual diploid viability selection, and meiotic drive (where heterozygotes may produce gametes that deviate from 50:50 proportions). It should be noted that the model does not accommodate fertility or sexual selection.

Response One could interpret our diploid selection as fertility selection, or am I missing something? Sexual selection does seem like a different model though, with for example, male fitnesses depending on female frequencies; a note such as "We do not consider sexual selection" in the paragraph beginning near L111 might do it?

1.2

The "haplotypic growth rate" plays a really essential role in providing intuition about the results. Similarly the $\chi_{mi}^{(XY)}$ terms also are crucial. I suggest giving a short name for the chi terms (currently they are the "rate at which mutant haplotypes on background i recombine onto the other A locus background in heterozygotes"...). The importance of these becomes clear in Table 2, but I wonder if there is a graphical way to make their meaning even clearer.

Response We now call these terms the "dissociative force" as they break down linkage disequilibrium (L162).

1.3

It needs to be stated somewhere that the models are entirely deterministic, implying that the authors assume that the selective forces always exceed the force of random drift. It might be useful to discuss this a bit. Is there evidence that in no case has drift been a key factor in changes of sex determination system? The discussion mentions alleles of weaker effect, but does not mention that in this scenario, models of weak effects would need to consider drift as well.

Response We now mention that the model is deterministic, and hence ignores drift (L136). We later mention that drift can in fact be a key factor in transitions: "It should be noted, however, that the dynamics of sex-determining alleles with very weak effect will be influenced by genetic drift, which itself has been shown to cause trans-GSD transitions (Veller et al 2017)." (L511)

1.4

A glitch appears in the section "Loose linkage with the ancestral sex-determining region" on line 270, where the text reads, "To leading order in selection, the leading eigenvalues are..."

Response We have dropped the words "the leading eigenvalues are".

1.5

Gametic selection among sperm is a bit different from sperm competition, since the latter involves pair-wise (or multi-way) bouts, whereas gametic selection assigns constant fitnesses to each haplotype. I would replace occurrences of "sperm competition" with "male gametic selection", or maybe "sperm gametic competition". To be consistent, replace "egg competition" with "female/egg gametic competition".

Response We have made the suggested replacements.

1.6

I am not certain of this, but it seems that a potentially important difference between ESD and GSD has to do with the variance in proportion male? Since ESD (in a constant environment, as is implicitly modeled here) has a binomial sampling of sexes, but GSD might deviate from this. This might be an issue because Fisherian sex ratio selection acts on the mean and not the variance.

Response In our deterministic model (where the number of binomial trials is infinite), the variance in the fraction male is 0 with ESD, as it is with GSD. We now mention this on L392: "In our deterministic model this means the fraction female is exactly k, even when m is rare (exploring the effect of the variance induced by ESD would also be interesting)."

1.7

Full sharing of the Mathematica file is to be commended.

2 Reviewer 2

Scott et al. present a 3-locus model for sex-determination (SD) evolution (1 locus has an ancestral SD function; 1 has a derived SD function; 1 locus affects selection in the haploid or diploid phase of the life cycle). Their results converge on previously-identified conditions for the spread of new SD alleles, while also broadening the range of scenarios and conditions that can favour transitions between SD systems.

The paper is interesting and conceptually appropriate for PLoS Biology. The authors have done a great job of analysing a somewhat complicated model and drawing out a relatively complete picture of the conditions favouring the evolution of new sex determination systems, and I expect their expert treatment will go over well with people that are close to the research context of the new theory.

On the other hand, I am much less sure that this paper will reach beyond experts in this subject area, though the authors should seek to broaden the appeal. This is not because the results are narrow – I think they can have a broad appeal. Rather, the paper is written in a technical manner that is likely to limit the appeal beyond the expert class. I am reasonably comfortable with models like this, but still found the presentation to be very hard going in a variety of places, particularly in the results. I offer some suggestions for presentation that may help improve the presentation and broaden the potential appeal of the paper.

2.1

I think the introduction could be simplified so that the context of the study is more obvious. The authors essentially need to make three key points, which are there, but could be better drawn out. First, transitions between sex-determining systems are common, and recent empirical work has given us a much greater appreciation for just how common these transitions are. Second, there are two major mechanisms that are thought induce evolution of new sex-determination mechanisms: (1) selection to stabilize the sex ratio, and (2) hitchhiking between (tightly linked) sex-determination and sexually antagonistic alleles. Third, the authors should clearly outline the major limitations of current theory as a means of motivating their mega-model that captures an array of genetic and selection scenarios that may lead to transitions in sex determination. As I mentioned, these elements can all be found in the introduction, but the pitch can be substantially streamlined to allow readers to easily see: (1) what is the context? (2) What do we currently think drives transitions between SD systems? And (3) what are the holes in the theory and how will the authors address them?

Response The introduction has been simplified to be as follows. The first paragraph discusses how common transitions are (context). The second and third paragraphs discuss the two major mechanisms currently hypothesized to drive the observed transitions, respectively (current theory). The fourth paragraph explains how assuming ancestrally-tight sex-linkage produces previously unexpected transitions (one hole in current theory, and how we address it). The fifth paragraph discusses the potential complications of haploid selection for transitions (a second hole in current theory). The sixth paragraph describes previous studies on transitions with haploid selection (attempts to address the second hole). The final, seventh, paragraph states how our approach differs from previous attempts and the major findings we uncover (how we address the second hole).

2.2

Likewise, the results yield three major insights:
(1) SD transitions can, counterintuitively, potentially INCREASE sex ratio bias
(2) Tight linkage and sexual antagonism are not required for transitions to occur
(3) Under haploid selection, conditions for the spread of new sex-determining alleles are extremely permissive.
Again, all of these points are there, but they could be better drawn out than they currently are. The results, as written, are hard-going, and I expect will be particularly challenging for readers that are not heavily immersed in evolutionary theory.

One potential way to help readers – particularly those interested in what the paper has to say, but are not mathematically inclined – is to conspicuously highlight each important result and briefly place it into context of earlier theory. But at the same time, the authors have done a great job of drawing out general mathematical results from a relatively complicated model – their mathematical insights ARE the results and I am not suggesting that they be minimized or shunted into appendices.

One tactic for including analytical details which specialists will want to see, while allowing less mathematically inclined readers to easily find each main result, is to place short and accessible summaries at the close of each section of the Results. Such a tactic has been put to good use in a hugely influential paper by Allen Orr (1998 Evolution), which I expect the authors will be familiar with. Orr presents the major mathematical results of his analysis alongside prominently labelled punchline paragraphs that summarize each of his main results in a concise and easily understandable manner. If the authors choose to take this approach, please be clear to place the take-home messages in context: clearly state whether the result confirms previous theory, or represents a new result or contradiction of an earlier result.

Response We now give 4 main conclusions in the results, in the style of Orr 1998:
(1A) ancestrally-tight sex-linkage allows looser sex-linkage to evolve in trans-GSD transitions (with or without haploid selection),
(1B) haploid selection allows looser sex-linkage to evolve in trans-GSD transitions,
(2) transitions can occur when new sex-determining alleles increase linkage with haploid selected loci,
(3) when selection is weak relative to recombination, sex-ratio selection is exactly equal in magnitude to the selection imposed by associations with selected alleles, and

(4) transitions from genetic to environmental sex-determination are not straightforwardly predicted by selection to balance the zygotic sex ratio when haploid selection is present.

As (1A), (1B), and (2) have some antecedents in the literature, we discuss these immediately below each conclusion.

2.3 MINOR COMMENTS

2.3.1

L3: "of diploid sexes" can be deleted

Response Deleted.

2.3.2

L12: missing a ")"

Response Fixed.

2.3.3

L35-37: sentence could use an edit for ease of reading.

Response We have replaced "Thus, if the population sex ratio is biased towards one sex, the average per-individual contribution of genetic material to the next generation from the opposite sex is greater" with "Thus, if the sex ratio is biased, an individual of the rarer sex will, on average, contribute more genetic material to the next generation".

2.3.4

Immediately preceding the paragraph beginning line 44: It would be worth establishing the limitations of previous work. What exactly motivated you to do this mathematical modelling?

Response We have rearranged and rewritten the introduction to better motivate our model (see Response 2.1). In particular, paragraphs 6 and 7 explain the previous attempts and our approach, respectively.

2.3.5

L56-57: Rephrase for clarity "individual cases of meiotic drive are generally sex-limited, and exclusively effect male or female gametes". And on line 57, "sex specific" could be changed to "sex-limited".

Response Rewritten as suggested.

2.3.6

Lines 80-81: This primary finding is completely aligned with the earlier views of theory that are summarized above. Are you sure you want to lead with this as the main emphasis of the study? It understates what is new.

Response We have rewritten the introduction, as well as our main conclusions (see Responses 2.1 and 2.2).

2.3.7

M locus is dominant to X. How important is this assumption? Is this compatible with any data (e.g., on feminizing or masculinizing factors in Musca domestica)?

Response This is an important assumption. As discussed in the supplementary of van Doorn & Kirkpatrick (2007, Nature), partial dominance and incomplete penetrance (partial epistatic dominance) reduce the range parameter values that favour transitions. However, this reduction in parameter space is smooth and does not preclude transitions, meaning the qualitative conclusions are robust. Further, there are many systems in which one sex-determining allele is epistatically dominant. And finally, our Mathematica file allows for arbitrary epistatic dominance coefficients. We now discuss the empirical evidence for epistatic dominance, our general Mathematica file, and the analysis of van Doorn and Kirkpartick when introducing our model (L117).

2.3.8

Paragraph beginning L104: the sex symbols are not ideal. The combined male/female symbol has been used In other contexts to refer to "male and female" rather than representing either/or

Response We have replaced the male/female symbol with a circle.

2.3.9

Table 2: a footnote to the table would be useful in defining the terms in the equations, some of which are far less obvious than others.

Response We have extended the footnote to define all terms in Table 2.

2.3.10

L169-170. This statement makes sense, but I wonder when such an initial condition would arise, since invasion criteria are evaluated at an equilibrium state? What ancestral conditions lead to the equilibrium sex-ratio bias, upon which the invasion results are based? This is touched on later, but a statement up front will help to not leave the reader hanging.

Response We have flipped the statement to talk about male biased sex-ratios instead, and mention that this is possible at equilibrium under the more familiar scenario of meiotic drive in males.

2.3.11

L195: define the epsilon character, which has not appeared before, or specify the size of the selection parameters relative to recombination, since this is the key point of contrast (e.g., s << r).

Response We have replaced this instance of ϵ with the more familiar $s << r$ style assumption. When we introduce ϵ in the weak selection section we now verbally explain that it is just a number that is much less than one.

2.3.12

Fig. 2, panel C. You might consider using log scale on the y-axis of the inset, since the blue and green curves do not really show.

Response The blue and green curves should not really show as they are not expected to increase from a very small initial frequency. We have kept the original scale but now state in the legend that the blue and green loci do not invade.

2.3.13

Eq. (2): the use of V_A is not really useful here. Much more straightforward to place the p(1 - p) in the main equation.

Response We have kept the V_A as we reuse this shorter, strictly positive, term throughout the text. This is also consistent with the well-known van Doorn and Kirkpatrick 2007, 2010 models.

3 Reviewer 3

This work (henceforth [SOO]) explores the role of both diploid and haploid selection on the evolution of new sex-chromosomes and or transitions between sex-determination systems. For that purpose, the authors develop a population genetics model of three loci with two alleles in each locus. The first locus X is the ancestral sex-determining locus, the second locus A is the locus under diploid and haploid selection. The third locus M is the novel sex-determining locus.

3.1

I find the article is not clearly written and could benefit from improving the presentation of the main results. I also find the article claims that: (a) the modeling work is novel and (b) the findings are unexpected to be only partially justified. The works of Kozielska 2014 and Ubeda 2015 model the combined effects of both haploid and diploid selection on the evolution of new sex-chromosomes. The work of [SOO] is thus not novel in considering both haploid and diploid selection on the evolution of new sex-chromosomes. It does generalize previous work by considering sex-specific viability selection and get some nice analytical results.

Response We have performed an extensive re-arrangement (e.g., Responses 2.1 and 2.2 and below) to improve the clarity of our writing, especially regarding the main results. We believe that our new presentation makes the novel and unexpected features of our results much more apparent. In particular, as described at length below, we now give more weight to the two previous papers suggested (Kozielska et al 2010 and Ubeda et al 2015) and more precisely indicate which of our model features and results are new and unexpected in light of these previous results. To be clear, we do not claim to be the first to include both haploid and diploid selection to a model investigating the evolution of new sex-chromosomes and we do not consider the addition of sex-specific selection or analytical results for previously numerical models to be our most significant contributions (again, see Responses 2.1 and 2.2 and below for more detail on what these are).

3.1.1

The findings in [SOO] claimed to be surprising ("Surprisingly, we find that neither force (sex ratio selection nor associations with genes that have sex-specific effects) dominates the spread of new sex-determining systems alone.") ... [but this result is rather similar to those in Ubeda et al 2015].

Response Here, we were referring to the result that $\lambda_{W'}^{XY} = \lambda_{Y'}^{ZW}$ with haploid selection, even when sex-ratio biases evolve to become either stronger or weaker (e.g., $\lambda_{Y'}^{ZW}$ vs. $\lambda_{W'}^{XY}$ with $R < r$ and male meiotic drive) or when sex-ratio biases are either present or absent (e.g., $\lambda_{W'}^{XY}$ vs. $\lambda_{Y'}^{ZW}$ with $r < 1/2$, $R = 1/2$, and male meiotic drive). The Ubeda et al. (2015) step 2 result shows that associations with a haploid selected locus can favour a new sex-determining allele despite causing biased sex ratios. Nevertheless, it was difficult for us to intuit whether the presence/absence of ancestral sex-ratio bias would have a negligible or overwhelming effect on the evolution of new sex-determining alleles. For example, step 3 of Ubeda et al. (2015) involves the spread of a autosomal meiotic drive suppressor favoured by Fisherian sex-ratio selection and Kozielska et al. (2010) show that ancestral sex-ratio bias can favour new sex determining systems that balance the sex ratio (now discussed in the Introduction, lines 56-66). We found our result, that these forces are exactly equal (with selection weak relative to recombination), such that there is no difference between the propensity for XY-ZW or ZW-XY transitions, to be surprising. To clarify our meaning we have rephrased (in Author summary): "Surprisingly, we find the two forces (sex-ratio selection and associations with genes that have sex-specific effects) will often be equally strong, and thus neither dominates the spread of new sex-determining systems alone."

3.1.2

and unexpected ("Even more unexpectedly, we find that, to spread, new sex-determining alleles do not necessarily have to arise in closer linkage with genes that are differentially selected in males and females"), are rather similar to those in Ubeda et al 2015. This is a problem that repeats itself over the paper

Response The Ubeda et al 2015 results do indeed show that differential selection between male and female diploids is not required for new sex-determining alleles to spread; they show that a new genetic sex-determining allele can spread from ancestral ESD when it is linked to a locus that experiences sex-specific meiotic drive. In this sentence, we were attempting to stress that new sex determining alleles can spread when they are *less closely linked or completely unlinked* to loci under differential male/female haploid and/or diploid selection. To our knowledge, this has only been shown for the special case of male meiotic drive in an ancestral XY system with $r = 0$ and $R = 1/2$ with a neo-W, which is driven by sex-ratio selection (Kozielska et al 2010). We therefore found the large parameter space allowing invasion with $r < R$, sometimes in the absence of sex-ratio selection (or even in the absence of haploid selection), to be very unexpected. To communicate this we have rephrased: "Even more unexpectedly, we find that new sex-determining alleles can spread despite being less closely linked to selected loci under a wide range of conditions." We believe that the changes to the presentation of the Introduction and Results should also help to clarify this point further (e.g., paragraph, lines 40-47, and more clearly highlighted conclusions on lines XXX; see Responses 2.1 and 2.2). We discuss the related results of Kozielska and Ubeda on lines 261 and 350.

3.1.3

For example in: Page 2. The authors claim that predominant theories of the evolution of sex-determining systems are two: sexually-antagonistic selection and sex-ratio selection. They ignore other two: inbreeding (Charlesworth 1978) and sex-different drive (Ubeda et al 2015).

Response Charlesworth and Charlesworth 1978 (Am Nat and Heredity) discuss the role of inbreeding (and particularly inbreeding depression) in transitions from hermaphroditism to dioecy. As noted on lines 468-472 (original submission), we have only considered GSD-GSD transitions and GSD-ESD transitions. For clarity, we have moved this description into the introduction (lines XXX). In addition, where we draw a parallel between ESD and hermaphroditism on lines 451-455 XXX (revision), we now bring up the limitation that our model of ESD does not include inbreeding.

The line referred to used sexually-antagonistic selection as an example of "selective differences between the sexes". We believe the phrase 'selective differences between the sexes' includes sex-differences in drive (Ubeda et al 2015), which just occur during haploid rather than diploid selection (see also Response 3.2.6).

We have rephrased the line referred to here to clarify our focus on GSD: "Predominant theories accounting for transitions between GSD systems by selection involve sex-ratio selection or selective differences between the sexes (e.g., sexually antagonistic selection)".

3.1.4

Page 3 "Here we use mathematical models to find the conditions under which new sex-determining systems spread when individuals experience selection at both diploid and haploid stages, which allows fitness differences between the sexes and sex ratio biases to occur simultaneously". This would be the same model as Ubeda et al 2015 adding sex-specific viability.

Response As we now make clear, we do not look at the same situation as Ubeda et al 2015 and our contribution is not just to add sex-specific viability. In particular, Ubeda starts from ESD. Therefore, associations with an ancestral sex-determining locus cannot build up. These lead to some of our most surprising results (e.g., haploid selection favours trans-GSD transitions for a wider variety of genomic locations than diploid selection alone; ESD does not invade despite initial sex-ratio bias). Further, our model and intuition-giving analytical solutions explore a much wider range of scenarios than the numerical results of Ubeda, allowing, for example, sex-specific selection (e.g., sex-antagonism) and various genomic arrangements (any order/linkage of the three loci). Our model is thus much more general and clarifies just when one should expect to see the numerical results of Ubeda et al 2015. We have

rephrased to make this clear (after discussing Ubeda et al 2015, see Response 2.1): "Here, we analytically find the conditions under which new GSD or ESD systems spread with generic linkage between the loci involved (and generic sex-specific haploid and diploid selection)." (L67-69).

3.1.5

Lines 68-69: "If we assume that haploid selection at any particular locus predominantly occurs in one sex (e.g., meiotic drive during spermatogenesis), then such loci experience a form of sex-specific selection."

This is a stretch and creates confusion. I consider selection acting on individuals with different sex-specific selection, now when selection is acting on gametes (which are neither males or females) males or females are just different environments in which selection may take place. Neither the dynamics nor the constraints are the same.

Response In this study, we are referring to organisms, such as animals and angiosperms, in which the type of gamete depends on the sex of the diploid that produced it. In this respect, one could consider gametic selection among sperm, for example, to be a form of male-specific selection. However, as noted here, the dynamics of selection during gametic selection cannot be captured by a diploid selection coefficient, which is why we model selection upon haploid genotypes specifically in two ways. We have attempted to clarify by expanding our explanation of the mating system and the way we model haploid selection (see Response 3.2).

3.1.6

Lines 70-71: "In this respect, we might expect that haploid selection would affect transitions between sex-determining systems in a similar manner to sex-specific diploid selection (as explored in [31,32])"

Following up my previous comment Ubeda 2015 explores this and shows that both forms of selection are different. For example sex-antagonistic selection is needed for the invasion of a new sex-determining gene with diploid selection while antagonistic drive in each of the sexes is not needed for the invasion of a new sex-determining gene with haploid selection.

Response In the absence of sex-differences in selection it is true that antagonistic drive is not needed, but *differences* in haploid selection between the sexes are needed (e.g., drive in only one sex, maintained by ploidally-antagonistic selection). If drive was the same in both sexes, $\alpha^m = \alpha^f$ (and all other male/female selection terms also equal), we show that the new sex-determining allele would not invade (e.g., equation 2 and 3). In this sense haploid selection is similar to sex-specific diploid selection, and together haploid and diploid selection determine the differences in male vs. female fitness, which can drive sex-determination transitions (see Response 3.2.4). This sentence meant to introduce the 'sex-specific' aspect of haploid selection in the way Ubeda et al (2015) considers it; i.e., associations with alleles favoured in one sex (or the gametes produced by that sex) should favour a new sex-determining allele. We have rephrased to make this clear: "Therefore, one might expect new sex-determining systems to benefit from close linkage with haploid selected loci, as found for sex-differences in diploid selection" (L51-53). We now follow this sentence up with a paragraph that clarifies its meaning by comparing with the results of Kozielska et al (2010) and Ubeda et al (2015).

3.1.7

Lines 77-78: "It is not immediately clear how the spread of new sex-determining systems would be influenced by the combination of sex ratio biases and associations with haploid selected allele"

This is indeed the focus of Ubeda et al 2015.

Response While Ubeda et al 2015 did focus on sex ratio biases and haploid selected alleles, the system started with ESD, where there are no associations (see Response 3.1.4). Further, the restricted scope of that numerical study prevents us from generalizing beyond its special case (see Response 3.1.4). Lines 48-66 present a refurbished argument for why it is difficult to intuit the implications of haploid selection, while summarizing the results of Ubeda and Kozielska.

3.1.8

I will not provide further examples, but it is surprising thatI the work of Ubeda et al 2015, which is closely related to the one presented here, is cited in page 12 supporting a statement only tangential to the work of Ubeda et al 2015.

Response Ubeda et al 2015 was already cited on line 396 of the original submission, where we mentioned that it showed that sex-ratio biases could become more severe during a transition. Nevertheless, we now discuss Ubeda et al 2015 in the introduction, results, and discussion (see Responses above).

3.2

In terms of modeling the distinction between meiotic drive and gametic competition is unnecessary and only complicates notation. Furthermore, from biological perspective the division seems blurry with molecular biologists considering that meiotic drive only happens in females and never in males and what is often called meiotic drive in males is gamete competition. I would suggest using a single parameter measuring transmission distortion.

Response We do not agree. Given our definition of meiotic drive and gametic competition in the Introduction, the two processes are different. In particular, meiotic drive is assumed to occur during meiosis, and thus is "gametic competition" amongst the gametes produced by a single individual. As such, meiotic drive can only have an effect in heterozygotes. Gametic competition on the other hand, is competition amongst all the gametes produced by one sex (e.g., pollen from many male plants). Thus allele frequency changes can occur by gametic competition even if all individuals were homozygous (as long as there is a polymorphism). We have rephrased our definitions in the Introduction to read: "We use the term 'meiotic drive' to refer to the biased (non-Mendelian) segregation of genotypes during gamete production (from one parent only) and the term 'gametic competition' to refer to selection upon haploid genotypes within a gamete/gametophyte pool (from many parents)."