Dear Pr. Baer,

Please find enclosed a revision of our manuscript (PCI Evol Biol #484) entitled “Masculinization of the X-chromosome in aphid soma and gonads”.

As recommended, we have taken at heart to consider the comments, and made appropriate modifications. Our responses appear below.

We thank you for the useful comments and for considering this revised manuscript.

Sincerely,

J. Jaquiéry and co-authors

Round #2

by Charles Baer, Tanja Schwander and Tanja Schwander, 05 Jun 2022 18:35
Manuscript: https://doi.org/10.1101/2021.08.13.453080 version 2

Masculinization of the X-chromosome in aphid soma and gonads

I only have one very specific criticism, which I don't think warrants additional review, but it does require either a rebuttal from the authors or a modest revision, which would not require additional work, only additional thinking and/or scholarship.

Minor comments on Jaquiéry et al., "Masculinization of the X-chromosome in aphid soma and gonads"

Sexual antagonism (SA), wherein the fitness interests of the sexes do not align, is inherent to organisms with two (or more) sexes, because sperm are cheap and eggs (and parental care) are expensive. SA leads to intra-locus sexual conflict, where an allele that confers higher fitness in one sex reduces fitness in the other. This situation leads to what has been referred to as "gender load" (why not "sex load" is unclear, but try typing those keywords into Google and see what you get), resulting from the segregation of SA alleles in the population. Gender load can be reduced by the evolution of sex-specific (or sex-biased) gene expression, in which the expression of the deleterious allele is suppressed in the sex in which it is deleterious. A specific prediction is that gene-duplication can lead to sub- or neo-functionalization, in which the two duplicates partition the function in the different sexes. The conditions for invasion by a SA allele differ between sex-chromosomes and autosomes, leading to the prediction that (in XY or XO systems) the X should accumulate recessive male-favored alleles and dominant female-favored alleles; similar considerations apply in ZW systems.

Aphids present an interesting special case, for several reasons: they have XO sex-determination, and three distinct reproductive morphs (sexual females, parthenogenetic females, and males). Previous theoretical work by the lead author predict that the X should be optimized for male function, which was borne out by whole-animal transcriptome analysis.

Here, the authors extend that work to investigate tissue-specific, sex-specific gene expression. They argue that, if intra-locus SA is the primary driver of sex-biased gene expression, it should be generally true in all tissues. They set up as an alternative the possibility that sex-biased gene expression could also be driven by dosage compensation. They cite references supporting their argument that "dosage compensation (could be) stronger in the brain", although the underlying motivation for that argument appears to be based on empirical evidence rather than theoretical predictions.

At any rate, the results are clear: all tissues investigated show masculinization of the X. Further, X-linked copies of gene duplicates were more frequently male-biased than duplicated autosomal genes or X-linked single-copy genes.

To sum up, this is a nice empirical study with clearly interpretable (and interpreted) results. The prediction that sex-biased gene expression resulting from some selective force other than SA should lead to variation among tissues, whereas SA should lead to uniform variation is not justified on clear theoretical principles; if such principles exist, they should be explicitly-stated. For example, I could imagine a situation in which all of the sex-biased gene expression was the outcome of sexual antagonism, but for some reason only some tissues experienced SA selection. If such principles are not forthcoming, the stated motivation for the study has the feel of a straw man.

Thank you for this evaluation and for your comments.

The main criticism is about the lack of theory behind the prediction of the chromosomal location of sex-biased genes in the different tissues. We agree with your comment but the reason we could not present clear theoretical predictions that would have included sexual antagonism (SA) and other factors (such as dosage compensation and meiotic sex chromosome inactivation) is simply because such an integrative and general model does not exist.

Various factors can lead to sex-biased gene expression, among which are 1) sex-specific selection/sexual antagonism, 2) dosage compensation and 3) meiotic sex chromosome inactivation (MSCI). These factors may also result in a non-random distribution of sex-biased genes among sex chromosomes and autosomes (because either sex chromosomes may be more or less invaded by SA variants, or imperfect dosage compensation may lead to an excess or deficit of sex-biased genes on sex chromosomes, or genes important for gamete production cannot locate on sex chromosomes if MSCI occurs).

SA has been the focus of considerable theoretical development (see next paragraph), but the role of dosage compensation and MSCI on the non-random chromosomal distribution of sex-biased gene is mostly known from empirical observations in the few best-studied species. Note also that - when present - a diversity of mechanisms of dosage compensation exists depending on species, and dosage compensation may also be partial or absent and vary between tissues/organs. MSCI has only been studied in a very limited number of model species. For these reasons, it is difficult to consider these two factors in our theoretical predictions, as very little is known about dosage compensation in aphids, and even less for MSCI. Indeed, the present study is the first one to perform RNAseq on different tissues of male aphids).

SA has been the focus of several theoretical studies in species with XX/XY and ZZ/ZW sex determination systems (e.g. Rice 1984 Evolution, Connallon & Clark 2010 Evolution, Fry 2010 Evolution, Connallon & Clark 2011 Genetics, Connallon & Clark 2012 Genetics, Connallon & Clark 2014 Proceedings of the Royal Society London, McGlothlin, Cox & Brodie 2019 Journal of Heredity, …). However, to our knowledge,

none of them has explicitly considered different types of tissues. The idea that some tissues (especially sexually dimorphic tissues) are more likely to have evolved under sexual antagonism or to be currently under the influence of sexual antagonism is appealing and often assumed in the literature, though empirical demonstrations remain scarce (Ingleby et al 2015 Cold Spring Harbor Perspective in Biology, Mank 2017 Nature Ecology and Evolution). Empirical data also show that sex-biased genes are more frequent in sexually dimorphic tissues, though determining whether sex-biased expression is the cause or the consequence of dimorphism remains challenging (Mank 2017 Nature Ecology and Evolution). In any case, these theoretical models do not apply to aphids, because of the particular inheritance of their X chromosome and their cyclical parthenogenesis, which requires the development of specific models (Jaquiéry et al 2013 Plos Genetics).

As a result, given the lack of data on dosage compensation and MSCI in aphids, we were not able to account for these factors in our theoretical predictions regarding the chromosomal location of sex-biased genes in different tissues. We now clearly mention these limitations in the introduction (lines 121-134). We have also removed a part in the abstract that was ambiguous (line 29).
As suggested, we now mention that some tissues might be more prone to sexual antagonism, and that we observe that sex-biased genes are more frequent in sexually dimorphic tissues (lines 138-139, 196-197, 420-424, 437-439). We also point out that, regardless of the extent of sexual antagonism (which might vary across tissues), our empirical data are consistent with the prediction that male-beneficial alleles tend to locate to the X, and that some of the intra-locus conflicts appears to have been mitigated by the evolution of sex-biased gene expression (lines 139-142).

We hope that we have adequately addressed your concerns, and we believe that this has improved the clarity of the manuscript.