Peer Community In Evolutionary Biology

Dosage compensation by upregulation of maternal X alleles in both males and females in young plant sex chromosomes

Tatiana Giraud *and* ***Judith Mank*** *based on peer reviews by 3 anonymous reviewers*

Aline Muyle, Niklaus Zemp, Cecile Fruchard, Radim Cegan, Jan Vrana, Clothilde Deschamps, Raquel Tavares, Franck Picard, Roman Hobza, Alex Widmer, Gabriel Marais (2018) Genomic imprinting mediates dosage compensation in a young plant XY system. bioRxiv, ver. 1, peer-reviewed and recommended by Peer Community in Evolutionary Biology. 10.1101/179044

Submitted: 20 September 2017, Recommended: 19 February 2018

Cite this recommendation as:
Giraud, T. and Mank, J. (2018) Dosage compensation by upregulation of maternal X alleles in both males and females in young plant sex chromosomes. *Peer Community in Evolutionary Biology*, 100044. 10.24072/pci.evolbiol.100044

Published: 19 February 2018

Sex chromosomes evolve as recombination is suppressed between the X and Y chromosomes. The loss of recombination on the sex-limited chromosome (the Y in mammals) leads to degeneration of both gene expression and gene content for many genes [1]. Loss of gene expression or content from the Y chromosome leads to differences in gene dose between males and females for X-linked genes. Because expression levels are often correlated with gene dose [2], these hemizygous genes have a lower expression levels in the heterogametic sex. This in turn disrupts the stoichiometric balance among genes in protein complexes that have components on both the sex chromosomes and autosomes [3], which could have serious deleterious consequences for the heterogametic sex. To overcome these deleterious effects of degeneration, the expression levels of dosage sensitive X-linked genes, and in some organisms, entire X chromosomes, are compensated, the expression of the single copy of in the heterogametic sex being increased. Dosage compensation for such genes has evolved in several species, restoring similar expression levels as in the ancestral state in males and/or equal gene expression in males and females [4-8]. The mechanisms for dosage compensation are variable among species and their evolutionary paths are not fully understood, as the few model sex chromosomes studied so far have old, and highly degenerate sex chromosomes [4-7]. Muyle et al. [9] studied the young sex chromosomes of the plant *Silene latifolia*, which has young sex chromosomes (4 MY) and highly variable dosage compensation [10, 11]. The authors used both an outgroup species without sex chromosomes for obtaining a proxy for ancestral expression levels before Y degeneration, and implemented methods to identify

sex-linked genes and disentangle paternal versus maternal allele expression [12]. Using these elements, Muyle et al. [9] reveal upregulation of maternal X alleles in both males and females in the young *S. latifolia* sex chromosomes [9], possibly by genomic imprinting. The upregulation in both sexes of the maternal X alleles likely yields non-optimal gene expression in females, which is strikingly consistent with the theoretical first step of dosage compensation as postulated by Ohno [8], which predicts restoration of ancestral expression in males, over-expression in females, and unequal expression in the two sexes. These findings provide surprising insight into the earliest stages of dosage compensation, one of the most intriguing aspects of evolutionary biology.

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Reviews

Evaluation round #2

DOI or URL of the preprint: **10.1101/179044**
Version of the preprint: 3

Authors' reply, 07 February 2018

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Decision by Tatiana Giraud, posted 07 February 2018

Revise

We have now received two referee reports for your resubmitted manuscript. Both referees are positive in their assessment of the revised version of your paper, although one has some additional suggestions. In particular, the referee would like more discussion on some findings that are only in supplementary material while not completely fitting the message of the main text, such as the findings on the X-hemizygous contigs and on the validated contigs.

We have read through the paper ourselves and found also that the previous concerns were satisfactorily addressed, but we had the following suggestions: - It would be useful to explain how you dealt with multiple SNPs in X/Y contigs. We understand from the equation E = r/ (n * l) that Y and X expression is a composite, but have the authors looked to see whether all SNPs in X/Y contigs show concordant parent-of-origin imprinting, or whether the significant is driven by one or two SNPs in a contig? Lack of concordance across SNPs in a single coding sequence has been an issue in previous studies of parent of origin imprinting (Gregg, Zhang, Weissbourd, Luo, Scroth, Haig, Dulac Science 2010 & Gregg, Zhang, Butler, Haig, Dulac Science 2010). Related to this, is the composite nature of Y vs X expression the cause of the wide confidence intervals in Fig 1?
-Another question lies with the different categorization of dosage compensation in Silene over the past few years. Initial reports by these authors indicated "rapid evolution of dosage compensation" (Muyle et al PLOS Biology 2012), followed by reports by other teams of "largely absent dosage compensation" in Silene (Bergero et al. Current Biology 2015), "highly variable dosage compensation" (Papadopulos et al. PNAS 2015) and most recently "incomplete dosage compensation" (Zemp et al. Nature Plants 2016). It would be helpful to explain why these characterizations lack concordance. Is it due to methodological differences, or different sets of X/Y genes? Or is it just the result of different operational definitions?
-Please make sure all data are made available (both new genomic and transcriptomic data, as well as the identity of sex-linked contigs, with accession numbers given in the text when applicable). Al Supplementary tables should be either in the main PDF or with an associated doi number. -1st paragraph P2: replace "Although, sensu stricto" by "Yet, sensu stricto"
-1st paragraph P2 : I would find useful for a broad audience to have a brief explanation about the rationale of Ohno's hypothesis on the evolution of dosage compensation. Why would it be more advantageous to have a suboptimal gene expression in females than in males?
-1st paragraph P4: a reference is needed in the sentence about convergence with marsupials.
-P3: "validated contigs": unclear enough

After you have satisfactorily addressed these minor concerns, we will be happy to write a recommendation for your preprint.

Tatiana Giraud and Judith Mank

Reviewed by anonymous reviewer 1, 05 December 2017

After reading the response letter and the revised manuscript thoroughly, I am satisfied that my concerns have been thoroughly addressed. In particular, the discussion of dosage compensation in the introduction is much improved, and the results well justified. I am also impressed with the detail devoted to methods in the supplementary material. I believe that this manuscript has the potential to have considerable influence within the field of sex chromosome evolution.

Reviewed by anonymous reviewer 3, 12 December 2017

I have read with attention the revised manuscript of Muyle and co-workers entitled "Genomic imprinting mediates dosage compensation in a young plant XY system". They studied the expression patterns of the sex-linked genes in Silene latifolia, and showed that the reduced expression from the degenerated Y chromosome is compensated by the upregulation of the maternal X chromosome is both sexes. This scenario is reminiscent of the early steps of the X inactivation in mammals proposed by Ohno. I would like to express satisfaction concerning the quality of the manuscript. I really enjoyed reviewing this paper: it is clearly written, competently analysed I guess (I am not a specialist in bioinformatics), and of high interest.

On the whole, I agree with all comments given by the two previous referees and the PCI Evol Biol recommender, and I am satisfied with most responses from the authors. It is a beautiful story which I want to believe, but I have one main concern. The pattern does not seem to be as clear as the main text suggests.

1) For some reasons, X-hemizygous contigs have been analysed separately, and only discussed in supplementary text. They showed that X-hemizygous contigs have poor dosage compensation, and that the parental origin of the X chromosome has limited to no effect on female X-linked gene expression (i.e., no upregulation of the maternal X). These results are strikingly surprising, as we should expect the opposite pattern, with stronger dosage compensation in the oldest strata, since (i) the Y copies have been lost, and thus the balance of gene products needs to be restored, (ii) there was enough time for dosage compensation to be recruited. Indeed, this is what we observe in the mammalian dosage compensation. The authors give some explanations, with which I mostly agree, but they are not completely satisfactory for me, as these hypotheses alone could not explain the complete absence of dosage compensation in this class of genes. More troubling, although this difference in dosage compensation between X hemizygous and X/Y genes is very important to understand the system, it is only discussed in supplementary data. In the same way, the categorie 0 has been removed from the Figures 1 and 2, but kept in the supplementary figures. As a reader, my feeling is that the authors tried to hide this part which does not fit the general conclusions of the paper.

2) The scenario is not that clear for the validated contigs (no significant upregulation of the maternal X), and again this part is downgraded to the supplementary text.

3) Figure 1 shows an excess of expression of female maternal alleles relative to an outgroup in the last two categories only

Hence, my main conclusion is that the results (and the title) should not be overstated.

Evaluation round #1

DOI or URL of the preprint: https://doi.org/10.1101/179044

Version of the preprint: 2

Authors' reply, 04 December 2017

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Decision by Tatiana Giraud, posted 04 December 2017

Revise

This manuscript has been evaluated by two referees, who agree that the findings represent an important advance in our understanding of the early stages of dosage compensation in sex chromosomes, an important topic in evolutionary biology. The second referee nevertheless raises concerns about the lack of a reverse cross, which impedes disentangling maternal/paternal imprinting from strain effects. The second referee also suggests that the discussion should be more balanced, highlighting the differences in pattern strength found between figures and experiments. Both referees further suggest some improvements regarding clarity, mainly on the hypotheses in the introduction and discussion, on the outgroup and the methods, and they have a list of questions which the revised manuscript should answer to. The main figures are missing and should be added in the PDF. In addition to these referees' suggestions, I also have the following recommendations for improving the manuscript. As a non-specialist of dosage compensation, I found the main text hard to understand and had to read the text several times to fully understand hypotheses and findings. If the authors target a broad audience, it would be useful to expose more clearly hypotheses and inferences, using less specific jargon. If room is needed, I would move the discussion on proximal mechanisms to supplementary text, and also the discussion about buffering explaining sex equality, as this pattern is not found in the present study. In addition, I wondered whether "using the ratio of Y over X expression levels in males as a proxy for Y degeneration" could not be circular. Indeed, the ratio of Y over X expression levels in males includes both the effect of Y degeneration and X compensation in males, and not only degeneration. Looking at dosage compensation as a function of a measure of Y degeneration that integrates dosage compensation may sound circular. I do not think the inferences are circular though, but I would be more careful in the definitions in the text and in the figures, and it may be better to take as a measure of Y degeneration differences or ratio of expression between Y and the outgroup autosome? About the main figures, why are they plotted and statistically analysed as discrete classes rather than as continuous variables? To sum up, there is potential for a highly interesting and novel contribution to the field of evolutionary biology. However, the paper needs careful revision along the lines above. If you are able to accommodate these points, I would encourage resubmission to PCI Evol Biol for recommendation.

Reviewed by anonymous reviewer 1, 28 November 2017

Muyle et al aim to address an important question in sex chromosome research, the mechanism by which dosage compensation evolves. They study gene expression patterns across sex-linked genes in Silene latifolia, a plant with young sex chromosomes. They show that Y degeneration is associated with upregulation of the maternal X allele, presumably to compensate for unequal gene dose, and provide support for the steps proposed by Ohno in his theory of dosage compensation. Not only does this research provide further insight into the status of dosage compensation in plants, which have been relatively understudied relative to animals, but also represents an important advance in our understanding of the early stages of sex chromosomes evolution. I very much enjoyed reading this paper; it was very clear and represents an important advance. I have some suggestions that should help to improve the study.

The discussion of the steps associated with the evolution of dosage compensation in the introduction are a little confusing. Currently, the introduction reads that equal expression between the sexes is a form of dosage compensation. This has been debated (Mank & Ellegren 2009 Heredity; Melamed et al 2009 Heredity) however, according to the definition proposed by Ohno, expression equality between the sexes is just a side effect of dosage compensation, and maintaining dose between the X and autosomes is the key step. Unequal

gene dosage will select for the upregulation of the single X in the heterogametic sex in order to re-establish ancestral expression levels, and then compensatory evolution in the homogametic sex will restore balanced gene expression. Following this, it would be helpful if the introduction were revised to more clearly outline these steps. In addition, it might be useful here to mention briefly why unequal gene dose between the X and autosomes is thought to negatively affect fitness.

It would be useful to have information in the main text about the outgroups and when they diverged from S. latifolia. When did the sex chromosomes in S. latifolia evolve? This would also be helpful in understanding why the authors averaged expression level across the two outgroups – are they equally diverged from S. latifolia? It is difficult to interpret how this may have influenced the results. Can the authors show that expression is S. viscosa and S. vulgaris is highly correlated for all genes and for Y/X genes with Y degeneration?

Figure 1 and Figure 2 appear to be missing. However, the supplementary figures were very clear and detailed. There appears to be a bracket missing on the Y axis.

The difference in dosage compensation between X hemizygous and X/Y genes is very interesting but only discussed in the supplementary text. I would recommend moving this to the main text and discussing there the potential dosage insensitivity of X hemizygous genes. The authors don't report whether X genes in general are significantly depleted in ribosomal protein coding genes. This is an important test, is it just X hemizygous genes, is there a significant difference between X/Y genes? Related to this, it would be useful to mention in the figure legends that X hemizygous genes have a mean Y/X expression ratio in males = 0 whereas all other categories are X/Y. For example, on page 3 it says 'for X/Y contigs, the difference between maternal and paternal X in females increases with Y degeneration', but clearly this is not the case for genes with mean Y/X expression ratio of 0.

For genes with no Y decay (mean Y/X expression ratio in males = 1-1.5), the Y allele appears to be upregulated in males relative to the ancestral allele. Is this because of sex-specific selection?

It would be useful to mention in the methods why it is important for SEX-DETector that X and Y sequences are assembled in the same contig. This is mentioned in the reference transcriptome section but not explained.

It would be good to mention dosage compensation in Tribolium, which seems to have a parallel mechanism to the pattern the authors find. The female X chromosome is hyper transcribed relative to autosomal expression levels but the X and autosomes in males are compensated. Prince, E. G., Kirkland, D. & Demuth, J. P. Hyperexpression of the X chromosome in both sexes results in extensive female bias of X-linked genes in the Flour Beetle. Genome Biol. Evol. 2, 336–346 (2010).

I assume that the genes shown in Figure 1, Supplementary Figures are genes with unbiased expression (as sex-biased genes were removed). It would be useful to have another supplementary graph showing the magnitude of expression differences between the sexes for these genes. I assume that the expression equality is achieved despite upregulation of the maternal X in females, because the upregulation of the maternal X in males is much greater?

Reviewed by anonymous reviewer 2, 28 November 2017

Mule et al. look at allele-specific expression of S. latifolia, a plant whose young sex chromosomes have acquired partial dosage compensation. They find that, contrary to the expectation if this was simply due to general buffering mechanisms, the maternally derived X chromosome of females is also up-regulated relative to the outgroups. They interpret this as evidence for an imprinting-based mechanism of dosage compensation. S. latifolia is a great model in which to study the evolution of dosage compensation, and the examination of paternal and maternal allele expression is an important step forward.

My main concern is that, as far as I understand, the authors did not perform the experiments on the reverse cross (Leuk144-3*mother x U10*37_father), and therefore cannot control for strain of origin effects. My impression is that this would be essencial for imprinting to be conclusively detected. As it is, the results are very suggestive but difficult to interpret. Since they pick genes that:

-do not have sex-biased expression

-do not differ in expression with the outgroup

-whose expression is lower for the Y copy than the X copy

Then in males these genes will by definition have increased X (maternal) expression. Since the same maternal X (or at least an X from the same strain) is being transmitted to the daughters, increased maternal expression may be expected even if there is no imprinting.

Another issue is that the patterns are not as clear as figure 2 and the main text seem to imply:

*The 0.75-1 category looks very different in figure 1 (no difference between maternal/paternal allele) and figure 2 (very significant difference).

*For 0.5-0.75 there seems to be a difference in figure 1, but not due to overexpression of the maternal allele.

*in flower buds the effect is stronger for the 0.25-0.5 category than 0-0.25.

*in leaf and flower bud an excess of maternal allele expression is detected for the autosomes in females, and in leaf an excess of paternal expression is detected in males for the 0.57-1 category.

*there is generally no significant excess of female maternal allele expression for the validated contigs (figures s4 to s6).

I understand that these could simply be due to noise/lack of power, but they should be addressed more openly in the text. It was particularly confusing that even though flower bud data was used for the sequencing of the parents, and for the reference transcriptome, seedling is the tissue shown in the main figures. This should be justified.

Other:

*It seems surprising to me that there is no differential expression between S. latifolia and its outgroups - such differences are usually found even between strains of the same species.

*Random selection of 200 autosomes: I think an important additional control would be to use the smallest sample size (79) and run a bootstrapping procedure to get a probability of being different from 0 in female.

*"Autosomal normalised expression levels in the two outgroups (S. vulgaris and S. viscosa) were averaged together." –> this does not seem like a standard approach. Was the analysis checked using each separately?

*The numbering in the supplementary methods is inconsistent (e.g. 4) Validation of sex-linked contigs and 4) Expression level estimates).

*Normalized fpkm: was this normalized any further than just calculating fpkm, which is often not sufficient? If not, did you check the distribution of fpkm between samples to exclude biases?

*The text could do with a few more details, even if most of the methods are supplementary. For instance:

-you don't mention the crossing scheme that you used at all.

-In page 2, a sentence explaining how you defined sex biased genes would have been useful.

-The number and type of tissues used is never mentioned in the main text.

-In page 3, it would be useful to have a qualification of the close relatedness of the species.