Response to the referees/editors – 2nd round

Decision

by Tatiana Giraud, 2018-01-18 16:18
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PCI recommendation for the preprint "Genomic imprinting mediates dosage compensation in a young plant XY system"

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.

R: We are thankful to the referees and editor for reviewing and recommending our manuscript by PCI Evol Biol. We address additional suggestions below.

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?

R: Indeed, Figure 1 shows an average of SNPs for each contig. The confidence intervals are large because for some contigs there are few SNPs and also because within one group of X/Y expression ratio, contigs have different levels of dosage compensation.
Figure 2 shows SNP by SNP analysis and clearly shows a consistent pattern across all SNPs with small confidence intervals. There is therefore concordance across SNPs for imprinting in our data. Fig. 2 legend has been modified to make it clear that it shows a SNP-wise analysis.

-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?

R: The first papers on dosage compensation in S. latifolia were indeed contradictory because they focused on different gene sets. Muyle et al. 2012* focused on X/Y gene pairs while other papers (Chibalina & Filatov 2011, Bergero et al. 2015) focused on X-hemizygous genes. However, the X-hemizygous gene sets returned by the RNA-seq approach used in those papers is much less reliable than the X/Y gene sets (Blavet et al. 2015). A gene might be inferred as X-hemizygous simply because the Y copy is not expressed in the tissue sampled for RNA-seq. In S.

latifolia, X-hemizygous genes tend to be less expressed than X/Y genes and are less likely to be detected by segregation analysis (as efficient SNP calling require a certain read depth, Blavet et al. 2015). In Papadopulos et al. 2015, 25% of the Y/X chromosomes were sequenced using a genomic approach. A much higher fraction of X-hemizygous genes was found than in previous RNA-seq papers. Papadopulos et al. 2015 did find evidence for dosage compensation in X-hemizygous genes (see their figure 3 A-D, X-hemizygous genes are shown in 3D). We feel that this question (the difference between X/Y gene pair and X-hemizygous genes) has already been addressed in Papadopulos et al. 2015 and it has been widely discussed in a recent review (Muyle et al. 2017). We don't think it is useful to address it again in this paper, which is why the data for X-hemizygous genes are in the supplementary material. We have nevertheless tried to make it clearer in the R2 version by changing both the main text and supplementary text 1.

* and later in Zemp et al. 2016, although dosage compensation is not the question addressed by this paper.

-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.

R: Data will be submitted during revision of the manuscript for publication in a Journal.

-1st paragraph P2: replace "Although, sensu stricto" by "Yet, sensu stricto"

R: Done

-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?

R: Done

-1st paragraph P4: a reference is needed in the sentence about convergence with marsupials.

R: We added a reference for the mechanism of X-inactivation in marsupials

-P3: "validated contigs": unclear enough

R: We now explain what we mean by validated

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

Reviews

Reviewed by anonymous reviewer, 2017-12-05 11:02
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.

R: We thank referee 1 for her/his positive feedback

Reviewed by anonymous reviewer, 2017-12-12 17:43

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.

R: see our reply to the editors' similar comment. The issue with the X-hemizygous genes is very technical and has already been addressed previously (Blavet et al. 2015; Papadopulos et al. 2015; Muyle et al. 2017). We have nevertheless changed the section on the X-hemizygous genes in the main text to make clear why these genes are analysed separately.

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.

R: The trend is the same for the validated and all contigs. The results are not as significant for the validated contigs compared to all contigs, which is not surprising as the dataset is divided by half and therefore we lose statistical power (see error bars in Supplementary Figures S5,S6,S7). Please bear in mind that our gene sets include genes with dosage compensation and genes without dosage compensation (as in S. latifolia dosage compensation does not affect all the genes, see Papadopulos et al. 2015), which explains why error bars are large. This is already indicated in the main text: "although statistical power is sometimes lacking due to the low number of validated contigs, Supplementary Figures S2-S7". The same is true for the linear model analysis and we now repeat it when we mention Figures S8-S13.

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.

R: Figure 1 shows a gradual increase of the delta expression for maternal X allele. For intermediate categories the values are close to zero, and it is not surprising that it is not significant. Note that in other tissues (leaf, flower buds), significant differences are found for the same intermediate categories (see Figures S3, S4). Moreover, figure 2 shows significant differences for all categories.