Commentary

Not all sex-biased genes are the same

Sexual antagonism has long fascinated biologists. It has been proposed to be the driver behind sex-chromosome evolution, it is a major influencer in the generation of biodiversity and it is responsible for the fastest rates of molecular evolution known (Swanson & Vacquier, 2002). The ideas of sexual antagonism and sexual conflict (Parker, 1979) developed back in the days when one of the main ways university students learned about biology was through observation and dissection of organisms, and computers were programmed for biological tasks by punching cards. With the massively parallel sequencing revolution (Koboldt *et al.*, 2013), much of the thinking behind biological processes, such as sexual antagonism, is now done by computational biologists, who can readily access genes with a possible involvement in sexual conflict. For example, there has been an explosion of studies of sex-biased genes (Grath & Parsch, 2016) and anyone interested in the consequences of sexual antagonism and sexual conflict can study their effects on gene expression in any organism.

'Sex-biased genes can be very different, depending on the tissue and species studied, and not all of them evolve under the same constraints.'

In this issue of the *New Phytologist*, Sanderson *et al.* (pp. 527–539) characterize sexual dimorphism in gene expression in a long-lived dioecious tree (*Populus balsamifera*), adding a rare type of datapoint to the literature. The authors compared gene expression between the sexes from both reproductive (catkins) and somatic tissues (leaves). They found very limited sexual dimorphism in leaves, while reproductive tissues showed higher sexual dimorphism – *c.* 30% of the genes expressed in catkins were sex-biased. Sanderson *et al.* did not include genes that were only expressed in one sex, which should exclude most gene expression arising from sex-specific tissues.

Unlike most similar studies in other organisms, including dioecious willows (Darolti *et al.*, 2017), there were twice as many female-biased genes compared to male-biased genes. This result demonstrates the usefulness of studying species from many domains of life, reproductive modes, and life histories. It nicely illustrates that very different genes can be sex-biased when different tissues or species are compared, and suggests that not all sex-biased genes evolve under the same constraints. In this context, the recent suggestion to split sexual dimorphism into primary and secondary, based on direct association with gamete production or not (Charlesworth, 2018), is a welcome step in the right direction. However, separate analysis of smaller subsets of sex-biased genes is likely to better inform independent biological processes that may produce results that cancel out when all sex-biased genes are analysed together.

Sex-biased genes are often assumed to evolve fast, because of their potential direct involvement in sexual antagonism (Zhang *et al.*, 2004). In this context, the finding of Sanderson *et al.* that female-biased genes in catkins had atypically slow evolutionary rates is surprising. A similar result was obtained in the basket willow and may be attributed to haploid selection (the expression of many genes in haploid cells, as part of the normal life cycle of a plant), which would result in strong purifying selection (Darolti *et al.*, 2017). To understand why these genes evolved slowly, the function of the sex-biased genes needs to be taken into account. Many of the female-biased genes identified by Sanderson *et al.* were associated with photosynthesis, which is an evolutionarily conserved process. Other explanations for slow evolutionary rates – such as the authors' suggestion involving a combination of the old age of the sex chromosomes, prolonged dioecy and the assumption that rapid sequence evolution only occurs early in sex-chromosome evolution – may still be valid. However, these factors would be better suited to a subset of female-biased genes that excludes the evolutionarily conserved, female-biased, photosynthesis genes, such as those associated with immunity, which were also enriched in the female-biased genes identified by Sanderson *et al.*

The differentially expressed genes between the sexes will differ between species with different sexually dimorphic tissues. This is obvious to anyone who has held a *P. balsamifera* catkin and a *Silene latifolia* flower. However, when discussing sex-biased genes, cross-species comparisons in the numbers or rates of evolution of sex-biased genes are common. It is unlikely that all the genes differing in expression between sexes have the same evolutionary constraints, as illustrated in *P. balsamifera*, where many female-biased genes have resulted from a reduction in photosynthesis in male catkins. Treating sex-biased genes as a homogeneous group of genes, and investigating their evolution in different tissues and organisms, does not capture the complexity of differences in male and female biology.

The example of photosynthesis also illustrates the limitations of removing sex-specific genes and employing $\log_2$ fold change thresholds when defining sex bias, to avoid the effects of tissue composition, as Sanderson *et al.* did. While such methods are recommended to reduce the effects of allometric differences between samples (Montgomery & Mank, 2016), they do not help with comparisons between sexually dimorphic tissues, because

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fundamental differences between the sexes are due to sex-specific tissues. One solution is to avoid gene-expression comparisons between reproductive tissues, but this would remove tissues with genes showing high evolutionary rates, such as pollen in *Arabidopsis thaliana* (Gossmann *et al.*, 2014) or accessory gland proteins in *Drosophila* (Swanson & Vacquier, 2002). Instead it is more important to accept that the comparison of two samples arising from the two sexes will never capture the complexity of sexual antagonism in a whole organism, such as sex allocation in hermaphrodites, sex-specific growth rates, differences in stress and investment in immunity.

One way to encompass these biological realities relating to sexual antagonism is to embrace the fact that the assumption that most parameters are unchanged when comparing two samples is not valid when two sexes are compared. Other than tissue composition, the two sexes often differ in the timing of gene expression. For example developmental time is different between animal meiotic tissue, with meiosis being ongoing or stalled in male and female animals, respectively. In addition, the sexes frequently differ in their overall pace of life, with males often adopting a 'live fast, die young' strategy (Immonen *et al.*, 2018). Plants have the potential to inform our understanding of sexual dimorphism in many phenotypes associated with the pace of life, because they represent many independent data points of different life cycles, with trees representing one extreme, and annual plants the other. The availability of a plethora of sexually dimorphic traits makes plants particularly well suited to the study of sexual antagonism in biologically realistic conditions. These include some sexually antagonistic traits such as flowering time (Meagher & Delph, 2001) and other traits whose sexual antagonism interacts with environmental conditions (such as specific leaf area, which is sexually dimorphic only in sites with high water availability in *S. latifolia*; Delph *et al.*, 2011).

It is time to incorporate more biology in the discussion of sex-biased genes. One such case is when accounting for tissue specificity in gene expression, when investigating the evolutionary rate of sex-biased genes, which finds high tissue specificity in gene expression to explain fast protein-coding evolution better than sex bias (Meisel, 2011). The influence of genes associated with photosynthesis on the evolutionary rate of female-biased genes in *P. balsamifera* catkins is, essentially, such an example. Another parameter to consider involves the proportion of genes that are expressed in the haploid phase, which prevents degeneration of the allele restricted to the heterogametic sex (Chibalina & Filatov, 2011). The fact that such diverse cases of interesting biology can be studied through sex-biased gene expression, illustrates that grouping of genes into male- and female-biased is too broad. Sex-biased genes can be very different, depending on the tissue and species studied, and not all of them evolve under the same constraints.

The rationale for studying non-model organisms is often to understand how the tweaks in textbook biology that our species of interest represents affect evolutionary processes. Realizing and understanding the underlying biology behind sex-biased genes is essential before understanding the evolutionary forces that have resulted in their observed state of expression and sequence evolution. Gene expression studies such as Sanderson *et al.* are only the first step towards understanding the contribution of sexual antagonism to the observed differences between species.

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