THE TRANSCRIPTIONAL ARCHITECTURE OF PHENOTYPIC DIMORPHISM

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PREFACE: The profound differences between the sexes in gene expression are increasingly used to study the molecular basis of sexual dimorphism, sexual selection and sexual conflict. Studies of transcriptional architecture, based on comparisons of gene expression, have been implemented on a wide variety of other intra-specific variation. These efforts are based on key assumptions regarding the relationship between transcriptional architecture, phenotypic variation and the target of selection. Some of these assumptions are better supported by available evidence than others. In all cases, the evidence is largely circumstantial, leaving considerable gaps in our understanding of the relationship between transcriptional and phenotypic dimorphism.

KEYWORDS: Sexual selection; sexual conflict; regulatory variation; organismal complexity

A major focus of evolutionary biology is concerned with identifying and understanding the selection pressures shaping phenotypic variation, and this often requires identifying the underlying genes. There are excellent examples where the genetic basis of phenotypic variation has been identified via genetic mapping approaches (e.g.[1,2]), but more complex phenotypes have often been more difficult to map. The democratization of RNA-Seq has made it possible to cheaply and quickly quantify RNA abundance for all expressed loci in virtually any organism. As a result, gene expression comparisons are increasingly used to identify transcriptional architecture, genes with expression differences between phenotypic variants. Ideally, functional genetic assays can be used to confirm the phenotypic effect of differential expression (for example[3,4]). However this is not always possible, either because the study organism lacks functional genetic tools, or because the number of differentially expressed genes is simply too large. This means that we often must assume that genes expressed differently between phenotypes are related to, and perhaps even responsible for, these complex phenotypic differences.

These methods have been used in studies of a wide array of phenotypes, including lip morphology in cichlids[5,6], castes in eusocial insects[7-9], behavioural adaptations in guppies[10], among many others. However, transcriptional architecture has perhaps been most often studied in the context of sexual dimorphism, arguably the most pervasive form of intra-specific diversity in the animal kingdom. The prevalence of sexual dimorphism in animals was sufficiently striking to prompt Darwin's conjecture of sexual selection as a force distinct from natural selection[11]. Indeed, beyond primary sex differences, sexual dimorphism is seen in a broad range of complex phenotypes, affecting morphology, physiology, behavior and life history, among many other traits. And yet, sexual dimorphism is in many ways a form of

polyphenism, or even just an extreme form of phenotypic plasticity, and the study of sexual dimorphism has implications to a broad array of intra-specific variation.

Central to the study of castes[7-9], sexes, or other forms of polyphenism and phenotypic plasticity, is the problem of how a single genome encodes often radically divergent phenotypes[12]. In addition, studies of regulatory adaptation based on underlying genetic differences often use similar approaches, based on the assumption that fixed regulatory differences cause expression differences of underlying genes (e.g.[5,6,10]). In the case of males and females, some aspects of dimorphism result from genes restricted to the sex chromosomes[13]. However, many organisms with pronounced dimorphisms lack sex chromosomes entirely[14], and even in those species that do have sex chromosomes, it is clear the majority of dimorphism results from genes that are present in both sexes. Expression is one way that genes can be deployed differently, and many loci show profound differences in male and female expression, referred to as sex-biased genes. Gene expression differences between males and females therefore offer a possible route to the genomic study of sexual dimorphism and sexual selection, but only if it is indeed true that sex-biased genes encode sexually dimorphic traits and are shaped by sex-specific selection. From this framework, studies of the transcriptional architecture of sexual dimorphism have progressed over the past ten years or so, and several assumptions about the relationship have solidified. First, it is often stated that sex-biased expression underlies phenotypic sexual dimorphism, and it remains unclear what proportion of sex-biased genes are relevant at the phenotypic level. Second, many assume that sex-biased genes represent resolved sexual conflict over optimal expression, as it is this resolution that permits transcriptional, and therefore phenotypic, sexual dimorphism. Finally, we often search for the footprint of sexual selection in the

sequence and expression characteristics of sex-biased genes, assuming that we can find it there. But does the evidence support any of these assumptions?

Some of these assumptions are better supported by available evidence than others, but in all cases, the evidence is arguably circumstantial. My goal here is to explore these assumptions with the evidence in hand in order to identify gaps in our understanding of the relationship between transcriptional and phenotypic dimorphism. Moreover, the number and diversity of organisms that have been studied in this context is remarkable, spanning models such as *Drosophila*[15,16], non-model invertebrates[17] and vertebrates[18,19], as well as plants[20,21], fungi[22] and algae[23]. This diversity presents an inherent power to identify convergent signals that transcend species-specific idiosyncrasies.

I. Sex-biased genes as an aggregate underlie sexual dimorphism

It is often assumed that some proportion of sex-biased genes are collectively responsible for phenotypic dimorphism, and the assumed connection between transcriptional and phenotypic variation is not unique to studies of sexual dimorphism, as studies of other forms of intra-specific diversity[7-9] use similar approaches. A staggeringly large proportion of coding sites exhibit different expression levels in males and females, depending on where in the body and when in the life cycle transcription is measured. Adult gonad samples, or whole-organism RNA preparations that include the gonad, can show significant differences in expression between the sexes for more than half of all expressed genes[24,25]. Samples based purely on somatic tissue show fewer expression differences, but even then there is substantial variation[26,27].

Is the aggregate pattern of sex-biased expression responsible for sexual dimorphism? In other words, what proportion of sex-biased genes important in sexual dimorphism, and how direct

is the relationship between RNA abundance and phenotypic dimorphism? The answer to this relatively simple question has major implications to whether we can use expression screens to identify genes underlying phenotypic variation of any kind, as well use those genes for downstream evolutionary analyses.

Although we often think of sex as a dichotomous trait, there is substantial intra-sexual variation, and this has been used to test for correlations between sex-biased genes as an aggregate and phenotypic dimorphism. The results are broadly consistent. For example, work in birds has shown that more dimorphic tissues within the body exhibit greater levels of transcriptional dimorphism[28]. Work in birds and *Drosophila* concordantly shows that the magnitude of sex-biased expression amplifies through development, and is most manifest in adults, mirroring the ontogeny of phenotypic dimorphism[24,29]. Studies of alternative male mating morphs in the wild turkey show that the magnitude of phenotypic dimorphism for each morph tracks roughly with the magnitude sex-biased expression[19]. In *Drosophila*, sex-biased expression exhibits condition-dependence[30], much like many sexually selected traits[31]. Finally, sex reversal due to infection in *Silene latifolia* produces the expected inversion of sex-biased expression that matches phenotypic sex, rather than sex chromosome complement[21]. The above studies measure a correlation between transcriptional and phenotypic dimorphism, but they do not identify the causal from dependent variable. Proving sex differences in expression cause, and are not a consequence of, phenotypic dimorphism requires additional functional analysis, and functional studies have revealed that sex-biased expression is not always a perfect indicator of sex-specific phenotypic effects. For example, *distal-less*, a developmental gene, is responsible for dimorphism in antennae shape in a water strider. Although this gene is expressed in both sexes, its phenotypic effects differ between males and females[4], perhaps due to sex-specific regulatory interactions, or perhaps subtle

expression differences between males and females that were not detected. Additionally, we might expect mutations in sex-biased genes to have sex-specific phenotypic effects, and although this is broadly true in *Drosophila*[32], mutations in many loci with substantial differences in expression affect both sexes, suggesting the relationship between sex-biased expression and phenotypic dimorphism is often indirect, or even counter to expectations.

Implications

There is clearly a relationship of some sort between total sex-biased expression and phenotypic dimorphism when averaged across the genome. However, there is significant variation, and not all loci show a direct or additive phenotypic effect. This suggests that a subset of sex-biased genes are at least connected with sexual dimorphism, but the exact proportion of this subset is unclear. Moreover, transcriptional comparisons between males and females may not always identify all loci underlying phenotypic dimorphism.

Even if only a subset of sex-biased loci are important for phenotypic dimorphism, the large proportion of the genome that exhibits sex-biased expression implies an extraordinary complexity to dimorphic phenotypes. As a result, we must expect that many sexually dimorphic traits are encoded by a large array of loci, many of potentially small effects. This is supported by QTL and GWAS studies of complex dimorphisms[33-36], as well as eQTL studies of sex-bias[27,37-39], all of which indicate that these traits are extremely polygenic.

If many small effect loci do indeed underlie these traits, there are several important implications. First, our evolutionary genetic models of sexual dimorphism, sexual selection and sexual conflict tend to involve one or two loci, and their predictions, particularly regarding genomic distributions on the sex chromosomes[40,41] will need to be carefully reconsidered. Additionally, this type of complexity suggests that non-additive, interactions among loci may create unpredictable phenotypic consequences, and this complex transcriptional architecture

could lead to variation in selective coefficients depending on genomic background. Finally, this complexity may well confound mapping efforts based on quantitative genetic approaches[42], particularly in wild populations.

Outstanding questions

Studies often assess sex-biased expression in adult tissues, and this logically follows the fact that sexual dimorphism is most manifest in adult, sexually reproductive individuals. However, particularly in invertebrates[4,31], but also other organisms[43], sexual dimorphism is at least in part a developmental process. This implies that sex-biased expression during larval, juvenile or otherwise immature stages may be most important, at least for some traits, to adult-manifest dimorphisms. In line with this, developmental time-series studies have shown that sex-biased expression varies substantially over ontogeny[24,29]. How these patterns relate to adult dimorphisms is not yet known.

Similarly, if many sexual dimorphisms result from juvenile expression patterns, what exactly are we measuring in studies of adult transcription? It is possible that some studies, particularly when whole adult bodies are used for RNA preparation, are simply measuring the transcriptional effects of allometry and cell type abundance differences (BOX 1) resulting from different developmental programs of females and males. Future experimental designs should account for developmental processes in sexual dimorphism. It would be particularly useful to know which adult phenotypes are the product of juvenile expression patterns, and which ontogenetic expression stages contribute most to adult dimorphisms. Additionally, it will be interesting to see how many genes show sex-biased expression in single-cell transcriptome analyses[44].

Finally, not all sex-bias is the same, and some loci show statistically significant yet small expression differences, while expression differences in other genes can be orders of

magnitude different (Figure 1). It remains unclear how the magnitude of sex-bias scales with phenotypic dimorphism, although there is some evidence that loci with greater levels of transcriptional dimorphism might be more important in phenotypic differences[19].

II. Sex-biased genes represent resolved sexual conflict over optimal expression

Males and females have different optimal measures for many traits. For example, in humans, the relationship between height and overall fitness differs substantially between the sexes[45]. At the same time, many traits show strong correlations between males and females (often referred to as intersexual correlation, or r_{MF})[46-48], as we might expect if they are based on a shared genetic architecture[49,50]. In these instances, differences in fitness optima between the sexes can lead to sexual conflict, or contradictory selection pressures on the underlying genes[46-48].

Intersexual correlation is typically viewed as a constraint[51] on the evolution of sexual dimorphism. We assume that only by breaking these correlations, a result of conflicting selection between the sexes, can males and females reach separate optima for a given trait or expression level. Therefore, dimorphism, whether in phenotypic traits such as human height or in gene expression, is thought to represent at least partially resolved sexual conflict over fitness optima[52,53].

Similar correlations are not limited to studies of sexual dimorphism, and would be expected in many other cases of polyphenism[54-56], or ecological morphs[57], and studies of genetic correlations are important for understanding genomic flexibility in encoding multiple phenotypes in general. The extent of genetic correlations among castes, morphs and sexes, and the speed to which it can be broken down, governs how effectively a single genome can encode multiple phenotypes, and whether there are costs to this genetic strategy.

Because it has been difficult to identify sexually antagonistic alleles within the genome, sex-biased gene expression has been used as a proxy for at least partially resolved sexual conflict over optimal expression[52,58]. This is supported by the overall high correlations observed between male and female expression (Figure 1), suggesting that contradictory forces acting on males and females is needed to generate sex-biased expression. It is important to note that although the assumptions for gene expression mirror findings from phenotypic studies[46,47,49], there has thus far been no direct test of whether sex-biased genes were originally under conflicting selection for expression in females and males. There is, however, substantial circumstantial evidence that supports the analogy between phenotypic traits and expression.

Consistent with the assumption that sex-biased gene expression results from the breakdown in intersexual correlation for transcription, convergent studies across populations in Drosophila[51] and birds[59] show that loci with differences in expression between the sexes tend to have lower intersexual correlation than genes with similar expression levels in males and females[51,59]. It remains unclear however whether sex-biased expression emerges as intersexual correlation decreases, or if sex-biased expression is simply more likely to evolve at loci with low inter-sexual correlations to start with. Importantly, some genes with large expression differences between females and males show high correlations between the sexes, suggesting that the intersexual correlation does not always need to be completely degraded to achieve sex-biased expression [59]. Additionally, pleiotropy, or multiple functionality, appears to hinder the evolution of sex-biased expression[60,61], or at least sex-biased genes tend to have more restricted expression than unbiased genes, consistent with the idea that loci under fewer functional constraints are more able to respond to conflicting selection between the sexes in any one functionality.

Other evidence comes from comparisons across the genome. Sexual conflict theory[62-64] predicts that the unique inheritance patterns of sex chromosomes will lead to the accumulation of sexually antagonistic variation in these regions. Consistent with this, sex chromosomes in a variety of organisms, including Drosophila[65], mammals [63], aphids[62], birds[66], flour beetles[67] have been shown to have an excess of sex-biased genes. Although there are other potential causes, these results suggest that sexually antagonistic regulatory variation could have accumulated on the sex chromosomes, and that this has to some extent been resolved through sex-biased expression. Along these lines, we might also expect older sex chromosomes, or older regions of sex chromosomes, to have greater sex-bias if the process is cumulative. Work in birds fits with this prediction, and shown that sexualization of expression accumulates over time, as older regions of sex chromosomes show a greater magnitude of sex-biased expression[68,69] compared to younger regions.

Finally, we might expect that gene expression differences between males and females would shift if sex-specific selection pressures shift to new male and female optima. This has indeed been shown to the be the case, and altering the direction and magnitude of sexual conflict produces the changes we would predict in male and female transcription in both birds[70] and *Drosophila*[15,71].

Implications

Intersexual genetic correlations in expression for any type of gene, sex-biased or not, is typically viewed as a constraint on the evolution of dimorphism[51], and this is likely true to at least some degree. However, not all loci are under conflicting selection pressure between the sexes, and so it is likely that in genomic surveys all genes, many, if not most loci with strong genetic correlations do not represent intersexual compromise, but rather loci under convergent selection in males and females.

To understand the degree of the constraint imposed by intersexual correlation we need to know how quickly it can be reduced once selection pressures diverge between males and females. The direct data are sparse, but evidence suggests that phenotypic intersexual correlations can be reduced in a few generations when divergent selection between the sexes is strong[46]. Indirect evidence is more abundant. The rapid turnover of sex-biased expression observed among closely related species[18,25] implies that if intersexual genetic variation is a substantial constraint, it is one that is easily and quickly navigated if sexual conflict is sufficient. The turnover of sex-bias across closely related species[18,25] also suggests that there is a substantial amount of sex-specific regulatory variation present in the genome, permitting different regulatory architectures in males and females and therefore reducing intersexual correlation for expression. This is also consistent with the speed at which sexually dimorphic traits evolve across closely related species in response to sexual selection[72].

Studies of sexual conflict arguably often focus on the sources of conflict, i.e. constraints and antagonism, rather than routes to resolution. That means that the potential for abundant sex-specific regulatory variation in reducing intersexual correlation may be sometimes overlooked[59]. However, it is worth noting that regulatory variation can act in very targeted ways, often far more complex than just two sexes (Figure 2). For example, caste-specific patterns of expression are abundant within social insects[7,9,73], and the number of castes nearly always exceeds the number of sexes. Additionally, tissue-specific regulatory variation permits organismal complexity[74] , and can alter expression within specific regions of the body plan without affecting it in others over short evolutionary time-scales[2,75]. Also, many genes show remarkable regulatory differences due to environmental interactions, even permitting different morphs depending on the season[57,76]. Given these forms of regulatory diversity, perhaps the abundance of sex-specific regulatory variation is not so surprising.

Outstanding questions

Nothing at this point is inconsistent with the idea that sex-biased expression represents at least partially resolved sexual conflict via the breakdown of intersexual correlation. However, our data remains circumstantial at this point, albeit increasingly substantial. It is also important to note that even within sexes, the expression of many genes are highly correlated with others, ensuring that selection on expression of one gene will produce a correlated response in others [77-79]. This suggests that sex-biased expression for some genes is due to selection at other, upstream loci. Finally, attempts to link sex-biased expression and sex-specific fitness more directly implicate relatively few loci[16], and this might suggest that sex-biased genes largely represent past resolved conflict, but could also be the product of non-additive polygenic traits.

With a few notable exceptions[80,81], conjectures about how expression is decoupled between males and females remain largely mechanism-free. Additionally, most studies of intersexual correlation in expression have not accounted for sex-specific patterns of alternative splicing, which can be common[82], and isoforms may offer an important route to resolution of conflict over expression of specific exons within a gene. Moreover, we have relatively little understanding of how quickly intersexual correlation in expression can be reduced, as well as the strength of selection required. The key to breaking down intersexual correlation for expression is sex-specific regulatory variation[83], and at this point we have hardly any estimates of what proportion of regulatory variation is sex-specific in its effects, or how such variation acts. Genome-wide association studies for medically relevant traits in human have revealed largely separate regulatory architectures in males and females[35,84-87]. If this is true more broadly across the Tree of Life, then it suggests the intersexual correlation is quickly broken down, as the regulation of many genes is largely independent in each sex.

III. The rapid rate of evolution for sex-biased gene expression and sequence results from sexual selection.

Early studies of sex-biased genes in *D. melanogaster* reported that male-biased genes show elevated rates of sequence and expression evolution[88,89], and this was interpreted as a result of the fact that sexual selection acts primarily on males[53] in many mating systems. The link between sex-specific expression levels and rates of evolution was based in part on earlier observations that highly-expressed genes tend to show stronger signatures of evolutionary constraint[90,91], perhaps indicating greater phenotypic importance. By analogy, genes expressed more in males should be more often shaped by male-specific selection, and genes expressed more in females shaped by female-specific selection. Because sexual selection often acts more forcefully on male traits[92], which in turn evolve more rapidly[93], it seems logical that male-biased genes should show convergent patterns to male traits. But is sexual selection indeed responsible for rapid rates of evolution for male-biased genes? Key to this is whether these fast rates of evolution are indeed adaptive.

There is a long list of species that show elevated rates of evolution for male-biased genes, including *D. melanogaster*[88,89,94], mammals[95], *C. elegans*[96] and adult birds[18]. Elevated rates of sequence evolution can result from positive selection and adaptive evolution, however it is worth keeping in mind that relaxed constraint can also lead to elevated rates of protein evolution, and this latter cause is not consistent with sexual selection. Because of this, it is important that rates of evolution (often measured by comparing the rate of non-synonymous divergence to the rate of synonymous divergence, designated as d_N/d_S, K_A/K_S or ω) be tested further to differentiate adaptive from non-adaptive signatures. Work in *D. melanogaster* has recovered stronger signatures of selection in male-biased genes[94]. However, recent work in

adult birds revealed that the rapid rate of evolution is more consistent with relaxed evolutionary constraint and genetic drift[18], and work in humans has shown that many non-synonymous changes in male-biased genes are mildly deleterious[97].

It is worth keeping in mind that the rate of evolution often scales with the degree of sex-bias. For example, the degree to which gene expression is limited to males is a strong predictor of its rate of evolution[18], and genes with lower levels of sex-biased expression do not tend to show elevated rates of evolution. This is important because recent models suggest that the mutation-selection equilibrium is different for strongly sex-biased genes[98]. In essence, genes that are effectively sex-limited in expression experience the mutational input from both sexes, but are only selected in one sex. This can give a false signal of positive selection for studies using more standard assumptions.

There are some additional problems with the assumption that sexual selection underlies the rapid rate of evolution for male-biased genes. First, the rate of evolution for male-biased genes across related bird species does not scale with the strength of sexual selection[18], as we might expect. Second, unlike *D. melanogaster*[88,89], *D. pseudoobscura* does not show a convergent pattern of rapid rate of evolution for male-biased genes, despite having a comparable level of sexual selection acting on males[99]. In brown algae, both male- and female-biased genes show elevated rates of evolution[23], and in yeast, rapid evolution is observed only for female-biased genes[22]. Finally, the rates of evolution for female-biased genes in embryonic birds is actually higher than the rate for male-biased genes in adults[24]. These counter-examples do not fit with any reasonable expectation for sexual selection.

Implications

Outside of *D. melanogaster*, the data do not seem to support the idea that sexual selection is driving rapid rates of evolution for male-biased genes. This may be at least partly due to the

fact that positive selection seems to act on a much larger fraction of amino acids in *D. melanogaster* than many other organisms [100]. Interestingly, work in fire ants on caste-biased gene expression has recovered similar elevated rates of evolution for caste-biased genes compared to genes expressed in all castes[8], but crucially, these genes also show elevated rates of evolution in related lineages without castes. This suggests that genes under relaxed purifying selection may be more likely to adopt caste-biased expression.

We might expect a similar phenomenon for sex-biased expression. If true, this suggests that rapid rates of evolution for male-biased genes are not due to sexual selection, but rather genes with rapid rates of evolution are simply more likely to become male-biased in expression. This is also consistent with the rapid turn-over of male-biased gene expression observed in both *Drosophila*[25] and birds[18], which is not seen for female-biased genes, suggesting male-biased genes are simply under fewer constraints to start with.

Outstanding questions

In addition to the possibility that the underlying models of evolution for sex-biased genes are simply not the same as for genes expressed in both sexes[8,98], there are several other potentially confounding variables that are often not accounted for. First, many studies of sex-biased gene expression are based either on gonad tissue or whole-organism preparations, creating different mixtures of gametic and somatic cells, and therefore different haploid versus diploid selection regimes. Second, in many species, reproductive biology differs between males and females, with female gametes programmed and then arrested in development and male gametes only formed in adults. This means that samples from any one life stage may not in fact identify genes involved in gametogenesis, which are likely under the strongest selection regimes. Third, studies of genic evolution tend to focus on conserved orthologs across species, and these may be less important than recent gene duplicates, which

are more common for testes-expressed, and therefore male-biased, genes[101]. Finally, the turnover of sex-bias in some lineages[18,25] may be faster than the accumulation of adaptive signatures in sequence data, or positive selection may act more often on expression level instead of coding sequence. How any or all of these variables influence perceived rates of evolution remains to be seen.

Concluding remarks

The three assumptions related to the transcriptional architecture of sexual dimorphism show substantial differences in the degree to which they are supported by empirical data. The data do seem to support some relationship between broad patterns of sex-biased expression and sexual dimorphism, although it is difficult to know at this point whether sex-biased genes identified in any particular study encode sexual dimorphism, or are a consequence of it (Box 1). There is also substantial evidence, albeit circumstantial, that sex-biased genes represent at least partially resolved sexual conflict over optimal expression. Again though, it is difficult to differentiate cause from consequence, and is not clear whether genes with low inter-sexual correlation tend to develop sex-biased expression, or whether inter-sexual correlation is reduced as sex-bias becomes more prominent. Finally, the hypothesized link between rates of evolution for male-biased genes and sexual selection seems somewhat at odds with the bulk of the data in most organisms, and it is unclear why *D. melanogaster* exhibits a seemingly unique adaptive signature. In other organisms, these patterns may be due to differences in underlying mutation-selection balance[98], or may represent relaxed functional constraints that predispose certain loci to male-biased expression[8,97].

Studies of the transcriptional architecture of sexual dimorphism are useful to the broader community of evolutionary biology because many of these assumptions carry over directly to

other studies of intra-specific variation. It is clear that we need to think carefully about how development and allometry may affect our results when designing studies of phenotypic variation of any type. Also, sex-specific (or morph- or caste-specific) phenotypic effects of regulatory variation need to be mapped more carefully, and this will also help us determine how quickly genetic correlations can be reduced, and by what routes. Finally, as transcriptome profiling becomes quicker and cheaper, it soon may be possible to actually select on male and female expression levels, in order to see how quickly expression responds to sexual conflict and chart the phenotypic consequences.

Box 1 Allometry, cell type abundance and the perils of proportional measures like RNA-Seq.

Measuring gene expression differences in the context of sexual dimorphism implies that there is some phenotypic dimorphism worth studying. In many organisms, males and females show dimorphism in the relative size of constituent body parts (Figure 3). When entire organisms are used in RNA preparations, as is often the case for insects and other small animals, this could lead to significant differences in perceived expression, even if the absolute expression within a constituent body part remains the same[102]. This is because RNA-Seq is a proportional measure, and measures of expression are actually based on the relative abundance of a given gene in the pool of all the sequence reads. Differences in the relative proportions of the organisms used for RNA preparation can therefore alter relative abundance in the RNA pool, even if expression within a given part of the organism is the same in both sexes. Although this is less of a concern for studies that measure expression on specific organs or tissues, males and females may differ in the allometric scaling among constituent parts[103], or even the relative abundance of different cell types within constituent parts[104].

The effect of allometry and cell type abundance will be strongest for genes that are specific to specific parts of the body or cell types. For genes expressed at different levels in many tissues, as commonly the case[74], the effects are less pronounced. In this latter case, the effects can be countered by using strict fold-change thresholds in addition to statistical significance alone. For example, even though allometric or cell-type abundance differences can create statistically significant differences, the magnitude will often be relatively small. In these cases, standard expression thresholds, such requiring that a gene show doubled expression between the sexes, as is common[18,25,30], may reduce the effect of differences in allometry.

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References

1 Linnen, C. R. *et al.* Adaptive Evolution of Multiple Traits Through Multiple Mutations at a Single Gene. *Science* **339**, 1312-1316 (2013).

2 Shapiro, M. D. *et al.* Genetic and developmental basis of evolutionary pelvic reduction in threespine sticklebacks. *Nature* **428**, 717-723 (2004).

3 Chen, Y. C. *et al.* Expression change in Angiopoietin-1 underlies change in relative brain size in fish. *Proceedings of the Royal Society, B* **282**, 9 (2015).

4 Khila, A., Abouheif, E. & Rowe, L. Function, Developmental Genetics, and Fitness Consequences of a Sexually Antagonistic Trait. *Science* **336**, 585-589 (2012).

5 Colombo, M. *et al.* The ecological and genetic basis of convergent thick-lipped phenotypes in cichlid fishes. *Molecular Ecology* **22**, 670-684 (2013).

6 Manousaki, T. *et al.* Parsing parallel evolution: ecological divergence and differential gene expression in the adaptive radiations of thick-lipped Midas cichlid fishes from Nicaragua. *Molecular Ecology* **22**, 650-669 (2013).

7 Ferreira, P. G. *et al.* Transcriptome analyses of primitively eusocial wasps reveal novel insights into the evolution of sociality and the origin of alternative phenotypes. *Genome Biology* **14** (2013).

8 Hunt, B. G. *et al.* Relaxed selection is a precursor to the evolution of phenotypic plasticity. *Proceedings of the National Academy of Sciences, USA* **108**, 15936-15941 (2011).

9 Patalano, S. *et al.* Molecular signatures of plastic phenotypes in two eusocial insect species with simple societies. *Proceedings of the National Academy of Sciences, USA* **112**, 13970-13975 (2015).

10 Ghalambor, C. K. *et al.* Non-adaptive plasticity potentiates rapid adaptive evolution of gene expression in nature. *Nature* **525**, 372-375 (2015).

11 Darwin, C. *The descent of man, and selection in relation to sex*. (John Murray, 1871).

12 Lande, R. Sexual dimorphism, sexual selection and adaptation in polygenic characters. *Evolution* **34**, 292-305 (1980).

13 Rice, W. R. Sex chromosomes and the evolution of sexual dimorphism. *Evolution* **38**, 735-742 (1984).

14 Bachtrog, D. *et al.* Sex Determination: Why So Many Ways of Doing It? *Plos Biology* **12**, e101899 (2014).

15 Hollis, B., Houle, D., Yan, Z., Kawecki, T. J. & Keller, L. Evolution under monogamy feminizes gene expression in *Drosophila melanogaster*. *Nature Communications* **5** (2014).

16 Innocenti, P. & Morrow, E. H. The Sexually Antagonistic Genes of *Drosophila melanogaster*. *Plos Biology* **8**, e1000355 (2010).

17 Stuglik, M. T., Babik, W., Prokop, Z. & Radwan, J. Alternative reproductive tactics and sex-biased gene expression: the study of the bulb mite transcriptome. *Ecology and Evolution* **4**, 623-632 (2014).

18 Harrison, P. W. *et al.* Sexual selection drives evolution and rapid turnover of male gene expression. *Proceedings of the National Academy of Sciences, USA* **112**, 4393-4398 (2015).

19 Pointer, M. A., Harrison, P. W., Wright, A. E. & Mank, J. E. Masculinization of Gene Expression Is Associated with Exaggeration of Male Sexual Dimorphism. *Plos Genetics* **9**, e1003697 (2013).

20 Gossmann, T. I., Schmid, M. W., Grossniklaus, U. & Schmid, K. J. Selection-Driven Evolution of Sex-Biased Genes Is Consistent with Sexual Selection in *Arabidopsis thaliana*. *Molecular Biology and Evolution* **31**, 574-583 (2014).

21 Zemp, N., Tavares, R. & Widmer, A. Fungal Infection Induces Sex-Specific Transcriptional Changes and Alters Sexual Dimorphism in the Dioecious Plant *Silene latifolia*. *PLoS Genetics* **11** (2015).

22 Whittle, C. A. & Johannesson, H. Evolutionary Dynamics of Sex-Biased Genes in a Hermaphrodite Fungus. *Molecular Biology and Evolution* **30**, 2435-2446 (2013).

23 Lipinska, A. *et al.* Sexual Dimorphism and the Evolution of Sex-Biased Gene Expression in the Brown Alga *Ectocarpus*. *Molecular Biology and Evolution* **32**, 1581-1597 (2015).

24 Mank, J. E., Nam, K., Brunstrom, B. & Ellegren, H. Ontogenetic Complexity of Sexual Dimorphism and Sex-Specific Selection. *Molecular Biology and Evolution* **27**, 1570-1578 (2010).

25 Zhang, Y., Sturgill, D., Parisi, M., Kumar, S. & Oliver, B. Constraint and turnover in sex-biased gene expression in the genus *Drosophila*. *Nature* **450**, 233-U232 (2007).

26 Mank, J. E., Hultin-Rosenberg, L., Axelsson, E. & Ellegren, H. Rapid evolution of female-biased, but not male-biased, genes expressed in the avian brain. *Molecular Biology and Evolution* **24**, 2698-2706 (2007).

27 Yang, X. *et al.* Tissue-specific expression and regulation of sexually dimorphic genes in mice. *Genome Research* **16**, 995-1004 (2006).

28 Mank, J. E., Hultin-Rosenberg, L., Webster, M. T. & Ellegren, H. The unique genomic properties of sex-biased genes: Insights from avian microarray data. *BMC Genomics* **9**, 148 (2008).

29 Perry, J. C., Harrison, P. W. & Mank, J. E. The Ontogeny and Evolution of Sex-Biased Gene Expression in *Drosophila melanogaster*. *Molecular Biology and Evolution* **31**, 1206-1219 (2014).

30 Wyman, M. J., Agrawal, A. F. & Rowe, L. Condition-dependence of the sexually dimorphic transcriptome in *Drosophila melanogaster*. *Evolution* **64**, 1836-1848 (2010).

31 Emlen, D. J., Warren, I. A., Johns, A., Dworkin, I. & Lavine, L. C. A Mechanism of Extreme Growth and Reliable Signaling in Sexually Selected Ornaments and Weapons. *Science* **337**, 860-864 (2012).

32 Connallon, T. & Clark, A. G. Association between sex-biased gene expression and mutations with sex-specific phenotypic consequences in Drosophila. *Genome Biology and Evolution* **3**, 151-155 (2011).

33 Mackay, T. F. C., Stone, E. A. & Ayroles, J. F. The genetics of quantitative traits: challenges and prospects. *Nature Reviews Genetics* **10**, 565-577 (2009).

34 Ober, C., Loisel, D. A. & Gilad, Y. Sex-specific genetic architecture of human disease. *Nature Reviews Genetics* **9**, 911-922 (2008).

35 Randall, J. C. *et al.* Sex-stratified Genome-wide Association Studies Including 270,000 Individuals Show Sexual Dimorphism in Genetic Loci for Anthropometric Traits. *Plos Genetics* **9**, e1003500 (2013).

36 Winkler, T. W. *et al.* The Influence of Age and Sex on Genetic Associations with Adult Body Size and Shape: A Large-Scale Genome-Wide Interaction Study. *Plos Genetics* **11**, e1005378 (2015).

37 Derome, N. *et al.* Pervasive sex-linked effects on transcription regulation as revealed by expression quantitative trait loci mapping in lake whitefish species pairs (*Coregonus* sp., *sahnonidae*). *Genetics* **179**, 1903-1917 (2008).

38 van Nas, A. *et al.* Expression Quantitative Trait Loci: Replication, Tissue- and Sex-Specificity in Mice. *Genetics* **185**, 1059-1068 (2010).

39 Whiteley, A. R. *et al.* The phenomics and expression quantitative trait locus mapping of brain transcriptomes regulating adaptive divergence in lake whitefish species pairs (Coregonus sp.). *Genetics* **180**, 147-164 (2008).

40 Kirkpatrick, M. & Hall, D. W. Sexual selection and sex linkage. *Evolution* **58**, 683-691 (2004).

41 Reeve, H. K. & Pfennig, D. W. Genetic biases for showy males: Are some genetic systems especially conducive to sexual selection? *Proceedings of the National Academy of Sciences, USA* **100**, 1089-1094 (2003).

42 Slate, J. From Beavis to beak color: A simulation study to examine how much QTL mapping can reveal about the genetic architecture of quantitative traits. *Evolution* **67**, 1251-1262 (2013).

43 Badyaev, A. V. Growing apart: an ontogenetic perspective on the evolution of sexual size dimorphism. *Trends in Ecology & Evolution* **17**, 369-378 (2002).

44 Tang, F. C. *et al.* mRNA-Seq whole-transcriptome analysis of a single cell. *Nature Methods* **6**, 377-U386 (2009).

45 Stulp, G., Barrett, L., Tropf, F. C. & Mills, M. Does natural selection favour taller stature among the tallest people on earth? *Proceedings of the Royal Society, B* **282** (2015).

46 Delph, L. F., Steven, J. C., Anderson, I. A., Herlihy, C. R. & Brodie, E. D. Elimination of a genetic correlation between the sexes via artificual correlation selection. *Evolution* **65**, 2872-2880 (2011).

47 Gosden, T. P. & Chenoweth, S. F. The evolutionary stability of cross-sex, cross-trait genetic correlations. *Evolution* **68**, 1687-1697 (2014).

48 Stearns, S. C., Govindaraju, D. R., Ewbank, D. & Byars, S. G. Constraints on the coevolution of contemporary human males and females. *Proceedings of the Royal Society, B* **279**, 4836-4844 (2012).

49 Fairbairn, D. J., Blanckenhorn, W. & Szekeley, T. *Sex, size and gender roles: evolutionary studies of sexual size dimorphism*. (Oxford University Press, 2007).

50 Lande, R. The genetic covariance between characters maintained by pleiotropic mutations. *Genetics* **94**, 203-215 (1980).

51 Griffin, R. M., Dean, R., Grace, J. L., Ryden, P. & Friberg, U. The Shared Genome Is a Pervasive Constraint on the Evolution of Sex-Biased Gene Expression. *Molecular Biology and Evolution* **30**, 2168-2176 (2013).

52 Connallon, T. & Knowles, L. L. Intergenomic conflict revealed by patterns of sex-biased gene expression. *Trends in Genetics* **21**, 495-499 (2005).

53 Ellegren, H. & Parsch, J. The evolution of sex-biased genes and sex-biased gene expression. *Nature Reviews Genetics* **8**, 689-698 (2007).

54 Ament, S. A., Corona, M., Pollock, H. S. & Robinson, G. E. Insulin signaling is involved in the regulation of worker division of labor in honey bee colonies. *Proceedings of the National Academy of Sciences, USA* **105**, 4226-4231 (2008).

55 Smith, C. R., Toth, A. L., Suarez, A. V. & Robinson, G. E. Genetic and genomic analyses of the division of labour in insect societies. *Nature Reviews Genetics* **9**, 735-748 (2008).

56 Whitfield, C. W., Cziko, A. M. & Robinson, G. E. Gene expression profiles in the brain predict behavior in individual honey bees. *Science* **302**, 296-299 (2003).

57 Windig, J. J., Brakefield, P. M., Reitsma, N. & Wilson, J. G. M. Seasonal polyphenism in the wild: survey of wing patterns in five species of *Bicyclus* butterflies in Malawi. *Ecological Entomology* **19**, 285-298 (2008).

58 Connallon, T. & Clark, A. G. Sex linkage, sex-specific selection and the role of recombination in the evolution of sexually dimorphic gene expression. *Evolution* **64**, 3417-3442 (2010).

59 Dean, R. & Mank, J. E. Tissue-specificity and sex-specific regulatory variation permits the evolution of sex-biased gene expression. *American Naturalist* (2016).

60 Mank, J. E., Hultin-Rosenberg, L., Zwahlen, M. & Ellegren, H. Pleiotropic constraint hampers the resolution of sexual antagonism in vertebrate gene expression. *American Naturalist* **171**, 35-43 (2008).

61 Meisel, R. P. Towards a More Nuanced Understanding of the Relationship between Sex-Biased Gene Expression and Rates of Protein-Coding Sequence Evolution. *Molecular Biology and Evolution* **28**, 1893-1900 (2011).

62 Jaquiery, J. *et al.* Masculinization of the X Chromosome in the Pea Aphid. *Plos Genetics* **9**, e1003690 (2013).

63 Khil, P. P., Smirnova, N. A., Romanienko, P. J. & Camerini-Otero, R. D. The mouse X chromosome is enriched for sex-biased genes not subject to selection by meiotic sex chromosome inactivation. *Nature Genetics* **36**, 642-646 (2004).

64 Sturgill, D., Zhang, Y., Parisi, M. & Oliver, B. Demasculinization of X chromosomes in the *Drosophila* genus. *Nature* **450**, 238-241 (2007).

65 Meisel, R. P., Malone, J. H. & Clark, A. G. Disentangling the relationship between sex-biased gene expression and X-linkage. *Genome Research* **22**, 1255-1265 (2012).

66 Mank, J. E. & Ellegren, H. Sex-linkage of sexually antagonistic genes is predicted by female, but not male, effects in birds. *Evolution* **63**, 1464-1472 (2009).

67 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 Biology and Evolution* **2**, 336-346 (2010).

68 Wright, A. E., Moghadam, H. K. & Mank, J. E. Trade-off Between Selection for Dosage Compensation and Masculinization on the Avian Z Chromosome. *Genetics* **192**, 1433-1445 (2012).

69 Wright, A. E., Zimmer, F., Harrison, P. W. & Mank, J. E. Conservation of Regional Variation in Sex-Specific Sex Chromosome Regulation. *Genetics* **201**, 587-598 (2015).

70 Moghadam, H. K., Pointer, M. A., Wright, A. E., Berlin, S. & Mank, J. E. W chromosome expression responds to female-specific selection. *Proceedings of the National Academy of Sciences, USA* **109**, 8207-8211 (2012).

71 Immonen, E., Snook, R. R. & Ritchie, M. G. Mating system variation drives rapid evolution of the female transcriptome in *Drosophila pseudoobscura*. *Ecology and Evolution* **4**, 2186-2201 (2014).

72 Seddon, N., Botero, C.A., Tobias, J.A., Dunn, P.O., MacGregor, H.E.A., Rubenstein, D.R., Uy, J.A.C., Weir, J.T., Whittingham, L.A. Sexual selection accelerates signal evolution during speciation in birds. *Proceedings of the Royal Society, B* **280**, 20131065 (2013).

73 Ometto, L., Shoemaker, D., Ross, K. G. & Keller, L. Evolution of Gene Expression in Fire Ants: The Effects of Developmental Stage, Caste, and Species. *Molecular Biology and Evolution* **28**, 1381-1392 (2011).

74 Brawand, D. *et al.* The evolution of gene expression levels in mammalian organs. *Nature* **478**, 343-348 (2011).

75 Shapiro, M. D., Bell, M. A. & Kingsley, D. M. Parallel genetic origings of pelvic reductions in vertebrates. *Proceedings of the National Academy of Sciences , USA* **103**, 13573-13758 (2006).

76 Brakefield, P. M. *et al.* Development, plasticity and evolution of butterfly eyespot patterns. *Nature* **384**, 236-242 (1996).

77 Blows, M. W., Alen, S. L., Collet, J. M., Chenoweth, S. F. & McGuigan. The phenome-wide distribution of genetic variance. *American Naturalist* **186**, 15-30 (2015).

78 Collet, J. M. Transcriptome-wide effects of seuxal selection on the fate of new mutations. *Evolution* **96**, 2905-2916 (2015).

79 McGuigan, K., Aguirre, J. D. & Blows, M. W. Simultaneous estimation of additive and mutational genetic variance in an outbred population of Drosophila serrata. *Genetics* **201**, 1239-1251 (2015).

80 Gompel, N., Prud'homme, B., Wittkopp, P. J., Kassner, V. A. & Carroll, S. B. Chance caught on the wing: cis-regulatory evolution and the origin of pigment patterns in Drosophila. *Nature* **433**, 481-487 (2005).

81 Prud'homme, B. *et al.* Repeated morphological evolution through cis-regulatory changes in a pleiotropic gene. *Nature* **440**, 1050-1053 (2006).

82 Blekhman, R., Marioni, J. C., Zumbo, P., Stephens, M. & Gilad, Y. Sex-specific and lineage-specific alternative splicing in primates. *Genome Research* **20**, 180-189 (2010).

83 Mullon, C., Wright, A. E., Reuter, M., Pomiankowski, A. & Mank, J. E. Evolution of dosage compensation under sexual selection differs between X and Z chromosomes. *Nature Communications* **6**, 7720 (2015).

84 Locke, A. E. *et al.* Genetic studies of body mass index yield new insights for obesity biology. *Nature* **518**, 197-206 (2015).

85 Wen, W. Q. *et al.* Meta-analysis of genome-wide association studies in East Asian-ancestry populations identifies four new loci for body mass index. *Human Molecular Genetics* **23**, 5492-5504 (2014).

86 Wood, A. R. *et al.* Defining the role of common variation in the genomic and biological architecture of adult human height. *Nature Genetics* **46**, 1173-1186 (2014).

87 Yao, C. *et al.* Sex- and age-interacting eQTLs in human complex diseases. *Human Molecular Genetics* **23**, 1947-1956 (2014).

88 Ranz, J. M., Castillo-Davis, C. I., Meiklejohn, C. D. & Hartl, D. L. Sex-dependent gene expression and evolution of the *Drosophila* transcriptome. *Science* **300**, 1742-1745 (2003).

89 Zhang, Z., Hambuch, T. M. & Parsch, J. Molecular evolution of sex-biased genes in *Drosophila*. *Molecular Biology and Evolution* **21**, 2130-2139 (2004).

90 Pal, C., Papp, B. & Hurst, L. D. Highly expressed genes in yeast evolve slowly. *Genetics* **158**, 927-931 (2001).

91 Urrutia, A. O. & Hurst, L. D. The signature of selection mediated by expression on human genes. *Genome Research* **13**, 2260-2264 (2003).

92 Andersson, M. *Sexual selection*. (Princeton University Press, 1994).

93 Lande, R. Models of speciation by sexual selection on polygenic traits. *Proceedings of the National Academy of Sciences , USA* **78**, 3721-3725 (1981).

94 Proschel, M., Zhang, Z. & Parsch, J. Widespread adaptive evolution of *Drosophila* genes with sex-biased expression. *Genetics* **174**, 893-900 (2006).

95 Khaitovich, P. *et al.* Parallel patterns of evolution in the genomes and transcriptomes of humans and chimpanzees. *Science* **309**, 1850-1854 (2005).

96 Cutter, A. D. & Ward, S. Sexual and temporal dynamics of molecular evolution in *C. elegans* development. *Molecular Biology and Evolution* **22**, 178-188 (2005).

97 Gershoni, M. & Pietrokovski, S. Reduced selection and accumulation of deleterious mutations in genes expressed exclusively in men. *Nature Communications* **5**, 4438 (2014).

98 Dapper, A. L., Wade, M.J. The evolution of sperm competition genes: The effect of mating system on levels of genetic variation within and between species. *Evolution* **70** (2016).

99 Cobbs, G. Multiple inseminations and male sexual selection in natural populations of Drosophila pseudoobscura. *American Naturalist* **111**, 641-656 (1977).

100 Smith, N. G. C. & Eyre-Walker, A. Adaptive protein evolution in Drosophila. *Nature* **415**, 1022-1024 (2002).

101 Wyman, M. J., Cutter, A. D. & Rowe, L. Gene duplication inthe evolution of sexual dimorphism. *Evolution* **66**, 1556-1566 (2012).

102 Montgomery, S. H. & Mank, J. E. Inferring regulatory change from gene expression: The confounding effects of tissue scaling. *Molecular Ecology* (in press).

103 Voje, K. L. Scaling of Morphological Characters across Trait Type, Sex, and Environment: A Meta-analysis of Static Allometries. *American Naturalist* **187**, 89-98 (2016).

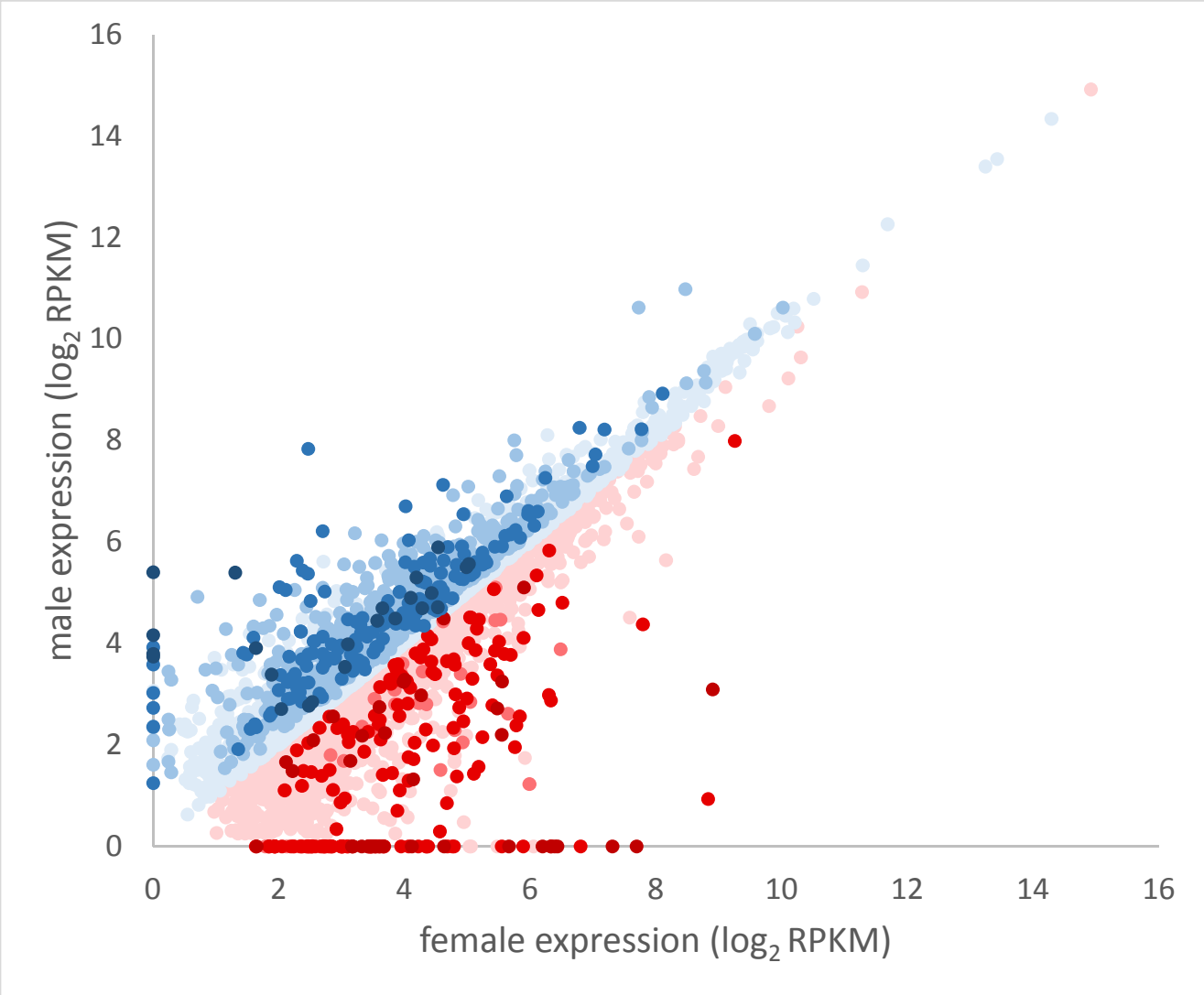
104 Gur, R. C. *et al.* Sex differences in brain gray and white matter in healthy young adults: correlations with cognitive performance. *Journal of Neuroscience* **15**, 4065-4072 (1999).

Figures

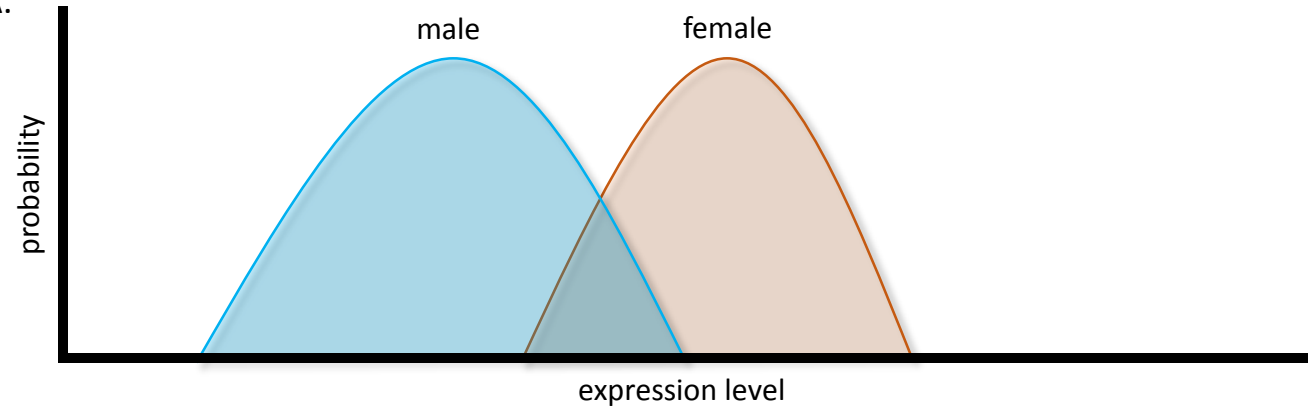
Figure 1. Correlation between male and female expression in Red Jungle Fowl. Shown is relative expression (RPKM, reads per kilobase of million mappable reads) for autosomal genes[70]. Genes expressed more in females (female-biased) are in red, and shading indicates significant difference from male expression. Genes expressed more in males are in blue, again with shading indicating significance. P-values have been adjusted for multiple comparisons. Genes with non-significant differences in expression show higher intersexual correlation than significantly sex-biased genes [51,59]. Additionally, many genes show small differences in expression between males and females that are nonetheless highly significant. The phenotypic effects of these genes remains unclear.

Figure 2. Regulatory complexity. Many genes show pronounced differences in expression. Shown are hypothetical density plots of gene expression. Although it often seems remarkable that expression can vary substantially for so many loci by sex (A), similar variation is also observed among social insect castes (B), and among tissues within the body (C).

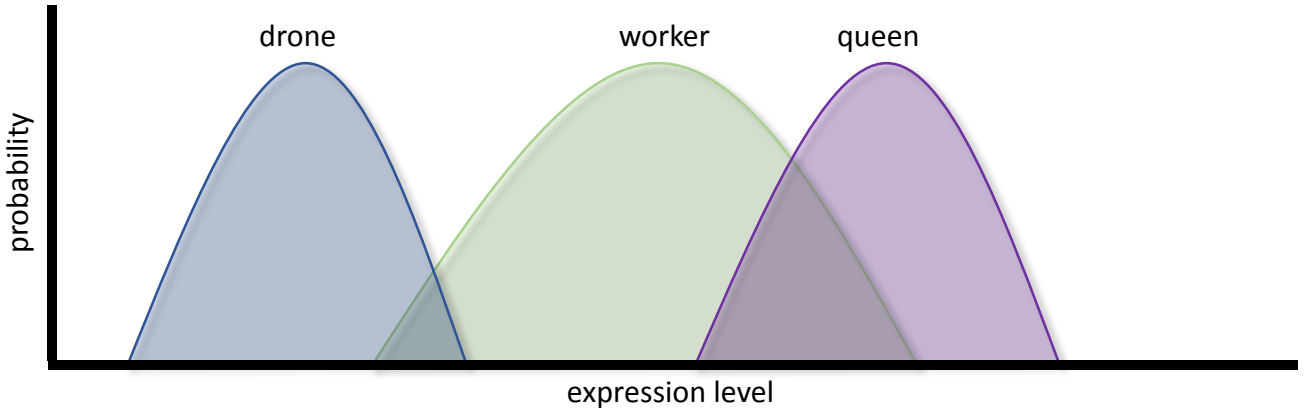
Figure 3. Differences in relative volume for a hypothetical insect with head sexual size dimorphism. In this case, males have a larger head than females, but all other body parts are equal in size. This leads to differences in relative volume. Because RNA-Seq is a relative measure, this could lead to perceived differences in expression for genes with uneven distribution across different body parts. In this case, head-expressed genes (orange) would appear to have higher expression in males, and genes in the thorax (green), eye (grey) and abdomen (blue) would appear to have lower expression in males, even though absolute expression within each of these constituent tissues is the same.


16
14
12
10
8
6
4
2
0
male expression (log2 RPKM)
0
2
4
6
8
10
12
14
16
female expression (log2 RPKM)

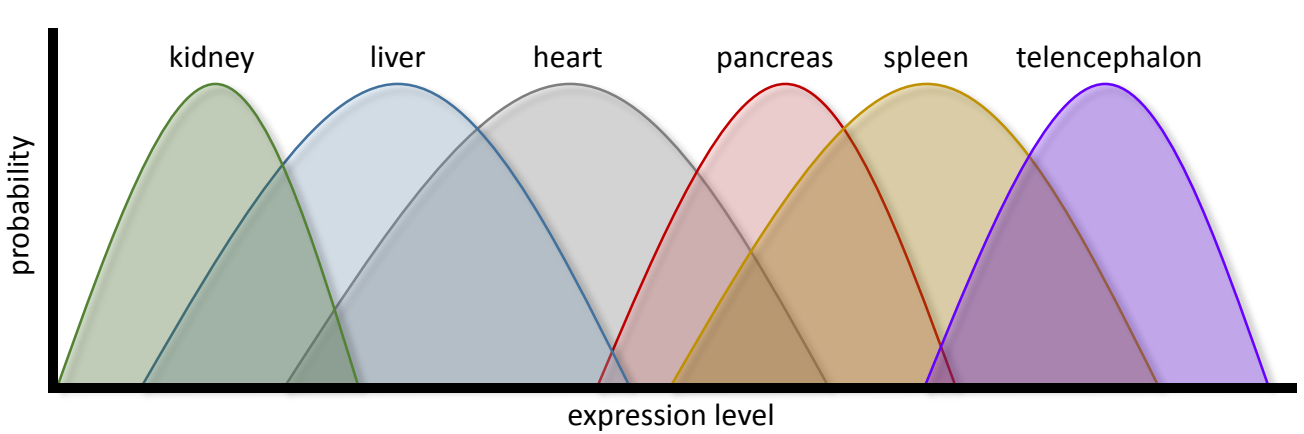
A.

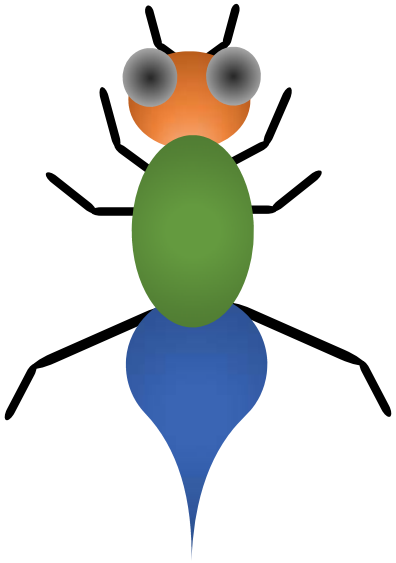

male
female
probability
expression level

B.

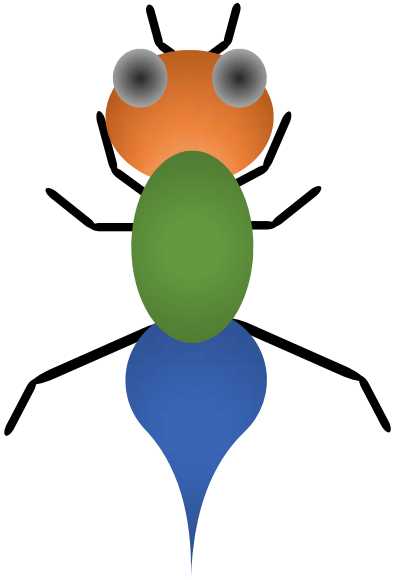

drone
worker
queen
probability
expression level

C.


kidney
liver
heart
pancreas
spleen
telencephalon
probability
expression level



female



male