Article:
Price, P.D., Parkus, S.M. and Wright, A.E. (2023) Recent progress in understanding the genomic architecture of sexual conflict. Current Opinion in Genetics & Development, 80. 102047. ISSN: 0959-437X

https://doi.org/10.1016/j.gde.2023.102047

Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Genetics & Development

Recent progress in understanding the genomic architecture of sexual conflict

Peter D. Price*, Sylvie M. Parkus and Alison E. Wright*

Genomic conflict between the sexes over shared traits is widely assumed to be resolved through the evolution of sex-biased expression and the subsequent emergence of sexually dimorphic phenotypes. However, while there is support for a broad relationship between genome-wide patterns of expression level and sexual conflict, recent studies suggest that sex differences in the nature and strength of interactions between loci are instead key to conflict resolution. Furthermore, the advent of new technologies for measuring and perturbing expression means we now have much more power to detect genomic signatures of sexual conflict. Here, we review our current understanding of the genomic architecture of sexual conflict in the light of these new studies and highlight the potential for novel approaches to address outstanding knowledge gaps.

Address
Ecology and Evolutionary Biology, School of Biosciences, University of Sheffield, United Kingdom

Corresponding author: Wright, Alison E (a.e.wright@sheffield.ac.uk)
* Twitter account: @PeterDPrice, @alielw

Current Opinion in Genetics & Development 2023, **80**:102047

This review comes from a themed issue on **Evolutionary Genetics**

Edited by **Harmit Singh Malik** and **Judith Mank**

Available online xxxx

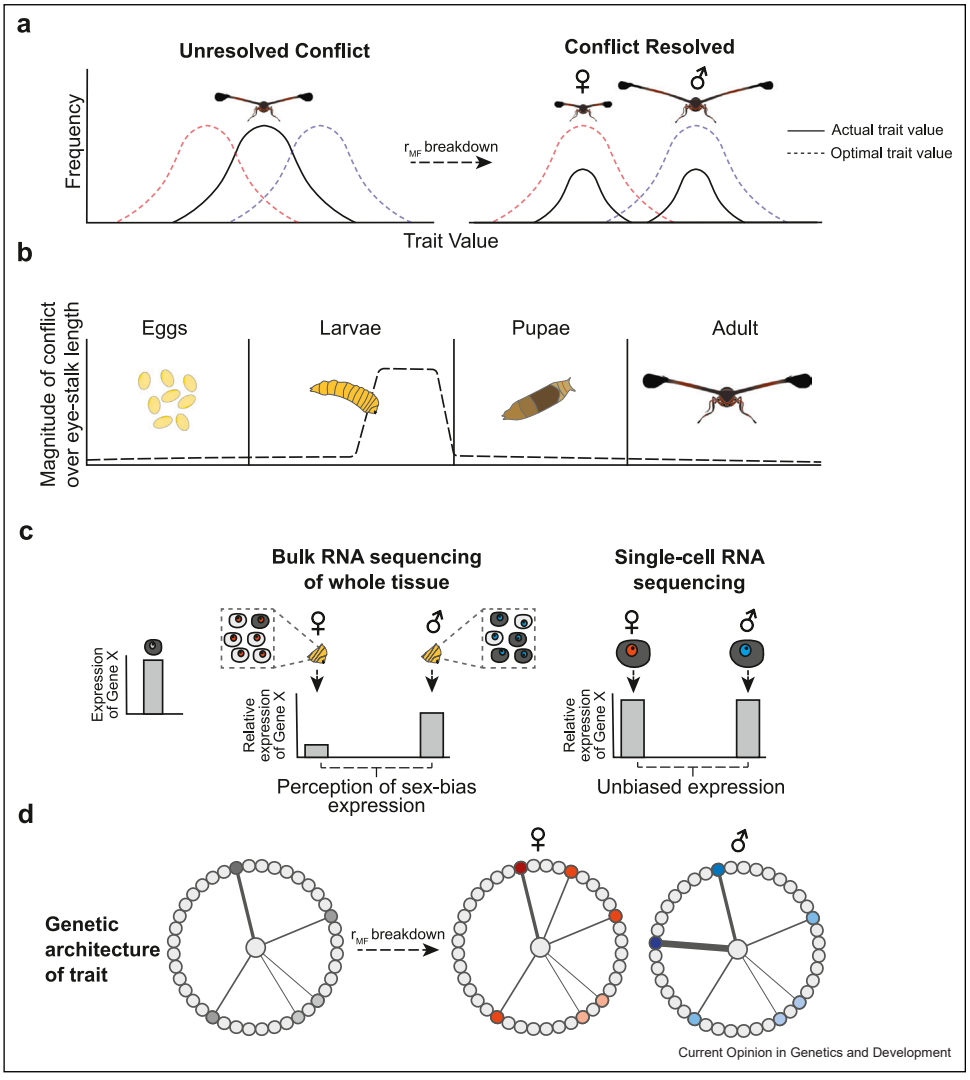
https://doi.org/10.1016/j.gde.2023.102047

Introduction

Males and females often experience sex-specific selection pressures towards divergent fitness optima across a range of traits [1]. However, when the genomic basis of these traits is shared between the sexes, intralocus sexual conflict can arise, where the trait in males, females, or both will be inhibited from evolving towards its fitness optima. This conflict has profound implications for multiple aspects of genome and trait evolution, including adaptability and levels of genetic variation [2–4]. Sexual conflict can ultimately be resolved via a decoupling of genetic architectures and subsequent reduction in the intersexual genetic correlation (Figure 1a), and the sex-specific loss or gain of a trait. Within a species, a single genome can encode multiple distinct phenotypes by varying expression levels of the underlying loci. Given that males and females share the majority of their genomes, transcriptomic analyses are increasingly used to study the genes that underlie sexual dimorphisms and the selective regimes acting on them [5]. In principle, sex differences in expression allow the genome to evolve in a sex-specific manner as selection can act independently on the sexes, circumventing constraints posed by sharing a genome and resolving sexual conflict. While some aspects of sexual dimorphism do result from genes located on the sex chromosomes [6], sex-linked genes are often few or absent in many species with pronounced sexually selected traits. It is therefore clear that the majority of sexual dimorphisms must arise from differential expression of genes present in both sexes.

To date, there is a large body of evidence in support of the relationship between broad, genome-wide patterns of expression and proxies for resolved sexual conflict. Differential expression both within [7–9] and across species [10–12] mirrors phenotypic sexual dimorphism with male-biased genes exhibiting greater levels of standing genetic variance in expression [13], consistent with sexual conflict theory. Male-biased gene expression also shows rapid turnover across lineages [14,15], however, this pattern might also represent relaxed pleiotropic constraints [16,17]. Furthermore, targets of ongoing sexually antagonistic selection are not typically enriched for sex differences in expression [18,19], suggesting that sex-biased genes are the footprint of resolved conflict. We also now have several examples directly linking sex-biased genes to sex-specific phenotypes [20]. In many insects, the expression of *doublesex* (*dsx*), the master regulator of sexual differentiation, is central to the evolution of sexually dimorphic traits [21]. For instance, its knockdown in male-horned beetles (*Onthophagus taurus*) reduces head horns, but induces the development of horns in females [22]. Similarly, in a closely related species (*Digitonthophagus gazella*), *dsx* expression is responsible for sex differences in the length of the fore tibia, used by males to grasp females during mating [23]. Whilst these examples directly link sexually dimorphic phenotypes and sex differences in expression, there are only a limited number of studies to do so and these are restricted to certain taxa, namely insects. This

Figure 1



Detecting the genomic architecture of intralocus sexual conflict. **(a)** A hypothetical pair of stalk-eyed fly species with a sexually selected trait, eye span, that exhibits a shared genomic architecture between males and females. Solid lines indicate the realised trait value, whereas the dotted lines indicate the optimal fitness value. The trait may exhibit a unimodal distribution (left) if it is under ongoing, unresolved sexual conflict or a bimodal distribution (right) where there is a breakdown of the intersexual correlation (r_{MF}) allowing males and females to evolve towards their fitness optima. **(b)** To accurately describe the molecular basis of ongoing and resolved conflict, it is essential to analyse the genetic basis of the trait at the appropriate developmental stage. Although dimorphism may be most striking in the adult phase, its manifestation can occur during development, where each sex will be subject to sex-specific selection pressures and exhibit divergent regulatory patterning. For instance, eye-stalks in stalk-eyed flies develop from eye-antennal imaginal discs and sexual conflict over cell proliferation likely manifests most strongly during the third-instar larval stage [33,81,82]. **(c)** Traditionally, to determine the molecular basis of such traits, whole tissues will be used to measure gene expression in bulk. This can, however, lead to perceptions of differential gene expression that are solely products of differences in the cellular composition of the tissue. This is especially important in the case of sexual ornamentation, where sexually selected structures may differ dramatically in size and cellular composition between the sexes. Single-cell RNA-seq accounts for this by removing the compounding effect of tissue heterogeneity and allowing the comparison of equivalent cell types. **(d)** Network-based approaches are also critical for studying sex-specific architectures. Grey circles represent loci that can contribute to phenotypic variation in eye span. Lines represent loci that do contribute to variation in the trait, and the width of the line corresponds to the size of the effect. This could be mediated by distinct male and female genetic architectures that differ in the number and identity of loci (right-hand side), where the male architecture includes a greater number or more strongly connected condition-dependent loci (e.g. hormonal and growth pathways) than in females.

is in part because the functional identification of the genetic basis of sex-specific adaptation is challenging, especially for more complex phenotypes.

On the other hand, recent research has shown that the genes responsible for some sexually selected traits are either not differentially expressed between males and females or display subtle patterns of expression change [24,25]. For instance, male water striders (*Microvelia longipes*) have exaggerated third legs used to fight and dominate egg-laying sites. Despite similar expression of *Ultrabithorax* (*Ubx*) in both male and female third legs, knockdown of *Ubx* during development results in significantly reduced leg length in males, but has only mild phenotypic effects in females [25]. This research indicates that the relationship between sex-biased expression and sexual conflict is complex, and it remains unclear what magnitude of sex-biased gene expression is necessary to fully resolve sexual conflict. For instance, it is possible that subtle expression differences between the sexes have large phenotypic effects and this is likely to differ on a gene-by-gene basis. Alternatively, sex differences in expression might be limited to specific cell types and so masked from detection using traditional RNA-seq approaches [26,27]. Here, we identify recent advances in efforts to study the role of differential expression in the resolution of sexual conflict, review our current understanding of the genomic architecture of sexual conflict and identify key outstanding questions for the field to address.

Considering the developmental context of sexual conflict

Many adult sexual dimorphisms are the product of differences in growth rate and cell-type proliferation between males and females through development, particularly for exaggerated sexual ornaments [28]. This includes rhinoceros beetle horns (*Trypoxylus dichotomus*) [29], stag beetle mandibles (*Cyclommatus metallifer*) [30], weapons in water striders (*Microvelia longipes*) [25], swordtail caudal fins (*Xiphophorus*) [31,32], and eye-stalks in stalk-eyed flies (*Teleopsis dalmanni*) [33]. Therefore, in many cases, we might expect sexual conflict to manifest most strongly over growth rates during development. For instance, eye-stalks in stalk-eyed flies)develop from eye-antennal imaginal discs. Experimental manipulation indicates that these discs are sensitive to changes in hormone signalling during the third-instar larval stage [33,34], suggesting that sexual conflict over cell proliferation is likely greatest at this point of development (Figure 1b). Such sex differences in growth and cell proliferation are likely due to differential gene expression in males and females, however, these changes in expression will not be detected if transcriptomes are measured after development is completed. This in part, may explain the inconsistencies between studies in the relationship between differential expression and signatures of sexual conflict.

An increasing number of studies are incorporating an ontogenetic perspective to the study of sexual dimorphism, either through measuring expression across multiple developmental stages [8,35,36] or perturbing expression directly during development [23,25]. Together, this research suggests that the magnitude of conflict likely varies across different developmental stages and strategies. For instance, hemimetabolous stick insects (*Timema californicum*) demonstrate a gradual increase in sex-biased expression during development, while holometabolous fruit flies (*Drosophila melanogaster*) have a burst of differential expression in the adult stage [35]. Patterns of expression in these two species closely reflect the development of sexual dimorphism, where *D. melanogaster* has monomorphic larval and pupal stages, and sexual dimorphism manifests abruptly after eclosion, whereas *T. californicum* exhibits a gradual increase in sexually dimorphic traits after its hatchling stage throughout development. Understanding the relative contribution of distinct developmental stages to adult sexual dimorphisms across species is a key priority for pinpointing the genomic architecture of conflict.

Reassessing how we measure differential expression

Key to studying the genomics of sexual conflict is the ability to distinguish whether sex-biased expression is due to regulatory differences or developmental changes in cellular composition between males and females. This is because sexual conflict can be resolved by a decoupling of male and female expression via a reduced intersexual genetic correlation, producing sex-biased genes. In turn, the resolution of conflict permits the evolution of sexual dimorphisms and sex differences in cellular composition. Therefore, only sex-biased expression arising from regulatory differences and not variation in cellular composition between males and females is informative for understanding how selection to resolve sexual conflict directly operates.

Traditional approaches of measuring expression meant it was difficult to distinguish between these two scenarios. This is because bulk RNA-seq approaches measure expression in aggregate across tissues or entire organisms, which, in practice, represents average expression across entire populations of distinct cell types. Therefore, samples that vary in tissue composition can produce patterns of differential expression that are mistaken as evidence of regulatory change or even mask genuine regulatory differences [26,27,37,38] (Figure 1c). This is especially relevant for sexual dimorphisms, which are often complex phenotypes composed of many cell types with variable expression profiles and, by definition, vary

Box 1 Outstanding questions

What magnitude of sex-biased gene expression is necessary to fully resolve sexual conflict, and how does this differ across genes?

How do genomic architectures evolve from shared to sex-specific, and vice versa, and what are the underlying regulatory networks and loci?

Are convergent patterns of sex-specific network rewiring responsible for conflict resolution across distantly diverged species?

Is loss or gain of sexually selected traits more common and how does this manifest in properties of sex-specific regulatory networks?

How do different types of sexual selection, such as Fisherian runaway selection and 'good genes' models, alter the genomic outcome of sexual conflict?

When is condition-dependent ornamentation in females a product of signalling and when is it a sign of incomplete conflict resolution?

in size, structure and composition between males and females [39]. Significant sex differences in cell type abundance seem to be the norm, even for somatic tissues [27]. Unfortunately, this makes it challenging to establish whether sex-biased genes, identified using bulk approaches, are products of regulatory change or simply sex differences in cellular composition. The problem is further confounded if the developmental perspective discussed above is not taken, as we might not expect the targets of sexual conflict to be expressed in the adult phenotype.

New advances in single-cell transcriptomics (scRNA-seq) circumvent issues of tissue composition variation by permitting direct comparisons of male and female expression across equivalent cell types (Figure 1c). To date, a handful of studies have employed single-cell approaches to test the role of differential expression in the evolution of within- [27,40,41] and across-species [42–45] phenotypic variation, however, only one explicitly addresses sex differences [27]. This study found that single-cell versus bulk approaches identify independent sets of sex-biased genes in the guppy (*Poecilia reticulata*) in both somatic and reproductive tissue and these distinct groups of genes exhibit different patterns of coding sequence evolution. Importantly, the exact proportion of genes incorrectly identified as differentially expressed is highly tissue-specific. This could explain some of the inconsistencies across studies in whether sex-biased genes exhibit genomic signatures of resolved or ongoing sexual conflict [18,19,46–48]. As single-cell approaches are increasingly applied to the study of sexual conflict, it will become possible to ascertain how many and what type of genes are truly differentially expressed, and how this relates to proxies of sexual conflict (see Box 1). This is particularly relevant for somatic tissues, where males and females are expected to contain equivalent cell types in different proportions.

Studying sex differences in gene interactions

Genes do not operate in isolation, but in multi-dimensional networks, and there is increasing evidence that sex differences in the nature and strength of interactions between loci are common [49–51]. This likely explains the growing evidence that loci expressed at similar levels in both sexes can have distinct sex-specific effects [25,52–55], consistent with separate male and female genetic architectures (Figure 1d). The evolution of sex-specific genetic architectures potentially alleviates conflict by circumventing constraints imposed by a shared genome and facilitates the evolution of sexual dimorphism [18]. Therefore, shifting focus to studying sex differences in co-expression networks is more informative for understanding how conflict can be resolved than current approaches where genes are typically studied independently. Sexual dimorphisms evolve rapidly, with frequent losses and gains [56,57], but it remains unclear if the underlying loci mirror this pattern. Important next steps include identifying the underlying regulatory networks and loci responsible for male and female genetic architectures, establishing how genetic architectures evolve from shared to sex-specific and vice versa and how frequently (see Box 1). For instance, for traits encoded by sex-specific factors expressed during the early stages of sex-determination pathways, such as the *dsx* gene, the construction of separate male and female architectures is relatively straightforward and sexual conflict could be easily mitigated.

Notably, it remains unclear whether convergent patterns of sex-specific network rewiring are responsible for conflict resolution across distantly diverged species, although the repeated involvement of *dsx* in sexual traits across insects [21,22,30,58,59] suggests similar processes might be operating. *dsx*, in particular, has distinct sex- and tissue-specific target loci due to alternative splicing into male and female isoforms. This allows regulation of the same genes in opposite directions in males and females in the dung beetle (*Onthophagus taurus*) and likely many other insects [60]. In the future, single-cell approaches are particularly important to address these questions as differences in cellular composition between males and females can affect the measurement of gene co-expression due to key differences in gene networks across cell types [61,62]. Unfortunately, many of the available methods of regulatory network inference are currently not effective for single-cell transcriptome data due to its intrinsic sparsity and high technical variation [61,63].

Identifying the evolutionary drivers of sexual conflict

Studies into the genomic basis of sexual conflict typically do not consider the mode of sexual selection. However, the type of sexual selection has important consequences for the strength of sexual conflict and how we expect conflict to manifest and be resolved across the genome. For instance, under the 'good genes' model of sexual selection, sexually dimorphic traits are predicted to evolve as honest signals of male genetic quality, where only high-condition males can afford to invest in elaborate sexual ornaments [64,65]. In contrast, under Fisherian runaway, sexually selected traits are not linked to individual condition [66]. This distinction is important as, in principle, sex-specific condition dependence restricts the expression of the costly trait to only those individuals who have sufficient resources, serving as a potential mechanism that aids in resolving sexual conflict.

There is now considerable evidence across many organisms that honest male sexual traits have evolved repeatedly in a range of phenotypes [28,67–70] and that the genetic architecture of these traits is sex-specific and condition-dependent [21], but see Ref. [71]. Recent studies have started to elucidate the precise genomic and physiological processes that link honest traits to condition and nutritional status in a sex-specific manner. For instance, conserved growth and hormonal pathways have been identified as common mechanisms regulating condition dependence of several male sexual traits, such as juvenile hormone signalling in stalk-eyed flies (*Teleopsis dalmanni*) [33] and stag beetles (*Cyclommatus metallifer*) [30], and insulin signalling in several beetle species (*O. taurus* and *T. dichotomus*) [29,72]. Often, these pathways are intrinsically linked to sex-determination factors meaning that the male but not the female trait can be linked to individual condition [23,30].

Recent evidence suggests that exaggerated traits in females can also be highly associated with individual condition [73–75]. If so, this suggests that plastic resource allocation [65] in both sexes could be key to alleviating sexual conflict for certain traits. However, whilst a 'good genes' model provides a framework for conflict resolution, it is unclear whether occurrences of female ornamentation are the active signalling of condition [74], or instead a product of correlated evolution arising from incomplete conflict resolution where male and female traits exhibit a similar genomic architecture. In this instance, high-condition females will pay a greater fitness cost [76].

Interestingly, in turn, the evolution of condition-dependent genetic architectures that exhibit variable expression across individuals may actually act to exacerbate the strength of sexual conflict [77]. This can be because high-fitness males produce low-fitness daughters and high-fitness mothers produce low-fitness sons [78,79] or because certain environments are more favourable for males than for females [80]. This sets the stage for a feedback loop, where 'good genes' processes might only be possible once sexual conflict has been resolved via the evolution of condition-dependent traits.

Together, these results suggest that the genomic architecture of sexual conflict may vary quite profoundly under different modes of sexual selection, however, this is rarely considered when testing for signatures of conflict across the genome. Establishing specific predictions for the types of loci and their interactions responsible for conflict resolution under 'good genes' versus Fisherian models of sexual selection is a major priority for the future.

Conclusion

The development of novel technologies for measuring and perturbing expression has shed new light on our understanding of how sexual conflict manifests across the genome and whether the differential gene expression we perceive is a signature of ongoing conflict or conflict resolved. It is also now apparent that sex differences in the nature and strength of gene interactions are key to conflict resolution. However, a number of outstanding questions regarding the genomic architecture of conflict remain unanswered (see Box 1). Solving these will require the effective integration of single-cell approaches across development with phenotypic studies that quantify the underlying drivers of conflict.

Data Availability

No data were used for the research described in the article.

Conflict of interest statement

The authors declare no conflict of interest.

Acknowledgements

This work was funded by a Natural Environment Research Council (NERC), UK, Independent Research Fellowship to A.E.W. (NE/N013948/1) and an NERC ACCE DTP to P.D.P.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Parker GA: **Sexual selection and sexual conflict**. Sexual Selection and Reproductive Competition in Insects. Elsevier; 1979:166.

2. • Kaufmann P, Howie JM, Immonen E: **Sexually antagonistic selection maintains genetic variance when sexual dimorphism evolves**. *Proc R Soc B* 2023, **290**:20222484.

This paper on the seed beetle (*Callosobruchus maculatus*) demonstrates that sexually antagonistic selection has the propensity to maintain

autosomal additive genetic variance longer than sex-limited directional selection.

3. Arnqvist G, Rowe L: **Sexual Conflict**. Princeton University Press; 2005.

4. Ruzicka F, Holman L, Connallon T: **Polygenic signals of sex differences in selection in humans from the UK Biobank**. *PLoS Biol* 2022, **20**:e3001768.

5. Mank JE: **The transcriptional architecture of phenotypic dimorphism**. *Nat Ecol Evol* 2017, **1**:0006.

6. Rice WR: **Sex chromosomes and the evolution of sexual dimorphism**. *Evolution* 1984, **38**:735-742.

7. Pointer MA, Harrison PW, Wright AE, Mank JE: **Masculinization of gene expression is associated with exaggeration of male sexual dimorphism**. *PLoS Genet* 2013, **9**:e1003697.

8. Ingleby FC, Flis I, Morrow EH: **Sex-biased gene expression and sexual conflict throughout development**. *Cold Spring Harb Perspect Biol* 2014, **7**:a017632.

9. Zemp N, Tavares R, Widmer A: **Fungal infection induces sex-specific transcriptional changes and alters sexual dimorphism in the dioecious plant *Silene latifolia***. *PLoS Genet* 2015, **11**:e1005536.

10. Harrison PW, Wright AE, Zimmer F, Dean R, Montgomery SH, Pointer MA, Mank JE: **Sexual selection drives evolution and rapid turnover of male gene expression**. *PNAS* 2015, **112**:4393-4398.

11. Parker DJ, Bast J, Jalvingh K, Dumas Z, Robinson-Rechavi M, Schwander T: **Sex-biased gene expression is repeatedly masculinized in asexual females**. *Nat Commun* 2019, **10**:1-11.

12. Wright AE, Rogers TF, Fumagalli M, Cooney CR, Mank JE: **Phenotypic sexual dimorphism is associated with genomic signatures of resolved sexual conflict**. *Mol Ecol* 2019, **28**:2860-2871.

13. Allen SL, Bonduriansky R, Chenoweth SF: **Genetic constraints on microevolutionary divergence of sex-biased gene expression**. *Philos Trans R Soc* 2018, **373**:20170427.

14. Zhang Y, Sturgill D, Parisi M, Kumar S, Oliver B: **Constraint and turnover in sex-biased gene expression in the genus Drosophila**. *Nature* 2007, **450**:233-237.

15. Rogers TF, Palmer DH, Wright AE: **Sex-specific selection drives the evolution of alternative splicing in birds**. *Mol Biol Evol* 2021, **38**:519-530.

16. Scharmann M, Rebelo AG, Pannell JR: **High rates of evolution preceded shifts to sex-biased gene expression in Leucadendron, the most sexually dimorphic angiosperms**. *eLife* 2021, **10**:e67485.

17. Mank JE, Ellegren H: **Are sex-biased genes more dispensable?** *Biol Lett* 2009, **5**:409-412.

18. Wright AE, Fumagalli M, Cooney CR, Bloch NI, Vieira FG, Buechel SD, Kolm N, Mank JE: **Male-biased gene expression resolves sexual conflict through the evolution of sex-specific genetic architecture**. *Evol Lett* 2018, **2**:52-61.

19. Ruzicka F, Hill MS, Pennell TM, Flis I, Ingleby FC, Mott R, Fowler K, Morrow EH, Reuter M: **Genome-wide sexually antagonistic variants reveal long-standing constraints on sexual dimorphism in fruit flies**. *PLoS Biol* 2019, **17**:e3000244.

20. Galouzis CC, Prud'homme B: **Transvection regulates the sex-biased expression of a fly X-linked gene**. *Science* 2021, **371**:396-400.

21. Mank JE: **Sex-specific morphs: the genetics and evolution of intra-sexual variation**. *Nat Rev Genet* 2023, **24**:44-52.

22. Kijimoto T, Moczek AP, Andrews J: **Diversification of doublesex function underlies morph-, sex-, and species-specific development of beetle horns**. *PNAS* 2012, **109**:20526-20531.

23. •• Rohner PT, Linz DM, Moczek AP: **Doublesex mediates species-, sex-, environment- and trait-specific exaggeration of size and shape**. *Proc R Soc B: Biol Sci* 2021, **288**:20210241.

This study applied RNAseq and RNAi-knockdown to the sexually dimorphic dung beetle and found that the gene networks and developmental pathways that underlie tibia exaggeration, as well as the *dsx* gene that differentially alters phenotypic exaggeration, function in a context-dependent manner.

24. Khila A, Abouheif E, Rowe L: **Function, developmental genetics, and fitness consequences of a sexually antagonistic trait**. *Science* 2012, **336**:585-589.

25. • Toubiana W, Armisén D, Viala S, Decaras A, Khila A: **The growth factor BMP11 is required for the development and evolution of a male exaggerated weapon and its associated fighting behavior in a water strider**. *PLoS Biol* 2021, **19**:e3001157.

This study used comparative transcriptomics and RNA interference to identify genes responsible for the exaggerated third leg found in males across many water strider species. Notably, despite similar expression of *Ubx* in both male and female third legs, knockdown of *Ubx* during development results in significantly reduced leg length in males but has only mild phenotypic effects in females, indicating that the relationship between sex-biased expression and sexual conflict is complex.

26. Price P, Palmer D, Taylor J, Kim D, Place E, Rogers T, Mank J, Cooney C, Wright A: **Detecting signatures of selection in regulatory variation**. *Nat Ecol Evol* 2022, **6**:1035-1045.

27. •• Darolti I, Mank JE: **Sex-biased gene expression at single-cell resolution: cause and consequence of sexual dimorphism**. *Evolution Letters* 2023,qrad013.

This is the first study to explicitly apply single-cell RNA sequencing to test the extent to which sex-biased expression is a consequence of regulatory change between the sexes or simply differences in cell type abundance. They found that single-cell versus bulk approaches identify independent sets of sex-biased genes, and these distinct groups of genes exhibit different patterns of coding sequence evolution.

28. Lavine L, Gotoh H, Brent CS, Dworkin I, Emlen DJ: **Exaggerated trait growth in insects**. *Annu Rev Entomol* 2015, **60**:453-472.

29. Emlen DJ, Warren IA, Johns A, Dworkin I, Lavine LC: **A mechanism of extreme growth and reliable signaling in sexually selected ornaments and weapons**. *Science* 2012, **337**:860-864.

30. Gotoh H, Miyakawa H, Ishikawa A, Ishikawa Y, Sugime Y, Emlen DJ, Lavine LC, Miura T: **Developmental link between sex and nutrition; doublesex regulates sex-specific mandible growth via juvenile hormone signaling in stag beetles**. *PLoS Genet* 2014, **10**:e1004098.

31. Powell DL, Payne C, Banerjee SM, Keegan M, Bashkirova E, Cui R, Andolfatto P, Rosenthal GG, Schumer M: **The genetic architecture of variation in the sexually selected sword ornament and its evolution in hybrid populations**. *Curr Biol* 2021, **31**:923-935 .e911.

32. Schartl M, Kneitz S, Ormanns J, Schmidt C, Anderson JL, Amores A, Catchen J, Wilson C, Geiger D, Du K, *et al.*: **The developmental and genetic architecture of the sexually selected male ornament of swordtails**. *Curr Biol* 2021, **31**:911-922 e914.

33. Fry CL: **Juvenile hormone mediates a trade-off between primary and secondary sexual traits in stalk-eyed flies**. *Evol Dev* 2006, **8**:191-201.

34. Cotton S, Fowler K, Pomiankowski A: **Condition dependence of sexual ornament size and variation in the stalk-eyed fly *Cyrtodiopsis dalmanni***. *Evolution* 2004, **58**:1038-1046.

35. Djordjevic J, Dumas Z, Robinson-Rechavi M, Schwander T, Parker DJ: **Dynamics of sex-biased gene expression during development in the stick insect *Timema californicum***. *Heredity* 2022, **129**:113-122.

36. Perry JC, Harrison PW, Mank JE: **The ontogeny and evolution of sex-biased gene expression in *Drosophila melanogaster***. *Mol Biol Evol* 2014, **31**:1206-1219.

37. Montgomery SH, Mank JE: **Inferring regulatory change from gene expression: the confounding effects of tissue scaling**. *Mol Ecol* 2016, **25**:5114-5128.

38. Hunnicutt KE, Good JM, Larson EL: **Unraveling patterns of disrupted gene expression across a complex tissue**. *Evolution* 2022, **76**:275-291.

39. •• Mank JE, Rideout EJ: **Developmental mechanisms of sex differences: from cells to organisms**. *Development* 2021, **148**:dev199750.
This review highlights the importance of incorporating both sexes for robust developmental studies in order to understand how sex differences contribute to the developmental mechanisms that underlie sexual dimorphism.

40. Fuess LE, Bolnick DI: **Single-cell RNA sequencing reveals microevolution of the stickleback immune system**. *Genome Biol Evol* 2023, **15**:evad053.

41. Wei KH-C, Chatla K, Bachtrog D: **Single cell RNA-seq in Drosophila testis reveals evolutionary trajectory of sex chromosome regulation**. *bioRxiv* 2022,519494.

42. Shami AN, Zheng X, Munyoki SK, Ma Q, Manske GL, Green CD, Sukhwani M, Orwig KE, Li JZ, Hammoud SS: **Single-cell RNA sequencing of human, macaque, and mouse testes uncovers conserved and divergent features of mammalian spermatogenesis**. *Dev Cell* 2020, **54**:529-547 e512.

43. Segami JC, Mugal CF, Cunha C, Bergin C, Schmitz M, Semon M, Qvarnström A: **The genomic basis of hybrid male sterility in Ficedula flycatchers**. *bioRxiv* 2022,508503.

44. Segami JC, Semon M, Cunha C, Bergin C, Mugal CF, Qvarnström A: **Single-cell transcriptomics reveals relaxed evolutionary constraint of spermatogenesis in two passerine birds as compared to mammals**. *bioRxiv* 2022,477241.

45. Murat F, Mbengue N, Winge SB, Trefzer T, Leushkin E, Sepp M, Cardoso-Moreira M, Schmidt J, Schneider C, Mößinger K, *et al.*: **The molecular evolution of spermatogenesis across mammals**. *Nature* 2023, **613**:308-316.

46. Cheng C, Kirkpatrick M: **Sex-specific selection and sex-biased gene expression in humans and flies**. *PLoS Genet* 2016, **12**:e1006170.

47. Dutoit L, Mugal CF, Bolívar P, Wang M, Nadachowska-Brzyska K, Smeds L, Yazdi HP, Gustafsson L, Ellegren H: **Sex-biased gene expression, sexual antagonism and levels of genetic diversity in the collared flycatcher (*Ficedula albicollis*) genome**. *Mol Ecol* 2018, **27**:3572-3581.

48. Sayadi A, Martinez Barrio A, Immonen E, Dainat J, Berger D, Tellgren-Roth C, Nystedt B, Arnqvist G: **The genomic footprint of sexual conflict**. *Nat Ecol Evol* 2019, **3**:1725-1730.

49. Sutherland BJG, Prokkola JM, Audet C, Bernatchez L: **Sex-specific co-expression networks and sex-biased gene expression in the Salmonid Brook Charr *Salvelinus fontinalis***. *G3* 2019, **9**:955-968.

50. •• Lopes-Ramos CM, Chen CY, Kuijjer ML, Paulson JN, Sonawane AR, Fagny M, Platig J, Glass K, Quackenbush J, DeMeo DL: **Sex differences in gene expression and regulatory networks across 29 human tissues**. *Cell Rep* 2020, **31**:107795.
This paper describes the abundance of sex-specific regulatory networks in humans, highlighting that transcription factors are often not differentially expressed yet target distinct and different sets of genes in males and females.

51. Rago A, Werren JH, Colbourne JK: **Sex biased expression and co-expression networks in development, using the hymenopteran *Nasonia vitripennis***. *PLoS Genet* 2020, **16**:e1008518.

52. Randall JC, Winkler TW, Kutalik Z, Berndt SI, Jackson AU, Monda KL, Kilpeläinen TO, Esko T, Mägi R, Li S: **Sex-stratified genome-wide association studies including 270,000 individuals show sexual dimorphism in genetic loci for anthropometric traits**. *PLoS Genet* 2013, **9**:e1003500.

53. Rawlik K, Canela-Xandri O, Tenesa A: **Evidence for sex-specific genetic architectures across a spectrum of human complex traits**. *Genome Biol* 2016, **17**:1-8.

54. Karp NA, Mason J, Beaudet AL, Benjamini Y, Bower L, Braun RE, Brown SDM, Chesler EJ, Dickinson ME, Flenniken AM, *et al.*: **Prevalence of sexual dimorphism in mammalian phenotypic traits**. *Nat Commun* 2017, **8**:15475.

55. van der Bijl W, Mank JE: **Widespread cryptic variation in genetic architecture between the sexes**. *Evol Lett* 2021, **5**:359-369.

56. Wiens JJ: **Widespread loss of sexually selected traits: how the peacock lost its spots**. *Trends Ecol Evol* 2001, **16**:517-523.

57. Oliver JC, Monteiro A: **On the origins of sexual dimorphism in butterflies**. *Proc R Soc B: Biol Sci* 2011, **278**:1981-1988.

58. Williams TM, Selegue JE, Werner T, Gompel N, Kopp A, Carroll SB: **The regulation and evolution of a genetic switch controlling sexually dimorphic traits in Drosophila**. *Cell* 2008, **134**:610-623.

59. • Hendrickx F, De Corte Z, Sonet G, Van Belleghem SM, Köstlbacher S, Vangestel C: **A masculinizing supergene underlies an exaggerated male reproductive morph in a spider**. *Nat Ecol Evol* 2022, **6**:195-206.
There are two male morphs in *Oedothorax gibbosus* spider, the 'hunched' morph and the 'flat' morph, with the flat morph more closely resembling the female spider form. This study found that the hunched-determining allele has roughly an extra three megabases, containing several masculinizing genes, that are notably absent from the flat-determining allele and are responsible for the male reproductive hunched morph.

60. Ledón-Rettig CC, Zattara EE, Moczek AP: **Asymmetric interactions between doublesex and tissue- and sex-specific target genes mediate sexual dimorphism in beetles**. *Nat Commun* 2017, **8**:14593.

61. Cha J, Lee I: **Single-cell network biology for resolving cellular heterogeneity in human diseases**. *Exp Mol Med* 2020, **52**:1798-1808.

62. Ribeiro DM, Ziyani C, Delaneau O: **Shared regulation and functional relevance of local gene co-expression revealed by single cell analysis**. *Commun Biol* 2022, **5**:876.

63. Chen S, Mar JC: **Evaluating methods of inferring gene regulatory networks highlights their lack of performance for single cell gene expression data**. *BMC Bioinform* 2018, **19**:1-21.

64. Iwasa Y, Pomiankowski A, Nee S: **The evolution of costly mate preferences II. The "handicap" principle**. *Evolution* 1991, **45**:1431-1442.

65. •• Fromhage L, Henshaw JM: **The balance model of honest sexual signaling**. *Evolution* 2022, **76**:445-454.
This paper presents a new model of sexual signalling, which considers the diminishing returns of investment into individual fitness components, thereby arguing that honest sexual signals are in balance with the allocation of resources to other components of the individual's fitness.

66. Fisher RA: **The Genetical Theory of Natural Selection: A Complete Variorum Edition**. Oxford University Press; 1999.

67. Emlen DJ: **Environmental control of horn length dimorphism in the beetle *Onthophagus acuminatus* (Coleoptera: Scarabaeidae)**. *Proc R Soc B: Biol Sci* 1994, **256**:131-136.

68. Warren IA, Gotoh H, Dworkin IM, Emlen DJ, Lavine LC: **A general mechanism for conditional expression of exaggerated sexually-selected traits**. *Bioessays* 2013, **35**:889-899.

69. Macartney EL, Crean AJ, Nakagawa S, Bonduriansky R: **Effects of nutrient limitation on sperm and seminal fluid: a systematic review and meta-analysis**. *Biol Rev* 2019, **94**:1722-1739.

70. • Dougherty LR: **Meta-analysis reveals that animal sexual signalling behaviour is honest and resource based**. *Nat Ecol Evol* 2021, **5**:688-699.
This meta-analysis demonstrates that sexual signalling is overwhelmingly an honest indicator of individual state, with high-condition individuals, predominantly males, tending to signal more.

71. Bath E, Rostant W, Ostridge HJ, Smith S, Mason JS, Rafaluk-Mohr T, Mank JE, Chapman T, Perry JC: **Sexual selection and the evolution of condition-dependence: an experimental test at two resource levels**. *Evolution* 2023, **77**:776-788 Evolution, 58, 2004, 1038–1046.

72. Casasa S, Moczek AP: **Insulin signalling's role in mediating tissue-specific nutritional plasticity and robustness in the horn-polyphenic beetle *Onthophagus taurus***. *Proc R Soc B: Biol Sci* 2018, **285**:20181631.

73. Takeda K, Furuta T, Hamada M, Sato Y, Taniguchi K, Tanizawa A, Yagi T, Adachi-Yamada T: **Handicap theory is applied to females**

but not males in relation to mate choice in the stalk-eyed fly *Sphyracephala detrahens*. *Sci Rep* 2020, **10**:19684.

74. Hernández A, Martínez-Gómez M, Beamonte-Barrientos R, Montoya B: **Colourful traits in female birds relate to individual condition, reproductive performance and male-mate preferences: a meta-analytic approach**. *Biol Lett* 2021, **17**:20210283.

75. Nolazco S, Delhey K, Nakagawa S, Peters A: **Ornaments are equally informative in male and female birds**. *Nat Commun* 2022, **13**:5917.

76. Parrett JM, Chmielewski S, Aydogdu E, Łukasiewicz A, Rombauts S, Szubert-Kruszyńska A, Babik W, Konczal M, Radwan J: **Genomic evidence that a sexually selected trait captures genome-wide variation and facilitates the purging of genetic load**. *Nat Ecol Evol* 2022, **6**:1330-1342.

77. •• Flintham EO, Savolainen V, Mullon C: **Male harm offsets the demographic benefits of good genes**. *Proc Natl Acad Sci* 2023, **120**:e2211668120.

The paper models the consequences of sexual selection on a population with condition dependent sexual traits. They find that sexual conflict is stronger when individuals are in better condition and that good genes selection exacerbates sexual conflict and reduces population size.

78. Pischedda A, Chippindale AK: **Intralocus sexual conflict diminishes the benefits of sexual selection**. *PLoS Biol* 2006, **4**:e356.

79. Pischedda A, Chippindale AK: **Direct benefits of choosing a high-fitness mate can offset the indirect costs associated with intralocus sexual conflict**. *Evolution* 2017, **71**:1710-1718.

80. Harts AM, Schwanz LE, Kokko H: **Demography can favour female-advantageous alleles**. *Proc R Soc B: Biol Sci* 2014, **281**:20140005.

81. Hurley I, Pomiankowski A, Fowler K, Smith H: **Fate map of the eye-antennal imaginal disc in the stalk-eyed fly *Cyrtodiopsis dalmanni***. *Dev Genes Evol* 2002, **212**:38-42.

82. Singh A, Gogia N, Chang CY, Sun YH: **Proximal fate marker homothorax marks the lateral extension of stalk-eyed fly *Cyrtodopsis whitei***. *Genesis* 2019, **57**:e23309.