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REVIEW

The locus of sexual selection: moving sexual selection studies into the post-genomics era

G. S. WILKINSON*, F. BREDEN†, J. E. MANK‡, M. G. RITCHIE§, A. D. HIGGINSON¶, J. RADWAN**, J. JAQUIERY††, W. SALZBURGER‡‡, E. ARRIERO§§, S. M. BARRIBEAU¶¶,[1], P. C. PHILLIPS***, S. C. P. RENN††† & L. ROWE‡‡‡

*Department of Biology, University of Maryland, College Park, MD, USA
†Department of Biological Sciences, Simon Fraser University, Burnaby, BC, Canada
‡Department of Genetics Evolution and Environment, University College London, London, UK
§Centre for Biological Diversity, University of St Andrews, St Andrews, Fife, UK
¶School of Biological Sciences, University of Bristol, Bristol, UK
**Institute of Environmental Biology, Adam Mickiewicz University, Poznan, Poland
††University of Rennes 1, CNRS UMR 6553 EcoBio, Rennes, France
‡‡Zoological Institute, University of Basel, Basel, Switzerland
§§Departamento de Zoologia y Antropologia Fisica, Universidad Complutense de Madrid, Madrid, Spain
¶¶Institute of Integrative Biology, ETH Zürich, Zürich, Switzerland
***Institute of Ecology and Evolution, University of Oregon, Eugene, OR, USA
†††Department of Biology, Reed College, Portland, OR, USA
‡‡‡Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, ON, Canada

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Abstract

Sexual selection drives fundamental evolutionary processes such as trait elaboration and speciation. Despite this importance, there are surprisingly few examples of genes unequivocally responsible for variation in sexually selected phenotypes. This lack of information inhibits our ability to predict phenotypic change due to universal behaviours, such as fighting over mates and mate choice. Here, we discuss reasons for this apparent gap and provide recommendations for how it can be overcome by adopting contemporary genomic methods, exploiting underutilized taxa that may be ideal for detecting the effects of sexual selection and adopting appropriate experimental paradigms. Identifying genes that determine variation in sexually selected traits has the potential to improve theoretical models and reveal whether the genetic changes underlying phenotypic novelty utilize common or unique molecular mechanisms. Such a genomic approach to sexual selection will help answer questions in the evolution of sexually selected phenotypes that were first asked by Darwin and can furthermore serve as a model for the application of genomics in all areas of evolutionary biology.

Introduction

Sexual selection is a powerful evolutionary force that can drive trait diversification within and among species (Darwin, 1871; Andersson, 1994), accelerate rates of molecular evolution (Swanson & Vacquier, 1995, 2002; Aguade, 1999) and promote speciation (Kraaijeveld *et al.*, 2011; Panhuis *et al.*, 2001; Ritchie, 2007; but see Servedio & Bürger, 2014). Sexual selection arises from competition for mates or their gametes when individuals with some trait variants outcompete members of the same sex, either directly or by virtue of being more attractive to the opposite sex (Darwin, 1871; Parker, 1970). These processes may lead to the evolution of sexually selected traits, usually in the male, leading to increased attractiveness, such as vivid coloration, vigorous courtship behaviours or extravagant body modifications, or increased competitiveness through enlarged

Correspondence: Gerald S. Wilkinson, Department of Biology, University of Maryland, College Park, MD 20742, USA. Tel.: +1 301 405 6942; fax: +1 301 314 9358; e-mail: wilkinso@umd.edu
[1]Present address: Department of Biology, East Carolina University, Greenville, NC, USA

body size, weapons or armour (Andersson, 1994). These structures and behaviours often differ conspicuously among males within populations and between closely related species, and female preferences for these male characters sometimes vary in parallel with them (Gray & Cade, 2000; Brooks, 2002; Grace & Shaw, 2011; Oh *et al.*, 2012), suggesting that evolution of both trait and preference can occur rapidly.

Darwin (1871) was the first to conceptualize sexual selection as a force distinct from natural selection. Because of the distinction between natural and sexual selection – the former generated by the direct action of the environment on survival and reproduction and the latter by variation in mating success – theoretical models have been crucial for separating their individual effects. For example, verbal and mathematical models have been particularly critical for explaining how traits and female preferences can evolve (Fisher, 1930; Lande, 1981; Kirkpatrick, 1982; Grafen, 1990; Pomiankowski *et al.*, 1991; Kirkpatrick & Hall, 2004b; Bernhard & Hamelin, 2013) and how the evolution of these traits might aid or impede diversification and speciation (Lande, 1981; Pomiankowski & Iwasa, 1998; Gavrilets, 2000; Servedio & Bürger, 2014). In general, most models of sexual selection that present possible scenarios for the evolution and maintenance of sexually selected traits, including mating preferences, are based on simple assumptions (e.g. two autosomal loci or simple quantitative genetic models of two or three traits). In many areas of evolutionary ecology, incorporation of mechanistic details into theoretical models is needed (McNamara & Houston, 2009) to overcome a mismatch between the assumptions of theory and the complexities of natural systems. Sexual selection theory is a leading case where mechanisms, namely the genetic details of specific systems, impose limitations to adaptation (Kirkpatrick & Hall, 2004a). To determine appropriate assumptions for sexual selection models, we require a better understanding of the genetic variants that give rise to sexually selected traits and enable their evolution. Recent advances in genomic approaches, coupled with the availability of genome sequences for a rapidly increasing number of species (Haussler *et al.*, 2009; Bernardi *et al.*, 2012; Evans *et al.*, 2013; Brawand *et al.*, 2014; Zhang *et al.*, 2014), provide opportunities for gaining insight into the genetic mechanisms underlying sexually selected traits. A major purpose of this review is to explore how new genomes and genomic approaches could be used to uncover the loci encoding sexually selected phenotypes so as to increase our understanding of the patterns of convergence and diversification of these traits in diverse species.

A long-standing goal of evolutionary biology has been to understand the genetic basis of evolutionary change (Dobzhansky, 1970; Lewontin, 1974). The recent explosion of genomic data and approaches has enabled progress towards this goal in several areas of evolutionary biology. For example, comparing the genomes of recently diverged species has made it possible to test alternative models of speciation (reviewed in Seehausen *et al.*, 2014) and to identify the genetic mechanisms underlying phenotypic adaptations (reviewed in Barrett & Hoekstra, 2011; Savolainen *et al.*, 2013), in some cases pinpointing the exact genomic locations under selection (Jones *et al.*, 2012). However, the genomic revolution has yet to infiltrate empirical studies of sexual selection to the same degree as other areas of evolutionary biology. Although key genes have been identified that influence the development of some sexually selected traits (Moczek & Rose, 2009; Williams & Carroll, 2009; Emlen *et al.*, 2012; Khila *et al.*, 2012; Kijimoto *et al.*, 2012; Santos *et al.*, 2014), the underlying sequence variants that cause differences in sexually selected traits within or between the sexes (which we will refer to as the 'locus of sexual selection') remain largely unidentified, with a few notable exceptions (Johnston *et al.*, 2011). As a result, most studies of sexual selection lack a precise genetic foundation, which hampers progress in the evaluation of the role of sexual selection in trait elaboration and diversification, molecular evolution and speciation.

Below, we discuss several reasons why it is likely to be more difficult to identify genes involved in sexual selection than in ecological adaptation. We then describe possible genomic approaches for revealing the sequence differences that underlie the morphological, physiological and behavioural diversity found within and between the sexes of many animals. We suggest alternative hypothesis-testing frameworks and organisms that have particular potential for accelerating our understanding of how sexual selection produces evolutionary change. Finally, we explain how identifying the genetic differences that determine intrasexual variation in attractiveness or underlie variation in trait sexual dimorphism within and between species can help us understand the process of sexual selection.

Challenges of a genomic approach to sexual selection

Although understanding the genetic basis of adaptive traits can be difficult (Rockman, 2012; Travisano & Shaw, 2013), notable progress has been made by studying model genetic organisms (e.g. Keane *et al.*, 2011), or closely related species for which existing genomic tools can be applied (Barrett & Hoekstra, 2011; Savolainen *et al.*, 2013). As difficult as this task may be for adaptive characters, genomic analyses of sexually selected traits pose at least three additional challenges. First, if Williams & Carroll (2009) are correct, then the majority of sexually dimorphic traits can be expected to develop as a consequence of differences in gene regulation rather than differences in coding sequences of genes. This is because gene regulation enables pheno-

typic differences to develop between the sexes, despite the fact that the two sexes largely share identical genomes. The exceptions to the shared genome are the sex-specific regions (Table 1) of the Y or W sex chromosomes. However, in animals with chromosomal sex determination, these regions appear to contain only a minority of the loci underlying sexually selected traits or female preferences (reviewed in Dean & Mank, 2014). Furthermore, many animals with sexually selected traits lack sex chromosomes altogether (reviewed in Beukeboom & Perrin, 2014). Gene regulation systems inherently depend on both DNA (or RNA) binding site motifs and trans-acting binding factors whose motif affinities we are only beginning to understand (e.g. Payne & Wagner, 2014). Because such systems may involve multiple short genomic regions that respond to sex-specific signals, such as alternatively spliced transcripts, detecting the underlying genetic cause of regulatory differences is challenging (although not impossible, Glaser-Schmitt *et al.*, 2013) using population genomic comparisons. These difficulties are multiplied many fold if regulation involves post-transcriptional or post-translational changes in protein abundance, which is currently much more difficult to study (Breker & Schuldiner, 2014). Once regulatory sequences are identified, they may be scrutinized as candidates for causing trait differences between the sexes or variation in elaboration within a sex (e.g. Loehlin *et al.*, 2010; Loehlin & Werren, 2012).

The second additional challenge is that sexually selected traits, by definition, experience different forms of selection in the two sexes (see Fig. 1). For example, strong directional selection on a male phenotype, such as tail length, could be accompanied by stabilizing selection in females, resulting in the possibility of substantial sexual conflict. Depending on how (or if) such

Table 1 Glossary of terms.

Term	Definition
Alternative splicing	Production of multiple messenger RNA variants from a single gene through different combinations of exons
Binding site motif	A short sequence (typically 4–30 bp) of DNA that is bound by molecules such as transcription factors
Candidate gene	A gene already known, or suspected (e.g. through homology), to be involved in the development of a phenotypic trait
Cis-acting element	A region of DNA that influences the expression of nearby genes
Differential gene expression	Comparison of the expression level for a given gene between samples Here, this is either between males and females or between individuals of the same sex that differ in a sexually selected phenotype
Forward genetics	Identifies genes that influence phenotypes by associating phenotypic variation with genetic sequence variation either by mapping or by cloning
GWAS	Genomewide association studies involve testing for an association between variable markers, such as a single nucleotide polymorphisms, and the expression of a phenotypic trait, across the entire genome
Locus of sexual selection	The underlying sequence variants that cause differences in sexually selected traits within or between the sexes
QTL(N)	Quantitative trait locus (nucleotide), a region of the genome that significantly associates with phenotypic variation present among lines or strains
Nonsynonymous substitution	A single nucleotide change that alters the amino acid sequence of a protein
Regulatory network	A set of genes that interact via RNA, proteins or other molecules to control the expression of RNA or protein
RADseq	Restriction-site-associated DNA sequencing, a reduced representational library (RRL) method for locating a large number of genetic markers (e.g. SNPs) throughout the genome that utilizes only those sequences flanking restriction sites where a particular restriction enzyme cuts DNA
Reverse genetics	Disrupts or modifies a target gene to determine its phenotypic effect
Sex-specific nonrecombining region	Region of the Y or W sex chromosome that never recombines during meiosis and is either only present in males (Y chromosome) or females (W chromosome)
SNP	Single nucleotide polymorphism, a population characteristic in which more than one nucleotide (C,A,T or G) is present within or between individuals at a single genomic site.
Synonymous substitutions	A nucleotide substitution in a codon that does not alter the amino acid sequence of the translated protein
Selective sweep	Reduction of polymorphism in a genomic region caused by recent positive selection on an allele, resulting in rapid increase in frequency
Transcription factor	Protein that controls the expression pattern of a gene by binding to regulatory elements
Transcriptome	All of the expressed genes within an individual's genome at a given time or condition
Transposable element	A genomic sequence that can change its location within the genome either by an RNA intermediate or by excision and insertion of DNA
Trans-acting element	A protein or RNA molecule that influences gene regulation elsewhere in the genome

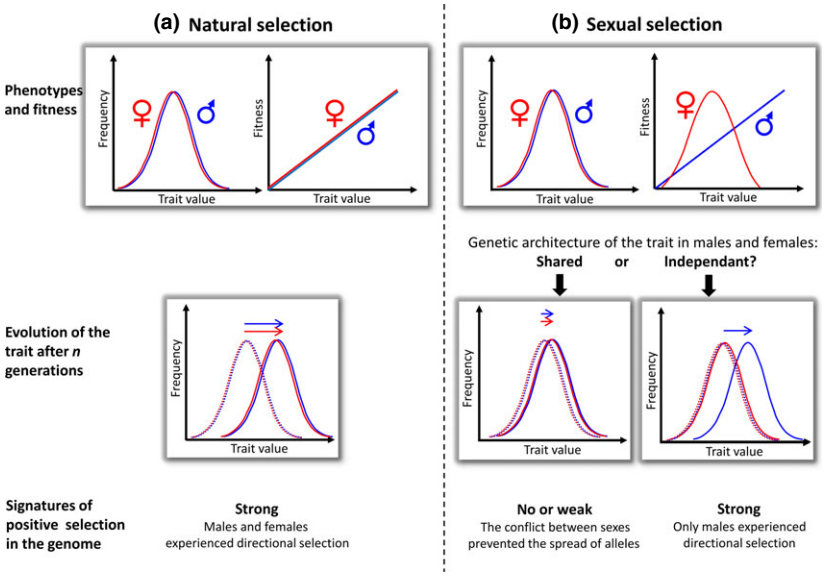

Fig. 1 Comparison of the effects of natural (a) and sexual (b) selection on the evolution of male and female phenotypes. The arrows denote the change in average phenotype after several generations for males (blue) and females (red).

conflicts are resolved, molecular signatures of selection could be less obvious than in cases where selection acts congruently in both sexes, or difficult to distinguish from other forms of balancing selection. Moreover, this difficulty can be compounded by pleiotropic gene expression in which selection varies additionally by tissue type (Mank *et al.*, 2008). Further, frequency-dependent selection, which may often be an important component of sexual selection, is likely to generate different signatures of selection than accounted for in classic sweep models (Takahata & Nei, 1990; Olendorf *et al.*, 2006).

The third additional challenge is that signal–receiver systems involved in sexual selection often comprise one or more behavioural traits. Finding the genetic basis of any behavioural trait is notoriously difficult due to high levels of within-individual phenotypic variation. Nevertheless, genetic polymorphisms for behaviour have been successfully identified (Boake *et al.*, 2002) and genomic approaches can be used to identify alternative strategies (Aubin-Horth & Renn, 2009; Rittschof & Robinson, 2014). Quantifying sexually selected behavioural traits is, however, doubly challenging because receiver responses may depend on a variety of conditions, including motivational state, receptivity and the type of conspecifics used to elicit a response. For example, the number and range of male phenotypes offered can influence the type of mate choice exhibited by a female. As a consequence, female preference functions should be quantified using a variety of male phenotypes even though considerable effort may be required (e.g. Murphy & Gerhardt, 2000; Ritchie, 2000; Shaw & Herlihy, 2000; McGuigan *et al.*, 2008). As in all whole-genome approaches, phenotypic heterogeneity is a major barrier to identifying the genetic basis of traits (Evangelou & Ioannidis, 2013).

Thus, finding the genetic factors associated with sexually selected phenotypes in males or females may require more integrative or novel approaches than are typically used to locate genes involved in speciation or adaptation, and these approaches have generally been lacking from many sexual selection studies. Below, we describe several different genomic approaches that have been or could be used to discover genetic variants underlying variation in sexually selected phenotypes, and identify methods and experimental designs that may be best suited for making progress in sexual selection research in the future.

Genomic methods for studying sexual selection

Studies of the genetic basis of a sexually selected phenotype, either within or between species, can be carried out using two types of analyses (Fig. 2). One type of analysis, which we refer to below as differential gene expression, involves identifying genes that differ in expression either between males and females or between ornamented and nonornamented males, and therefore might give rise to a sexually selected phenotype. These loci can be identified either by quantifying genomewide patterns of inter- or intrasexual gene expression to identify genes with differential transcription or by testing specific candidate genes that may be critically involved in trait development due to their presence in a particular gene regulatory network. The second type of analysis, which we refer to below as either trait-based or anonymous forward genetics, involves finding the underlying sequence variant that

putatively controls variation in the sexually selected trait, that is the locus of sexual selection. Confirmation that sequence change has the inferred phenotypic effects requires sequence or expression manipulation, that is reverse genetics. For both types of analyses, genomic approaches on either model or nonmodel species can provide important information regarding the genetics underlying sexually selected phenotypes.

Differential gene expression

Transcriptional dimorphism, often termed sex-biased gene expression, where a gene is expressed more in one sex than the other sex, is pervasive across a broad array of taxa, and sex often explains most of the variation in gene expression in adult tissues (Yang *et al.*, 2006; Baker *et al.*, 2011; Viguerie *et al.*, 2012; Böhne *et al.*, 2014). The extent of sex-biased expression across taxa, combined with recent evidence of widespread change in sex-biased expression as a consequence of experimental manipulation of sexual selection in *Drosophila* (Hollis *et al.*, 2014; Immonen *et al.*, 2014) and comparative analyses of sex-biased expression among related species across a gradient of sexual selection (Harrison *et al.*, 2015), suggests that patterns of transcription across the genome are strongly influenced by sexual selection. Numerous studies on a broad array of organisms using first microarrays and more recently RNAseq, some of which we review below, are congruent with expectations from sexual selection.

In many cases, male-biased genes exhibit higher variance in expression and are more likely than nonbiased genes to have a duplicate (Gallach *et al.*, 2010; Wyman *et al.*, 2012). Moreover, species-restricted (often referred

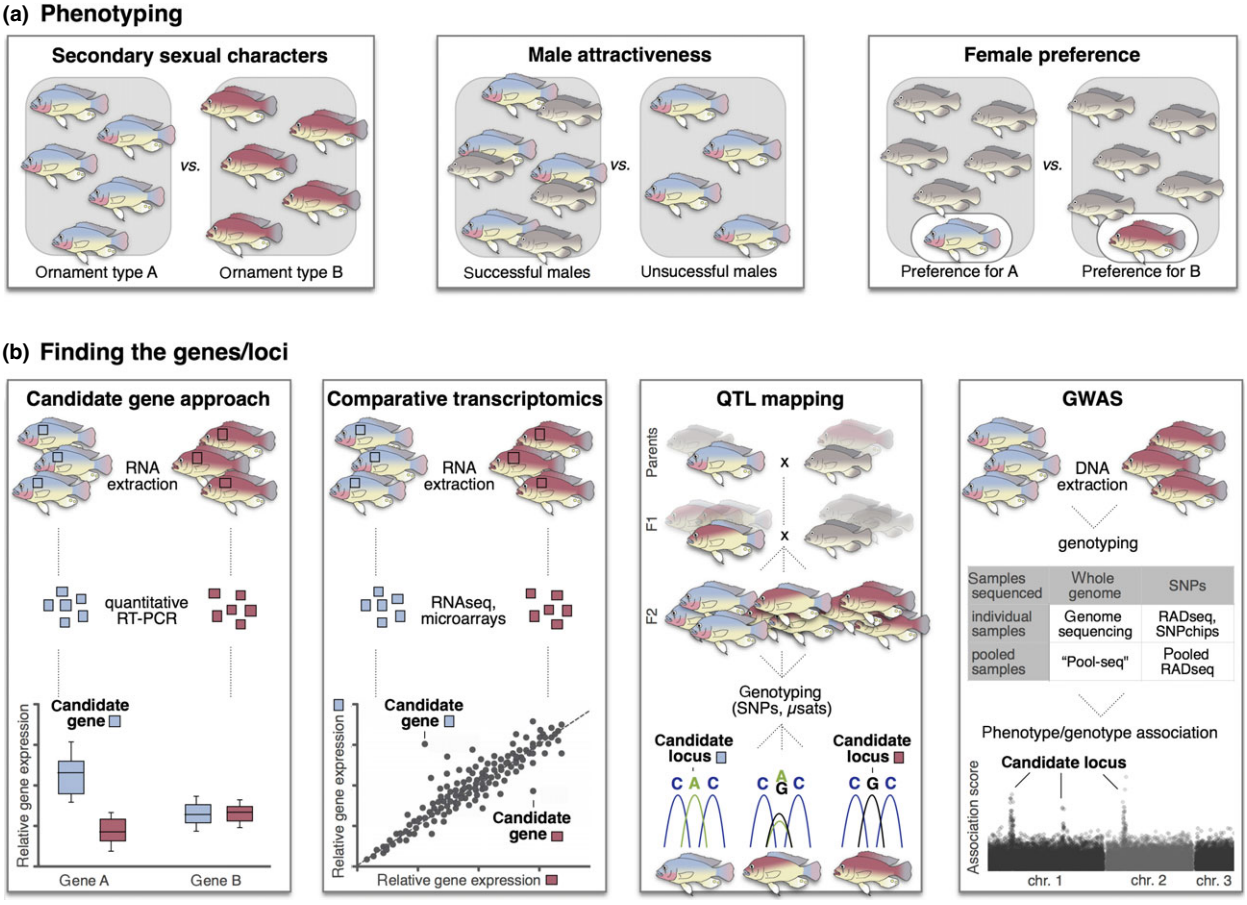

Fig. 2 Overview of forward genetic approaches for identifying genes that control expression of traits involved in sexual selection. The trait used to group individuals may be, for example, a male secondary sexual character, any measure of male attractiveness (e.g. mating success), or female preferences (panel a). Comparisons can be limited to a set of candidate genes (e.g. left panel in b, where expression levels of one candidate and one control gene are assessed) or performed at the scale of the whole genome (the three other panels in b), taking advantage of high-throughput sequencing methods (available for RNA and DNA). Comparative transcriptomics can be used to identify genes that are expressed at different levels between individuals with contrasted phenotypes, whereas QTL (quantitative trait locus) mapping and GWAS (genomewide association studies) pinpoint allelic variants at a locus associated with phenotypic variation.

to as young) genes are more likely to exhibit male-biased than female-biased expression (Zhang *et al.*, 2007). Although these patterns are broadly congruent with a history of strong sexual selection acting on male-specific traits, they may also be the product of high transcription rates in the male germ line or greater functional pleiotropy of genes expressed in females, the latter of which would be expected to constrain their expression and rates of evolution (Zhang *et al.*, 2007).

Interestingly, with some exceptions (Mank *et al.*, 2010; Whittle & Johannesson, 2013), genes with male-biased expression tend to have elevated rates of evolution compared to genes with female-biased expression (reviewed in Parsch & Ellegren, 2013). Although this has been suggested to be the product of positive selection for male traits due to sexual selection (Ellegren & Parsch, 2007), sexual selection does not seem to underlie the evolutionary patterns of coding sequence evolution for male-biased genes. Rather, relaxed evolutionary constraint seems to result in elevated levels of genetic drift for these loci (Moran & Poetrokovski, 2014; Harrison *et al.*, 2015), possibly due to their tissue- and sex-specific expression patterns (Zhang *et al.*, 2007). The incongruence between sexually selected traits and coding sequence evolution of male-biased genes illustrates the need to remain cautious in drawing direct connections between the transcriptome and the phenotype.

Although sexual selection is clearly an important source of sex-specific selection, without additional functional genetic analysis it is not possible to determine whether the genes that show significant sex-biased expression also encode or influence identifiable sexually selected phenotypes. Functional genetic analysis can be complicated because gene expression differences between females and males vary substantially throughout development (Mank *et al.*, 2010; Wilkinson *et al.*, 2013; Perry *et al.*, 2014) as well as across tissues (Yang *et al.*, 2006; Baker *et al.*, 2011); therefore, ontogenetic trajectories of sexually selected phenotypes must be determined to identify when and where differential gene expression triggers the development of sexually selected traits. Nevertheless, studies of gene expression in species with intrasexual variation in male phenotypes indicate that sexual selection does contribute substantially to sex-biased gene expression patterns. For example, in turkeys (Pointer *et al.*, 2013), horned beetles (Snell-Rood *et al.*, 2011) and bulb mites (Stuglik *et al.*, 2014), more dimorphic, sexually selected morphs are characterized by widespread elevated male-biased expression compared to less sexually dimorphic morphs. Furthermore, related avian species with elevated levels of sexual dimorphism resulting from sexual selection show increased levels of male-biased expression compared to monomorphic species (Harrison *et al.*, 2015). These results indicate that patterns of sex-biased gene expression are congruent with phenotypic differences. Although the large numbers of differentially expressed genes in these species suggest that candidate gene approaches may fail in some cases to identify many of the genes involved in these phenotypes, these approaches do indicate that detailed tissue-specific expression studies might be useful in reconstructing sexually dimorphic gene networks in other species with male dimorphisms, such as found in sheep (Johnston *et al.*, 2011), ruff (Lank *et al.*, 1995, 2013), blue-headed wrasse (Alonzo & Warner, 2000), side-blotched lizards (Sinervo & Lively, 1996) or sponge isopods (Shuster & Wade, 1991; Shuster & Sassaman, 1997), to give a few possible examples.

When traits are controlled by relatively few loci, candidate gene approaches may be useful. Such candidates may be chosen either through knowledge of existing gene regulatory networks or by detection of differential expression in a transcriptome experiment as described above. This approach has revealed, for example, that *doublesex* (Kijimoto *et al.*, 2012) and insulin growth factors are associated with sexually dimorphic horn development in beetles (Emlen *et al.*, 2012), *distalless* is associated with sexually dimorphic antennae in water striders (Khila *et al.*, 2012), and the transcription factor *fruitless* is involved in determining the gender of the central nervous system of *Drosophila* and together with *doublesex* influences many elements of the behavioural courtship repertoire (Demir & Dickson, 2005; Rideout *et al.*, 2007). This type of candidate gene or candidate pathway approach is ideal for finding genes that are conserved across taxa, such as *doublesex,* which is associated with sexual differentiation in a variety of insect species (Gempe & Beye, 2010), but may fail to recover rapidly evolving genetic regions (Wilkins, 2014). Finding the genetic differences that underlie inter- or intraspecific variation in sexually selected traits requires an approach that can detect DNA sequence changes that have morph-specific or sex-specific effects.

Trait-based forward genetics

The classical approach to identifying the genetic basis of a particular trait is to associate phenotypic variation with genetic markers in a mapping population of individuals in which both phenotype and genotype are segregating in predictable patterns, usually as a consequence of a line cross or pedigree relationship (Liu, 1998; Lynch & Walsh, 1998). In organisms with an annotated genome and with sufficient mapping resolution, quantitative trait loci (QTL) can then be examined for candidate gene regions to determine potential genetic mechanisms. Large numbers of markers can now be obtained relatively quickly and easily using restriction-site-associated DNA (RAD) markers and related methods (Miller *et al.*, 2007; Baird *et al.*, 2008; Hohenlohe *et al.*, 2010). As long as the phenotype is heritable, genetic differences can be directly linked to

phenotypic variation both within and between sexes. Several examples of this approach exist for sexually selected traits (e.g. Johns *et al.*, 2005; Shaw *et al.*, 2007; Chenoweth & McGuigan, 2010; Schielzeth *et al.*, 2012), but relatively few have been able to connect phenotypic variation to genotypic variation at the sequence level. Exceptions include cases in which the genome is well characterized, and large-scale mapping studies are possible, such as in *Drosophila* (e.g. Kopp *et al.*, 2000, 2003). However, some studies of QTLs for behaviours in *Drosophila*, including male courtship song, suggest that these traits are highly polygenic with few genes of large effect (Turner & Miller, 2012), which makes identifying QTL difficult without very large sample sizes.

The availability of low-cost, high-throughput genotyping and sequencing methods has made genomewide association studies (GWAS) a practical, and in many cases preferable, alternative to QTL mapping. GWAS involve identifying causal regions from whole-genome typing or resequencing of multiple individuals or pools of individuals that differ by phenotype and contain informative single nucleotide polymorphisms (SNPs). A clear advantage of this approach over other mapping techniques based on experimental crossing is that it can utilize most of the natural genetic diversity in a population, rather than some subset, such as found in a set of inbred lines, to locate genetic differences that underlie natural phenotypic variation. Furthermore, GWAS make use of all recombination events that occurred in the past to separate causal and physically linked variants; the amount of recombination possible can otherwise limit resolution with other mapping techniques. For animals with small family sizes or long generation times, GWAS approaches permit study of the quantitative genetics of sexually selected traits in vertebrates and other systems where QTL approaches that require inbreeding or controlled pedigrees are intractable. On the other hand, the added precision provided by GWAS typically comes at the cost of genotyping more individuals at more markers than in a QTL study because the probability of linkage between an anonymous marker and a causal locus is much lower. Recent results from human GWAS raise a particularly strong cautionary tale, as it appears that for many diseases, the full genomes of many tens of thousands of individuals might be necessary for a reasonable chance of success (Visscher *et al.*, 2012). However, there is reason to be more optimistic for the study for sexually selected traits. Rather, than being maintained by mutation–selection balance, as is probably the case for most human disease traits, selection on secondary sexual traits is likely to be strong and, importantly, recent. This history of selection provides an opportunity for alleles of large effect to sort from alleles of smaller effect, especially in comparisons between populations that display divergence in sexually selected traits and particularly if these populations are linked by periodic migration. Similarly, if sexual selection generates frequency-dependent selection at the level of individual alleles, then segregating effect sizes could potentially be larger and allele frequencies higher than expected under mutation–selection balance.

Furthermore, in contrast to studies in humans, it is possible in some animals to generate multiple measurements on the same genotype, which greatly reduces the contribution of sampling variance to estimation errors. Nevertheless, successful application of GWAS requires appropriate experimental design, explicit consideration of genetic background and, when possible, modelling of underlying pathways (Korte & Farlow, 2013; Marjoram *et al.*, 2014).

Although resequencing large numbers of individuals remains prohibitively expensive for many researchers, resequencing pooled samples that contain multiple individuals matched for divergent phenotypes is much more affordable. This pool-seq approach (Sham *et al.*, 2002) relies on past recombination in large populations to find variants that associate with extreme phenotypes and has been referred to as fast-forward genetics (Schneeberger & Weigel, 2011; Leshchiner *et al.*, 2012). By analysing multiple independent sample pools, sampling variance effects can also be reduced. For example, Bastide *et al.* (2013) selected 1000 each of the darkest and lightest individuals from 8000 female offspring produced by large samples of *Drosophila melanogaster* collected in Italy and Austria. Site-specific comparisons of single nucleotide polymorphisms (SNPs) between five replicate dark and light pooled samples identified two small cis-regulatory regions near pigment genes, *tan* and *bric-a-brac 1*, known to be involved in sexually dimorphic abdominal pigmentation. Similarly, a meta-analysis of multiple GWAS based on 2.8 million SNPs for nine sexually dimorphic traits related to body size in 270 000 humans identified seven loci that exhibited sexually dimorphic associations with one of the traits (Randall *et al.*, 2013). A similar approach can be used in experimental populations, such as those that manipulate the strength and pattern of sexual selection using experimental evolution, in which ancestral and selected populations can be compared using pooled sequencing approaches (Schlötterer *et al.*, 2014).

Thus, in principle, genomic approaches can use a virtually unlimited number of SNPs for mapping traits in any organism, such that the search for anonymous marker-based QTLs can now be theoretically replaced with genomic scans for quantitative trait nucleotides (QTNs), that is the nucleotide substitutions associated with variation in quantitative traits. However, QTN approaches applied to nonsexual traits have so far yielded surprisingly few cases in which a sequence variant can be associated with phenotypic variation, even though the traits investigated were known to be heritable (reviewed in, Rockman, 2012; Travisano & Shaw, 2013). This 'missing heritability problem' most likely results from the highly polygenic character of the traits

investigated, such that effects of single nucleotide substitutions can be detected only with large sample sizes (Rockman, 2012) and, if detected, may overestimate the effect size of weak associations (Slate, 2013). The extent to which these issues apply to sexually selected traits depends on the number of genes involved and their relative effect sizes. The existence of at least some cases of major gene effects on male sexually selected traits (e.g. Johnston *et al.*, 2011) suggests that this problem is not universal, but it may be substantial in some systems.

Anonymous forward genetics

A disadvantage of trait-based approaches is that phenotypic measurements are typically conducted independent of the mechanism of sexual selection, *that is* the degree to which a particular phenotype influences reproductive success is not taken into account. In many species, phenotypic differences between successful and unsuccessful mating individuals are not immediately obvious. In these cases, a trait-based approach cannot be easily applied. Two alternative approaches, scanning the genome to find regions that exhibit signatures of recent selection or using variation in mating success to identify different categories of individuals for GWAS analyses, may provide solutions in some circumstances, although the limitations of these approaches also need to be recognized.

Signatures of selection in genome sequences manifest in several ways that can be detected by comparing sequences between species or between populations within species (Akey *et al.*, 2004; Hurst, 2009). For example, one can detect possible positive selection on a gene by calculating the ratio of normalized nonsynonymous to synonymous substitution rates, between two or more species. Alternatively, one can calculate measures of genetic diversity across the genome within a population and compare them to neutral expectations (e.g. Tajima's D, Tajima, 1989) or between different populations (e.g. FST, Wright, 1951). Strong directional selection is then revealed by evidence of a recent selective sweep that locally reduces variation within, or increases divergence between, populations. In contrast, balancing selection should increase diversity within populations and might also decrease divergence between them (Nielsen *et al.*, 2005). Genes involved in sexual competition that have sex-limited expression, such as male accessory gland proteins, can be expected to have characteristic molecular signatures of strong positive selection. However, genes that are expressed in both sexes might not produce the same type of signature of genomic change as that produced solely by natural selection, because sexual selection acts differently on males than females in the same population or a trait is conditionally expressed (Van Dyken & Wade, 2010). In some cases, this may produce signatures of positive selection, but in other cases of conflicting selection between the sexes, signatures of weak balancing selection may result (Connallon & Clark, 2012, 2013; Mullon *et al.*, 2012).

However, regions of the genome display signatures of positive or balancing selection unrelated to sexual selection. It is therefore quite important to note that genomic scans in themselves cannot differentiate natural from sexual selection, as they simply reveal the molecular signature, rather than the cause, of selection. Consequently, detecting evidence of sexual selection requires demonstrating that genetic differences among individuals associate with sex-specific phenotypic effects. In the absence of sex-specific allelic associations, it can be difficult to tell whether the molecular signal of selection is due to natural selection, sexual selection, a genomic conflict such as segregation distortion, or some combination (e.g. Patton, 2014). Thus, signatures of selection by themselves are unlikely to provide unequivocal evidence of sexual selection. One potential exception is when sex-specific alternatively spliced gene transcripts show differing signatures of selection. Such a case has recently been described for *fruitless* in *Drosophila* and suggests that male functions have been under stronger divergent selection, most likely due to sexually dimorphic selection pressures (Parker *et al.*, 2014).

Also, rather than focusing on the specific traits thought to be under sexual selection, if the mating success of large numbers of individuals can be determined, then a GWAS could be conducted on mating success itself. Any genomic regions identified in this way should be functionally coupled to traits that are by definition the targets of sexual selection. In this way, the GWAS approach would be anonymous to the specific traits and could, in fact, be used to help identify the meaningful set of intermediate traits (sensu 'reverse ecology', Levy & Borenstein, 2012). If such a GWAS analysis were coupled with measurements of gene expression in males and females, assuming the appropriate tissues were examined, then it should also be possible to determine the underlying cause of sex-biased gene expression and relate this to sexually selected phenotypic variation. For example, an explosive breeding frog (Wells, 1977) or lekking fly (Wilkinson & Johns, 2005) would be ideal for such a GWAS of mating success.

Reverse genetics

Once candidate genes or regulatory regions are identified, direct genetic manipulation and functional confirmation are typically required before concluding that a sequence variant is truly causal. Historically, such gene manipulation involved constructing and testing transgenic organisms, which in many cases is difficult and time-consuming although in some cases manipulation

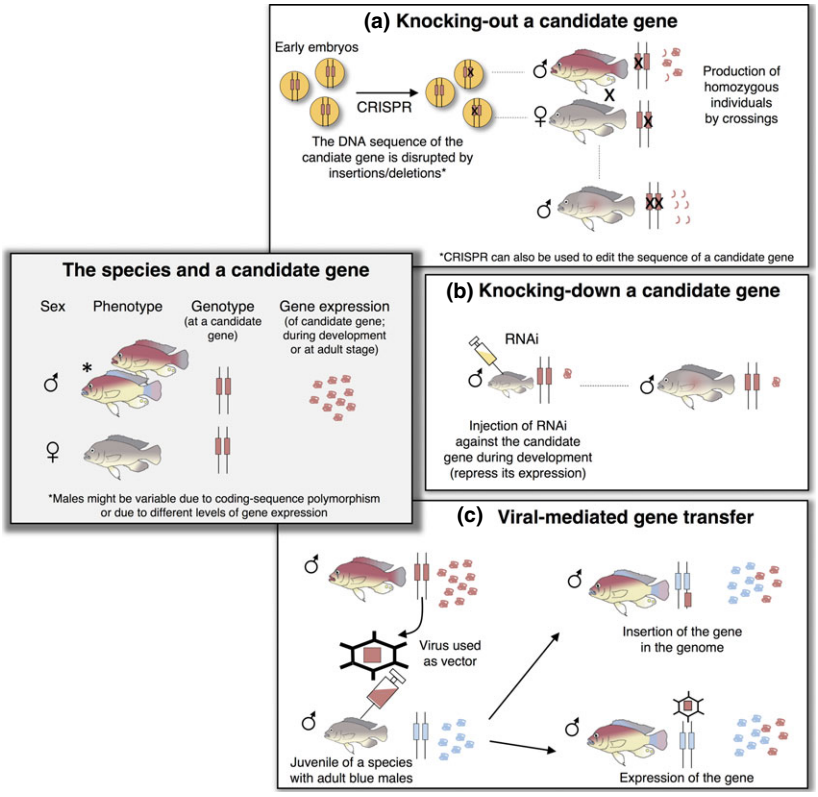

Fig. 3 Overview of reverse genetic approaches for functional validation of a candidate gene. In the species considered, the candidate gene controls variation in a male secondary sexual character with the variation among males resulting either from a genetic polymorphism (e.g. different alleles at a locus encode different male phenotypes) or from the amount of gene product (e.g. the amount of protein determines alternative male phenotypes). Knocking-out such a gene using CRISPR technology (Panel a) leads to a nonfunctional protein because of frameshifts or premature stop codons and confirms that males homozygous for the disrupted allele have an altered phenotype. CRISPR approaches can also be used to edit allelic variants in order to evaluate the phenotypic effect of different alleles in the same genetic background. For genes with pleiotropic effects, knocking-down candidate gene expression with RNA interference (Panel b) can be used to test causation at a specific developmental stage without genome editing. Alternatively, viral-mediated transfer (Panel c) provides a way to express a candidate gene (or its different alleles) in another genetic background or species to evaluate its phenotypic effect in adults.

of a related model organism can be informative. For example, transformed zebrafish have been used to confirm that a novel sexually selected phenotype of haplochromine cichlid fish, anal fin egg spots, is due to a rapidly evolving paralog of a pigmentation gene whose expression has been modified by insertion of a transposable element (Santos *et al.*, 2014). In cases where model organisms cannot be used, several techniques are now available that permit gene sequence or expression modification (see Fig. 3). RNA interference and morpholinos (e.g. Marshall *et al.*, 2009; Khila *et al.*, 2012) can be used to decrease gene expression. In some systems, the effect can be modulated or activated to occur at a specific time or place during development (Mohr, 2014). Viral-mediated gene transfer (e.g. Bennett *et al.*, 1999; Young & Wang, 2004) can be used to introduce novel gene sequences into brain tissues of adult vertebrates to modify behaviour (Harris & Hofmann, 2014). Direct sequence editing using clustered regularly interspaced short palindromic repeats (CRISPR) can be used to selectively modify DNA (Xue *et al.*, 2014) or RNA (O'Connell *et al.*, 2014). These techniques now make it possible to do reverse genetics on a wide range of species.

Experimental paradigms for inferring sexual selection

Although the methods described above will identify genetic variants that influence phenotypes, the degree to which those phenotypes are caused by sexual selection is likely to remain in doubt, as any kind of association study of natural variation is necessarily correlational in nature. In particular, effects due to sexual selection could often be conflated with effects due to viability selection. Thus, separating sexual selection from viability selection requires either taking advantage of a natural experiment in which sexual selection varies

across populations and/or morphs or using experimental evolution in which sexual selection is manipulated directly.

Several types of natural experiments can be informative. Species in which individuals change sex over their lifetime, such as in many teleost fishes, or are simultaneously hermaphroditic, such as some nematode worms, provide situations where male and female traits could be measured in the same individual. Similarly, clonal organisms, such as *Daphnia*, where both sexes occur in the same genotype, allow for simultaneous testing of SNP variants with traits from either sex, as well as comparison of gene expression changes between the sexes. Alternatively, closely related species that can still interbreed or isolated populations that differ in mating systems and/or in sexually dimorphic traits (Houde, 1993) provide opportunities to detect the underlying genetic causes using a GWAS approach between populations.

For organisms that can be reared in captivity, experimental evolution provides a powerful technique for studying the dynamics of beneficial alleles, as populations evolving in the laboratory experience natural and sexual selection in a replicated, controlled manner. Thus, manipulating the mating system in replicate lines is one way to measure the effect of sexual selection on the phenotype. Possible mating regimes include choice (mating in a group) versus no choice (random pair mating), which permits assessment of the effect of pre-mating sexual selection, or single mating versus multiple mating, which can reveal effects of post-mating sexual selection (caused by either sperm competition or cryptic female choice). Whole-genome resequencing, obtained over the course of sustained laboratory selection, could potentially provide insights into the mutational dynamics that most likely occur in natural populations under similar circumstances for organisms with short generation times. To date, whole-genome data are available for only a few evolution experiments (Burke *et al.*, 2010; Burke, 2012; Pespeni *et al.*, 2013). Recent RNA sequencing of evolved lines of *Drosophila* has demonstrated that sexual dimorphism of the transcriptome may rapidly respond to sexual selection, with female *D. melanogaster* showing a more 'feminized' transcriptome when they have been reared under monogamy for several generations (Hollis *et al.*, 2014). Furthermore, genes that are sexually dimorphic in expression are more likely to respond to artificial manipulation of the intensity of sexual selection in female *D. pseudoobscura* (Immonen *et al.*, 2014).

With sequencing costs continuing to fall, such approaches will become increasingly feasible and the number and nature of genes showing species-specific responses to sexual selection will become clearer. Limitations may shift from obtaining sufficient genomic sequence information to obtaining reliable phenotypic information. Methods for automating phenotype measurements, such as running, fighting and flying in *Drosophila* (Dankert *et al.*, 2009; Babcock & Ganetzky, 2014; Bath *et al.*, 2014; Pérez-Escudero *et al.*, 2014) enable collection of phenotypes from large numbers of individuals in short periods of time and, as a consequence, could be used to increase statistical power in GWAS analyses.

What we can learn from a genomic approach to sexual selection

As our ability to apply genomic approaches to questions in sexual selection rapidly advances, it is important to consider the overarching goals, and how these should help prioritize questions to which genomics are applied. As noted above, theoretical models have been critical for understanding how female preference evolution could occur, and finding the genetic basis of both female preferences and sexually selected male traits can be key to evaluating the relative importance of alternative models for female preference evolution. For example, mapping the genetic differences responsible for trait variation onto phylogenies could be used to test whether the genetic differences responsible for male trait exaggeration evolve before or after those for female preference. The latter supports a pre-existing sensory bias mechanism for female preference evolution (Endler, 1992; Ryan & Keddy-Hector, 1992). In contrast, co-evolutionary models of sexual selection assume that female preferences evolve in response to selection on male traits. In addition, these female–male co-evolutionary processes depend on various additive genetic covariances arising between female preference, male trait and offspring viability (Mead & Arnold, 2004; Kokko *et al.*, 2006). Traditionally, quantitative genetic approaches have been used to measure these covariances in breeding designs or selection experiments (Blows, 1999; Qvarnström *et al.*, 2006) but have not identified loci underlying these traits. Finding the actual genes involved would help reveal how pleiotropy and linkage promote or constrain each of these covariances. For example, an important pheromonal polymorphism in *Drosophila* is influenced by the gene *desat-1,* which influences both signalling and receiving. This gene shows tissue-specific alternative splicing, with one isoform in the pheromone-producing tissues responsible for the pheromone change, and another isoform expressed in antennal neurons important for pheromone recognition (Bousquet *et al.*, 2012).

Determining the molecular mechanisms underlying variation in sexually selected traits can also reveal whether recurrent cases of trait elaboration stem from a common genetic or developmental mechanism or involve derived but convergent causes. For example, the insulin-signalling pathway has been proposed as a mechanism that links organism condition to the development of sexually selected ornaments and weapons in

a variety of species, from insects to mammals (Emlen *et al.*, 2012; Warren *et al.*, 2013). Identifying causal genetic variants influencing ornament expression in additional organisms would provide a test of this hypothesis and perhaps reveal other important developmental pathways that have been utilized by different taxa.

Another conundrum in sexual selection arises because strong selection is expected to rapidly deplete genetic variation for mating preferences, attractive male traits and offspring viability indicated by a male ornament. Given that sexual selection has rapidly shaped morphological and behavioural diversity in many species, genetic variation in these characters must have been, and apparently still is (Prokop *et al.*, 2012; Prokuda & Roff, 2014), present. This seeming contradiction is often referred to as the paradox of the lek (Taylor & Williams, 1982; Kirkpatrick & Ryan, 1991). Although a number of theoretical solutions to the lek paradox have been offered (Pomiankowski & Møller, 1995; Rowe & Houle, 1996; Kotiaho *et al.*, 2001; Kokko & Heubel, 2008; Higginson & Reader, 2009), understanding the genetic basis for a sexually selected trait and how it interacts with environmental variation can help determine what maintains genetic variation and, in conjunction with estimates of selection, enable predictions of evolutionary dynamics (Radwan, 2008). For example, identifying the genetic polymorphism responsible for variation in horn morphology in wild Soay sheep revealed that sexual selection favouring large horn size is countered by viability selection favouring smaller horns (Johnston *et al.*, 2013). The resulting heterozygote advantage at a single locus leads to a balanced polymorphism, which is inconsistent with genic capture or other good genes models of sexual selection.

Furthermore, the amount of genetic variation expected for any trait depends on the underlying mutational mechanism, as well as the number of genes contributing to trait expression. The magnitude and directionality of mutational effects on phenotypic variance and covariance could differ dramatically depending on whether new variation in the trait is caused, for example by gene duplication (Izsvak *et al.*, 2009; Kuhn *et al.*, 2014), changes in transcription factor binding sites (Fondon & Garner, 2004; Pearson *et al.*, 2005) or changes in intronic regulatory regions due to transposable element insertions (Faulkner *et al.*, 2009; Wang *et al.*, 2013). Incorporating explicit assumptions about these processes can alter evolutionary predictions. For example, both mutation bias (Pomiankowski *et al.*, 1991) and sex linkage (Kirkpatrick & Hall, 2004b) can influence the outcome of alternative co-evolutionary models for the evolution of female preference. Thus, incorporating explicit genetic mechanisms for sexually selected phenotypes will enable development of models with the potential to provide greater insight into the degree of evolutionary constraint in different systems.

The identification of allelic variants that underlie variation in sexually selected traits could also be used to measure fitness in natural habitats, as has been done for putative adaptations (Le Rouzic *et al.*, 2011; Gompert *et al.*, 2014; Soria-Carrasco *et al.*, 2014). At present, the strength of sexual selection is measured as the relationship between phenotype and reproductive success within generations. By measuring change in the frequency of alleles known to control a sexually selected phenotypic variant, it would be possible to measure long-term fitness consequences of these phenotypes. The lack of examples of this type of approach for sexually selected phenotypes presumably is explained by our lack of knowledge of connections between genetic differences and variation in sexually selected phenotypes. Such studies would provide a way to circumvent a limitation hampering the testing of models of sexual selection: the difficulty of measuring fitness consequences of the expression of sexual traits (Kokko *et al.*, 2003), as well as provide a more integrative measure that can span generations.

Finally, identifying the loci underlying sexually selected traits can help us understand how sexual conflicts can be resolved in the genome. For example, one potential mechanism to resolve sexual conflict is for a gene to undergo duplication and then have the paralogs acquire sex- and tissue-specific expression (Gallach & Betran, 2011). Sex-specific expression can also arise via the acquisition of sex-specific cis-regulatory elements, or, in insects, alternative splicing of transcripts. The degree to which sexual conflict is resolved can have significant biomedical implications, in that understanding the genetic bases underlying the striking differences between females and males in behaviour, physiology and form can have important implications for sex-specific rates of ageing and mortality (Berg & Maklakov, 2012; Maklakov & Lummaa, 2013), and sex differences in response to therapies and treatments have recently become an area of major biomedical concern (Clayton & Colling, 2014). The causes of these differences are largely a product of gene expression differences between males and females, yet there is a strong intersexual correlation between males and females for transcription levels (Griffin *et al.*, 2013). Identifying the genetic basis of sexually selected traits will help reveal the regulatory complexity required to break down intersexual correlations in order to encode sexual dimorphisms.

Conclusions

Sexual selection research has a strong history of building mathematical models that explore the possible paths to diversity and speciation due to exaggerated male traits and female preferences in a variety of species. In an attempt to test these models, many research programmes have focused on using quantitative or func-

tional genetics to find the genetic variants that cause variation in sexually selected traits. However, despite this effort, few sexually selected characters have been mapped to specific loci in the genome. This could be because many of these differences involve changes in gene regulation mechanisms, given that trait differences between the sexes often are encoded by a genome they share. Additionally, our ability to identify regulatory regions and link sequence variants in them to transcriptional and phenotypic variation remain quite limited. Nevertheless, some genomic approaches have been applied to species exhibiting strong sexual dimorphism or intrasexual variation in sexually selected phenotypes. A number of studies have successfully measured sex-specific differences in gene expression, and quantified effects of sex chromosomes, where the initiating polymorphisms for sexual dimorphism may lie. Very few, however, have succeeded in identifying the underlying sequence differences that are responsible for phenotypic evolution due to sexual selection.

We believe this gap can be closed using genomic approaches, such as fast-forward genomic scans, and contrasting either recently diverged species or populations, replicate lines in an experimental evolution paradigm that manipulates sexual selection intensity, or sexually dimorphic phenotypes from a clonal species. New techniques for manipulating gene sequence or expression in nonmodel organisms provide opportunities for confirming causation through direct genetic manipulation that were not previously possible.

Progress in many aspects of evolutionary and behavioural ecology will require greater integration of mechanistic (e.g. genomics) and functional (e.g. co-evolutionary models) approaches (McNamara & Houston, 2009). This is especially the case for sexual selection because shared genomes, sexual conflict and signal–receiver interactions all introduce complexities in how sexually selected traits develop over ontogeny and evolve among species, meaning that simple co-evolutionary models will often fail to predict real-world observations. Identification of causal variants will enable a new generation of theoretical models that allow for the constraints and contingencies of the genomic systems in which sexual selection operates. The post-genomic era provides exciting opportunities to overcome these long-standing obstacles.

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References

Aguade, M. 1999. Positive selection drives the evolution of the Acp29Ab accessory gland protein in *Drosophila*. *Genetics* **152**: 543.

Akey, J.M., Eberle, M.A., Rieder, M.J., Carlson, C.S., Shriver, M.D., Nickerson, D.A. *et al.* 2004. Population history and natural selection shape patterns of genetic variation in 132 genes. *PLoS Biol.* **2**: E286.

Alonzo, S.H. & Warner, R.R. 2000. Female choice, conflict between the sexes and the evolution of male alternative reproductive behaviours. *Evol. Ecol. Res.* **2**: 149–170.

Andersson, M. 1994. *Sexual Selection*. Princeton University Press, Princeton.

Aubin-Horth, N. & Renn, S.C. 2009. Genomic reaction norms: using integrative biology to understand molecular mechanisms of phenotypic plasticity. *Mol. Ecol.* **18**: 3763–3780.

Babcock, D.T. & Ganetzky, B. 2014. An improved method for accurate and rapid measurement of flight performance in Drosophila. *J. Vis. Exp.* **84**: e51223.

Baird, N.A., Etter, P.D., Atwood, T.S., Currey, M.C., Shiver, A.L., Lewis, Z.A., *et al.* 2008. Rapid SNP discovery and genetic mapping using sequenced RAD markers. *PLoS ONE* **3**: e3376.

Baker, D.A., Nolan, T., Fischer, B., Pinder, A., Crisanti, A. & Russell, S. 2011. A comprehensive gene expression atlas of sex-and tissue-specificity in the malaria vector, *Anopheles gambiae*. *BMC Genom.* **12**: 296.

Barrett, R.D.H. & Hoekstra, H.E. 2011. Molecular spandrels: tests of adaptation at the genetic level. *Nat. Rev. Genet.* **12**: 767–780.

Bastide, H., Betancourt, A., Nolte, V., Tobler, R., Stobe, P., Futschik, A. *et al.* 2013. A genome-wide, fine-scale map of natural pigmentation variation in Drosophila melanogaster. *PLoS Genet.* **9**: e1003534.

Bath, D.E., Stowers, J.R., Hörmann, D., Poehlmann, A., Dickson, B.J. & Straw, A.D. 2014. FlyMAD: rapid thermogenetic control of neuronal activity in freely walking Drosophila. *Nat. Methods* **11**: 756–762.

Bennett, J., Maguire, A.M., Cideciyan, A.V., Schnell, M., Glover, E., Anand, V. *et al.* 1999. Stable transgene expression in rod photoreceptors after recombinant adeno-associated virus-mediated gene transfer to monkey retina. *Proc. Natl. Acad. Sci. USA* **96**: 9920–9925.

Berg, E.C. & Maklakov, A.A. 2012. Sexes suffer from suboptimal lifespan because of genetic conflict in a seed beetle. *Proc. R. Soc. Lond. Ser. B: Biol. Sci.* **279**: 4296–4302.

Bernardi, G., Wiley, E.O., Mansour, H., Miller, M.R., Orti, G., Haussler, D. *et al.* 2012. The fishes of Genome 10K. *Mar. Genomics* **7**: 3–6.

Bernhard, P. & Hamelin, F.M. 2013. Simple signaling games of sexual selection (Grafen's revisited). *J. Math. Biol.* **69**: 1719–1742.

Beukeboom, L.W. & Perrin, N. 2014. *The Evolution of Sex Determination*. Oxford University Press, Oxford.

Blows, M.W. 1999. Evolution of the genetic covariance between male and female components of mate recognition:

an experimental test. *Proc. R. Soc. Lond. Ser. B: Biol. Sci.* **266**: 2169–2174.

Boake, C.R.B., Arnold, S.J., Breden, F., Meffert, L.M., Ritchie, M.G., Taylor, B.J. *et al.* 2002. Genetic tools for studying adaptation and the evolution of behavior. *Am. Nat.* **160**: S143–S159.

Böhne, A., Sengstag, T. & Salzburger, W. 2014. Comparative transcriptomics in East African cichlids reveals sex- and species-specific expression and new candidates for sex differentiation in fishes. *Genome Biol. Evol.* **6**: 2567–2585.

Bousquet, F., Nojima, T., Houot, B., Chauvel, I., Chaudy, S., Dupas, S. *et al.* 2012. Expression of a desaturase gene, *desat1*, in neural and nonneural tissues separately affects perception and emission of sex pheromones in Drosophila. *Proc. Natl. Acad. Sci. USA* **109**: 249–254.

Brawand, D., Wagner, C.E., Li, Y.I., Malinsky, M., Keller, I., Fan, S. *et al.* 2014. The genomic substrate for adaptive radiation in African cichlid fish. *Nature* **513**: 375–381.

Breker, M. & Schuldiner, M. 2014. The emergence of proteome-wide technologies: systematic analysis of proteins comes of age. *Nat. Rev. Mol. Cell Biol.* **15**: 453–464.

Brooks, R. 2002. Variation in female mate choice within guppy populations: population divergence, multiple ornaments and the maintenance of polymorphism. *Genetica* **116**: 343–358.

Burke, M.K. 2012. How does adaptation sweep through the genome? Insights from long-term selection experiments *Proc. R. Soc. Lond., Ser. B: Biol. Sci.* **279**: 5029–5038.

Burke, M.K., Dunham, J.P., Shahrestani, P., Thornton, K.R., Rose, M.R. & Long, A.D. 2010. Genome-wide analysis of a long-term evolution experiment with Drosophila. *Nature* **467**: 587–590.

Chenoweth, S.F. & McGuigan, K. 2010. The genetic basis of sexually selected variation. *Annu. Rev. Ecol. Evol. Syst.* **41**: 81–101.

Clayton, J.A. & Colling, F.S. 2014. NIH to balance sex in cell and animal studies. *Nature* **509**: 282–283.

Connallon, T. & Clark, A.G. 2012. A general population genetic framework for antagonistic selection that accounts for demography and recurrent mutation. *Genetics* **190**: 1477–1489.

Connallon, T. & Clark, A.G. 2013. Antagonistic versus nonantagonistic models of balancing selection: characterizing the relative timescales and hitchhiking effects of partial selective sweeps. *Evolution* **67**: 908–917.

Dankert, H., Wang, L., Hoopfer, E.D., Anderson, D.J. & Perona, P. 2009. Automated monitoring and analysis of social behavior in Drosophila. *Nat. Methods* **6**: 297–303.

Darwin, C.R. 1871. *The Descent of Main and Selection in Relation to Sex*. John Murray, London.

Dean, R. & Mank, J.E. 2014. The role of sex chromosomes in sexual dimorphism: discordance between molecular and phenotypic data. *J. Evol. Biol.* **27**: 1443–1453.

Demir, E. & Dickson, B.J. 2005. Fruitless splicing specifies male courtship behavior in Drosophila. *Cell* **121**: 785–794.

Dobzhansky, T. 1970. *Genetics of the Evolutionary Process*. Columbia University Press, New York.

Ellegren, H. & Parsch, J. 2007. The evolution of sex-biased genes and sex-biased gene expression. *Nat. Rev. Genet.* **8**: 689–698.

Emlen, D.J., Warren, I.A., Johns, A., Dworkin, I. & Lavine, L.C. 2012. A mechanism of extreme growth and reliable signaling in sexually selected ornaments and weapons. *Science* **337**: 860–864.

Endler, J.A. 1992. Signals, signal conditions and the direction of evolution. *Am. Nat.* **139**: S125–S153.

Evangelou, E. & Ioannidis, J.P.A. 2013. Meta-analysis methods for genome-wide association studies and beyond. *Nat. Rev. Genet.* **14**: 379–389.

Evans, J.D., Brown, S.J., Hackett, K.J., Robinson, G., Richards, S., Lawson, D. *et al.* 2013. The i5K initiative: advancing arthropod genomics for knowledge, human health, agriculture, and the environment. *J. Hered.* **104**: 595–600.

Faulkner, G.J., Kimura, Y., Daub, C.O., Wani, S., Plessy, C., Irvine, K.M. *et al.* 2009. The regulated retrotransposon transcriptome of mammalian cells. *Nat. Genet.* **41**: 563–571.

Fisher, R.A. 1930. *The Genetical Theory of Natural Selection*. Clarendon, Oxford.

Fondon, J.W. & Garner, H.R. 2004. Molecular origins of rapid and continuous morphological evolution. *Proc. Natl. Acad. Sci. USA* **101**: 18058–18063.

Gallach, M. & Betran, E. 2011. Intralocus sexual conflict resolved through gene duplication. *Trends Ecol. Evol.* **26**: 222–228.

Gallach, M., Chandrasekaran, C. & Betran, E. 2010. Analyses of nuclearly encoded mitochondrial genes suggest gene duplication as a mechanism for resolving intralocus sexually antagonistic conflict in *Drosophila*. *Genome Biol. Evol.* **2**: 835–850.

Gavrilets, S. 2000. Rapid evolution of reproductive barriers driven by sexual conflict. *Nature* **403**: 886–889.

Gempe, T. & Beye, M. 2010. Function and evolution of sex determination mechanisms, genes and pathways in insects. *BioEssays* **33**: 52–60.

Glaser-Schmitt, A., Catalan, A. & Parsch, J. 2013. Adaptive divergence of a transcriptional enhancer between populations of *Drosophila melanogaster*. *Philos. Trans. R. Soc. Lond., Ser. B: Biol. Sci.* **368**: 20130024.

Gompert, Z., Comeault, A.A., Farkas, T.E., Feder, J.L., Parchman, T.L., Buerkle, C.A. *et al.* 2014. Experimental evidence for ecological selection on genome variation in the wild. *Ecol. Lett.* **17**: 369–379.

Grace, J.L. & Shaw, K.L. 2011. Coevolution of male mating signal and female preference during early lineage divergence of the Hawaiian cricket, *Laupala cerasina*. *Evolution* **65**: 2184–2196.

Grafen, A. 1990. Sexual selection unhandicapped by the Fisher process. *J. Theor. Biol.* **144**: 473–516.

Gray, D.A. & Cade, W.H. 2000. Sexual selection and speciation in field crickets. *Proc. Natl. Acad. Sci. USA* **97**: 14449–14454.

Griffin, R.M., Dean, R., Grace, J.L., Ryden, P. & Friberg, U. 2013. The shared genome is a pervasive constraint on the evolution of sex-biased gene expression. *Mol. Biol. Evol.* **30**: 2168–2176.

Harris, R.M. & Hofmann, H.A. 2014. Neurogenomics of behavioral plasticity. In: *Ecological Genomics* (C.R. Landry & N. Aubin-Horth, eds), pp. 149–168. Springer Science, Dondrecht.

Harrison, P.W., Wright, A.E., Zimmer, F., Dean, R., Montgomery, S.H., Pointer, M.A. *et al.* 2015. Sexual selection drives evolution and rapid turnover of male-biased genes. *Proc. Natl. Acad. Sci. USA.* pnas.1501339112.

Haussler, D., O'Brien, S.J., Ryder, O.A., Barker, F.K., Clamp, M., Crawford, A.J. *et al.* 2009. Genome 10K: a proposal to obtain whole-genome sequence for 10 000 vertebrate species. *J. Hered.* **100**: 659–674.

Higginson, A.D. & Reader, T. 2009. Environmental heterogeneity, genotype-by-environment interactions and the reliability

of sexual traits as indicators of mate quality. *Proc. R. Soc. Lond. Ser. B: Biol. Sci.* **276**: 1153–1159.

Hohenlohe, P.A., Bassham, S., Etter, P.D., Stiffler, N., Johnson, E.A. & Cresko, W.A. 2010. Population genomics of parallel adaptation in threespine stickleback using sequenced RAD tags. *PLoS Genet.* **6**: e1000862.

Hollis, B., Houle, D., Yan, Z., Kawecki, T.J. & Keller, L. 2014. Evolution under monogamy feminizes gene expression in *Drosophila melanogaster*. *Nat. Comm.* **5**: 3482.

Houde, A.E. 1993. Evolution by sexual selection: what can population comparisons tell us? *Am. Nat.* **141**: 796–803.

Hurst, L.D. 2009. Fundamental concepts in genetics and the understanding of selection. *Nat. Rev. Genet.* **10**: 83–93.

Immonen, E., Snook, R.R. & Ritchie, M.G. 2014. Mating system variation drives rapid experimental evolution of the female transcriptome in *Drosophila pseudoobscura*. *Ecol. Evol.* **4**: 2182–2201.

Izsvak, Z., Wang, Y. & Ivics, Z. 2009. Interactions of transposons with the cellular DNA repair machinery. In: *Transposons and the Dynamic Genome* (D.-H. Lankenau & J.-N. Volff, eds), pp. 133–176. Springer-Verlag, Berlin.

Johns, P.M., Wolfenbarger, L.L. & Wilkinson, G.S. 2005. Genetic linkage between a sexually selected trait and X chromosome meiotic drive. *Proc. R. Soc. Lond. Ser. B: Biol. Sci.* **272**: 2097–2103.

Johnston, S.E., McEwan, J.C., Pickering, N.K., Kijas, J.W., Beraldi, D., Pilkington, J.G. *et al.* 2011. Genome-wide association mapping identifies the genetic basis of discrete and quantitative variation in sexual weaponry in a wild sheep population. *Mol. Ecol.* **20**: 2555–2566.

Johnston, S.E., Gratten, J., Berenos, C., Pilkington, J.G., Clutton-Brock, T.H., Pemberton, J.M. *et al.* 2013. Life history trade-offs at a single locus maintain sexually selected genetic variation. *Nature* **502**: 93–95.

Jones, F.C., Grabherr, M.G., Chan, Y.F., Russell, P., Mauceli, E., Johnson, J. *et al.* 2012. The genomic basis of adaptive evolution in threespine sticklebacks. *Nature* **484**: 55–61.

Keane, T.M., Goodstadt, L., Danecek, P., White, M.A., Wong, K., Yalcin, B. *et al.* 2011. Mouse genomic variation and its effect on phenotypes and gene regulation. *Nature* **477**: 289–294.

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

Kijimoto, T., Moczek, A.P. & Andrews, J. 2012. Diversification of doublesex function underlies morph-, sex-, and species-specific development of beetle horns. *Proc. Natl. Acad. Sci. USA* **109**: 20526–20531.

Kirkpatrick, M. 1982. Sexual selection and the evolution of female choice. *Evolution* **36**: 1–12.

Kirkpatrick, M. & Hall, D.W. 2004a. Male-biased mutation, sex linkage, and the rate of adaptive evolution. *Evolution* **58**: 437–440.

Kirkpatrick, M. & Hall, D.W. 2004b. Sexual selection and sex linkage. *Evolution* **58**: 683–691.

Kirkpatrick, M. & Ryan, M.J. 1991. The evolution of mating preferences and the paradox of the lek. *Nature* **350**: 33–38.

Kokko, H. & Heubel, K. 2008. Condition-dependence, genotype-by-environment interactions and the lek paradox. *Genetica* **132**: 209–216.

Kokko, H., Brooks, R., Jennions, M.D. & Morley, J. 2003. The evolution of mate choice and mating biases. *Proc. R. Soc. Lond. Ser. B: Biol. Sci.* **270**: 653–664.

Kokko, H., Jennions, M.D. & Brooks, R. 2006. Unifying and testing models of sexual selection. *Annu. Rev. Ecol. Evol. Syst.* **37**: 43–66.

Kopp, A., Duncan, I., Godt, D. & Carroll, S.B. 2000. Genetic control and evolution of sexually dimorphic characters in Drosophila. *Nature* **408**: 553–559.

Kopp, A., Graze, R.M., Xu, S.Z., Carroll, S.B. & Nuzhdin, S.V. 2003. Quantitative trait loci responsible for variation in sexually dimorphic traits in *Drosophila melanogaster*. *Genetics* **163**: 771–787.

Korte, A. & Farlow, A. 2013. The advantages and limitations of trait analysis with GWAS: a review. *Plant Methods* **9**: 29.

Kotiaho, J.S., Simmons, L.W. & Tomkins, J.L. 2001. Towards a resolution of the lek paradox. *Nature* **410**: 684–686.

Kraaijeveld, K., Kraaijeveld-Smit, F.J.L. & Maan, M.E. 2011. Sexual selection and speciation: the comparative evidence revisited. *Biol. Rev.* **86**: 367–377.

Kuhn, A., Ong, Y.M., Cheng, C.-Y., Wong, T.Y., Quake, S.R. & Burkholder, W.F. 2014. Linkage disequilibrium and signatures of positive selection around LINE-1 retrotransposons in the human genome. *Proc. Natl. Acad. Sci. USA* **111**: 8131–8136.

Lande, R. 1981. Models of speciation by sexual selection on polygenic traits. *Proc. Natl. Acad. Sci. USA* **78**: 3721–3725.

Lank, D.B., Smith, C.M., Hanotte, O., Burke, T. & Cooke, F. 1995. Genetic polymorphism for alternative mating behavior in lekking male ruff *Philomachus pugnax*. *Nature* **378**: 59–62.

Lank, D.B., Farrell, L.L., Burke, T., Piersma, T. & McRae, S.B. 2013. A dominant allele controls development into female mimic male and diminutive female ruffs. *Biol. Lett.* **9**: 20130653.

Le Rouzic, A., Ostbye, K., Klepaker, T.O., Hansen, T.F., Bernatchez, L., Schluter, D. *et al.* 2011. Strong and consistent natural selection associated with armour reduction in sticklebacks. *Mol. Ecol.* **20**: 2483–2493.

Leshchiner, I., Alexa, K., Kelsey, P., Adzhubei, I., Austin-Tse, C.A., Cooney, J.D. *et al.* 2012. Mutation mapping and identification by whole-genome sequencing. *Genome Res.* **22**: 1541–1548.

Levy, R. & Borenstein, E. 2012. Reverse ecology: from systems to environments and back. In: *Evolutionary Systems Biology* (O.S. Soyer, ed.), pp. 329–345. Springer, New York.

Lewontin, R.C. 1974. *The Genetic Basis of Evolutionary Change*. Columbia University Press, New York.

Liu, B. 1998. *Statistical Genomics: Linkage, Mapping and QTL Analysis*. CRC Press, Boca Raton, FL.

Loehlin, D.W. & Werren, J.H. 2012. Evolution of shape by multiple regulatory changes to a growth gene. *Science* **335**: 943–947.

Loehlin, D.W., Oliveira, D.C., Edwards, R., Giebel, J.D., Clark, M.E., Cattani, M.V. *et al.* 2010. Non-coding changes cause sex-specific wing size differences between closely related species of Nasonia. *PLoS Genet.* **6**: e1000821.

Lynch, M. & Walsh, B. 1998. *Genetics and Analysis of Quantitative Traits*. Sinauer, Sunderland, MA.

Maklakov, A.A. & Lummaa, V. 2013. Evolution of sex differences in lifespan and aging: causes and constraints. *BioEssays* **35**: 717–724.

Mank, J.E., Hultin-Rosenberg, L., Zwahlen, M. & Ellegren, H. 2008. Pleiotropic constraint hampers the resolution of sexual antagonism in vertebrate gene expression. *Am. Nat.* **171**: 35–43.

Mank, J.E., Nam, K., Brunstrom, B. & Ellegren, H. 2010. Ontogenetic complexity of sexual dimorphism and sex-specific selection. *Mol. Biol. Evol.* **27**: 1570–1578.

Marjoram, P., Zubair, A. & Nuzhdin, S.V. 2014. Post-GWAS: where next? More samples, more SNPs or more biology? *Heredity* **112**: 79–88.

Marshall, J.L., Huestis, D.L., Hiromasa, Y., Wheeler, S., Oppert, C., Marshall, S.A. *et al.* 2009. Identification, RNAi knockdown, and functional analysis of an ejaculate protein that mediates a postmating, prezygotic phenotype in a cricket. *PLoS ONE* **4**: e7537.

McGuigan, K., Van Homrigh, A. & Blows, M.W. 2008. Genetic analysis of female preference functions as function-valued traits. *Am. Nat.* **172**: 194–202.

McNamara, J.M. & Houston, A.I. 2009. Integrating function and mechanism. *Trends Ecol. Evol.* **24**: 670–675.

Mead, L.S. & Arnold, S.J. 2004. Quantitative genetic models of sexual selection. *Trends Ecol. Evol.* **19**: 264–271.

Miller, M.R., Dunham, J.P., Amores, A., Cresko, W.A. & Johnson, E.A. 2007. Rapid and cost-effective polymorphism identification and genotyping using restriction site associated DNA (RAD) markers. *Genome Res.* **17**: 240–248.

Moczek, A.P. & Rose, D.J. 2009. Differential recruitment of limb patterning genes during development and diversification of beetle horns. *Proc. Natl. Acad. Sci. USA* **106**: 8992–8997.

Mohr, S.E. 2014. RNAi screening in Drosophila cells and *in vivo*. *Methods* **68**: 82–88.

Moran, G. & Poetrokovski, S. 2014. Reduced selection and accumulation of deleterious mutations in genes exclusively expressed in men. *Nat. Comm.* **5**: 4438.

Mullon, C., Pomiankowski, A. & Reuter, M. 2012. The effects of selection and genetic drift on the genomic distribution of sexually antagonistic alleles. *Evolution* **66**: 3743–3753.

Murphy, C.G. & Gerhardt, H.C. 2000. Mating preference functions of individual female barking treefrogs, *Hyla gratiosa*, for two properties of male advertisement calls. *Evolution* **54**: 660–669.

Nielsen, R., Williamson, S., Kim, Y., Hubisz, M.J., Clark, A.G. & Bustamante, C. 2005. Genomic scans for selective sweeps using SNP data. *Genome Res.* **15**: 1566–1575.

O'Connell, M.R., Oakes, B.L., Sternberg, S.H., East-Seletsky, A., Kaplan, M. & Doudna, J.A. 2014. Programmable RNA recognition and cleavage by CRISPR/Cas9. *Nature* **516**: 263–266.

Oh, K.P., Fergus, D.J., Grace, J.L. & Shaw, K.L. 2012. Interspecific genetics of speciation phenotypes: song and preference coevolution in Hawaiian crickets. *J. Evol. Biol.* **25**: 1500–1512.

Olendorf, R., Rodd, F.H., Punzalan, D., Houde, A.E., Hurt, C., Reznick, D.N. *et al.* 2006. Frequency-dependent survival in natural guppy populations. *Nature* **441**: 633–636.

Panhuis, T.M., Butlin, R., Zuk, M. & Tregenza, T. 2001. Sexual selection and speciation. *Trends Ecol. Evol.* **16**: 364–371.

Parker, G.A. 1970. Sperm competition and its evolutionary consequences in the insects. *Biol. Rev.* **45**: 525–567.

Parker, D.J., Gardiner, A., Neville, M.C., Ritchie, M.G. & Goodwin, S.F. 2014. The evolution of novelty in conserved genes; evidence of positive selection in the Drosophila fruitless gene is localised to alternatively spliced exons. *Heredity* **112**: 300–306.

Parsch, J. & Ellegren, H. 2013. The evolutionary causes and consequences of sex-biased gene expression. *Nat. Rev. Genet.* **14**: 83–87.

Patton, M.M. 2014. Meiotic drive influences the outcome of sexually antagonistic selection at a linked locus. *J. Evol. Biol.* **27**: 2360–2370.

Payne, J.L. & Wagner, A. 2014. The robustness and evolvability of transcription factor binding sites. *Science* **343**: 875–877.

Pearson, C.E., Edamura, K.N. & Cleary, J.D. 2005. Repeat instability: mechanisms of dynamic mutations. *Nat. Rev. Genet.* **6**: 729–742.

Pérez-Escudero, A., Vicente-Page, J., Hinz, R.C., Arganda, S. & de Polavieja, G.G. 2014. idTracker: tracking individuals in a group by automatic identification of unmarked animals. *Nat. Methods* **11**: 743–748.

Perry, J.C., Harrison, P.W. & Mank, J.E. 2014. The ontogeny and evolution of sex-biased gene expression in Drosophila melanogaster. *Mol. Biol. Evol.* **31**: 1206–1219.

Pespeni, M.H., Sanford, E., Gaylord, B., Hill, T.M., Hosfelt, J.D., Jaris, H.K. *et al.* 2013. Evolutionary change during experimental ocean acidification. *Proc. Natl. Acad. Sci. USA* **110**: 6937–6942.

Pointer, M.A., Harrison, P.W., Wright, A.E. & Mank, J.E. 2013. Masculinization of gene expression Is associated with exaggeration of male sexual dimorphism. *PLoS Genet.* **9**: 1003697.

Pomiankowski, A. & Iwasa, Y. 1998. Runaway ornament diversity caused by Fisherian sexual selection. *Proc. Natl. Acad. Sci. USA* **95**: 5106–5111.

Pomiankowski, A. & Møller, A.P. 1995. A resolution of the lek paradox. *Proc. R. Soc. Lond. Ser. B: Biol. Sci.* **260**: 21–29.

Pomiankowski, A., Iwasa, Y. & Nee, S. 1991. The evolution of costly male preferences. I. Fisher and biased mutation. *Evolution* **45**: 1422–1430.

Prokop, Z.M., Michalczyk, L., Drobniak, S.M., Herdegen, M. & Radwan, J. 2012. Meta-analysis suggests choosy females get sexy sons more than "good genes". *Evolution* **66**: 2665–2673.

Prokuda, A.Y. & Roff, D.A. 2014. The quantitative genetics of sexually selected traits, preferred traits and preference: a review and analysis of the data. *J. Evol. Biol.* **27**: 2283–2296.

Qvarnström, A., Brommer, J.E. & Gustafsson, L. 2006. Testing the genetics underlying the co-evolution of mate choice and ornament in the wild. *Nature* **441**: 84–86.

Radwan, J. 2008. Maintenance of genetic variation in sexual ornaments: a review of the mechanisms. *Genetica* **134**: 113–127.

Randall, J.C., Winkler, T.W., Kutalik, Z., Berndt, S.I., Jackson, A.U., Monda, K.L. *et al.* 2013. Sex-stratified genome-wide association studies including 270,000 individuals show sexual dimorphism in genetic loci for anthropometric traits. *PLoS Genet.* **9**: e1003500.

Rideout, E.J., Billeter, J.C. & Goodwin, S.F. 2007. The sex-determination genes fruitless and doublesex specify a neural substrate required for courtship song. *Curr. Biol.* **17**: 1473–1478.

Ritchie, M.G. 2000. The inheritance of female preference functions in a mate recognition system. *Proc. R. Soc. Lond. Ser. B: Biol. Sci.* **267**: 1–6.

Ritchie, M.G. 2007. Sexual selection and speciation. *Annu. Rev. Ecol. Evol. Syst.* **38**: 79–102.

Rittschof, C.C. & Robinson, G.E. 2014. Genomics: moving behavioural ecology beyond the phenotypic gambit. *Anim. Behav.* **92**: 263–270.

Rockman, M.V. 2012. The QTN program and the alleles that matter for evolution: all that's gold does not glitter. *Evolution* **66**: 1–17.

Rowe, L. & Houle, D. 1996. The lek paradox and the capture of genetic variance by condition-dependent traits. *Proc. R. Soc. Lond. Ser. B: Biol. Sci.* **263**: 1415–1421.

Ryan, M.J. & Keddy-Hector, A. 1992. Directional patterns of female mate choice and the role of sensory biases. *Am. Nat.* **139**: s4–s35.

Santos, M.E., Braasch, I., Boileau, N., Meyer, B.S., Sauteur, L., Böhne, A. *et al.* 2014. The evolution of cichlid fish egg-spots is linked with a cis-regulatory change. *Nat. Comm.* **5**: 5149.

Savolainen, O., Lascoux, M. & Merilä, J. 2013. Ecological genomics of local adaptation. *Nat. Rev. Genet.* **14**: 807–820.

Schielzeth, H., Kempenaers, B., Ellegren, H. & Forstmeier, W. 2012. QTL linkage mapping of zebra finch beak color shows an oligogenic control of a sexually selected trait. *Evolution* **66**: 18–30.

Schlötterer, C., Tobler, R., Kofler, R. & Nolte, V. 2014. Sequencing pools of individuals - mining genome-wide polymorphism data without big funding. *Nat. Rev. Genet.* **15**: 749–763.

Schneeberger, K. & Weigel, D. 2011. Fast-forward genetics enabled by new sequencing technologies. *Trends Plant Sci.* **16**: 282–288.

Seehausen, O., Butlin, R.K., Keller, I., Wagner, C.E., Boughman, J.W., Hohenlohe, P.A. *et al.* 2014. Genomics and the origin of species. *Nat. Rev. Genet.* **15**: 176–192.

Servedio, M.R. & Bürger, R. 2014. The couterintuitive role of sexual selection in species maintenance and speciation. *Proc. Natl. Acad. Sci. USA* **111**: 8113–8118.

Sham, P., Bader, J.S., Craig, I., O'Donovan, M. & Owen, M. 2002. DNA pooling: a tool for large-scale association studies. *Nat. Rev. Genet.* **3**: 862–871.

Shaw, K.L. & Herlihy, D.P. 2000. Acoustic preference functions and song variability in the Hawaiian cricket Laupala cerasina. *Proc. R. Soc. Lond Ser. B: Biol. Sci.* **267**: 577–584.

Shaw, K.L., Parsons, Y.M. & Lesnick, S.C. 2007. QTL analysis of a rapidly evolving speciation phenotype in the Hawaiian cricket *Laupala*. *Mol. Ecol.* **16**: 2879–2892.

Shuster, S.M. & Sassaman, C. 1997. Genetic interaction between male mating strategy and sex ratio in a marine isopod. *Nature* **388**: 373–377.

Shuster, S.M. & Wade, M.J. 1991. Equal mating success among male reproductive strategies in a marine isopod. *Nature* **350**: 608–610.

Sinervo, B. & Lively, C.M. 1996. The rock-paper-scissors game and the evolution of alternative male strategies. *Nature* **380**: 240–243.

Slate, J. 2013. 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.

Snell-Rood, E.C., Cash, A., Han, M.V., Kijimoto, T., Andrews, J. & Moczek, A.P. 2011. Developmental decoupling of alternative phenotypes: insights from the transcriptomes of horn polyphenic beetles. *Evolution* **65**: 231–245.

Soria-Carrasco, V., Gompert, Z., Comeault, A.A., Farkas, T.E., Parchman, T.L., Johnston, J.S. *et al.* 2014. Stick insect genomes reveal natural selection's role in parallel speciation. *Science* **344**: 738–742.

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

Swanson, W.J. & Vacquier, V.D. 1995. Extraordinary divergence and positive Darwinian selection in a fusagenic protein coating the acrosomal process of abalone spermatozoa. *Proc. Natl. Acad. Sci. USA* **92**: 4957–4961.

Swanson, W.J. & Vacquier, V.D. 2002. The rapid evolution of reproductive proteins. *Nat. Rev. Genet.* **3**: 137–144.

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* **123**: 585–595.

Takahata, N. & Nei, M. 1990. Allelic genealogy under overdominant and frequency-dependent selection and polymorphism of major histocompatibility complex loci. *Genetics* **124**: 967–978.

Taylor, P.D. & Williams, G.C. 1982. The lek paradox is not resolved. *Theor. Popul. Biol.* **22**: 392–409.

Travisano, M. & Shaw, R.G. 2013. Lost in the map. *Evolution* **67**: 305–314.

Turner, T.L. & Miller, P.M. 2012. Investigating natural variation in Drosophila courtship song by the evolve and resequence approach. *Genetics* **191**: 633–642.

Van Dyken, J.D. & Wade, M.J. 2010. The genetic signature of conditional expression. *Genetics* **184**: 557–570.

Viguerie, N., Montastier, E., Maoret, J.-J., Roussel, B., Combes, M., Valle, C. *et al.* 2012. Determinants of human adipose tissue gene expression: impact of diet, sex, metabolic status, and cis genetic regulation. *PLoS Genet.* **8**: e1002959.

Visscher, P.M., Brown, M.A., McCarthy, M.I. & Yang, J. 2012. Five years of GWAS discovery. *Am. J. Hum. Genet.* **90**: 7–24.

Wang, X., Weigel, D. & Smith, L.M. 2013. Transposon variants and their effects on gene expression in Arabidopsis. *PLoS Genet.* **9**: e1003255.

Warren, I.A., Gotoh, H., Dworkin, I.M., Emlen, D.J. & Lavine, L.C. 2013. A general mechanism for conditional expression of exaggerated sexually-selected traits. *BioEssays* **35**: 889–899.

Wells, K.D. 1977. The social behaviour of anuran amphibians. *Anim. Behav.* **25**: 666–693.

Whittle, C.A. & Johannesson, H. 2013. Evolutionary dynamics of sex-biased genes in a hermaphrodite fungus. *Mol. Biol. Evol.* **30**: 2435–2446.

Wilkins, A.S. 2014. "The genetic tool-kit": the life-history of an important metaphor. In: *Advances in Evolutionary Developmental Biology* (J.T. Streelman, ed.), pp. 1–14. John Wiley and Sons Inc, New York.

Wilkinson, G.S. & Johns, P.M. 2005. Sexual selection and the evolution of mating systems in flies. In: *The Evolutionary Biology of Flies* (B.M. Wiegmann & D.K. Yeates, eds), pp. 312–329. Columbia University Press, New York.

Wilkinson, G.S., Johns, P.M., Metheny, J.D. & Baker, R.H. 2013. Sex-biased gene expression during head development in a sexually dimorphic stalk-eyed fly. *PLoS ONE* **8**: e59826.

Williams, T.M. & Carroll, S.B. 2009. Genetic and molecular insights into the development and evolution of sexual dimorphism. *Nat. Rev. Genet.* **10**: 797–804.

Wright, S. 1951. The genetical structure of populations. *Ann. Eugen.* **15**: 323–354.

Wyman, M.J., Cutter, A.D. & Rowe, L. 2012. Gene duplication in the evolution of sexual dimorphism. *Evolution* **66**: 1556–1566.

Xue, Z., Ren, M., Wu, M., Dai, J., Rong, Y.S. & Gao, G. 2014. Efficient gene knock-out and knock-in with transgenic Cas9 in Drosophila. *G3* **4**: 925–929.

Yang, X., Schadt, E.E., Wang, S., Wang, H., Arnold, A.P., Ingram-Drake, L. *et al.* 2006. Tissue-specific expression and regulation of sexually dimorphic genes in mice. *Genome Res.* **16**: 995–1004.

Young, L.J. & Wang, Z. 2004. The neurobiology of pair bonding. *Nat. Neurosci.* **7**: 1048–1054.

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

Zhang, G., Li, C., Li, Q., Li, B., Larkin, D.M., Lee, C. *et al.* 2014. Comparative genomics reveals insights into avian genome evolution and adaptation. *Science* **346**: 1311–1320.

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