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

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Keywords:
candidate gene;
cis-regulation;
forward genetics;
genomewide association studies;
resequeing;
reverse genetics;
transcriptome.

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
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 |
| Selective sweep                   | A nucleotide substitution in a codon that does not alter the amino acid sequence of the translated protein |
| Transcription factor              | Protein that controls the expression pattern of a gene by binding to regulatory elements |
| Transcripctome                    | 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 |
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; Ritschof & 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

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**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.

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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 Parch & Ellegrén, 2013). Although this has been suggested to be the product of positive selection for male traits due to sexual selection (Ellegren & Parch, 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 intra-specific 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
Several examples of this approach exist for sexually
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 pheno-
typic 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 identi-
fying QTL difficult without very large sample sizes.

The availability of low-cost, high-throughput geno-
typing 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 popula-
tion, 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 vari-
ants; the amount of recombination possible can other-
wise limit resolution with other mapping techniques.
For animals with small family sizes or long generation
times, GWAS approaches permit study of the quantita-
tive 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 individ-
uals 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 ge-
nome of many tens of thousands of individuals might
be necessary for a reasonable chance of success (Vis-
scher 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 selec-
tion 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 measure-
ments 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 indivi-
duals 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 (Sch-
neberger & Weigel, 2011; Leshchiner et al., 2012). By
analysing multiple independent sample pools, sampling
variance effects can also be reduced. For example, Bas-
tide et al. (2013) selected 1000 each of the darkest and
lightest individuals from 8000 female offspring pro-
duced by large samples of Drosophila melanogaster col-
lected 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 manip-
ulate 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 vir-
tually 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 nonssexual traits have so far
yielded surprisingly few cases in which a sequence vari-
ant can be associated with phenotypic variation, even
though the traits investigated were known to be herita-
ble (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; Mullan 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
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 & Möller, 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; Gompper 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.

Acknowledgments

This paper arose from a workshop held at the Wissenschaftskolleg zu Berlin (Wiko). We thank the Wiko and the Otto and Martha Fischbeck Stiftung for financial and logistical support and R. Meyer-Kalkus for encouragement. H. Kokko, J. Reinhardt, K. Paczolt, K. Miller-Hesed and two anonymous reviewers provided comments that helped improve the manuscript. A.D.H. was supported by the European Research Council (Advanced Grant 250209 to Alasdair Houston) and a NERC Independent Research Fellowship. G.S.W. was funded by the National Science Foundation (DEB-0952260).

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*Received 1 December 2014; revised 4 March 2015; accepted 6 March 2015*