THE LOCUS OF SEXUAL SELECTION: MOVING SEXUAL SELECTION STUDIES INTO THE POST-GENOMICS ERA

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Keywords:	Transcriptome, candidate gene, resequencing, forward genetics, reverse genetics, cis-regulation, GWAS

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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 behaviors, 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.

Keywords: Transcriptome, candidate gene, resequencing, forward genetics, reverse genetics, cis-regulation, GWAS

Introduction

Sexual selection is a powerful evolutionary force that can drive trait diversification within and among species (Andersson, 1994, Darwin, 1871), accelerate rates of molecular evolution (Aguade, 1999, Swanson & Vacquier, 1995, Swanson & Vacquier, 2002), 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 behaviors, or extravagant body modifications, or increased competitiveness through enlarged body size, weapons or armor (Andersson, 1994). These structures and behaviors 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 (Brooks, 2002, Grace & Shaw, 2011, Gray & Cade, 2000, 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 (Bernhard & Hamelin, 2013, Fisher, 1930, Grafen, 1990, Kirkpatrick, 1982, Kirkpatrick & Hall, 2004b, Lande, 1981, Pomiankowski et al., 1991), and how the evolution of these traits might aid or impede diversification and

speciation (Gavrilets, 2000, Lande, 1981, Pomiankowski & Iwasa, 1998, 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). In order 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 (Bernardi et al., 2012, Brawand et al., 2014, Evans et al., 2013, Haussler et al., 2009, 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 toward 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. While key genes have been identified that influence the development of some sexually selected traits (Emlen et al., 2012, Khila et al., 2012, Kijimoto et al., 2012, Moczek & Rose, 2009, Santos et al., 2014, Williams & Carroll, 2009), 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 behavioral 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 sexual dimorphism, intrasexual variation in attractiveness, or underlie variation in trait exaggeration within and between species can help us understand the process of sexual selection.

Challenges of a genomic approach to sexual selection

While 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 and 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 phenotypic 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 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, e.g. 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

146 selection in females, resulting in the possibility of substantial sexual conflict. Depending on how
147 (or if) such conflicts are resolved, molecular signatures of selection could be less obvious than
148 in cases where selection acts congruently in both sexes, or difficult to distinguish from other
149 forms of balancing selection. Moreover, this difficulty can be compounded by pleiotropic gene
150 expression in which selection varies additionally by tissue type (Mank et al., 2008). Further,
151 frequency dependent selection, which may often be an important component of sexual
152 selection, is likely to generate different signatures of selection than accounted for in classic
153 sweep models (Olendorf et al., 2006, Takahata & Nei, 1990).
154
155 (Figure 1 here)
156
157 The third additional challenge is that signal-receiver systems involved in sexual selection often
158 comprise one or more behavioral traits. Finding the genetic basis of any behavioral trait is
159 notoriously difficult due to high levels of within-individual phenotypic variation. Nevertheless,
160 genetic polymorphisms for behavior have been successfully identified (Boake et al., 2002) and
161 genomic approaches can be used to identify alternative strategies (Aubin-Horth & Renn, 2009,
162 Rittschof & Robinson, 2014). Quantifying sexually selected behavioral traits is, however, doubly
163 challenging because receiver responses may depend on a variety of conditions, including
164 motivational state, receptivity, and the type of conspecifics used to elicit a response. For
165 example, the number and range of male phenotypes offered can influence the type of mate
166 choice exhibited by a female. As a consequence, female preference functions should be
167 quantified using a variety of male phenotypes even though considerable effort may be required
168 (e.g. Mcguigan et al., 2008, Murphy & Gerhardt, 2000, Ritchie, 2000, Shaw & Herlihy, 2000). As
169 in all whole-genome approaches, phenotypic heterogeneity is a major barrier to identifying the
170 genetic basis of traits (Evangelou & Ioannidis, 2013).
171

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

Studying 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 non-ornamented males, and therefore might give rise to a sexually selected phenotype. These loci can be identified either by quantifying genome-wide patterns of inter- or intra-sexual 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, *i.e*. the locus of sexual selection. Confirmation that sequence change has the inferred phenotypic effects requires sequence or expression manipulation, i.e. reverse genetics. For both types of analyses genomic approaches on either model or non-model species can provide important information regarding the genetics underlying sexually selected phenotypes.

(Figure 2 here)

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 (Baker et al., 2011, Böhne et al., 2014, Viguerie et al., 2012, Yang et al., 2006). 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 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 germline 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 (Harrison et al., 2015, Moran & Poetrokovski, 2014), 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.

While sexual selection is clearly an important source of sex-specific selection, without additional functional genetic analysis it is not possible to determine if 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, Perry et al., 2014, Wilkinson et al., 2013) as well as across tissues (Baker et al., 2011, Yang et al., 2006), therefore ontogenetic trajectories of sexually selected phenotypes must be determined to identify when and where differential gene expression triggers development of sexually selected traits. Nevertheless, studies of gene expression in species with intra-sexual 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., 2013, Lank et al., 1995), blue-headed wrasse (Alonzo & Warner, 2000), side-blotched lizards (Sinervo & Lively, 1996), or sponge isopods (Shuster & Sassaman, 1997, Shuster & Wade, 1991), 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 behavioral 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 (Baird et al., 2008, Hohenlohe et al., 2010, Miller et al., 2007). 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. Chenoweth & Mcguigan, 2010, Johns et al., 2005, Schielzeth et al., 2012, Shaw et al., 2007), 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, Kopp et al., 2003). However, some studies of QTLs for behaviors 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 genome-wide 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

299 natural genetic diversity in a population, rather than some subset, such as found in a set of
300 inbred lines, to locate genetic differences that underlie natural phenotypic variation.
301 Furthermore, GWAS make use of all recombination events that occurred in the past to separate
302 causal and physically linked variants, while the amount of recombination possible can otherwise
303 limit resolution with other mapping techniques. For animals with small family sizes or long
304 generation times, GWAS approaches permit study of the quantitative genetics of sexually
305 selected traits in vertebrates and other systems where QTL approaches that require inbreeding
306 or controlled pedigrees are intractable. On the other hand, the added precision provided by
307 GWAS typically comes at the cost of genotyping more individuals at more markers than in a
308 QTL study because the probability of linkage between an anonymous marker and a causal
309 locus is much lower. Recent results from human GWAS raise a particularly strong cautionary
310 tale, as it appears that for many diseases the full genomes of many tens of thousands of
311 individuals might be necessary for a reasonable chance of success (Visscher et al., 2012).
312 However, there is reason to be more optimistic for the study for sexually selected traits. Rather,
313 than being maintained by mutation-selection balance, as is probably the case for most human
314 disease traits, selection on secondary sexual traits is likely to be strong and, importantly, recent.
315 This history of selection provides an opportunity for alleles of large effect to sort from alleles of
316 smaller effect, especially in comparisons between populations that display divergence in
317 sexually selected traits and particularly if these populations are linked by periodic migration.
318 Similarly, if sexual selection generates frequency dependent selection at the level of individual
319 alleles, then segregating effect sizes could potentially be larger and allele frequencies higher
320 than expected under mutation-selection balance.
321
322 Furthermore, in contrast to studies in humans, it is possible in some animals to generate
323 multiple measurements on the same genotype, which greatly reduces the contribution of
324 sampling variance to estimation errors. Nevertheless, successful application of GWAS requires

appropriate experimental design, explicit consideration of genetic background, and, when possible, modeling 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 (Leshchiner et al., 2012, Schneeberger & Weigel, 2011). By analyzing multiple independent sample pools, sampling variance effects can also be reduced. For example, Bastide and colleagues (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 (see below), 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), *i.e*. the nucleotide substitutions associated with variation in quantitative traits. However, QTN

approaches applied to non-sexual 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, *i.e*. 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, Connallon & Clark, 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 if 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 is 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. Khila et al., 2012, Marshall et al., 2009) 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 behavior (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.

(Figure 3 here)

Experimental paradigms for inferring sexual selection

While the methods described above will identify genetic variants that influence phenotypes, the degree to which those phenotypes are caused by sexual selection are 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 premating sexual selection, or single mating versus multiple mating, which can reveal effects of postmating 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, 2012, Burke et al., 2010, 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* (Babcock & Ganetzky, 2014, Bath et al., 2014, Dankert et al., 2009, 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 coevolutionary processes depend on various additive genetic covariances arising between female preference, male trait, and offspring viability (Kokko et al., 2006, Mead & Arnold, 2004). 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 signaling 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-signaling pathway has been proposed as a mechanism that links organism condition to 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 behavioral 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 (Kirkpatrick & Ryan, 1991, Taylor & Williams, 1982). While a number of theoretical solutions to the lek paradox have been offered

(Higginson & Reader, 2009, Kokko & Heubel, 2008, Kotiaho et al., 2001, Pomiankowski & Møller, 1995, Rowe & Houle, 1996), 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 favoring large horn size is countered by viability selection favoring 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 coevolutionary 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

(Gompert et al., 2014, Le Rouzic et al., 2011, 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 behavior, physiology, and form can have important implications for sex-specific rates of aging 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 inter-sexual correlation between males and females for transcription levels (Griffin et al., 2013). Identifying the genetic basis of sexually selected traits will help reveal the

581 regulatory complexity required to break down intersexual correlations in order to encode sexual
582 dimorphisms.
583

584 Conclusions

585 Sexual selection research has a strong history of building mathematical models that explore the
586 possible paths to diversity and speciation due to exaggerated male traits and female
587 preferences in a variety of species. In an attempt to test these models, many research programs
588 have focused on using quantitative or functional genetics to find the genetic variants that cause
589 variation in sexually selected traits. However, despite this effort, few sexually selected
590 characters have been mapped to specific loci in the genome. This could be because many of
591 these differences involve changes in gene regulation mechanisms, given that trait differences
592 between the sexes often are encoded by a genome they share. Additionally, our ability to
593 identify regulatory regions and link sequence variants in them to transcriptional and phenotypic
594 variation remain quite limited. Nevertheless, some genomic approaches have been applied to
595 species exhibiting strong sexual dimorphism or intra-sexual variation in sexually selected
596 phenotypes. A number of studies have successfully measured sex-specific differences in gene
597 expression, and quantified effects of sex chromosomes, where the initiating polymorphisms for
598 sexual dimorphism may lie. Very few, however, have succeeded in identifying the underlying
599 sequence differences that are responsible for phenotypic evolution due to sexual selection.
600

601 We believe this gap can be closed using genomic approaches, such as fast-forward genomic
602 scans, and contrasting either recently diverged species or populations, replicate lines in an
603 experimental evolution paradigm that manipulates sexual selection intensity, or sexually
604 dimorphic phenotypes from a clonal species. New techniques for manipulating gene sequence

or expression in non-model organisms provide opportunities for confirming causation through direct genetic manipulation that were not previously possible.

Progress in many aspects of evolutionary and behavioral 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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1044 **Table 1** Glossary of terms
1045

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 cloning
GWAS	genome-wide 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 non-recombining 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

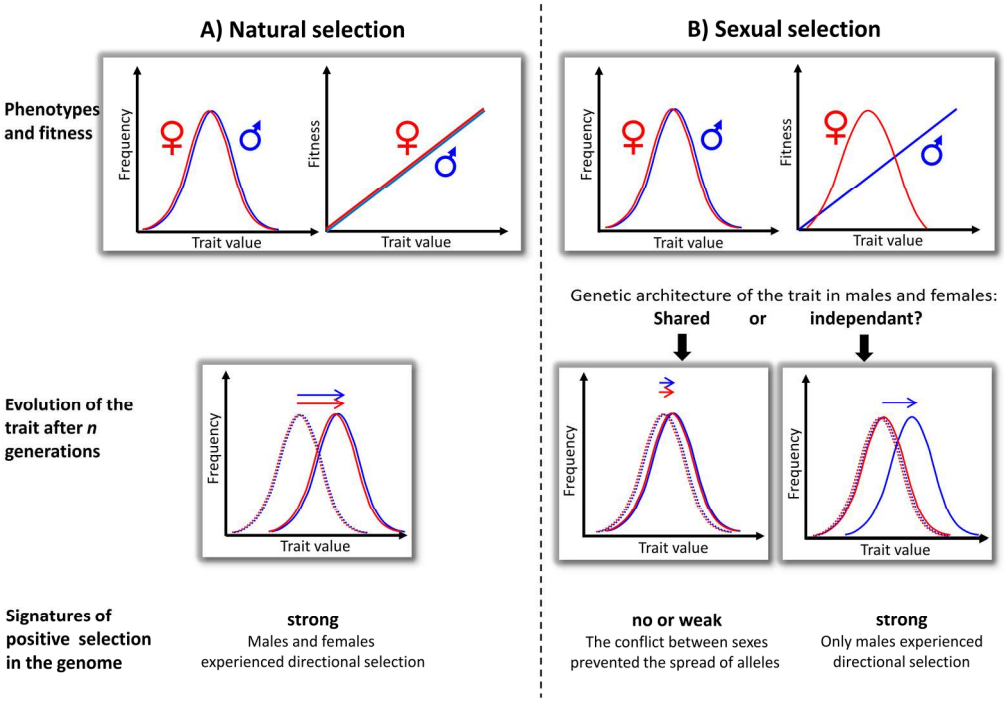
Figure Legends

Figure 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).

Figure 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, while QTL (quantitative trait locus) mapping and GWAS (genome-wide association studies) pinpoint allelic variants at a locus associated with phenotypic variation.

Figure 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 non-functional 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.



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).
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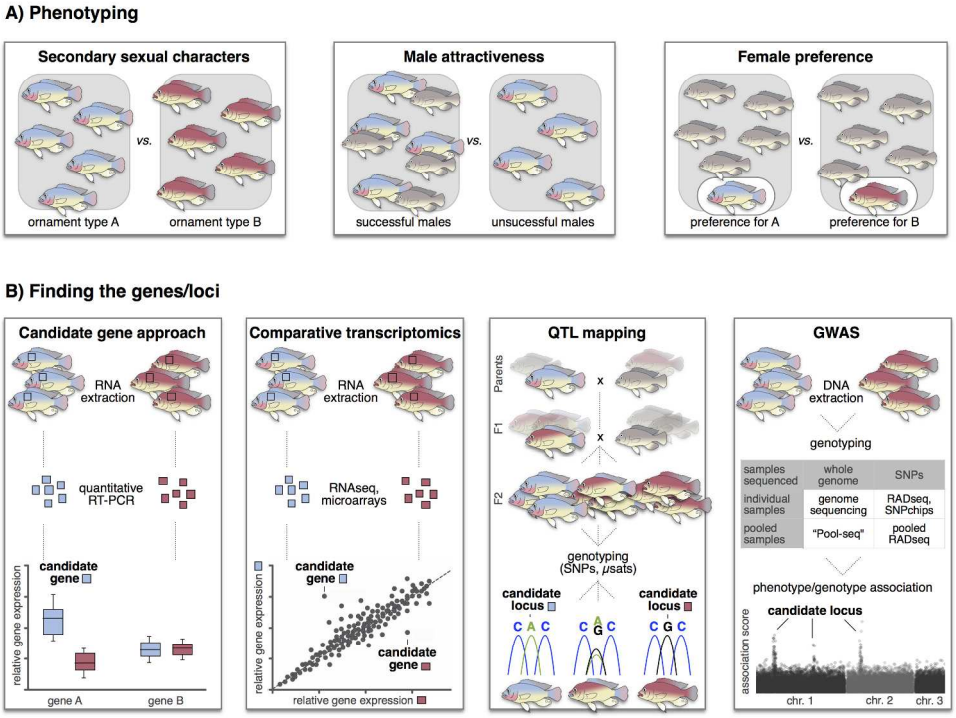
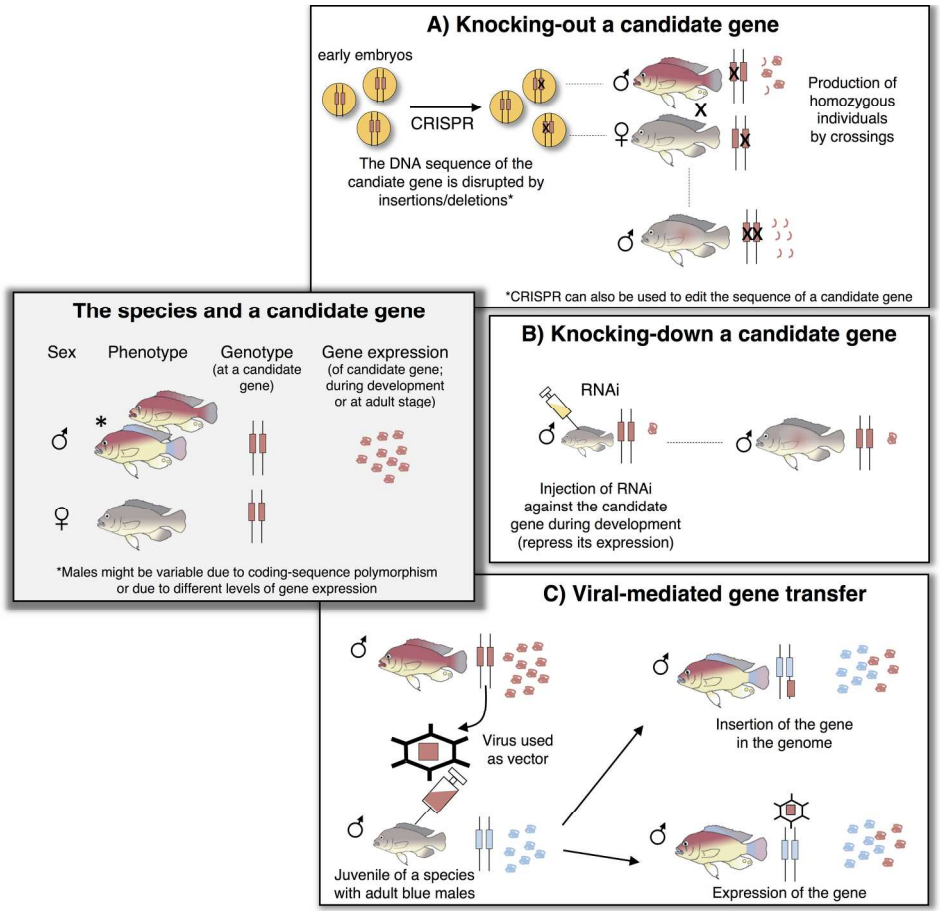


Overview of forward genetic approaches for identifying genes that control expression of traits involved in sexual selection. The trait used to group individuals may be, for example, a male secondary sexual character, any measure of male attractiveness (e.g. mating success), or female preferences (panel 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, while QTL (quantitative trait locus) mapping and GWAS (genome-wide association studies) pinpoint allelic variants at a locus associated with phenotypic variation.
254x191mm (300 x 300 DPI)



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 non-functional 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.
190x172mm (300 x 300 DPI)