JOURNAL OF Evolutionary Biology

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

Journal:	*Journal of Evolutionary Biology*
Manuscript ID:	JEB-2014-00753.R2
Manuscript Type:	Reviews
Date Submitted by the Author:	n/a
Complete List of Authors:	Wilkinson, Gerald; University of Maryland, Biology Breden, Felix; Simon Fraser University, Biological Sciences Mank, Judith; University College London, Department of Genetics, Evolution and Environment Ritchie, Mike; University of St Andrews, School of Biology Higginson, Andrew; University of Bristol, Biological Sciences Radwan, Jacek; Adam Mickiewicz University, Institute of Environmental Biology; Jaquiery, Julie; Universityof Rennes 1, Institute of Environmental Biology Salzburger, Walter; University of Basel, Zoological Institute Arriero, Elena; Universidad Complutense de Madrid, Departamento de Zoologia y Antropologia Fisica Barribeau, Seth; ETH Zürich, Institute of Integrative Biology; East Carolina University, Biology Phillips, Patrick; University of Oregon, Center for Ecology and Evolutionary Biology Renn, Susan; Reed College, Biology Rowe, Locke; University of Toronto, Ecology and Evolutionary Biology
Keywords:	Transcriptome, candidate gene, resequencing, forward genetics, reverse genetics, cis-regulation, GWAS

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

Gerald S. Wilkinson[1†], Felix Breden[2], Judith E. Mank[3], Michael G. Ritchie[4], Andrew D. Higginson[5], Jacek Radwan[6], Julie Jaquiery[7], Walter Salzburger[8], Elena Arriero[9], Seth M. Barribeau[10.11], Patrick C. Phillips[12], Suzy C.P. Renn[13], and Locke Rowe[14]

[1]Department of Biology, University of Maryland, College Park, MD 20742 USA; [2]Department of Biological Sciences, Simon Fraser University, Burnaby, B.C., V5A 1S6, Canada; [3]Department of Genetics, Evolution and Environment, University College London, London, WC1E 6BT, UK; [4]Centre for Biological Diversity, University of St Andrews, St Andrews, Fife, Scotland KY16 9TH, UK; [5]School of Biological Sciences, University of Bristol, Bristol BS8 1TQ, UK; [6]Institute of Environmental Biology, Adam Mickiewicz University, 61-614 Poznan, Poland; [7]University of Rennes 1, CNRS UMR 6553 EcoBio, 35042 Rennes, France; [8]Zoological Institute, University of Basel, 4051 Basel, Switzerland; [9]Departamento de Zoologia y Antropologia Fisica, Universidad Complutense de Madrid, 28040 Madrid, Spain; [10]Institute of Integrative Biology, ETH Zürich, 8092, Zürich, Switzerland; [11]current address: Department of Biology, East Carolina University, Greenville, NC 27858 USA; [12]Institute of Ecology and Evolution, University of Oregon, Eugene, OR 97403 USA; [13]Department of Biology, Reed College, Portland, OR 97202 USA; [14]Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, M5S 3B2 Canada

[†]Corresponding author: Department of Biology, University of Maryland, College Park, MD 20742 USA; email: wilkinso@umd.edu; tel: 301-405-6942; fax: 301-314-9358

Running title: Genomics of sexual selection

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.

Acknowledgements

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.

References

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

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

630 Alonzo, S. H. & Warner, R. R. 2000. Female choice, conflict between the sexes and the
631 evolution of male alternative reproductive behaviours. *Evol. Ecol. Res.* **2**: 149-170.
632 Andersson, M. 1994. *Sexual Selection*. Princeton University Press, Princeton.
633 Aubin-Horth, N. & Renn, S. C. 2009. Genomic reaction norms: using integrative biology to
634 understand molecular mechanisms of phenotypic plasticity. *Mol. Ecol.* **18**: 3763-80.
635 Babcock, D. T. & Ganetzky, B. 2014. An improved method for accurate and rapid measurement
636 of flight performance in Drosophila. *JoVE (Journal of Visualized Experiments)*: e51223-
637 e51223.
638 Baird, N. A., Etter, P. D., Atwood, T. S., Currey, M. C., Shiver, A. L., Lewis, Z. A., et al. 2008.
639 Rapid SNP discovery and genetic mapping using sequenced RAD markers. *PloS One* **3**:
640 e3376.
641 Baker, D. A., Nolan, T., Fischer, B., Pinder, A., Crisanti, A. & Russell, S. 2011. A comprehensive
642 gene expression atlas of sex-and tissue-specificity in the malaria vector, Anopheles
643 gambiae. *BMC Genomics* **12**: 296.
644 Barrett, R. D. H. & Hoekstra, H. E. 2011. Molecular spandrels: tests of adaptation at the genetic
645 level. *Nat. Rev. Genet.* **12**: 767-780.
646 Bastide, H., Betancourt, A., Nolte, V., Tobler, R., Stobe, P., Futschik, A., et al. 2013. A genome-
647 wide, fine-scale map of natural pigmentation variation in Drosophila melanogaster. *PLoS*
648 *Genet.* **9**: e1003534.
649 Bath, D. E., Stowers, J. R., Hörmann, D., Poehlmann, A., Dickson, B. J. & Straw, A. D. 2014.
650 FlyMAD: rapid thermogenetic control of neuronal activity in freely walking Drosophila.
651 *Nat. Methods* **11**: 756-762.
652 Bennett, J., Maguire, A. M., Cideciyan, A. V., Schnell, M., Glover, E., Anand, V., et al. 1999.
653 Stable transgene expression in rod photoreceptors after recombinant adeno-associated
654 virus-mediated gene transfer to monkey retina. *Proc. Natl. Acad. Sci. USA* **96**: 9920-
655 9925.

656 Berg, E. C. & Maklakov, A. A. 2012. Sexes suffer from suboptimal lifespan because of genetic
657 conflict in a seed beetle. *Proc. R. Soc. Lond., Ser. B: Biol. Sci.* **279**: 4296-4302.
658 Bernardi, G., Wiley, E. O., Mansour, H., Miller, M. R., Orti, G., Haussler, D., et al. 2012. The
659 fishes of Genome 10K. *Marine Genomics* **7**: 3-6.
660 Bernhard, P. & Hamelin, F. M. 2013. Simple signaling games of sexual selection (Grafen's
661 revisited). *J. Math. Biol.* **69**: 1719-1742.
662 Beukeboom, L. W. & Perrin, N. 2014. *The Evolution of Sex Determination*. Oxford University
663 Press, Oxford.
664 Blows, M. W. 1999. Evolution of the genetic covariance between male and female components
665 of mate recognition: an experimental test. *Proc. R. Soc. Lond., Ser. B: Biol. Sci.* **266**:
666 2169-2174.
667 Boake, C. R. B., Arnold, S. J., Breden, F., Meffert, L. M., Ritchie, M. G., Taylor, B. J., et al.
668 2002. Genetic tools for studying adaptation and the evolution of behavior. *Am. Nat.* **160**:
669 S143-S159.
670 Böhne, A., Sengstag, T. & Salzburger, W. 2014. Comparative transcriptomics in East African
671 cichlids reveals sex- and species-specific expression and new candidates for sex
672 differentiation in fishes. *Genome Biol. Evol.* **6**: 2567-2585.
673 Bousquet, F., Nojima, T., Houot, B., Chauvel, I., Chaudy, S., Dupas, S., et al. 2012. Expression
674 of a desaturase gene, *desat1,* in neural and nonneural tissues separately affects
675 perception and emission of sex pheromones in Drosophila. *Proc. Natl. Acad. Sci. USA*
676 **109**: 249-254.
677 Brawand, D., Wagner, C. E., Li, Y. I., Malinsky, M., Keller, I., Fan, S., et al. 2014. The genomic
678 substrate for adaptive radiation in African cichlid fish. *Nature* **513**: 375-381.
679 Breker, M. & Schuldiner, M. 2014. The emergence of proteome-wide technologies: systematic
680 analysis of proteins comes of age. *Nat. Rev. Mol. Cell Biol.* **15**: 453–464.

681 Brooks, R. 2002. Variation in female mate choice within guppy populations: population
682 divergence, multiple ornaments and the maintenance of polymorphism. *Genetica* **116**:
683 343-358.
684 Burke, M. K. 2012. How does adaptation sweep through the genome? Insights from long-term
685 selection experiments. *Proc. R. Soc. Lond., Ser. B: Biol. Sci.*: rspb20120799.
686 Burke, M. K., Dunham, J. P., Shahrestani, P., Thornton, K. R., Rose, M. R. & Long, A. D. 2010.
687 Genome-wide analysis of a long-term evolution experiment with Drosophila. *Nature* **467**:
688 587-590.
689 Chenoweth, S. F. & McGuigan, K. 2010. The genetic basis of sexually selected variation. *Annu.*
690 *Rev. Ecol., Evol. Syst.* **41**: 81-101.
691 Clayton, J. A. & Colling, F. S. 2014. NIH to balance sex in cell and animal studies. *Nature* **509**:
692 282-283.
693 Connallon, T. & Clark, A. G. 2012. A general population genetic framework for antagonistic
694 selection that accounts for demography and recurrent mutation. *Genetics* **190**: 1477-89.
695 Connallon, T. & Clark, A. G. 2013. Antagonistic versus nonantagonistic models of balancing
696 selection: characterizing the relative timescales and hitchhiking effects of partial
697 selective sweeps. *Evolution* **67**: 908-17.
698 Dankert, H., Wang, L., Hoopfer, E. D., Anderson, D. J. & Perona, P. 2009. Automated
699 monitoring and analysis of social behavior in Drosophila. *Nat. Methods* **6**: 297-303.
700 Darwin, C. R. 1871. *The Descent of Main and Selection in Relation to Sex*. John Murray,
701 London.
702 Dean, R. & Mank, J. E. 2014. The role of sex chromosomes in sexual dimorphism: discordance
703 between molecular and phenotypic data. *J. Evol. Biol.* **27**: 1443-1453.
704 Demir, E. & Dickson, B. J. 2005. Fruitless splicing specifies male courtship behavior in
705 Drosophila. *Cell* **121**: 785-794.

706 Dobzhansky, T. 1970. *Genetics of the Evolutionary Process*. Columbia University Press, New
707 York.
708 Ellegren, H. & Parsch, J. 2007. The evolution of sex-biased genes and sex-biased gene
709 expression. *Nat. Rev. Genet.* **8**: 689-698.
710 Emlen, D. J., Warren, I. A., Johns, A., Dworkin, I. & Lavine, L. C. 2012. A mechanism of
711 extreme growth and reliable signaling in sexually selected ornaments and weapons.
712 *Science* **337**: 860-864.
713 Endler, J. A. 1992. Signals, signal conditions and the direction of evolution. *Am. Nat.* **139**: S125-
714 S153.
715 Evangelou, E. & Ioannidis, J. P. A. 2013. Meta-analysis methods for genome-wide association
716 studies and beyond. *Nat. Rev. Genet.* **14**: 379–389.
717 Evans, J. D., Brown, S. J., Hackett, K. J., Robinson, G., Richards, S., Lawson, D., et al. 2013.
718 The i5K initiative: advancing arthropod genomics for knowledge, human health,
719 agriculture, and the environment. *J. Hered.* **104**: 595-600.
720 Faulkner, G. J., Kimura, Y., Daub, C. O., Wani, S., Plessy, C., Irvine, K. M., et al. 2009. The
721 regulated retrotransposon transcriptome of mammalian cells. *Nat. Genet.* **41**: 563-571.
722 Fisher, R. A. 1930. *The Genetical Theory of Natural Selection*. Clarendon, Oxford.
723 Fondon, J. W. & Garner, H. R. 2004. Molecular origins of rapid and continuous morphological
724 evolution. *Proc. Natl. Acad. Sci. USA* **101**: 18058-18063.
725 Gallach, M. & Betran, E. 2011. Intralocus sexual conflict resolved through gene duplication.
726 *Trends Ecol. Evol.* **26**: 222-228.
727 Gallach, M., Chandrasekaran, C. & Betran, E. 2010. Analyses of nuclearly encoded
728 mitochondrial genes suggest gene duplication as a mechanism for resolving intralocus
729 sexually antagonistic conflict in *Drosophila*. *Genome Biol. Evol.* **2**: 835-850.
730 Gavrilets, S. 2000. Rapid evolution of reproductive barriers driven by sexual conflict. *Nature*
731 **403**: 886-889.

732 Gempe, T. & Beye, M. 2010. Function and evolution of sex determination mechanisms, genes
733 and pathways in insects. *Bioessays* **33**: 52-60.
734 Glaser-Schmitt, A., Catalan, A. & Parsch, J. 2013. Adaptive divergence of a transcriptional
735 enhancer between populations of Drosophila melanogaster. *Philos. Trans. R. Soc.*
736 *Lond., Ser. B: Biol. Sci.* **368**: 20130024.
737 Gompert, Z., Comeault, A. A., Farkas, T. E., Feder, J. L., Parchman, T. L., Buerkle, C. A., et al.
738 2014. Experimental evidence for ecological selection on genome variation in the wild.
739 *Ecol. Lett.* **17**: 369-379.
740 Grace, J. L. & Shaw, K. L. 2011. Coevolution of male mating signal and female preference
741 during early lineage divergence of the Hawaiian cricket, *Laupala cerasina*. *Evolution* **65**:
742 2184-2196.
743 Grafen, A. 1990. Sexual selection unhandicapped by the Fisher process. *J. Theor. Biol.* **144**:
744 473-516.
745 Gray, D. A. & Cade, W. H. 2000. Sexual selection and speciation in field crickets. *Proc. Natl.*
746 *Acad. Sci. USA* **97**: 14449-14454.
747 Griffin, R. M., Dean, R., Grace, J. L., Ryden, P. & Friberg, U. 2013. The shared genome is a
748 pervasive constraint on the evolution of sex-biased gene expression. *Mol. Biol. Evol.* **30**:
749 2168-2176.
750 Harris, R. M. & Hofmann, H. A. (2014) Neurogenomics of behavioral plasticity. In: *Ecological*
751 *Genomics*, (Landry, C. R. & Aubin-Horth, N., eds.). pp. 149-168. Springer Science,
752 Dondrecht.
753 Harrison, P. W., Wright, A. E., Zimmer, F., Dean, R., Montgomery, S. H., Pointer, M. A., et al.
754 2015. Sexual selection drives evolution and rapid turnover of male-biased genes. *Proc.*
755 *Natl. Acad. Sci. USA.* **in press**.

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

Higginson, A. D. & Reader, T. 2009. Environmental heterogeneity, genotype-by-environment interactions and the reliability of sexual traits as indicators of mate quality. *Proc. R. Soc. Lond., Ser. B: Biol. Sci.* **276**: 1153-1159.

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

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

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

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

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

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

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

780 Johnston, S. E., Gratten, J., Berenos, C., Pilkington, J. G., Clutton-Brock, T. H., Pemberton, J.
781 M., et al. 2013. Life history trade-offs at a single locus maintain sexually selected genetic
782 variation. *Nature* **502**: 93-95.
783 Johnston, S. E., McEwan, J. C., Pickering, N. K., Kijas, J. W., Beraldi, D., Pilkington, J. G., et al.
784 2011. Genome-wide association mapping identifies the genetic basis of discrete and
785 quantitative variation in sexual weaponry in a wild sheep population. *Mol. Ecol.* **20**: 2555-
786 2566.
787 Jones, F. C., Grabherr, M. G., Chan, Y. F., Russell, P., Mauceli, E., Johnson, J., et al. 2012.
788 The genomic basis of adaptive evolution in threespine sticklebacks. *Nature* **484**: 55-61.
789 Keane, T. M., Goodstadt, L., Danecek, P., White, M. A., Wong, K., Yalcin, B., et al. 2011. Mouse
790 genomic variation and its effect on phenotypes and gene regulation. *Nature* **477**: 289-
791 294.
792 Khila, A., Abouheif, E. & Rowe, L. 2012. Function, developmental genetics, and fitness
793 consequences of a sexually antagonistic trait. *Science* **336**: 585-589.
794 Kijimoto, T., Moczek, A. P. & Andrews, J. 2012. Diversification of doublesex function underlies
795 morph-, sex-, and species-specific development of beetle horns. *Proc. Natl. Acad. Sci.*
796 *USA* **109**: 20526-20531.
797 Kirkpatrick, M. 1982. Sexual selection and the evolution of female choice. *Evolution* **36**: 1-12.
798 Kirkpatrick, M. & Hall, D. W. 2004a. Male-biased mutation, sex linkage, and the rate of adaptive
799 evolution. *Evolution* **58**: 437-440.
800 Kirkpatrick, M. & Hall, D. W. 2004b. Sexual selection and sex linkage. *Evolution* **58**: 683-691.
801 Kirkpatrick, M. & Ryan, M. J. 1991. The evolution of mating preferences and the paradox of the
802 lek. *Nature* **350**: 33-38.
803 Kokko, H., Brooks, R., Jennions, M. D. & Morley, J. 2003. The evolution of mate choice and
804 mating biases. *Proc. R. Soc. Lond., Ser. B: Biol. Sci.* **270**: 653-664.

805 Kokko, H. & Heubel, K. 2008. Condition-dependence, genotype-by-environment interactions
806 and the lek paradox. *Genetica* **132**: 209-216.
807 Kokko, H., Jennions, M. D. & Brooks, R. 2006. Unifying and testing models of sexual selection.
808 *Annu. Rev. Ecol., Evol. Syst.* **37**: 43-66.
809 Kopp, A., Duncan, I., Godt, D. & Carroll, S. B. 2000. Genetic control and evolution of sexually
810 dimorphic characters in Drosophila. *Nature* **408**: 553-559.
811 Kopp, A., Graze, R. M., Xu, S. Z., Carroll, S. B. & Nuzhdin, S. V. 2003. Quantitative trait loci
812 responsible for variation in sexually dimorphic traits in Drosophila melanogaster.
813 *Genetics* **163**: 771-787.
814 Korte, A. & Farlow, A. 2013. The advantages and limitations of trait analysis with GWAS: a
815 review. *Plant methods* **9**: 29.
816 Kotiaho, J. S., Simmons, L. W. & Tomkins, J. L. 2001. Towards a resolution of the lek paradox.
817 *Nature* **410**: 684–686.
818 Kraaijeveld, K., Kraaijeveld-Smit, F. J. L. & Maan, M. E. 2011. Sexual selection and speciation:
819 the comparative evidence revisited. *Biol. Rev.* **86**: 367-377.
820 Kuhn, A., Ong, Y. M., Cheng, C.-Y., Wong, T. Y., Quake, S. R. & Burkholder, W. F. 2014.
821 Linkage disequilibrium and signatures of positive selection around LINE-1
822 retrotransposons in the human genome. *Proc. Natl. Acad. Sci. USA* **111**: 8131-8136.
823 Lande, R. 1981. Models of speciation by sexual selection on polygenic traits. *Proc. Natl. Acad.*
824 *Sci. USA* **78**: 3721-3725.
825 Lank, D. B., Farrell, L. L., Burke, T., Piersma, T. & McRae, S. B. 2013. A dominant allele
826 controls development into female mimic male and diminutive female ruffs. *Biol. Lett.* **9**:
827 20130653.
828 Lank, D. B., Smith, C. M., Hanotte, O., Burke, T. & Cooke, F. 1995. Genetic polymorphism for
829 alternative mating behavior in lekking male ruff *Philomachus pugnax*. *Nature* **378**: 59-62.

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

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

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

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

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

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

Loehlin, D. W. & Werren, J. H. 2012. Evolution of shape by multiple regulatory changes to a growth gene. *Science* **335**: 943-7.

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

Maklakov, A. A. & Lummaa, V. 2013. Evolution of sex differences in lifespan and aging: Causes and constraints. *Bioessays* **35**: 717-724.

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

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

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

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

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

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

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

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

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

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

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

Mullon, C., Pomiankowski, A. & Reuter, M. 2012. The effects of selection and genetic drift on the genomic distribution of sexually antagonistic alleles. *Evolution* **66**: 3743-53.

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

881 Nielsen, R., Williamson, S., Kim, Y., Hubisz, M. J., Clark, A. G. & Bustamante, C. 2005.
882 Genomic scans for selective sweeps using SNP data. *Genome Res.* **15**: 1566-1575.
883 O'Connell, M. R., Oakes, B. L., Sternberg, S. H., East-Seletsky, A., Kaplan, M. & Doudna, J. A.
884 2014. Programmable RNA recognition and cleavage by CRISPR/Cas9. *Nature advance*
885 *online*: 10.1038/nature13769.
886 Oh, K. P., Fergus, D. J., Grace, J. L. & Shaw, K. L. 2012. Interspecific genetics of speciation
887 phenotypes: song and preference coevolution in Hawaiian crickets. *J. Evol. Biol.* **25**:
888 1500-1512.
889 Olendorf, R., Rodd, F. H., Punzalan, D., Houde, A. E., Hurt, C., Reznick, D. N., et al. 2006.
890 Frequency-dependent survival in natural guppy populations. *Nature* **441**: 633-636.
891 Panhuis, T. M., Butlin, R., Zuk, M. & Tregenza, T. 2001. Sexual selection and speciation.
892 *Trends Ecol. Evol.* **16**: 364-371.
893 Parker, D. J., Gardiner, A., Neville, M. C., Ritchie, M. G. & Goodwin, S. F. 2014. The evolution
894 of novelty in conserved genes; evidence of positive selection in the Drosophila fruitless
895 gene is localised to alternatively spliced exons. *Heredity* **112**: 300-306.
896 Parker, G. A. 1970. Sperm competition and its evolutionary consequences in the insects. *Biol.*
897 *Rev.* **45**: 525-567.
898 Parsch, J. & Ellegren, H. 2013. The evolutionary causes and consequences of sex-biased gene
899 expression. *Nat. Rev. Genet.* **14**: 83-87.
900 Patton, M. M. 2014. Meiotic drive influences the outcome of sexually antagonistic selection at a
901 linked locus. *J. Evol. Biol.*: doi: 10.1111/jeb.12493.
902 Payne, J. L. & Wagner, A. 2014. The robustness and evolvability of transcription factor binding
903 sites. *Science* **343**: 875-877.
904 Pearson, C. E., Edamura, K. N. & Cleary, J. D. 2005. Repeat instability: mechanisms of
905 dynamic mutations. *Nat. Rev. Genet.* **6**: 729-742.

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

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

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

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

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

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

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

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

Prokuda, A. Y. & Roff, D. A. 2014. The quantitative genetics of sexually selected traits, preferred traits and preference: a review and analysis of the data. *J. Evol. Biol.* **27**: 2283-96.

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

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

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

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

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

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

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

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

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

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

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

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

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

957 Schlötterer, C., Tobler, R., Kofler, R. & Nolte, V. 2014. Sequencing pools of individuals - mining
958 genome-wide polymorphism data without big funding. *Nat. Rev. Genet.* **15**: 749-763.
959 Schneeberger, K. & Weigel, D. 2011. Fast-forward genetics enabled by new sequencing
960 technologies. *Trends Plant Sci.* **16**: 282-288.
961 Seehausen, O., Butlin, R. K., Keller, I., Wagner, C. E., Boughman, J. W., Hohenlohe, P. A., et
962 al. 2014. Genomics and the origin of species. *Nat. Rev. Genet.* **15**: 176-192.
963 Servedio, M. R. & Bürger, R. 2014. The couterintuitive role of sexual selection in species
964 maintenance and speciation. *Proc. Natl. Acad. Sci. USA* **111**: 8113-8118.
965 Sham, P., Bader, J. S., Craig, I., O'Donovan, M. & Owen, M. 2002. DNA pooling: A tool for
966 large-scale association studies. *Nat. Rev. Genet.* **3**: 862-871.
967 Shaw, K. L. & Herlihy, D. P. 2000. Acoustic preference functions and song variability in the
968 Hawaiian cricket Laupala cerasina. *Proc. R. Soc. Lond., Ser. B: Biol. Sci.* **267**: 577-584.
969 Shaw, K. L., Parsons, Y. M. & Lesnick, S. C. 2007. QTL analysis of a rapidly evolving speciation
970 phenotype in the Hawaiian cricket *Laupala*. *Mol. Ecol.* **16**: 2879-2892.
971 Shuster, S. M. & Sassaman, C. 1997. Genetic interaction between male mating strategy and
972 sex ratio in a marine isopod. *Nature* **388**: 373-377.
973 Shuster, S. M. & Wade, M. J. 1991. Equal mating success among male reproductive strategies
974 in a marine isopod. *Nature* **350**: 608-610.
975 Sinervo, B. & Lively, C. M. 1996. The rock-paper-scissors game and the evolution of alternative
976 male strategies. *Nature* **380**: 240-243.
977 Slate, J. 2013. From Beavis to beak color: a simulation study to examine how much QTL
978 mapping can reveal about the genetic architecture of quantitative traits. *Evolution* **67**:
979 1251-1262.
980 Snell-Rood, E. C., Cash, A., Han, M. V., Kijimoto, T., Andrews, J. & Moczek, A. P. 2011.
981 Developmental decoupling of alternative phenotypes: insights from the transcriptomes of
982 horn polyphenic beetles. *Evolution* **65**: 231-245.

983 Soria-Carrasco, V., Gompert, Z., Comeault, A. A., Farkas, T. E., Parchman, T. L., Johnston, J.
984 S., et al. 2014. Stick insect genomes reveal natural selection's role in parallel speciation.
985 *Science* **344**: 738-742.
986 Stuglik, M. T., Babik, W., Prokop, Z. & Radwan, J. 2014. Alternative reproductive tactics and
987 sex-biased gene expression: the study of the bulb mite transcriptome. *Ecol. Evol.* **4**: 623-
988 632.
989 Swanson, W. J. & Vacquier, V. D. 1995. Extraordinary divergence and positive Darwinian
990 selection in a fusagenic protein coating the acrosomal process of abalone spermatozoa.
991 *Proc. Natl. Acad. Sci. USA* **92**: 4957–4961.
992 Swanson, W. J. & Vacquier, V. D. 2002. The rapid evolution of reproductive proteins. *Nat. Rev.*
993 *Genet.* **3**: 137-144.
994 Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA
995 polymorphism. *Genetics* **123**: 585-95.
996 Takahata, N. & Nei, M. 1990. Allelic genealogy under overdominant and frequency-dependent
997 selection and polymorphism of major histocompatibility complex loci. *Genetics* **124**: 967-
998 78.
999 Taylor, P. D. & Williams, G. C. 1982. The lek paradox is not resolved. *Theor. Popul. Biol.* **22**:
1000 392-409.
1001 Travisano, M. & Shaw, R. G. 2013. Lost in the map. *Evolution* **67**: 305-314.
1002 Turner, T. L. & Miller, P. M. 2012. Investigating natural variation in Drosophila courtship song by
1003 the evolve and resequence approach. *Genetics* **191**: 633-U516.
1004 Van Dyken, J. D. & Wade, M. J. 2010. The genetic signature of conditional expression. *Genetics*
1005 **184**: 557-70.
1006 Viguerie, N., Montastier, E., Maoret, J.-J., Roussel, B., Combes, M., Valle, C., et al. 2012.
1007 Determinants of human adipose tissue gene expression: impact of diet, sex, metabolic
1008 status, and cis genetic regulation. *PLoS Genet.* **8**: e1002959.

1009 Visscher, P. M., Brown, M. A., McCarthy, M. I. & Yang, J. 2012. Five years of GWAS discovery.
1010 *The American Journal of Human Genetics* **90**: 7-24.
1011 Wang, X., Weigel, D. & Smith, L. M. 2013. Transposon variants and their effects on gene
1012 expression in Arabidopsis. *PLoS Genet.* **9**: e1003255.
1013 Warren, I. A., Gotoh, H., Dworkin, I. M., Emlen, D. J. & Lavine, L. C. 2013. A general
1014 mechanism for conditional expression of exaggerated sexually-selected traits. *Bioessays*
1015 **35**: 889-899.
1016 Wells, K. D. 1977. The social behaviour of anuran amphibians. *Anim. Behav.* **25**: 666-693.
1017 Whittle, C. A. & Johannesson, H. 2013. Evolutionary dynamics of sex-biased genes in a
1018 hermaphrodite fungus. *Mol. Biol. Evol.* **30**: 2435-2446.
1019 Wilkins, A. S. (2014) "The genetic tool-kit": The life-history of an important metaphor. In:
1020 *Advances in Evolutionary Developmental Biology*, (Streelman, J. T., ed.). pp. 1-14. John
1021 Wiley and Sons, Inc., New York.
1022 Wilkinson, G. S. & Johns, P. M. (2005) Sexual selection and the evolution of mating systems in
1023 flies. In: *The Evolutionary Biology of Flies*, (Wiegmann, B. M. & Yeates, D. K., eds.). pp.
1024 312-329. Columbia University Press, New York.
1025 Wilkinson, G. S., Johns, P. M., Metheny, J. D. & Baker, R. H. 2013. Sex-biased gene
1026 expression during head development in a sexually dimorphic stalk-eyed fly. *Plos One* **8**:
1027 e59826.
1028 Williams, T. M. & Carroll, S. B. 2009. Genetic and molecular insights into the development and
1029 evolution of sexual dimorphism. *Nat. Rev. Genet.* **10**: 797-804.
1030 Wright, S. 1951. The genetical structure of populations. *Annals of Eugenics* **15**: 323-354.
1031 Wyman, M. J., Cutter, A. D. & Rowe, L. 2012. Gene duplication in the evolution of sexual
1032 dimorphism. *Evolution* **66**: 1556-1566.
1033 Xue, Z., Ren, M., Wu, M., Dai, J., Rong, Y. S. & Gao, G. 2014. Efficient gene knock-out and
1034 knock-in with transgenic Cas9 in Drosophila. *G3* **4**: 925-929.

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

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

Zhang, G., Li, C., Li, Q., Li, B., Larkin, D. M., Lee, C., et al. 2014. Comparative genomics reveals insights into avian genome evolution and adaptation. *Science* **346**: 1311-20.

Zhang, Y., Sturgill, D., Parisi, M., Kumar, S. & Oliver, B. 2007. Constraint and turnover in sex-biased gene expression in the genus Drosophila. *Nature* **450**: 233-U2.

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

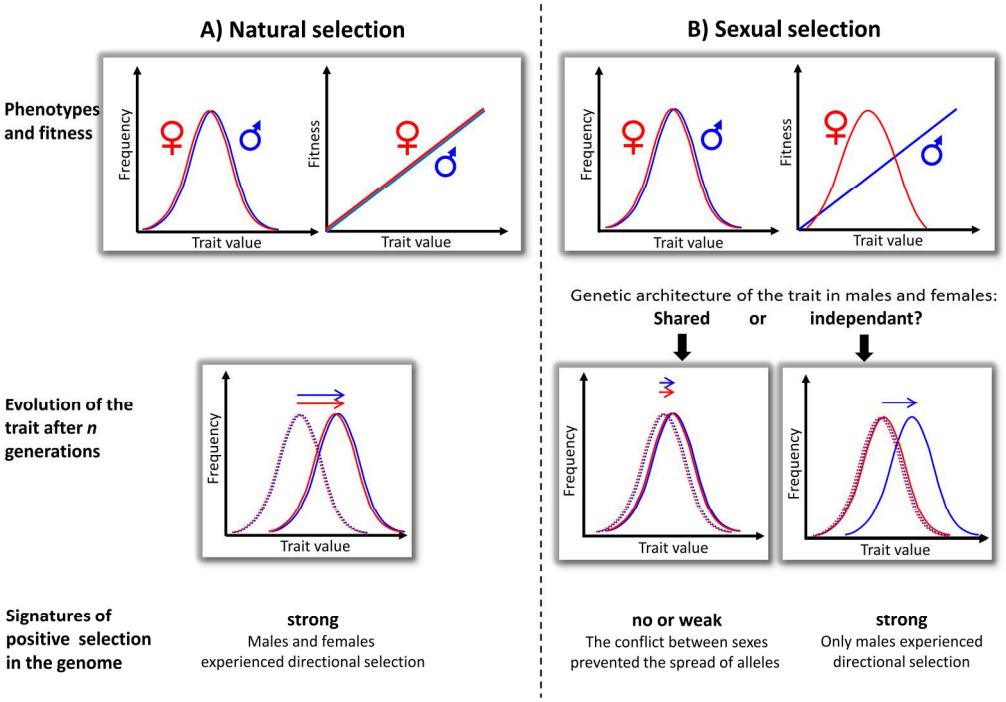
Figure Legends

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).

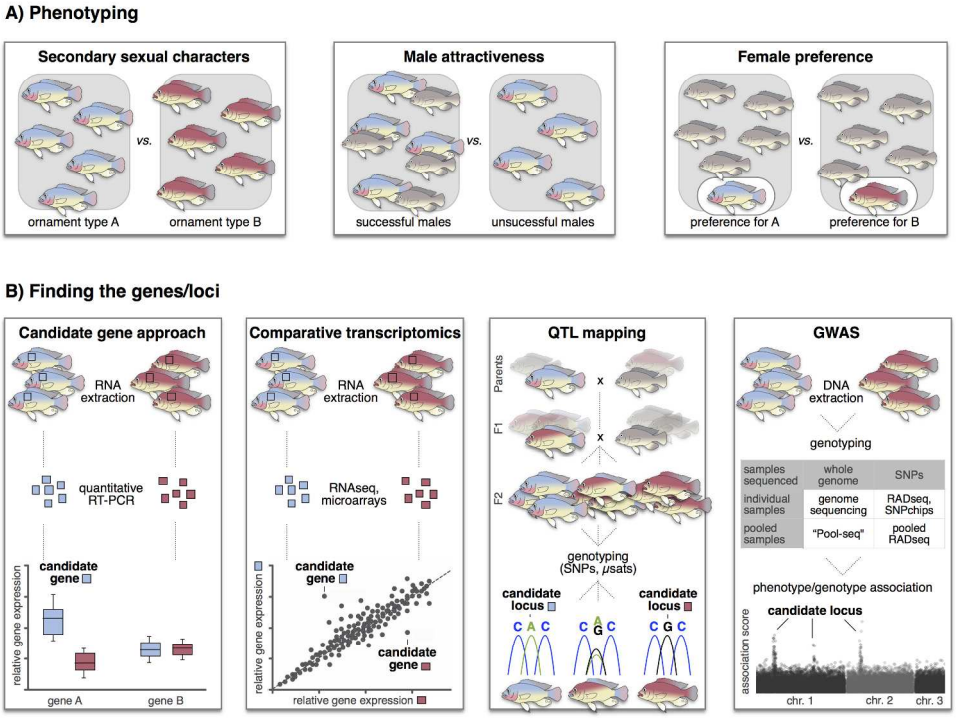
Figure 2. Overview of forward genetic approaches for identifying genes that control expression of traits involved in sexual selection. The trait used to group individuals may be, for example, a male secondary sexual character, any measure of male attractiveness (e.g. mating success), or female preferences (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

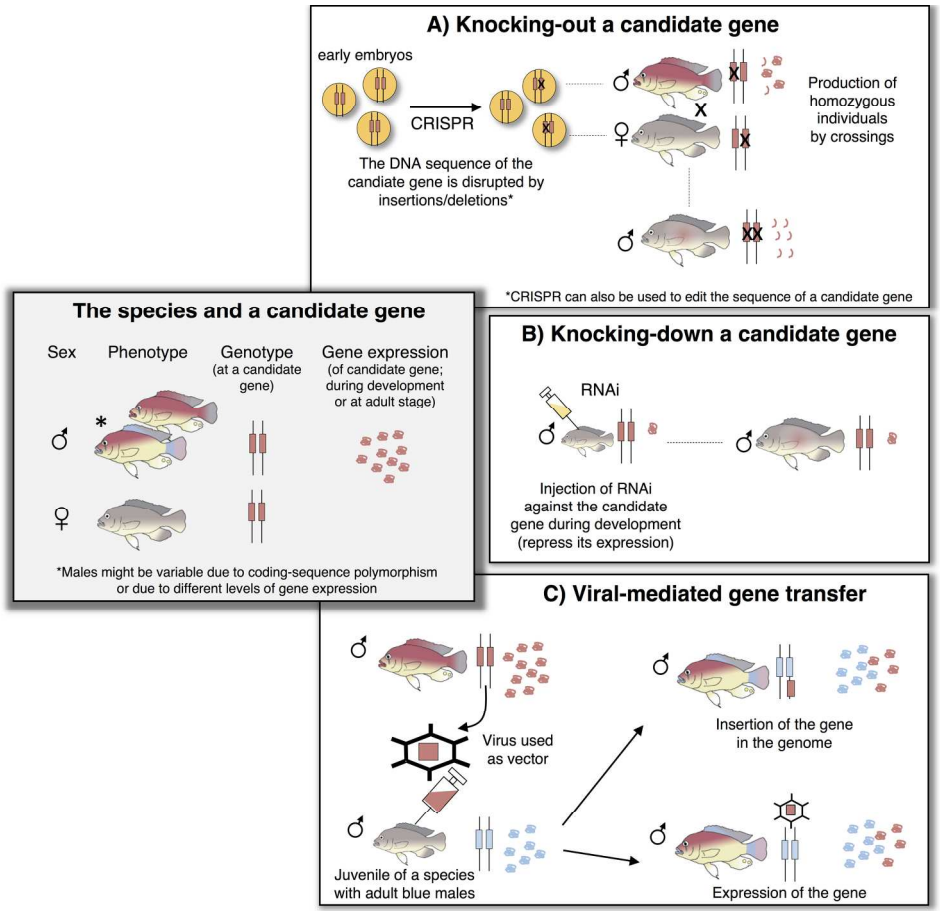
allele have an altered phenotype. CRISPR approaches can also be used to edit allelic variants in order to evaluate the phenotypic effect of different alleles in the same genetic background. For genes with pleiotropic effects, knocking-down candidate gene expression with RNA interference (Panel B) can 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).
190x142mm (300 x 300 DPI)



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)