Chapter 2
The Genomics of Sexual Ornaments, Gene Identification and Pleiotropy

Martin Johnsson

Abstract Sexual ornaments, which are traits that make an individual attractive to potential mates, have a long history in evolutionary biology. These adaptations to mate choice have been the subject of research from the perspective of genetics, ecology and theoretical biology. The rapid development of genomic methods has equipped modern genetics with new tools to answer old questions and open up new areas of analysis. For research into sexual ornamentation, this has meant the application of genetic mapping, in particular quantitative trait locus (QTL) methods, and transcriptomics to search for genes and biological pathways affecting ornamental traits and investigate pleiotropy between ornamental traits, ornament preference and fitness-related traits. Examples come from QTL studies of beak colour in the zebra finch, colocalisation between loci for ornaments and other traits in crickets and moths, QTL mapping and population genomics of colour in guppies and cichlids, genetical genomics and pleiotropy mapping of comb size in the chicken, transcriptomic studies of handicap mechanisms in the grouse, and genetics and molecular evolution of several sexual traits in *Drosophila*. Genomic methods help reveal the variety of mechanisms involved in sexual ornamentation and are complementary to quantitative genetics, population genetics and organismal studies.

2.1 Introduction

Sexual ornaments are traits that help make an individual attractive to potential mates of the opposite sex. Here, ornaments are taken to include secondary sexual traits favoured by mate choice, but not sexual weaponry used in same-sex competition. Traits may serve a dual purpose, and in actual cases, the function of a trait is an empirical question that may be difficult to answer definitively. Ornamental

M. Johnsson (✉)
Department of Physics, Chemistry and Biology, Linköping University, Linköping, Sweden
e-mail: martin.johnsson@liu.se

P. Pontarotti (ed.), *Evolutionary Biology: Biodiversification from Genotype to Phenotype*, DOI 10.1007/978-3-319-19932-0_2

traits may be morphological or behavioural, such as exaggerated structures, colourful displays, acoustic signals and sexual behaviours.

Genomic methods allow the simultaneous investigation of genetic variants, transcripts and many other molecular features on a genome-wide scale. Such methods successively become available to evolutionary biologists and geneticists studying evolutionary phenomena, such as sexual ornamentation. With the exception of *Drosophila* and possibly the chicken, the study organisms for sexual ornaments are not model organisms for genetics. Hence, the genomic resources are comparatively limited and often have to be constructed by the researchers themselves. Improvements in sequencing technology have made this kind of work easier and will most likely continue to do so. Genomic research on sexual ornaments largely consists of genetic mapping studies in the linkage mapping framework. There is great promise in combining this kind of mapping with genome-scale molecular work and population genetics, made possible by recent advances in genomics.

There are different hypotheses about the basis of sexual ornamentation. Moreover, there is empirical support for several of them in different organisms. In the Fisher process, mate preference, once established, builds up genetic correlations between preference alleles and ornament alleles and runaway selection for ornament exaggeration (Mead and Arnold 2004). Except the Fisher process, most mechanistic hypotheses about sexual ornaments presuppose some form of pleiotropy between variants affecting the ornament and variants affecting other traits. In all cases, sufficiently tight linkage disequilibrium can substitute for genuine pleiotropy. The difference between hypotheses is what other traits that are coupled with the ornament and how they relate to fitness. In "good genes"-related hypotheses, variants affecting ornaments are expected to also pleiotropically affect fitness-related traits. More specifically, the "genic capture" variety of good gene hypotheses predicts many small-effect loci with pleiotropic effects on overall condition (Rowe and Houle 1996). Finally, "handicap hypotheses" add a layer of mechanistic detail, predicting these effects to be mediated by effects on physiological traits such as immunocompetence (Hamilton and Zuk 1982). In contrast, direct benefit hypotheses would predict no effect link between ornamentation and offspring genetic quality, but instead links between ornamentation and direct provisioning of resources by ornamented individuals to their mates.

Pleiotropy is a commonly used term with several slightly different meanings. Paaby and Rockman (2013) make a useful distinction between three different perspectives. The first is molecular gene pleiotropy, which means that a molecular gene, gene product or sequence element has several biological roles. While molecular gene pleiotropy is a prerequisite for any genetic variant to affect multiple traits, what is most interesting for the study of the evolution of any trait is when genetic variants have multiple phenotypic effects. This is what Paaby and Rockman call developmental pleiotropy. Finally, selectional pleiotropy is when a variant affects multiple fitness components. This perspective is also crucial for

understanding of sexual selection. Ornaments that act by means of handicap signalling or antagonistic pleiotropy would be clear examples of selectional pleiotropy. However, even when handicap signalling is not the central mechanism of an ornament, exaggerated displays are expected to be associated with trade-offs.

The establishment of a trait as a sexual ornament requires studies of mate preference and knowledge of the ecology and behaviour of the study organism. When it comes to the genetic basis of ornamentation, genomic approaches are complementary to quantitative and population genetic methods. Statistical quantitative genetic methods are based on the measurements of traits and pedigrees, and population genetic methods consider variants in populations. For example, a quantitative genetic technique to investigate pleiotropy is to estimate the genetic correlation between two traits. One genomic approach is to map quantitative trait loci associated with both traits and see whether they overlap. In one sense, these experiments study the same phenomenon but from different angles. However, the genomic analysis also raises new questions. Are the co-localising loci genuinely pleiotropic or are they built up of closely linked variants that effectively act as pleiotropic? What is the molecular basis of pleiotropic effects at this locus? Does the variant affect several biological pathways? Genomic analysis can help test old hypotheses about the mechanisms behind sexual ornaments, but they also raise new levels of analysis that are largely independent of the classical questions.

2.2 Genetic Mapping and Transcriptomic Techniques

Genetic mapping is localisation of genetic variants affecting a trait by means of statistical models that use genetic markers as predictors and phenotypic traits as response variables. When the trait in question is measured on some quantitative scale, rather than presence/absence, this is called quantitative trait locus (QTL) mapping (Soller et al. 1976; Lander and Botstein 1989). All kinds of genetic mapping rely on genetic markers that are linked to the causative variants. In linkage mapping, one uses related individuals with a known pedigree with markers that are informative of the segregation of chromosomes in these particular families. Linkage mapping can be undertaken either with natural pedigrees and using variance component methods or with experimental line crosses. QTL mapping in experimental crosses finds variants that differ between the founder individuals and are usually constructed from divergent populations, ideally from inbred founder individuals, to maximise the genetic variation that can be detected. Line crosses can be applied to within-population variation by, for example, repeated QTL mapping from inbred individuals or crosses of selection lines established from the same population.

As genotyping and sequencing technology have progressed and a high marker density has become easier to achieve, genome-wide association studies (GWASs) have risen to prominence (Risch and Merikangas 1996). GWAS relies on linkage

disequilibrium on the population level, rather than linkage within a family, and hence has much higher mapping resolution. Linkage disequilibrium blocks can be short enough for GWAS to even isolate a single candidate gene for an association. Also, GWAS works on unrelated individuals, so natural populations can be investigated without the need to construct a pedigree. However, a GWAS is a major undertaking in terms of both sample size and construction of marker maps, if such genomic resources are not already available for the species in question.

A complementary approach to genetic mapping is transcriptome-wide gene expression measurement. Expressed sequence tag (EST) sequencing and microarrays have been available for some time, and with the rise of massively parallel sequencing, RNA sequencing will likely be more common in the future. Gene expression studies can be applied both to the environmental and to the genetic mechanisms of ornamental traits, by either comparing individuals of different genotype or exposed to different environments. The application of genetic mapping to gene expression values is called expression QTL or eQTL mapping (Jansen and Nap 2001). eQTL mapping allows screening for genes that are affected by segregating variants in the population. The co-localisation of eQTL and QTL can be used as a way to filter the genes under the QTL to find those that could be a causative gene affected by a regulatory variant. When the genomic location of the gene being measured is known, eQTL can be divided into local eQTL that map back to the location of the gene and distal eQTL that map to other regions of the genome. Local eQTL are putative cis-eQTL, that is QTL affected by variants in some regulatory sequence such as a promoter, enhancer or insulator. Distal eQTL are likely trans-acting, meaning that they affect some upstream gene in a regulatory pathway. While trans-eQTL often have relatively smaller effect sizes and are more difficult to detect, this distinction in principle can allow one to find downstream consequences of causative genes. Gene expression comparisons between genetically distinct stocks such as different populations or selection lines are similar in spirit to eQTL mapping. These experimental designs also find genes affected by genetic variants differing between populations or lines. However, they do not give any genomic localisation of the regulatory variant and do not distinguish local and distal regulatory effects.

2.3 Beak Colour in the Zebra Finch

Beak colour in the zebra finch is one ornamental trait that has been investigated in quantitative genetic and QTL mapping studies. The redness of the beak is sexually dimorphic, and females of some populations prefer males with darker red beaks. Beak redness is one example of a carotenoid-based colour, which is suggested to be involved in trade-offs between colouration and immune function (Blount et al. 2003). Schielzeth et al. (2012) mapped QTL for beak colour as measured with spectrometry. They used variance component mapping in a within-population design, which allows mapping naturally segregating variants. In this case, the

population was a pedigree of captive birds. They report four QTL at the suggestive threshold level that still appear to explain most of the genetic variation within the population: 29 %, when the heritability was estimated to 34 %. They take this to mean that beak colour is oligogenic, with these four loci explaining the bulk of the additive genetic variance. This is at odds with genic capture, because genic capture implies a polygenic architecture built up of many small-effect loci. They hypothesise that antagonistic pleiotropy could be at work. However, there is an alternative interpretation, which has to do with the statistical limitations of QTL mapping, even in large studies such as this one. If a study is underpowered, that is it has a small sample size in relation to the expected size of the effect to be estimated, it is likely to result in overestimates. The problem with genetic mapping is that under a polygenic model, genetic effects are expected to be very small. Furthermore, variance component-based genetic mapping using natural pedigrees requires much larger sample sizes than line-cross QTL mapping to achieve good power. In a simulation study, Slate (2013) suggested that the results of the beak colour study are consistent with a polygenic architecture.

2.4 Eye Span and Meiotic Drive in the Stalk-Eyed Fly

The stalk-eyed fly *Cyrtodiopsis dalmanni* provides an example of how an ornamental trait can reflect genetic quality of a mate in the form of the absence of or protection against a meiotic driver. In this species, some males carry an X-linked variant that makes them produce offspring with very skewed sex ratios. The X-linked driver causes them to pass on itself, rather than the Y chromosome, producing female offspring that also carry the driver. This effect causes the driver chromosome to spread in the population because of this advantage. Females who avoid mating with driver-carrying males, however, can gain a reproductive advantage in that they produce sons. In these flies, eye span is associated with brood sex ratio and hence seems to work as a signal of meiotic drive (Wilkinson et al. 1998). Johns et al. (2005) used QTL mapping to investigate the genomic overlap between drive and eye span, investigating whether the traits are linked and in particular if there is an X-linked eye locus linked to the driver. They did find five eye stalk QTL, the largest of which was X-linked. It appears that the driver is indeed linked with a variant affecting eye span. They also investigated the offspring sex ratio of males in relation to their X driver genotype. They found an association between one autosomal QTL and sex ratio of the offspring. This suggests that the eye stalks not only signal the presence or absence of the meiotic driver, but also at least one autosomal modifier locus that counteracts the effect of the driver. The length of the eye stalk allows mate choice before mating, but there is also a potential for post-copulatory sexual selection. In particular, promiscuity in these flies opens the door to sperm competition, where variation in sperm traits might be important for the mating success of the males. In a later QTL study, Johns and

Wilkinson (2007) found that the X-linked driver and one autosomal eye stalk QTL also co-localised with QTL for sperm tail length.

There is also a study (Baker et al. 2009) that applies transcriptomics to the study of eye span in the stalk-eyed fly. The authors measured gene expression in eye stalk tissue at the larval, pupal and adult stage in individuals from lines selected for long or short eye span by means of EST cloning and sequencing. They measured differential expression between selection lines and also investigated gene duplication and protein coding gene evolution with alignment against *Drosophila melanogaster* genes. If a reference genome for the stalk-eyed fly were to be constructed, comparisons of differentially expressed and quickly evolving genes against the eye stalk QTL identified in previous studies could aid in identifying quantitative trait genes for these QTL.

2.5 Acoustic Signalling in Crickets and Moths

Even though Fisherian runaway selection does not presuppose physical linkage between variants for the ornament and the preference, mate preference and ornament expression QTL sometimes co-localise, revealing direct functional or physical links between preference and ornament. In the case of behavioural ornamental traits, such as certain patterns of sound production, one might hypothesise that the faculties required to produce the signal and receive it might be the same and affected by the same genetic variants. In the Hawaiian cricket genus *Laupala*, there is indeed direct linkage or pleiotropy of male song and female song preference loci. This was investigated in a series of QTL mapping studies (Shaw et al. 2007; Shaw and Lesnick 2009; Wiley and Shaw 2010) with crosses of closely related species *L. paranigra* and *L. kohalensis*. When performing genome-wide scan for female preference (Shaw and Lesnick 2009), they detected one preference QTL. Investigating preference, however, is in general more difficult and time-consuming than investigating the signalling trait. A strength in this case is that the preference was measured using synthesised songs and hence avoided the variation introduced by interactions with actual stimulus males. In follow-up studies, the preference effects were tested in directed crosses (Wiley and Shaw 2010) and introgression lines (Wiley et al. 2012) using the five male song QTL. Four out of five pulse rate QTL also affected female song preference. The fact that these QTL were readily detected in between-species crosses suggests that they have indeed been fixed by selection. Possibly, the fixation of preference for and production of songs of different pulse rate are part of the rapid speciation of these crickets. Direct genetic linkage between preference and signal may make Fisherian runaway selection even more effective, since it ensures high genetic correlation from the outset.

In the acoustic moth *Achroia grisella*, on the contrary, QTL mapping with inbred lines based on two populations from Florida and Kansas, USA, did not find any evidence of shared architecture between female preference and male signalling with ultrasound pulses (Limousin et al. 2012). A following study (Alem et al. 2013)

investigated whether song preference and response to bat echolocation signals share overlapping loci. They found no overlap. However, there are complications with trying to learn the evolutionary history of a trait from current genetic variation. Even if male acoustic signalling did evolve by co-opting mechanisms from predator avoidance, which does seem likely despite the lack of overlap, the pleiotropic variants involved in the early evolution of this signal must still be different between populations for QTL mapping to detect them. The authors interpret the lack of overlap to mean the independent architecture might reflect later modifications of the female responses.

2.6 Comb Size in the Chicken

The chicken comb is an example of a mutual sexual ornament that is preferred by both sexes. It is used by females to guide largely post-copulatory mate choice and by males to allocate sperm (Pizzari and Birkhead 2000; Pizzari et al. 2003). Comb size reflects status and reproductive investment (Cornwallis and Birkhead 2007). There is also evidence of handicap effects on males (Von Schantz et al. 1995). Because relative comb size has increased under chicken domestication, the genetic and molecular basis of comb size can be studied in intercrosses of wild and domestic chickens. QTL mapping in two F_2 crosses and one eighth-generation advanced intercross (Wright et al. 2008; Johnsson et al. 2012, 2014) found six replicable QTL regions with additive moderate to large effects. The advanced intercross is a way of increasing the mapping density of experimental intercrosses. Starting from the F_2 intercross, the intercross line is interbred for a number of generations, accumulating recombinations. The QTL regions of the advanced intercross are considerably tighter than the F_2 study (Johnsson et al. 2012), but most QTL still have many positional candidate genes. The exception in this case is one major comb QTL that covers the non-coding region between the genes *bone morphogenetic protein 2* and *hydroxyacid oxidase 1*. A local eQTL study of these two genes in comb base tissue suggests that both of them are regulated by genetic variants at the QTL. A microarray-based eQTL study (Johnsson et al. 2014) targeted to five of the replicated comb QTL found an additional three putative quantitative genes that display local eQTL effects and an association between comb gene expression and relative comb mass.

These QTL studies also provide evidence that comb size reflects different mate quality traits. Egg production in the female chicken is physiologically coupled with bone metabolism, since bone mineralisation and eggshell production share a common calcium reserve. Female chicken bone is continually being remodelled with the laying cycle (Kerschnitzki et al. 2014). In line with this phenomenon, comb mass QTL overlapped with both egg production and bone mineral density QTL (Rubin et al. 2007). Additionally, QTL for female onset of sexual maturity was found to overlap with comb QTL at all three loci that were detected (Wright et al. 2012). Moreover, multivariate QTL models were used to help disentangle

pleiotropy from close linkage and found that in several cases, the data were better explained by multiple linked QTL. This analysis is subject to the statistical uncertainties of model selection and limited by the recombinations available in the cross. However, it does suggest that tight linkage as well as genuine pleiotropy plays a role in the ornamental function of the chicken comb. The presence of clusters of linked QTL seems to be a general feature in the genetic architecture of chicken domestication (Wright et al. 2010). In the case of sexual ornaments, linkage between fitness-influencing variants and ornament variants could be a way for ornaments to capture variation in different traits. This mechanism of coupling requires no functional connection but the contingencies of genome organisation.

2.7 Comb Size in the Red Grouse

Studies in the field suggest that the size of the comb of the red grouse *Lagopus lagopus scoticus* is a condition-dependent sexual ornament dependent on testosterone signalling (Mougeot et al. 2004). Males that were implanted with testosterone displayed larger combs, more parasites and a reduction in T-cell-mediated immunity. This is consistent with immunocompetence handicap, where the cost of a large comb is a steroid-induced impairment of the immune system. Subsequent transcriptomic studies (Webster et al. 2011; Wenzel et al. 2013) investigated the mechanistic basis of testosterone effects on red grouse males. The authors manipulated the testosterone as well as the caecal parasite load of the birds in a factorial design under natural conditions (Webster et al. 2011). They measured gene expression in caecal tissue with microarrays and found genes affected by parasite load and testosterone. The treatment of high testosterone and high parasite load is particularly relevant to males with a large comb, who should suffer the handicap effect of testosterone and parasite load. Fifty-two transcripts were differentially expressed under these conditions, and 51 of them were down-regulated. These putative handicap-related transcripts were not necessarily from immune-related genes. A later analysis of the same field experiment (Wenzel et al. 2013) explicitly compared two mechanistic hypotheses for the handicap effect: immunocompetence handicap and oxidative stress handicap, where the testosterone increase is thought to cause either immunosuppression or increased oxidative stress. The authors compiled one set of immune system genes and one of oxidative stress genes based on Gene Ontology annotation. More genes from the immune set than the oxidative stress set were differentially expressed, but most of the detected genes fell in neither category. In short, the transcriptome evidence was not clear in favour of either hypothesis. This could be due to the limits of available gene annotation, but also raises the possibility that handicap in this species is mediated by a third unknown mechanism or a mix of physiological mechanisms.

2.8 Pigmentation in Guppies and Cichlids

The guppy, *Poecilia reticulata*, displays heritable genetic variation in male colour pattern, which is polymorphic within populations. Experiments with mate choice in the laboratory and with constructed populations in the field have found negative frequency-dependent selection for male colour (Hughes et al. 2013; Zajitschek et al. 2006). The number of offspring produced favoured males with rare colour patterns. This indicates that male colour polymorphisms are maintained by balancing selection, something that is theoretically known to be able to maintain variation. The mechanism favouring the preference for rare males is not known. Colour polymorphism in guppies has been the subject of QTL mapping in a cross of two distinct populations (Tripathi et al. 2009). The authors mapped 12 aspects of male colouration and found between two and eleven QTL per trait. This suggests a polygenic architecture with several QTL on the linkage group corresponding to the sex chromosome. A population genetic study of guppies from different populations (Willing et al. 2010) found evidence for selection at two of these loci. While variation can be maintained within a population, these between-population studies are evidence of directional selection as well.

Cichlid fishes are another example of diverse colour variation both within and between species. Cichlid colour has also been studied with QTL mapping. Albertson et al. (2014) crossed two related species from Lake Malawi that have different colour patterns and measured black and red yellow pixel counts with digital photography on 12 regions of the body. Mapping resulted in 41 QTL for various aspects of pigmentation. They genotyped the fishes by massively parallel sequencing of restriction site associated regions. In addition to the F_2 intercross, genotyping included wild-caught fishes from each population that were used for population genomics of the detected QTL regions. They found scaffolds of the reference cichlid genome matching their QTL regions and estimated genetic differentiation between the populations. Seventeen out of 366 of the single nucleotide variants found in one large QTL for red yellow colour had a high degree of differentiation. One of them was fixed between species and located within the 5' untranslated region of the *pax3a* gene. Levels of *pax3a* expression, as measured with qPCR, correlated with number of pigment-bearing xanthophores, and *pax3a* displayed allele-specific expression in the F_1 generation. Taken together, this suggests that this QTL is explained by a cis-acting regulatory variant acting on *pax3a* expression. It also demonstrates the power of combining QTL mapping with population genomics and gene expression for gene identification.

2.9 Sexual Ornaments in *Drosophila*

The fruit flies *Drosophila* spp. are genetic model organisms par excellence but also have sophisticated systems of mate choice. Studies on *Drosophila* highlight the importance of integrating multiple ornamental traits for an understanding of how

preference and ornamentation work in a natural setting. Cuticular hydrocarbons, one class of sexually selected trait in *Drosophila*, are inherently multivariate, since the signal is made up of a complex chemical mixture. However, *Drosophila* also utilise courtship dance and song and pigment patterns to guide mate choice.

Related species of *Drosophila* provide a model for how sexually selected traits contribute to isolation between species. Interspecific crosses of *D. simulans* and *D. sechellia* make up such a system. Gleason and Richie (2004) measured the interpulse distance in male courtship song in backcrosses and found six QTL. None of them overlapped the within-species QTL detected for the same trait detected in *D. melanogaster* (Gleason et al. 2002). However, the latter study was based on two laboratory strains and found three QTL, which can only cover a limited sampling of the variation within *D. melanogaster*. Similarly, Gleason et al. (2005, 2009) mapped QTL for cuticular hydrocarbons between *D. simulans* and *D. sechellia*. These studies estimate rather large effect sizes, explaining between 30 and 80 % of the variance in the crosses with few QTL per trait. Since very small-effect sizes are quite possible for polygenic traits, there is always a risk of QTL effect overestimation. One also needs to keep in mind that the proportion of variance explained in an intercross does not necessarily reflect the variance explained by that QTL in any natural setting. However, there is at least the potential for large-effect genes contributing to species isolation. As a counterpoint, consider a QTL study of within-population genetic variation in cuticular hydrocarbons in *D. melanogaster* (Foley et al. 2007). The authors used recombinant inbred lines based on a single pair of files derived from field-caught females. They identified 25 QTL in females and 15 in males, indicative of polygenic within-population variation.

Several, but by no means all, of the QTL for cuticular hydrocarbon production that has been found in the above studies may be explained by variants in genes of the desaturase gene family. *desatF* (Shirangi et al. 2009) is potentially one of the genes responsible for the differences between *D. simulans* and *D. sechellia*. It is also sexually dimorphic in some *Drosophila* species and monomorphic in some. The authors use in situ hybridisation and reporter assays to find regulatory sequences that drive *desatF* expression. They find that sexually dimorphic expression is caused by the presence of a binding site for the transcription factor *doublesex* and find a single nucleotide difference in this motif that abolishes its function in *D. takahasii*. Arguably, these differences have more to do with mate recognition than sexual selection, but they are still examples of the things that can be achieved when causative genes have been isolated.

2.10 The Value of Genome-Wide Studies Compared to Candidate Gene Studies

The QTL mapping of beak colouration (Schielzeth et al. 2012) also exemplifies the difficulties of candidate applying gene approaches to sexual ornaments. The authors compile a list of zebra finch orthologs of known carotenoid-related genes and

searched for these genes under their QTL. There was no evidence for any association with the candidate genes in this population. Similarly, enrichment testing of Gene Ontology annotation categories is a popular method to try to extract biological knowledge from gene sets derived from genomic experiments. Gene Ontology annotation enrichment on the genes located in QTL regions in this study found only an enrichment of the "metabolic process" category, which is extremely general. QTL regions are usually broad and contain many more genes than the expected number of causative genes. Hence, Gene Ontology enrichment on QTL regions is unlikely to yield anything but noise, except possibly in the most high-resolution studies such as GWAS. The gene expression study on stalk-eyed flies (Baker et al. 2009) also illustrates the difficulty of Gene Ontology enrichment in gene expression studies. The comparison between flies selected for long and short eye span resulted in 367 differentially expressed genes, but no overrepresented categories. The study found a handful of particularly promising candidates based on both gene expression and sequence evolution; however, at least two of the five candidates highlighted by the authors have little to no functional literature. This emphasises the value of genome-wide approaches such as these, even if genome-wide top-down studies require more work than targeted candidate gene studies.

The functions of genes are often unknown, particularly since molecular pleiotropy means that a given molecular gene may participate in processes it is not currently known for. Candidate gene approaches may be applicable in certain cases when the functional biology of the trait is well known or when high-quality candidate gene data from mutation studies in the same species are available. For example, Kottler et al. (2013) performed a successful candidate gene study of pigment patterns in the guppy based on genes from the zebrafish, and some of the QTL found in the abovementioned *Drosophila* QTL studies overlaps mutational candidate genes for those traits. This level of functional information is usually not the available when investigating complex traits in non-model organisms.

2.11 Maintenance of Genetic Variation

A recurring question in sexual selection is how, if at all, genetic variance in sexually selected traits is maintained. If a sexual ornament is under directional selection, the genetic variance should be depleted eventually (Taylor and Williams 1982). Several mechanisms of maintenance are possible and have empirical support in different cases. For instance, frequency-dependent selection that favours rare variants can maintain variation, and this is the case for colour morphs in the guppy (Hughes et al. 2013). Another mechanism is heterozygote advantage, where selection favours heterozygotes with intermediary trait levels, possibly because of life history trade-offs between ornamentation and survival. Sexual weaponry in the form of horn shape in the Soay sheep of St Kilda is an example of heterozygote advantage in the wild (Johnston et al. 2013).

However, quantitative trait variation is not necessarily maintained. The genic capture model of good genes suggests that variation is continually replenished, because ornaments reflect polygenic variation in fitness. When between-population QTL mapping works and reveals loci, it works best for differences that are fixed between populations. This could reflect local adaptation, but also that different populations go through and fix different subsets of the possible mutations that affect an ornament. Moreover, even if there is detectable genetic variation in a trait, selection could still have exhausted all available genetic variation. Whether apparent genetic variation in a single trait is available to selection depends on the interactions with other genetically correlated traits. This curse of dimensionality potentially affects all traits subject to multivariate selection, which in a naturalistic setting may be most ornamental traits. Quantitative genetic studies of cuticular hydrocarbons in *D. serrata* suggest that sexual selection has actually depleted the variance in the direction of selection and that univariate estimates of genetic variance that suggest maintenance can be misleading in this case (Hine et al. 2004; Blows et al. 2004).

2.12 Promises and Limitations of Genomic Methods

Sexual ornaments are a diverse set of morphological and behavioural traits that provide case studies for functional and evolutionary genomics. Researchers have used genomics, principally QTL mapping and microarrays, to investigate the genetic architecture of ornaments, to search for the underlying causative genes and to study mechanisms of ornament function. All three perspectives will likely benefit from improvements in genomic sequencing, transcriptomics and other genome-wide molecular assays. Also, laboratory and field studies of phenotypes and fitness as well as pedigree-based quantitative genetic studies will remain crucial.

The main limiting factors to untangling the genetic architecture of a trait through mapping are power and resolution. Low power prevents one from finding loci and leads to overestimation of the ones identified. Low resolution prevents one from distinguishing linkage from pleiotropy. Both effects are related to sample size and bias mapping towards making genetic architecture look simpler than it is. Mapping resolution in a genome-wide association study is superior to linkage mapping and can often cut associated regions down to a handful of genes. Therefore, GWAS is an attractive alternative for within-population mapping studies. GWAS requires much higher marker density than linkage mapping. With modern sequencing and genotyping methods, marker discovery is still a major undertaking, but achievable. However, the main limitation will be collecting and phenotyping sufficient samples of individuals.

Genomics opens up for the top-down identification of genes and genetic variants affecting ornamental traits without relying on previous knowledge of molecular mechanisms and candidate genes, which is often not available. However, such gene identification studies require high mapping resolution and likely the combination of

multiple techniques. A promising approach that several groups have taken is to integrate mapping with gene expression or population genomics. Other functional genomic data, such as chromatin immunoprecipitation of transcription factors or chromatin marks, could be also be overlaid on top of QTL and genetic variant data to aid the search for causative variants. Genetic variants and molecular genes that have been found through such methods can then be the starting point for molecular evolution studies that trace the evolution of ornaments through extant populations and phylogenetic lineages. Evolutionary studies of *desat* genes (Shirangi et al. 2009; Keays et al. 2011; Fang et al. 2009) in *Drosophila* provide tantalising examples.

A lot of the value of genomics comes from harnessing functional information about genes and pathways. While databases such as RefSeq, Ensembl, UniProt, the Gene Ontology Annotation project and many others are vast troves of information, much is still unknown about any given biological process. The path from molecular function to organismal trait is often uncharted. This complicates any inference that relies heavily on annotation derived from other species and contexts. Additionally, the final demonstration of any causative variant is experimental manipulation in transgenic organisms. Such molecular genetic tools take time and effort to develop and are not readily available for most species. However, these limitations are not specific to this field. Even human genomics has to rely on study systems such as laboratory mice for molecular testing of causative genes.

2.13 Conclusions

Sexual ornaments have long stimulated evolutionary research from genetical, ecological and theoretical perspectives. It appears that a multitude of mechanisms is at work in sexual ornamentation in different species and possibly within the same populations. Some ornamental traits have been revisited several times with different approaches over decades of work. Lately, genetic mapping and gene expression methods have been brought to bear on ornamentation and many quantitative trait loci have been mapped for different ornamental traits. However, going from quantitative trait loci to genes and molecular mechanisms is not an easy feat. There is good reason to think that future developments in genomics will improve the chances of isolating causative genes and help illuminate the basis of pleiotropy between ornaments and other traits.

References

Albertson RC, Powder KE, Hu Y, Coyle KP, Roberts RB, Parsons KJ (2014) Genetic basis of continuous variation in the levels and modular inheritance of pigmentation in cichlid fishes. Mol Ecol 23(21):5135–5150

Alem S, Streiff R, Courtois B, Zenboudji S, Limousin D, Greenfield M (2013) Genetic architecture of sensory exploitation: QTL mapping of female and male receiver traits in an acoustic moth. J Evol Biol 26(12):2581–2596

Baker RH, Morgan J, Wang X, Boore JL, Wilkinson GS (2009) Genomic analysis of a sexually-selected character: EST sequencing and microarray analysis of eye-antennal imaginal discs in the stalk-eyed fly *Teleopsis dalmanni* (Diopsidae). BMC Genom 10(1):361

Blount JD, Metcalfe NB, Birkhead TR, Surai PF (2003) Carotenoid modulation of immune function and sexual attractiveness in zebra finches. Science 300(5616):125–127

Blows MW, Chenoweth SF, Hine E (2004) Orientation of the genetic variance-covariance matrix and the fitness surface for multiple male sexually selected traits. Am Nat 163(3):329–340

Cornwallis CK, Birkhead TR (2007) Experimental evidence that female ornamentation increases the acquisition of sperm and signals fecundity. Proc R Soc B Biol Sci 274(1609):583–590. doi:10.1098/rspb.2006.3757

Fang S, Ting C-T, Lee C-R, Chu K-H, Wang C-C, Tsaur S-C (2009) Molecular evolution and functional diversification of fatty acid desaturases after recurrent gene duplication in *Drosophila*. Mol Biol Evol 26(7):1447–1456

Foley B, Chenoweth S, Nuzhdin S, Blows M (2007) Natural genetic variation in cuticular hydrocarbon expression in male and female *Drosophila melanogaster*. Genetics 175:1465–1477

Gleason JM, Ritchie MG (2004) Do quantitative trait loci (QTL) for a courtship song difference between *Drosophila simulans* and *D. sechellia* coincide with candidate genes and intraspecific QTL? Genetics 166(3):1303–1311

Gleason J, Nuzhdin S, Ritchie M (2002) Quantitative trait loci affecting a courtship signal in *Drosophila melanogaster*. Heredity 89(1):1–6

Gleason JM, Jallon J-M, Rouault J-D, Ritchie MG (2005) Quantitative trait loci for cuticular hydrocarbons associated with sexual isolation between *Drosophila simulans* and *D. sechellia*. Genetics 171(4):1789–1798

Gleason J, James R, Wicker-Thomas C, Ritchie M (2009) Identification of quantitative trait loci function through analysis of multiple cuticular hydrocarbons differing between *Drosophila simulans* and *Drosophila sechellia* females. Heredity 103(5):416–424

Hamilton WD, Zuk M (1982) Heritable true fitness and bright birds: a role for parasites? Science 218(4570):384–387

Hine E, Chenoweth SF, Blows MW (2004) Multivariate quantitative genetics and the lek paradox: genetic variance in male sexually selected traits of *Drosophila serrata* under field conditions. Evolution 58(12):2754–2762

Hughes KA, Houde AE, Price AC, Rodd FH (2013) Mating advantage for rare males in wild guppy populations. Nature 503(7474):108–110

Jansen R, Nap J (2001) Genetical genomics: the added value from segregation. Trends Genet 17 (7):388–391

Johns P, Wilkinson G (2007) X chromosome influences sperm length in the stalk-eyed fly *Cyrtodiopsis dalmanni*. Heredity 99(1):56–61

Johns PM, Wolfenbarger LL, Wilkinson GS (2005) Genetic linkage between a sexually selected trait and X chromosome meiotic drive. Proc R Soc B Biol Sci 272(1576):2097–2103

Johnsson M, Gustafson I, Rubin C-J, Sahlqvist A-S, Jonsson KB, Kerje S, Ekwall O, Kämpe O, Andersson L, Jensen P, Wright D (2012) A sexual ornament in chickens is affected by pleiotropic alleles at HAO1 and BMP2, selected during domestication. PLoS Genet 8(8): e1002914. doi:10.1371/journal.pgen.1002914

Johnsson M, Rubin CJ, Höglund A, Sahlqvist AS, Jonsson K, Kerje S, Ekwall O, Kämpe O, Andersson L, Jensen P (2014) The role of pleiotropy and linkage in genes affecting a sexual ornament and bone allocation in the chicken. Mol Ecol 23(9):2275–2286

Johnston SE, Gratten J, Berenos C, Pilkington JG, Clutton-Brock TH, Pemberton JM, Slate J (2013) Life history trade-offs at a single locus maintain sexually selected genetic variation. Nature

Keays MC, Barker D, Wicker-Thomas C, Ritchie MG (2011) Signatures of selection and sex-specific expression variation of a novel duplicate during the evolution of the *Drosophila* desaturase gene family. Mol Ecol 20 (17):3617–3630

Kerschnitzki M, Zander T, Zaslansky P, Fratzl P, Shahar R, Wagermaier W (2014) Rapid alterations of avian medullary bone material during the daily egg-laying cycle. Bone

Kottler VA, Fadeev A, Weigel D, Dreyer C (2013) Pigment pattern formation in the guppy, *poecilia reticulata*, involves the kita and csf1ra receptor tyrosine kinases. Genetics 194(3):631–646

Lander ES, Botstein D (1989) Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. Genetics 121:185–199

Limousin D, Streiff R, Courtois B, Dupuy V, Alem S, Greenfield MD (2012) Genetic architecture of sexual selection: QTL mapping of male song and female receiver traits in an acoustic moth. PLoS ONE 7(9):e44554

Mead LS, Arnold SJ (2004) Quantitative genetic models of sexual selection. Trends Ecol Evol 19 (5):264–271

Mougeot F, Irvine JR, Seivwright L, Redpath SM, Piertney S (2004) Testosterone, immunocompetence, and honest sexual signaling in male red grouse. Behav Ecol 15(6):930–937

Paaby AB, Rockman MV (2013) The many faces of pleiotropy. Trends Genet 29(2):66–73

Pizzari T, Birkhead TR (2000) Female feral fowl eject sperm of subordinate males. Nature 405:787

Pizzari T, Cornwallis CK, Lovlie H, Jakobsson S, Birkhead TR (2003) Sophisticated sperm allocation in male fowl. Nature 426:70–73

Risch N, Merikangas K (1996) The future of genetic studies of complex human diseases. Science 273(5281):1516–1517

Rowe L, Houle D (1996) The Lek paradox and the capture of genetic variance by condition dependent traits. Proc R Soc Lond B Biol Sci 263(1375):1415–1421. doi:10.1098/rspb.1996.0207

Rubin C-J, Brändström H, Wright D, Kerje S, Gunnarsson U, Schutz K, Fredriksson R, Jensen P, Andersson L, Ohlsson C, Mallmin H, Larsson S, Kindmark A (2007) Quantitative trait loci for BMD and bone strength in an intercross between domestic and wildtype chickens. JBMR 22 (3):375–384

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(1):18–30

Shaw KL, Lesnick SC (2009) Genomic linkage of male song and female acoustic preference QTL underlying a rapid species radiation. Proc Natl Acad Sci 106(24):9737–9742

Shaw KL, Parsons YM, Lesnick SC (2007) QTL analysis of a rapidly evolving speciation phenotype in the Hawaiian cricket Laupala. Mol Ecol 16(14):2879–2892

Shirangi TR, Dufour HD, Williams TM, Carroll SB (2009) Rapid evolution of sex pheromone-producing enzyme expression in *Drosophila*. PLoS Biol 7(8):e1000168

Slate J (2013) From Beavis to beak color: a simulation study to examine how much QTL mapping can reveal about the genetic architecture of quantitative traits. Evolution 67(5):1251–1262

Soller M, Brody T, Genizi A (1976) On the power of experimental designs for the detection of linkage between marker loci and quantitative loci in crosses between inbred lines. Theor Appl Genet 47(1):35–39

Taylor PD, Williams GC (1982) The lek paradox is not resolved. Theor Popul Biol 22(3):392–409

Tripathi N, Hoffmann M, Willing E-M, Lanz C, Weigel D, Dreyer C (2009) Genetic linkage map of the guppy, *Poecilia reticulata*, and quantitative trait loci analysis of male size and colour variation. Proc R Soc B Biol Sci 276(1665):2195–2208

Von Schantz T, Tufvesson M, Göransson G, Grahn M, Wilhelmson M, Wittzell H (1995) Artificial selection for increased comb size and its effects on other sexual characters and viability in *Gallus domesticus* (the domestic chicken). HEREDITY-LONDON 75:518–518

Webster L, Paterson S, Mougeot F, MARTINEZ-PADILLA J, Piertney J (2011) Transcriptomic response of red grouse to gastro-intestinal nematode parasites and testosterone: implications for population dynamics. Mol Ecol 20(5):920–931

Wenzel MA, Webster LM, Paterson S, Mougeot F, Martínez-Padilla J, Piertney SB (2013) A transcriptomic investigation of handicap models in sexual selection. Behav Ecol Sociobiol 67 (2):221–234

Wiley C, Shaw KL (2010) Multiple genetic linkages between female preference and male signal in rapidly speciating Hawaiian crickets. Evolution 64(8):2238–2245
Wiley C, Ellison CK, Shaw KL (2012) Widespread genetic linkage of mating signals and preferences in the Hawaiian cricket Laupala. Proc R Soc B Biol Sci 279(1731):1203–1209
Wilkinson GS, Presgraves DC, Crymes L (1998) Male eye span in stalk-eyed flies indicates genetic quality by meiotic drive suppression. Nature 391(6664):276–279
Willing E, Bentzen P, Van Oosterhout C, Hoffmann M, Cable J, Breden F, Weigel D, Dreyer C (2010) Genome-wide single nucleotide polymorphisms reveal population history and adaptive divergence in wild guppies. Mol Ecol 19(5):968–984
Wright D, Kerje S, Brändström H, Schütz K, Kindmark A, Andersson L, Jensen P, Pizzari T (2008) The genetic architecture of a female sexual ornament. Evolution 62:86–98
Wright D, Rubin CJ, Martinez Barrio A, Schütz K, Kerje S, Brändström H, Kindmark A, Jensen P, Andersson L (2010) The genetic architecture of domestication in the chicken: effects of pleiotropy and linkage. Mol Ecol 19(23):5140–5156
Wright D, Rubin C, Schutz K, Kerje S, Kindmark A, Brandström H, Andersson L, Pizzari T, Jensen P (2012) Onset of sexual maturity in female chickens is genetically linked to loci associated with fecundity and a sexual ornament. Reprod Domest Anim 47:31–36
Zajitschek SR, Evans JP, Brooks R (2006) Independent effects of familiarity and mating preferences for ornamental traits on mating decisions in guppies. Behav Ecol 17(6):911–916

http://www.springer.com/978-3-319-19931-3

Evolutionary Biology: Biodiversification from Genotype to Phenotype

Pontarotti, P. (Ed.)
2015, IX, 409 p. 68 illus., 40 illus. in color., Hardcover
ISBN: 978-3-319-19931-3