CHAPTER 27

Sexual system genomics and speciation

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27.1 In the beginning: Darwin and Wallace on sexual selection and speciation

The grand theory of natural selection that Charles Darwin and Alfred Wallace jointly proposed was meant to explain the origin and evolution of biological diversity among all living things. Both men shared a common vision on precisely how natural selection operated and, for the remainder of their lives, maintained a healthy connection that included a mutual appreciation and reciprocal endorsement of each other’s work. However, Darwin and Wallace remained unconvinced of the other’s selective explanations for two of nature’s most visible and widespread biological features: the presence of spectacular secondary sexual dimorphic characters, and the phenomenon of hybrid sterility that commonly occurs when plant and animal breeders cross closely related species.

Darwin explained the evolution of secondary and seemingly maladaptive sexual traits such as the peacock’s long tail (Fig. 27.1a) by sexual selection, i.e. sex-biased selection as the result of each sex possessing different strategies to increase their reproductive output (Darwin 1871). Darwin distinguished sexual from natural selection in order to explain how boldly dimorphic traits were the byproduct of selecting mates by females and outcompeting other males. In contrast, Wallace thought that the spectacular sexual dimorphisms found in nature could be best explained by natural selection for protective adaptation. For Wallace, sexual dimorphism in bird species with bright conspicuous male plumages evolved by gradually selecting for dull-coloration in females in response to predation pressure (Wallace 1890).

To explain why hybrid sterility occurs so frequently, Darwin and Wallace again evoked different models for selection to act upon. Since it would be counter-productive, in the context of a population’s overall fitness, for sterility to evolve within a species by natural selection, Darwin explained it as merely an incidental outcome of natural selection on diverging populations (Darwin 1859). Wallace, on the contrary, held that natural selection could increase the degree of sterility in hybrids, ultimately proposing a mechanism that is the precursor of what is now known as sympatric speciation and reinforcement.

It is interesting to note that disagreements still exist over the explanations of these two phenomena among evolutionary biologists, more than a century after Darwin and Wallace’s con genital correspondences. The genetic basis of hybrid sterility is a well-studied field of inquiry and a variety of explanations have been put forth (for a review, see Kulathinal and Singh 2008). Ecological and genetic studies of sexual selection now cover a full range of hypotheses from game theory to good-genes models (Shuster and Wade 2003). While it is most certainly true that neither Darwin nor Wallace comprehended the importance of hybrid sterility and sexual selection in the context of species formation, both these phenomena currently represent two of the most active areas of research in the field of speciation.
27.2 The Modern Synthesis and the development of speciation theory

After the Modern Evolutionary Synthesis of the 1930s and 1940s introduced species as reproductively isolated units (Dobzhansky 1937; Mayr 1942), post-Synthesis theories of speciation were based on two prevailing views in population genetics: that the genetic basis of most adaptive traits is complex and multigenic, and that gene flow is a powerful force of homogenization such that population differentiation cannot take place without geographical or temporal isolation (Dobzhansky 1951; Mayr 1963). Ernst Mayr’s allopatric geographic theory of speciation was built on the basis of these two assumptions. Mayr emphasized the importance of geographic isolation and considered reproductive isolation as a byproduct of genetic divergence in isolation, much like Darwin did a century earlier (Mayr 1963). Theodosius Dobzhansky, on the other hand, focused on the evolution of reproductive isolating mechanisms proposing the role of natural selection in their perfection (Dobzhansky 1937, 1951). While Wallace had argued for the importance of natural selection under sympatric conditions, Dobzhansky extended the role of natural selection during secondary contact between species as an additional stage in the allopatric model of speciation that allowed for the reinforcement of premating isolation (Dobzhansky 1951).
Two of the most fruitful approaches in understanding the genetics of speciation include the Mendelian analysis of hybrid incompatibility genes and expanding the role of sexual selection from morphological to molecular levels. Dobzhansky first made use of genetic crosses to investigate the genetic basis of hybrid sterility between species, employing two closely related fruit fly species, *Drosophila pseudoobscura* and *D. persimilis* (Dobzhansky 1937). By backcrossing fertile F₁ hybrids to either parent, he showed that the X chromosome produced the largest effect on hybrid male sterility. Not much was done for 40 years until a new generation of speciation geneticists resurrected the technique *en force* (Coyne and Orr 1989) to study the genetic basis of Haldane’s rule, i.e. the observation that hybrid inviability and sterility tend to occur more commonly in the heterogametic than homogametic sex (Haldane 1922). Observations from crosses between different species paved the way to new theoretical developments such as the faster-X (Charlesworth et al. 1987) and dominance (Orr and Turelli 1996) theories of hybrid incompatibilities. On the empirical side, a handful of genes affecting hybrid inviability and sterility have been identified (reviewed in Presgraves 2010). Indeed, the use of trans-species Mendelian genetics and its associated theoretical developments have taught us more about the genetic basis of speciation during the last 25 years than over the previous 150 years.

The second approach, pursued independently and in parallel, has allowed theories of speciation based on sexual selection and mating behavior to grow in scope (Fig. 27.1b,c). During the last three decades, formal mathematical models (e.g. Lande 1981; Kirkpatrick 1982) and empirical examples spanning a wide range of organisms (Arnqvist and Rowe 2005) have propelled the field of sexual selection into the forefront of evolutionary biology. An emphasis on classic male features can be seen in such early models as runaway selection which attempted to explain Darwin’s original paradoxic observation that outwardly maladaptive traits are the evolutionary consequence of increasing male fitness (Fisher 1930; Lande 1981). Later theories of sexual selection emphasized the benefits of female discrimination through direct resource-based advantages (Maynard Smith 1991), the indirect appraisal of males by the good-genes model (Andersson 1994), and coevolutionary arms conflict between male and female strategies (Rice 1996).

With new molecular evidence, a new and encompassing view of sexual selection has begun to materialize (Coulthart and Singh 1988; Civetta and Singh 1999; Swanson and Vacquier 2002). This broadened perspective allows mechanisms of sexual selection to be extended beyond classical examples of female choice and exaggerated male phenotypes that have been limited to male secondary sexual traits and extreme male behavior (Darwin 1871). Foremost, the extension of sexual selection to the molecular level finally presents a direct link to the genetics of speciation by providing a common functional pool of genes and traits. This systems-based framework enables us to understand the origin of species-specific characters alongside species formation and divergence. In addition, microevolutionary processes that are rapidly evolving can be viewed to directly impact macroevolutionary patterns of diversity.

### 27.3 A new paradigm: the genomics of sexual systems and the origin of species

Our view of how species form has changed dramatically over the past 150 years. Early theories of speciation were shaped more by population dynamics, i.e. how populations split in both space and time and ultimately become species, than by the kind of genes or genetic mechanisms involved. A shift in emphasis to studying sex and reproduction-related systems holds the potential of providing us with greater insight on how populations diverge and ultimately become reproductively isolated. Using empirical evidence from the literature, we briefly outline the following framework for this shift in paradigm with highlights from the recent literature: (1) genomes can be broadly organized into sexual and non-sexual functional components, (2) sexual systems are generally more variable and possess higher rates of evolutionary change, (3) sexual selection is generally strong and repetitive, (4) sexual coevolutionary forces and interacting systems can drive rapid evolution, and (5) reprodu-
tive systems are generally the first to break down in interspecific hybrids.

27.3.1 Functional genomics: organization into sexual and non-sexual systems

Sexual systems are comprised of genes involved in male and female fertility in addition to morphological and behavioral traits involved in sexual selection. By being directly or indirectly involved in some aspect of reproductive function, sex genes are prone to sexual selective pressures. In contrast, non-sex genes primarily affect viability and are subject mostly to natural selection. This binary classification provides a heuristic framework to treat reproduction and survival as separate but coupled evolutionary systems open to independent modification at different evolutionary rates (Singh 2000). Ultimately, the basis of this dichotomy has its origin in the concept of individual fitness. As integrated components of fitness, fertility and viability are difficult to tease apart, yet historically, the viability component has received disproportionate attention. From the handful of studies that quantify both components of fitness, fertility appears more prominent. For example, in a meta-analysis of studies from the literature where selection was quantified in the wild, fertility fitness components were found to be more significant than viability and sexual selection stronger than natural selection (Kingsolver et al. 2001). It was also found that sex-specific variance in reproductive success produces a much larger effect than differences between males and females in survivability (Charlesworth 2001).

Modern genomic tools provide growing support for a functional treatment of the genomic landscape. Genomic approaches can quickly assay for sex-biased gene expression on a genome-wide scale for both male- and female-specific tissues in addition to somatic ones. Microarray, EST (expressed sequence tag), and now RNAseq studies reveal that a surprisingly large fraction of the genome is involved in reproductive function. In *Drosophila*, upwards to one half of all genes are expressed in the male testis (Ranz et al. 2003; Parisi et al. 2004). In addition, testes harbor a much greater fraction of tissue-specific genes than other tissues (Singh and Kulathinal 2005) suggesting the availability of a large number of targets for sexual selection to act upon.

Of course, any dichotomous perspective that classifies genes, traits, and systems as sex versus non-sex may be too simplistic due to the multifunctional and pleiotropic nature of most genes and traits. A more realistic representation pictures somatic non-sexual roles at one end of the functional spectrum and purely reproductive roles at the opposite end. This functional grouping allows us to better visualize how different selective processes act upon each genetic system with respect to mechanism (Fig. 27.2: sexual vs. natural selection, positive vs. purifying selection, rapid vs. slow evolution).

27.3.2 Higher variation among reproductive systems

Mounting evidence reveals that traits involved in pre- and postzygotic mechanisms of isolation are evolving rapidly, suggesting that sex and

![Figure 27.2](image-url) The relative contribution of natural versus sexual selection on various genetic systems. The effectiveness (right-hand side triangles) of purifying selection (negative selection on deleterious characters) and positive selection (eventual fixation of advantageous variants) differs between systems since genes related to sex and reproductive allow for more latitude on an organism's genetic endowment. Also indicated are expected correlated differences in evolutionary rates.
reproduction-related (SRR) genes are preferentially involved in speciation (Singh and Kulatilaka 2000). Two pioneering studies of sexual systems set the stage for studies of reproductive characters and their association to speciation. William Eberhard's broad survey of animal taxa found male genitalia to be among the most disparate and diagnostic (Eberhard 1985). Of course, entomologists had distinguished related species on the basis of genitalia for centuries (for example, sexual characters were the basis for the Linnaean system of classification), however, Eberhard's comprehensive compilation of male gonadal morphology underscored the connection of high gonadal diversity to sexual selection. In the second pioneering study, Hampton Carson demonstrated that over a very short period of time (~6 million years), a founding population of fruit flies diverged to over 500 species across an expanding Hawaiian archipelago, and that much of the remarkable morphological diversity between species was driven by the evolution of behavioral preferences for conspecific mates in newly founded populations (Carson 1997; Fig. 27.1d). These two case studies point to the power of sexual selection driving the variability of both reproductive systems and species diversity.

Recent genomics studies are also finding that the genetic components of SRR systems are generally more liable to rapid evolutionary change. Various lines of evidence are summarized as follows: (1) among developmental programs, reproductive systems including sex determination (Hodgkin 1990), mating strategies (Shuster and Wade 2003), and floral pollination (Charlesworth et al. 2005; Cozzolino and Widmer 2005) are among the most rapidly evolving. (2) Reproductive genes show faster rates of sequence evolution (Civetta and Singh 1998; Wyckoff et al. 2000) and a more rapid loss of orthology (Haerty et al. 2007). Sex genes, by virtue of their non-ubiquitous expression and action, and not because of their dispensability (Torgerson et al. 2005), appear to be more open to mutational, selectional and neutral change than other genes. (3) Reproductive genes, particularly those expressed in the male testes, show higher rates of retention in the testis after evolving off the X chromosome onto an autosome via retroposition (Emerson et al. 2004). (4) Sexual systems are more likely to evolve novel genes (Dorus et al. 2008), thus, molecular diversity may be directly associated with morphological specialization in sexual systems. (5) Reproductive tissues exhibit greater variance in gene expression. Computational and microarray approaches using Drosophila point towards a complex set of transcripts (Telonis-Scott et al. 2009). Furthermore, in a large number of cases, ubiquitously expressed genes show significantly higher levels of expression in the testis when compared to ovary or somatic tissue (Parisi et al. 2004).

27.3.3 Strength of sexual selection

Why do SRR genes, traits, and systems tend to evolve faster? Male competition and female choice form the basis of Darwin's original hypothesis (Darwin 1871). Recent theories have focused on the antagonistic interaction (conflict) that arises between opposing male and female strategies. These mechanisms of conflict may be behavioral, morphological, or physiological. That females continually respond and coevolve to counter male strategies has been demonstrated in many taxa. In a now classic experimental evolution study, it was found that Drosophila females that were not given the opportunity to coevolve with males were less fit than females allowed to coevolve with those same males (Rice 1996). Such experiments reveal that what biologists actually observe in both nature and the laboratory is most likely the tip of the iceberg. Due to the coevolutionary and recurrent nature of sexual selection, the majority of directional change will most likely be cryptic. Furthermore, the cycle of inconspicuous change in the male's and female's sexual machinery does not cease to take place.

Empirically, the rate of speciation between groups of phylogenetically similar species of insects that differ in their capacity for sexual conflict has also been compared (Arnqvist et al. 2000). Species with multiple partners, allowing for sexually antagonistic strategies to develop, were compared to related species with monogamous mating systems. Results show that speciation rates are four times higher in species where conflict is present, providing evidence that sexual conflict is an important driver of evolutionary change, and speciation. While sexual conflict has become an important
topic in the field of speciation, the rapid evolution of reproductive systems presents an alternative to the metaphor of 'fitness conflicts.' In this view, reproduction and fitness do not belong to males or females alone but to both sexes together, and battles (interactions) between males and males, females and females, and males and females are all part of a broader form of sexual selection (known as broad-sense sexual selection; Civetta and Singh 1999).

A more recent theory explains rapid sexual systems evolution as the direct result of male sexual drive (Singh and Kulathinal 2005; also known as male-driven sexual selection) whereby all aspects of males are under intense sexual selection due to their leading role in initiating mating and doing everything possible—from molecules to morphology—to increase their chances of mating with females, and thus increasing their overall fitness. An important consequence of male sexual drive theory is how the genome itself becomes masculinized by sexual selection. In general, genomes appear to maintain a disproportionate number of reproductive genes, an influx of new male-specific genes, and a biased distribution of harbored male versus female genes on sex chromosomes (Singh and Kulathinal 2005). Female reproductive systems often coevolve with these male genes into a rapidly evolving trajectory.

27.3.4 Sexual systems interaction, coevolution, and rapid change

Using an expanded role of sexual selection, it is highly probable that we will find natural and sexual selection to often interact and reinforce each other’s effects. How often natural and sexual selection reinforce or oppose each other is an area of current interest. Selection on secondary sexual traits most likely affects survival. Similarly, natural selection on nonsexual traits, such as the shape and size of beaks in Darwin’s finches, may affect mate choice and reproduction through their effect on song (Podos 2001) (Fig. 27.1e). Since sexual systems are more closely allied with fitness, and are more prone to be affected by natural and sexual selection, they have the potential to evolve the fastest. Perpetual sexual interactions, coupled with inequalities in sex allocation, differences in selection intensity (due to males generally being more active and passionate), and sexual conflict in fitness interests, make sexual traits more liable to rapid, often exaggerated, and seemingly ‘maladaptive’ evolutionary change. The exaggerated tail length of peacocks and the wastage of reproductive gametes (such as the excessive production of pollen in plants and sperm in animals) most likely represent only a fraction of the maladaptive change with regard to reproduction in these organisms.

While pleiotropic effects across different genetic systems may yield greater selective pressure and constraint, inter-molecular coevolution between male and female components can generate rapid change. These components may include signaling systems such as pheromones and visual cues used in mating rituals as well as gametic interactions in fertilization. A growing number of intermolecular examples from the coevolution of sperm-egg proteins in marine invertebrates (Swanson and Vacquier 2002) to male-specific sperm accessory proteins and their targets in female Drosophila (Ram and Wolfer 2009) have been characterized.

The extension of sexual selection to beyond their original precopulatory scope also allows its inclusion in other taxa and processes that have not been part of traditional sexual selection literature. For example, reproductive mechanisms not aligned with the classic notion of female choice on male traits may provide a forum for sexual selection to prevail in plants. Because many angiosperms release pollen in massive amounts, pollination can be considered analogous to sperm competition (Delph and Havens 1998). Insect-plant pollination interactions may also be viewed to help orchestrate male-female coevolutionary interactions found in sexual selection models (for example, Fig. 27.1f). A growing number of examples between plants and animals are discovering such coevolving partners of rapid evolutionary change (e.g. Whittall and Hodges 2007). It is intriguing to consider that not only does strong and recurring selection maintain many of the dramatic sexual dimorphisms observed within species, but that it may also play a role in creating the complex web of species-to-species interactions in many ecosystems by evolving innovative new traits that are specific to a particular species and its partners.
27.3.5 Rapid breakdown of sexual systems in species hybrids

Darwin devoted an entire chapter in *Origins* to the evolution of hybrid sterility (Darwin 1859) and it is generally now observed that hybrid sterility evolves faster than hybrid inviability (Wu et al. 1996). This pattern contrasts the distribution of within-species mutations: in *Drosophila*, there are many more incidences of mutations affecting viability than sterility (Lindsley and Lifschytz 1972). This contrast suggests that the genetic basis of hybrid sterility is of a different nature than within-species sterility (Kulathinal and Singh 2008). Of course, the rapid evolution of reproductive genes may account for this pattern by generating a higher rate of deleterious interactions in the hybrid. In addition, sequence divergence is correlated to gene expression divergence as shown in *Drosophila* species hybrids (Artieri et al. 2007). However, the contrast of gene expression profiles between species and gene expression breakdown in their hybrids suggests that regulatory divergence in response to stabilizing selection alone may be capable of producing hybrid incompatibility. Indeed, the role of regulatory divergence as a result of stabilizing selection, at present, remains unappreciated.

Applying a sexual/non-sexual gene pool dichotomy also allows species concepts and competing genetic theories of speciation to be related directly to one or more components of genetic systems. The result of this sexual systems approach is that speciation theories may not be as different to each other as they appear. For example, as an explanation to species formation on small isolated island populations—observations that inspired Darwin on his seminal voyage on the *Beagle*—Mayr proposed founder (also known as peripatric) speciation models. Various founder effect models prominently feature sexual traits and sexual selection (Carson 1989). Non-founder models include those that employ the 'Recognition' species concept (Paterson 1985), which applies to only the subset of genes affecting species-specific sexual recognition signals and does not constrain the variation and evolution of genes affecting other functional components. Allopatric speciation with reinforcement and sympatric models also rely on a phase that involves homogamic mating to complete incipient reproductive isolation (Noor 1995). Table 27.1 summarizes the implications of a dichotomous gene pool concept to various speciation theories.

An understanding of sexual systems evolution may help resolve a host of controversies associated with the general problem of speciation. For example, the faster evolution of reproductive genes, along with the mechanics of dominance and hemizygosity, may provide a more unitary explanation of Haldane's rule (Haldane 1922) than the faster-male hypothesis by including cases in which the heterogametic sex is either male or female (Kulathinal and Singh 2008). The Dobzhansky-Muller model of reproductive incompatibility, representing the general framework used by speciation geneticists today, can be extended to include gene function (Fig. 27.2; Kulathinal and Singh 2008). For example, by introducing a simple binary parameter of sexual versus non-sexual function to loci in the Dobzhansky-Muller model, we may begin to envision a wider scope to the resolution of why hybrid sterility evolves faster than hybrid inviability. A focus on the study of sexual system genes may also offer new perspectives on other such phenomena as the evolution of sexual dimorphism, sex

27.4 Towards a post-genomics synthesis of speciation

Molecular studies of sexual systems are providing a general framework to explore genetic mechanisms of speciation among sexually reproducing organisms. By categorizing genes according to their function, the relative roles of natural versus sexual selection, as well as the importance of purifying versus positive selection and their associated evolutionary rates among functional classes, can be established (Fig. 27.2). As we extend our approach to studying speciation from a Mendelian one to a genomics one, it will be exciting to see how different genomes and transcriptomes from a range of species have evolved depending on such biological parameters as mating strategies, the strength of sexual isolation, as well as the genetic bases of evolved reproductive networks.
allocation, life-history traits, and the maintenance of sex (Singh and Artieri 2010).

27.5 Future prospects: sex as a major force in evolution

Molecular and evolutionary genomic studies of sexual systems are beginning to connect two of Darwin’s greatest theories: sexual selection at the microevolutionary level, and species formation at the macroevolutionary level. Functional genomics provides a direct way to sort through which selective mechanism—natural or sexual—a gene or trait will be most prone to be acted upon. At present, genetic systems remain poorly characterized. For example, how pleiotropic are genes in sexual versus non-sexual systems? What proportion of genes from each genetic system has evolved de novo? What epigenetic landscape does a typical gene lie in? At a higher level of genetic organization, how different are each of the systems’ networks in terms of size, robustness, redundancy, and degree of epistasis? Further experiments and exploration of these systems will inform us to whether sexual systems have indeed different intrinsic properties, and how these properties would impact the evolution of reproductive traits as well as incompatibilities in the hybrid.

In a historical context, the Evolutionary Synthesis that occurred over half a century ago furnished the first unification of biological principles (e.g. Dobzhansky 1937; Mayr 1940). Sexual system genomics and a molecular re-appraisal of sexual selection are offering a new perspective: while natural selection is responsible for survival and maintenance of multitude of characters, sexual selection is primarily responsible for some of the most spectacular aspects of organismal diversity, including sexual dimorphism and possibly the origin of species. Subsuming sexual selection under natural selection, although technically correct, tends to rob us of the rich vista spanned by the evolutionary dynamics of sexual and reproductive systems. Studying the genomic consequences of rapidly evolving sexual systems at both the organismal and molecular levels holds much promise in our quest to understand one of the greatest of all mysteries: the origin of species.

References


