Sexual selection and mate choice

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The past two decades have seen extensive growth of sexual selection research. Theoretical and empirical work has clarified many components of pre- and postcopulatory sexual selection, such as aggressive competition, mate choice, sperm utilization and sexual conflict. Genetic mechanisms of mate choice evolution have been less amenable to empirical testing, but molecular genetic analyses can now be used for incisive experimentation. Here, we highlight some of the currently debated areas in pre- and postcopulatory sexual selection. We identify where new techniques can help estimate the relative roles of the various selection mechanisms that might work together in the evolution of mating preferences and attractive traits, and in sperm–egg interactions.

Introduction

Twenty years ago, when sexual selection was reviewed in the first issue of TREE [1], it was a rapidly growing field. Darwin’s idea of female preferences for male ornaments was still controversial, although his theory had received new support from two directions. First, empirical studies showed that male ornaments are favoured by female choice in some fishes and birds [1]. Second, a major problem left open by Darwin, the reasons why females prefer ornamented males, was clarified when genetic models [2,3] verified the logical coherence of Fisherian self-reinforcing coevolution of male ornaments and female preferences (Box 1).

These results posed as many interesting questions as they answered, and inspired much new research. In the two decades since 1986, sexual selection theory has been corroborated [4] and enriched with exciting new ideas and discoveries, some of which we highlight here. We also point to new possibilities for testing genetic mechanisms of sexual selection in the era of functional genomics. Genetic analyses of sexual selection by mate choice have worked so far on a mainly top-down basis, inferring genetic causes from phenotypic patterns, based on few-locus genetics or quantitative genetics theory [5]. These remain excellent tools for the analysis of preference–display coevolution and for other purposes in sexual selection research. However, quantitative trait locus (QTL) identification and sequencing combined with functional genomics now provide the opportunity for bottom-up approaches, based on the precise characterization of genes and their effects, from DNA sequences via protein to phenotypic expression at the level of the individual, with possible consequences at the population level and above.

Evolution of mate choice

Although mate choice occurs in males and females [4], for convenience we refer here to female choice of male traits. As experimental evidence accumulated, mate choice became widely recognized, but the genetic mechanisms underlying its evolution remain the subject of debate (Box 1). Showing how mating preferences evolve genetically is harder than showing that they exist, and the problem is aggravated by the possibility that several mechanisms co-occur (Box 1). Moreover, conflicts between the sexes can add further selection pressures on preference and the preferred trait [6,7]. Opinions differ over the relationships between Fisherian and genetic indicator mechanisms of mate choice (e.g. [5,8–13]). Given that there are qualitative differences between them, we think there are good reasons to keep the distinction clear [9,11,12].

Costs of mate choice, such as the loss of energy and time, might prevent Fisherian self-reinforcing coevolution of the trait and the preference for it, but need not do so; the outcomes of models depend on the details of several assumptions, for instance about sex linkage [10] (reviewed in [5]). In spite of being theoretically plausible, the Fisherian genetic mechanism has been difficult to demonstrate empirically. There is corroborating phenotypic evidence (e.g. [14,15]), and this is also the case for indicator mechanisms (e.g. reviewed in [5,11,16]). These and other mechanisms (Box 1) are compatible and might co-occur (e.g. [4,12]), and thus a challenging task is to gauge their relative roles. Estimating the effects of the Fisherian sexy son mechanism might help us to decide whether it explains why male ornaments and displays are often extreme, and differ more than do other traits between closely related species, apparently evolving rapidly and perhaps being involved in speciation [2,17,18]. These questions have been with us since Charles Darwin’s time and have yet to be answered satisfactorily.

Testing the evolution of trait and preference

Molecular genetic and genomic tools enable the detailed characterization of genes and their effects (e.g. reviewed in [19–21]). Combined with selection experiments that quantify genetic evolution over generations, they offer tools for detailed genetic analyses and tests of whether...
Box 1. Mechanisms of mate choice evolution

Several mechanisms have been put forward to explain mate choice: (i) Direct phenotypic effects. Female preference for a male ornament can evolve as a result of direct phenotypic benefits if the ornament reflects the ability of the male to provide material advantages, such as a high-quality territory, nutrition, parental care or protection. There is considerable empirical support for this mechanism [76]. Female choice might also evolve as a result of resistance to direct costs imposed by males [7,38].

(ii) Sensory bias. Female preference favouring a male ornament can initially evolve under natural selection for other reasons, for instance in the context of foraging or predator avoidance (e.g. [22,77]). Males evolving traits that exploit this bias then become favoured by mate choice. There is increasing phenotypic evidence that some male ornaments initially evolved through female sensory biases [22,77,78], but the evolution of female sensory bias itself requires more testing [12].

(iii) Fisherian sexual selection. If there are genetic components to variance in female preference and male trait, a female choosing a male with a large trait bears daughters and sons that can both carry alleles for a large trait, and for the preference for it. This genetic coupling might lead to self-reinforcing coevolution between trait and preference [2,3,5,10,12,17]. Direct critical testing of this mechanism is difficult, but molecular genetics offers new possibilities (see main text).

(iv) Indicator mechanisms. ‘Good genes’ or ‘handicap mechanisms’) suggest that attractive male traits reflect broad genetic quality. Inherent in such mechanisms is the maintenance of genetic variation, the ‘paradox of the lek’, and parasite- and pathogen-mediated mechanisms have been suggested as potential solutions (Box 4 [79,80]). In addition, other advantageous genes and relative freedom from deleterious mutations might lead to high male condition and expression of sex traits (e.g. [11,81–83]). Female preference for such traits can provide genetic benefits to those of her offspring that inherit favourable alleles from their father (e.g. reviewed in [5,11,13,84], but see [85]). The resolution of the lek paradox remains a crucial area for sexual selection research.

(v) Genetic compatibility mechanisms. As well as additive genetic benefits reflected by indicator traits, traits might be non-additive benefits from choosing a mate with alleles that complement the genome of the chooser [37,65–88,84]. Examples have been found for instance in major histocompatibility complex genes, and compatibility advantages might be one adaptive reason for multiple mating by females (see main text).

The evolution of mate choice is based either on direct selection of a preference that gives a fitness advantage (mechanisms i–ii) or on indirect selection of a preference as it becomes genetically correlated with directly selected trait [mechanisms iii,iv] [4,5,10,12]. In addition, rather than favouring any particular display trait, mate choice might evolve because it conveys non-additive genetic benefits (mechanism v) [65,66]. These mechanisms are mutually compatible and can occur together, rendering the evolution of mating preferences a multiple-causation problem, and calling for estimation of the relative roles of individual mechanisms [4]. Several diagnostic differences among the mechanisms suggest ways in which they can be tested by quantitative genetic analyses [5,12].

Bottom-up genetic testing

One possible approach to testing is to identify, sequence, and characterize in functional detail a gene locus with several alleles that influence the expression of an attractive male trait, and to do likewise for the corresponding female preference. Although there are many difficulties, a combination of molecular genetic tools can make such analyses feasible in suitable model organisms (Box 2). Chromosomal regions hosting genetic variation with substantial influence on the expression of trait or preference can be identified by QTL analysis [19,25], followed by nucleotide characterization of the alleles at the loci. Techniques such as DNA microarrays, which can detect expression-level polymorphism, are useful in gene hunting for variable loci that influence the phenotypic traits of interest (e.g. [19,21,26]), as are candidate gene approaches [20,26].

If several alleles can be sequenced that encode for different degrees of expression of the trait, and likewise for the preference, genetic tests of the Fisherian process might be possible, for example by finding out whether alleles for trait and preference coevolve as predicted. Selective breeding over several generations from those males with greatest (or smallest) trait expression could show whether and how preference alleles coevolve with trait alleles, and vice versa. In combination with microsatellite analysis of parentage, it might also be possible to measure mating and fertilization success, and to quantify over several generations the Fisherian sexy sons advantage in relation to male trait size.

How can the genetic effects of Fisherian and indicator processes be distinguished in such experiments? In species where only males express the attractive trait and do not provide parental care or other phenotypic resources, daughters can be tested for genetic viability effects that are correlated with the indicator trait of their father, to find out whether it reflects a viability advantage, which is not predicted by the Fisherian mechanism. Maternal half-sib designs [27] can be particularly useful. Experimental designs could also be used where Fisherian mating advantages are prevented, enabling a male to mate only with a single female. There are other possibilities; for example, if an attractive male trait is also expressed to some extent in females, although at a cost, the added effect of intralocus sexual conflict [7] might
analyses have identified loci that cause variation in
available (also see[28]). For example, in
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be estimated by measuring the fitness effect of the trait in
females as well as in males.
Prospects for success in such tests seem best in model
species (Box 2), where much genetic information is already
available (also see [28]). For example, in Drosophila, QTL
analyses have identified loci that cause variation in
courtship traits, such as male song and female mate
recognition [29,30]. Ecologically interesting phylogenetic
relatives of genomic model species could also be used by
taking advantage of a candidate gene approach, based on
knowledge of the model species [19,20], because gene
function is sometimes phylogenetically conserved. For
instance, information from the genome databases of
pufferfish Tetraodon nigroviridis and zebrafish Danio
rerio could be useful in searching for trait and/or
preference loci in two frequent targets of sexual and
natural selection analyses, the guppy (e.g. [23,31–35,
Box 2] and the threespined stickleback (e.g. [24,27]).

Mate choice and sexual conflict
The importance of conflict between the sexes is becoming
increasingly clear through a flood of exciting recent
research [7]. Early insights of Williams [36], Trivers [37]
and, especially Parker [6], have recently received much
support, and it now appears that sexual conflict between
males and females is the rule rather than the exception,
sometimes leading to sexually antagonistic coevolution
(e.g. [38,39]).

In terms of mate-choice evolution (Box 1), sexual
conflict can impose direct selection on the female
preference that leads to increased female resistance to
the male trait, because its consequences are negative for
females [40]. Many aspects of sexually antagonistic
coevolution, such as its co-occurrence with indirect
selection on mate choice (Box 1), are debated (e.g.
[7,9,13,38,39,41]). Combinations of genetic and phenotypic
approaches might help resolve these issues (reviewed
in [7]).

Mate choice or paternity choice?
One of the main advances in sexual selection theory since
Darwin was the realization by Parker [42] that selection
can continue after copulation. If a female mates with more
than one male, there will be competition among sperm of
different males to fertilize available ova. In the 35 years
since Parker’s paper, sperm competition has become a
major branch of sexual selection research [43].

Initially, the evolution of traits such as male genital
morphology and ejaculate size were attributed to sexual
selection for male engagement in sperm competition.
Traits such as mating plugs, seminal fluid peptides that
influence female reproductive physiology, and postcopia-
lar mate guarding, were interpreted as male adaptations
for the avoidance of sperm competition. Multiple mating
by females is a prerequisite for sperm competition, yet it is
only in the past decade that postcopulatory sexual
selection has been considered from the female perspective
[44,45]. Bateman [46] suggested that female fitness is
maximised by mating with one or a few males. However,
this view is now shifting rapidly to one in which females
are thought to often obtain direct phenotypic and indirect
benefits from multiple mating [47]. Whereas
sperm competition is viewed as the postcopulatory
equivalent of male contest competition, the corresponding
equivalent of female choice is referred to as cryptic female
choice, because we are unable to observe directly female
preferences for sperm from particular males. Rather, they manifest as biases in paternity (Box 3).

Thus, mate choice by females can be viewed as the first step in the process of paternity choice. The incorporation of female perspectives into postcopulatory sexual selection has generated theoretical models for the evolution of multiple mating by females that in some ways parallel the mechanisms of mate choice evolution in Box 1. For example, the ‘sexy-sperm’ hypothesis parallels the Fish-erian sexy sons mechanism in proposing that multiply mating females will have sons with high fertilization success, because they inherit traits that caused high fertilization success for their fathers. These traits can include genital morphology, number and/or quality of sperm, or seminal fluid composition [48].

Similar to Fisherian sexy sons, the evolutionary potential of the sexy-sperm mechanism depends on the genetic architecture of traits that contribute to fertilization success [49]. Nevertheless, it has been argued that such a Fisherian mechanism might underlie the rapid and divergent evolution of male genital morphology, if females are more likely to store and use sperm from males able to provide the appropriate genital stimulation during copulation [50]. Under this scenario, male genitalia, traditionally viewed as primary sexual traits, can become postcopulatory equivalents of the peacock’s train, questioning the utility of a distinction between primary and secondary sexual traits. There is increasing experimental support for such a notion [51,52].

The ‘good-sperm’ hypothesis suggests that the ability of a male to gain high fertilization success is correlated with his underlying genetic quality, so that males successful in sperm competition sire offspring with generally high viability [53]. The good-sperm hypothesis in this respect parallels indicator mechanisms for the evolution of male sexual ornaments. Male sexual ornaments subject to precopulatory sexual selection are often condition-dependent indicators, developing in proportion to male condition [11,16]. Perhaps directional postcopulatory sexual selection might likewise favour condition-dependent traits that influence paternity and thereby provide indirect genetic benefits for offspring (Box 4). There are few tests of this ‘good-sperm’ hypothesis, but the males of bulb mites [54] and dung flies [55] that are most successful in sperm competition sire offspring of higher fitness, and quantitative genetic studies of traits thought to be important in sperm competition suggest condition dependence in a beetle species [56].

Interacting pre- and postcopulatory choice
Although theoretically possible, there is currently little evidence that sperm competition and/or cryptic female choice can amplify precopulatory mate choice. Suggestive evidence comes from studies of guppies in which males with more orange body colouration, a trait subject to precopulatory female choice, have a paternity advantage following artificial insemination of females with equal numbers of sperm from two competing males [32]. Given that females had not met the sperm donors in these experiments, the most parsimonious explanation seems to be that males of intrinsically high quality have attractive sexual traits and also produce highly competitive sperm.
(but see Box 3). Later behavioural experiments found that females accept more sperm from attractive males [33]. Both sperm competition and cryptic female choice might amplify precopulatory sexual selection in guppies. Measurement of the relative sexual selection intensities during pre- and postcopulatory phases of paternity choice seems a worthwhile endeavour, yet has rarely been attempted [57,58].

Molecular genetic studies of parentage have revolutionised our views of avian mating systems, and multiple mating by avian females is known to result in widespread and highly variable rates of extra-pair paternity (EPP) [59]. In several species, EPP is most common in broods of females whose social mate is relatively unattractive and/or of lower social status in precopulatory sexual selection [59,60]. If females engage in extra-pair copulations to trade up from their social mate and maximize offspring fitness, we might expect pre- and postcopulatory mate choice to work synergistically in favouring the evolution of male sexual ornaments. Comparative evidence suggests that sexual dichromatism correlates with rates of EPP across bird species, particularly with respect to structural colours used in sexual display [61]. But this relationship can also be explained by females timing their copulation behaviour to ensure that offspring are sired by extra-pair males. Thus, patterns of EPP might reflect precopulatory choice of extra-pair males rather than postcopulatory mate choice. An exciting new avenue for research on EPP in birds would be to explore correlations between male display traits and the fertilization capacity of their sperm in controlled experiments, to determine the relative importance of extra-pair copulations compared with postcopulatory processes in determining EPP. Artificial insemination techniques offer a promising approach.

Postcopulatory sexual selection can attenuate precopulatory sexual selection. It is now recognized that, counter to Bateman’s principle [46], the reproductive success of a male is sometimes limited by the number of sperm that he can produce. Males who are successful in precopulatory sexual selection can have higher costs of sperm production and become sperm depleted, so that female fertility is sperm limited, and successful males can suffer reduced competitive fertilization success [62–64]. Moreover, one of the putative genetic benefits of postcopulatory female choice is the avoidance of genetic incompatibility between maternal and paternal haplotypes, so that the preferred sire of a female depends on her own genotype [65,66]. Thus, although attractive males might gain more copulations, their paternity can be reduced among females with whom their genes are incompatible. As a result, the strength of sexual selection on male ornamental traits can be reduced [67]. Integrating the effects of precopulatory female choice of males carrying good genes with postcopulatory choice of compatible genes is a new challenge in mate choice evolution [68].

Postcopulatory sexual conflict
Despite an early focus on male perspectives in sperm competition, Parker [42] recognized that females are not inert environments in which male adaptations to sperm competition arise. Traits such as male genital morphology, or accessory gland products that enhance male fitness in sperm competition, can impose costs on female fitness [69] and generate antagonistic coevolution between the sexes (e.g. [7,38,39]). Molecular genetic and genomic tools are now being used to clarify the proximate mechanisms of potentially strong postcopulatory sexual selection and conflict, for example by identifying gene loci and proteins of Drosophila seminal fluids. These proteins induce changes in the female that are beneficial to the male, such as increased egg laying and decreased receptivity to other males. Some of the substances are, however, harmful to and reduce life span in females, and so are generating selection via sexual conflict (reviewed in [7,70]). Nonetheless, such harmful male adaptations might also evolve via a Fisherian sexy sons process, if the net fitness benefit to the female from producing harmful sons outweighs the costs of harm to her (e.g. [41]).

Many male and female reproductive proteins involved in sperm–egg recognition and fertilization evolve rapidly, and differ strongly between closely related taxa (reviewed in [7,18,71]). For example, among 987 genes expressed at different times during mouse spermatogenesis, protein evolution was fastest for testis-specific genes expressed during late stages of sperm maturation and involved in sperm–egg interactions [72]. Although the mechanisms are not yet known, many of these genes show evidence of positive selection [72], as expected if there is sexual selection leading to rapid coevolution of male and female reproductive proteins [7,72]. Genetically engineered lines of Drosophila enable the estimation of the relative strengths of selection via antagonistic coevolution and indirect genetic benefits to females through their sons [73–75]. These studies illustrate how combinations of behavioural, biochemical and genetical experiments can produce further insights into sexual selection and conflict and their potential roles in speciation [18,40].

Conclusions
Twenty years on we no longer think of sexual selection simply acting on male sexual ornaments. Traits as diverse as behavioural and structural displays, genital morphology and reproductive proteins can be subject to a multitude of selection pressures imposed by mate choice. The genetic mechanisms of pre- and postcopulatory sexual selection are still far from being fully understood, but molecular genetic and genomic tools enable their detailed experimental testing. This goal is likely to be achieved first in model organisms with sequenced genomes, or in some of their phylogenetic relatives. Progress will not be quick and easy, but we would not be surprised if the 40th anniversary issue of TREE is able to review detailed genetic clarification of Fisherian, indicator, mate compatibility, sperm competition, cryptic female choice, sexual conflict and other mechanisms of mate choice evolution. We thus look forward to the future of sexual selection with much curiosity and expectation.

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