POSTCOPULATORY SEXUAL SELECTION

Timothy R. Birkhead* and Tommaso Pizzari‡

The female reproductive tract is where competition between the sperm of different males takes place, aided and abetted by the female herself. Intense postcopulatory sexual selection fosters inter-sexual conflict and drives rapid evolutionary change to generate a startling diversity of morphological, behavioural and physiological adaptations. We identify three main issues that should be resolved to advance our understanding of postcopulatory sexual selection. We need to determine the genetic basis of different male fertility traits and female traits that mediate sperm selection; identify the genes or genomic regions that control these traits; and establish the coevolutionary trajectory of sexes.

EVOLUTION OF SEX

SEXUALLY ANTAGONISTIC GENE
A gene whose expression or the effect of whose expression is beneficial in one sex but harmful in the other.

*Department of Animal and Plant Sciences, University of Sheffield, Sheffield S10 2TN, UK. ‡Section of Ethology, Department of Animal Environment and Health, Swedish Agricultural University, Skara, PO Box 234, SE-532 23, Sweden. Correspondence to T.R.B. e-mail: t.r.birkhead@sheffield.ac.uk

Sexual selection is the evolutionary process that favours the increase in frequency of genes that confer a reproductive advantage. Darwin[1] thought of this process as exclusively precopulatory because he assumed females to be sexually monogamous. Only in the past 30 years has it become apparent that females are far from monogamous, and more recently it has been shown that females of many species actively seek multiple copulation partners[2,3]. Female promiscuity, or polyandry, has important biological implications: it means that sexual selection persists after copulation up to the point of fertilization, and in some cases beyond[4]. Postcopulatory sexual selection comprises both male–male competition in the form of sperm competition, and cryptic female choice. Sperm competition is the competition between the sperm of different males to fertilize the ova of a female[5–7]. Cryptic female choice is the ability of a female to bias the fertilization success of the males that copulate with and inseminate them[8]. It is 'cryptic' because the choice takes place hidden in the female reproductive tract. Both forms of postcopulatory sexual selection create powerful, if subtle, evolutionary forces, and have important consequences at both a population level and a molecular level. For example, the fact that female promiscuity results in offspring of mixed paternity is likely to affect gene frequencies over generations, the genetic diversity of a population and the fate of additive genetic variation (for example, REF. 9). In addition, because the maternal genome in the offspring of promiscuous females combines with the genomes of several males, there are important implications for sibling conflict[10] and for the evolution (and the study) of maternal genetic (including within-genotype and among-genotypes epistasis[11]) and environmental[12] effects. Postcopulatory sexual selection, arising from sexual promiscuity, is also a potent broker of rapid molecular evolution, high inter-sexual specialization and population divergence. In particular, post-insemination sexual selection can create the potential for conflict between the sexes, favouring the spread of SEXUALLY ANTAGONISTIC GENES, the sex limitation of their expression and sex-biased control of their transmission.

In this review, we discuss, first, the causes and consequences of male and female promiscuity; second, male aspects of postcopulatory sexual selection (sperm competition); and third, female aspects (cryptic female choice). We finish by identifying some of the major genetic and evolutionary issues still to be resolved in this active area of research.

Sexual promiscuity

Evolutionary biologists have examined the issue of promiscuity primarily from an adaptive standpoint,

Box 1 | **Potential benefits of polyandry**

The benefits to females of copulating with more than one male are usually divided into direct (those that a female obtains for herself) and indirect (benefits that a female obtains for her offspring).

Direct benefits are uncontroversial, and include the following:
- nutrient acquisition through courtship or nuptial feeding, in which females trade food for copulations;
- fertility benefits — for example, adequate sperm supply is a common reason for female polyandry among insects[106,107];
- change in partner, in which females use copulations as part of pair formation to obtain a new (and better) partner;
- reduced risk of sexual harassment.

Indirect benefits include the following:
- offspring diversity — the production of genetically diverse offspring;
- offspring attractiveness — females copulate with several males and the most attractive sperm fertilize her eggs and produce sons with attractive sperm (see also BOX 3);
- offspring viability — females copulate with several males and the sperm from the most viable male fertilize most eggs and produce viable offspring;
- compatibility between sperm and either the female reproductive tract or the ova — females copulate with several males to find the most genetically compatible sperm/partner.

Indirect benefits remain controversial because the underlying genetic (and physiological) mechanisms remain unclear.

asking why it is advantageous for each sex to have several copulation partners during a single reproductive cycle. The answer for males is obvious: ever since Bateman's classic *Drosophila* experiments[13], it has been clear that the more females a male inseminates, the more offspring he fathers, and the greater is his reproductive fitness. The benefits of promiscuity to females are less obvious, and until relatively recently it was assumed either that females were coerced into copulating with multiple males or that female promiscuity was a non-adaptive by-product of the positive selection for promiscuity genes in males[14] (but see also REF. 15), and so females had little to gain. More recently, it has been shown that females actively seek inseminations from several males, indicating that they might benefit from doing so[16]. Sexual selection is assumed to operate more intensively on males than on females because the reproductive potential of males is so much greater than that of females[6,17–19]; nevertheless, it is now clear that this does not preclude the possibility that females benefit from multiple copulation partners. These benefits might be direct or indirect (genetic) (BOX 1).

Sperm competition has its origins in the evolution of sex itself[20]. If gametes had originally shown a normal distribution of sizes, selection would have been disruptive, favouring either large or small ones and the fusion of unlike types. Small, highly mobile gametes, which could be produced in large numbers, had a competitive advantage in seeking and fertilizing larger ones that, in turn, were immobile but had large energy stores that facilitated their survival[20]. Small gametes are what we now call sperm, and the individuals that produce them are defined as males; the large

ANISOGAMY
The condition in which the male and female gametes are of different sizes.

ones are ova or eggs, and they are produced by females. In other words, sperm competition had a fundamental role in the evolution of the state of ANISOGAMY and the evolution of the sexes. Sperm competition has persisted ever since, in both externally and internally fertilizing species, and is now recognized to be almost ubiquitous across the animal kingdom[3]. Its botanical equivalent, pollen competition, is also widespread[21]. Sperm competition results in conflicting selection pressures on males, simultaneously favouring both the ability to usurp any sperm previously inseminated by other males, and also the ability to prevent any female they inseminate from being inseminated by other males[6]. These opposing aspects of sperm competition, referred to as 'offence' and 'defence', are nicely illustrated by *Drosophila* species. When male fruitflies inseminate females they do so with sperm in a cocktail of seminal substances that include prohormones, peptides and modification enzymes, which are released from the accessory glands of males. Among their other functions, these substances deactivate sperm that are already stored in the reproductive tract of the female (offence), and act as an anti-aphrodisiac, discouraging the female from copulating with other males (defence)[22]. Sperm competition therefore results in intense male–male competition and strong selection on male fertilizing ability. Sperm competition also creates potential for the evolution of the ability of females to discriminate between the ejaculates of different males (cryptic female choice) and generates a conflict between individual partners over which male fertilizes the ova of a female. In mating systems in which females can be coerced into mating, most females will typically be inseminated by several males, precluding any overt mate choice by females, and so favouring females that can discriminate between, and manipulate the ejaculates of, different males. As soon as females adapt to restore some control over who fertilizes their ova, there will be selection on males to evolve counterstrategies. In addition, sperm competition can result in the evolution of traits that increase male fertilizing efficiency at the expense of female fitness, thereby creating an evolutionary conflict between the sexes. Evolutionary biologists are now interested in understanding the underlying mechanisms of insemination, sperm use and fertilization, as well as the evolutionary implications of sexual conflict. For example, an extensive expressed sequence tag (EST) screen has identified many of the accessory gland products (Acps) produced by male *Drosophila melanogaster* and *Drosophila simulans*. One of the identified Acps, Acp26Aa, has been shown to stimulate female ovulation, possibly by acting at the base of the female ovary, where changes in exocytosis and levels of signalling molecules occur after insemination[23]. Increasing the number of eggs produced by a female might increase male reproductive success by diluting the intensity of sperm competition. However, increasing egg production beyond an optimal level might reduce female longevity and ultimately lower her lifetime reproductive

Box 2 | **Post-insemination sexual selection and speciation**

Post-insemination sexual selection drives the evolution of many male and female reproductive traits. Because a certain degree of cooperation between partners is required in sexual reproduction, the evolution of sexual traits in one sex typically triggers an evolutionary response in the other, generating a process of inter-sexual coevolution that leads to increased inter-sexual specialization. In addition, the discrepancy in the phenotypic optima of males and females can generate antagonistic coevolution between the sexes, whereby the spread of alleles that allow one sex to approach its phenotypic optimum by driving away the other sex from its own optimum are counteracted by the spread of alleles with opposite effects. One consequence of inter-sexual coevolution might be reproductive isolation between populations, resulting in prezygotic isolation, population divergence and, ultimately, speciation.

   The extent to which inter-sexual coevolution is a catalyst of speciation is the subject of much current debate[108–112]. Theoretically, males are expected to gain from promiscuity and it is therefore in their evolutionary interest to maintain gene flow between populations, but inter-population mating might also involve some fitness costs. For example, it could break up mechanisms of sex limitation of sexually antagonistic alleles or result in the production of less viable offspring[109]. Because females typically invest more than males in individual gametes, females are expected to gain from reproductive isolation, and female reproductive strategies might contribute to the promotion of speciation. Therefore, the potential for speciation generated by post-insemination sexual selection is partly dependent on the magnitude of inter-sexual conflict and on which sex has more control over reproduction at any given stage of the coevolutionary process between males and females of a population[110–112]. Two lines of evidence are consistent with the idea that post-insemination sexual selection fosters speciation: (i) the relatively rapid molecular evolution of traits targeted by post-insemination (for example, accessory gland products in *Drosophila* spp.[84,108] and sea urchins[113]), and (ii) the relatively high speciation rate of CLADES, in which the potential for post-insemination sexual selection is quite high[114–116].

success[24,25]. One outcome of research in this field is that many previously unexplained behavioural, physiological or anatomical traits now make evolutionary sense in the light of sperm competition and/or cryptic female choice and the process of inter-sexual coevolution that these generate. For example, the promiscuous tortoise beetle *Chelymorpha alternans* has an extraordinarily long penis, and the female has an extraordinarily long and convoluted spermathecal duct: it has been proposed that such extreme features evolved in response to continuing sexual conflicts as each sex attempted to retain control over fertilization[8]. Such coevolution between the sexes results in the rapid evolution of extreme traits[26,27] and might drive extreme specialization in the opposite sex, leading to population divergence, reproductive isolation and, ultimately, speciation (BOX 2).

Sperm competition

An early prediction of sperm competition theory was that, in species or situations in which sperm competition was intense, it would pay males to produce ejaculates that contain more sperm[5,6]. The subsequent discovery that, across a wide range of taxa including mammals, birds, butterflies and fish, species that experience more sperm competition had relatively large testes is entirely consistent with this theory[3]. Theory also predicted that because males of all species experience sperm depletion,

CLADE
A lineage of organisms or alleles that comprises an ancestor and all its descendants.

PROTURANS
(or Protura). Minute soil-inhabiting insects (hexapods) that are characterized by a lack of eyes and antennae, a 12-segmented abdomen and development by the indefinite addition of segments at each moult.

they would be selected to allocate sperm to females in a strategic manner. The most obvious form of strategic sperm allocation is the allocation of ejaculates that contain more sperm in the presence of sperm competition, as occurs in crabs[28] and birds[29–30] (T. Pizzari *et al.*, unpublished data). More is not always better, however, and theory predicts that, once the intensity of sperm competition becomes very high, as it does with some externally spawning fish, it pays individual males to invest fewer sperm in individual ejaculates, because for each male the probability of fertilization is reduced; this is exactly what is observed[31]. Sperm allocation can also result in sexual conflict: in the bluehead wrasse *Thalassoma bifasciatum*, territorial males are visited by spawning females throughout the day and males carefully allocate sperm to maximize the number of females they can spawn with. To achieve this, males reduce the number of sperm they release at each spawning, resulting in a fertilization success of only 95–98% of eggs, and hence the wastage of some of the female's eggs[32].

   In addition to selecting for sperm numbers, sperm competition selects for sperm form and function. Across species, sperm vary enormously in design, from the amoeboid sperm of *Caenorhabditis elegans*, the immotile disc-like sperm of PROTURANS, to the more typical 'tadpole-like' sperm of vertebrates. Sperm size, which varies from a few micrometres in some insects to the giant sperm (>5 cm) of certain *Drosophila* species[33], seems to be directly related to sperm competition in certain taxa. In *Caenorhabditis* spp., larger sperm are more competitive[34], and a comparative study of birds revealed that longer sperm occur in species with more intense sperm competition[35]. Sperm 'quality' also seems to be sexually selected. Polyandrous insects, for example, produce a higher proportion of live sperm than monandrous species[36]. Moreover, within species, individual males show consistent and repeatable differences in the fertilizing efficiency of their sperm, which predicts fertilization success when there is sperm competition[37]. Individual males show considerable variation in sperm morphology within their ejaculates. This variation has traditionally been explained in terms of 'production errors', but it might also be adaptive. In the *Drosophila pseudoobscura* species group, males produce two or more sperm sizes[38], and, in molluscs and lepidopterans, males produce both nucleate and anucleate (non-fertilizing) sperm whose role seems to be a form of paternity defence: by cheaply filling the sperm store of the female, she is discouraged from remating, therefore reducing the likelihood that the male's sperm will have to compete with those of others[39]. Human ejaculates comprise a diverse mixture of sperm 'types' and it has been suggested that some of these are 'kamikaze' sperm, which are designed to destroy themselves and the sperm of rival males[40]; however, there is little evidence to support this[41].

   The outcome of sperm competition can be predicted from sperm numbers (FIG. 1), the fertilizing ability of sperm, and the timing of inseminations relative to each other (FIG. 2a) and to when a female ovulates (FIG. 2b).
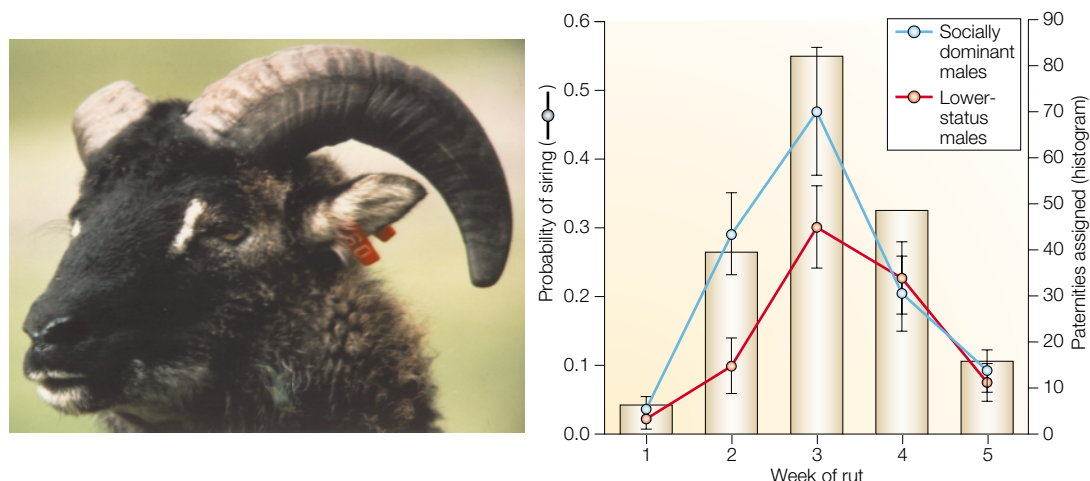
Figure 1 | **Sperm numbers influence the outcome of sperm competition in Soay sheep.** During the first half of the rut, socially dominant males sire most offspring, but later in the season (during weeks 4 and 5) socially dominant rams become sperm depleted, allowing lower-status males (with more available sperm) to gain paternities[132]. Photo courtesy of Ian Stevenson, University of Stirling, UK.

However, the mechanisms of sperm competition differ across taxa, as expected. In many insects (and in birds; see FIG. 2a), the second of two or the last of several males fertilizes most eggs, a phenomenon referred to as last-male sperm precedence. The sheer number of insect species means that a great diversity of mechanisms exists; at the simplest level (for example, in dragonflies[42]), males use a specially modified penis to remove any previously stored sperm before introducing their own; or repeatedly inseminate a female between the laying of successive eggs to ensure that only their sperm are used for fertilization[43]. In the yellow dungfly

Scathophaga stercorcaria, one of the best-studied insects in terms of sperm competition, last-male sperm precedence is mediated through a male–female interaction[44] (FIG. 3). In *Drosophila*, sperm competition is mediated by Acps in the seminal fluid, which determine the ability to displace and neutralize previously stored sperm. Acps result in (i) a reduced female receptivity to future copulations[24,45], and (ii) a reduced hatching rate of eggs that are fertilized by previous males[46], as well as inducing oogenesis and ovulation. The effect of some seminal fluid products, such as the glycoprotein Acp36DE, on sperm displacement is stronger when both the protein
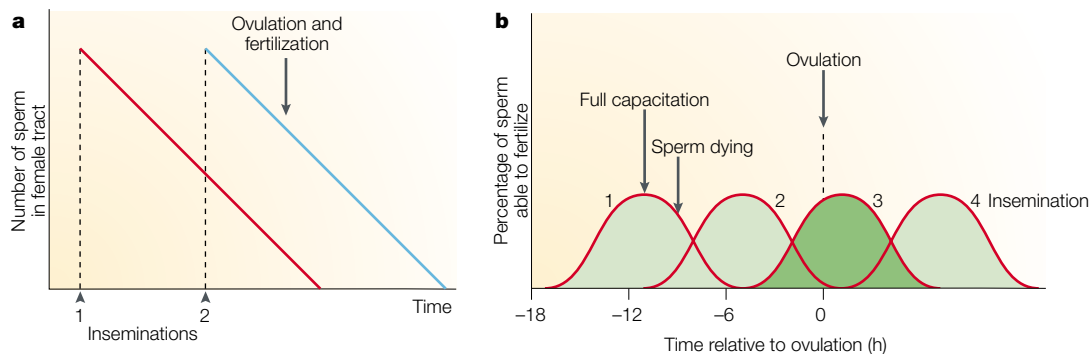

Figure 2 | **Mechanism of sperm competition in birds and mammals. a** | The diagram illustrates the passive sperm-loss model that results in last-male sperm precedence in birds. Sperm are stored in the numerous sperm storage tubules (at the utero-vaginal junction) of the female from which they leak out into the oviduct at a constant rate. All else being equal, the longer the interval between two inseminations (indicated by the arrowheads), the greater the proportion of sperm from the first insemination that have been lost or used, so the sperm of the second male are numerically dominant at the time of fertilization. This is the most basic and general mechanism of sperm competition in birds and is modified by many other factors, including sperm numbers, timing of insemination relative to the time of laying, interval between inseminations, the fertilizing ability of sperm and sperm ejection[37,92,133,134]. **b** | In mammals, there are no consistent order effects and, all else being equal (for example, sperm numbers or 'quality'), the outcome of sperm competition depends on an interaction between the timing of ovulation, the timing of insemination and the time taken for SPERM CAPACITATION in the oviduct[134]. The most successful male is the one that copulates at a time that results in his capacitated sperm reaching the recently shed ova. In the diagram, the third insemination (dark green) takes place ~6 h before ovulation; as a consequence, the sperm reach their peak close to the time of ovulation and are therefore more likely to result in fertilization.

SPERM CAPACITATION
The state of physiological readiness to fertilize an ovum. Freshly ejaculated mammalian sperm are incapable of fertilization and require a period of time in the female tract to acquire this ability.

Figure 3 | **Sperm competition and cryptic female choice in the yellow dungfly** *Scathophaga stercorcaria.*

S. stercorcaria is one of the best-studied insects in terms of sperm competition. In this insect, the last male to inseminate a female has a fertilizing advantage[73]. Last-male sperm precedence occurs as a result of the way in which the male transfers sperm to the female and the way in which the female takes it up into her sperm store: with every new insemination, previously stored sperm are pushed out of the store and replaced by those of the most recent male, although the effectiveness with which this occurs depends partly on how well the genitals of the male 'fit' those of the female. The dungfly also shows a rather complex form of non-directional cryptic female choice. An analysis of the variation of the paternity of the second male (P2) under experimental conditions revealed that when females (but not males) were raised under stable environmental conditions, P2 was higher if both the second male and the female were homozygous for the same allele at the *phosphoglucomutase* (*Pgm*) locus. When females were raised under variable environmental conditions, however, there was no effect of genetic similarity at the *Pgm* locus on P2 (REF. 89). Females that are homozygous for the two *Pgm* alleles lay eggs in different microhabitats, and offspring viability for both genotypes is maximized in the microhabitat that is preferentially selected by the females[135]. It is therefore plausible that, under stable environmental conditions, females favour fertilization from genetically similar males, whereas under more variable conditions, heterozygous offspring might, on average, have a better chance of survival. Photo courtesy of Paul Ward, University of Zürich, Switzerland. Reproduced with permission from REF. 16 © (2000) Faber & Faber.

and sperm are inseminated, which indicates cooperation between sperm and Acps to displace the ejaculate of another male[47]. An interaction between sperm and seminal fluid products to displace the sperm of other males is also observed in the nematode *C. elegans*, in which a group of genes (spe) controls spermatogenesis. Spermatogenesis-defective male mutants have abnormal sperm–egg interactions, but their sperm are able to displace hermaphroditic sperm in hermaphrodites[48]. Furthermore, the interaction between the seminal fluid (but not the sperm) of these mutants and the hermaphroditic sperm of normal males results in an increase in fertilizing efficiency[49,50], which indicates that seminal fluids might promote fertilizing efficiency through mechanisms not necessarily related to sperm displacement. The spe group of genes, and in particular *spe-12*,

blocks spermatid activation by encoding for a transmembrane protein that might be important in the signalling cascade system activated by signals from the seminal fluid[51].

Cryptic female choice

Until recently, the idea that, in sexually promiscuous species, female choice continues after insemination through cryptic female choice had received relatively little attention. Several factors contributed to the reluctance to investigate cryptic female choice. First, female choice is typically more subtle and less obvious than male–male competition and, therefore, mechanisms of female choice are often masked by male-driven processes and are harder to detect. Second, in species with internal fertilization, post-insemination mechanisms of sexual selection are difficult to study and often have to be inferred from indirect measures (for example, variation in paternity or number of sperm stored in the female sperm-storage organs). These measures are often ambiguous and difficult to interpret, so exacerbating the problems of explicitly showing cryptic female choice[52–55]. In addition, the idea that females had an active role in sexual selection was historically regarded with some scepticism, as was the idea that females actively chose their copulation partners and solicited copulations from several males. A cultural bias therefore discouraged an initial interest in cryptic female choice. Although first proposed in 1983 (REF. 56, but see REF. 57), the catalyst for the current interest in cryptic female choice was the extensive review by Eberhard[8], which explored the potential mechanisms that females could adopt to bias sperm storage and use in favour of certain males and against others. In many taxa, female sperm-storage organs are complex, are highly differentiated structurally and functionally, and rapidly coevolve with sperm or ejaculate traits, which indicates that they allow females some control over sperm use and fertilization[58–60]. However, copulation, insemination and the subsequent performance of an inseminated ejaculate depend on the complex interaction between male-driven and female-driven processes, the effects of which are difficult to disentangle. In some species, male copulatory behaviours condition the biased use of sperm by the female, as in the red flour beetle *Tribolium castaneum*. Males rub their legs on the lateral edges of the female wing cases; the intensity with which a male carries out this behaviour is positively associated with the fertilizing success of his ejaculate when in competition with the ejaculate of a control male[61]. Therefore, because cryptic female choice can be manipulated or conditioned by males, at a functional level it is important to establish not only which sex directly controls sperm use and fertilization, but also which sex gains from this control. In general, the conditions for the evolution of female choice at a post-insemination level are most favourable when (i) choice of partners is costly, and (ii) female choice is based not only on male phenotype, but also on the compatibility of the genotypes of their partners. Cryptic female choice can result in directional or non-directional sexual selection.

Figure 4 | ***Gallus gallus domesticus.*** Feral fowl provide an example of directional cryptic female choice. Although *Gallus* females cannot avoid some inseminations from subdominant males, they can expel the ejaculates of these lower-ranking males immediately after insemination. Photo courtesy of Charles Cornwallis, University of Sheffield, UK. Reproduced with persmission from REF. 63 *Nature* © (2000) Macmillan Magazines Ltd.

Directional cryptic female choice. In directional cryptic female choice, the criteria by which a female chooses her partner are predicted to be consistent with the criteria of cryptic female choice. In other words, we expect females to bias sperm use in favour of the male phenotypes that are also favoured in partner choice. To the extent to which the traits targeted by female choice are underlined by additive genetic variance, cryptic female choice is expected to generate directional sexual selection that favours the spread of the attractive alleles. Very few examples are consistent with directional cryptic female choice. In feral fowl, *Gallus gallus domesticus*, females prefer socially dominant copulation partners, but cannot avoid some inseminations from subdominant males (FIG. 4). They can, however, expel ejaculates immediately after insemination, and the probability of sperm ejection by females is significantly and negatively correlated with the social status of a male[62]. Directional cryptic female choice has the advantage of reducing the costs of partner choice, but it might be a less effective way of avoiding some of the costs that are associated with an insemination (for example, toxins and pathogens in seminal fluid, ecological and physiological costs of copulation, and the risk that some sperm of the unwanted male might escape female selection and achieve fertilization).

Non-directional cryptic female choice. In non-directional cryptic female choice, females are predicted to favour the sperm of the males with compatible genotypes regardless of their phenotype. This process is expected to result in non-directional sexual selection and might buffer the effect of the selection that occurs before insemination (see below). Several mechanisms that result in non-directional cryptic female choice have been described in sessile hermaphroditic organisms in which the risk of self-fertilization is typically high[63]. A striking example of cryptic female choice is the selection by the female pronucleus of sperm pronuclei in the comb jelly *Beroë ovata*[64] (FIG. 5). Similarly, some examples of differential fertilizing ability that are related to the interaction with the partner's genotype, rather than to the partners' genotypes as such, indicate that non-directional cryptic female choice might be a widespread (but not ubiquitous[65,66]) process[67,68]; it might be particularly relevant in DIOECIOUS species in which the risk of inbreeding is associated with the expression of detrimental recessive alleles[4]. How females identify the genotype of their partners from their sperm is unknown. Prime candidates are non-recombining regions of the genome, such as the major histocompatibility complex, a gene complex that is responsible for disease resistance and immune function[4]. Members of this complex might be expressed on the surface of spermatozoa[69], which facilitates a differential response by the female or egg to sperm of different male genotypes. It is also possible that sperm haplotypes are sometimes expressed[70–72]. A more complex situation might exist in the yellow dungfly *S. stercoraria*, in which the last male to inseminate a female has a fertilizing advantage[73] (FIG. 3).

Unresolved evolutionary issues

Despite the recent interest in the mechanisms of post-insemination sexual selection, our knowledge of the evolutionary implications of these mechanisms is limited. In particular, the following issues must be addressed: (i) the relationship between mechanisms of sexual selection that occur before and after copulation, (ii) the interactions between different mechanisms of postcopulatory sexual selection, and (iii) the rate of evolutionary change caused by mutualistic or antagonistic inter-sexual coevolution driven by postcopulatory sexual selection.

Pre/postcopulatory sexual selection. The fact that episodes of pre-insemination sexual selection continue after insemination indicates that the phenotypic and genetic relationship between pre-insemination and post-insemination mechanisms might have profound repercussions on the way in which sexual selection affects variation in reproductive fitness and the way in which it changes gene frequencies over generations. Pre-insemination sexual selection typically favours the reproductive success of a subsample of the male population. It favours genes that confer an advantage in male copulation success (for example, through aggression and/or competitive ability, and/or through attractiveness to females) and the ability to select copulation partners in females. Promiscuity means that fertilization is biased in favour of certain males within this subsample, which then enjoy disproportionate genetic representation in the next generation. Post-insemination sexual selection mainly targets genes that convey a fertilizing advantage in males (for example, testes mass and the competitive ability of ejaculates), and the ability to discriminate between, and differentially use, the ejaculates of different males in females (for example, number and

DIOECIOUS
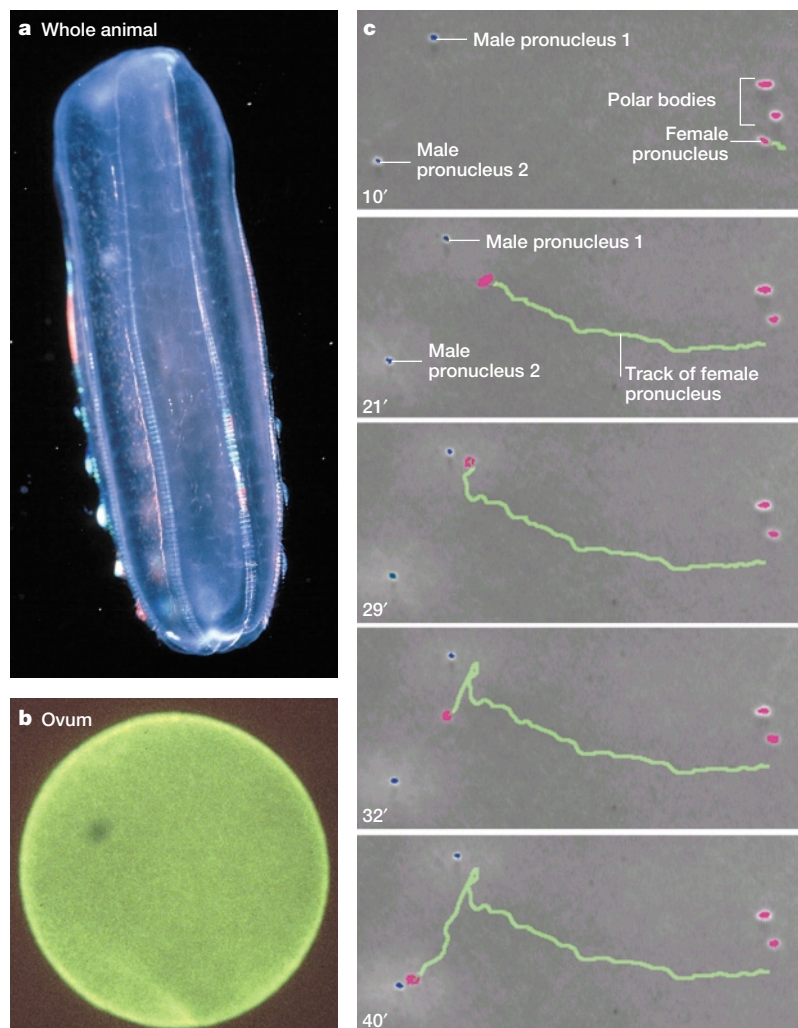Species in which the sexes are in separate individuals.

Figure 5 | **Cryptic female choice in the comb jelly *Beroë ovata*. a, b** | *B. ovata* provides one of the most remarkable and visible cases of cryptic female choice. Typically more than one sperm penetrates the egg (which is about 1 mm in diameter (**b**)), a phenomenon known as 'polyspermy'. **b** | The sequence of events during the 40 min after penetration of the egg by two sperm (the pronuclei of which are the two blue dots at the left). The female pronucleus moves through the cytoplasm to assess the two male pronuclei, checking one at 29 min, rejecting it and moving on to fuse with the other one at 40 min. Images courtesy of Claude Carré, Danielle Carré, Evelyn Houliston, Chistian Rouvière and Christian Sardet, Station Zoologique, Villefranche sur Mer, France. Reproduced with permission from REF. 16 © (2000) Faber & Faber.

favoured in post-insemination sexual selection[62,75]. In this model, the different component of fertilizing efficiency (that is, traits that influence copulation success and traits that influence the fertilizing ability of inseminations) are expected to be under positive correlational selection[76], and become genetically and phenotypically integrated[77].

Postcopulatory mechanisms. Different fertility traits can be prevented from genetic integration, and pre-insemination and post-insemination mechanisms of sexual selection often operate in different directions. For example, the defensive and offensive abilities of *Drosophila* ejaculates are controlled by different genomic regions[23,25]. Similarly, sexual promiscuity can provide unattractive males (that is, males of phenotypes that are disadvantaged in pre-insemination episodes of sexual selection) with the opportunity to compensate for impaired attractiveness or impaired competitive ability through the production of more competitive ejaculates. In many species, males can adopt alternative reproductive strategies: they can either invest in (i) competitive ability and attractive traits (for example, ornaments, visual, acoustic and olfactory displays), therefore securing privileged access to numerous females, or (ii) the production of highly competitive ejaculates, therefore decreasing copulation success but increasing the fertilizing efficiency of any inseminations they achieve. In some species, male alternative reproductive strategies are condition dependent[19] and possibly maintained by selection for genes that allow phenotypic plasticity. However, in other species, different male genotypes result in strikingly different reproductive tactics[78–80]. The fitness pay-offs of the different strategies are frequency dependent, and genetic polymorphism is maintained by the fact that when different strategies reach an evolutionarily stable proportion in a population they result in similar fitness pay-offs. In this model, post-insemination sexual selection might be fundamental to the maintenance of genetic polymorphism. To this end, it is crucial to establish the genetic and phenotypic relationship between male traits that are favoured under pre-insemination and under post-insemination episodes of sexual selection. Investigating the link between male phenotypic traits that are selected by pre-insemination sexual selection, and fertilizing efficiency selected by post-insemination sexual selection, has been the focus of considerable research in evolutionary biology (see BOX 3). Part of the interest stems from the need to investigate the adaptive significance of female choice of partners: an association between the preferred male phenotype and fertilizing efficiency would indicate that the evolution of female choice might be driven by the pursuit of fertility, as predicted by the phenotype-linked hypothesis (BOX 3) and/or by the production of sons with superior fertilizing efficiency, as predicted by the sexually selected sperm hypothesis (BOX 3). Both hypotheses are based on the assumption that females benefit from having their eggs fertilized by the most

morphology of sperm-storage organs). When pre-insemination and post-insemination mechanisms operate in unison, sexual promiscuity might function as a catalyst in the selection of male phenotypes and of the genes associated with different male phenotypes. For example, in many socially monogamous but sexually promiscuous species (as in many birds), females prefer to establish a pair bond with males of a particular phenotype. However, those females that obtain social partners of suboptimal phenotypic quality can seek copulations outside the pair bond (extra-pair copulations) with more attractive males[74]. Similarly, in some polyandrous species, males that are favoured by female choice and in competition over copulation opportunities can produce ejaculates that are

Box 3 | **The 'sexually selected sperm' and the 'phenotype-linked fertility' hypotheses**

These two interrelated hypotheses are concerned with the evolution of sexy sperm and sexy males — that is, sperm and males that females find attractive.

The sexually selected sperm hypothesis
This[117] provided an explanation for female promiscuity, by proposing that it evolved to increase the likelihood of females producing sons with superior fertilizing ability. The hypothesis predicts that promiscuous females will produce sons whose fertilizing ability is high relative to those whose mothers are monogamous. This is a difficult hypothesis to test and so far there is limited support for it. However, the prediction is complicated by two factors: (i) sex-biased transmission of traits in male efficiency in fertilizing, and (ii) sexual antagonism. In fact, if traits in male efficiency in fertilizing are inherited maternally and/or have detrimental effects on females they might not increase female fitness. However, both sex-biased mechanisms and sexual antagonism might help to explain the maintenance of genetic variance and selection potential of fertilizing efficiency.

The phenotype-linked fertility hypothesis
This[118] links precopulatory and postcopulatory male success by proposing that attractive males (those with well-developed secondary sexual traits) also have high fertilizing efficiency. Intuitively, this seems obvious: high-quality males should be good at both attracting females and fertilizing their eggs. Although 'good genes' models of sexual selection assume positive genetic correlations between fitness-related traits[19], there is no reason *a priori* why this should be true. Indeed, it is more likely that negative genetic correlations exist between fitness-related traits (antagonistic pleiotropy) for two reasons: first, antagonistic pleiotropy provides an explanation for the unexpectedly high variance observed in such traits[119,120], and second, good evidence exists for trade-offs between other life-history traits[121]. However, it is difficult at this stage to predict which particular precopulatory and postcopulatory sexually selected traits we expect to show positive or negative genetic correlations. Consistent with this is the fact that some studies have found male fitness-related traits to covary positively, as with body ornamentation and sperm traits in guppies, *Poecilia reticulata*[74]. By contrast, in feral fowl, males whose sperm have superior fertilizing ability are more likely to be socially subordinate and less successful in obtaining copulations[92].

fertile male genotype. However, theoretical work[81] and recent experimental studies[24,82–84] have indicated that this need not be so.

Rate of evolutionary change. Intense post-insemination sexual selection is a powerful generator of inter-sexual conflict. A divergence in the reproductive interests of males and females can trigger rapid inter-sexual coevolution based on antagonism (for example, female resistance and male coercion) rather than on cooperation (for example, female choice of beneficial male genotype). Either way, when post-insemination sexual selection acts directionally and in unison with other selective episodes (that is, natural and pre-insemination sexual selection), or is more intense and therefore overrides other mechanisms, rapid inter-sexual coevolution occurs[23,59,60,84], which can lead to population divergence, reproductive isolation and possibly speciation (see BOX 2). For example, *D. melanogaster* and *D. simulans* Acps have disproportionately high rates of molecular sequence evolution, which is consistent with rapid adaptive evolution[85]. Mean heterozygosity at REPLACEMENT SITES is considerably higher for Acp genes than for control non-Acp genes[85]. Similarly, and crucially, the divergence between *D. melanogaster* and *D. simulans* is much higher at replacement sites of Acp rather than non-Acp genes, which indicates that molecular divergence between species is also associated with high levels of within-species polymorphism[85]. This process of rapid evolution can be constrained by the sex-biased inheritance of the genes that are targeted by post-insemination sexual selection. Precisely because these genes might be sexually antagonistic they find an ideal reservoir in regions of the genome that are under the preferential

control of one sex[25,81]. For example, mitochondrial genes, which are exclusively maternally transmitted in many species, can be selected for their expression in females but not in males. Consistent with this is the observation that mtDNA and maternally transmitted symbionts, such as *Wolbachia* spp., are responsible for male infertility, feminization and the maintenance of additive variation in the fertilizing ability of sperm[25,86,87]. In addition, sexually antagonistic interactions between mitochondrial and nuclear genes have been theoretically predicted and experimentally shown in *D. melanogaster*[88]. Sex-biased transmission therefore stems from, and interferes with, inter-sexual coevolution and might limit the ability of one sex to counteract sexually antagonistic alleles that benefit the opposite sex[25]. Another factor that contributes to buffer the effect of pre-insemination sexual selection is the fact that post-insemination mechanisms might not generate directional selection. Non-directional cryptic female choice is driven by genetic incompatibility between partners[4,53]; it might interfere with selection acting on different male genotypes and might contribute to the maintenance of genetic polymorphism at a stable equilibrium[89]. For example, in some flowering plants, the fertilizing efficiency of pollen depends on pollen tube growth, which in turn is determined by the Ga/Ga alleles of both the pollen grain and the female style[90]. Furthermore, most discussions of post-insemination sexual selection ignore the potential for competition among the different sperm genotypes that are present in the ejaculate of a single male. However, it is possible that the sperm haplotype not only might be expressed but also might influence the outcome of both sperm competition and cryptic female choice, which could have important

REPLACEMENT SITE
Any position within a gene at which a point mutation alters the encoded amino-acid sequence.

QUANTITATIVE TRAIT LOCUS (QTL). A genetic locus that is identified through the statistical analysis of complex traits (such as plant height or body weight). These traits are typically affected by more than one gene and also by the environment.

implications for the evolution of conflict between the male and the sperm genotypes and between different sperm haplotypes[70–72,91].

Genetics and the future

Genetic and molecular tools will be increasingly important in advancing our understanding of

post-insemination sexual selection, in particular: (i) to determine the genetic basis of different male fertility traits and female traits that mediate sperm selection, (ii) to identify the genes or the genomic regions that control these traits, and (iii) to infer microevolutionary and macroevolutionary patterns (BOX 4).

Assessing the genetic basis of traits that are selected by post-insemination sexual selection is often difficult. Many of these traits are likely to be sex linked or exclusively under the genetic control of one sex[25]. For example, sperm efficiency can be inherited through exclusively maternally transmitted mitochondrial genes[86,92]. In addition, inbreeding, which can covary with the degree of sexual promiscuity and therefore with the intensity of post-insemination sexual selection in a population[93], can also confound estimates of genetic variance that underlie traits selected by post-insemination sexual selection[94]. Restricted maximum-likelihood (REML) analysis provides a promising statistical tool for overcoming these problems, using an animal model to partition phenotypic variance across its components of additive genetic value and to include random and fixed effects[95]. REML analysis is considerably more powerful than more conventional ANOVA (analysis of variance) models because it allows researchers to use all known pedigree relationships between individuals and because it can be used to analyse highly unbalanced data sets[95], which provides estimates of components of variance that are unbiased by population size or by inbreeding in subsequent generations[96]. Although REML analysis is a powerful tool for disentangling maternal and paternal genetic effects, it is becoming increasingly obvious that environmental effects and particularly environmental maternal effects might have profound repercussions on the phenotypic variance of sexually selected traits[97]. Experiments should be designed to test for these effects — for example, by exposing full-sibs to different environmental conditions (see, for example, REF. 98) and by comparing the progeny of the same female with different males.

The genetic and molecular dissection of post-insemination sexual selection traits has been greatly improved by the establishment of many DNA polymorphic markers in several species, providing the potential for finding trait loci by linkage analysis, whole-genome scanning and QUANTITATIVE TRAIT LOCUS (QTL) analysis (see, for example, REFS 99,100). In addition, the development of ESTs and cDNA libraries in mice and humans might allow comparative mapping through fluorescent *in situ* hybridization to study regions of conserved synteny between humans or mice and other organisms[100]. However, the identification of genes does not reveal their function; neither does it reveal how such genes act at an organismal and molecular level. Moreover, epistasis between QTL might also have important — if little-known — effects[101]. A promising breakthrough in identifying both the genes that underlie traits targeted by post-insemination sexual selection and their function is the use of model species, such as *C. elegans* and

---

Box 4 | **Microevolutionary and macroevolutionary patterns**

Molecular studies need to address five main issues to further our understanding of postcopulatory sexual selection, by doing the following tests.

• They must test whether substitution rate is relatively high in regions that control traits targeted by post-insemination sexual selection, as predicted by both mutualistic and antagonistic models of inter-sexual coevolution driven by post-insemination sexual selection.

• They must test whether high substitution rates are due to Darwinian selection rather than to high mutation rate. Several studies have shown recently that both male and female reproductive proteins that are important determinants of reproductive fitness at a post-insemination level evolve rapidly[122–125], possibly as a result of positive Darwinian selection[124].

• They must determine the fitness consequences for the other sex of traits favoured by post-insemination sexual selection in one sex. In other words, they must assess the potential for sexual antagonism generated by post-insemination sexual selection. Intra-locus sexual antagonism can be tested for by showing the negative genetic inter-sexual correlation of post-insemination fitness components. This was elegantly achieved by Chippindale *et al.*[126], who cloned most of the haploid genomes of *Drosophila melanogaster* (chromosome IV excluded). They have also shown[127] a negative genetic correlation between adult fitness of males and females: genotypes that produced males with high reproductive success also produced females with impaired adult fitness, and vice versa. The demonstration of inter-locus sexual conflict is based on the analysis of the fitness consequences of sex-limited traits for the opposite sex. The use of male mutant *D. melanogaster* that were unable to produce specific accessory gland products allowed researchers to show that some accessory gland products are responsible for increased female mortality in this species[24,128]. To this end, it is also important to establish the relative evolutionary rate of male and female reproductive traits. Post-insemination models of sexual selection based on inter-locus conflict (see, for example, REF. 108) assume that females can counteract female-detrimental/male-beneficial mutations. However, the mutation rate in males might be higher than in females owing to a higher number of cell divisions during spermatogenesis than in oogenesis[129], and this could have important consequences for the coevolutionary trajectories of the sexes.

• They must investigate the origin of chromosomal regions that are responsible for traits selected by post-insemination sexual selection. Theory predicts that sexually antagonistic genes should accumulate on sex-linked regions of the genome (see, for example, REF. 130). Molecular studies of different genes might shed light not only on the current location of different genes promoted by post-insemination sexual selection, but also on the region of their origin. For example, the closest paralogues of male fertility genes on the Y chromosome of *D. melanogaster* are autosomal rather than X linked, indicating that, at least in this species, autosomal, male-beneficial genes tend to move and accumulate on the Y chromosome[131].

• Finally, an additional challenge is to apply molecular techniques to establish the level of gametic haploid expression in species in which the gametic phenotype is generally assumed to depend mainly on the diploid genome. Not only is this crucial for an understanding of the mechanisms that drive the evolution of gametic traits, but also it has important evolutionary implications (see section entitled 'Unresolved evolutionary issues' on p.267). Several studies have recently adopted Northern blot techniques with cDNA to identify sperm proteins that are transcribed exclusively at the haploid stage[70–72]. The versatility of Northern blot analysis facilitates this task by allowing the use of probes with only partial homology, such as cDNA from other species[70].

D. melanogaster, in which mutants can be isolated that show specific defects in post-insemination performance due to the knockout of specific genes[22,102,103]. This allows the identification of genes that are associated with post-insemination fitness and, crucially, of their specific functions. However, care must be taken when inferring the causal relationship between particular genes and specific traits from gene knockout data, because of the pleiotropic effects and overlapping functions of different genes, for example[104]. Identifying the proteins that are encoded by mutated genes can shed light on the mechanisms that mediate individual genetic effects. This can be achieved by testing whether phenotypic defects in mutants can be rescued by cell-by-cell expression of cloned wild-type cDNA in the mutants during the development of specific traits[102]. Determining the biochemical activity of specific proteins in the cell is often complicated by the fact that identified proteins produced by mutated genes are novel, but this can be addressed either by searching for partner proteins with known functions that interact directly with these proteins or by isolating second-site mutations that strengthen or weaken the mutant phenotype, which allows the identification of genes that encode upstream or downstream, or of partner elements that contribute to the encoding of these proteins[105]. The molecular analysis and comparisons of different genes will also shed light on how post-insemination sexual selection drives molecular evolution, and ultimately on the evolution of male fertility and female selectivity (BOX 4).

Conclusions

Generating directional testable predictions of the evolutionary response that is triggered by post-insemination sexual selection will require quantitative information on the following: (i) the intensity of individual episodes of post-insemination sexual selection; (ii) the selective mechanisms with which post-insemination mechanisms interact, namely natural and pre-insemination sexual selection; (iii) the degree of cooperation and conflict between male-driven and female-driven processes (for example, the degree of toxicity of traits that affect the fertilizing efficiency of males, the direction of sperm competition and cryptic female choice); (iv) the efficiency of individual post-insemination mechanisms (for example, the efficiency of male genotypes in maximizing fertilization success under sperm competition, of female storage organs in separating the ejaculates of different males, and of the female reproductive tract in ejecting or neutralizing unwanted ejaculates); and (v) the selection potential and the mode of genetic transmission of genes that are targeted by post-insemination sexual selection (for example, autosomal transmission and sex-biased transmission). Only by measuring these parameters in individual populations can we further our understanding of post-insemination sexual selection, the coevolutionary trajectories of the sexes and the evolution of reproductive traits and strategies.

1. Darwin, C. *The Descent of Man and Selection in Relation to Sex* (John Murray, London, 1871).
2. Smith, R. L. *Sperm Competition and the Evolution of Animal Mating Systems* (Academic, London, 1984).
3. Birkhead, T. R. & Møller, A. P. *Sperm Competition and Sexual Selection* (Academic, London, 1998). **A comprehensive review of postcopulatory sexual selection across the animal kingdom, together with a chapter on pollen competition and several conceptual chapters.**
4. Tregenza, T. & Wedell, N. Genetic compatibility, mate choice and patterns of parentage: invited review. *Mol. Ecol.* **9**, 1013–1027 (2000).
5. Parker, G. A. Sperm competition and its evolutionary consequences in the insects. *Biol. Rev.* **45**, 525–567 (1970).
6. Parker, G. A. in *Sperm Competition and the Evolution of Animal Mating Systems* (ed. Smith, R. L.) 1–60 (Academic, London, 1984).
7. Parker, G. A. in *Sperm Competition and Sexual Selection* (eds T. R. Birkhead & A. P. Møller) 3–54 (Academic, London, 1998).
8. Eberhard, W. G. *Female Control: Sexual Selection by Cryptic Female Choice* (Princeton Univ. Press, Princeton, New Jersey, 1996).
9. Coltman, D. W., Pilkington, J. G., Kruuk, L. E., Wilson, K. & Pemberton, J. M. Positive genetic correlation between parasite resistance and body size in a free-living ungulate population. *Evolution* **55**, 2116–2125 (2001).
10. Hurst, L. D. Embryonic growth and the evolution of the mammalian Y chromosome. I. The Y as an attractor for selfish growth factors. *Heredity* **73**, 223–232 (1994).
11. Wolf, J. B. Gene interactions from maternal effects. *Evolution* **54**, 1882–1898 (2000).
12. Pizzari, T. Sexual selection: separating genes from imprinting. *Trends Ecol. Evol.* **14**, 399 (1999).
13. Bateman, A. J. Intra-sexual selection in *Drosophila*. *Heredity* **2**, 349–368 (1948).
14. Halliday, T. R. & Arnold, S. J. Multiple mating by females: a perspective from quantitative genetics. *Anim. Behav.* **35**, 939–941 (1987).
15. Ketterson, E. D. *et al.* The relative impact of extra-pair fertilizations on variation in male and female reproductive success in dark-eyed juncos (*Junco hyemalis*). *Ornithol. Monogr.* **49**, 81–101 (1997).
16. Birkhead, T. R. *Promiscuity: An Evolutionary History of Sperm Competition and Sexual Conflict* (Faber & Faber, London, 2000).
17. Trivers, R. L. in *Sexual Selection and the Descent of Man 1871–1971* (ed. Campbell, B.) 136–179 (Aldine–Atherton, Chicago, Illinois, 1972).
18. Clutton-Brock, T. H. & Vincent, A. C. J. Sexual selection and the potential reproductive rates of males and females. *Nature* **351**, 58–60 (1991).
19. Andersson, M. *Sexual Selection* (Princeton Univ. Press, Princeton, New Jersey, 1994).
20. Parker, G. A., Baker, R. R. & Smith, V. G. F. The origin and evolution of gamete dimorphism and the male–male phenomenon. *J. Theor. Biol.* **36**, 529–553 (1972).
21. Delph, L. & Havens, K. in *Sperm Competition and Sexual Selection* (eds Birkhead, T. R. & Møller, A. P.) 150–173 (Academic, London, 1998).
22. Chapman, T. Seminal fluid-mediated fitness traits in *Drosophila*. *Heredity* **87**, 1–11 (2001). **An overview of the effect of male seminal proteins on the female reproductive process. Seminal protein genes are expressed only in males but it remains to be discovered how and why they affect female reproduction.**
23. Swanson, W. J., Clark, A. G., Waldrip-Dail, H. M., Wolfner, M. F. & Aquadro, C. F. Evolutionary EST analysis identifies rapidly evolving male reproductive proteins in *Drosophila*. *Proc. Natl Acad. Sci. USA* **98**, 7375–7379 (2001).
24. Chapman, T., Liddle, L. F., Kalb, J. M., Wolfner, M. F. & Partridge, L. Cost of mating in *Drosophila melanogaster* females is mediated by male accessory gland products. *Nature* **373**, 241–244 (1995).
25. Pizzari, T. & Birkhead, T. R. The sexually selected sperm hypothesis: sex-biased inheritance and sexual antagonism. *Biol. Rev.* (in the press). **A detailed review of traits that influence male reproductive success at a post-copulatory stage and of the mechanisms of their inheritance. Reproductive traits are likely to have sex-biased inheritance and sexually antagonistic effects.**
26. Swanson, W. J. & Vacquier, V. D. Concerted evolution in an egg receptor for a rapidly evolving abalone sperm protein. *Science* **281**, 710–712 (1998). **Compares cDNA sequences of a sperm protein, lysin, which mediates fertilization, with that of its egg receptor, VERL, in several species of marine molluscs (such as abalone and *Haliotis* sp.). Proteins evolve in unison and at a high rate, providing molecular evidence that postcopulatory sexual selection drives rapid intersexual specialization, leading to reproductive isolation and possibly speciation.**
27. Palumbi, S. All males are not created equal: fertility differences depend on gamete recognition polymorphisms in sea urchins. *Proc. Natl Acad. Sci. USA* **96**, 12632–12637 (1999).
28. Jivoff, P. & Hines, A. H. Female behaviour, sexual competition and mate guarding in the blue crab, *Callinectes sapidus*. *Anim. Behav.* **55**, 589–603 (1998).
29. Hunter, F. M., Harcourt, R., Wright, M. & Davis, L. Strategic allocation of ejaculates by male Adélie penguins. *Proc. R. Soc. Lond.* B **267**, 1541–1545 (2000).
30. Nicholls, E. H., Burke, T. & Birkhead, T. R. Ejaculate allocation by male sand martins *Riparia riparia*. *Proc. R. Soc. Lond.* B **268**, 1265–1270 (2001).
31. Stockley, P., Gage, M. J. G., Parker, G. A. & Møller, A. P. Sperm competition in fish: the evolution of testis size and ejaculate characteristics. *Am. Nat.* **149**, 933–954 (1997).
32. Warner, R. R., Shapiro, D. Y., Marcanato, A. & Petersen, C. W. Sexual conflict: males with highest mating success convey the lowest fertilization benefits to females. *Proc. R. Soc. Lond.* B **262**, 135–139 (1995).

33. Pitnick, S., Spicer, G. S. & Markow, T. A. How long is a giant sperm? *Nature* **375**, 109 (1995).
34. LaMunyon, C. W. & Ward, S. Evolution of sperm size in nematodes: sperm competition favours larger sperm. *Proc. R. Soc. Lond.* B **266**, 263–267 (1999).
35. Briskie, J. V., Montgomerie, E. & Birkhead, T. R. The evolution of sperm size in birds. *Evolution* **51**, 937–945 (1997).
36. Hunter, F. M. & Birkhead, T. R. Sperm viability and sperm competition in insects. *Curr. Biol.* **12**, 121–123 (2002).
37. Birkhead, T. R., Martinez, J. G., Burke, T. & Froman, D. P. Sperm mobility determines the outcome of sperm competition in the domestic fowl. *Proc. R. Soc. Lond.* B **266**, 1759–1764 (1999).
38. Snook, R. R. & Karr, T. L. Only long sperm are fertilization-competent in six sperm-heteromorphic *Drosophila* species. *Curr. Biol.* **8**, 291–298 (1998).
39. Cook, P. A. & Wedell, N. Non-fertile sperm delay female remating. *Nature* **397**, 486 (1999).
    Shows that non-fertilizing (apyrene) sperm in a butterfly serve as a 'cheap filler' of the female's sperm store and hence delay remating, decreasing the risk of sperm competition for males.
40. Baker, R. R. & Bellis, M. *Human Sperm Competition* (Chapman & Hall, London, 1995).
41. Moore, H. D. M., Martin, M. & Birkhead, T. R. No evidence for killer sperm or other selective interactions between human spermatozoa in ejaculates of different males *in vitro*. *Proc. R. Soc. Lond.* B **266**, 2343–2350 (1999).
42. Waage, J. K. Dual function of the damselfly penis: sperm removal and transfer. *Science* **203**, 916–918 (1979).
43. Smith, R. L. Repeated copulation and sperm precedence: paternity assurance for a male brooding water bug. *Science* **205**, 1029–1031 (1979).
44. Hosken, D., Meyer, E. & Ward, P. Internal female reproductive anatomy and genitalic interactions during copula in the yellow dungfly *Scathophaga stercoraria* (Diptera: Scathophagidae). *Can. J. Zool.* **77**, 1975–1983 (1999).
45. Chen, P. S. *et al.* A male accessory gland peptide that regulates reproductive behaviour of female *D. melanogaster*. *Cell* **54**, 291–298 (1988).
46. Prout, T. & Clark, A. G. Seminal fluid causes temporarily reduced egg hatch in previously mated females. *Proc. R. Soc. Lond.* B **267**, 201–203 (2000).
47. Chapman, T., Neubaum, D. M., Wolfner, M. F. & Partridge, L. The role of male accessory gland protein Acp36DE in sperm competition in *Drosophila melanogaster*. *Proc. R. Soc. Lond.* B **267**, 1097–1105 (2000).
48. Singson, A., Hill, K. L. & L'Hernault, S. W. Sperm competition in the absence of fertilization in *Caenorhabditis elegans*. *Genetics* **152**, 201–208 (1999).
49. L'Hernault, S. W., Shakes, D. C. & Ward, S. Developmental genetics of chromosome I spermatogenesis-defective mutants in the nematode *Caenorhabditis elegans*. *Genetics* **120**, 435–442 (1988).
50. Shakes, D. C. & Ward, S. Mutations that disrupt the morphogenesis and localization of a sperm-specific organelle in *Caenorhabditis elegans*. *Dev. Biol.* **134**, 307–316 (1989).
51. Nance, J., Minniti, A. N., Sadler, C. & Ward, S. *spe-12* encodes a sperm cell surface protein that promotes spermiogenesis in *Caenorhabditis elegans*. *Genetics* **152**, 209–220 (1999).
52. Briskie, J. V. & Montgomerie, R. Sperm size and sperm competition in birds. *Proc. R. Soc. Lond.* B **247**, 89–95 (1992).
53. Birkhead, T. R. Cryptic female choice: criteria for establishing female sperm choice. *Evolution* **52**, 1212–1218 (1998).
54. Birkhead, T. R. Defining and demonstrating postcopulatory female choice — again. *Evolution* **54**, 1057–1060 (2000).
55. Pitnick, S & Brown, W. D. Criteria for demonstrating female sperm choice. *Evolution* **54**, 1052–1056 (2000).
56. Thornhill, R. Cryptic female choice and its implications in the scorpionfly *Harpobittacus nigriceps*. *Am. Nat.* **122**, 765–788 (1983).
57. Childress, D. & Hartl, D. L. Sperm preference in *Drosophila melanogaster*. *Genetics* **71**, 417–427 (1972).
58. Hellriegel, B. & Ward, P. I. Complex female reproductive tract morphology: its possible use in postcopulatory female choice. *J. Theor. Biol.* **190**, 179–186 (1998).
59. Pitnick, S., Markow, T. & Spicer, G. S. Evolution of multiple kinds of female sperm-storage organs in *Drosophila*. *Evolution* **53**, 1804–1822 (1999).
60. Presgraves, D. C., Baker, R. H. & Wilkinson, G. S. Coevolution of sperm and female reproductive tract morphology in stalk-eyed flies. *Proc. R. Soc. Lond.* B **266**, 1041–1047 (1999).
61. Edvardsson, M. & Arnqvist, G. Copulatory courtship and cryptic female choice in red flour beetles *Tribolium castaneum*. *Proc. R. Soc. Lond.* B **267**, 559–563 (2000).
62. Pizzari, T. & Birkhead, T. R. Female fowl eject sperm of subdominant males. *Nature* **405**, 787–789 (2000).
63. Bishop, J. D. D., Jones, C. S. & Noble, L. R. Female control of paternity in the internally fertilizing compound ascidian *Diplosoma listerianum*. II. Investigation of male mating success using RAPD markers. *Proc. R. Soc. Lond.* B **263**, 401–407 (1996).
64. Carré, D. & Sardet, C. Fertilization and early development in *Beroë ovata*. *Dev. Biol.* **105**, 188–195 (1984).
65. Stockely, P. No evidence of sperm selection by female common shrews. *Proc. R. Soc. Lond.* B **264**, 1497–1500 (1997).
66. Cunningham, E. J. A. & Cheng, K. M. Biases in sperm utilization in the mallard: no evidence for selection by females based on sperm genotype. *Proc. R. Soc. Lond.* B **266**, 905–910 (1999).
67. Wilson, N., Tubman, S. C., Eady, P. E. & Robertson, G. W. Female genotype affects male success in sperm competition. *Proc. R. Soc. Lond.* B **264**, 1491–1495 (1997).
68. Prout, T. & Clark, A. G. Polymorphism in genes that influence sperm displacement. *Genetics* **144**, 401–408 (1996).
    Addresses the issue of what maintains genetic variation in sperm displacement despite directional postcopulatory sexual selection. A single locus model shows that genetic polymorphism can be evolutionarily stable when alleles that control sperm displacement have pleiotropic effects on fecundity and mating ability.
69. Martin-Villa, J. M., Longas, J. & Arnaiz-Villena, A. Cyclic expression of HLA class I and II molecules on the surface of purified human spermatozoa and their control by serum inhibin B levels. *Biol. Reprod.* **61**, 1381–1386 (1999).
70. Catalano, R. D., Hillhouse, E. W. & Vlad, M. Developmental expression and characterization of *FS39*, a testis complementary DNA encoding an intermediate filament-related protein of the sperm fibrous sheath. *Biol. Reprod.* **65**, 277–287 (2001).
71. Dahle, M. K., Reinton, N., Orstavik, S., Tasken, K. A. & Tasken, K. Novel alternatively spliced mRNA (1c) of the protein kinase A RIα subunit is implicated in haploid germ cell specific expression. *Mol. Reprod. Dev.* **59**, 11–16 (2001).
72. Uchida, K. *et al.* Cloning and characterization of a complementary deoxyribonucleic acid encoding haploid-specific alanine-rich acidic protein located on chromosome-X. *Biol. Reprod.* **63**, 993–999 (2000).
73. Simmons, L. W., Stockley, P., Jackson, R. L. & Parker, G. A. Sperm competition or sperm selection: no evidence for female influence over paternity in yellow dung flies *Scatophaga stercoraria*. *Behav. Ecol. Sociobiol.* **38**, 199–206 (1996).
74. Kempenaers, B. *et al.* Extra-pair paternity results from female preference for high quality males in the blue tit. *Nature* **357**, 494–496 (1992).
75. Matthews, I. M., Evans, J. P. & Magurran, A. E. Male display rate reveals ejaculate characteristics in the Trinidadian guppy *Poecilia reticulata*. *Proc. R. Soc. Lond.* B **264**, 695–700 (1997).
76. Lande, R. & Arnold, S. J. The measurement of of selection on correlated characters. *Evolution* **37**, 1210–1226 (1983).
77. Cheverud, J. M. Developmental integration and the evolution of pleiotropy. *Am. Zool.* **36**, 44–50 (1996).
78. Lank, D. B., Smith, C. M., Hannote, O., Burke, T. & Cooke, F. Genetic polymorphism for alternative mating behaviour in lekking male ruff *Philomachus pugnax*. *Nature* **378**, 59–62 (1995).
79. Shuster, S. M. & Sassaman, C. A. Genetic interaction between male mating strategy and sex ratio in a marine isopod. *Nature* **388**, 373–377 (1997).
80. Shuster, S. M. & Wade, M. J. Equal mating success among male reproductive strategies in a marine isopod. *Nature* **350**, 608–610 (1991).
81. Parker, G. A. in *Sexual Selection and Reproductive Competition in Insects* (eds Blum, M. S. & Blum, N. A.) 123–166 (Academic, New York, 1979).
82. Rice, W. R. Sexually antagonistic male adaptation triggered by experimental arrest of female evolution. *Nature* **381**, 232–234 (1996).
83. Holland, B. & Rice, W. R. Experimental removal of sexual selection reverses intersexual antagonistic coevolution and removes reproductive load. *Proc. Natl Acad. Sci. USA* **96**, 5083–5088 (1999).
    A spectacular demonstration that sexual selection drives the evolution of male traits that are detrimental to females. This could be mediated by seminal fluid products, which determine male postcopulatory performance but have toxic effects on females.
84. Hosken, D. J., Garner, T. W. J. & Ward, P. I. Sexual conflict selects for male and female reproductive characters. *Curr. Biol.* **11**, 1–20 (2001).
85. Begun, D. J., Whitley, P., Todd, B., Waldrip-Dail, H. & Clark, A. G. Molecular population genetics of male accessory proteins in *Drosophila*. *Genetics* **156**, 1879–1888 (2000).
86. Frank, S. A. & Hurst, L. D. Mitochondria and male disease. *Nature* **383**, 224 (1996).
87. Ruiz-Pesini, E. *et al.* Human mtDNA haplogroups associated with high or reduced spermatozoa motility. *Am. J. Hum. Genet.* **67**, 543–548 (2000).
88. Rand, D. M., Clark, A. G. & Kann, L. M. Sexually antagonistic cytonuclear fitness interactions in *Drosophila melanogaster*. *Genetics* **159**, 173–187 (2001).
89. Ward, P. Cryptic female choice in the yellow dungfly *Scathophaga stercoraria* (L.). *Evolution* **54**, 1680–1686 (2000).
90. Ottaviano, E. & Mulcahy, D. L. Genetics of angiosperm pollen. *Adv. Genet.* **26**, 1–65 (1989).
91. Haig, D. & Bergstrom, C. T. Multiple mating, sperm competition and meiotic drive. *Genetics* **121**, 129–138 (1995).
92. Froman, D. P., Pizzari, T., Feltmann, A. J., Castillo–Juarez, H. & Birkhead, T. R. Sperm mobility: mechanisms of fertilizing efficiency, genetic variation and phenotypic relationship with male status in the fowl, *Gallus g. domesticus*. *Proc. R. Soc. Lond.* B **269** (in the press).
93. Snook, R. R. Conflict, kindness and chicanery. *Curr. Biol.* **11**, R337–R341 (2001).
94. Hughes, K. A. Quantitatuve genetics of sperm precedence in *Drosophila melanogaster*. *Genetics* **145**, 139–151 (1997).
95. Lynch, M. & Walsh, B. *Genetics and Analysis of Quantitative Traits* (Sinauer Associates, Sunderland, Massachusetts, 1998).
96. Van der Werf, J. H. J. & der Boer, I. J. M. Estimation of additive genetic variance when base populations are selected. *J. Anim. Sci.* **68**, 3124–3132 (1990).
97. Sheldon, B. C. Differential allocation: tests, mechanisms and implications. *Trends Ecol. Evol.* **15**, 397–402 (2000).
98. David, P., Bjorksten, P., Fowler, K. & Pomiankowski, A. Condition-dependent signalling of genetic variation in stalk-eyed flies. *Nature* **406**, 186–188 (2000).
99. Takahashi, J. S., Pinto, L. H. & Vitaterna, M. H. Forward and reverse genetic approaches to behavior in the mouse. *Science* **264**, 1724–1733 (1994).
100. Andersson, L. Genetic dissection of phenotype diversity in farm animals. *Nature Rev. Genet.* **2**, 130–138 (2001).
101. Mackay, T. F. C. Quantitative trait loci in *Drosophila*. *Nature Rev. Genet.* **2**, 11–21 (2001).
102. Yamamoto, D. & Nakano, Y. Genes for sexual behaviour. *Biochem. Biophys. Res. Commun.* **246**, 1–6 (1998).
103. Singson, A. Every sperm is sacred: fertilisation in *Caenorhabditis elegans*. *Dev. Biol.* **230**, 101–109 (2001).
104. Pfaff, D. Precision in mouse behavior genetics. *Proc. Natl Acad. Sci. USA* **98**, 5957–5960 (2001).
105. Karim, F. D. *et al.* A screen for genes that function downstream of *Ras1* during *Drosophila* eye development. *Genetics* **143**, 315–329 (1996).
106. Ridley, M. Mating frequency and fecundity in insects. *Biol. Rev.* **63**, 509–549 (1988).
107. Simmons, L. W. & Siva-Jothy, M. T. in *Sperm Competition and Sexual Selection* (ed. Birkhead, T. R. & Møller, A. P.) 341–434 (Academic, London, 1998).
108. Rice, W. R. Male fitness increases when females are eliminated from gene pool: implications for the Y chromosome. *Proc. Natl Acad. Sci. USA* **95**, 6217–6221 (1998).
109. Parker, G. A. & Partridge, L. Sexual conflct and speciation. *Phil. Trans. R. Soc. Lond.* B **353**, 261–274 (1998).
110 Gavrilets, S. Rapid evolution of reproductive barriers driven by sexual conflict. *Nature* **403**, 886–889 (2000).

111. Tregenza, T., Butlin, R. K. & Wedell, N. Evolutionary biology — sexual conflict and speciation. *Nature* **407**, 149–150 (2000).

112. Panhuis, T. M., Butlin, R., Zuk, M. & Tregenza, T. Sexual selection and speciation. *Trends Ecol. Evol.* **16**, 364–371 (2001).

113. Metz, E. C. & Palumbi, S. R. Positive selection and sequence rearrangements generate extensive polymorphism in the gamete recognition protein bindin. *Mol. Biol. Evol.* **13**, 397–406 (1996).

114. Arnqvist, G. Comparative evidence for the evolution of genitalia by sexual selection. *Nature* **393**, 784–786 (1998).

115. Møller, A. P. & Cuervo, J. J. Speciation and feather ornamentation in birds. *Evolution* **52**, 859–869 (1998).

116. Arnqvist, G. Comparative evidence for the evolution of genitalia by sexual selection. *Nature* **393**, 784–786 (1998).

117. Keller, L. & Reeve, H. K. Why do females mate with multiple males? The sexually selected sperm hypothesis. *Adv. Study Behav.* **24**, 291–315 (1995).

118. Sheldon, B. C. Male phenotype, fertility and the pursuit of extra-pair copulations by female birds. *Proc. R. Soc. Lond.* B **257**, 25–30 (1994).

119. Roff, D. A. The estimation of genetic correlations from phenotypic correlations: a test of Cheverud's conjecture. *Heredity* **74**, 481–490 (1995).

120. Møller, A. P. & Alatalo, R. V. Good-genes effects in sexual selection. *Proc. R. Soc. Lond.* B **266**, 85–91 (1999).

121. Stearns, S. *The Evolution of Life Histories* (Oxford Univ. Press, Oxford, 1992).

122. Vacquier, D. Concerted evolution in an egg receptor for a rapidly evolving abalone sperm protein. *Science* **281**, 710–712 (1998).

123. Swanson, W. J., Clark, A. G., Waldrip-Dail, H. M., Wolfner, M. F. & Aquadro, C. F. Evolutionary EST analysis identifies rapidly evolving male reproductive proteins in *Drosophila*. *Proc. Natl Acad. Sci. USA* **98**, 7375–7379 (2001).
   A comparative EST analysis of male accessory glands of *D. melanogaster* and their orthologues in *D. simulans,* showing that many male reproductive genes undergo faster rates of selection than regions that control non-reproductive traits in the same species.

124. Swanson, W., Yang, Z., Wolfner, M. F. & Aquadro, C. Positive Darwinian selection drives the evolution of several reproductive proteins in mammals. *Proc. Natl Acad. Sci. USA* **98**, 2509–2514 (2001).

125. Swanson, W. J. & Vacquier, V. D. The rapid evolution of reproductive proteins. *Nature Rev. Genet.* **3**, 137–144 (2002).

126. Chippindale, A. K. & Rice, W. R. Y chromosome polymorphism is a strong determinant of male fitness in *Drosophila melanogaster*. *Proc. Natl Acad. Sci. USA* **98**, 5677–5682 (2001).

127. Chippindale, A. K., Gibson, J. R. & Rice, W. R. Negative genetic correlation for adult fitness between sexes reveals ontogenetic conflict in *Drosophila*. *Proc. Natl Acad. Sci. USA* **98**, 1671–1675 (2001).

128. Civetta, A. & Clark, A. G. Correlated effects of sperm competition and postmating female mortality. *Proc. Natl Acad. Sci. USA* **97**, 13162–13165 (2000).

129. Montell, H., Fridolfsson, A. K. & Ellegren, H. Contrasting levels of nucleotide diversity on the avian Z and W chromosome. *Mol. Biol. Evol.* **18**, 2010–2016 (2001).

130. Rice, W. R. Sex chromosomes and the evolution of sexual dimorphism. *Evolution* **38**, 735–742 (1984).

131. Carvalho, A. B., Lazzaro, B. P. & Clark, A. G. Y chromosomal fertility factors kl-2 and kl-3 of *Drosophila melanogaster* encode dynein heavy chain polypeptides. *Proc. Natl Acad. Sci. USA* **97**, 13239–13244 (2000).

132. Preston, B. T., Stevenson, I. R., Pemberton, J. M. & Wilson, K. Dominant rams lose out by sperm depletion. *Nature* **409**, 681–682 (2001).

133. Birkhead, T. R. Mechanisms of sperm competition in birds. *Am. Sci.* **84**, 254–262 (1996).

134. Huck, U. W., Tonias, B. A. & Lisk, R. D. The effectiveness of competitive male inseminations in golden hamsters, *Mesocricetus auratus*, depends on an interaction of mating order, time delay between males and the time of mating relative to ovulation. *Anim. Behav.* **37**, 674–680 (1989).

135. Ward, P. A possible explanation for cryptic female choice in the yellow dung fly *Scathophaga stercoraria* (L.). *Ethology* **104**, 97–110 (1997).

◉⟩ Online links