

Choosy Females

Exploring the role of cryptic female choice in sexual selection and battles over paternity

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The image of two males engaged in a dramatic battle over the right to mate with a female is a staple of television nature documentaries. Such male-male competition leads to intrasexual selection among males—evolutionary selection for traits that increase a male's ability to compete with other males for reproductive access to females. But females are not necessarily passive participants in these contests. The ability of females to pick and choose among competing males provides the opportunity for another form of sexual selection—intersexual selection for male traits that can influence female mate choice.

This basic picture of sexual selection, first outlined by Charles Darwin in 1871, has since grown more complex. One significant piece that has been added to the still-incomplete jigsaw puzzle of sexual selection comes from the growing realization over the past 30 years that in many species, females copulate with more than one male during a single reproductive cycle and can store and use sperm from more than one male to fertilize their eggs. Biologists now recognize that, as a result, sexual selection can continue to operate during and after copulation.

But just as biologists have grappled for many years with the question of the extent to which female choice before copulation influences male-male com-

petition and selects for the elaborate and often cumbersome male traits that attract mates—such as the peacock's large and flamboyant tail—some researchers are now engaged in a vigorous debate over the question of the extent to which female choice can influence the outcome of male-male competition after copulation.

The possibility that female choice might continue during and after copulation has been discussed by researchers for a long time. According to William Eberhard, of the Smithsonian Tropical Research Institute and the University of Costa Rica, females are more likely to be able to affect the outcome of male-male competition after copulation than before, because “the female's body constitutes the field on which males compete, and her behaviour and physiology set some of the rules by which they must abide,” he writes in *Sperm Competition and Sexual Selection* (T. R. Birkhead, A. P. Møller, eds., Academic Press, 1998). Therefore, he argues, “small changes in female morphology, behaviour or physiology can tilt the playing field and change the rules, thus biasing paternity.”

However, sorting out the relative contributions of postcopulatory male-male competition per se and female choice to the evolution of male traits is far from simple. “It's not uncommon that the same characters that are effective in female choice are also effective in male-male competition,” says Jane Brockmann, a behavioral ecologist at the University of Florida. “It takes special experiments to separate out those effects.”

Sperm competition and cryptic female choice

For many years, most behavioral ecologists studying postcopulatory sexual selection focused on the phenomenon of sperm competition (in the strict sense; see box page 8), in which the sperm from two or more males compete to fertilize a given set of eggs. As discussed in the first part of this series (*BioScience* 49: 951–956), sperm competition leads to a host of behavioral, anatomical, and physiological adaptations in males that serve to increase their sperm's ability to compete for paternity. Therefore, sperm competition can be viewed as the postcopulatory equivalent of intrasexual selection via direct male-male competition.

In this male-focused view of postcopulatory sexual selection, females were seen as passive vessels in which the ejaculates of different males vied to fertilize as many eggs as possible. More recently, however, biologists have become increasingly interested in the notion that, through a phenomenon now known as cryptic female choice, females may influence which of several copulatory partners father their offspring. Cryptic female choice can thus be seen as the postcopulatory version of sexual selection via female choice.

The term cryptic female choice was coined in 1983 by Randy Thornhill, of the University of New Mexico, and is broadly defined as any postcopulatory ability of females to favor one male of the same species over another. Thornhill called it cryptic, he says, because “it is hidden when researchers only measure variation among males in mating



The male Australian hanging fly (*Harpobittacus nigriceps*; top/right) provides his mate (below/left) with a "nuptial gift" of food—in this case, a blowfly—on which to dine during copulation. Female hanging flies allow males who provide larger nuptial gifts to copulate longer and thus transfer more sperm, thereby biasing paternity. Photo: Randy Thornhill.

success," as in the classical sperm competition experiment, in which researchers determine the proportion of offspring fathered by each of two males when they are mated in succession with a single female.

Eberhard, who is one of the chief proponents of the significance of cryptic female choice in postcopulatory sexual selection, has defined it in greater detail as the ability of female traits—whether behavioral, morphological, or physiological—to consistently favor those copulatory partners that possess a particular trait that is lacking or less

fully developed in other males. By Thornhill and Eberhard's definition, cryptic female choice can operate at many points in the often complex postcopulatory reproductive process, including egg laying, remating, and even differential female investment in offspring sired by different males—not just at the level of ejaculates competing within the female reproductive tract.

Furthermore, Eberhard emphasizes that sperm competition and cryptic female choice "are not [necessarily] an either-or situation." Indeed, says behavioral ecologist Tim Birkhead, of the University of Sheffield, "we now realize that the way fertilization takes place is often through an interaction between male processes and female processes such as cryptic female choice."

Female choice in action

As is the case for sperm competition, many examples of possible cryptic female choice come from studies of insects. In insect species in which males present females with a "nuptial gift" of prey on which to feed during copulation, females may exercise mate choice in a most materialistic manner. For example, in the first demonstration of female influence over insemination, Thornhill showed that female hanging flies (*Hylobittacus apicalis*) allow males who provide larger nuptial gifts to copulate longer, and thus to transfer more sperm, thereby biasing paternity. Males who provide gifts that are deemed inadequate are cast off by the fickle female before insemination is complete.

Female choice over insemination may also occur in the field cricket *Gryllus bimaculatus*, in which the male transfers his ejaculate to the female by attaching his spermatophore (a packet of sperm encased in male reproductive

gland secretions) at the entrance to her reproductive tract. After copulation—and often before insemination is complete—the female removes and consumes the spermatophore. Work by Leigh Simmons, of the University of Western Australia, has shown that female field crickets may bias the paternity of their offspring in favor of certain males by removing the spermatophores of some males before insemination and leaving those of other males attached even longer than is necessary for insemination. Females also copulate repeatedly with some males, allowing them to transfer multiple ejaculates.

Although a male field cricket will try to guard the female after attaching his spermatophore and thus prevent her from removing it prematurely and seeking other mates, he is not always successful. Some researchers have suggested that females may judge a male's "quality" by his success at postcopulatory mate guarding, so that female interests—which favor quality over quantity when it comes to mates—may be served by cryptic choice for insemination by males with superior mate-guarding abilities.

An intriguing behavioral adaptation in the fly *Dryomyza anilis* provides a case of possible cryptic female choice involving sperm storage. Studies by Merja Otronen, of the University of Turku, in Finland, and Mike Siva-Jothy, of the University of Sheffield, show that in the intervals between multiple copulations, the male *D. anilis* repeatedly taps the female's external genitalia with his genital claspers and females emit a droplet of sperm. Tapping mobilizes sperm stored in the female's spermatheca (sperm storage organ) from previous matings into an adjacent chamber called the bursa copulatrix, where they mix with the current male's ejaculate. Sperm then reenter the spermatheca, and approximately 50

Some Notes on Terminology

Sperm competition in the strict sense, as the term is used in this article, refers to the competition among the ejaculates of different males for fertilization of a given set of eggs. The term can also be used more broadly to include all of the behavioral, physiological, and morphological adaptations in males that arise as a result of this competition.

The terms **mating** and **copulating** (and **to mate** and **to copulate**) are used synonymously in this article.

percent of the sperm left in the bursa copulatrix are emitted in the droplet.

The more tapping sequences a male performs, the more his sperm displaces that of previous mates and the lower the proportion of his sperm in the emitted droplet, thereby increasing the likelihood that his sperm will fertilize the eggs. The potential for female choice arises because female *D. anilis* flies actively try to resist tapping by all males, but larger males are able to perform more tapping sequences. Thus, by resisting all males, the female may be indirectly choosing in favor of those males that are big and strong enough to subdue her.

Weighing the evidence

Many phenomena that have been interpreted as evidence of cryptic female choice, however, can also be interpreted in terms of direct male-male sperm competition—and vice versa. In the case of *D. anilis*, for instance, the evolution of male tapping behavior could be viewed as the result of intrasexual selection due to direct male-male competition over control of sperm storage, with larger males outcompeting smaller ones, or of intersexual selection via the ability of females to selectively resist the tapping attempts of larger males. Alternatively, it could be viewed as a reflection of the combined inputs of sperm competition and cryptic female choice, with neither sex having complete control over the outcome.

Demonstrating postcopulatory female choice at the level of selective use of sperm from different males is particularly challenging because it may involve subtle mechanisms that take place within the female reproductive tract and because sperm competition and female choice can occur simultaneously. Although Birkhead and others are skeptical of some of the purported evidence, one example that Birkhead says “provides some of the very best evidence for cryptic female choice” at this level is a study of male and female influences on sperm competition in the fruit fly *Drosophila melanogaster*, reported in the 8 January 1999 issue of

Science by Andrew Clark, of Pennsylvania State University, and his colleagues.

Although the second of two male fruit flies to copulate with a female fathers over 80 percent of the offspring on average, the ability of male fruit flies to compete for fertilization has been shown to vary among males of different genotypes. In an earlier study, reported in the July 1998 issue of *Genetics*, Clark and David Begun, of the University of Texas–Austin, showed that the proportion of offspring fathered by each of two male fruit flies mated with a single female also varies substantially, depending on the female's genotype. Their January 1999 article showed that interactions between various male and female fruit fly genotypes affect the outcome of sperm competition both in terms of the ability of one male's sperm to displace the stored sperm of a male who mated previously and in terms of a male's ability to prevent sperm displacement by a subsequent mate. Taken together, the results suggest that, through variation in certain traits, both sexes can influence the ability of a particular male's sperm to achieve fertilization.

Cryptic female choice and male genitalia

One male trait that is likely to play a role in determining fertilization success is male genital morphology. In a 1985 book, *Sexual Selection and Animal Genitalia* (Harvard University Press), Eberhard showed that rapid divergent evolution of male genitalia among closely related species is a common trend in animals with internal fertilization. “If you compare closely related species, the male genitalia are often extraordinarily different,” Eberhard explains, “and they are often more different than are the other structures in [the animals'] bodies.” As



In the wild, female zebra finches appear to be particularly choosy when it comes to copulating with males other than their regular partner. Studies of these birds in captivity have shown that female zebra finches prefer males with certain traits, such as high song rates. Researchers have found that females do not gain direct benefits from such extra-pair copulations, suggesting that they may instead obtain indirect, or genetic, benefits. Photo: Tim Birkhead.

a result, he notes, “taxonomists have learned that that's the ‘magic’ character that they can use to recognize species differences.”

The evolutionary processes behind this pattern of rapid divergence, however, remain controversial. One long-standing theory, known as the lock and key hypothesis, suggests that variation among male genitalia evolved through natural selection as a way to prevent mating between closely related species. Other researchers have proposed that sperm competition, cryptic female choice, or other mechanisms of postcopulatory sexual selection are responsible for the rapid divergence.

In the 25 June 1998 issue of *Nature*, Göran Arnqvist, of the University of Umeå, in Sweden, reported that genital morphology—analyzed using a new approach known as geometric morphometrics—diverges more than twice as much among species in groups of insects in which females mate multiply



The 113 known species of the drosophilid fly genus *Cladochaeta* are largely nondescript yellowish and brownish small flies; the most conspicuous differences among them are their elaborate male genitalia. Male genitalia of four *Cladochaeta* species are illustrated here: *C. albifrons* (upper left), *C. trauma* (upper right), *C. venebula* (lower left), *C. mexinversa* (lower right). Illustration from D. Grimaldi and T. Nguyen, 1999. Monograph on the spittlebug flies, genus *Cladochaeta* (Diptera: Drosophilidae: Cladochaetini). Bulletin of the American Museum of Natural History, Vol. 241.

than in related groups in which females mate only once. These findings support the idea that some type of postcopulatory sexual selection is responsible for the rapid divergent evolution of male genitalia. In both his 1985 book and a more recent book (*Female Control: Sexual Selection by Cryptic Female Choice*, Princeton University Press, 1996), Eberhard has argued that cryptic female choice is the most likely mechanism behind this rapid divergence.

A key component of Eberhard's hypothesis is that cryptic female choice can select for traits that provide males with a superior ability to trigger a series of preexisting female reproductive processes. After all, he notes, the simple transfer of sperm into the female is not enough to ensure the success of a given copulation. The sperm may need to be transported to a storage site within the female, nourished, transferred to the site of fertilization, and used to fertilize the eggs. Other necessary steps may include ovulation, egg maturation, egg laying (oviposition), and avoidance of remating. These and other female reproductive processes provide the opportunity for cryptic female choice.

According to Eberhard, through natural selection "females are likely to

evolve the ability to trigger these [reproductive] processes on the basis of stimuli that are associated with copulation," thereby avoiding wasted effort on the female's part when mating does not occur or providing her with other survival benefits. Males whose genital structures are better able to trigger these responses—for example, by generating increased friction in the female reproductive tract—will be more likely to succeed in male-male contests over fertilization of the female's eggs.

Because females often respond to copulation with incomplete responses, he says, "all the male has to do is emphasize the same stimuli that the female is already using" to trigger these responses. For example, envision a situation in which the female "is under [natural] selection to sense something about her own mating that tells her nervous system 'okay, I've mated, now I can trigger oviposition.'" At this point, Eberhard says, cryptic female choice comes into play. "Once she's now using some male stimuli to trigger oviposition, and if she's not always triggering her oviposition completely—as seems to be very typically the case—then there's competition among males to be better at triggering." For example, a substance in the seminal fluid of *D. melanogaster* can increase the female's oviposition rate.

Sexual selection of male traits occurs because those males with genital structures (or other traits, such as seminal fluid components) that permit improved triggering of the necessary

female responses will father more offspring than their competitors. In turn, Eberhard believes, females are likely to evolve in ways that ratchet up their response threshold to accentuate the biasing effects that females have on triggering by competing males. These female adaptations will lead to further opportunities for cryptic female choice in favor of refinements in male genital morphology or other traits that give some males a competitive edge in "persuading" females to use their sperm for fertilization.

According to Eberhard, in addition to causing the rapid divergence of male genital morphology, sexual selection via cryptic female choice can account for the rapid divergent evolution of other male traits, as well as female traits, that are associated with the biasing of sperm use. Such divergence has been well documented for what Eberhard calls male copulatory courtship behaviors—such as the tapping behavior described for *D. anilis*—that occur, paradoxically, after copulation has already begun. Males of different insect species, Eberhard writes in *Sperm Competition and Sexual Selection*, "lick, tap, rub, push, kick, stroke, shake, squeeze, feed, sing to, and vibrate the female during copulation." Like male genitalia themselves, these behaviors may serve as signals to trigger reproductive responses in females.

Components of male seminal fluid have also been reported to diverge rapidly in some insects, and Eberhard has argued that these proteins—which are known to affect several processes associated with reproduction, including egg laying and remating—are also subject to selection via cryptic female choice.

Costs and benefits

In Eberhard's model of sexual selection via cryptic female choice, male and female traits associated with reproduction coevolve, and both males and females may derive a net benefit from the outcome of male-male competition. Males benefit by fathering a larger number of offspring than their competitors, and females benefit by increasing the quality of their male offspring. But other researchers who study postcopulatory sexual selection view the process differently, referring to an "evolutionary arms race" between the sexes. In this view of sexual selection, male and female traits also continually coevolve, but conflicts between male and female interests select for adaptations in one sex that impose a net cost on the other.

The question of who benefits (or loses), and how, when females copulate with more than one male is therefore of central importance. Although the benefit that males derive from copulating with as many females as possible is obvious (they father more offspring), the benefits that females obtain from multiple mating are less clear. In some cases, males may force copulations on females, and the female presumably does not benefit at all. In many cases, however, females actively choose additional mates, suggesting that they do benefit from multiple mating.

The possible benefits for females fall into two general (and not necessarily mutually exclusive) categories—material benefits and indirect (genetic) benefits. Material benefits increase the female's survival or fecundity and can include an adequate sperm supply, nutrient acquisition through male nuptial gifts, and avoidance of harassment by other males through mate guarding. Genetic benefits—the nature and even the existence of which are controversial—include the opportunity to acquire "good genes" that enhance the survival of her offspring or "attractiveness genes" that enable her sons to be better at competing for fertilization; the avoidance of inbreeding; and the avoidance of fertilization by genetically incompatible sperm,

which can lead to the formation of non-viable embryos.

Male-female conflict

Biologists who study sexual selection agree that, unless a species is truly monogamous, a conflict between males and females over reproduction exists, in the sense that males want to copulate with as many females as possible and fertilize as many eggs as possible, whereas females want only the "best" males to fertilize their eggs.

But is postcopulatory sexual selection driven primarily by cryptic female choice in favor of male traits that ultimately benefit both males and females, according to an evolutionary cost-benefit analysis? Or is it driven by male-female conflicts that lead to so-called antagonistic coevolution, in which male adaptations evolve that induce females to mate even when mating can harm females in some way, and female counteradaptations evolve that minimize potential harm from males?

What is probably the best evidence for such male-female conflict over postcopulatory sexual selection comes from the work of William Rice, of the University of California-Santa Barbara. In the 16 May 1996 issue of *Nature*, Rice described studies in which *D. melanogaster* females were artificially prevented from coevolving with males, while "adapting males" could continue to evolve in response to females. After only 41 generations of promiscuous mating, the fitness of the adapting males was greater than that of a group of control males: Adapting males produced more male offspring and were better at obtaining matings with previously mated females as well as at preventing the females with whom they had mated from producing offspring by later males—most likely due to effects that have been attributed to male seminal fluid components. But these favorable adaptations occurred at the expense of females. Females had higher death rates when exposed to adapting males than when exposed to control males, at least in part because males evolved to be better able to "persuade" females to remate with them

despite the fact that seminal fluid has toxic effects on females. Additionally, in one of two adapting male fruit fly lines studied, the seminal fluid itself apparently evolved to be more toxic.

Further support for the concept of antagonistic coevolution was provided by a recent study by Rice and his former graduate student, Brett Holland, in which sexual selection was experimentally removed for both males and females through enforced monogamous mating and random mate assignment. As Rice and Holland reported in the 27 April 1999 issue of *Proceedings of the National Academy of Sciences*, under these mating conditions the pattern that Rice had observed in his 1996 study was reversed. That is, males evolved to be less harmful to females, and females evolved to be less resistant to male-induced harm.

In particular, seminal fluid evolved to be less toxic to females, and the frequency of aggressive male courtship behavior was reduced. In addition, monogamous females were harmed more than the promiscuous control females when they mated with promiscuous males, suggesting that female resistance to the toxic effects of seminal fluid diminishes in the absence of sexual selection.

Eberhard concedes that Rice and Holland's results show that a male trait (seminal fluid components) that is subject to sexual selection can exert a cost on females. But, he says, so far this is only a single trait in a single species that occurs under one particular set of circumstances. Although Rice agrees that the general applicability of his findings remains to be determined, he says that "there's nothing that in my mind makes this result idiosyncratic to flies. I see the same conflicts of interest in virtually any male-female system."

"There is a conflict in that the male wants to fertilize all the eggs of all the females he ever finds, and the female doesn't necessarily want that particular male to fertilize all her eggs," Eberhard says. "But the question of who's gaining what in this interaction is open to debate." Rather than seeing females as

being hurt by male manipulations and responding by evolving ways to resist this harm, Eberhard believes that, in the face of male-male competition, females will often win by "selective cooperation"—cooperation with males that have certain traits and rejection of other males.

Taking stock

The relative importance of sperm competition, cryptic female choice, and antagonistic coevolution in driving the rapid evolution of male traits such as genital morphology and seminal fluid components remains a question for the future. Eberhard's 1996 book helped raise biologists' consciousness regarding cryptic female choice as a potentially significant factor in paternity determination and sexual selection, and it helped stimulate research and discussion on the subject. As a result, Eberhard says, "my sense is that people are less opposed to or outraged by the possibility of females actually having effects on these processes than they were several years ago."

However, although Birkhead says he has "an open mind that females probably do control things much more than we currently appreciate," he says that

cryptic female choice may be more important in some species, such as insects, than in other species, such as birds, in which precopulatory behaviors are the main way that females choose among mates.

"There's quite good evidence from a number of [bird] species now that females seem to actively choose their extra-pair copulation partners," he says. "Often, but not always, these are birds who are of better quality than her social partner. That strongly suggests that females are making very active choices about who their extra-pair copulation partner is going to be. And if they can do that, and if they can time these extra-pair copulations well relative to when their eggs would be fertilized, then this might be sufficient for female birds to control paternity to the extent that they have to."

When it comes to the importance of cryptic female choice in relation to sperm competition mechanisms, Robert Smith, of the University of Arizona, believes that "there will be lots of demonstrations of [female manipulation of competing ejaculates] in the future." The study of cryptic female choice, he says, "is a natural extension of the study of sperm competition that

is certainly a frontier for the next decade in the field."

Geoffrey Parker, of the University of Liverpool, who pioneered the field of sperm competition research, has long been skeptical of the role of cryptic female choice in determining the outcome of male-male battles over paternity. But now, he says, "I think that the balance probably is that some species show very little female control at all and are almost entirely male controlled, other species are probably the reverse, and most species are probably in some rather boring hinterland between the two extremes."

Although in the view of some researchers in the field, Eberhard may overemphasize the female's role, his work "helps put the balance right," Parker says, after many researchers, including Parker, focused mainly on males and underplayed the female's role for many years. "The basic objective of my 1996 book was to stimulate people to take the idea seriously and to design experiments and observations to see whether it is or isn't happening," Eberhard says. "On that scale this is about how I'd hoped it would go," he adds. "Though I hadn't anticipated that it would be quite so controversial." □