The evolution of polyandry II: post-copulatory defences against genetic incompatibility

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SUMMARY

Fundamental to the recently-proposed hypothesis that females mate with more than one male as a hedge against genetic incompatibility is the premise that mechanisms are available to polyandrous females which enable them to safeguard their reproductive investment against the threat of incompatibility between maternal and paternal genomes. Accumulation of sperm from several males shifts the arena for sexual selection from the external environment to the female reproductive tract where, we suggest, interactions at the molecular and cellular levels provide females with direct mechanisms for assessing genetic compatibility. We present examples from the literature to illustrate how sperm competition and female choice of sperm can enable polyandrous females to minimize the risk of fertilization by genetically-incompatible sperm. Polyandry and multiple paternity also create the opportunity to reduce the cost of genetic incompatibility by reallocation of maternal resources from defective to viable offspring. This is likely to be a critically important post-copulatory mechanism for viviparous females whose intimate immunological relationship with developing embryos makes them particularly vulnerable to genetic incompatibility arising from intragenomic conflict and other processes acting at the suborganismal level.

1. INTRODUCTION

In his classic paper on sexual selection, Trivers (1972) recognized genetic complementarity as a potentially important criterion for female choice of mate. However, the full significance of genetic incompatibility as a force driving female mating strategies is only now becoming apparent, as evidence accumulates that cellular endosymbionts, transposable elements, segregation distorters, maternal-effect lethals, hypervariable DNA and imbalances between genomically-imprinted genes can all undermine female fitness by rendering certain combinations of maternal and paternal haplotypes incompatible within the developing embryo (Zeh & Zeh 1996). Sexual reproduction thus involves the merging in embryos of parental genomes likely to vary in the extent to which they are genetically compatible.

Unlike other ideas presented in Trivers' (1972) paper, female choice based on genetic complementarity has received little attention, at least in part because it has not been obvious how females could recognize genetically incompatible males. Pre-copulatory mate choice based on male phenotype appears to provide little scope for females to match male genotype against their own (Parker 1992; but see Drickamer & Lenington (1987) and Lenington *et al.* (1994) for an important exception). Similarly, strong mating order effects on sperm utilization (Birkhead & Hunter 1990) appeared to provide little opportunity for mechanisms operating at the post-copulatory stage. However, this view is now being called into question by increasing molecular evidence that multiple paternity is widespread in nature, with data currently available on many species of birds (reviewed in Birkhead & Møller 1995), several mammals (Inoue *et al.* 1990; Tegelström *et al.* 1991; Amos *et al.* 1993; Schenk & Kovacs 1995), as well as some snakes (e.g. Stille *et al.* 1986; Schwartz *et al.* 1989), turtles (Galbraith 1993), isopods (Heath *et al.* 1990), insects (Gromko *et al.* 1984; Moritz *et al.* 1995; Oldroyd *et al.* 1995), spiders (Martyniuk & Jaenike 1982; Oxford 1993), and pseudoscorpions (Zeh & Zeh 1994). In addition, recent research has shown that last-male sperm precedence can be an artifact of two-male, laboratory mating experiments (Zeh & Zeh 1994), and that mating order effects can vary with mating context (Siva-Jothy & Tsubaki 1989; Radwan 1991; Bauer 1994; Otronen 1994). This relaxation of mating order constraints on sperm utilization suggests that the opportunity for post-copulatory sexual selection may be much greater in nature than was previously supposed.

In a recent paper, we proposed that females mate with more than one male as a hedge against genetic incompatibility arising as a secondary consequence of various agents of intragenomic conflict and other forces acting at the suborganismal level (Zeh & Zeh 1996). Fundamental to this hypothesis is the premise that post-copulatory mechanisms are available to polyandrous females which enable them to safeguard their reproductive investment against the threat of genetic incompatibility. Were this not the case, a polyandrous

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female would, on average, suffer the same reproductive cost of incompatibility as a female randomly mated to a single male. Here, we propose that accumulation of sperm from several males shifts the arena for sexual selection from the external environment to the female reproductive tract, where interactions at the molecular and cellular levels can provide females with direct mechanisms for assessing genetic compatibility. We present examples from the literature to illustrate how sperm competition and female choice of sperm, as well as reallocation of maternal resources from defective to viable offspring, can serve as post-copulatory mechanisms for minimizing the risk and/or cost of fertilization by genetically-incompatible sperm.

2. POST-COPULATORY DEFENCES AGAINST INCOMPATIBILITY

(*a*) *Sperm competition*

While behavioural ecologists have generally viewed sperm competition as a mechanism enhancing female reproductive success (henceforth, RS) through selection for sperm of high genetic quality (e.g. Madsen *et al.* 1992; Birkhead *et al.* 1993), sperm competition can also enable females to reduce the probability of fertilization by genetically incompatible sperm. In *Drosophila* meiotic drive systems, for example, heterozygous males may be at a disadvantage in sperm competition since they produce up to 50 % fewer viable sperm per ejaculate than males not carrying the drive allele (Wu 1983). Haig & Bergstrom (1995) have argued that if females mate with several males, this handicap restrains the spread of drive alleles, with genes that promote polyandry being selected to reduce the advantage of the distorter allele. From the standpoint of meiotic drive's negative impact on female RS, sperm competition is most advantageous for females who are themselves heterozygous at the drive locus. In addition to producing heterozygous sons of lower competitive ability, heterozygous females face the more immediate risk of mating with a heterozygous male and producing offspring which are homozygous at the drive locus, and consequently inviable or infertile (see Zeh & Zeh 1996).

(*b*) *Female choice of sperm*

Crosses between closely-related and partially reproductively compatible species of grasshoppers (Hewitt *et al.* 1989), crickets (Howard & Gregory 1993) and beetles (Wade *et al.* 1994) suggest that post-copulatory sexual selection may play an important role in preventing the production of defective offspring. In these studies, although at least some viable, hybrid offspring were produced from heterospecific crosses, any mating order effects were overridden when females mated with both a conspecific and a heterospecific male, and eggs were fertilized by conspecific sperm (the most genetically compatible). This could result from sperm competition in which conspecific sperm are better adapted to negotiate the female reproductive tract (Eberhard 1996). Alternatively, females may recognize differences between sperm genotypes and either actively choose sperm to be used in fertilization or bias against certain genotypes through inhibition or preferential sperm loss (Zimmering *et al.* 1970). Although few studies have directly investigated the mechanisms of non-random sperm utilization, there is evidence for compatibility-based discrimination against sperm genotypes in the female reproductive tracts of *Drosophila* (Zimmering & Fowler 1968; Childress & Hartl 1972), flour beetles (Lewis & Austad 1990), mice (Bateman 1960), rabbits (Cohen & Werrett 1975), Swedish sand lizards (Olsson *et al.* 1996) and humans (Dondero *et al.* 1978).

How might such female choice of sperm genotype occur? It is now known that, in mammals, several cell-surface proteins of spermatozoa are synthesized through haploid gene expression during spermiogenesis (e.g. Klemm *et al.* 1989; Erickson 1991; Penttilä *et al.* 1995; Choudhary *et al.* 1995). These macromolecules can stimulate production of auto-antibodies in males, and are normally sequestered from the immune system by the blood-testis barrier (Bellvé *et al.* 1990). After transfer to the female, sperm are perceived as antigens and must run the gauntlet of a female reproductive tract populated by large numbers of anti-sperm leucocytes and antibodies (see Birkhead *et al.* 1993). Of the 40 to 1800 million sperm deposited, for example, in the human vagina, approximately only 300 reach the site of fertilization (Austin 1995). The sperm antigens responsible for anti-sperm immune infertility in humans have been identified as a small group (3–5) of sperm-surface glycoproteins (Primakoff *et al.* 1990). In mice, sperm antigens induce cell-mediated immune factors that decrease sperm motility and affect embryonic development (Naz & Mehta 1989). In addition, anti-sperm antibodies can impair sperm function both at the level of cervical mucus-penetrating ability (Bronson *et al.* 1987; Jager *et al.* 1987) and gamete interaction (Clarke *et al.* 1985; Mandelbaum *et al.* 1987; D'Almeida *et al.* 1989).

The ability of the immune system to distinguish between proteins differing by only a single amino acid, or even between optical isomers of the same protein (Alberts *et al.* 1994), makes it highly likely that, in any particular female, sperm from different males may differ in the extent to which they are perceived as non-self. Strong support for this hypothesis is provided by clinical testing of apparently infertile human couples in which the male produced normal semen with no anti-sperm auto-antibody. In one third of such couples, the female's cervical mucus agglutinated her partner's spermatozoa but not donor spermatozoa (Dondero *et al.* 1978). While an invertebrate analogue to T- and B-cell immune recognition has not been found, experiments on metazoans ranging from sponges to colonial tunicates have documented a natural invertebrate immunity capable of rapid allorecognition and have shown that the processes involved in invertebrate immunity are dependent on an 'exquisite recognition specificity' (Humphreys & Reinherz 1994).

Birkhead *et al.* (1993) have proposed that female anti-sperm responses provide mechanisms by which females ensure that their eggs are 'fertilized by the fittest sperm, or minimize the risk of being fertilized by

the 'worst' sperm in the population'. Whereas their hypothesis posits female choice based on inherent male genetic quality, the genetic incompatibility hypothesis asserts that sperm quality is a relative characteristic which depends, at least in part, on the genotype of the female herself. Consistent with the hypothesis that the female anti-sperm immune response discriminates against genetically-incompatible sperm is the fact that some spermatozoan cell-surface antigens are the products of loci critically important in embryonic development (Van Blerkom 1977). In mice, for example, F9 antigen, present on spermatozoa and also expressed by pre-implantation embryos, is associated with the abnormal development of primitive teratocarcinoma cells and is thought to be the product of the developmentally critical mouse *t*-locus (see Van Blerkom 1977). Since meiotic drive alleles sabotage alternative alleles during spermiogenesis, it is not surprising that genes known to exhibit haploid expression in spermatids include genes located within the mouse *t*-complex meiotic drive region (Schimenti *et al.* 1988). Particularly intriguing from the point of view of cell recognition is the finding that also included in the *t*-complex region is a gene encoding a polypeptide likely to facilitate the species-specific binding of sperm to eggs (Silver 1993).

Histocompatibility genes also exhibit parent-of-origin-dependent patterns of expression, with maternal non-H2 alloantigens evident at all stages of mouse embryogenesis from the two-cell to the 4.5-day-old blastocyst stage, but with paternal antigens only becoming obvious at the six- to eight-cell stage (Muggleton-Harris & Johnson 1976). As Van Blerkom (1977) points out, such differential expression could have a central role in establishing cell-to-cell communication within the embryo and between embryonic and maternal cells. In humans, the presence in females of circulating anti-sperm antibodies is associated with an increased incidence of spontaneous abortion, while in female cattle, guinea-pigs, mice and rabbits, immunization with sperm caused an increased incidence of post-fertilization infertility resulting from pre-implantation embryo mortality (reviewed in Menge 1980). Prefertilization interaction between sperm genotype and the female immune system may thus provide a reliable indicator of post-fertilization complementarity between maternal and paternal genotypes.

The extent to which female mammals can discriminate between individual sperm produced by a single male remains controversial (see Austin 1995). Although haploid gene expression does occur, immunocyto-chemical analyses have established that gene products can move through the intercellular bridges connecting spermatids developing within a common syncytium (Braun *et al.* 1989). However, this study has demonstrated only that products diffuse down a concentration gradient to spermatids which completely lack a gene. It did not show that alternative forms of the same haploid-expressed gene product are mixed and shared equally between all the member spermatids of a syncytium (Barratt 1995). The finding that X- and Y-bearing sperm in mice exhibit pronounced variation in quantity of histocompatibility-Y (H-Y) antigen present on the sperm head strongly suggests that haploid-expressed gene products are not equally shared (reviewed in Koo *et al.* 1977). Whether sperm phenotype reflects haploid or diploid gene expression is, in any case, relatively unimportant vis-à-vis the post-copulatory potential for females to recognize genetic incompatibility generated by selfish genetic elements. Essentially all the viable sperm produced by a male heterozygous for a meiotic drive allele carry the drive allele (Lyttle 1991). Similarly, the modifications to sperm genotype caused by transposable elements and cellular endosymbionts are likely to affect all the sperm produced by a male carrying such a genetic element.

In theory, choice of sperm could enable females to minimize the risk to their RS posed by cellular endosymbionts (Zeh & Zeh 1996). For example, in the presence of feminizing agents or male killers, selection on nuclear genes should favour mutations which suppress the activity of the cytoplasmic sex ratio distorters (Hurst 1991), as occurs in the isopod, *Armadillidium vulgare* (Juchault *et al.* 1993) and several neotropical *Drosophila* species (Williamson & Poulson 1979). Infected females could therefore enhance their RS through polyandry and choice of sperm carrying neutralizing nuclear alleles. In the case of *Wolbachia*-generated cytoplasmic incompatibility, the endosymbiont appears to cause protein composition changes in the reproductive tissues of infected males (Karr 1994). Polyandrous, uninfected females could conceivably recognize these endosymbiont effects on sperm phenotype and discriminate against such sperm. Polyandry, however, appears to have the reverse effect in *Tribolium confusum* beetles (Wade & Chang 1995). In uninfected females, post-copulatory sexual selection apparently favours sperm from males infected with *Wolbachia pipiens* over sperm from antibiotically cured males, even though such fertilization results in embryo inviability. However, interpretation of these results is complicated by potential antibiotic effects on sperm mitochondrial function in cured males. Moreover, the female founders of the infected laboratory stock presumably became infected through an inability to respond to the cellular endosymbiont. Consequently, the uninfected females derived from this stock by antibiotic treatment (Wade & Chang 1995) would not be expected to exhibit a response to the bacterium.

Particularly intriguing is the evidence that female choice of sperm may occur even after sperm have penetrated eggs. In the ctenophore, *Beroe ovata*, egg penetration by several sperm (polyspermy) is common and can delay first cleavage by several hours. During this interval, each sperm remains immobilized at its point of entry while the egg pronucleus '... acts as if it was choosing a mate' (Carré & Sardet 1984; Carré *et al.* 1991). It may fuse with the first sperm pronucleus encountered or may migrate back and forth between the maturation pole and as many as three sperm penetration sites before fusing with one sperm pronucleus. In birds and reptiles, a similar process may occur: several sperm may be allowed to penetrate the egg and form pronuclei but only one fuses with the egg

pronucleus (Birkhead *et al.* 1993). Although infrequent, polyspermy also occurs in mammals (Kovacs *et al.* 1991; Navara *et al.* 1994). Further evidence of the ability of eggs to discriminate between sperm genotypes comes from crosses between closely-related species of mice. Kaneda *et al.* (1995) present compelling evidence that elimination of paternal mitochondria is triggered by the egg cytoplasm recognizing species-specific, nuclear encoded proteins in the sperm midpiece.

A final example, involving one of the few known cases of meiotic drive in females, suggests that a general feature of vertebrate meiosis, postponement of the second meiotic division in eggs until after fertilization, may provide females with an additional opportunity for incompatibility avoidance. In female mice heterozygous for a meiotic drive locus on chromosome 1, chromatid segregation depends on the haplotype of the fertilizing sperm (Agulnik *et al.* 1993). While egg penetration by wild-type sperm results in strong meiotic drive, with 85% of wild-type chromatids being diverted to polar bodies, segregation normalizes to 50:50 when a drive-haplotype sperm enters the egg. This ability to modify segregation patterns in response to sperm genotype has major fitness benefits for heterozygous females since it reduces the proportion of offspring which fail because they are homozygous for the drive allele.

(*c*) *Reallocation of maternal investment*

Compatibility between maternal and paternal genomes is likely to be particularly critical for species in which both fertilization and embryonic development occur within the female. For a viviparous female, the optimal sperm genotype is likely to be one which interacts with her reproductive tract without generating a strong immunological anti-sperm response yet, at the same time, is sufficiently different at critical recognition loci to establish the immunological détente between mother and foetus essential for normal development (Beer *et al.* 1982). In their review, Beer *et al.* (1982) discuss several lines of evidence that incompatibility can result from a lack of distinction between maternal and paternal genotypes. For example, it has been shown that a significantly higher proportion of women experiencing repeated miscarriages shared common major histocompatibility complex (MHC) antigens with their husbands when compared to control groups, indicating that foetuses not possessing alleles distinct from their mothers' may be less capable of triggering a protective blocking antibody response. Similarly, the finding that, in couples with recurrent spontaneous abortion of karyotypically-normal foetuses, there was a significantly depressed response of the female's lymphocytes when stimulated by the respective spouse's lymphocytes but not when stimulated by the donor lymphocytes, led to the suggestion that this resulted from a failure of the mother's cellular immune system to respond to the paternal histocompatibility antigens. This hypo-responsiveness was not detected in abortions involving karyotypically abnormal foetuses. These clinical data support the controversial view (see Pusey & Wolf 1996) proposed by Shields (1982) and Bateson (1983) that optimal outbreeding, a phenomenon known to occur in plant populations (reviewed in Marshall & Folsom 1991), may also be a factor favouring polyandry in animals.

Polyandry provides females of viviparous species with a mechanism for reducing the cost of fertilization by incompatible sperm which is not available to females that lay eggs. By mating with several males and producing mixed paternity litters, viviparous females have the opportunity to shunt resources from genetically-defective to viable embryos. This mechanism is likely to be particularly effective if females typically produce more zygotes per litter than can survive to birth. Consider, for example, the intriguing case of asymmetrical, reproductive incompatibility exhibited by the DDK mouse strain, in which the embryos of DDK females mated to non-DDK males failed before or soon after implantation (see Renard *et al.* 1994). Since female mice eliminate as many as one third of their fertilized eggs without affecting total litter size (Hull 1964), reallocation of maternal resources should, on average, result in a DDK female mated to both a DDK and a non-DDK male, suffering a negligible impact on her RS compared to a randomly mated, monogamous DDK female.

Ironically, it is this capacity of viviparous females to reallocate resources which also makes them especially vulnerable to intragenomic conflict. Indeed, Haig & Graham (1991) have argued that genomic imprinting can only evolve in the context of post-zygotic maternal investment and multiple paternity. A theoretical model by Hurst (1991) has also shown that redirection of nutrients to female offspring following death of male embryos is critical for the spread of cytoplasmic male killers. In addition, in meiotic drive systems, the ability of females to reallocate resources may have the counter-intuitive effect of generating selection that favours recessive, lethal alleles at loci closely linked to the drive locus. In the *t*-complex system, for example, since any sons homozygous for the distorter allele would be completely sterile, early homozygote death and reallocation can be to the benefit of both the female and the drive allele (Lyttle 1991; Charlesworth 1994).

3. CONCLUSIONS

In this review of data from diverse biological and clinical sources, we have found extensive circumstantial evidence that post-copulatory mechanisms do exist which may enable polyandrous females to reduce the threat of genetic incompatibility. Elsewhere, we have shown that the cumulative effects of intragenomic conflict and other processes operating at the sub-organismal level may significantly undermine the reproductive cohesiveness of natural populations (Zeh & Zeh 1996). This raises the question of why all females do not engage in polyandry. Whether or not females opt to mate with more than one male will depend, of course, on whether the reproductive benefits to polyandry outweigh the costs. Such costs include increased time allocated to mating and increased risk

of predation, greater exposure to sexually-transmitted diseases and potentially harmful seminal products, as well as retribution and/or withdrawal of paternal care by the female's first mate (see Keller & Reeve 1995). It is increasingly evident from molecular data showing high levels of mixed paternity in the offspring of females previously thought to be monogamous that females can reduce these latter costs by concealing their polyandrous behaviour from males. The discreet nature of polyandry may explain why, although complementarity has long been recognized as a important factor influencing gamete competition and differential abortion in plants (reviewed in Marshall & Folsom 1991), the implications of genetic incompatibility for female mating behaviour have not been fully appreciated in animals.

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