Sexy sons: a dead end for cytoplasmic genes

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Critics of sexual conflict theory argue that females may gain a net reproductive benefit from mating with manipulative males because the direct costs that they suffer may be offset by the production of sexy, i.e. manipulative, sons. However, this exclusive focus on nuclear gene effects represents an incomplete view of female fitness. Females differ fundamentally from males in transmitting not only nuclear genes but also a wide range of cytoplasmic genetic elements (CGEs) that can have profound effects, from male killing to influencing development of the nervous system and cognitive ability. Maternal transmission of CGEs has two major implications for sexual selection. First, the evolution of male fitness traits, such as sperm competitive ability, may be constrained because response to selection on mitochondrial genomes can occur only through the female line. Second, CGEs bear the direct costs of male manipulation but gain no indirect benefits when females produce sexy sons. This should result in perpetual antagonistic coevolution between nuclear genes involved in male manipulation and CGEs that promote female resistance to male sexually selected traits. Explicit consideration of the consequences of selection acting on CGEs is therefore necessary for a better understanding of the relationship between sexual selection and sexual conflict.

Keywords: antagonistic coevolution; cytoplasmic genes; mitochondria; nucleocytoplasmic conflict; sexy sons; *Wolbachia*

1. INTRODUCTION

Traditional sexual selection theory is being challenged by growing evidence that the reproductive interests of males and females frequently differ and therefore generate sexual conflict rather than cooperation (Chapman *et al.* 2003). According to the good-genes and Fisherian runaway models, choosy females benefit indirectly from mate choice by producing high-viability offspring and/or sexy sons (Kokko *et al.* 2002). With few exceptions (e.g. Parker 1979), this view of cooperative male–female interactions has, until recently, pervaded mathematical formulations of the sexual selection process (Holland & Rice 1998). However, the importance of conflict in sexual selection is now gaining recognition with the realization that polyandry is a pervasive feature of natural populations. Theory predicts that mating system is a critical determinant of the intensity of sexual conflict (Zeh & Zeh 2003). With strict monogamy,

male and female reproductive interests coincide and selection favours cooperative males that maximize their mates' lifetime reproductive success. By contrast, when both sexes copulate with multiple partners, male and female interests diverge, and conflicts occur over, for example, mating frequency, timing and pattern of fertilization, relative parental effort, female re-mating behaviour, female reproductive rate and clutch size (Chapman *et al.* 2003).

In the debate over the importance of sexually antagonistic coevolution, criticisms have focused on the failure to (i) consider alternative hypotheses in interpreting empirical patterns (Pizzari & Snook 2003), and (ii) incorporate the indirect benefits that females may gain from producing manipulative sons (Cordero & Eberhard 2003). It has been suggested that an indirect, sexy-son benefit could outweigh direct costs, resulting in an overall benefit for females (Cordero & Eberhard 2003). Apparent male–female conflict could thus form part of a reproductive interaction whose net coevolutionary effect is cooperative and therefore in accordance with traditional sexual selection theory (Pizzari & Snook 2003). This suggestion is, however, not supported by theoretical analyses indicating that the production of manipulative sons cannot alone outweigh the direct costs to females (Cameron *et al.* 2003).

Here, I propose that understanding the relationship between sexual selection and sexual conflict requires incorporating the effects of selection acting not only on nuclear genes (as indirect, sexy-son benefits) but also on genes present in the cytoplasm. Females differ fundamentally from males in transmitting both nuclear genes and a variety of cytoplasmic genetic elements (CGEs). The latter can have profound phenotypic effects, from male killing by cellular endosymbionts (Charlat *et al.* 2003) to mitochondrial haplotype influence on the development of the nervous system and cognitive ability (Roubertoux *et al.* 2003). It has long been recognized that the differing modes of inheritance of nuclear and CGEs create the potential for nucleocytoplasmic conflict (e.g. Eberhard 1980; Cosmides & Tooby 1981; Hurst 1993). However, the general implications of maternal inheritance of CGEs for sexual selection and sexual conflict have received little attention. Maternal transmission of CGEs has two major consequences for sexual selection. First, the evolution of male fitness traits, such as sperm competitive ability, may frequently be constrained because response to selection on mitochondrial genomes can occur only through the female line. Second, CGEs bear the direct costs of male manipulation but gain no indirect benefits when females produce sexy sons. This should result in perpetual antagonistic coevolution between nuclear genes involved in male manipulation and CGEs that promote female resistance to male sexually selected traits.

2. CYTOPLASMIC GENES AND SEX RATIO DISTORTION

Whereas males produce large numbers of small, inexpensive sperm, females produce relatively few, large and costly eggs. The economic implications of anisogamy for sexual selection were first recognized by Trivers (1972) who identified differential investment in gametes as a fundamental force driving the evolution of divergent mating tactics in males and females. The genetic implications of anisogamy for sexual selection, however, have been largely ignored. In most animal species, anisogamy is coupled

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with the strictly maternal inheritance of CGEs and males are effectively a dead end for any non-nuclear genetic elements. These include cellular organelles, such as mitochondria in plants and animals and chloroplasts in plants, as well as cellular endosymbionts, such as protists, bacteria and viruses. As a consequence of maternal inheritance, selection promotes CGEs that increase their transmission rate by biasing sex ratio in favour of females (Eberhard 1980; Cosmides & Tooby 1981). Female bias, in turn, generates selection on biparentally inherited nuclear genes to restore the sex ratio to 1 : 1, triggering an evolutionary arms race over optimal sex ratio.

Sex ratio distortion is widespread in invertebrates and angiosperms, and is achieved through a variety of mechanisms by a diversity of CGEs. The most intensively studied are members of the bacterial genus, *Wolbachia*. By some estimates, these intracellular bacteria occur in 76% of insect species (Jeyaprakash & Hoy 2000), and have also been isolated from nematodes, crustaceans and arachnids (Charlat *et al.* 2003). *Wolbachia* achieve female bias by either killing male embryos, feminizing diploid males or inducing parthenogenesis in haplodiploid species (Charlat *et al.* 2003). Other cellular endosymbionts, such as *Rickettsia* and *Spiroplasma* bacteria, and microsporidian, unicellular eukaryotes, have also been implicated in male killing and feminization (Hurst 1993; Werren *et al.* 1994). Cellular organelles can also manipulate sex ratios to enhance their own transmission. In angiosperms, mitochondrial mutants can convert hermaphrodites into females by undermining functional pollen production, resulting in a gynodioecious mating system in which hermaphrodites and females co-occur. An estimated 7.5% of European angiosperms are gynodioecious, and loss of male function has been linked to mitochondrial mutations in many species (Budar & Pelletier 2001). The causes of male sterility range from complete absence of male organs to dysfunctional pollen. Reallocation of resources from male to female function may result in increased transmission of CGEs.

High frequencies of certain CGEs can produce heavily female-biased populations, with dramatic and very apparent consequences for sexual selection. For example, the shortage of males resulting from *Wolbachia* infection rates exceeding 90% has caused some populations of the butterfly, *Acraea encedon*, to shift to a sex-role-reversed mating system in which virgin females congregate in lekking swarms to solicit matings (Jiggins *et al.* 2000).

3. UNIPARENTAL INHERITANCE OF CYTOPLASMIC GENES: IMPLICATIONS FOR THE EVOLUTION OF SPERM PERFORMANCE

Even without sex ratio distortion, maternal inheritance of CGEs has potentially important, albeit less obvious, implications for sexual selection. First, although there is likely to be intense selection for sperm competitive ability, response may be constrained, insofar as mitochondrial haplotype contributes to sperm performance. In domestic fowl, for example, sperm mobility is correlated with sperm competitive ability and breeding experiments indicate that '… a maternally transmitted genetic element, independent from autosomal genes, is involved in determining sperm mobility' (Froman *et al.* 2002, p. 609). Although direct,

sequence-based evidence is currently lacking, these patterns are consistent with mitochondrial influence on sperm competitive ability. Moreover, there is compelling evidence that mitochondrial mutations that cause sperm dysfunction may be maintained in populations because mitochondria are maternally inherited. In humans, a common cause of male infertility in which a high percentage of sperm are immotile (asthenozoospermia) has been linked to mitochondrial DNA (mtDNA) haplotypes with an impaired oxidative phosphorylation system (Ruiz-Pesini *et al.* 2000). Recognizing that maternal inheritance of mitochondria may limit sexy-sperm effects, Pizzari & Birkhead (2002) suggest that this barrier may be circumvented by male choice of females carrying mitochondria with superior fertilization capability. However, given that mitochondria are selected in females to function in somatic and female germ-line cells, it seems unlikely that this mechanism could draw on mitochondria optimally adapted for sperm competition.

The inability of selection to hone mitochondria for sperm performance may have far-ranging implications. It may, for example, be a factor that contributes to the great taxonomic diversity in sperm morphology. With mitochondrial function unavailable as an avenue for adaptive evolution of sperm phenotype, selection is likely to be particularly intense on sperm traits encoded by nuclear genes that can respond to selection. Female-limited response to selection on mitochondria provides a possible explanation for the observation that homo-population males sometimes lose in sperm competition with males from closely related populations, as occurs in dung flies (Hosken *et al.* 2002). While the sperm of insect species are equipped with mitochondrial derivatives whose contribution to sperm motility is poorly understood (Werner *et al.* 2002), population differences in sperm competitive ability could theoretically be the indirect consequence of divergent selection for mitochondrial function acting on females from the two populations.

An intriguing exception to strict maternal mtDNA inheritance occurs in some bivalve molluscs in which mitochondria are transmitted both maternally (F mtDNA) and paternally (M mtDNA) (Zouros *et al.* 1994). Females transmit F mtDNA to both daughters and sons. Sons also inherit the M mtDNA from their fathers. Of particular interest is the distribution of these mitochondrial types in sexually mature males. Whereas F mtDNA is predominant in somatic tissues, the M mtDNA predominates in the testes. Sperm contain only M mtDNA. Such doubly uniparental inheritance circumvents constraints associated with maternal mtDNA transmission, and could facilitate adaptive evolution of paternally inherited mtDNA for sperm performance. Consistent with this interpretation is the fact that sequence divergence between closely related bivalve species is much greater for M mtDNA than for F mtDNA (e.g. Stewart *et al.* 1996). This pattern holds for synonymous and non-synonymous substitutions, and has been attributed to greater selective constraints on F mtDNA that must function in both somatic and female germ-line cells (Stewart *et al.* 1996). The higher rate of sequence divergence in M mtDNA could also result from species differences in selection pressures on sperm, stemming from differences in the abiotic environment or in the intensity of sperm competition. The selection on sperm

performance hypothesis was rejected for the *Mytilus edulis–M. trossulus* species pair by Stewart *et al.* (1996), based on the higher level of sequence polymorphism in the M lineage than in the F lineage (in *M. edulis* but not in *M. trossulus*). However, spatially varying selection, nucleo–mitochondrial interactions and differing patterns of multi-level selection in the M and F lineages (see Rand 2001) could maintain M mtDNA polymorphism in an externally fertilizing species, despite selection for sperm performance. Directly testing this hypothesis requires analysis of sperm performance under varying environmental and competitive regimes.

4. UNIPARENTAL INHERITANCE OF CYTOPLASMIC GENES: IMPLICATIONS FOR SEXUAL CONFLICT

Maternal inheritance of CGEs also has previously unrecognized implications for the relationship between sexual selection and sexual conflict. Full assessment of the relative contributions of conflict and cooperation between the sexes must necessarily involve consideration of the reproductive interests of the entire cast of nuclear genes and cytoplasmic genomes that are represented by the female. Because of strict maternal inheritance, CGEs suffer the direct costs of male manipulation but receive no indirect benefits from the production of sexy sons. As a consequence of this nucleocytoplasmic difference in reproductive interests, there is likely to be perpetual antagonistic coevolution between nuclear genes encoding male manipulation and CGEs that promote resistance by the female to male manipulation of her physiology and control of her reproductive options.

The above hypothesis predicts involvement of cytoplasmic factors and/or nucleocytoplasmic interactions in genetic control of female resistance behaviour. Unfortunately, to my knowledge, no studies have yet been designed to test this prediction. Indeed, the importance of CGEs has been ignored in sexual selection theory, as a consequence of the focus of the 'modern synthesis' on nuclear genes and Mendelian genetics. Moreover, the individual has traditionally been viewed as the product of a genome in which selfish activity of individual genes is held in check by selection favouring integrated functioning of the genome as a whole. However, as Cosmides & Tooby (1981, p. 88) point out: 'within the causal network found in cells, the complexities of biochemical pathways, the sensitivity of morphogenesis, the differential effects of environmental fluctuations …, the selection which occurs within an organism between various cytoplasmic genes, and so on, provide a situation dense with asymmetries that give advantage to some genetic factors at the expense of others. It is highly implausible that for every possible mutation there exists an immediate and reciprocal reversible selection possibility which would exactly cancel the exploitative phenotypic effect.'

Although the potential contribution of nucleo-cytoplasmic conflict to sexually antagonistic coevolution remains to be investigated, there is accumulating evidence for strong nucleocytoplasmic interaction effects on female fitness (Rand 2001). Moreover, support for the plausibility of CGE involvement in the evolution of female resistance behaviour is provided by very recent research demonstrating a profound effect of mitochondrial haplotype on development of the nervous system and cognitive

ability (Roubertoux *et al.* 2003). In this study, total substitution of mtDNA was achieved by 20 repeated backcrosses between two strains of mice with different mtDNA origins. Learning, exploration, sensory development and brain anatomy were all modified by interactions between mitochondrial and nuclear DNA. These results suggest a developmental pathway by which mitochondrial mutations could directly influence female behavioural responses to male manipulative traits.

5. CONCLUSIONS

With polyandry now known to be a pervasive feature of natural populations, there has been an explosion of interest in the factors influencing female reproductive success and the importance of male–female conflict in sexual selection (Zeh & Zeh 2003). The main thesis of this paper is that the debate over the importance of antagonistic coevolution between the sexes cannot be adequately resolved without due consideration of selection acting on maternally inherited CGEs. Anisogamy and uniparental inheritance of CGEs may not only place constraints on the adaptive evolution of male function but also seem likely to result in irreconcilable conflicts of interest between the sexes.

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