IDEAS IN ECOLOGY AND EVOLUTION 4: 14-23, 2011
doi:10.4033/iee.2011.4.3.n

Received 12 April 2011; Accepted 30 August 2011

New Idea

Post-plasmogamic pre-karyogamic sexual selection: mate choice inside an egg cell

Root Gorelick[1,2], Lindsay Jackson Derraugh[1], Jessica Carpinone[1], and Susan M. Bertram

Root Gorelick (Root_Gorelick@carleton.ca), Dept. of Biology, and School of Mathematics & Statistics, Carleton University, 1125 Colonel By, Ottawa, Ontario, CANADA K1S 5B6

Lindsay Jackson Derraugh (LindsayDerraugh@gmail.com), Dept. of Biology, Carleton University, 1125 Colonel By, Ottawa Ontario, CANADA K1S 5B6

Jessica Carprinone (JessicaCarpinone@gmail.com), Dept. of Biology, Carleton University, 1125 Colonel By, Ottawa Ontario, CANADA K1S 5B6

Susan M. Bertram (Sue_Bertram@carleton.ca), Dept. of Biology, Carleton University, 1125 Colonel By, Ottawa Ontario, CANADA K1S 5B6

[1] The first three authors contributed equally to the manuscript.
[2] Corresponding author

Abstract

Intersexual selection is often categorized as pre-copulatory or post-copulatory mate choice by individuals of one sex over showy individuals of the other sex. We extend the framework of post-copulatory choice to include post-plasmogamic pre-karyogamic sexual selection. That is, selection of haploid nuclei within the microcosm of a single fertilized egg cell after sperm has entered an egg cell but before fusion of their nuclear membranes, in which an egg nucleus chooses a sperm nucleus with which to fuse. The role of sexual selection at this nuclear level is probably small in monospermic out-crossers, but large in polyspermic out-crossers. Post-plasmogamic pre-karyogamic sexual selection may also explain how the sperm nucleus is rejected in gynogens, and how the egg nucleus is rejected in androgens. We propose that male and female nuclei behave independently within an egg cell, and that post-plasmogamic pre-karyogamic mate choice is largely based on either (1) a pre-existing sensory bias for certain characteristics of the sperm pronuclear membrane, or (2) detection of good genes through signal (mRNA) detection.

Key Words: Sexual selection, cryptic female choice, pronucleus, polyspermy, gynogenesis, androgenesis.

Introduction

Charles Darwin (1871) first introduced the idea of sexual selection to account for the differences in secondary sexual characteristics that could not be explained by natural selection. He defined sexual selection as differential reproductive success that results from variation among individuals in their success at obtaining mates. Darwin (1871) subdivided sexual selection into intrasexual selection, where one sex (typically males) competes aggressively with other members of the same sex for access to the opposite sex, and intersexual selection, where one sex (again typically males) competes with each other for the other sex's attention. Intrasexual selection usually results in the evolution of elaborate weaponry, aggressive behaviors, and defensive characteristics, while intersexual selection usually results in the evolution of exaggerated sexual signals and female preferences for those signals (Andersson 1994). Although Darwin (1871) proposed his ideas to account for elaborate pre-copulatory sexual displays and preferences, his ideas have since been expanded to include forms of post-copulatory choice, such as sperm competition (Parker 1970) and cryptic female choice (Thornhill 1983). Sperm competition occurs when sperm from multiple males compete with each other for the opportunity to fertilize an ovum (Parker 1970, Birkhead and Møller 1998). The result of sperm competition is evolution of male traits that increase the sperm's fertilization success including increased sperm number, increased sperm size, seminal fluids, sperm removal, and copulatory plugs or mate guarding (reviewed by Birkhead and Møller 1998, Simmons 2001, Snook 2005). Sperm competition can be thought of as the post-copulatory (pre-fertilization) equivalent to intrasexual selection.

Cryptic female choice is the post-copulatory equivalent to intersexual selection (Thornhill 1983, 1984). Eberhard (1996) defines cryptic female choice as the ability of females to consistently favor the copulatory partners that possess a particular trait that is lacking or less fully developed in other males. Cryptic female choice operates at several levels, including dumping sperm from previous mates (reviewed in Eberhard 1996) and increasing the duration of copulation (Hoikkala and Crossley 2000, Hoikkala et al. 2000). In the post-copulatory reproductive process, cryptic female choice can include increased sperm precedence (Edvardsson and Arnqvist 2000). Cryptic female choice also operates after zygote formation, including at the egg laying stage (laying more eggs; Barbosa 2009), at the developmental stage (through differential abortion of embryos; Hull 1964, Willson and Burley 1983), at the re-mating stage (enhanced resistance to future mates; King and Fischer 2005), and at the offspring stage through differential investment into offspring who were sired by different males (Willson and Burley 1983, Simmons 1987, Burley 1988, Eberhard 1996).

Here we extend this framework of sexual selection to include post-plasmogamic pre-karyogamic cryptic female choice of haploid nuclei within the microcosm of a single egg cell after entry of the sperm nucleus or nuclei into the egg (after plasmogamy), but before fusion of the egg and sperm nucleus (before karyogamy). Technically, egg and sperm nuclei (or pronuclei) do not usually fuse with one another, but instead their two nuclear membranes dissolve and are replaced with a single new nuclear membrane. Nonetheless, we will use the common vernacular of referring to karyogamy as nuclear or pronuclear fusion. We propose that in polyspermic outcrossers (multiple sperm nuclei within one egg), egg nuclei may be able to consistently favor sperm that possess a particular trait that is lacking or less fully developed in other sperm. Post-plasmogamic pre-karyogamic female choice within the microcosm of an egg cell would occur if the egg nucleus were to move about the cell and discriminate between multiple male nuclei before selecting one to fuse with. We hypothesize that this intersexual post-plasmogamic pre-karyogamic choice may regularly occur in polyspermic out-crossers based on tantalizing evidence from ctenophores (comb jellies; Carré and Sardet 1984). While it is theoretically possible for intrasexual selection and choice to exist inside an egg cell, conspicuously we do not see evidence for male-male nor female-female interactions within this egg microcosm.

Post-plasmogamic pre-karyogamic sexual selection has been very briefly mentioned by Tim Birkhead, but never fully developed. Birkhead (1998) includes a paragraph alluding to the possibility of post-plasmogamic pre-karyogamic sexual selection based on the intriguing work of Carré and Sardet (1984), which we also discuss below. Even more explicitly, albeit more succinctly, Birkhead and Brillard (2007) include two sentences on pronuclei choosing one another. A recent review of egg-sperm interactions (Karr et al. 2009) mentions nothing about post-plasmogamic pre-karyogamic sexual selection except to say that sperm nuclei may emit mRNA that egg nuclei can detect. None of the other major reviews of post-copulatory sexual selection or sexual choice—such as Eberhard (1996), Simmons (2001), Pizzari and Birkhead (2002), or Arnqvist and Rowe (2005)—allude to anything even remotely resembling post-plasmogamic pre-karyogamic sexual selection. Therefore, in this paper, we develop the theoretical framework by which post-plasmogamic pre-karyogamic female choice of haploid nuclei may occur. We present evidence showing the conditions of the framework, including independently behaving nuclei and differences between nuclei within an egg cell, especially with polyspermy. We also show how post-plasmogamic pre-karyogamic sexual selection may explain how the sperm nucleus is rejected in gynogens, and how the egg nucleus is rejected in androgens. And, finally, we briefly discuss how post-plasmogamic pre-karyogamic choice may make sexual selection more common than originally thought.

Post-plasmogamic pre-karyogamic sexual selection has the potential to exist

Two requirements must be met in order for post-plasmogamic pre-karyogamic sexual selection to occur. First, nuclei must be able to behave independently. Based on differences between egg and sperm nuclei in (1) presence of pores, (2) timing of nuclear membrane breakdown, and (3) timing of duplication and reduction events, we argue that male and female nuclei are controlled separately by nuclear rather than cytoplasmic factors. Because of this differential control between the two haploid sexes, egg nuclei may have the ability to choose sperm nuclei. Second, there must be detectable differences between sperm nuclei for post-plasmogamic pre-karyogamic female choice to have selective consequences. We argue these detectable differences are based on either the proportion of original male membrane that is replaced during pronuclear formation and/or differential mRNA signals (see Karr et al. 2009 and references therein, which we discuss below).

Can different nuclei within a single egg cell behave independently?

To consider selection inside an egg cell, we first must establish whether the nuclei inside an egg cell behave individually. If the nuclei inside an egg—both egg and sperm nuclei—are all controlled in the same manner by egg cytoplasmic factors, it would be inaccurate to characterize the male and female nuclei as being capable of independent actions and selection. Mate choice requires independent control by one individual doing the choosing and another individual being chosen, where the individuals here are haploid nuclei.

When the timing of chromatin condensation and decondensation, and the timing of nuclear envelope breakdown of the egg and sperm nuclei are considered, it becomes apparent that nuclei and pronuclei within an egg cell do in fact behave as individuals. In outcrossing animals, sperm almost always inseminate the egg at an arrested stage of meiosis (Wilson 1925, Austin 1965). The stage at which meiosis is arrested varies depending on the taxon; most vertebrates are arrested at metaphase II of meiosis at the time of plasmogamy (fusion of cellular membranes). At plasmogamy, egg chromosomes are condensed and lined up at the metaphase plate. When a sperm enters an egg, the sperm nucleus almost immediately undergoes a dispersal of its chromatin, while egg chromatin remains condensed (Longo 1987, Wright and Longo 1988). Wright and Longo (1988: 155) noted, “The sperm nucleus undergoes dispersion following its incorporation, resulting in a significant expansion of its volume. Concomitantly, the maternal chromatin, which is condensed, completes meiosis. How the paternal and maternal genomes are regulated to undergo such divergent processes, dispersion versus condensation, within a common cytoplasm has not been determined.” Both male and female nuclei in the same cytoplasm act independently with respect to when their nuclear envelopes breakdown and their chromosomes decondense. We propose that the divergent processes of the egg and sperm nuclei prior to karyogamy can be explained by the presence/absence of nuclear pores. Because the sperm nucleus lacks pores upon plasmogamy, it can behave independently of the egg’s cytoplasm.

We assert that sperm and egg nuclei act as truly separate individuals in that they are under separate (nuclear) control, as opposed to cytoplasmic control. It is important to note that sperm nuclei are usually only effective at karyogamy if they form a pronucleus. The process of pronuclear formation encompasses the breakdown of the poreless male nuclear membrane followed by the reformation of a new nuclear membrane composed mostly of porous female endoplasmic reticulum material. This event is the only instance in nature when nuclear membrane breakdown occurs in the absence of chromosomal reduction or duplication. We reject the usage of the term “pronucleus” to describe the egg nucleus prior to karyogamy because, unlike sperm nuclear envelopes, the egg nuclear envelopes only are replaced as part of a nuclear division, a parsing of half the chromosomes into two separate nuclei (Longo 1985). The fact that the only place that nuclear envelope breakdown is ever observed prior to DNA replication occurs in males during pronuclear formation, shortly after it enters into the egg, confirms how sperm nuclei are able to evade the egg’s cell cycle control. After, or sometimes during, chromatin dispersion, the sperm sheds its nuclear membrane (Longo 1985). Normally, the tight constraints of the cell cycle, specifically the G_2/M-phase checkpoint, would not allow for nuclear envelope breakdown to occur prior to DNA replication (Hartwell and Weinert 1989, Murray 1992, Sluder et al. 1995). However, the sperm evades the egg’s intrinsic controls, likely because of its poreless membrane.

While formation of the sperm pronucleus is probably mediated at least in part by cytoplasmic factors originating from the egg’s germinal vesicle (Moriya and Katagiri 1976, Skoblina 1976), a male pronuclear growth factor is also thought to exist (Thibault and Gérard 1973, Motlik and Fulka 1974, Trounson et al. 1977). Nuclear envelope breakdown is under cytoplasmic control in mitotic and meiotic cell divisions. Mitosis-promoting factor is a cytoplasmic factor that normally drives a cell into a division by forcing nuclear envelope breakdown. However, this is not the case for pronuclei within an egg. Nuclear envelope breakdown of the male pronucleus and the female nucleus is under nuclear, *not* cytoplasmic, control (Sluder et al. 1995). Thus, from the time of sperm entry, to the formation of the sperm pronucleus, to the breakdown of the pronuclear envelope, sperm nuclei are under different controls from egg nuclei.

The fact that male and female nuclei are controlled separately within a single egg cell is essential to recognize if a discussion of post-plasmogamic pre-karyogamic sexual selection is to take place.

Do nuclei within a single egg cell have detectable differences?

For post-plasmogamic pre-karyogamic sexual selection, there must be some kind of detectable differences between male nuclei/pronuclei upon which a choosy egg nucleus can make a choice. We propose that differences between the multiple sperm nuclei/pronuclei in an egg cell may reside in the nuclear envelopes of the sperm pronuclei or in the signals sperm pronuclei send.

We hypothesize that sexual selection within an egg is most likely to occur when there are multiple sperm pronuclei within an egg cell, i.e. polyspermy. Polyspermy occurs in a wide variety of taxa including birds, reptiles, elasmobranch fishes, urodele amphibians, mollusks, arachnids, insects, bryozoans, and ctenophores (Nelson 1953, Rothschild 1954, Wourms 1977, Carré and Sardet 1984, Iwamatsu 2000). Polyspermic organisms have evolved one or more mechanisms to allow multiple sperm, sometimes well over twenty and occasionally hundreds of sperm (Dean 1906), to participate in plasmogamy with a single egg, yet still only allow one male pronucleus to participate in karyogamy with the female nucleus. Although it is often assumed that female nuclei simply fuse with the closest male pronucleus in polyspermic situations, there is strong evidence to suggest otherwise. In at least one species of comb jelly (*Beroe ovata*), the female nucleus within the polyspermic egg does not fuse with the first male nucleus that enters the cell, nor does it fuse with the closest. Instead, upon plasmogamy, the female *Beroe ovata* nucleus migrates within the egg cell—from male pronucleus to pronucleus—before finally fusing with just one (Carré and Sardet 1984). The only data showing a female nucleus querying of multiple male pronuclei comes from this one species of comb jelly, and the purpose of this migration is unknown.

Why does the female not simply fuse with the closest male pronucleus or the male pronucleus that enters the cell first? This question is really the same as the more general one that has plagued evolutionary biologists for decades: why should females be choosy when they receive no obvious benefits from males except for their genetic contribution? Based on existing well-developed hypotheses for the evolution of female preference, we propose two hypotheses as likely candidates to explain evolution of post-plasmogamic pre-karyogamic cryptic female choice that occurs within the microcosm of a fertilized egg cell: (1) Females base their choice on a pre-existing bias for self, based on the mosaic characteristics of sperm pronuclear membrane, and (2) Females detect good genes via mRNA signals.

Our first hypothesis is based on sensory bias: that the chosen sperm contains the most female-like nuclear membrane. The sensory bias hypothesis suggests that female preferences are by-products of the underlying physiology of the female's sensory systems. Natural selection has molded the female's sensory systems, and males are proposed to have evolved traits that match the female's sensory system characteristics (Basolo 1990, 1995, Endler and Basolo 1998, Ryan 1998). When sperm enter an egg cell, they shed their nuclear membrane and replace it with egg membrane material. In the process, some taxa, such as the sea urchin *Arbacia* (Longo 1973) and the hamster (Yanagimachi and Noda 1970), retain parts of their sperm nuclear envelope, thus, the newly formed pronucleus is a mosaic. Sperm retain part of their original nuclear membrane, with variation between sperm being a function of the amount and/or composition of nuclear membrane retained (Yanagimachi and Noda 1970, Longo 1976). There is a noticeable gap in the literature with regards to the function of replacing a sperm nucleus with a pronucleus. We argued elsewhere that sperm shed their nuclear membrane and replace it with one made from egg material because they need nuclear pores to complete DNA replication (Gorelick et al. 2012, Carpinone and Gorelick in review). Here we also hypothesize that the pronucleus may have a secondary function to deliver specific information to the female nucleus that aids in her mate choice. Sperm pronuclear membranes may provide the variation necessary for sexual selection to be possible. We suspect that in polyspermic organisms, the female selects the male that retains the least amount of sperm nuclear membrane (poreless verses porous membrane). The idea that there is selection pressure on sperm to be better female mimics comes from analyzing the conditions faced by the first sexual organisms. The first sexual organisms probably had sex with themselves (i.e. were automictic; Gorelick and Carpinone 2009) and did not use sperm, but instead restored diploidy by either simply replicating all their chromosomes (endomitosis) or by the fusion of two haploid products of egg meiosis with one another. In such an ancestral condition, any invasion of the egg by another nucleus, a sperm nucleus, would have been detrimental. Early sexual organisms would have had eggs that fused with their own polar nuclei (Gorelick and Carpinone 2009), which are virtually identical to the egg nucleus. This could be why females still prefer to fuse with something similar to self. In monospermic out-crossers, female nuclei have a choice between fusing with a polar nucleus or the sperm pronucleus. Because of self-incompatibility, however, eggs preferentially fuse with something that is similar to self, but not identical. We call this the Trojan sperm hypothesis: males don pronuclear membranes to 'mimic' females in order to be chosen for karyogamy, due to a pre-existing bias for the females to fuse with themselves.

We digress to discuss our somewhat unconventional definition of sex as either meiosis or karyogamy, especially in light of automixis. With this definition, most or possibly even all eukaryotes become facultatively sexual, at least if we accept reports of cryptic meiosis, especially in basal taxa (Solari 2002, Gandolfi et al. 2003, Signorovitch et al. 2005, Cooper et al. 2007, Gorelick and Carpinone 2009). This definition also means that parasex should count as sex (Forche et al. 2008, Gorelick and Carpinone 2009). There is no difference between autogamy and complete automixis, except that automixis contains diploid mitotic divisions between karyogamy and meiosis. The notion that fusion of gametes defines sex is an anachronism, described before meiosis was elucidated. For example, Spencer (1863 [1890]) described how fusion of gametes defined individuals, while Braun (1853 [1851]) and Bütschli (1876) described how fusion of gametes rejuvenated a lineage, and they did this long before Hertwig (1890) elucidated that meiosis was a reduction division. We have also demonstrated that karyogamy is a modified form of meiosis (Gorelick and Carpinone 2009), making the definition of sex as either meiosis or karyogamy both simple and general.

Our second hypothesis regarding the evolution of female choice on the nuclear level is based on the good genes hypothesis, with a positive genetic covariance between male displays and their viability (Iwasa et al. 1991, Jennions and Petrie 2000, Houle and Kondrashov 2002). The good genes hypothesis also assumes that there is genetic variation in female preference, which results in covariance between preference and viability (Iwasa et al. 1991, Jennions and Petrie 2000, Houle and Kondrashov 2002). Preferences start out at a naturally selected optimum, but are then pushed from the optimum by selection for high viability. Males with very attractive displays indicate their higher overall viability to females more than males with less attractive displays. By preferring these males, females receive better genes that may be passed on to their offspring. The good genes hypothesis suggests that there is no direct selection on the preference. Instead, there is only direct selection for high viability. What we add to this existing sexual selection theory is that males could simply be individual sperm nuclei or pronuclei. In polyspermic organisms, we hypothesize that the female nucleus migrates to several pronuclei before selecting one. The female nucleus does not come into contact with male pronuclei, but enters into the “pronuclear zone”, an area close to the pronucleus (Carré and Sardet 1984). Once the female nucleus enters into the pronuclear zone, it commits to karyogamy with that particular pronucleus. During the period of female nuclear migration, male pronuclei contain decondensed DNA (Longo 1985). It is plausible that male pronuclei are actively transcribing mRNA and that female nuclei can detect these mRNA signals and are using the quality or nature of these signals as a basis for their choice. There is also evidence that sperm nuclei, before they lose their nuclear membrane and decondense their chromosomes, emit mRNAs (Ostermeier et al. 2004, Krawetz 2005, Miller et al. 2005, Ostermeier et al. 2005, Dadoune 2009). In this case, males are actively advertising to females. We call this the good haploid genes hypothesis, which is like the sexy sperm hypothesis, but with the individuals being sperm nuclei or pronuclei, rather than entire sperm. Sexual selection for individual sperm nuclei/pronuclei was briefly alluded to by Birkhead (1998) and Birkhead and Brillard (2007).

Implications of post-plasmogamic pre-karyogamic sexual selection for alternative sexual systems

We next outline the implications of sexual selection inside the microcosm of an egg cell for polyspermic, gynogenetic, and androgenetic taxa, which may shed additional observability into post-plasmogamic pre-karyogamic sexual selection. The major implications for the theory developed herein are for polyspermy, which occur in virtually every animal group. Post-plasmogamic pre-karyogamic sexual selection is also applicable to gynogenesis, which occurs in almost 60 metazoan species spanning seven phyla, including chordates, mollusks, arthropods, rotifers, platyhelminthes, nematodes, and annelids (Beukeboom and Vrijenhoek 1998), and androgenesis, which only occurs in two metazoan genera, *Corbicula* freshwater clams and *Bacillus* stick insects (Mantovani and Scali 1992, Komaru et al. 1998). We focus our discussion on gynogenesis and androgenesis because they may be the easiest place to find evidence for post-plasmogamic pre-karyogamic sexual selection.

Implications of post-plasmogamic pre-karyogamic sexual selection for polysperms

Polyspermic organisms may be the most informative species in which to study post-plasmogamic pre-karyogamic sexual selection. Not only can there be hundreds of male nuclei within a single egg cell, but these male nuclei may even be from different diploid males. Sexual selection theory predicts that when the number of males in a population is high and the variation between these males is great, females should be choosier (Emlen 1976). Females should survey more individual males before choosing one with which to mate. Sexual selection should be low in monogamous systems, while high in polygamous systems (Emlen and Oring 1977). These ideas can be applied to the microcosm of an egg cell, the only difference here being that the individuals involved are haploid nuclei and pronuclei, and that the population includes only those individuals within a given egg cell. Thus, in post-plasmogamic pre-karyogamic sexual selection, we predict that polyspermic polygamous female nuclei will be choosier and therefore migrate around the egg more compared to polyspermic monogamous female nuclei. Good test species include those from clades in which organisms show polyspermy in most species, but have varying degrees of (diploid) polygamous and monogamous matings. Amongst polyspermic vertebrates, female salamanders and birds mate with multiple males (Jordan 1893, Blount 1909, Fankhauser 1932, Fankhauser and Moore 1941, Petrie and Kempenaers 1998, Gopurenko et al. 2006). Amongst polyspermic invertebrates, females mate with multiple males in those taxa that broadcast their sperm, such as bryozoans (Rothschild 1954). These test species would enable a comparison of haploid female migration patterns between species that have (1) eggs containing multiple sperm from the same male and (2) eggs containing multiple sperm coming from multiple males.

Implications of post-plasmogamic pre-karyogamic sexual selection for gynogens

Formation of a pronucleus is a prerequisite for karyogamy between egg and sperm nuclei, however, failure to form a sperm pronucleus does not always lead to a lack of a viable zygote. Egg cells sometimes restore diploidy without incorporating a sperm's chromosomes. While egg meiosis in all animals (except cnidarians, sea urchins, and some starfish; Tarín 2000) requires plasmagamy, zygote formation in over 50 species across seven phyla does not require karyogamy between male and female haploid nuclei (Beukeboom and Vrijenhoek 1998). This situation is termed gynogenesis, where sperm are needed for the resumption of arrested egg meiosis (and maybe some epigenetic factors?), but where sperm chromosomes do not get passed on to the zygote. Gynogens are unisexual female hybrids whose eggs participate in plasmogamy but not karyogamy, with sperm. During gynogenesis, two egg nuclei can fuse to form a zygote, with no contribution from the sperm. Restitutional meiosis or pre-meiotic doubling of egg nuclei is also possible. In gynogenetic eggs, like the eggs of polysperms, and in all eggs that have arrested meiosis, there are multiple nuclei—both female and male nuclei/pronuclei—with which the female nuclei can participate in karyogamy. With polyspermy, female nuclei choose a male nucleus to associate/fuse with, whereas with gynogenesis, female nuclei choose another female nucleus (its own polar body or polar nucleus) to associate/fuse with.

Why are gynogens not fusing with the sperm that fertilizes the egg, like their close relatives do? In at least one species of gynogenetic carp (*Carassius auratus langsdorfii*), the sperm that fertilizes the egg fails to form a pronucleus and is subsequently ejected (Yamashita et al. 1990). Sperm that fertilize gynogens should be able to form a pronucleus insofar as studies have shown that pronuclear formation is not a species-specific process (see Longo 1985 for a review). However, for some reason, in *Carassius auratus langsdorfii*, the pronucleus does not form. We hypothesize that all sperm fertilizers of gynogens are rejected by the egg (at least in part) because they fail to form pronuclei, which (as previously discussed) may be the basis of female choice. Sperm that fertilize gynogenetic eggs are compatible enough to induce completion of egg meiosis. If post-plasmogamic pre-karyogamic sexual selection is occurring in gynogens, we predict that gynogenetic egg nuclei migrate, like in polyspermic amphimicts, to one or more nuclei within the egg cell. The male nuclei, however, have not formed pronuclei and therefore are not chosen for karyogamy because they are neither (1) exploiting the females pre-existing bias for a porous membrane, nor (2) sending out enticing mRNA signals due to lack of nuclear pores. The gynogen rejects all male nuclei and restores ploidy either by endoduplication or by choosing another haploid female to fuse with (i.e. her polar body).

Implications of post-plasmogamic pre-karyogamic sexual selection for androgens

Female choice on the nuclear level seems plausible and maybe even likely for both polyspermic and gynogenetic organisms. Female nuclei can distinguish between different male nuclei (with polyspermy) and between male and female nuclei (with gynogenesis and outcrossing). Are female nuclei the only nuclei that choose between mates? This leads to questions surrounding androgenesis, where sexual role reversal may occur within an egg cell. So far, the only known obligate androgens found in the animal kingdom are in the genus *Corbicula* of freshwater clams. The eggs of these clams are readily fertilized by diploid, triploid, or tetraploid biflagellate sperm (biflagellate sperm is very peculiar in unikonts). Upon meiosis, the egg's genetic material is extruded as two (presumably) diploid polar bodies (Komaru et al. 1998). Androgenesis also occurs obligately in one conifer, *Cupressus dupreziana*, by means of diploid sperm (Pichot et al. 2001), and facultatively in *Bacillus* stick insects (Mantovani and Scali 1992). Facultative androgens are fertilized by haploid sperm, and diploidy is restored, like in gynogens, either by endomitosis, or karyogamy between two haploid male pronuclei. Spontaneous androgenesis is rare, typically occurring via endomitosis of a single haploid sperm nucleus in some fish and angiosperms. In androgenesis, all egg nuclei are discarded as polar bodies or degenerate. Why are the female nuclei being rejected in these species? We hypothesize that male nuclei can occasionally exert choice over female nuclei by rejecting the female nucleus. Somehow, male nuclei within an egg manipulate the egg to reject itself. The mechanics behind this antithetical rejection of egg nuclei is unknown. We take the liberty here to hypothesize that male nuclei still take female endoplasmic reticulum material to form the pore-rich pronuclei needed for genome duplication, and, if there is selection of haploid male pronuclei by one another, pronuclei would also form the basis of sexual choice. However, we are not so adventurous to speculate whether haploid male pronuclei evaluate other male pronuclei based on proportion of female membrane, mRNA signals, or some other completely different system, or how it is settled which male does the choosing.

Closing Remarks

Pre-copulatory and post-copulatory sexual selection prior to plasmogamy have been extensively studied, however to the best of our knowledge, post-plasmogamic pre-karyogamic sexual selection has never been considered, except for a few short sentences by Birkhead. This lack of study is probably due to technical difficulties in observing the behaviours of the nuclei and pronuclei within a single egg cell. We predict that the prevalence of post-plasmogamic pre-karyogamic sexual selection will be proportional to the number of sperm nuclei that enter an egg cell. It is easy enough to measure rates of polyspermy. The problem is that we know virtually nothing about how haploid nuclei inside of an egg cell move and how they might possibly choose one another. While it appears that it is usually the haploid egg nuclei that do most of the moving around the egg cell, as the haploid sperm nuclei remain around the periphery, there is too little data to know how general this pattern is. Do haploid egg nuclei prefer certain male mRNAs? If so, are these mRNA produced before or after sperm pronuclear formation? Do haploid egg nuclei prefer certain male pronuclear membranes? Does the ratio of native female-to-male content of these nuclear envelopes matter? Does it vary? Until data addressing these questions is amassed, it will remain impossible to make more specific testable predictions about the interaction of haploid nuclei inside a fertilized egg cell based on standard sexual selection theory.

We propose post-plasmogamic pre-karyogamic sexual selection exists based on previously recorded movements of female nuclei toward multiple male nuclei prior to karyogamy within a single egg cell (Carré and Sardet 1984). Post-plasmogamic pre-karyogamic sexual selection is indeed plausible because female and male nuclei within an egg are under their own nuclear control rather than under the control of the cytoplasm, and there is opportunity for differences between male nuclear/pronuclear membranes and signals. We should not summarily dismiss post-plasmogamic pre-karyogamic sexual selection, especially when analyzing organisms that have little or no pre-copulatory nor pre-plasmogamic selection, despite no definitive evidence for this theoretically reasonable construct. Instead of avoidance of polyspermy a lá Eberhard (1996) and Arnqvist and Rowe (2005), individual female eggs might tolerate or facilitate polyspermy and then choose sperm nuclei at a slightly later ontological date, after plasmogamy but before karyogamy. For example, post-plasmogamic pre-karyogamic sexual selection could easily exist in sessile organisms, such as bryozoans and plants—organisms in which sexual selection is thought to play relatively minor evolutionary roles (Morgan 1927, Andersson 1994). Therefore, sexual selection has the potential to be much more universal, occurring in many more organisms than generally believed. The implications of post-plasmogamic pre-karyogamic intersexual selection are the same as the implications for other forms of intersexual selection: the evolution of exaggerated sexual signals and female preferences for those signals (Andersson 1994). In the case of post-plasmogamic pre-karyogamic sexual selection, these implications would include male nuclei being selected to either don more of the female’s pronuclear membrane to ‘mimic’ females (Trojan sperm hypothesis) or male nuclei being selected to signal their good genes through enhanced mRNA emission (good haploid genes hypothesis), thereby actively advertising themselves to females. In turn, female nuclei would be selected to exhibit enhanced preferences for these pronuclear or mRNA signals.

Acknowledgments

Thanks to Simone Immler and Hanne Løvlie, who provided many constructive comments. Thanks to the Natural Sciences and Engineering Research Council of Canada (NSERC) for Discovery Grants to RG and SMB.

Referees

Simone Immler – simone.immler@ebc.uu.se
Uppsala University

Hanne Løvlie – hanne.lovlie@zoologi.su.se
Stockholm University

References

Andersson, M.B. 1994. Sexual selection. Princeton New Jersey Press, Princeton, NJ.

Arnqvist, G. and L. Rowe. 2005. Sexual conflict. Princeton University Press, Princeton.

Austin, C.R. 1965. Fertilization. Prentice-Hall, Edgewood Cliffs.

Barbosa, F. 2009. Cryptic female choice by female control of oviposition timing in a soldier fly. Behavioral Ecology 20: 957-960. CrossRef

Basolo, A.L. 1990. Female preference predates the evolution of the sword in swordtail fish. Science 250: 808-810. CrossRef

Basolo, A.L 1995. Phylogenetic evidence for the role of pre-existing bias in sexual selection. Proceedings of the Royal Society of London Series B-Biological Sciences 259: 307-311. CrossRef

Beukeboom, L.W. and R.C. Vrijenhoek. 1998. Evolutionary genetics and ecology of sperm-dependent parthenogenesis. Journal of Evolutionary Biology 11: 755-782. CrossRef

Birkhead, T.R. 1998. Cryptic female choice: criteria for establishing female sperm choice. Evolution 52: 1212-1218. CrossRef

Birkhead, T.R. and A.P. Møller. 1998. Sperm competition and sexual selection. Academic Press, London.

Birkhead, T.R. and J-P. Brillard. 2007. Reproductive isolation in birds: postcopulatory prezygotic barriers. Trends in Ecology & Evolution 22: 266-272. CrossRef

Blount, M. 1909. The early development of the pigeon's egg, with special reference to polyspermy and the origin of the periblast nuclei. Journal of Morphology 20: 1-64. CrossRef

Braun, A. 1853 [1851]. Reflections on the phenomenon of rejuvenescence in nature especially in the life and development of plants. Pages 1-341 *in* A. Henfrey, editors. Biological and physiological memoirs, Ray Society, London.

Burley, N. 1988. The differential allocation hypothesis: an experimental test. *American Naturalist* 132: 611-628. CrossRef

Bütschli, O. 1876. Studien über die ersten entwicklungvorgange der eizelle, die zelltheilung und die conjungation der infusorien. Abhandlungen herausgegeben von der Senckenbergischen Naturforschenden Gesellschaft 10: 213-452.

Carpinone, J. and R. Gorelick. in review. Evolution of syngamy.

Carré, D. and C. Sardet. 1984. Fertilization and early development in *Beroe ovata*. Developmental Biology 105: 188-195.

Cooper, M.A., Adam, R.D., Worobey, M., and C.R. Sterling. 2007. Population genetics provides evidence for recombination in *Giardia*. Current Biology 17: 1984-1988. CrossRef

Dadoune, J-P. 2009. Spermatozoal RNAs: What about their functions? Microscopy Research and Technique 72: 536-551. CrossRef

Darwin, C.R. 1871. The descent of man and selection in relation to sex. John Murray, London.

Dean, B. 1906. Chimaeroid fishes and their development. Carnegie Institution of Washington., Washington DC.

Eberhard, W.G. 1996. Female control: sexual selection by cryptic female choice. Princeton University Press., Princeton.

Edvardsson, M. and G. Arnqvist. 2000. Copulatory courtship and cryptic female choice in red flour beetles *Tribolium castaneum*. Proceedings of the Royal Society of London Series B-Biological Sciences 267: 559-563. CrossRef

Emlen, S.T. 1976. Lek organization and mating strategies in the bullfrog. Behavioral Ecology and Sociobiology 1: 283-313. CrossRef

Emlen, S.T. and L.W. Oring. 1977. Ecology, sexual selection, and evolution of mating systems. Science 197: 215-223. CrossRef

Endler, J.A. and A.L. Basolo. 1998. Sensory ecology, receiver biases and sexual selection. Trends in Ecology & Evolution 13: 415-420. CrossRef

Fankhauser, G. 1932. Cytological studies on egg fragments of the salamander *Triton*. II. The history of the supernumerary sperm nuclei in normal fertilization and cleavage of fragments containing the egg nucleus. Journal of Experimental Zoology 62: 185-235. CrossRef

Fankhauser, G. and C. Moore. 1941. Cytological and experimental studies of polyspermy in the newt *Triturus viridescens*. I. Normal fertilization. Journal of Morphology 68: 347-385. CrossRef

Forche, A., Alby, K., Schaefer, D., Johnson, A.D., Berman, J., and R.J. Bennet. 2008. The parasexual cycle in *Candida albicans* provides an alternative pathway to meiosis for the formation of recombinant strains. PLoS Biology 6: e110. CrossRef

Gandolfi, A., Sanders, I.R., Rossi, V., and P. Menozzi. 2003. Evidence of recombination in putative ancient asexuals. Molecular Biology and Evolution 20: 754-761. CrossRef

Gopurenko, D., Williams, R.N., McCormick, C.R., and J.A. DeWoody. 2006. Insights into the mating habits of the tiger salamander (*Ambystoma tigrinum tigrinum*) as revealed by genetic parentage analyses. Molecular Ecology 15: 1917-1928. CrossRef

Gorelick, R. and J. Carpinone. 2009. Origin and maintenance of sex: the evolutionary joys of self sex. Biological Journal of the Linnean Society 98: 707-728. CrossRef

Gorelick, R., Carpinone, J., and L.J. Derraugh. 2012. Fundamental differences between females and males? Pages *in* M. Ah-King and A. Lee, editors. Challenging popular myths of sex, gender, and biology.

Hartwell, L.H. and T.A. Weinert. 1989. Checkpoints: controls that ensure the order of cell cycle events. Science 246: 629-634. CrossRef

Hertwig, O. 1890. Vergleich der ei- und samenbildung bei nematoden: eine grundlage für cellülare streitfragen. Archiv für mikroscopische Anatomie 36: 1-138.

Hoikkala, A. and S. Crossley. 2000. Copulatory courtship in *Drosophila*: behavior and songs of *D. birchii* and *D. serrata*. Journal of Insect Behavior 13: 71-86. CrossRef

Hoikkala, A., Crossley, S., and C. Castillo-Melendez. 2000. Copulatory courtship in *Drosophila birchii* and *D. serrata*, species recognition and sexual selection. Journal of Insect Behavior 13: 361-373. CrossRef

Houle, D. and A.S. Kondrashov. 2002. Coevolution of costly mate choice and condition-dependent display of good genes. Proceedings of the Royal Society of London Series B-Biological Sciences 269: 97-104. CrossRef

Hull, P. 1964. Partial incompatibility not affecting total litter size in the mouse. Genetics 50: 563-570.

Iwamatsu, T. 2000. Fertilization in fishes. Pages 89-145 *in* J.J. Tarín and A. Cano, editors. Fertilization in protozoa and metazoan animals: cellular and molecular aspects, Springer-Verlag, Berlin.

Iwasa, Y., Pomiankowski, A., and S. Nee. 1991. The evolution of costly mate preferences. II. The 'handicap' principle. Evolution 45: 1431-1442. CrossRef

Jennions, M.D. and M. Petrie. 2000. Why do females mate multiply? A review of the genetic benefits. Biological Reviews 75: 21-64. CrossRef

Jordan, E.O. 1893. The habits and development of the newt (*Diemyctylus viridescens*). Journal of Morphology 8: 269-366. CrossRef

Karr, T.L., Swanson, W.J., and R.R. Snook. 2009. The evolutionary significance of variation in sperm-egg interactions. Pages 305-365 *in* T.R. Birkhead, D.J. Hosken, and S. Pitnick, editors. Sperm biology: an evolutionary perspective, Academic Press, Burlington.

King, B.H. and C.R. Fischer. 2005. Males mate guard in absentia through extended effects of postcopulatory courtship in the parasitoid wasp *Spalangia endius* (Hymenoptera: Pteromalidae). Journal of Insect Physiology 51: 1340-1345. CrossRef

Komaru, A., Kawagishi, T., and K. Konishi. 1998. Cytological evidence of spontaneous androgenesis in the freshwater clam *Corbicula leana* Prime. Development, Genes and Evolution 208: 46-50. CrossRef

Krawetz, S.A. 2005. Paternal contribution: new insights and future challenges. Nature Reviews Genetics 6: 633-642. CrossRef

Longo, F.J. 1973. Fertilization: a comparative ultrastructural review. Biology of Reproduction 9: 149-215.

Longo, F.J. 1976. Derivation of membrane comprising male pronuclear envelope in inseminated sea urchin eggs. Developmental Biology 49: 347-368. CrossRef

Longo, F.J. 1985. Pronuclear events during fertilization. Pages 251-298 *in* C.B. Metz and A. Monroy, editors. Biology of fertilization. Volume 3. The fertilization response of the egg, Academic Press, Orlando.

Longo, F.J. 1987. Fertilization. Chapman & Hall, London.

Mantovani, B. and V. Scali. 1992. Hybridogenesis and androgenesis in the stick-insect *Bacillus rossius-grandii benaazzi* (Insecta, Phasmotdea). Evolution 46: 783-796. CrossRef

Miller, D., Ostermeier, G.C., and S.A. Krawetz. 2005. The controversy, potential and roles of spermatozoal RNA. Trends in Molecular Medicine 11: 156-163. CrossRef

Morgan, T.H. 1927. Experimental embryology. Columbia University Press, New York.

Moriya, M. and C. Katagiri. 1976. Microinjection of toad sperm into oocytes undergoing maturation division. Development, Growth & Differentiation 18: 349-356. CrossRef

Motlik, J. and J. Fulka. 1974. Fertilization of pig follicular oocytes cultivated in vitro. Journal of Reproduction and Fertility 36: 235-237. CrossRef

Murray, A.W. 1992. Creative blocks: cell-cycle checkpoints and feedback controls. Nature 359: 599-604. CrossRef

Nelson, O. 1953. Comparative embryology of the vertebrates. Blackstone, New York.

Ostermeier, G.C., Miller, D., Huntriss, J.D., Diamond, M.P., and S.A. Krawetz. 2004. Reproductive biology: delivering spermatozoan RNA to the oocyte. Nature 429: 154-154. CrossRef

Ostermeier, G.C., Goodrich, R.J., Moldenhauer, J.S., Diamond, M.R., and S.A. Krawetz. 2005. A suite of novel human spermatozoal RNAs. Journal of Andrology 26: 70-74.

Parker, G.A. 1970. Sperm competition and its evolutionary consequences in insects. Biological Reviews of the Cambridge Philosophical Society 45: 525-567. CrossRef

Petrie, M. and B. Kempenaers. 1998. Extra-pair paternity in birds: explaining variation between species and populations. Trends in Ecology & Evolution 13: 52-58. CrossRef

Pichot, C., Maâtaoui, M., Raddi, S., and P. Raddi. 2001. Surrogate mother for endangered *Cupressus*. Nature 412: 39-39. CrossRef

Pizzari, T. and T.R. Birkhead. 2002. The sexually-selected sperm hypothesis: sex-biased inheritance and sexual antagonism. Biological Reviews 77: 183-209. CrossRef

Rothschild. 1954. Polyspermy. Quarterly Review of Biology 29: 332-342.

Ryan, M.J. 1998. Sexual selection, receiver biases, and the evolution of sex differences. Science 281: 1999-2003. CrossRef

Signorovitch, A.Y., Dellaporta, S.L., and L.W. Buss. 2005. Molecular signatures for sex in the Placozoa. Proceedings of the National Academy of Sciences of the United States of America 102: 15518-15522. CrossRef

Simmons, L.W. 1987. Heritability of a male character chosen by females in the field crickets, *Gryllus bimaculatus*. Behavioral Ecology and Sociobiology 21: 129-133. CrossRef

Simmons, L.W. 2001. Sperm competition and its evolutionary consequences in the insects. Princeton University Press, Princeton.

Skoblina, M.N. 1976. Role of karyoplasm in the emergence of capacity of egg cytoplasm to induce DNA synthesis in transplanted sperm nuclei. Journal of Embryology and Experimental Morphology 36: 67-72.

Sluder, G., Thompson, E.A., Rieder, C.L., and F.J. Miller. 1995. Nuclear envelope breakdown is under nuclear not cytoplasmic control in sea urchin zygotes. Journal of Cell Biology 129: 1447-1458. CrossRef

Snook, R.R. 2005. Sperm in competition: not playing by the numbers. Trends in Ecology & Evolution 20: 46-53. CrossRef

Solari, A.J. 2002. Primitive forms of meiosis: the possible evolution of meiosis. BioCell 26: 1-13.

Spencer, H. 1863 [1890]. The principles of biology (volume 1). Williams & Norgate [Appleton], London [New York].

Tarín, J.J. 2000. Fertilization in protozoa and metazoan animals: a comparative overview. Pages 277-314 *in* J.J. Tarín and A. Cano, editors. Fertilization in protozoa and metazoan animals: cellular and molecular aspects, Springer-Verlag, Berlin.

Thibault, C. and M. Gérard. 1973. Cytoplasmic and nuclear maturation of rabbit oocytes in vitro. Annales De Biologie Animale Biochimie Biophysique 13: 145-156. CrossRef

Thornhill, R. 1983. Cryptic female choice and its implications in the scorpionfly *Harpobittacus nigriceps*. American Naturalist 122: 765-788. CrossRef

Thornhill, R. 1984. Alternative female choice tactics in the scorpion fly *Hylobittacus apicalis* (Mecoptera) and their implications. American Zoologist 24 367-383.

Trounson, A.O., Willadsen, S.M., and L.E.A. Rowson. 1977. Fertilization and development capability of bovine follicular oocyte matured in vitro and in vivo and transferred to the oviducts of rabbits and cows. Journal of Reproduction and Fertility 51: 321-327. CrossRef

Willson, M.F. and N. Burley. 1983. Mate choice in plants. Princeton University Press, Princeton.

Wilson, E.B. 1925. The cell in development and heredity (3rd edition). Macmillan, New York.

Wourms, J.P. 1977. Reproduction and development in chondrichthyan fishes. American Zoologist 17: 379-410.

Wright, S.J. and F.J. Longo. 1988. Sperm nuclear enlargement in fertilized hamster eggs is related to meiotic maturation of the maternal chromatin. Journal of Experimental Zoology 247: 155-165. CrossRef

Yamashita, M., Onozato, H., Nakanishi, T., and Y. Nagahama. 1990. Breakdown of the sperm nuclear envelope is a prerequisite for male pronucleus formation: direct evidence from the gynogenetic crucian carp *Carassius auratus langsdorfii*. Developmental Biology 137: 155-160. CrossRef

Yanagimachi, R. and Y.D. Noda. 1970. Ultrastructural changes in the hamster sperm head during fertilization. Journal of Ultrastructure Research 31: 465-485. CrossRef