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Comment

Mitochondrial activity in gametes and uniparental inheritance — a comment on 'What can we infer about the origin of sex in early eukaryotes?'

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In his insightful article, Speijer [1] discussed the origin of sex under multiple points of view, providing a comprehensive and balanced overview of different theories and hypotheses. However, we think that the section "Higher mutational loads in one gamete type and retention of uniparental mitochondrial inheritance" needs some clarification. Speijer [1] elaborates on the correlation between gamete metabolic and physiological differences and their organellar contribution across generations. Specifically, he quotes the "division of labour" hypothesis which postulates that male gametes maximize energy production for motility by sacrificing mitochondrial DNA (mtDNA) to oxidative phosphorylation (OXPHOS) and its mutagenic by-products, while non-motile female gametes repress OXPHOS, thus being somewhat inactive [2]. Basically, we would like to clarify two discussion points: 1) the exceptions to the strictly maternal inheritance (SMI) of mitochondria, and 2) the claim that mtDNA is highly mutated in sperm and the supposed causal relationship between such damage and OXPHOS.

Exceptions to the rule

Exceptions to SMI by which bioenergetically active mitochondria are stably inherited through generations might represent a challenge for the division of labour hypothesis. Doubly uniparental inheritance (DUI) is the only known evolutionarily stable exception to

the SMI typical of Metazoa. In DUI animals (~100 species of gonochoric bivalve molluscs identified so far [3]), two mitochondrial lineages are inherited, one through eggs (F-type), the other through sperm (M-type). Eggs are homoplasmic for the F-type, while spermatozoa are homoplasmic for the M-type. These “mother-to-daughter” and “father-to-son” mitochondrial lineages have evolved independently for million years (e.g. >200 Myr in unionids), accumulating up to 40% of DNA sequence divergence. Since eggs do not transmit the M-type, germ line mitochondria of DUI males are apportioned from the four/five mitochondria of the fertilizing spermatozoon, which carry mtDNA that must be functional and successfully inherited [4]. It is clear that the long evolutionary persistence of DUI—as inferred from the nucleotide divergence between conspecific sex-linked mtDNAs—indicates that the mtDNA transmitted through sperm can be a viable genetic template. Speijer [1] highlighted that *"upon loss of the need for highly active sperm cells during the life cycle of an organism the strong purging of paternal mitochondria might on occasion be relaxed"*, and—referring to DUI species of the family Mytilidae (sea mussels) and Unionidae (freshwater mussels)—claimed that the presence of paternal mtDNA inheritance in such animals can be explained by the absence of mitochondrial activity in sperm. According to Speijer [1] *"Mussel sperm is taken along by water currents, and the energy for the final entry of the female is provided by the female incurrent siphon"*, thus actually avoiding the proposed reactive oxygen species (ROS) generation and its consequences. In this respect, we have to clarify that spermatozoa of DUI bivalves have a well-formed flagellum, a midpiece that contains mitochondria with normal appearance (Fig. 1; see also [5]), and, most importantly, they actively swim. Bivalve sperm is motile—as can be easily assessed by optical microscope observation—irrespective of the type of reproduction (oviparous or larviparous), and sperm motility is needed for fertilization to take place [6, 7]. Moreover, OXPHOS is required to sustain the long motility phase of Pacific oyster spermatozoa [8], and spermatozoa swim to reach eggs guided by egg-produced chemoattractants and not simply carried by water currents. In the DUI marine mussel *Mytilus galloprovincialis*, Evans et al. [9] showed that spermatozoa exploit such chemical cues to preferentially swim towards eggs, and the presence of chemoattractants not only in bivalves, but also in several marine organisms, supports this as a shared feature of broadcast spawning animals [9-17] (Table 1).

Mitochondrial activity and mtDNA mutations.

The foundation of the division of labour hypothesis is the assumption that high ATP generation in sperm leads to high ROS formation and thus to mtDNA mutations, with a

consequent strong selection for the exclusion of the highly mutated paternal mtDNA from the filial generation. We would like to point out that the correlation between OXPHOS, ROS formation, and mtDNA mutations is still matter of debate, and that no clear-cut evidence is unambiguously supporting it. In the last decade, many studies failed to support a causal link between high OXPHOS activity and generation of hazardous amounts of ROS, so caution is advised (see [18] for a detailed discussion). The high energy demand for flagellar movements may even produce a lower amount of ROS (compared to “basal” ROS production), as documented during high exercise activity [19]. In addition, it is important to mention that high levels of ROS production may inhibit sperm motility [20, 21]. In DUI animals, it appears that the mtDNA is not accumulating damage faster in motile gametes, thus not being degraded on evolutionary time scales. Accordingly, there is no sign of genetic decay in the sperm-transmitted mitochondrial genome such as, for example, nonsense mutations or pseudogenization, even though multiple studies found that M-type mitochondria are clearly functional—e.g. they show a high membrane potential [18], and active replication [22], transcription [18, 23], and translation ([24, 25] and Ghiselli & Milani, unpublished, for OXPHOS proteins)—and they do OXPHOS (Bettinazzi et al. unpublished). Interestingly, Ghiselli et al. [23] used a high-throughput approach to assess the amount and type of polymorphism in the gonadal mitochondrial populations of the DUI species *Ruditapes philippinarum*, showing that F- and M-type mtDNAs have about the same amount of single nucleotide polymorphisms (SNPs) (actually F-type has more SNPs than M-type), and, most strikingly, M-type has significantly less SNPs with highly-deleterious effects, compared to F-type. These results can be explained by two observations: a) sperm mitochondria in DUI species are subject to selection for fundamental male functions such as spermatogenesis and fertilization performance; and b) selection on sperm mitochondria is more effective due to a much lower mtDNA copy number per gamete (discussed also in [18]).

Lastly, Speijer [1] also cites the ‘parent switching’, a mechanism known, among people studying DUI, as ‘role-reversal’ or ‘masculinization’ of F-type mtDNA. Role-reversal consists of F genomes that invade the male gonad, assume the role of the M genome, and become sperm-transmitted. We want to point out that this phenomenon has been documented only in species of the *Mytilus edulis* complex which are known to hybridize frequently—with all the expected alteration of mitochondrial heredity mechanisms (see for example [26])—and for which DUI disruption has been reported [27] (for further details on this topic see the discussion with Nick Lane under “Reviewers’ comment and response” in

[18]).

Conclusions

Is the DUI system undermining the division of labour hypothesis? More work is needed to assess this point, and there are at least two possibilities by which this would not be the case: *i)* DUI species might use alternative energy-production pathways (see for example [28] and/or produce less ROS; *ii)* DUI species might have evolved specific mechanisms of ROS scavenging and/or mtDNA protection. Such possibilities are under investigation. If, on the other hand, conclusive evidence about the lack of causation between energy production in sperm mitochondria and generation of hazardous amounts of ROS is eventually provided, then the division of labour as a general hypothesis to explain the evolution of anisogamy and of two sexes would be falsified. What insights can an evolutionary derived trait as DUI provide about more general biological patterns such as the evolution of sex? Although DUI might look like a weird exception to a quite conserved biological “rule”, molecular and phylogenetic evidence suggests that it evolved from SMI (DUI can revert to SMI under some circumstances [3, 18, 29]) so, most likely, the two systems share the same basic molecular mechanism of mitochondrial inheritance. DUI has been proposed to be the result of a resolved genomic conflict triggered by a mitochondrial selfish element [30], and its apparently unusual mechanism of sperm mitochondria segregation into male germ line could be explained by their high membrane potential and other factors causing their retention, instead of the common degradation [31]. As stated by Speijer [1], the selective pressure behind the evolution of anisogamy could also be the avoidance of genomic conflicts [32] (but see [33] for additional, not mutually-exclusive explanations for the evolution of anisogamy). In SMI species the elimination of sperm mitochondria might have been selected to avoid genomic conflicts, whereas in DUI species the same effect might have been obtained by segregating the competing mitochondrial lineages in two gamete types (see [30] for a detailed discussion on DUI origin). The DUI system seems to favour the hypothesis of genomic conflicts as the trigger for the evolution of uniparental inheritance and anisogamy over other explanations. Biology is extremely complex, and a greater effort should be made to elucidate an important and, in our opinion, overlooked topic such as the role of mitochondria in germ line evolution. Such effort should include as much taxa as possible, and, under this light, DUI organisms can be helpful in a similar way as mutants are central to understand genetics.

List of abbreviations

DUI = doubly uniparental inheritance of mitochondria

mtDNA = mitochondrial genome

M-mtDNA = male-transmitted mitochondrial genome

OXPHOS = oxidative phosphorylation

ROS = reactive oxygen species

SMI = strictly maternal inheritance of mitochondria

SNP = single nucleotide polymorphism

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Figure 1. Morphology of spermatozoa in bivalves that transmit sperm mitochondria to the progeny. (A) Spermatozoon of a freshwater mussel (*Elliptio fumata*, family Unionidae). (B) Spermatozoon of a marine clam (*Ruditapes philippinarum*, family Veneridae). (C) Magnification of *R. philippinarum* mitochondrial midpiece (transversally and longitudinally sectioned). (n = nucleus; m = mitochondrion).

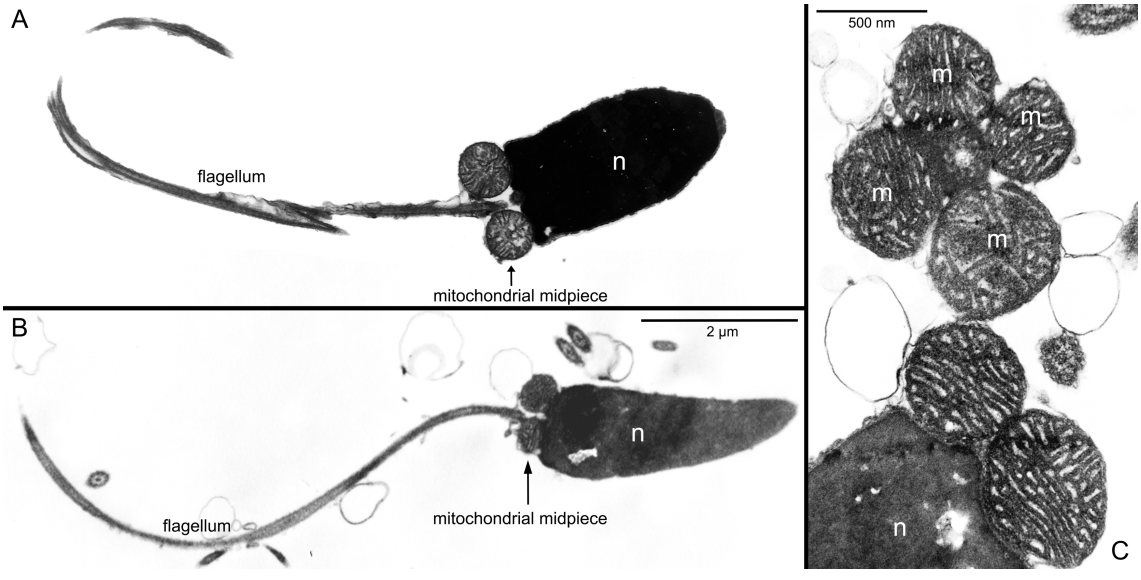

Table 1. Chemotaxis and sperm swimming

species	phenomenon	Reference
Molluscs		
Sepia officinalis	Species-specific sperm chemotaxis	[10]
Haliotis rufescens	Species-specific sperm chemotaxis	[11]
Mytilus edulis *Mytilus galloprovincialis*	Assortative mating through gamete preference	[12]
M. galloprovincialis	Sperm chemotaxis promoting sperm–egg encounters and facilitating species recognition; chemoattractants unforeseen role in sexual selection by enabling sperm to 'choose' between the eggs of different conspecific females - mediating mate choice for genetically compatible partners	[9]
M. galloprovincialis	Egg chemoattraction also promotes changes in sperm behavior and physiology; gamete 'preferences' - sperm from individual males consistently swim towards (and fertilize) the eggs of certain females	[13]
Other invertebrate broadcast spawners		
	Sperm chemotaxis - attractants and stimulators of sperm motility and respiration	[14]
	Chemotactic signaling of sperm from marine invertebrates - sperm adjust their swimming path in a gradient of a chemical factor released by the egg	[15]
	Species-specific sperm chemotaxis - preventing crossbreeding, especially in marine invertebrates with external fertilization	[16]
Strongylocentrotus purpuratus	Speract: egg peptide that regulates sperm motility and stimulates sperm mitochondrial metabolism	[17]