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The unusual system of doubly uniparental inheritance of mtDNA: isn't one enough?

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Mitochondria possess their own genetic material (mitochondrial DNA or mtDNA), whose gene products are involved in mitochondrial respiration and oxidative phosphorylation, transcription, and translation. In animals, mitochondrial DNA is typically transmitted to offspring by the mother alone. The discovery of 'doubly uniparental inheritance' (DUI) of mtDNA in some bivalves has challenged the paradigm of strict maternal inheritance (SMI). In this review, we survey recent advances in our understanding of DUI, which is a peculiar system of cytoplasmic DNA inheritance that involves distinct maternal and paternal routes of mtDNA transmission, a novel extension of a mitochondrial gene (*cox2*), recombination, and periodic 'role-reversals' of the normally male and female-transmitted mitochondrial genomes. DUI provides a unique opportunity for studying nuclear-cytoplasmic genome interactions and the evolutionary significance of different modes of mitochondrial inheritance.

Mitochondrial inheritance: rules for mussels

Mitochondria are multifunctional, DNA-bearing organelles found in eukaryotic cells. Animal mitochondrial DNA (mtDNA) is typically a circular molecule ~16.5-kilobase long (but linear and longer mtDNAs exist across eukaryotes, see Ref. [1]) that normally encodes ~37 genes [2]. Among them, 24 mitochondrial genes encode components involved in the mitochondrial translational machinery (22 tRNAs and two rRNAs). The remaining 13 genes encode protein subunits of the respiratory chain complexes and ATP synthase (see Glossary).

In animal species, mtDNA is exclusively maternally inherited [3] (but see Refs [4,5] for exceptions). Apart from rare mutants, all copies of mtDNA in each cell typically have identical DNA sequences, a situation known as homoplasmy. Disruption of mitochondrial homoplasmy (i.e. the mixing of different mtDNAs within a cell or heteroplasmy) results in genetic variance within an organism. The resulting potential for inter-mitochondrial competition could set the stage for the spread of deleterious, selfish mitochondrial elements [6,7]. Uniparental inheritance of mtDNA in animals is a mechanism that has apparently evolved to avoid such intracellular conflict [7–9]. However, an extreme deviation from this general rule, termed doubly uniparental inheritance (DUI) [10], occurs in marine mussels of the order Mytiloida, freshwater mussels of the superfamily Unionoidea, and marine clams of the order Veneroida [10–21]. Species possessing DUI are characterized by the presence of distinct gender-associated mtDNAs that are inherited either maternally or paternally. These female-transmitted

Glossary

Gonochoric: describes a sexually reproducing species in which there are two (at least) distinct sexes.
Heteroplasmy: the existence of two (or more) plastid variants (mitochondrial or chloroplast DNA) within an organelle, cell, tissue or individual.
Homoplasmy: the condition in which all plastid genomes (i.e. usually referring to genetic identity of mitochondria or chloroplasts) in an organelle, cell, tissue or individual are identical.
Hybrid zone: an area where two species come into contact and offspring are produced that are the result of interbreeding between the different species.
Masculinization: a female-transmitted mtDNA can become 'masculinized' (i.e. reverse its role and become transmitted paternally).
Nuclear isoforms: different forms of a nuclear-encoded protein that might be produced from different genes or from the same gene by alternative splicing (i.e. different versions of messenger RNA) or posttranslational modifications. Isoforms are usually tissue-specific.
Ovotestis: refers to a gonad that contains both ovarian and testicular tissues (i.e. hermaphroditism).
Oxidative phosphorylation (OXPHOS): the synthesis of ATP (i.e. the high energy source used for essentially all active metabolic processes within the cell) by phosphorylation of ADP for which energy is obtained by electron transport and which takes place in the mitochondria during aerobic respiration.
Respiratory chain complexes and ATP synthase: an elaborate system composed of five enzyme complexes situated on the inner mitochondrial membrane. The four first complexes act as an electron transport chain from reduced cofactors (e.g. the reduced form of nicotinamide adenine dinucleotide [NADH] or flavin adenine dinucleotide [$FADH_2$]) to molecular oxygen. The passage of electrons is linked to proton efflux across the mitochondrial inner membrane to establish a source of power for ATP synthase (complex V) to synthesize ATP. The mtDNA encodes seven subunits of the NADH: ubiquinone oxidoreductase (complex I; *ND1* to *ND6*, and *ND4L*), one subunit of the ubiquinone: cytochrome *c* oxidoreductase (complex III; Cyt *b*), three subunits of the cytochrome *c* oxidase (complex IV; *COX1* to *COX3*) and two subunits of the ATP synthase (complex V; *ATP6* and *ATP8*). Other subunits of complexes I, III, IV, and V, as well as all the components of complex II (succinate: ubiquinone oxidoreductase), the membrane transporters, the enzymes of the matrix, and the factors involved in other mitochondrial functions, e.g. mtDNA replication and mtDNA expression, are nuclear-encoded in animals.
Selfish mitochondrial elements: Potentially deleterious elements that enhance their own transmission relative to their allelic counterparts.

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and male-transmitted mitochondrial genomes (referred to for convenience as 'F genomes' and 'M genomes', respectively) often exhibit nucleotide divergences greater than 20%. Female mussels typically inherit their F genome only from their mother, but they transmit this F genome to both sons and daughters. Male zygotes inherit their mtDNA from both parents, but they sort the mixture of mitochondrial genomes present such that the M genome inherited from their father becomes established in the germ line. This paternal M genome will subsequently be passed on by way of the sperm where it will ultimately be retained only by sons, possibly as a consequence of limited replication of the M genome in females [22,23]. Females are essentially homoplasmic for the F genome whereas males are heteroplasmic for both F and M genomes. This peculiar situation challenges our traditional view of the strict maternal inheritance (SMI) of mtDNA. A striking difference between SMI and DUI systems is that the latter allows selection to act directly on the male mitochondrial genome; unlike most animals, male mussels do not represent an evolutionary dead-end for mitochondrial genomes [24,25].

Although the essential structure of the DUI system is known, we are still revising and refining our understanding of the evolutionary implications of DUI. Major questions about DUI into which we are just now gaining insight include whether DUI originated to avoid the deleterious effects of mitochondrial DNA mutations on sperm function or whether the phenomenon evolved because of a role in sex determination of selfish mitochondrial DNA elements. In addition to surveying recent studies of DUI, we discuss the latest ideas regarding the evolutionary origin of this atypical system of mtDNA inheritance and we highlight some fascinating questions that are beginning to emerge from studies of DUI.

The DUI system unveiled

DUI was discovered in 1990 when a high frequency of heteroplasmic individuals was detected in a study of mtDNA variation in a hybrid zone between *Mytilus edulis* and *M. galloprovincialis* mussels in southwest England [26]. The occurrence of two divergent mtDNAs in the same individuals was subsequently confirmed in other mytilid populations [27,28]. These findings, combined with previous cytological studies showing retention of paternal mitochondria in early embryos [29], led the authors to suggest biparental transmission of *Mytilus* mitochondrial DNA [27,28]. Subsequent studies showed that heteroplasmy in *Mytilus* was associated to a gender-associated mtDNA transmission system that required distinct paternal and maternal mitotypes [10,13]. For this reason, it was named doubly uniparental inheritance [10]. DUI was later documented in other (but not all) bivalve taxa as noted above (Figure 1). Given the broad taxonomic distribution of DUI observed within the Bivalvia [15–17,20,21,30–32] it was proposed that this system evolved once in an ancestral bivalve lineage and was subsequently lost in some descendants [19,33].

The transition from strict maternal inheritance (SMI) to DUI probably involved a modification of the recognition system of sperm mitochondria by eggs, and a specific mechanism ensuring a father-to-son transmission of M mtDNA [33]. Empirical evidences for such mechanisms came from studies on *Mytilus* embryos. Typically, the fate of sperm mitochondria depends on whether the embryo is destined to develop into a female or a male [34–38]. For example, Sutherland *et al.* [35] found that during fertilization, all eggs receive sperm mitochondria, which are eliminated or drastically reduced within 24–48 hrs in female embryos. Recent epifluorescence-based observations of embryos destined to become males demonstrated that sperm mitochondria tend to aggregate in a single blastomere that is thought to give rise to the male germ line. By contrast, in embryos destined to become female, sperm mitochondria are randomly dispersed among blastomeres [36,37]. This sex-specific difference in the embryonic aggregation of M versus F genomes, which is one factor responsible for the tissue specific differences in ratios of M and F genomes, appears to be dependent on the action of microtubules [39,40].

These studies suggest that heteroplasmy is the initial state in the early development of mytilid embryos. In the family Mytilidae, females normally shift from being heteroplasmic zygotes to essentially homoplasmic (F genome containing) adults. Mature male mytilids contain varying ratios of the F and M genomes in all tissues (i.e. testes contain predominantly M genome, somatic tissues contain predominantly F genome) [41,42]. By contrast, the venerid clam *Venerupis* (=*Ruditapes*) *philippinarum* has a strong predominance of M mtDNAs in somatic tissues [16]. Taken together, these results indicate that mechanisms for the sequestration of sperm mitochondria and the M genome into the male gonad are not perfect [33], or that M genomes have distinct functional repertoires in these two divergent marine taxa. Similarly, the mechanisms that limit the presence of sperm mitochondria and M genomes in developing females are also not perfect. Trace amounts of M genome have been found in tissues of adult females [41,42] and even in the unfertilized eggs of *M. galloprovincialis* [22]. By contrast, the male germ line seems to normally exclude the mitochondrial F mitochondrial genome and preferentially amplify the M genome [38]. Indeed, by forcing spawned sperm to swim through a solution of Percoll, and thus minimizing the probability of sperm contamination by somatic tissues or cells, Venetis *et al.* [38] recovered only distinct, paternally transmitted mtDNA genomes in the total DNA extractions from the 'washed' sperm of 36 *M. galloprovincialis* individuals. This precise male-specific transmission of the M mitochondrial genome is required for the stability of DUI [38].

Another unusual aspect of mussel genetics (and to date this has only been demonstrated in *Mytilus*) is that some females produce female-biased offspring whereas other females produce male-biased offspring, regardless of which male they mate with [34]. A model to explain the coupling of mtDNA inheritance and sex determination in mussels has been previously developed [33,34,37,43] (Box 1). According to this model, mtDNA inheritance in bivalves with DUI could be controlled by a maternally-encoded sex-determining gene or a gene linked to a sex-determining factor, as has been observed in the basidiomycete fungus *Cryptococcus neoformans* [4]. It is tempting to speculate that the sperm mitochondria might

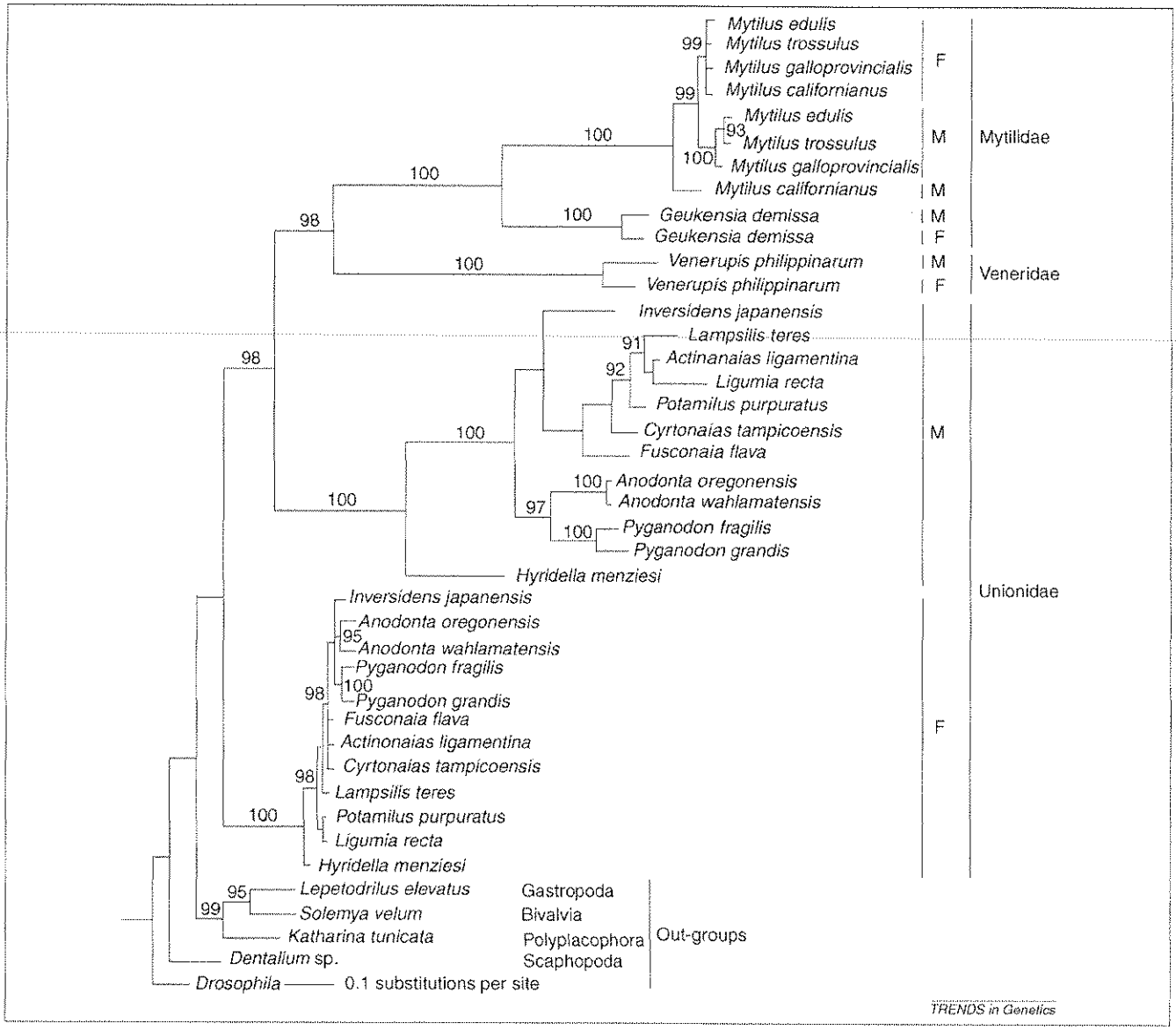

Figure 1. DUI phylogeny. The mitochondrial genome evolutionary relationships for the DUI-containing taxa showing that the male (M) and female (F) genomes in the freshwater mussels, the Unionidae, have been stable whereas there is evidence for occasional masculinization events (i.e. F genomes giving rise to M genomes) in the families Veneridae and Mytilidae. Topology and branch lengths are based on analysis of 199 amino acids from *cox*1 and posterior probabilities (×100) >90 are displayed. The taxonomic and sex-specific transmission affiliations of the individual sequences are indicated at the right. The Mr. Bayes analysis [73] used the Jones, Taylor and Thornton (JTT) model of amino acid substitution [74] and was run for 10 million generations with eight chains. Overall, 10 000 trees were saved during the course of the analysis but only the last 9000 were used to construct the consensus tree (i.e. the first 1000 trees were discarded as burn-in). The original nucleotide sequences (species and accessions numbers) were translated with the Drosophila mitochondrial genetic code and used according to Refs [19,47,48,75–79].

Box 1. DUI model

The model, based on sex-ratio bias of certain pair matings, proposes that the bias is under the control of the female parent and suggests that this control is exercised by the nuclear genotype of the mother rather than mitochondrial genotype [43]. It has been hypothesized that three genetic factors (i.e. W, X, and Z) are involved. According to this model, W is located in the outer surface of the sperm mitochondrion and is recognized by an egg nuclear-encoded cytoplasmic X factor. These two factors are suggested to be parts of the 'sperm mitochondria elimination system' that leads to maternal mtDNA inheritance, a mechanism that could be comparable to the ubiquitination system observed in mammals [43]. In addition to W and X, the model also implies a DUI-specific, one-locus two-allele, Z factor (i.e. the active Z and the inactive z), which occurs in the egg cytoplasm. The role of the factor Z is to suppress factor X, and thus prevent the elimination (or dispersion) of sperm mitochondria in fertilized eggs (Figure I). If only sperm mitochondria subsequently gain entrance into the primordial germ cell, or if the M genome has a replicative advantage over the F genome in these cells, it might explain the dominance of the M genome in the male gonad [37]. The Z factor, paternal mitochondria and the M genome herein are virtually linked to sex determination.

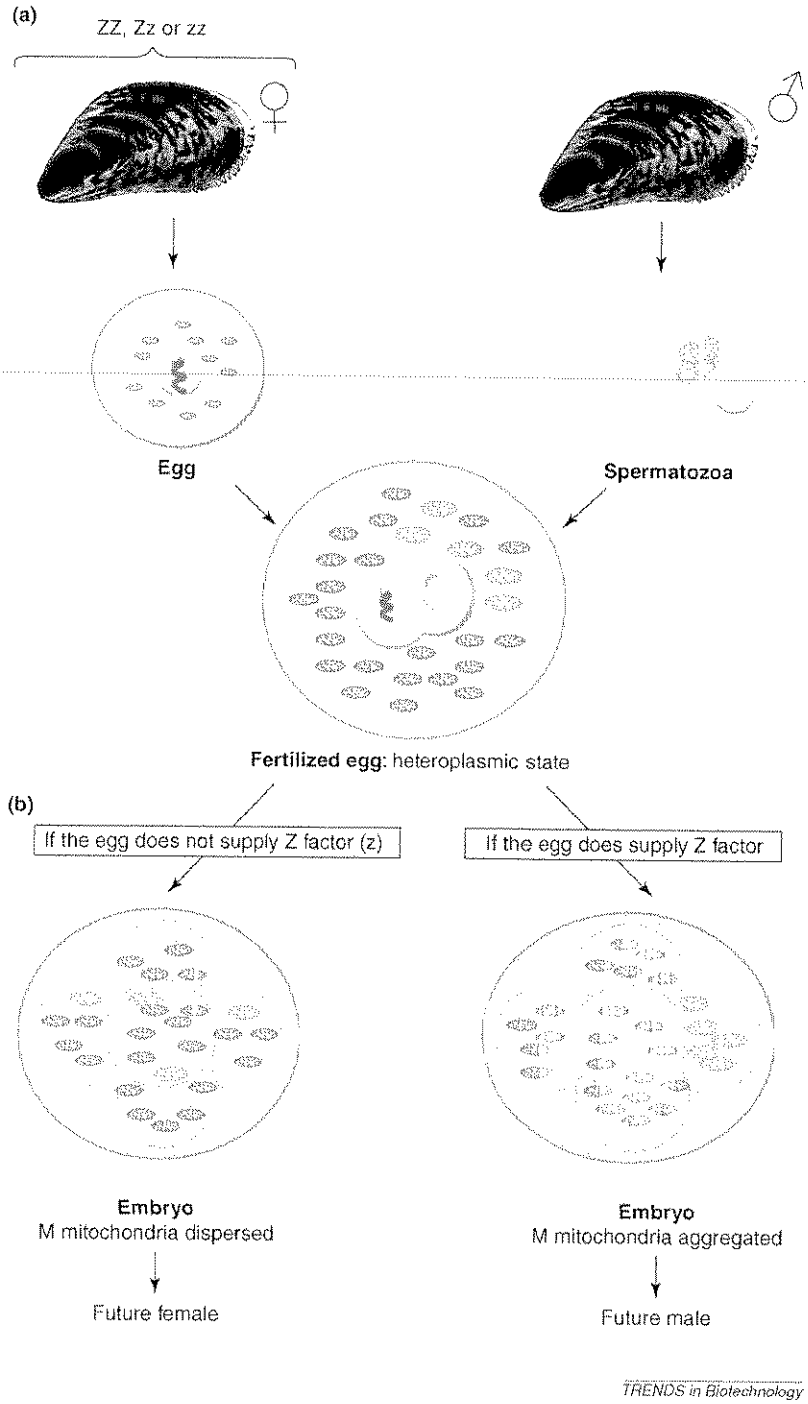

Figure I. Proposed genetic control of mitochondrial transmission under the DUI system. (a) Male and female gametes contribute to the mitochondrial population of the fertilized egg (b) Mother-dependant genetic models [33,34,37,43] predict that mothers carrying the Z allele will produce eggs with the Z factor, allowing the retention of sperm mitochondria and their subsequent aggregation in a single blastomere in embryos destined to become males. By contrast, zz mothers will produce eggs without the Z factor in which sperm mitochondria will be dispersed and/or lost, and embryos will become females.

be involved in differentiation of male reproductive tissue once they have been sequestered in the germ line. This hypothesis would imply functional differences between the mitochondrial M and F genomes and/or specific nucleo-mitochondrial interactions in the male gonad, although to our knowledge such interactions have not yet been demonstrated.

Molecular evolution of M and F mitochondrial genomes

Genetic analyses using partial mtDNA sequences of M and F mitotypes in species with DUI [14–18,20,30,31] indicated that (i) DUI appeared 200 million years ago, if not before; (ii) mussel mtDNA (both M and F lineages) has experienced an accelerated rate of mtDNA sequence divergence

compared with that of other animal taxa; and (iii) the M genome evolves more quickly than the F genome. A leading interpretation explaining why M evolves faster than F, and why both of them evolve faster than typical mtDNA, is the relaxation of selective constraints due to the unequal 'division of labour' in the DUI system [32,33]. Indeed, contrary to typical mtDNA, which has to perform fully in gonad and soma of both sexes, the F genome functions in female gonad and soma of both sexes, whereas the male genome serves primarily in the male gonad and only partially in male soma, where it occurs sporadically in conjunction with the more abundant F type [32,41,42].

The availability of 11 complete or nearly complete F and M mtDNA genome sequences has shown how species with DUI vary from the typical pattern of animal mtDNA gene content (Table 1). Most species with DUI [except *Lampsilis* (a unionoidean bivalve)] have lost the gene for ATPase subunit 8 (*atp8*) and some have a second tRNA gene for methionine (*trnM*) (e.g. *Mytilus* spp. and female *Venerupis*). However, other bivalve species, such as the sea scallop, *Placopecten magellanicus*, also lack *atp8* and have multiple *trnM* genes [44].

The mtDNA control region (i.e. the region of the mitochondrial genome in which replication and transcription are initiated) has been identified in mytilid bivalves [45], but, to our knowledge, no control region has been confirmed yet in veneroid or unionoidean bivalves (see Ref. [46]). With few exceptions, gene order and content of F and M genomes from the same species are well preserved. The freshwater mussel *Inversidens japanensis* has two gene-order inversions (in both the light and the heavy strands) and two tRNAs (*trnD* and *trnV*) encoded by opposite strands that are responsible for differences between F and M mtDNAs [47] (M. Okazaki and R. Ueshima, unpublished). The F genome of the marine clam *Venerupis philippinarum* contains a duplication of the gene for cytochrome *c* oxidase subunit II (*cox2*) and the M genome contains an extra *trnM* gene, distinguishing the two mtDNAs [47] (M. Okazaki and R. Ueshima, unpublished).

A recent comparative analysis of several mitochondrial genomes from three *Mytilus* species showed that the amino acid variability within mtDNA regions of the M genome was highly correlated with variability within the F genome (i.e. regions with high or low amino acid differences were similar in both lineages). This was interpreted as evidence of cyto-nuclear co-evolution (Box 2). The necessity of evolving in the same nuclear background has apparently forced the F and M mtDNA genomes to experience similar selective pressures. These selective pressures could result in the retention of a particular amino acid from the ancestral mtDNA genome at positions of structural importance to enzyme function, convergent amino acid changes at other sites, or even functionally equivalent (but different) amino acid substitutions in the M and F lineages. The key observation from this study is that the regions of variability in the distinct gender-associated mtDNA lineages are highly correlated [48].

When DUI breaks down: masculinization of F mtDNA

Phylogenetic analyses of cytochrome *c* oxidase subunit I (*cox1*) sequences have demonstrated that in marine (but not freshwater) mussels, the fidelity of DUI is sometimes compromised. Some males seem to lack a typical M genome [33,49–51], and F genomes seem to occasionally invade the male route of inheritance such that they become transmitted from generation to generation only through sperm [33,34,38,49]. However, the reverse (i.e. an M genome invading the F genome route of transmission) has not been observed. This phenomenon has been referred to as a 'masculinization' or 'role-reversal' event. The F genomes that have invaded the M genome's route of transmission are referred to as 'recently-masculinized' M types [33,49–51]. Several populations of *Mytilus* mussels are polymorphic for two classes of M mitochondrial genomes: an older, 'standard' M type and a 'recently-masculinized' M type. The genomes of the latter, particularly their protein coding regions, are highly similar to F genomes but they are transmitted as M genomes through sperm.

Table 1. Complete mitochondrial genomes of species with DUI

Species	Gender	Order	Genome size (bp)	Gene number (proteins–tRNAs–rRNAs)	GenBank accession number	Refs
Marine mussels						
Mytilus edulis	Female	Mytiloida	16 740	12 – 23 – 2	AY484747	[75,90]
M. galloprovincialis	Female	Mytiloida	16 744	12 – 23 – 2	AY497292	[47]
M. edulis	Male haplotype 1	Mytiloida	16 622	12 – 23 – 2	AY823623	[48]
M. edulis	Male haplotype 2	Mytiloida	16 624	12 – 23 – 2	AY823624	[48]
M. galloprovincialis	Male	Mytiloida	17 671	12 – 23 – 2	AY363687	[47]
M. trossulus	Masculinized	Mytiloida	18 652	12 – 23 – 2	AY823625	[48]
Marine clam						
Venerupis philippinarum	Female	Veneroida	22 676	13 – 23 – 2[b]	AB065375	M. Okazaki and R. Ueshima, unpublished
V. philippinarum	Male	Veneroida	21 441	12 – 24 – 2	AB065374	M. Okazaki and R. Ueshima, unpublished
Freshwater mussels						
Lampsilis ornata	Female	Unionoida	16 060	13 – 22 – 2[c]	AY365193	[46]
Inversidens japanensis	Female	Unionoida	16 826[a]	12 – 22 – 2	AB055625	M. Okazaki and R. Ueshima, unpublished
I. japanensis	Male	Unionoida	16 967[a]	12 – 22 – 2	AB055624	M. Okazaki and R. Ueshima, unpublished

[a]Incomplete mitochondrial sequences.
[b]Cox2 is duplicated.
[c]Contains atp8.

Box 2. DUI and the study of intergenomic interactions

Strong evidence for intergenomic co-evolution has been provided by direct manipulation of cells and/or embryos in culture or laboratory crosses where OXPHOS functional assays can be linked to amino acid substitutions in the interacting proteins encoded by mitochondrial and nuclear genes (see Refs [80] and [81] for reviews). Levels of intra-species mtDNA sequence divergences between M and F genomes in marine and freshwater male mussels are the highest intra-individual values yet reported (with sequence divergences in *cox1* often >20% and >30%, respectively), even higher than intra- or inter-species values reported in classical model systems used for the study of intergenomic co-evolution (Figure I) (see Ref. [82]). From a co-evolutionary perspective, one challenging question is: 'Do nuclear-encoded peptides function equally-well with either the M or F mtDNA?'. For example, there is the possibility that in somatic tissue of either sex the M genome is present but not serving any useful function. Another hypothesis is that the M genome might do its job even under a clumsy collaboration with the nuclear genes. A third assumption is the existence of nuclear isoforms that are only expressed and interact with either the M or F mtDNA genome. These isoforms might exhibit differences in biochemical activity that could have evolved to avoid potential within-individual intergenomic conflict. Indeed, testis-specific isoforms (e.g. nuclear-encoded mitochondrial cytochrome *c* and *COX* subunit VIb [complex IV]) have been discovered in mammals, suggesting a possible accommodation for the high energy demand of sperm motility [83].

Because of the important differences in the mutation rates of male and female mtDNAs, it has been hypothesized that male mtDNA should be either under relaxed constraints or positive selection pressures. In both cases, these differences can offer exceptional material to reveal, by comparative approaches (i.e. male and female mtDNAs of different species), the hot-spots of selective pressures under common nuclear backgrounds. Such studies could be of major significance because most models of mtDNA evolution consider neutral processes and purifying selection as the major forces shaping the pattern of sequence divergence among species.

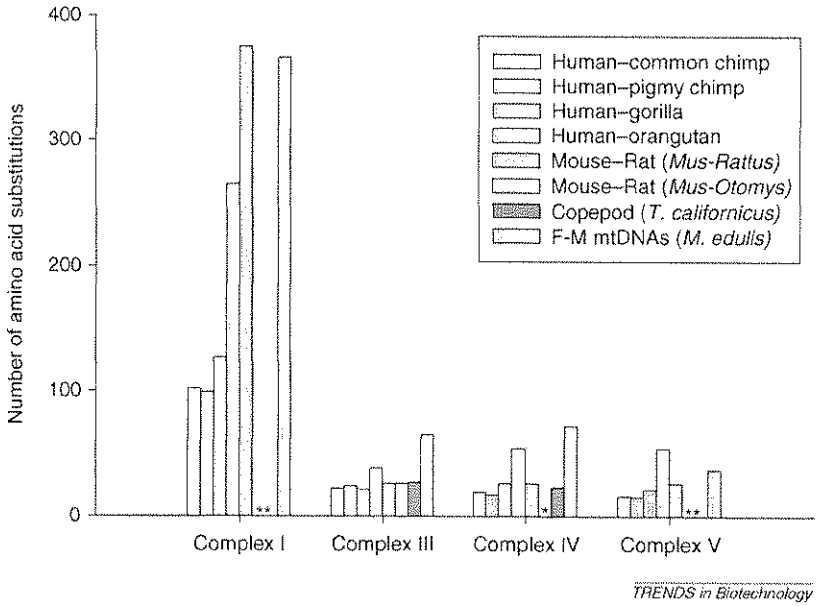

Figure I. Divergences in model systems for the study of co-evolution. Amino acid sequence differences of mtDNA-encoded peptides in model systems for analyses of cytonuclear co-evolution in which disruption of respiratory chain function has been observed. Human cells with mtDNA from chimpanzee and gorilla had impaired oxygen consumption (i.e. decreased, on average, by 20%, 34%, and 27%, respectively, compared with the human cells and/or human mtDNA control) that was attributable to a marked deficiency in respiratory chain complex I [84,85]. Activities of complexes I, III, and IV in the Rattus xenocybrid were 44%, 37%, and 78% of control mean, respectively, whereas the activity of complex III was 2% of control cybrid in the Otomys xenocybrid [86,87]. COX activities were decreased as mitochondria from a population were moved to a purer and purer nuclear background from a distant population [88,89].*, Data not available.

There are at least two potential explanations for the breakdown of DUI in *Mytilus* spp. According to one view, failure of DUI is associated with hybridization events between pairs of *Mytilus* species [18,19,52], leading to the disruption of nuclear–cytoplasmic interactions and DUI instability [18]. A second explanation for the existence of this masculinization phenomenon came with the discovery of intermolecular mitochondrial recombination within the *cox3* gene in male gonadal tissues from *Mytilus galloprovincialis* [53]. It should be noted that both hypotheses are not mutually exclusive; recombination might occur more frequently (or perhaps is more easily detected) in hybrid situations. These findings are highly significant because they provided direct evidence of recombination in animal mtDNA, a much debated subject (reviewed in Ref. [54]). Mitochondrial recombination was later confirmed in other mytilid and venerid species [20,48,55–57]. The observation that some PCR-amplified main control regions in male gonads of *M. trossulus* were a mosaic of F- and M-like sequences provided a potential link between the homologous recombination of F and M genomes and masculinization [55]. Specifically, because these recombinant variants were transmitted through sperm like the M genome, but showed high coding sequence similarity to the *M. edulis* F genome, Burzynski *et al.* [55] speculated that occasional invasions of the male transmission route by the F genome could be possible through the addition of M control region sequences to the control region of F genomes. The first complete sequence of a recently-masculinized *M. trossulus* mitochondrial genome also indicated that two control regions exist, one male-specific and the other female-specific [48]. At present, it is not known whether both potential control regions are functional, whether they act in a tissue-specific manner, or what their respective roles are in

mitotype transmission. Nevertheless, recent evidence of mitochondrial recombination within the control region in male and female *M. trossulus* mussels corroborates the hypothesis that an M-type control region sequence is necessary to confer the paternal role on genomes that are otherwise F-like [57].

Interestingly, there is no evidence of mitochondrial recombination or masculinization events during the evolutionary history of freshwater mussels (Unionoida) [19,21]. The absence of recombination could explain why gender-switching events are lacking in unionoidean bivalves [47]. The lack of masculinization in freshwater mussels also coincides with the presence of a unique M genome-specific 3′ extension of the mtDNA-encoded cytochrome *c* oxidase subunit II gene (M*cox2*) [17,18,58]. This extension, which is ~185 codons in length, is present in all unionoidean M genome *cox2* genes examined to date, including each of the three unionoidean bivalve families (i.e. Hyriidae, Margaritiferidae and Unionidae) [17,18]. Examination of the rates and patterns of substitution suggests that the extension (M*cox2*e) is evolving under relaxed purging selection relative to the upstream M*cox2* homologous region (M*cox2*h), that is, the region present in both F*cox2* and M*cox2* [17,18]. The M*cox2*e is likely to be the most rapidly evolving mitochondrial domain identified in animals [18]. Apparently, M*cox2*e is neither present in mytiloid M genomes, nor in other animal mitochondrial genomes [47,48].

A specific function for M*cox2*e has not yet been demonstrated. The *cox2* gene encodes a highly conserved subunit of cytochrome *c* oxidase, the terminal enzyme of the mitochondrial inner membrane that is responsible for the transfer of electrons from cytochrome *c* to oxygen [59]. MCOX2e antibody-based analyses indicated that the extended MCOX2 protein in *Venustaconcha ellipsiformis* (Unionoidea: Unionidae) is predominantly expressed in testes, weakly expressed in other male tissues, and not expressed in female tissues [58]. The immunohistochemistry-based localization of MCOX2 to sperm mitochondria combined with the predicted presence of five transmembrane helices in the *V. ellipsiformis* MCOX2e region suggest that the latter is located in the outer and/or inner mitochondrial membrane [58]. These characteristics suggest several potential functions for the MCOX2e; for example, within the sperm mitochondria and in developmental interactions. During spermatogenesis, M*cox2*e expression could be involved in apoptosis, which is an important physiological mechanism that regulates the number of sperm produced (e.g. [60]). One likely possibility for a developmental interaction functionality for MCOX2e is that an outer mitochondrial membrane localization could facilitate gender-specific movements of sperm-derived mitochondria within unionoidean embryos in a manner similar to that observed in *Mytilus* [36,37]. Although more studies are needed to elucidate the function(s) of the MCOX2e protein, its association with the absence of masculinization in unionoidean bivalves suggests that it has been selected either as a protective mechanism against gender-switching or for advantageous male reproductive function (which could also explain the lack of gender-switching).

Origin and evolution of DUI

Even if the occurrence of DUI in other taxa remains to be explored, the question persists why DUI evolved in bivalve mollusks [7,61]. Can the considerable variation of bivalve reproductive strategies provide a clue [62]? Although individuals of many bivalve species demonstrate stable gonochoric sexuality (i.e. once they become male or female, they remain that sex throughout their life), several species have simultaneous hermaphrodites that produce both male and female gametes in the ovotestis or sequential hermaphrodites that change sex as they age. In all cases, sex (or type of gamete produced) is not determined until germ cells are differentiated but the exact mechanism(s) of sexualization of the undifferentiated gonad are unknown [63]. Moreover, neither sex chromosomes nor sex-related genes have been identified, except for the detection of an esterase (Est)-like 'male-associated polypeptide' in the male gonad (or male reproductive tissue), and a fibronectin (Fn)-like polypeptide in the female gonad (or female reproductive tissue) of the gonochoric DUI-species *Mytilus galloprovincialis* and the hermaphroditic *Pecten maximus* [62,64].

A recent hypothesis suggests that DUI first emerged in a simultaneously hermaphroditic species in which both eggs and sperm were produced in an ovotestis [61]. This idea is based on the assumption that in species with (i) distinct sex chromosomes, (ii) maternal transmission of mtDNA, and (iii) heterogametic males, maternally sex-linked genes and maternally inherited mitochondria should be co-adapted (i.e. mitochondria are more frequently co-transmitted with maternally expressed X-linked genes than with autosomal genes, whereas they are never co-transmitted with paternally expressed Y-linked genes) [8]. In this context, it is not in the interest of maternally expressed sex-linked genes to 'allow' paternal inheritance to persist [61]. By contrast, no such conflict exists in hermaphrodite species, which do not have sex chromosomes and in which both male and female gametes are produced in the ovotestis. Perhaps paternally inherited mitochondria were retained for some time in a hermaphroditic ancestral species; however, to persist over the longer term, paternal M genomes would have been selected to secure more reliable transmission. This could have been achieved by the masculinization of the ovotestis (i.e. suppression of its ovarian aspects), so that paternal transmission of the M genome and a dioecious sex-determination system would become associated (see above) [61]. However, further studies on bivalve species with and without DUI, as well as on hermaphroditic and dioecious species will be essential to better understand the coupling of gender and mtDNA inheritance in the DUI system.

Potential adaptive evolution of M mtDNA

Currently, it is unclear whether a selective advantage favoured the retention of DUI in bivalves, but one possible mechanism could be related to the sex-antagonistic effects of mtDNA resulting from maternal inheritance [7–9]. In SMI, natural selection on mtDNA operates only in females because males do not transmit their cytoplasmic genes [24,25]. Consequently, mtDNA genotypes that have positive (or neutral, or even slightly deleterious) fitness effects in females but potentially deleterious effects in

males can theoretically be maintained in a population [8,65,66]. For example, it has been shown that the human mtDNA variant T, which is observed at a frequency of 20% in some European populations, yields significantly less motile sperm than the most common and best performing human mtDNA variant H [67]. Recent experimental studies of mice provide even more compelling evidence that mitochondrial mutations with comparatively small effects on female function can have profound impacts on spermatogenesis [68] and the incidence of major sperm abnormalities [69]. These findings provide strong support for the hypothesis that adaptive evolution of male function might be significantly constrained because of the maternal inheritance of mitochondria [65]. Doubly uniparental inheritance of mitochondria would thus be both an elegant strategy to avoid sex-specific constraints associated with maternal mtDNA transmission and an opportunity for mitochondria to evolve adaptively for male function [25,70,71].

An interesting approach to address the issue of adaptive evolution of M genome in males employs comparison of functional properties of mitochondria between 'standard' and 'recently-masculinized' mytilid mussel spermatozoa. Because these different male mtDNAs exhibit almost 9% amino acid sequence divergence, and that many of these amino acid substitutions are not conservative, it was hypothesized that these differences could affect mitochondrial functions, and thus sperm motility [72]. The initial test of this hypothesis indicated no significant differences in sperm swimming speeds between standard male and recently masculinized mitotypes in *Mytilus edulis* [72]. Swimming speed is, however, only one of the key parameters of sperm motility and fitness, and we can not exclude the possibility of more subtle effects on mitochondrial respiratory chain function or on other gamete characteristics (e.g. longevity, numbers of sperm). Additional comparative analyses of masculinized and standard M-type bearing-sperm are needed to clarify the potential impact associated with the amino acid substitutions observed between these mtDNAs.

Concluding remarks

Since its discovery in the early 1990s, much progress has been made in understanding the DUI system in bivalve species, in particular with regard to the mechanisms underlying the sex-specific behaviour of sperm mitochondria and the molecular evolution of M and F mitochondrial genomes. Future work will focus on unique features of the DUI system such as the potential adaptive evolution of the M genome on sperm motility, the role of recombination in masculinization events (and specifically the role of the control region in determining whether a particular mitochondrial genome will behave as an F or an M genome), functional aspects of the exclusive M*cox2* extension, and finally, the possible association between mtDNA inheritance and sex determination. As these examples illustrate, DUI provides an excellent opportunity to investigate evolutionary and functional consequences of alternative modes of mitochondrial inheritance.

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References

1 Burger, G. *et al.* (2003) Mitochondrial genomes: anything goes. *Trends Genet.* 19, 709–716
2 Boore, J.L. (1999) Animal mitochondrial genomes. *Nucleic Acids Res.* 27, 1767–1780
3 Birky, C.W., Jr (2001) The inheritance of genes in mitochondria and chloroplasts: laws, mechanisms, and models. *Annu. Rev. Genet.* 35, 125–148
4 Xu, J. (2005) The inheritance of organelle genes and genomes: patterns and mechanisms. *Genome* 48, 951–958
5 Elson, J.L. and Lightowlers, R.N. (2006) Mitochondrial DNA clonality in the dock: can surveillance swing the case? *Trends Genet.* 22, 603–607
6 Wallace, D.C. (2005) A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. *Annu. Rev. Genet.* 39, 359–407
7 Burt, A. and Trivers, R.L. (2006) *Genes in Conflict.* Belknap Press of Harvard University Press
8 Rand, D.M. (2001) The units of selection on mitochondrial DNA. *Annu. Rev. Ecol. Syst.* 32, 415–448
9 Ballard, J.W.O. and Whitlock, M.C. (2004) The incomplete natural history of mitochondria. *Mol. Ecol.* 13, 729–744
10 Zouros, E. *et al.* (1994) Mitochondrial DNA inheritance. *Nature* 368, 818
11 Skibinski, D.O.F. *et al.* (1994) Mitochondrial-DNA inheritance. *Nature* 368, 817–818
12 Skibinski, D.O.F. *et al.* (1994) Sex-limited mitochondrial DNA transmission in the marine mussel *Mytilus edulis*. *Genetics* 138, 801–809
13 Zouros, E. *et al.* (1994) An unusual type of mitochondrial-DNA inheritance in the blue mussel *Mytilus*. *Proc. Natl. Acad. Sci. U. S. A.* 91, 7463–7467
14 Liu, H.P. *et al.* (1996) Paternal mitochondrial DNA differentiation far exceeds maternal mitochondrial DNA and allozyme differentiation in the freshwater mussel, *Anodonta grandis grandis*. *Evolution Int. J. Org. Evolution* 50, 952–957
15 Hoeh, W.R. *et al.* (1996) Multiple origins of gender-associated mitochondrial DNA lineages in bivalves (Mollusca: Bivalvia). *Evolution Int. J. Org. Evolution* 50, 2276–2286
16 Passamonti, M. and Scali, V. (2001) Gender-associated mitochondrial DNA heteroplasmy in the venerid clam *Tapes philippinarum* (Mollusca Bivalvia). *Curr. Genet.* 39, 117–124
17 Curole, J.P. and Kocher, T.D. (2002) Ancient sex-specific extension of the cytochrome c oxidase II gene in bivalves and the fidelity of doubly-uniparental inheritance. *Mol. Biol. Evol.* 19, 1323–1328
18 Curole, J.P. and Kocher, T.D. (2005) Evolution of a unique mitotype-specific protein-coding extension of the cytochrome c oxidase II gene in freshwater mussels (Bivalvia: Unionoida). *J. Mol. Evol.* 61, 381–389
19 Hoeh, W.R. *et al.* (2002) High fidelity of mitochondrial genome transmission under the doubly uniparental mode of inheritance in freshwater mussels (Bivalvia: Unionoidea). *Evolution Int. J. Org. Evolution* 56, 2252–2261
20 Passamonti, M. *et al.* (2003) Molecular evolution and recombination in gender-associated mitochondrial DNAs of the manila clam *Tapes philippinarum*. *Genetics* 164, 603–611
21 Walker, J.M. *et al.* (2006) Taxonomic distribution and phylogenetic utility of gender-associated mitochondrial genomes in the Unionoida (Bivalvia). *Malacologia* 48, 265–282
22 Obata, M. *et al.* (2006) Sperm mitochondrial DNA transmission to both male and female offspring in the blue mussel *Mytilus galloprovincialis*. *Dev. Growth Differ.* 48, 253–261
23 Sano, N. (2007) Quantitation of the male and female types of mitochondrial DNA in a blue mussel, *Mytilus galloprovincialis*, using real-time polymerase chain reaction assay. *Dev. Growth Differ.* 49, 67–72
24 Zeh, J.A. (2004) Sexy sons: a dead end for cytoplasmic genes. *Proc. R. Soc. Lond. B. Biol. Sci.* 271, S306–S309
25 Zeh, J.A. and Zeh, D.W. (2005) Maternal inheritance, sexual conflict and the maladapted male. *Trends Genet.* 21, 281–286

26 Fisher, C. and Skibinski, D.O.F. (1990) Sex-biased mitochondrial-DNA heteroplasmy in the marine mussel *Mytilus*. *Proc. R. Soc. Lond. B. Biol. Sci.* 242, 149–156

27 Hoeh, W.R. *et al.* (1991) Heteroplasmy suggests limited biparental inheritance of *Mytilus* mitochondrial-DNA. *Science* 251, 1488–1490

28 Zouros, E. *et al.* (1992) Direct evidence for extensive paternal mitochondrial-DNA inheritance in the marine mussel *Mytilus*. *Nature* 359, 412–414

29 Longo, F.J. and Anderson, E. (1969) Cytological aspects of fertilization in the lamellibranch, *Mytilus edulis*. II. Development of the male pronucleus and the association of the maternally and paternally derived chromosomes. *J. Exp. Zool.* 172, 97–119

30 Rawson, P.D. and Hilbish, T.J. (1995) Evolutionary relationships among the male and female mitochondrial-DNA lineages in the *Mytilus edulis* species complex. *Mol. Biol. Evol.* 12, 893–901

31 Stewart, D.T. *et al.* (1995) Male and female mitochondrial-DNA lineages in the blue mussel (*Mytilus edulis*) species group. *Mol. Biol. Evol.* 12, 735–747

32 Stewart, D.T. *et al.* (1996) Degree of selective constraint as an explanation of the different rates of evolution of gender-specific mitochondrial DNA lineages in the mussel *Mytilus*. *Genetics* 143, 1349–1357

33 Zouros, E. (2000) The exceptional mitochondrial DNA system of the mussel family Mytilidae. *Genes Genet. Syst.* 75, 313–318

34 Saavedra, C. *et al.* (1997) Male-dependent doubly uniparental inheritance of mitochondrial DNA and female-dependent sex-ratio in the mussel *Mytilus galloprovincialis*. *Genetics* 145, 1073–1082

35 Sutherland, B. *et al.* (1998) The fate of paternal mitochondrial DNA in developing female mussels, *Mytilus edulis*: implications for the mechanism of doubly uniparental inheritance of mitochondrial DNA. *Genetics* 148, 341–347

36 Cao, L. *et al.* (2004) Differential segregation patterns of sperm mitochondria in embryos of the blue mussel *(Mytilus edulis)*. *Genetics* 166, 883–894

37 Cogswell, A.T. *et al.* (2006) Segregation of sperm mitochondria in two- and four-cell embryos of the blue mussel Mytilus edulis: implications for the mechanism of doubly uniparental inheritance of mitochondrial DNA. *Genome* 49, 799–807

38 Venetis, C. *et al.* (2006) No evidence for presence of maternal mitochondrial DNA in the sperm of *Mytilus galloprovincialis* males. *Proc. R. Soc. Lond. B. Biol. Sci.* 273, 2483–2489

39 Obata, M. and Komaru, A. (2005) Specific location of sperm mitochondria in mussel *Mytilus galloprovincialis* zygotes stained by MitoTracker. *Dev. Growth Differ.* 47, 255–263

40 Yaffe, M.P. (1999) Dynamic mitochondria. *Nat. Cell Biol.* 1, E149–E150

41 Garrido-Ramos, M.A. *et al.* (1998) The distribution of male-transmitted and female-transmitted mitochondrial DNA types in somatic tissues of blue mussels: implications for the operation of doubly uniparental inheritance of mitochondrial DNA. *Genome* 41, 818–882

42 Dalziel, A.C. and Stewart, D.T. (2002) Tissue-specific expression of male-transmitted mitochondrial DNA and its implications for rates of molecular evolution in *Mytilus* mussels (Bivalvia: Mytilidae). *Genome* 45, 348–355

43 Kenchington, E. *et al.* (2002) Genetics of mother-dependent sex ratio in blue mussels (*Mytilus* spp.) and implications for doubly uniparental inheritance of mitochondrial DNA. *Genetics* 161, 1579–1588

44 Smith, D.R. and Snyder, M. Complete mitochondrial DNA sequence of the scallop *Placopecten magellanicus*: evidence of transposition leading to an uncharacteristically large mitochondrial genome. *J. Mol. Evol.* (in press)

45 Cao, L. *et al.* (2004) Evidence that the large noncoding sequence is the main control region of maternally and paternally transmitted mitochondrial genomes of the marine mussel (*Mytilus* spp.). *Genetics* 167, 835–850

46 Serb, J.M. and Lydeard, C. (2003) Complete mtDNA sequence of the north American freshwater mussel, *Lampsilis ornata* (Unionidae): an examination of the evolution and phylogenetic utility of mitochondrial genome organization in bivalvia (Mollusca). *Mol. Biol. Evol.* 20, 1854–1866

47 Mizi, A. *et al.* (2005) The complete maternal and paternal mitochondrial genomes of the Mediterranean mussel Mytilus galloprovincialis: implications for the doubly uniparental inheritance mode of mtDNA. *Mol. Biol. Evol.* 22, 952–967

48 Breton, S. *et al.* (2006) Comparative analysis of gender-associated complete mitochondrial genomes in marine mussels (*Mytilus* spp.). *Genetics* 172, 1107–1119

49 Hoeh, W.R. *et al.* (1997) Phylogenetic evidence for role-reversals of gender-associated mitochondrial DNA in *Mytilus* (Bivalvia: Mytilidae). *Mol. Biol. Evol.* 14, 959–967

50 Quesada, H. *et al.* (1999) Interspecies transfer of female mitochondrial DNA is coupled with role-reversals and departure from neutrality in the mussel *Mytilus trossulus*. *Mol. Biol. Evol.* 16, 655–665

51 Quesada, H. *et al.* (2003) Heteroplasmy suggests paternal co-transmission of multiple genomes and pervasive reversion of maternally into paternally transmitted genomes of mussel (*Mytilus*) mitochondrial DNA. *J. Mol. Evol.* 57, S138–S147

52 Wood, A.R. *et al.* (2003) Disruption of doubly uniparental inheritance of mitochondrial DNA in hybrid mussels (*Mytilus edulis* × *M. galloprovincialis*). *Heredity* 91, 354–360

53 Ladoukakis, E.D. and Zouros, E. (2001) Direct evidence for homologous recombination in mussel (*Mytilus galloprovincialis*) mitochondrial DNA. *Mol. Biol. Evol.* 18, 1168–1175

54 Rokas, A. *et al.* (2003) Animal mitochondrial DNA recombination revisited. *Trends Ecol. Evol.* 18, 411–417

55 Burzynski, A. *et al.* (2003) Evidence for recombination of mtDNA in the marine mussel *Mytilus trossulus* from the Baltic. *Mol. Biol. Evol.* 20, 388–392

56 Rawson, P.D. (2005) Nonhomologous recombination between the large unassigned region of the male and female mitochondrial genomes in the mussel, *Mytilus trossulus*. *J. Mol. Evol.* 61, 717–732

57 Burzynski, A. *et al.* (2006) Doubly uniparental inheritance is associated with high polymorphism for rearranged and recombinant control region haplotypes in Baltic *Mytilus trossulus*. *Genetics* 174, 1081–1094

58 Chakrabarti, R. *et al.* (2006) Presence of a unique male-specific extension of C-terminus to the cytochrome c oxidase subunit II protein coded by the male-transmitted mitochondrial genome of *Venustaconcha ellipsiformis* (Bivalvia: Unionoidea). *FEBS Lett.* 580, 862–866

59 Rawson, P.D. and Burton, R.S. (2006) Molecular evolution at the cytochrome oxidase subunit 2 gene among divergent populations of the intertidal copepod, Tigriopus californicus. *J. Mol. Evol.* 62, 753–764

60 Chan, W.Y. *et al.* (2006) Transcriptome analyses of male germ cells with serial analysis of gene expression (SAGE). *Mol. Cell. Endocrinol.* 250, 8–19

61 Davison, A. (2006) The ovotestis: an underdeveloped organ of evolution. *Bioessays* 28, 642–650

62 Paz, M. *et al.* (2005) Esterase-like and fibronectin-like polypeptides share similar sex-cell-biased patterns in the gonad of hermaphroditic and gonochoric species of bivalve mollusks. *Cell Tissue Res.* 322, 475–489

63 Paz, M. *et al.* (2001) Sexual differentiation of the somatic gonad tissue in marine bivalve mollusks: esterase- and fibronectin-like recognition signals. *Int. J. Dev. Biol.* 45, S119–S120

64 Torrado, M. *et al.* (2003) Mussel MAP, a major gonad-duct esterase-like protein, is released into sea water as a dual constituent of the seminal fluid and the spermatozoon. *J. Exp. Biol.* 206, 313–326

65 Frank, S.A. and Hurst, L.D. (1996) Mitochondria and male disease. *Nature* 383, 224

66 Gemmell, N.J. *et al.* (2004) Mother's curse: the effect of mtDNA on individual fitness and population viability. *Trends Ecol. Evol.* 19, 238–244

67 Ruiz-Pesini, E. *et al.* (2000) Human mtDNA haplogroups associated with high or reduced spermatozoa motility. *Am. J. Hum. Genet.* 67, 682–696

68 Trifunovic, A. *et al.* (2004) Premature ageing in mice expressing defective mitochondrial DNA polymerase. *Nature* 429, 417–423

69 Nakada, K. *et al.* (2006) Mitochondria-related male infertility. *Proc. Natl. Acad. Sci. U. S. A.* 103, 15148–15153

70 Gemmell, N.J. and Braisher, T.L. (2001) Organelle genome evolution. *Trends Ecol. Evol.* 16, 489–490

71 Selosse, M. *et al.* (2001) Organelle genome evolution – Response from Selosse, Albert, and Godelle. *Trends Ecol. Evol.* 16, 490

72 Everett, E.M. *et al.* (2004) Mitochondrial DNA polymorphisms and sperm motility in *Mytilus edulis* (Bivalvia: Mytilidae). *J. Exp. Zool. A Comp. Exp. Biol.* 301A, 906–910

73 Huelsenbeck, J.P. *et al.* (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17, 754–755

74 Jones, D.T. *et al.* (1992) The rapid generation of mutation data matrices from protein sequences. *Comput. Appl. Biosci.* 8, 275–282
75 Boore, J.L. *et al.* (2004) Complete sequences of the highly rearranged molluscan mitochondrial genomes of the scaphopod *Graptacme eborea* and the bivalve *Mytilus edulis*. *Mol. Biol. Evol.* 21, 1492–1503
76 Mock, K.E. *et al.* (2004) Genetic diversity and divergence among freshwater mussel (Anodonta) populations in the Bonneville Basin of Utah. *Mol. Ecol.* 13, 1085–1098
77 Beagley, C.T. *et al.* (1997) Gender-associated diverse mitochondrial DNA molecules of the mussel *Mytilus californianus*. *Curr. Genet.* 31, 318–324
78 Hoeh, W.R. *et al.* (1998) Testing alternative hypotheses of Neotrigonia (Bivalvia: Trigonioida) phylogenetic relationships using cytochrome C oxidase subunit I DNA sequences. *Malacologia* 40, 267–278
79 Riginos, C. *et al.* (2004) Differential patterns of male and female mtDNA exchange across the Atlantic Ocean in the blue mussel, *Mytilus edulis*. *Evolution Int. J. Org. Evolution* 58, 2438–2451
80 Blier, P.U. *et al.* (2001) Natural selection and the evolution of mtDNA-encoded peptides: evidence for intergenomic co-adaptation. *Trends Genet.* 17, 400–406
81 Rand, D.M. *et al.* (2004) Cytonuclear coevolution: the genomics of cooperation. *Trends Ecol. Evol.* 19, 645–653
82 Blier, P.U. *et al.* (2006) Functional conservatism in mitochondrial evolution: insight from hybridization of arctic and brook charrs. *J. Exp. Zoolog. B. Mol. Dev. Evol.* 306B, 425–432
83 Das, J. (2006) The role of mitochondrial respiration in physiological and evolutionary adaptation. *Bioessays* 28, 890–901
84 Kenyon, L. and Moraes, C.T. (1997) Expanding the functional human mitochondrial DNA database by the establishment of primate xenomitochondrial cybrids. *Proc. Natl. Acad. Sci. U. S. A.* 94, 9131–9135
85 Barrientos, A. *et al.* (1998) Human xenomitochondrial cybrids - Cellular models of mitochondrial complex I deficiency. *J. Biol. Chem.* 273, 14210–14217
86 McKenzie, M. and Trounce, I. (2000) Expression of *Rattus norvegicus* mtDNA in *Mus musculus* cells results in multiple respiratory chain defects. *J. Biol. Chem.* 275, 31514–31519
87 McKenzie, M. *et al.* (2003) Functional respiratory chain analyses in murid xenomitochondrial cybrids expose coevolutionary constraints of cytochrome b and nuclear subunits of complex III. *Mol. Biol. Evol.* 20, 1117–1124
88 Burton, R.S. *et al.* (1999) Genetic architecture of physiological phenotypes: Empirical evidence for coadapted gene complexes. *Am. Zool.* 39, 451–462
89 Ellison, C.K. and Burton, R.S. (2006) Disruption of mitochondrial function in interpopulation hybrids of *Tigriopus californicus*. *Evolution* 60, 1382–1391
90 Hoffmann, R.J. *et al.* (1992) A novel mitochondrial genome organization for the blue mussel. *Mytilus edulis*. *Genetics* 131, 397–412