Bivalve molluscs as model systems for studying mitochondrial biology

Bivalve molluscs as model systems for studying mitochondrial biology

Fabrizio Ghiselli*, Mariangela Iannello, Giovanni Piccinini, and Liliana Milani

Department of Biological, Geological, and Environmental Sciences
University of Bologna, Italy

*Corresponding Author: fabrizio.ghiselli@unibo.it

Abstract

The class Bivalvia is a highly successful and ancient taxon including ~25,000 living species. During their long evolutionary history bivalves adapted to a wide range of physicochemical conditions, habitats, biological interactions, and feeding habits. Bivalves can have strikingly different size, and despite their apparently simple body plan, they evolved very different shell shapes, and complex anatomic structures. One of the most striking features of this class of animals is their peculiar mitochondrial biology: some bivalves have facultatively anaerobic mitochondria that allow them to survive prolonged periods of anoxia/hypoxia. Moreover, more than 100 species have now been reported showing the only known evolutionarily stable exception to the strictly maternal inheritance of mitochondria in animals, named doubly uniparental inheritance. Mitochondrial activity is fundamental to eukaryotic life, and thanks to their diversity and uncommon features, bivalves represent a great model system to expand our knowledge about mitochondrial biology, so far limited to a few species. We highlight recent works studying mitochondrial biology in bivalves at either genomic or physiological level. A link between these two approaches is still missing, and we believe that an integrated approach and collaborative relationships are the only possible ways to be successful in such endeavour.

Keywords: Doubly Uniparental Inheritance, mitochondrial genomics, mitochondrial inheritance, heteroplasmy, mitochondrial bottleneck, physiological adaptation.

1. Why Bivalves?

The paradigm of mitochondrial genome (mtDNA) as a single clock-like-evolving molecule, stable in both its content and organization has definitely fallen in the last decades. MtDNA shows considerable variability across Eukaryota (Kolesnikov and Gerasimov 2012; Sloan et al. 2018), but it was once considered having consistent genomic features across Metazoa. However, as new taxa were investigated, it became increasingly clear that several of such supposedly general genomic features—derived from a few model species or from taxonomically biased studies—were actually not shared across animals (Gissi et al. 2008; Breton et al. 2014; Lavrov and Pett 2016; Milani and Ghiselli 2020), also in taxa previously considered as stable in their mtDNA gene order and evolutionary rates (see for instance ascidians, holometabolous insects, but also vertebrates: Gissi et al. 2010; Kaltenpoth et al. 2012; Rubinstein et al. 2013; Macey et al. 2021; Zhang et al. 2021). Molluscs in general, and bivalves in particular, show a great diversity in numerous mtDNA features, and peculiar deviations from the "textbook descriptions" (Ghiselli et al. 2021; see Box 1 for details). The most peculiar feature concerning mitochondrial biology of bivalves is the Doubly Uniparental Inheritance (DUI), a unique mitochondrial inheritance system that has been reported, so far, in more than 100 species (Capt et al. 2020). Bivalves showing DUI have two mtDNAs following two separate, sex-specific inheritance routes: one lineage, called F-type, is inherited matrilinearly, the other, called M-type, is inherited patrilinearly. Eggs are homoplasmic for the F-type, sperm for the M-type; adult tissues show various degrees of heteroplasmy (see Section 2.1). F-type and M-type represent two completely segregated lineages and can reach considerable levels of nucleotide and amino acid divergence (respectively up to ~45% and ~53% in Unionida (Doucet-Beaupré et al. 2010); up to 53% amino acid divergence in Venerida: Capt et al. 2020). Moreover, the two genomes can differ in their gene order and/or gene content. So far, among animals DUI represents the only known evolutionarily stable exception to the typical Strictly Maternal Inheritance (SMI). To assess whether DUI originated in bivalves and whether it had a single origin or multiple origins inside the Class is a fundamental point to fully understand DUI evolutionary dynamics, but there is no clear answer to such questions. The main obstacle to the solution of this conundrum is the largely incomplete information we have about presence/absence of DUI because of missing data—namely, unsampled species and/or species in which DUI presence was not thoroughly investigated. DUI detection is particularly prone to false negatives, so it can easily be overlooked by studies that were not specifically designed for that purpose. Overall, the patchy distribution of DUI-positive species across bivalves could be either due to loss

of DUI in some taxa or multiple origins. The single origin hypothesis initially met the support of most DUI researchers, but now a multiple-origin hypothesis is gaining popularity (Milani et al. 2013; Zouros 2013; Milani et al. 2014; Milani et al. 2016; Ghiselli et al. 2017; Maeda et al. under review).

DUI bivalves represent a unique model for the study of mitochondrial dynamics: having two highly divergent mtDNA populations, characterized by different dynamics, selective pressure, and segregation pattern, can provide insights into mitonuclear coevolution and mitochondrial inheritance. One of the most asked questions about DUI is: why bivalves? Is there a reason why DUI has been observed so far only in bivalves? We actually do not know if DUI or some similar mechanism exists outside bivalves, because mitochondrial genetic variation and heteroplasmy have been not extensively investigated across invertebrates. Finding DUI is not a straightforward process, and it requires dedicated efforts and specific analyses (discussed in: Theologidis et al. 2008; Ghiselli et al. 2017). That said, there must be some reason behind the impressive mtDNA plasticity of bivalves, and we think it has something to do with the likewise remarkable plasticity that they show at the physiological level. Indeed, bivalves can resist periodic air exposure leading to cycles of hypoxia-reoxygenation by changing mitochondrial metabolism, they have alternative oxidase (AOX) proteins on inner mitochodrial membrane that allows them to limit reactive oxygen species (ROS) emission during stressful fluctuations of environmental parameters, they can live exceptionally long compared with other extant taxon (in particular, a DUI species is the longest-lived animal documented so far), and some of them (DUI species) are subject to sex-specific mitochondrial evolutionary pressures with female and male gametes showing different mechanisms of energy production when compared to SMI gametes (see Table 1 and Section 4).

But which is the cause, and which is the effect? Are bivalves physiologically plastic because of their genomic diversity, or is their peculiar physiology that allows the great variability at the genome level and the remarkable features described above? So far, a great deal of work was done at the mtDNA level, trying to unravel the features that could be associated with such unique traits, but much less work has been carried on at the level of nuclear genomes, transcriptomes, proteomes, and physiology. It is becoming clear how a single-discipline perspective is weak in unravelling such complex and diverse evolutionary mechanisms, so it is now necessary to build a bridge between genetics/genomics and physiology—namely between the genotype and the phenotype—to shed light on some of the most exciting and promising biological systems among animals (Ghiselli and Milani 2020).

In this work we highlight some topics concerning genomics and physiology that have been previously investigated, and some others that may stimulate future works. The two fields are still distant and not well-connected, and prompted by the SICB2021 Symposium “Genomic Perspectives in Comparative Physiology of Mollusks: Integration Across Disciplines”, we wanted to review some potentially interesting features of bivalve mitochondrial biology for future integrative, multidisciplinary research.

2. Within-individual variability of mtDNA

MtDNA is present in multiple copies within cells and, consequently, within individuals. Such copies are not identical to each other, therefore organisms—to various degrees—show a heteroplasmic population of mtDNA (Payne et al. 2013; Aryaman et al. 2018). The presence of different mitochondrial variants results in a competition among mitochondrial genomes (Klucnika and Ma 2019): on the one hand, selection may favor some variants over others (Fan et al. 2008), on the other hand, stochastic events may also have a crucial role in affecting the variability of the mitochondrial population (Wonnapinij et al. 2008). Both selection and genetic drift shape the within-individual mitochondrial variability (Milani and Ghiselli 2015), which in turn impacts the dynamics of mitonuclear coevolution (Rand et al. 2004), mtDNA inheritance, and mtDNA segregation during embryo development (Johnston and Burgstaller 2019). Mitonuclear coevolution hypothesis predicts that the two coexisting genomes have to coadapt because their products have to cofunction in order to provide proper cellular metabolism (Rand et al. 2004). Indeed, the strict coadaptation coupled with the crucial importance of the metabolic mechanisms involved were invoked to explain the high degrees of homoplasmy observed in animal cells, and the detrimental effects of heteroplasmy (Lane 2011, 2012). Experiments on xenomitochondrial cybrids revealed a direct relationship between the efficiency of cellular respiratory capacity, and the evolutionary divergence between the nuclear background and the foreign mtDNAs (McKenzie et al. 2003; Bayona-Bafaluy et al. 2005; Niehuis et al. 2008; Wang et al. 2017; Kong, Xiang, et al. 2020). This reduced mitonuclear fitness was observed also in natural interpopulation hybrids, allowing to hypothesize a key-role of cytoplasmic incompatibilities in speciation and hybrid breakdown (Ellison and Burton 2006; Burton et al. 2013; Hill 2017; Hill et al. 2019).

Variability in the mitochondrial population can be assessed in terms of Single Nucleotide Polymorphisms (SNPs), genome structure/architecture, and pattern of gene presence/absence. Even if only a few works investigated the within-individual variability of mtDNA in bivalves, the results reported so far are peculiar, and deserve attention. Here we briefly highlight some of the within-individual variability patterns observed in bivalves.

2.1 Distribution and abundance of M-type in male tissues

An intriguing characteristic found in some DUI species is the plastic pattern of presence/absence of the M-type mitochondrial genome in somatic tissues. It is indeed well established that some DUI species commonly possess the paternally-inherited mitochondrial genome not only in male gonads, but also in male and female somatic tissues (Obata et al. 2006; Kyriakou et al. 2010; Ghiselli et al. 2011, 2019; Breton et al. 2017). Still, this pattern shows both between and within species variability: the presence of M-type in somatic tissues can vary from being the predominant mitochondrial genome to being not detectable, and everything in between (see for example: Ghiselli et al. 2011; Breton et al. 2017; Iannello et al. 2021). The possibility for DUI species to manage not only two different populations of mtDNA (F- and M-type), but also variable patterns of presence/absence of M-type DNA in different tissues is fascinating. The main implication is that, differently from gonads—which are mostly homoplasmic for either F- or M-type—, somatic tissues are effectively heteroplasmic. The coexistence in the same tissue of two very divergent mtDNA populations opens questions about how replication, transcription, and translation of different mitochondrial genomes are coregulated by the nuclear genome (see Section 3).

2.2 Intraindividual variation of mtDNA

In bivalves we find also examples of variability in the mtDNA structure (see for example: Ghiselli et al. 2013, 2017; Guerra et al. 2014). More in detail, mtDNA length polymorphism has been reported across different individuals from the same species, and the differences are likely due to a variable number of repeated elements in the control region. Moreover, variability in mtDNA length spanning thousands of bases was reported within the same individual of *Ruditapes decussatus* (Ghiselli et al. 2017). The reason beneath such differences is unclear, especially the maintenance in the mtDNA population of longer variants. Indeed, it was reported that mtDNA length plays a role in mitochondrial dynamics: shorter genomes should be advantaged in transmission, since these variants would require a shorter time to replicate themselves (Diaz et

al. 2002). One intriguing explanation could lie in the nature of the repeated elements: if these elements could bring some sort of advantage in mtDNA replication—for example by carrying polymerase-binding motifs or secondary structures—this would explain the presence of longer mtDNA variants. In this case, such variants would not bring benefits in terms of fitness and what we observe would be the result of replication advantage allowing longer variants to be preferentially transmitted to the offspring. A similar scenario was reported in *Drosophila melanogaster* (Rand 2011). The presence of structural variants in bivalves has not been deeply investigated yet, and approaches like High-Throughput Sequencing (HTS) of mtDNA using long reads, will allow to better explore such interesting feature.

The within-individual variability at the SNP level has been so far investigated only in the DUI species *Ruditapes philippinarum*. A recent work analyzed the within-individual mtDNA variability (Iannello et al. 2021) by Illumina HTS: while the same pattern of shared SNPs was found for the F-type among tissues of the same individual, a different situation was observed for the M-type. Indeed, a high variability was found between somatic tissues (i.e.: male adductors) and gonads: with up to 200 different M-type SNPs, which is possibly the highest within-individual mtDNA variability observed so far.

2.3 Mitochondrial bottleneck

A peculiar feature of the paternally-inherited mtDNA population is that it comes from few (usually 4-5) sperm mitochondria (Cao et al. 2004; Milani et al. 2012). This implies that—even if there are no precise indications of the total number of mtDNA in spermatozoa yet—the population size of paternally-inherited mtDNA is likely considerably lower than the maternally-inherited one (Ghiselli et al. 2011). In addition, the population size of M-type does not increase during the first phases of embryo development (Guerra et al. 2016), so the M-type mtDNA faces a very narrow bottleneck during embryo development, narrower than that experienced by female-inherited organelles. This feature makes M-type mtDNA particularly suited for studying the effect of population size on genetic bottlenecks.

The within-individual variability in the mtDNA population depends on the genetic variation and sample size of the inherited mtDNA population (i.e.: the variability and size of mtDNA population in the oocyte for almost all Metazoans) (Johnston 2019). In particular, the higher the number of mtDNA that segregates in different cells during embryo development, the lower will be the cell-to-cell and tissue-to-tissue variability of mtDNA populations in the adult (Johnston and Burgstaller 2019). This was shown by mathematical simulations by Radzvilavicius et al. (2016),

and the authors hypothesized that the reason behind the high number of mitochondria in oocytes is to reduce mitochondrial variance across tissues.

In DUI species, the paternally-inherited mtDNA with low initial copy number provides a unique opportunity to investigate the above mentioned prediction about mitochondrial bottleneck. Iannello et al. (2021) analyzed the within-individual mtDNA variability in the DUI species *R. philippinarum* and found a remarkable difference in M-type SNPs across adductors and gonads in male samples, possibly the highest within-individual variability in a mtDNA population analyzed so far. Intriguingly, such variability was not detected in the F-type of the same male samples, nor in female samples. The authors argued that such variability is a consequence of the very small population size of the paternally-inherited mtDNA populations—which profoundly impact the segregation of mitochondrial variants in different tissues—and confirmed the mathematical predictions made by Radzvilavicius et al. (2016) and in Johnston et al. (2019).

Future studies using deep HTS of mtDNA in sperm and oocytes from DUI species could provide an exact indication of both the mtDNA population size and mtDNA variability in gametes. Similarly, HTS of mtDNA in both male and female adult tissues would give a more precise indication of the variability of paternally- and maternally-inherited mitochondrial genomes in the whole mtDNA population. If such works confirmed the observations by Iannello et al. (2021), the high level of M-type variability would add to the co-existence of paternally- and maternally-inherited mDNA populations, increasing the complexity of the heteroplasmic condition in these animals. How the same nuclear genome can handle all such mtDNA variability is an open question.

3. Mitonuclear coevolution in bivalves

MtDNA variance within an individual seems to be an unfavourable condition that is suppressed in multiple ways (Lane 2012). First, the exclusion of the paternal contribution to the mtDNA pool in the zygote should ensure a higher level of homosplamy in the individual (Sato and Sato 2017); in addition, the high number of mtDNA in the oocyte is believed to greatly reduce mitochondrial genetic variation across different tissues (Radzvilavicius et al. 2016; Johnston and Burgstaller 2019). So, homoplasmy seems to be a favourable trait (Lane 2012), even if low levels of heteroplasmy are commonly detected across animals (Dowling 2014). One reason could be that orchestrating multiple, very different mtDNA variants by the same nuclear genome, would be challenging (Lane 2011). Accordingly, the presence of heteroplasmic mtDNA population results

in a reduction of fitness in mice (Sharpley et al. 2012), and a strict coevolution between nuclear and mitochondrial genomes is thought to be necessary for healthy organisms (Rand et al. 2004; Bar-Yaacov et al. 2012). That said, most of the investigations have been conducted on a few mammals and on a handful of model species, so how mtDNA heteroplasmy affects organisms with a quite different mitochondrial dynamics and physiology is still unknown. The evolution and variability of mitochondrial genomes have been deeply investigated in bivalves, but studies on the nuclear counterpart are limited. Nowadays, sequencing is easier and cheaper: an increase in bivalve genomes (both showing DUI and not) in public databases will allow to investigate the evolution of nuclear genes involved in mitochondrial functions—among them oxidative phosphorylation, mtDNA replication, transcription, translation, and mito-nuclear signaling. In addition, in DUI species such data could help to understand how the three genomes (F-, M-type and nuclear) coexist. In the same way, a comparative analysis of DUI vs SMI genomes will allow to detect duplications and gene family expansions that could have a role in the functional interactions with multiple divergent mtDNA variants. Finally, transcriptomes, which are much more abundant in public databases than genomes, may be useful tools to investigate the nuclear response to the peculiar mitochondrial scenario we observe in DUI species. Indeed, RNA-Seq data provide information about differential transcription and alternative splicing, which play an important role in evolution and adaptation (Romero et al. 2012). Specific works on DUI species are still not available, but a comprehensive investigation of RNA-Seq data from DUI species could reveal a role of differential expression in handling different mitochondrial genomes in different tissues. In a still unpublished work on *R. philippinarum*, we found that F- and M-type mtDNAs are co-transcribed with different nuclear genes (Xu et al., in preparation).

3.1 First molecular mitonuclear coevolutionary data in bivalves

Given the features discussed above, it is clear how bivalves represent an exceptional resource for mitonuclear coevolutionary investigations. Different molecular aspects are involved in coevolutionary mitonuclear mechanisms, including heterogenomic protein-protein, protein-RNA, and protein-DNA interactions (Bar-Yaacov et al. 2012). The high evolutionary rates, the size variation, gene duplication events that happen to tRNAs, rRNAs, and protein coding genes, and the length variability of some OXPHOS proteins, represent natural experiments to test the coevolutionary dynamics between nuclear and mitochondrial genomes. The combination of the striking mitogenomic diversity and the widespread distribution of DUI in bivalves provides a natural model of high-level heteroplasmy well-suited to test for mitonuclear coevolutionary dynamics.

To our knowledge, a direct investigation on mitonuclear coevolution in bivalves was covered only in two recent works. Iannello et al. (2019) investigated the molecular evolution of mtOXPHOS and 59 nuclear OXPHOS (nuOXPHOS) subunits in two congeneric bivalve species (one of which shows DUI), reporting striking deviations from the evolutionary patterns observed in other animals. In metazoans, high nuOXPHOS dN/dS ratios are interpreted as signals of accelerated evolution driven by compensatory fixations in the nuclear genes to compensate for the faster-mutating mtDNA (nuclear compensation hypothesis; e.g.: Osada and Akashi 2012; Havird and Sloan 2016; Barreto et al. 2018; Hill et al. 2019). Interestingly, Iannello et al. (2019) observed very high dN/dS for mtOXPHOS subunits supporting a faster evolutionary rate of mitochondrial genes in bivalves, but these were not associated with a higher dN/dS of nuOXPHOS subunits. A second work analyzed OXPHOS genes in 31 bivalve species and retrieved similar dN/dS values, thus extending the evolutionary trends observed in Iannello et al. (2019) to the whole class (Piccinini et al. 2021). Moreover, strong signals of mitonuclear coevolution were assessed, but no unambiguous indication of nuclear compensatory evolution was observed, not even at the residue-specific level.

Most of the nuclear compensatory predictions were built on model taxa to explain how the higher mtDNA mutation rate could coexist with the preservation of the OXPHOS function: in Vertebrata, the ratio between mitochondrial and nuclear mutation rate can indeed be as high as 32 (Allio et al. 2017). In bivalves, however, the mitochondrial mutation rate is just around twice the nuclear one (Allio et al. 2017; Piccinini et al. 2021). Given the fast mtDNA evolutionary rate typical of the class, the lower mitonuclear mutational ratio might be due to a faster-mutating nuclear DNA (Plough 2016). This might be the reason behind the apparent lack of nuclear compensatory signals (Iannello et al. 2019; Piccinini et al. 2021): the mutational pressure acts on two genomes, therefore the need for compensation, or at least for a one-way nuclear compensation, is much lower (Havird and Sloan 2016). However, other non-mutually exclusive reasons could be behind it, including relaxed selection on OXPHOS genes. Bivalves are indeed sedentary, mostly filtrating animals. Bivalves show the capability to survive in hypoxic states thanks to alternative acceptors pathways (Müller et al. 2012), and the presence of an Alternative Oxidase (AOX) (McDonald and Vanlerberghe 2004; see Section 4.2). Thanks to such physiological features, the selective pressure on the respiratory complexes might be lower, allowing for higher degrees of mitonuclear mismatches in their structural assembly (see for instance similar conclusions on the plant genus Silene in Weaver et al. 2020).

Bivalve molecular coevolutionary data are quite intriguing and meet the expectations for such a mito-diverse taxon, but the surface of the matter has been just scratched. Class-wide deviations from animal mitonuclear coevolution patterns have been observed, however an OXPHOS-centric perspective might exclude the possibility to retrieve different and potentially interesting signals of coevolution. Protein-protein interactions are just one of the aspects involved (Bar-Yaacov et al. 2012), but, up to now, the only one investigated in bivalves. Furthermore, interactions between the nuclear-encoded ribosomal machinery and the mitochondrial-encoded RNAs, interactions between the mtDNA and the nuclear-encoded transcriptional/replicational complexes, and interactions between mtDNA and nuclear-encoded proteins involved in localisation and stability, represent promising research subjects (see for instance: van der Sluis et al. 2015; Sloan et al. 2017; Barreto et al. 2018). However, to address the question thoroughly, both higher-quality data and an integrative approach have to be implemented. More and better genomic data, but mostly protein 3D structures of closely related taxa are crucial to study the actual sites of coevolution.

3.2 DUI: a potential frontier for mitonuclear coevolution

The high degree of heteroplasmy in bivalves represents an interesting scenario for mitonuclear coevolutionary studies, especially regarding the compensatory role hypothesized for nuclear subunits: the fixation of compensatory variants in the nuclear genome might be advantageous for one of the two mtDNAs, but detrimental to the other. As mentioned above, bivalve metabolic plasticity might allow for a looser selection on OXPHOS proteins, and some degrees of mitonuclear mismatches could be tolerated because of a non-significant impact on the organism fitness. However, DUI can persist for hundreds of million years while keeping functional male gametes that rely exclusively on M-type mitochondria, therefore other hypotheses should be taken into account to explain the coevolutionary dynamics among the three genomes involved (F and M mtDNA, and the nuclear genome).

One conclusion could be that the M-type is simply not selected for high efficiency of OXPHOS functions. In this case, the mitonuclear coevolution would be biased toward the F-type genome, leading to the accumulation in nuclear genes of F-type-specific compensatory fixations that would eventually disrupt cytonuclear complexes built with M-type subunits. However, M-type genomes are under selection, they are functionally active, genes are transcribed and translated, sperm swimming relies on them, and M-type transcripts/proteins have been observed also in male

somatic tissues (Ghiselli et al. 2013, 2019; Milani et al. 2014; Milani and Ghiselli 2015; Breton et al. 2017; Lubośny et al. 2018; Bettinazzi et al. 2019, 2020; 2021). Spermatozoa in DUI species rely on OXPHOS for energy production (Bettinazzi et al. 2020; see Section 4.4) therefore there should be a certain level of constraints on M-type genes. The difference observed in OXPHOS performance of sperm mitochondria when compared to egg mitochondria could be due to sperm-specific selective pressures resulting in a modified mitochondrial metabolism (Bettinazzi et al. 2019, 2020), and/or to a nuclear compensatory mechanism "prioritizing" the functional efficiency of F-type complexes while keeping a sufficient level of functionality of M-type ones. The latter explanation, however, is undermined by the fact that in some lineages the two mitochondrial genomes have been separated for hundreds of million years (e.g.: in Unionida): after so much time of F-type-prioritized compensatory evolution the M-type complexes could become completely nonfunctional, and conflicts between the two genomes could seriously compromise either fitness or DUI itself.

Another explanation could be found in the relative contributions of the genomes to the fixation of compensatory alleles. As said before, according to the nuclear compensation hypothesis, the nuclear genome is generally identified as the primary site of compensatory substitutions balancing the fast-mutating mtDNA. Osada and Akashi (2012) observed that, in primates, nuclear substitutions usually appeared later in time with respect to the mitochondrial mutations they should putatively compensate, therefore supporting the nuclear compensation hypothesis. However, other works on that subject lack the temporal factor, therefore they cannot precisely address a specific form of coevolution (e.g.: Gershoni et al. 2010; Levin and Mishmar 2017; Li et al. 2017; Barreto et al. 2018). On the other hand, recent *in vivo* evidence in *Caenorhabditis elegans* suggested that the mitochondrial compensatory variants were able to retrieve functional recovery after deleterious nuclear gene mutations in 60 generations (Wernick et al. 2019). Mitochondrial compensation might not be uncommon in animals and it could represent a relatively fast mechanism to maintain function after a deleterious nuclear mutation. If this was the case in bivalves, the two lineages of mitochondrial genomes in DUI species could independently compensate for mutations in the nuclear genome, therefore relieving the latter of a two-way compensatory pressure, and representing, in our opinion, a more parsimonious hypothesis. Works directly addressing the direction of the compensations—either *in silico* with sequence reconstructions and investigations on recent DUI-loss/DUI-gain events, or with *in vitro* enzymatic assays of characterized mitotypes—will likely solve this issue.

A third explanation for the evolutionary stability of DUI might lie in the existence of a duplicated set of nuclear genes that specifically coevolve with the M-type genome and are differentially transcribed in a sex-specific manner. Examples of sex-specific transcription of nuOXPHOS genes are known in mammals and *Drosophila* (Gallach et al. 2010; Eslamieh et al. 2017; Havird and McConie 2019), and tissue-specific splice variants are present in humans (Barshad et al. 2018). However, no data suggested the presence of a complete set, or subset, of OXPHOS nuclear paralogues in bivalves, so far. Nuclear sequences available for DUI species are few and high-quality annotated genomes are lacking, and so far two duplicated OXPHOS genes were reported (e.g.: *cox5b* in *Crassostrea gigas*: XP_011413812.1 and XP_011413810.1; *cox4* in *Mytilus coruscus*, a non-DUI Mytilidae: CAC5406076.1 and CAC5401226.1). Transcriptomic data in *R. philippinarum* did not show evidence of duplicated nuOXPHOS genes with sex-specific transcription (Maeda et al. submitted; Xu et al. in preparation). To test this hypothesis more high-quality genomic data will be crucial. However, only “canonical” nuOXPHOS proteins have been investigated so far (Iannello et al. 2019; Piccinini et al. 2021); Maeda et al. under review; Xu et al. in preparation). It was proposed that the mitochondrial mutational load could be the reason behind the evolution of supernumerary, lineage-specific, nuclear-encoded proteins that are recruited to the core structure of OXPHOS complexes protecting it from damaging free radicals and/or providing structural stability as the mitochondrial subunits were degraded by mutational erosion (van der Sluis et al. 2015; Hill et al. 2019). Within-metazoan lineage-specific novel proteins involved in OXPHOS complexes and mitochondrial ribosomes have been indeed cautiously associated with different mtDNA evolutionary patterns (van Esveld and Huynen 2018). It could be worthwhile to look for similar lineage-specific acquisitions either across all bivalves, or in long-lasting DUI lineages, since that could be a way to “patch” for highly diversifying mtDNAs.

In the end, no interpretation on the mitonuclear coevolutionary mechanism assuring DUI maintenance can be confidently excluded so far, but data are partial because the matter has not been directly approached yet. Coupling respirometric assays with molecular sequencing and the detection of co-variating residues in mitochondrial and nuclear genes could shed light on the nature of this DUI tri-genomic coevolution, yielding fruitful contributions to the defining of mitonuclear coevolutionary mechanisms in general.

4. From genotype to phenotype: mtDNA and physiological traits

In bivalves, the mostly investigated phenotypic traits related to mitochondrial function include tolerance to hypoxia, thermal tolerance, and longevity (Table 1). What makes these animals so suitable to investigate resistance to a large variety of stressors? Is it the fluctuating environmental parameters under which they evolved that may have promoted the maintenance of plastic traits for oxygen consumption and energy production? Can this resistance be linked to the presence in bivalves of the highest level of heteroplasmy observed so far in living beings? Unfortunately, to date there is no study addressing phenotype descriptions coupled with a precise genetic characterization of the mitonuclear genetic variation from the same sample (be it specimen, population, or species), or quantifying mtDNA heteroplasmy. Some studies so far compared physiological traits in DUI and SMI species but without any information about the presence and the level of heteroplasmy (Table 1). Often such studies were performed on somatic tissues, thus being subject to the high variability in heteroplasmy described above (see Section 2.1). Without the analysis of mtDNA sequences, it is impossible to understand which genetic variants are associated with the observed phenotype because it is the result of a cumulative effect due to the whole mitochondrial population, with no means of clarifying the origin of a diverse organelle performance.

We think there is a need for simultaneous investigation of genetic variation and associated phenotypic traits, of expanding the research beyond correlational data gaining functional clues, to understand the causal relationship among the different observed features. Interestingly, in some plants, a high mutation rate appears to cause no more than subtle differences in mitochondrial physiology: lineages with dramatically different mitochondrial mutation rates may indeed underlie similar mitochondrial phenotypes at higher levels of biological organization (Table 1). It would be interesting to see if this can be the same in bivalve species with diverse levels of heteroplasmy.

4.1 Tolerance to fluctuating environmental factors

The regulation of mitochondrial processes in animals living in highly stressful environments allows them to maintain mitochondrial integrity and function despite intense and rapid fluctuating conditions. For example, oxygen fluctuations are an important mitochondrial stressor to aerobic organisms and can cause severe damage to mitochondria, energy deficiency

and cell death, thus having major consequences for organism fitness (Sokolova 2018; Sokolova et al. 2019). In hypoxia-tolerant organisms, mitochondrial tolerance appears to be achieved with the regulation of many processes, and even distantly related animal species revealed common physiological responses, including upregulation of electron transport system (ETS) capacity, suppression of ROS-producing processes, reversible suppression of OXPHOS, and the upregulation of mitochondrial quality control mechanisms (Sokolova 2018; Sokolova et al. 2019). In the case of bivalves, for example, modification in energy metabolism appears to allow the adaptation to a wide variety of environments, including intertidal zones, cold Arctic and Antarctic waters, estuaries and coastal waters, temporary freshwater bodies, and also areas with high anthropogenic impact (Sokolova et al. 2012). Mitochondria of animals living in the intertidal zone regulate energy production preventing cellular damage during the cycle of hypoxia and reoxygenation (H/R) (Ivanina et al. 2016; Sokolova 2018). Indeed, relevant differences were found when comparing bivalve species that live in intertidal zones with species in subtidal environments (Table 1): the former showing hypoxia-tolerance, the latter being hypoxia-sensitive.

Hypoxia-tolerant intertidal bivalves (e.g.: *Mercenaria mercenaria*, *Crassostrea virginica*, see Table 1) suppress ATP-demanding cellular functions during hypoxia, contributing to their high tolerance to H/R stress (Ivanina et al. 2016). Then, during reoxygenation, mitochondrial reorganizations sustain high oxidative phosphorylation flux and ETS capacity (Kurochkin et al. 2009; Sussarellu et al. 2013; Ivanina et al. 2016). Instead, in subtidal hypoxia-sensitive species (e.g.: *Argopecten irradians*, Table 1), mitochondrial ETS, OXPHOS capacity, and mitochondrial membrane potential collapse during reoxygenation (Ivanina et al. 2016), similarly to what observed during hypoxia-reoxygenation in mammalian tissues (Kalogeris et al. 2014; Korge et al. 2015; Sokolova 2018). In hypoxia-sensitive species, exposure to hypoxia followed by reoxygenation led also to elevated levels of oxidative stress in mitochondria (Ivanina et al. 2016). Notably, *Mytilus edulis* intertidal populations that experience H/R stress showed higher antioxidant defense capacities than their subtidal steadily submerged counterparts (Letendre et al. 2009; Sokolova et al. 2012).

Another kind of stress acting on mitochondrial metabolism is that due to temperature fluctuations. In intertidal bivalves (e.g.: *C. virginica*, *Mya arenaria* and in the Antarctic clam *Laternula elliptica*), an increased temperature resulted in reduced mitochondrial coupling and excess ROS generation (Sokolova 2018) (Table 1). A comparison between freshwater unionids (*Dreissena bugensis* and *Elliptio complanata*) also indicated that high temperature is linked to a

similar impairment of the OXPHOS machinery, characterized by a lower coupling and an increased proton leakage, maybe associated with an increase in ROS emission, with a lower tolerance linked to a higher aerobic metabolic depression (Hraoui et al. 2020). These data indicate that mitochondrial thermal sensitivity may affect the survival and distribution of animal species, and highlight the basic role played by mitochondria in determining thermal tolerance. This is true not only for aquatic ectotherms, but also, for example, for mice and humans (Lemieux et al. 2017; Mitov et al. 2017), indicating that differential thermal sensitivity of the mitochondrial OXPHOS and proton leak may be common in animal mitochondria (Sokolova 2018).

Organism resilience to stresses caused by temperature changes and hypoxia are not fully clarified in bivalves, although rapid modulation of the ETS capacity, upregulation of antioxidant defenses, and fast degradation of damaged mitochondrial proteins appear to be involved in these processes (Sokolova 2018). As suggested by many authors, the study of additional bivalve species with specific mitochondrial adaptations will help revealing the molecular pathways involved in tolerance capacity to environmental stress; at the same time, the concurrent analysis of mitochondrial genetic variability is fundamental to link the mitochondrial genotype to phenotype.

4.2 Alternative oxidase and protection from oxidative stress

The AOX enzyme is highly conserved in many non-vertebrate taxa and may protect cells against hypoxia and oxidative stress, decreasing the emission of ROS during stress conditions (Liu and Guo 2017). AOX is a mitochondrial inner-membrane oxidase that accepts electrons directly from ubiquinol and it reduces oxygen to water without involving cytochrome-linked electron transport chain (Liu and Guo 2017). Given the proposed role of AOX in mediating the transition from sulfide-rich to oxygen-rich environments, it is not surprising that many extant animals that experience hypoxic and/or sulfidic conditions have an AOX (Weaver 2019). For example, burrowing and benthic organisms, such as marine bivalves, have AOX sequences in their genomes (Tschischka et al. 2000; Munro et al. 2013; Robertson et al. 2016; Weaver 2019). It was proposed that animals that occupy environments with fluctuations in oxygen content have retained AOX for the protective role against ROS-based damage during the hypoxic–reoxygenation transition (McDonald and Gospodaryov 2019). This appears confirmed in the intertidal copepod *Tigriopus californicus*, whose AOX transcription is upregulated under stress conditions, resulting in increased AOX protein levels (Tward et al. 2019; Weaver 2019).

Interestingly, two AOX mRNAs were identified in an intertidal bivalve, the oyster *C. virginica* (Liu and Guo 2017). The gene *CvAOX* has 10 exons with a tandem duplication of exon 10, that, with alternative splicing, can produce the two variants *CvAOXB* or *CvAOXA*, using either the first or second exon 10, respectively. The second exon 10 is the most conserved across taxa, while the first exon 10, present in *CvAOXB*, shows novel mutations close to functional sites. Under stress (i.e.: air exposure) *CvAOXB* showed significantly higher transcription than *CvAOXA*. Interestingly, these data documented the evolution of a novel AOX variant adaptive to stress in a bivalve (once again, a novelty regarding mitochondrial biology discovered for the first time studying bivalves).

Notably, AOX increased activity has been reported in some plant species characterized, like bivalves, by an elevated rate of mtDNA mutations. In angiosperm plants of the genus *Silene*, ecologically similar, closely related species have either 'fast' or 'slow' mtDNA mutation rates, with "fast" species in which mtDNA evolves faster than nuDNA (as in mammals), and 'slow' species in which mtDNA evolves slower than nuDNA (similar to typical angiosperms) (Weaver et al. 2020). Interestingly, *Silene* mitochondria showed similar respiration profiles among species, regardless of them having 'fast' or 'slow' mtDNA mutation rates. Indeed, the overall mitochondrial function appears minimally impacted in *Silene* species showing elevated mtDNA mutation rates. Also, some fast species with lower respiration efficiency relied on AOX increased activity. Thus, *Silene* lineages with highly different mitochondrial mutation rates may underlie similar phenotypes at higher levels of biological organization (Weaver et al. 2020) and this can be due also to an increased reliance on AOX. These data on plants suggest the importance of coupling mtDNA sequence modification to the phenotype (e.g.: physiological outcomes) to make predictions on the effects of mtDNA mutations.

4.3 Ageing and lifespan: membrane peroxidation and ROS generation

Membrane susceptibility to peroxidation was supposed to be one main driver of lifespan (Rodríguez et al. 2019). Mitochondrial membrane composition has been studied in bivalves, given the extremely wide range of longevity inside the class. Indeed, the release of reactive carbonyls upon oxidation of polyunsaturated fatty acids of biological membranes are believed to foster cellular ageing. This was suggested by comparative studies in mammals and birds, in which membrane lipids peroxidation index (PI) negatively correlated with longevity (Munro and Blier

2012). Interestingly, a PI decrease with increasing longevity was also documented in marine bivalves. The PI-longevity relationship was addressed by comparing the clam *Arctica islandica*, having maximum reported longevity (MRL) of 507 years, to other sympatric bivalves with different longevity (in the range of 28-106 years). Membrane PI was found to decrease with increasing longevity, with PI decreasing faster in mitochondrial membranes than in other membranes (Munro and Blier 2012).

We discussed in previous sections that bivalves exposed to environmental stressors such as hypoxia/reoxygenation and temperature variations experience oxidative stress and that the presence of AOX can modulate ROS emission. In this connection, the emission of the ROS H_2O_2 in mitochondria isolated from mantle tissue was compared among the long-lived *A. islandica* and two short-lived species, *M. arenaria* and *Spisula solidissima* (MRL = 28 and 37 years, respectively), two species of similar size and taxonomically related (Munro et al. 2013). *A. islandica* mitochondria were found to produce significantly less H_2O_2 than those of the two short-lived species. While AOX activity does not seem to explain the observed differences, the lower ROS emission in *A. islandica* mitochondria was suggested to be due to reduced complex I and III activity (Munro et al. 2013). This is actually similar to what proposed for long-lived species that lack an AOX (e.g.: homeotherms), in which the rate of ROS emission was supposed to be lowered by maintaining a lower steady-state degree of electronic reduction of complexes I and III (Barja and Herrero 1998; Gredilla et al. 2001).

Population studies were also carried out to search for correlation between PI, ETS enzyme activities, and MRL. Six European populations of *A. islandica* showing a wide variation in MRL (from 36 to 507 years) were analyzed, but no relationship was found between membrane PI and MRL, as well as between ETS activities and MRL (Rodríguez et al. 2019). However, individuals from sites with a wide range of temperature and salinity showed markedly lower ETS enzyme activity (relative to citrate synthase activity), for which the authors suggested the presence of environmentally dependent remodeling of mitochondrial phenotypes (Rodríguez et al. 2019).

Future perspectives to clarify physiological adjustments in mitochondria under fluctuating environmental conditions should pertain ROS emission and management, the control of electron flux, the use of alternative electron transport pathways, as well as the rates of mtDNA mutations and differences in heteroplasmy levels in different populations, especially when considering DUI species, as *A. islandica* among the others. Also, as a general note, it is important to consider that

mitochondrial ROS metabolism includes both ROS production and ROS consumption, thus acting in the overall ROS level regulation (Munro and Treberg 2017). For example, mitochondria can produce and consume H_2O_2 with consequences for both oxidative stress and signalling pathways, with the production capacity being strongly influenced by membrane potential (Treberg et al. 2019). This consideration underlines the importance to combine multiple metrics to get a more comprehensive picture of mitochondrial energetics, and membrane potential estimate can be a valuable addition to oxygen consumption measurements (Treberg et al. 2018).

4.4 DUI vs SMI phenotypes

Few studies so far investigated the link between mitochondrial genotype and phenotype, taking advantage from the use of the natural heteroplasmic state of DUI species. For example, OXPHOS activity of different tissues in different species were compared through respirometry (Bettinazzi et al. 2019), and taxa were chosen in order to compare DUI and SMI species (Table 1). The data obtained showed that DUI species express sex-linked mitochondrial phenotypes. Specifically, eggs, homoplasmic for the F-type mtDNA, and female somatic tissues (i.e.: gills) expressed a common 'F-phenotype', whereas sperm, homoplasmic for the M-type, express a 'M-phenotype'. The M-phenotype was characterized by low OXPHOS/ETS rates, a strong limitation by the phosphorylation system, and a high flux control of complex IV over the upstream ETS complexes (with an almost null excess capacity of complex IV) (Bettinazzi et al. 2019). This represents the first description of a mitochondrial phenotype resulting from male-driven evolution of mtDNA, the first case of an mtDNA that can specifically adapt for male functions. Complex IV excess capacity was exclusively observed in the F-phenotype (Bettinazzi et al. 2019). The observed differences in metabolism between F- and M-type mitochondria could represent different adaptive traits since the evolution of two sex-linked mitochondrial lineages could have potentially allowed fulfillment of the different requirements of different gametes. Indeed, the existence of an M-type mtDNA, the only mtDNA present in DUI sperm, allows the study of a M-phenotype, possibly resulting from direct selection on sperm performance and potentially linking male-energetic adaptation with mitotype preservation and inheritance (Bettinazzi et al. 2019).

The comparison of DUI and SMI species was extended to the measure of sperm performance and sperm response to the presence of eggs (Bettinazzi et al. 2020). The sperm of DUI species showed low speed and low linearity, a strict dependence on OXPHOS for energy production, and a partial shift towards glycolysis and fermentation in the presence of eggs (Bettinazzi et al. 2020). In the absence of egg chemical cues, DUI sperm strictly rely on OXPHOS,

whereas sperm of SMI species combine both aerobic and anaerobic pathways of energy production. Instead, in the presence of egg chemoattractants, a partial metabolic shift in the DUI sperm was produced but did not affect SMI sperm. These results are based on distantly related bivalve species and are shown to be consistent across the DUI species analysed, suggesting a convergent evolution of sex-linked mtDNAs in DUI systems (Bettinazzi et al. 2019, 2020). Of course, further investigations are necessary, possibly extended to other DUI species, to further test the hypothesis of a link between male-specific mtDNA energetic adaptation, paternal mitochondria preservation and inheritance.

Acknowledgements

The authors would like to thank Dr. Omera Matoo and Prof. Maurine Neiman for organizing the SICB2021 Symposium “Genomic Perspectives in Comparative Physiology of Mollusks: Integration Across Disciplines”, and all the participants for the interesting and constructive interactions. We also thank two anonymous reviewers for their helpful comments and suggestions. This work was supported by the Canziani Bequest funded to FG, and the "Ricerca Fondamentale Orientata" (RFO) funding from the University of Bologna to FG and to LM.

Box 1 – MtDNA in a shell

From the very first handful of bivalve mtDNA data, it appeared clear that the extent of genome rearrangements is uncommonly high and associated with higher substitution rates, even compared to other Mollusca (Gissi et al. 2008). Bivalve mtDNA variability involves rearrangements of all genes (from tRNAs to protein coding genes), and this fluidity is observable at the subfamily level (e.g.: Brachidontinae (Lee et al. 2019); Psammobiidae (Sun et al. 2020)) and at the genus level (e.g.: *Laternula* (Williams et al. 2017); *Scapharca* (Li et al. 2020; Kong, Li, et al. 2020)). Genome size is also subject to wide deviations in this class: for instance, within the order of Arcida (Pteriomorphia), mitochondrial genomes range from 16 to 56 kb (as a result of multiple independent elongation/reduction events (Kong, Li, et al. 2020)), within Anomalodesmata (Euheterodonta) from 14 to 32 (possibly >40) kilobases (Williams et al. 2017), while *Dreissena polymorpha* (Euheterodonta) holds, with 67 kb, the metazoan record of single-molecule mtDNA length (McCartney et al. 2019). Most of these size variations are caused by shifts in non-coding region length, gene duplications are also common in Bivalvia. Without considering the erratic nature of the tRNA content, the ribosomal gene *rrnS* underwent two independent duplications in *Crassostrea* (Wu et al. 2010) and some Arcidae species (Kong, Li, et al. 2020), while the OXPHOS gene *cox2* has been duplicated at least three times. Until recently, *cox2* duplications were found exclusively in DUI species, one in the M-type mtDNA of *Musculista senhousia* (Passamonti et al. 2011), and one in the F-type of *R. philippinarum* (Ghiselli et al. 2013), and some sort of association to the peculiar mitochondrial inheritance mechanism was hypothesized (Capt et al. 2020). However, two copies of complete and divergent *cox2* have also been found within Arcida (Kong, Li, et al. 2020), in four species that are so far not considered DUI. Up to now, what appears to be a DUI-specific common pattern for *cox2* is the elongation of the ORF: all three Unionidae families share a 3' elongation in the M-type *cox2* (up to a gene length of 1,380bp in *Hyridella menziensii* (Guerra et al. 2017)); *M. senhousia* and *R. philippinarum* have a 3' elongation in the duplicated copy of M-type and F-type *cox2*, respectively (Passamonti et al. 2011; Ghiselli et al. 2013); *Meretrix lamarckii* has a 100-amino acid insertion within the ORF (Bettinazzi et al. 2016); *Scrobicularia plana* and *Limecola balthica* have an M-type-specific insertion of >4.5 and >3.5 kb, respectively (Capt et al. 2020). These latter species have a large insertion (curiously similar, even in the position of the insertion, to what observed in *Campsomeris*, an ectoparasitoid Hymenoptera (Szafranski 2017)) and, while the *L. balthica* insertion splits the gene in two ORFs, the insertion in *S. plana* mtDNA is in-frame, that if translated would make it the longest COX2 protein in Metazoa. Other peculiar variations in the canonical mtDNA genes include the splitting

of *rrnL* in Ostreidae (Ren et al. 2010), the translocation of a portion of *nad5* in *Anodonta cygnea* (a non-DUI Unionida (Chase et al. 2018)), and an intron-like ~600 bp insertion in the *cox1* of *Cucullea labiata* (Arcoida (Feng et al. 2017)). Last but not least, multiple lineage-specific ORFans have been found in DUI species (Breton et al. 2011; Ghiselli et al. 2013; Milani et al. 2013, 2014, 2015; Guerra et al. 2019; Ouimet et al. 2020). These ORFans are usually present in both F- and M-type mtDNAs, and evidence of a protein product was found: polypeptides are usually localized in mitochondria (*R. philippinarum* (Milani et al. 2014); *M. edulis* (Ouimet et al. 2020)), but have been observed also on the nuclear envelope (*Venustaconcha ellipsiformis* (Breton et al. 2011)) or even inside it (*R. philippinarum* (Milani et al. 2014)). These proteins, that might have a viral origin (Milani et al. 2013, 2014, 2015, 2016), were hypothesized to have a role in sex specification and/or mitochondrial inheritance (i.e.: DUI maintenance (Milani et al. 2013, 2014, 2015, 2016; Guerra et al. 2019)).

References

Allio R, Donega S, Galtier N, Nabholz B. 2017. Large Variation in the Ratio of Mitochondrial to Nuclear Mutation Rate across Animals: Implications for Genetic Diversity and the Use of Mitochondrial DNA as a Molecular Marker. Mol Biol Evol 34:2762–72.

Aryaman J, Johnston IG, Jones NS. 2018. Mitochondrial Heterogeneity. Front Genet 9:718.

Barja G, Herrero A. 1998. Localization at complex I and mechanism of the higher free radical production of brain nonsynaptic mitochondria in the short-lived rat than in the longevous pigeon. J Bioenerg Biomembr 30:235–43.

Barreto FS, Watson ET, Lima TG, Willett CS, Edmands S, Li W, Burton RS. 2018. Genomic signatures of mitonuclear coevolution across populations of *Tigriopus californicus*. Nat Ecol Evol 2:1250–57.

Bar-Yaacov D, Blumberg A, Mishmar D. 2012. Mitochondrial-nuclear co-evolution and its effects on OXPHOS activity and regulation. Biochim Biophys Acta 1819:1107–11.

Bayona-Bafaluy MP, Müller S, Moraes CT. 2005. Fast adaptive coevolution of nuclear and mitochondrial subunits of ATP synthetase in orangutan. Mol Biol Evol 22:716–24.

Bettinazzi S, Nadarajah S, Dalpé A, Milani L, Blier PU, Breton S. 2020. Linking paternally inherited mtDNA variants and sperm performance. Philos Trans R Soc Lond B Biol Sci 375:20190177.

Bettinazzi S, Plazzi F, Passamonti M. 2016. The Complete Female- and Male-Transmitted Mitochondrial Genome of *Meretrix lamarckii*. PLoS One 11:e0153631.

Bettinazzi S, Rodríguez E, Milani L, Blier PU, Breton S. 2019. Metabolic remodelling associated with mtDNA: insights into the adaptive value of doubly uniparental inheritance of mitochondria. Proc Biol Sci 286:20182708.

Breton S, Bouvet K, Auclair G, Ghazal S, Sietman BE, Johnson N, Bettinazzi S, Stewart DT, Guerra D. 2017. The extremely divergent maternally- and paternally-transmitted mitochondrial genomes are co-expressed in somatic tissues of two freshwater mussel species with doubly uniparental inheritance of mtDNA. PLoS One 12:e0183529.

Breton S, Milani L, Ghiselli F, Guerra D, Stewart DT, Passamonti M. 2014. A resourceful genome: updating the functional repertoire and evolutionary role of animal mitochondrial DNAs. Trends Genet 30:555–64.

Breton S, Stewart DT, Shepardson S, Trdan RJ, Bogan AE, Chapman EG, Ruminas AJ, Piontkivska H, Hoeh WR. 2011. Novel protein genes in animal mtDNA: a new sex determination system in freshwater mussels (Bivalvia: Unionoida)? Mol Biol Evol 28:1645–59.

Burton RS, Pereira RJ, Barreto FS. 2013. Cytonuclear Genomic Interactions and Hybrid Breakdown. Annu Rev Ecol Evol Syst 44:281–302.

Cao L, Kenchington E, Zouros E. 2004. Differential segregation patterns of sperm mitochondria in embryos of the blue mussel (*Mytilus edulis*). Genetics 166:883–94.

Capt C, Bouvet K, Guerra D, Robicheau BM, Stewart DT, Pante E, Breton S. 2020. Unorthodox features in two venerid bivalves with doubly uniparental inheritance of mitochondria. Sci Rep 10:1087.

Chase EE, Robicheau BM, Veinot S, Breton S, Stewart DT. 2018. The complete mitochondrial genome of the hermaphroditic freshwater mussel *Anodonta cygnea* (Bivalvia: Unionidae): in silico analyses of sex-specific ORFs across order Unionoida. BMC Genomics 19:221.

Cherkasov AA, Overton RA Jr, Sokolov EP, Sokolova IM. 2007. Temperature-dependent effects of cadmium and purine nucleotides on mitochondrial aconitase from a marine ectotherm, *Crassostrea virginica*: a role of temperature in oxidative stress and allosteric enzyme regulation. J Exp Biol 210:46–55.

Cherkasov AS, Biswas PK, Ridings DM, Ringwood AH, Sokolova IM. 2006. Effects of

acclimation temperature and cadmium exposure on cellular energy budgets in the marine mollusk *Crassostrea virginica*: linking cellular and mitochondrial responses. J Exp Biol 209:1274–84.
Cherkasov AS, Taylor C, Sokolova IM. 2010. Seasonal variation in mitochondrial responses to cadmium and temperature in eastern oysters *Crassostrea virginica* (Gmelin) from different latitudes. Aquat Toxicol 97:68–78.
Diaz F, Bayona-Bafaluy MP, Rana M, Mora M, Hao H, Moraes CT. 2002. Human mitochondrial DNA with large deletions repopulates organelles faster than full-length genomes under relaxed copy number control. Nucleic Acids Res 30:4626–33.
Doucet-Beaupré H, Breton S, Chapman EG, Blier PU, Bogan AE, Stewart DT, Hoeh WR. 2010. Mitochondrial phylogenomics of the Bivalvia (Mollusca): searching for the origin and mitogenomic correlates of doubly uniparental inheritance of mtDNA. BMC Evol Biol 10:50.
Ellison CK, Burton RS. 2006. Disruption of mitochondrial function in interpopulation hybrids of *Tigriopus californicus*. Evolution 60:1382–91.
Eslamieh M, Williford A, Betrán E. 2017. Few Nuclear-Encoded Mitochondrial Gene Duplicates Contribute to Male Germline-Specific Functions in Humans. Genome Biol Evol 9:2782–90.
Fan W, Waymire KG, Narula N, Li P, Rocher C, Coskun PE, Vannan MA, Narula J, Macgregor GR, Wallace DC. 2008. A mouse model of mitochondrial disease reveals germline selection against severe mtDNA mutations. Science 319:958–62.
Feng Y, Li Q, Yu H, Kong L. 2017. Complete mitochondrial genome sequence of *Cucullaea labiata* (Arcoida: Cucullaeidae) and phylogenetic implications. Genes Genomics 39:867–75.
Gallach M, Chandrasekaran C, Betrán E. 2010. Analyses of nuclearly encoded mitochondrial genes suggest gene duplication as a mechanism for resolving intralocus sexually antagonistic conflict in *Drosophila*. Genome Biol Evol 2:835–50.
Gershoni M, Fuchs A, Shani N, Fridman Y, Corral-Debrinski M, Aharoni A, Frishman D, Mishmar D. 2010. Coevolution predicts direct interactions between mtDNA-encoded and nDNA-encoded subunits of oxidative phosphorylation complex i. J Mol Biol 404:158–71.
Ghiselli F, Gomes-dos-Santos A, Adema CM, Lopes-Lima M, Sharbrough J, Boore JL. 2021. Molluscan Mitochondrial Genomes Break the Rules. Philosophical Transactions of the Royal Society B 376: 20200159.
Ghiselli F, Maurizii MG, Reunov A, Ariño-Bassols H, Cifaldi C, Pecci A, Alexandrova Y, Bettini S, Passamonti M, Franceschini V, Milani L. 2019. Natural Heteroplasmy and Mitochondrial Inheritance in Bivalve Molluscs. Integr Comp Biol 59:1016–32.
Ghiselli F, Milani L. 2020. Linking the mitochondrial genotype to phenotype: a complex endeavour. Philos Trans R Soc Lond B Biol Sci 375:20190169.
Ghiselli F, Milani L, Guerra D, Chang PL, Breton S, Nuzhdin SV, Passamonti M. 2013. Structure, transcription, and variability of metazoan mitochondrial genome: perspectives from an unusual mitochondrial inheritance system. Genome Biol Evol 5:1535–54.
Ghiselli F, Milani L, Iannello M, Procopio E, Chang PL, Nuzhdin SV, Passamonti M. 2017. The complete mitochondrial genome of the grooved carpet shell, *Ruditapes decussatus* (Bivalvia, Veneridae). PeerJ 5:e3692.
Ghiselli F, Milani L, Passamonti M. 2011. Strict sex-specific mtDNA segregation in the germ line of the DUI species *Venerupis philippinarum* (Bivalvia: Veneridae). Mol Biol Evol 28:949–61.
Gissi C, Iannelli F, Pesole G. 2008. Evolution of the mitochondrial genome of Metazoa as exemplified by comparison of congeneric species. Heredity 101:301–20.
Gissi C, Pesole G, Mastrototaro F, Iannelli F, Guida V, Griggio F. 2010. Hypervariability of ascidian mitochondrial gene order: exposing the myth of deuterostome organelle genome stability. Mol Biol Evol 27:211–15.
Gredilla R, Barja G, López-Torres M. 2001. Effect of short-term caloric restriction on H2O2 production and oxidative DNA damage in rat liver mitochondria and location of the free

radical source. J Bioenerg Biomembr 33:279–87.
Guerra D, Ghiselli F, Milani L, Breton S, Passamonti M. 2016. Early replication dynamics of sex-linked mitochondrial DNAs in the doubly uniparental inheritance species *Ruditapes philippinarum* (Bivalvia Veneridae). Heredity 116:324–32.
Guerra D, Ghiselli F, Passamonti M. 2014. The largest unassigned regions of the male- and female-transmitted mitochondrial DNAs in *Musculista senhousia* (Bivalvia Mytilidae). Gene 536:316–25.
Guerra D, Lopes-Lima M, Froufe E, Gan HM, Ondina P, Amaro R, Klunzinger MW, Callil C, Prié V, Bogan AE, Stewart DT, Breton S. 2019. Variability of mitochondrial ORFans hints at possible differences in the system of doubly uniparental inheritance of mitochondria among families of freshwater mussels (Bivalvia: Unionida). BMC Evol Biol 19:229.
Guerra D, Plazzi F, Stewart DT, Bogan AE, Hoeh WR, Breton S. 2017. Evolution of sex-dependent mtDNA transmission in freshwater mussels (Bivalvia: Unionida). Sci Rep 7:1551.
Havird JC, McConie HJ. 2019. Sexually Antagonistic Mitonuclear Coevolution in Duplicate Oxidative Phosphorylation Genes. Integr Comp Biol 59:864–74.
Havird JC, Sloan DB. 2016. The Roles of Mutation, Selection, and Expression in Determining Relative Rates of Evolution in Mitochondrial versus Nuclear Genomes. Mol Biol Evol 33:3042–53.
Heise K, Puntarulo S, Pörtner HO, Abele D. 2003. Production of reactive oxygen species by isolated mitochondria of the Antarctic bivalve *Laternula elliptica* (King and Broderip) under heat stress. Comp Biochem Physiol C Toxicol Pharmacol 134:79–90.
Hill GE. 2017. The mitonuclear compatibility species concept. Auk 134:393–409.
Hill GE, Havird JC, Sloan DB, Burton RS, Greening C, Dowling DK. 2019. Assessing the fitness consequences of mitonuclear interactions in natural populations. Biol Rev Camb Philos Soc 94:1089–1104.
Hraoui G, Bettinazzi S, Gendron AD, Boisclair D, Breton S. 2020. Mitochondrial thermo-sensitivity in invasive and native freshwater mussels. J Exp Biol 223.
Iannello M, Bettinazzi S, Breton S, Ghiselli F, Milani L. 2021. A naturally heteroplasmic clam provides clues about the effects of genetic bottleneck on paternal mtDNA. Genome Biol Evol.
Iannello M, Puccio G, Piccinini G, Passamonti M, Ghiselli F. 2019. The dynamics of mito-nuclear coevolution: A perspective from bivalve species with two different mechanisms of mitochondrial inheritance. J Zoolog Syst Evol Res 57:534–47.
Ivanina AV, Kurochkin IO, Leamy L, Sokolova IM. 2012. Effects of temperature and cadmium exposure on the mitochondria of oysters (*Crassostrea virginica*) exposed to hypoxia and subsequent reoxygenation. J Exp Biol 215:3142–54.
Ivanina AV, Nesmelova I, Leamy L, Sokolov EP, Sokolova IM. 2016. Intermittent hypoxia leads to functional reorganization of mitochondria and affects cellular bioenergetics in marine molluscs. J Exp Biol 219:1659–74.
Johnston I. 2019. Varied mechanisms and models for the varying mitochondrial bottleneck. Frontiers in Cell and Developmental Biology 7:294.
Johnston IG, Burgstaller JP. 2019. Evolving mtDNA populations within cells. Biochem Soc Trans.
Kalogeris T, Bao Y, Korthuis RJ. 2014. Mitochondrial reactive oxygen species: a double edged sword in ischemia/reperfusion vs preconditioning. Redox Biol 2:702–14.
Kaltenpoth M, Showers Corneli P, Dunn DM, Weiss RB, Strohm E, Seger J. 2012. Accelerated evolution of mitochondrial but not nuclear genomes of Hymenoptera: new evidence from crabronid wasps. PLoS One 7:e32826.
Klucnika A, Ma H. 2019. A battle for transmission: the cooperative and selfish animal mitochondrial genomes. Open Biol 9:180267.

Kolesnikov AA, Gerasimov ES. 2012. Diversity of mitochondrial genome organization. Biochemistry 77:1424–35.
Kong L, Li Y, Kocot KM, Yang Y, Qi L, Li Q, Halanych KM. 2020. Mitogenomics reveals phylogenetic relationships of Arcoida (Mollusca, Bivalvia) and multiple independent expansions and contractions in mitochondrial genome size. Mol Phylogenet Evol 106857.
Kong M, Xiang H, Wang J, Liu J, Zhang X, Zhao X. 2020. Mitochondrial DNA Haplotypes Influence Energy Metabolism across Chicken Transmitochondrial Cybrids. Genes 11.
Korge P, Calmettes G, Weiss JN. 2015. Increased reactive oxygen species production during reductive stress: The roles of mitochondrial glutathione and thioredoxin reductases. Biochim Biophys Acta 1847:514–25.
Kurochkin IO, Ivanina AV, Eilers S, Downs CA, May LA, Sokolova IM. 2009. Cadmium affects metabolic responses to prolonged anoxia and reoxygenation in eastern oysters (*Crassostrea virginica*). Am J Physiol Regul Integr Comp Physiol 297:R1262–72.
Kyriakou E, Zouros E, Rodakis GC. 2010. The atypical presence of the paternal mitochondrial DNA in somatic tissues of male and female individuals of the blue mussel species *Mytilus galloprovincialis*. BMC Res Notes 3:222.
Lane N. 2011. Mitonuclear match: optimizing fitness and fertility over generations drives ageing within generations. Bioessays 33:860–69.
Lane N. 2012. The problem with mixing mitochondria. Cell.
Lavrov DV, Pett W. 2016. Animal Mitochondrial DNA as We Do Not Know It: mt-Genome Organization and Evolution in Nonbilaterian Lineages. Genome Biol Evol 8:2896–2913.
Lee Y, Kwak H, Shin J, Kim S-C, Kim T, Park J-K. 2019. A mitochondrial genome phylogeny of Mytilidae (Bivalvia: Mytilida). Mol Phylogenet Evol 139:106533.
Lemieux H, Blier PU, Gnaiger E. 2017. Remodeling pathway control of mitochondrial respiratory capacity by temperature in mouse heart: electron flow through the Q-junction in permeabilized fibers. Sci Rep 7:2840.
Letendre J, Chouquet B, Manduzio H, Marin M, Bultelle F, Leboulenger F, Durand F. 2009. Tidal height influences the levels of enzymatic antioxidant defences in Mytilus edulis. Mar Environ Res 67:69–74.
Levin L, Mishmar D. 2017. The genomic landscape of evolutionary convergence in mammals, birds and reptiles. Nat Ecol Evol 1:41.
Liu M, Guo X. 2017. A novel and stress adaptive alternative oxidase derived from alternative splicing of duplicated exon in oyster *Crassostrea virginica*. Sci Rep 7:10785.
Li Y, Zhang R, Liu S, Donath A, Peters RS, Ware J, Misof B, Niehuis O, Pfrender ME, Zhou X. 2017. The molecular evolutionary dynamics of oxidative phosphorylation (OXPHOS) genes in Hymenoptera. BMC Evol Biol 17:269.
Lubośny M, Przyłucka A, Śmietanka B, Breton S, Burzyński A. 2018. Actively transcribed and expressed atp8 gene in *Mytilus edulis* mussels. PeerJ 6:e4897.
Macey JR, Pabinger S, Barbieri CG, Buring ES, Gonzalez VL, Mulcahy DG, DeMeo DP, Urban L, Hime PM, Prost S, Elliott AN, Gemmell NJ. 2021. Evidence of two deeply divergent co-existing mitochondrial genomes in the Tuatara reveals an extremely complex genomic organization. Commun Biol 4:116.
McCartney MA, Auch B, Kono T, Mallez S, Zhang Y, Obille A, Becker A, Abrahante JE, Garbe J, Badalamenti JP, Herman A, Mangelson H, Liachko I, Sullivan S, Sone ED, Koren S, Silverstein KAT, Beckman KB, Gohl DM. 2019. The Genome of the Zebra Mussel, *Dreissena polymorpha*: A Resource for Invasive Species Research. Cold Spring Harbor Laboratory.
McDonald AE, Gospodaryov DV. 2019. Alternative NAD(P)H dehydrogenase and alternative oxidase: Proposed physiological roles in animals. Mitochondrion 45:7–17.
McDonald A, Vanlerberghe G. 2004. Branched mitochondrial electron transport in the Animalia: presence of alternative oxidase in several animal phyla. IUBMB Life 56:333–41.

McKenzie M, Chiotis M, Pinkert CA, Trounce IA. 2003. Functional respiratory chain analyses in murid xenomitochondrial cybrids expose coevolutionary constraints of cytochrome b and nuclear subunits of complex III. Mol Biol Evol 20:1117–24.
Milani L, Ghiselli F. 2015. Mitochondrial activity in gametes and transmission of viable mtDNA. Biol Direct 10:22.
Milani L, Ghiselli F. 2020. Faraway, so close. The comparative method and the potential of non-model animals in mitochondrial research. Philos Trans R Soc Lond B Biol Sci 375:20190186.
Milani L, Ghiselli F, Guerra D, Breton S, Passamonti M. 2013. A comparative analysis of mitochondrial ORFans: new clues on their origin and role in species with doubly uniparental inheritance of mitochondria. Genome Biol Evol 5:1408–34.
Milani L, Ghiselli F, Maurizii MG, Nuzhdin SV, Passamonti M. 2014. Paternally transmitted mitochondria express a new gene of potential viral origin. Genome Biol Evol 6:391–405.
Milani L, Ghiselli F, Passamonti M. 2012. Sex-linked mitochondrial behavior during early embryo development in *Ruditapes philippinarum* (Bivalvia Veneridae) a species with the Doubly Uniparental Inheritance (DUI) of mitochondria. J Exp Zool B Mol Dev Evol 318:182–89.
Milani L, Ghiselli F, Passamonti M. 2016. Mitochondrial selfish elements and the evolution of biological novelties. Curr Zool 62:687–97.
Milani L, Ghiselli F, Pecci A, Maurizii MG, Passamonti M. 2015. The Expression of a Novel Mitochondrially-Encoded Gene in Gonadic Precursors May Drive Paternal Inheritance of Mitochondria. PLoS One 10:e0137468.
Mitov MI, Harris JW, Alstott MC, Zaytseva YY, Evers BM, Butterfield DA. 2017. Temperature induces significant changes in both glycolytic reserve and mitochondrial spare respiratory capacity in colorectal cancer cell lines. Exp Cell Res 354:112–21.
Müller M, Mentel M, van Hellemond JJ, Henze K, Woehle C, Gould SB, Yu R-YY, van der Giezen M, Tielens AGM, Martin WF. 2012. Biochemistry and evolution of anaerobic energy metabolism in eukaryotes. Microbiol Mol Biol Rev 76:444–95.
Munro D, Blier PU. 2012. The extreme longevity of *Arctica islandica* is associated with increased peroxidation resistance in mitochondrial membranes. Aging Cell 11:845–55.
Munro D, Pichaud N, Paquin F, Kemeid V, Blier PU. 2013. Low hydrogen peroxide production in mitochondria of the long-lived *Arctica islandica*: underlying mechanisms for slow aging. Aging Cell 12:584–92.
Munro D, Treberg JR. 2017. A radical shift in perspective: mitochondria as regulators of reactive oxygen species. J Exp Biol 220:1170–80.
Niehuis O, Judson AK, Gadau J. 2008. Cytonuclear genic incompatibilities cause increased mortality in male F2 hybrids of *Nasonia giraulti* and *N. vitripennis*. Genetics 178:413–26.
Obata M, Kamiya C, Kawamura K, Komaru A. 2006. Sperm mitochondrial DNA transmission to both male and female offspring in the blue mussel *Mytilus galloprovincialis*. Dev Growth Differ 48:253–61.
Osada N, Akashi H. 2012. Mitochondrial-nuclear interactions and accelerated compensatory evolution: evidence from the primate cytochrome C oxidase complex. Mol Biol Evol 29:337–46.
Ouimet P, Kienzle L, Lubosny M, Burzyński A, Angers A, Breton S. 2020. The ORF in the control region of the female-transmitted *Mytilus* mtDNA codes for a protein. Gene 725:144161.
Passamonti M, Ricci A, Milani L, Ghiselli F. 2011. Mitochondrial genomes and Doubly Uniparental Inheritance: new insights from *Musculista senhousia* sex-linked mitochondrial DNAs (Bivalvia Mytilidae). BMC Genomics 12:442.
Payne BAI, Wilson IJ, Yu-Wai-Man P, Coxhead J, Deehan D, Horvath R, Taylor RW, Samuels DC, Santibanez-Koref M, Chinnery PF. 2013. Universal heteroplasmy of human mitochondrial DNA. Hum Mol Genet 22:384–90.

Peck LS, Pörtner HO, Hardewig I. 2002. Metabolic demand, oxygen supply, and critical temperatures in the Antarctic bivalve *Laternula elliptica*. Physiol Biochem Zool 75:123–33.
Piccinini G, Iannello M, Puccio G, Plazzi F, Havird JC, Ghiselli F. 2021. Mitonuclear Coevolution, but Not Nuclear Compensation, Drives Evolution of OXPHOS Complexes in Bivalves. Mol Biol Evol.
Plough LV. 2016. Genetic load in marine animals: a review. Curr Zool 62:567–79.
Radzvilavicius AL, Hadjivasiliou Z, Pomiankowski A, Lane N. 2016. Selection for Mitochondrial Quality Drives Evolution of the Germline. PLoS Biol 14:e2000410.
Rand DM. 2011. Population genetics of the cytoplasm and the units of selection on mitochondrial DNA in *Drosophila melanogaster*. Genetica 139:685–97.
Rand DM, Haney RA, Fry AJ. 2004. Cytonuclear coevolution: the genomics of cooperation. Trends Ecol Evol 19:645–53.
Ren J, Liu X, Jiang F, Guo X, Liu B. 2010. Unusual conservation of mitochondrial gene order in *Crassostrea* oysters: evidence for recent speciation in Asia. BMC Evol Biol 10:394.
Robertson A, Schaltz K, Neimanis K, Staples JF, McDonald AE. 2016. Heterologous expression of the *Crassostrea gigas* (Pacific oyster) alternative oxidase in the yeast Saccharomyces cerevisiae. J Bioenerg Biomembr 48:509–20.
Rodríguez E, Dégletagne C, Hagen TM, Abele D, Blier PU. 2019. Mitochondrial Traits Previously Associated With Species Maximum Lifespan Do Not Correlate With Longevity Across Populations of the Bivalve *Arctica islandica*. Front Physiol 10:946.
Romero IG, Ruvinsky I, Gilad Y. 2012. Comparative studies of gene expression and the evolution of gene regulation. Nat Rev Genet 13:505–16.
Rubinstein ND, Feldstein T, Shenkar N, Botero-Castro F, Griggio F, Mastrototaro F, Delsuc F, Douzery EJP, Gissi C, Huchon D. 2013. Deep sequencing of mixed total DNA without barcodes allows efficient assembly of highly plastic ascidian mitochondrial genomes. Genome Biol Evol 5:1185–99.
Sato K, Sato M. 2017. Multiple ways to prevent transmission of paternal mitochondrial DNA for maternal inheritance in animals. J Biochem 162:247–53.
Sharpley MS, Marciniak C, Eckel-Mahan K, McManus M, Crimi M, Waymire K, Lin CS, Masubuchi S, Friend N, Koike M, Chalkia D, MacGregor G, Sassone-Corsi P, Wallace DC. 2012. Heteroplasmy of mouse mtDNA is genetically unstable and results in altered behavior and cognition. Cell 151:333–43.
Sloan DB, Havird JC, Sharbrough J. 2017. The on-again, off-again relationship between mitochondrial genomes and species boundaries. Mol Ecol 26:2212–36.
Sloan DB, Warren JM, Williams AM, Wu Z, Abdel-Ghany SE, Chicco AJ, Havird JC. 2018. Cytonuclear integration and co-evolution. Nat Rev Genet 19:635–48.
Sokolova I. 2018. Mitochondrial Adaptations to Variable Environments and Their Role in Animals' Stress Tolerance. Integr Comp Biol 58:519–31.
Sokolova IM. 2004. Cadmium effects on mitochondrial function are enhanced by elevated temperatures in a marine poikilotherm, *Crassostrea virginica* Gmelin (Bivalvia: Ostreidae). J Exp Biol 207:2639–48.
Sokolova IM, Sokolov EP, Haider F. 2019. Mitochondrial Mechanisms Underlying Tolerance to Fluctuating Oxygen Conditions: Lessons from Hypoxia-Tolerant Organisms. Integr Comp Biol 59:938–52.
Sokolova IM, Sukhotin AA, Lannig G. 2012. Stress effects on metabolism and energy budgets in mollusks. In: Oxidative stress in aquatic ecosystems Wiley-Blackwell. p. 263–80.
Sussarellu R, Dudognon T, Fabioux C, Soudant P, Moraga D, Kraffe E. 2013. Rapid mitochondrial adjustments in response to short-term hypoxia and re-oxygenation in the Pacific oyster, *Crassostrea gigas*. J Exp Biol 216:1561–69.
Szafranski P. 2017. Evolutionarily recent, insertional fission of mitochondrial cox2 into complementary genes in bilaterian Metazoa. BMC Genomics 18:269.

Theologidis I, Fodelianakis S, Gaspar MB, Zouros E. 2008. Doubly uniparental inheritance (DUI) of mitochondrial DNA in *Donax trunculus* (Bivalvia: Donacidae) and the problem of its sporadic detection in Bivalvia. Evolution 62:959–70.
Treberg JR, Braun K, Selseleh P. 2019. Mitochondria can act as energy-sensing regulators of hydrogen peroxide availability. Redox Biol 20:483–88.
Treberg JR, Braun K, Zacharias P, Kroeker K. 2018. Multidimensional mitochondrial energetics: Application to the study of electron leak and hydrogen peroxide metabolism. Comp Biochem Physiol B Biochem Mol Biol 224:121–28.
Tschischka K, Abele D, Pörtner HO. 2000. Mitochondrial oxyconformity and cold adaptation in the polychaete Nereis pelagica and the bivalve *Arctica islandica* from the Baltic and White Seas. J Exp Biol 203:3355–68.
Tward CE, Singh J, Cygelfarb W, McDonald AE. 2019. Identification of the alternative oxidase gene and its expression in the copepod *Tigriopus californicus*. Comp Biochem Physiol B Biochem Mol Biol 228:41–50.
van der Sluis EO, Bauerschmitt H, Becker T, Mielke T, Frauenfeld J, Berninghausen O, Neupert W, Herrmann JM, Beckmann R. 2015. Parallel Structural Evolution of Mitochondrial Ribosomes and OXPHOS Complexes. Genome Biol Evol 7:1235–51.
van Esveld SL, Huynen MA. 2018. Does mitochondrial DNA evolution in metazoa drive the origin of new mitochondrial proteins? IUBMB Life 70:1240–50.
Wang J-G, Zhang D, Jakovlić I, Wang W-M. 2017. Sequencing of the complete mitochondrial genomes of eight freshwater snail species exposes pervasive paraphyly within the Viviparidae family (Caenogastropoda). PLoS One 12:e0181699.
Weaver RJ. 2019. Hypothesized Evolutionary Consequences of the Alternative Oxidase (AOX) in Animal Mitochondria. Integr Comp Biol 59:994–1004.
Weaver RJ, Carrion G, Nix R, Maeda GP, Rabinowitz S, Iverson ENK, Thueson K, Havird JC. 2020. High mitochondrial mutation rates in Silene are associated with nuclear-mediated changes in mitochondrial physiology. Biol Lett 16:20200450.
Wernick RI, Christy SF, Howe DK, Sullins JA, Ramirez JF, Sare M, Penley MJ, Morran LT, Denver DR, Estes S. 2019. Sex and Mitonuclear Adaptation in Experimental *Caenorhabditis elegans* Populations. Genetics 211:1045–58.
Williams ST, Foster PG, Hughes C, Harper EM, Taylor JD, Littlewood DTJ, Dyal P, Hopkins KP, Briscoe AG. 2017. Curious bivalves: Systematic utility and unusual properties of anomalodesmatan mitochondrial genomes. Mol Phylogenet Evol 110:60–72.
Wonnapinij P, Chinnery PF, Samuels DC. 2008. The distribution of mitochondrial DNA heteroplasmy due to random genetic drift. Am J Hum Genet 83:582–93.
Wu X, Xu X, Yu Z, Wei Z, Xia J. 2010. Comparison of seven *Crassostrea* mitogenomes and phylogenetic analyses. Mol Phylogenet Evol 57:448–54.
Yusseppone MS, Rocchetta I, Sabatini SE, Luquet CM, Ríos de Molina MDC, Held C, Abele D. 2018. Inducing the Alternative Oxidase Forms Part of the Molecular Strategy of Anoxic Survival in Freshwater Bivalves. Front Physiol 9:100.
Zhang K, Zhu K, Liu Y, Zhang H, Gong L, Jiang L, Liu L, Lü Z, Liu B. 2021. Novel gene rearrangement in the mitochondrial genome of *Muraenesox cinereus* and the phylogenetic relationship of Anguilliformes. Sci Rep 11:2411.

Table 1 - Commonly investigated phenotypic traits related to mitochondrial function in bivalves (Section 4)

Species/Taxon	Taxonomy	SMI/DUI	References
Tolerance to fluctuating environmental factors			
Mercenaria mercenaria	Imparidentia, Veneridae	SMI	(Ivanina et al. 2016)
Argopecten irradians	Pteriomorphia, Pectinidae	SMI	
Crassostrea virginica	Pteriomorphia, Ostreidae	SMI	(Sokolova 2004, 2018; Cherkasov et al. 2006, 2007, 2010; Ivanina et al. 2012)
Crassostrea gigas	Pteriomorphia, Ostreidae	SMI	(Sussarellu et al. 2013; Sokolova et al. 2019)
Diplodon chilensis	Paleoheterodonta, Hyriidae	DUI?	(Yusseppone et al. 2018; Sokolova et al. 2019)
Mya arenaria	Imparidentia, Myidae	SMI	(Abele et al. 2002; Sokolova 2018)
Laternula elliptica	Anomalodesmata, Laternulidae	?	(Peck et al. 2002; Heise et al. 2003; Sokolova 2018)
Mytilus edulis	Pteriomorphia, Mytilidae	DUI	(Letendre et al. 2009; Müller et al. 2012; Sokolova et al. 2012)
Dreissena bugensis	Imparidentia, Dreissenidae	?	(Hraoui et al. 2020)
Elliptio complanata	Paleoheterodonta, Unionidae	DUI	
Alternative oxidase and protection from oxidative stress			
Crassostrea virginica	Pteriomorphia, Ostreidae	SMI	(Liu and Guo 2017)
Bivalves			(Weaver 2019)
Arctica islandica	Imparidentia, Arcticidae	DUI	(Tschischka et al. 2000; Munro et al. 2013)
Crassostrea gigas	Pteriomorphia, Ostreidae	SMI	(Robertson et al. 2016)
Ageing and lifespan: membrane peroxidation and ROS generation			
Arctica islandica	Imparidentia, Arcticidae	DUI	(Munro and Blier 2012)
Mya arenaria	Imparidentia, Myidae	SMI?	
Spisula solidissima	Imparidentia, Mactridae	SMI?	
Mactromeris polynyma	Imparidentia, Mactridae	SMI?	
Mercenaria mercenaria	Imparidentia, Veneridae	SMI?	
Arctica islandica	Imparidentia, Arcticidae	DUI	(Munro et al. 2013)
Mya arenaria	Imparidentia, Myidae	SMI?	
Spisula solidissima	Imparidentia, Mactridae	SMI?	
Arctica islandica	Imparidentia, Arcticidae	DUI	(Rodríguez et al. 2019)
DUI vs SMI phenotypes			
Arctica islandica	Imparidentia, Arcticidae	DUI	(Bettinazzi et al. 2019)
Mytilus edulis	Pteriomorphia, Mytilidae	DUI	
Placopecten magellanicus	Pteriomorphia, Pectinidae	SMI	
Mercenaria mercenaria	Imparidentia, Veneridae	SMI	
Mytilus edulis	Pteriomorphia, Mytilidae	DUI	(Bettinazzi et al. 2020)
Ruditapes philippinarum	Imparidentia, Veneridae	DUI	
Mercenaria mercenaria	Imparidentia, Veneridae	SMI	
Nuttallia obscurata	Imparidentia, Psammobiidae	SMI	
Placopecten magellanicus	Pteriomorphia, Pectinidae	SMI	