Université de Montréal

Energy metabolism in species with Doubly Uniparental Inheritance (DUI) of mitochondria: investigating the functioning, maintenance and evolutionary relevance of a naturally heteroplasmic system

Par
Stefano Bettinazzi

Département de sciences biologiques, Faculté des arts et des sciences

Thèse présentée en vue de l’obtention du grade de Philosophiae Doctor (Ph.D.) en sciences biologiques

June 2020

A été évaluée par un jury composé des personnes suivantes

Sandra Binning
Président-rapporteur

Sophie Breton
Directeur de recherche

Pierre Blier
Codirecteur

Annie Anger
Membre du jury

Ron Burton
Examinateur externe

RÉSUMÉ

Les mitochondries et leur génome, l'ADN mitochondrial (ADNmt), sont généralement transmis uniquement par la mère aux fils et aux filles chez les métazoaires (transmission strictement maternelle, SMI). Une exception à la règle générale de la SMI se trouve dans environ 100 espèces de bivalves, qui se caractérise par une double transmission uniparentale (DUI) des mitochondries. Chez les espèces DUI, deux lignées d'ADNmt très divergentes et liées au sexe coexistent. Une lignée mitochondriale maternelle (type F), présente dans les ovocytes et les tissus somatiques des individus femelles et males, et une lignée paternelle (type M), présente dans les spermatozoïdes. Dans les tissus somatiques mâles, les deux lignées coexistent parfois, une condition appelée hétéroplasmie. En sachant que les variations génétiques dans l'ADNmt peuvent avoir un impact sur les fonctions mitochondriales, et en donnant l'association stricte des ADNmt de type M et F avec différents gamètes, il est imaginable que la forte divergence entre les deux lignées DUI puisse entraîner des adaptations bioénergétiques avec répercussion sur la reproduction. Le système DUI apporte également la nécessité pour les mitochondries paternelles de préserver leur propre intégrité génétique, ainsi que pour les cellules somatiques de faire face à l'hétéroplasmie.

L'objectif de ma thèse était de lier le génotype mitochondrial des espèces bivalves DUI et SMI au phénotype. Plus précisément, j'ai exploré l'impact des variations de l'ADN mitochondrial spécifiques au sexe sur un large éventail de traits phénotypiques, allant de la bioénergétique mitochondriale et cellulaire à la performance des spermatozoïdes, en étudiant la valeur adaptative du système DUI à la lumière du fitness reproductif, de la sélection et de la transmission mitochondriales.

Les résultats issus de ce projet de thèse ont révélé une nette divergence phénotypique entre les espèces DUI et SMI, reflétant peut-être les différentes pressions sélectives agissant sur les deux lignées mitochondriales. Contrairement aux espèces SMI, l'évolution sexo-spécifique des variants d'ADNmt DUI entraîne l'expression de différents phénotypes bioénergétiques mâles et femelles. Au niveau de la fonctionnalité mitochondriale, les mitochondries DUI de type M présentent une phosphorylation oxydative (OXPHOS) remodelée, caractérisée par un contrôle respiratoire inhabituel à l'extrémité de la chaîne respiratoire. La réorganisation générale de la bioénergétique des spermes DUI entraîne également une variation de l'équilibre entre les principales voies de production d'énergie, incluant la glycolyse, la glycolyse anaérobique, le métabolisme des acides gras, le cycle de l'acide tricarboxylique, l'OXPHOS, ainsi que la capacité antioxydante. Enfin, les spermatozoïdes DUI comptent entièrement sur l'énergie produite par OXPHOS pour maintenir une motilité inhabituelle caractérisée par une vitesse lente et une trajectoire plus curviligne, traits potentiellement associés à un plus grand succès de reproduction chez les organismes marins sessiles. Aussi, ils conservent la capacité de passer à une stratégie de production d'énergie mixte (aérobique et anaérobie) après la détection des ovocytes. Dans l'ensemble, ces résultats suggèrent que la variation de l'ADNmt dans les espèces DUI pourrait être adaptative, incluant adaptation bioénergétique sexo-spécifiques avec un effet en aval sur la performance des spermatozoïdes, la capacité de reproduction, la sélection et transmission des mitochondries paternelles.

Mots-clés: mitochondries - DUI - SMI - bivalves - gamètes - hétéroplasmie - OXPHOS - métabolisme énergétique - coévolution mitonucléaire

ABSTRACT

Mitochondria and their genome, the mitochondrial DNA (mtDNA), are usually transmitted only by the mother to both sons and daughters in metazoan (i.e. strict maternal inheritance, SMI). An exception to the general rule of SMI is found in around 100 species of bivalves, which are characterized by a doubly uniparental inheritance (DUI) of mitochondria. In DUI species, two highly divergent and sex-linked mtDNA lineages coexist. One mitochondrial lineage is maternally inherited (F-type) and is present in oocytes and somatic tissues of both female and male individuals. The other lineage is paternally inherited (M-type) and is present in sperm. In male somatic tissues both lineages sometimes coexist, a condition named heteroplasmy. Knowing that variations in mitochondrially-encoded genes might impact mitochondrial functions, and giving the strict association of M and F-type mtDNAs with different gametes, it is conceivable that the variation between the two DUI lineages might result in sex-specific bioenergetic adaptations with repercussion on reproduction. Despite providing an unprecedented opportunity for the mtDNA to evolve for male functions, the DUI system also brings the need for sperm mitochondria to preserve their genetic integrity, as well as for somatic cells to deal with heteroplasmy.

The objective of my PhD was to link the mitochondrial genotype of DUI and SMI bivalve species to the phenotype. I explored the impact of sex-specific mtDNA variations upon a wide set of phenotypic traits, ranging from mitochondrial and cellular bioenergetics to sperm performance, investigating the adaptive value of DUI system in the light of reproductive fitness, mitochondrial selection, preservation and transmission.

The results stemming from this PhD project revealed a clear phenotypic divergence between DUI and SMI species, possibly reflecting the different selective pressures acting on their mitochondria as a result of their different mode of mitochondria transmission. Conversely to SMI species, the sex-specific evolution of DUI mtDNA variants results in the expression of different male and female bioenergetic phenotypes. At the level of mitochondrial functionality, M-type mitochondria exhibit a remodelled OXPHOS characterized by unusual respiratory control at the terminus of the respiratory chain. The general reorganization of DUI sperm bioenergetics also entails variation in the balance between the main energy producing pathways, including glycolysis, anaerobic glycolysis, fatty acid metabolism, tricarboxylic acid cycle, OXPHOS, as well as the antioxidant capacity. Finally, DUI sperm exhibit an unusual motility phenotype characterized by slow speed and high curvilinear trajectory, traits potentially associated with a higher reproductive success in sessile broadcast spawning marine organisms. They also completely rely on the energy produced by OXPHOS to sustain their performance, although maintaining the ability to switch to a more combined aerobic/anaerobic strategy of energy production after oocyte detection. Altogether, these results suggest that the mtDNA variation in DUI species might be adaptive, resulting in the expression of sex-specific bioenergetic adaptation with downstream effect on sperm performance, reproductive fitness, paternal mitochondria selection, preservation and transmission. The results also suggest that heteroplasmy has an impact onto the bioenergetics of male soma, and that a functional compensation between genomes might minimize any potential deleterious outcome.

Keywords: mitochondria – DUI – SMI – bivalves – gametes – heteroplasmy – OXPHOS – energy metabolism – mitonuclear coevolution

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ADP: adenosine diphosphate

ALH: amplitude of lateral head displacement

Ama: antimycin A

Ama$_E$: residual mitochondrial respiration after inhibition of complex III

AOX: alternative oxidase

Asc: ascorbate

ASW: artificial seawater

ATP: adenosine triphosphate

Azd: sodium azide

Bb: Balbiani body

BCA: bicinchoninic acid

BCF: beat-cross frequency

BSA: bovine serum albumin

c: cytochrome *c*

CASA: computer-aided sperm analyser

CAT: catalase

CETF: electron-transferring flavoprotein complex

CI: complex I, NADH-dehydrogenase

CII: respiratory complex II, succinate dehydrogenase

CIII: respiratory complex III, coenzyme Q: cytochrome *c* oxidoreductase

Cis: confidence intervals

CIV or CCO: respiratory complex IV, cytochrome *c* oxidase

CIV_E: ETS-state, $_E$ complex IV standalone capacity

Q: coenzyme Q, ubiquinone/ubiquinol

CoRR: colocation for redox regulation

CPT: carnitine palmitoyl transferase (CPT)

CS: citrate synthase

DAP: distance of average path

DCL: curvilinear distance

DNA: deoxyribonucleic acid

DSL: straight-line distance

DTNB: 5,5’-dithiobis(2-nitrobenzoic acid)

DUI: doubly uniparental inheritance

$_E$: ETS-state - mitochondrial respiratory state 3u

ETS: electron transport system or mitochondrial complex I + III

F_1F_0-ATPase: ATP-synthase complex

FAD/$FADH_2$: flavin adenine dinucleotide

FCCP: carbonyl cyanide p-(trifluoromethoxy)-phenylhydrazone

FCFs: flux control factors

FCRs: flux control ratios

FR: fumarate reductase

F-type mtDNA: DUI maternally inherited mtDNA lineage

G: glutamate

Gp CF: glycerophosphate control factor

Gp: glycerophosphate

GpDH: glycerophosphate dehydrogenase

GPx: glutathione peroxidase

H_2O: water

H_2O_2: hydrogen peroxide

HRR: high resolution respirometry

$j_{\approx P}$: OXPHOS coupling efficiency

j_{ExCIV}: apparent excess capacity of cytochrome *c* oxidase

j_{ExP}: ETS apparent excess capacity

$_L$: LEAK-state - mitochondrial respiratory state 4 or state 2'

LDH: lactate dehydrogenase (LDH)

LDH4: lactate dehydrogenase 4

LIN: linearity

M: malate

MDH: malate dehydrogenase (MDH)

MRL: maximum reported longevity

mt: mitochondrial

mtDNA: mitochondrial DNA

M-type mtDNA: DUI paternally inherited mtDNA lineage

N: NADH-linked substrates

NAD^+/NADH: nicotinamide adenine dinucleotide

nDNA: nuclear DNA

N_L: Leak-state, $_L$ with N substrates combination, no ADP

N_{P}: OXPHOS-state, $_{\mathrm{P}}$ with N substrates combination

$\mathrm{NPrc}_{\mathrm{P}}$: OXPHOS-state $_{\mathrm{P}}$ with NPrc substrates combination

$\mathrm{NPrcSGp}_{\mathrm{E}}$: ETS-state, $_{\mathrm{E}}$ with NPrcSGp substrates combination

$\mathrm{NPrcSGp}_{\mathrm{P}}$: OXPHOS-state, $_{\mathrm{P}}$ with NPrcSGp substrates combination

$\mathrm{NPrcS}_{\mathrm{P}}$: OXPHOS-state, $_{\mathrm{P}}$ with NPrcS substrates combination

$\mathrm{NPr}_{\mathrm{P}}$: OXPHOS-state, $_{\mathrm{P}}$ with NPr substrates combination

$O_2^{\cdot -}$: superoxide radical

O_2: molecular oxygen

$OH^{\cdot}$: hydroxyl radical

Omy: oligomycin

ORFs: open reading frames

Oxa: sodium oxamate

OXPHOS: oxidative phosphorylation

$_{\mathrm{P}}$: OXPHOS-state - mitochondrial respiratory state 3

P: pyruvate

PC1: first principal component

PC2: second principal component

PCA: principal component analysis

PCGs: protein coding genes

PK: pyruvate kinase (PK)

PMF: proton motive force

Pr: proline

ProDH: proline dehydrogenase

RNA: ribonucleic acid

ROS: reactive oxygen species

Rot: rotenone

ROX: residual oxygen consumption

rRNAs: ribosomal RNAs

s.e.m.: standard error of the mean

S: succinate

S-Cc: somatic-specific isoform of cytochrome *c*

Shm: salicylhydroxamic acid - SHAM

Shm$_E$: residual mitochondrial respiration after inhibition of complex III and AOX

SMI: strict maternal inheritance

SOD: superoxide dismutase

STR: straightness

Succinate CF: succinate control factor

SUIT: substrate-uncoupler-inhibitor titration protocol

T-Cc: testes-specific isoform of cytochrome *c*

Tm: N,N,N',N'-Tetramethyl-p-phenylenediamine dihydrochloride - TMPD

tRNAs: transfer RNAs

VAP: average path velocity

VCL: curvilinear velocity

VSL: straight-line velocity

WOB: wobble coefficient

Δψm: mitochondrial membrane potential

A María

Acknowledgements

A mia madre Aurora, mio padre Roberto, mio fratello Nicola, i miei zii Mariangela, Danilo, Egizia, Carla, Italo e mio cugino Emilio. Grazie per avermi sempre sostenuto ed aver creduto in me. Un ricordo speciale a mio zio Flavio. Gracias a María-Jesús, Ismael y Mari, Clara y toda mi familia y amigos de España que son demasiados para mencionarlos a todos.

Alla mia cara María, compagna di viaggio e di avventure. Grazie per l'amore che mi hai dato, per il sostegno e per l'aiuto. Per condividere con me tanti momenti belli ma anche difficili, per supportarmi e sopportarmi ;). Senza di te questo viaggio non sarebbe stato lo stesso. Te quiero.

Ai miei compagni di una vita Ale, Adriano, Fede, Samuele e a tutti gli amici trentini (e non): Bepi, Ele, Sebastian, Pietvo, Galli, Adriana, Lisa, Fradell, Leandro, James, Federica, Franz (e tutti gli altri amici che non ho spazio per menzionare in queste poche righe, pure il fico sacro). È sempre bello tornare a casa e sapere che ci siete. Un enorme grazie agli amici italo-montrealesi: Andrea, Diana, Francesca e Giovanna, grazie per le risate e le grandi abbuffate pasquali.

I would really like to thank all the UdeM friends that shared this path with me as colleagues. First, thank you Charlotte for all the beautiful and funny moments we shared along the way. Moreover, to the old good-looking guys Karim and Arthur, to Davide, Philip, Ariane, Marie Bd, Maire Lvt, George, Rudy, Guillaume, Jeremy, Andréanne, Etienne, Victoria, Joelle, Gabrielle, Suga and so on. Thanks for being there and sharing with me so many happy moments.

To my co-supervisor Pierre. Merci, Pierre, pour tous les bons conseils et pour ton aide pendant ces années. Furthermore, to my beloved Rimouski friends Enrique and Felix. You guys "du bist sehr". Thank you for all the beautiful moments. "El paño es preto".

Last but certainly not least, my supervisor. Sophie, je me considère vraiment chanceux de t'avoir eu comme superviseure et je te suis extrêmement reconnaissant pour ton soutien IMMENSE pendant ces années. En fait, le mot immense est probablement réductif. Merci pour tous les conseils e pour croire en moi, pour les belles discussions (scientifiques et non) et aussi pour les bières et les beaux moments :) !

Thanks to all of you!

CHAPTER I – GENERAL INTRODUCTION

Mitochondria, a cellular power plant

Mitochondria are membrane-enclosed cytoplasmic organelles contained in almost all eukaryotic cells (Karnkowska et al., 2016). They originated from once free-living α-proteobacteria which integrated into an archaeon host cell, progressively lost autonomy and ended fully integrated into the now eukaryotic cells (Margulis, 1970; Roger et al., 2017). The acquisition of mitochondria provided eukaryotes severalfold more energy availability compared to prokaryotes (Lane & Martin, 2010), in turn potentially supporting the evolution of eukaryotic complexity through genome expansion, higher regulatory complexity and increased capacity of protein synthesis (Lane, 2020). Although involved in different cellular mechanisms (e.g., calcium homeostasis, apoptosis), mitochondria are generally associated with cellular bioenergetics (Amaral et al., 2013; Nunnari & Suomalainen, 2012; Spinelli & Haigis, 2018).

Mitochondria are *de facto* oxygen-consuming electrochemical generators (Gnaiger et al., 2020), fulfilling most of the energy requirement in eukaryotic cells through a process known as oxidative phosphorylation (OXPHOS), a metabolic pathway accomplished by the electron transport system (ETS) plus the phosphorylation system (Saraste, 1999). Briefly, the energy released during cytosolic and mitochondrial substrate oxidation (e.g. glycolysis, fatty acid oxidation, tricarboxylic acid cycle) is stored as electrons in the respiratory cofactors nicotinamide adenine dinucleotide (NAD^+) and flavin adenine dinucleotide (FAD). Their resulting reduced forms (i.e. NADH and $FADH_2$) are crucial reducing equivalents that donate electrons to the mitochondrial respiratory system. The ETS is an elaborate system involving the activity of four main enzymatic complexes situated on the inner mitochondrial membrane. Due to the presence of tightly bound cofactors, the ETS complexes can undergo redox reactions and consequently shuttle the electrons coming from substrate oxidation along the entire pathway. The electrons stored in the NADH pool are transferred to complex I (NADH-dehydrogenase or CI), while complex II (succinate dehydrogenase or CII) catalyses the oxidation of succinate to fumarate, concomitantly forming $FADH_2$. The ETS further involves complex III (coenzyme Q: cytochrome *c* oxidoreductase or CIII) and finally complex IV (cytochrome *c* oxidase, CIV or CCO), which represents the final oxidase of the chain, catalysing the reduction of molecular oxygen (O_2) into water (H_2O). Additional external factors are also involved, such as coenzyme Q (ubiquinone or ubiquinol) and cytochrome *c*, which act as electron transporters between different complexes. The redox reaction chain and the consequent flow of electrons along the ETS couples with a proton efflux from the matrix across the inner membrane into the intermembrane space. As the protons accumulate in the external medium, they generate an electrochemical gradient across the membrane (Mitchell, 1961). This proton motive force (PMF) is in turn exploited by the ATP synthase complex (F_1F_0-ATPase) as the power source to synthetize adenosine triphosphate (ATP), the high-energy compound used for essentially all active metabolic processes within the cell (figure 1.1). In addition to the four "classic" ETS complexes, other enzymatic components such as the mitochondrial glycerophosphate dehydrogenase (GpDH), electron-transferring flavoprotein complex (CETF), proline dehydrogenase (ProDH), fumarate reductase (FR) and alternative oxidase (AOX) are also found as integral components of the respiratory chain, playing an important role for cell bioenergetics (Donaghy et al., 2015; Gnaiger, 2014; Gnaiger et al., 2020; Mracek et al., 2013; Muller et al., 2012). Other than energy production, the electrochemical potential generated by the ETS through respiration and/or by other mechanisms (e.g. ATP synthase reversal activity in anoxia-tolerant frogs (St-Pierre et al., 2000) plays a role in crucial mitochondrial functions such as protein import and calcium homeostasis (Amaral et al., 2013; Friedman & Nunnari, 2014; Neupert & Herrmann, 2007). Furthermore, as the mitochondrial membrane potential ($\Delta\psi m$), i.e. the electric part of the proton motive force, can reflect functional and healthy mitochondria, it has been proposed to be related with mitochondria selection, segregation and inheritance (Milani, 2015; Tworzydlo et al., 2020). Otherwise, mitochondria depolarization might trigger pathways that bring either mitochondria or cell to elimination (e.g. mitophagy, apoptosis) (Jin et al., 2010; Knorre, 2020; Twig et al., 2008; Westermann, 2010).

A predictable by-product of the redox reactions associated with mitochondrial respiration is the formation of reactive oxygen species (ROS). These free radicals originate from the partial reduction of oxygen following the escape of electrons out from their ideal ETS route. The result is the production of unstable molecules, susceptible to steal electrons to other compounds in order to stabilize themselves. These unstable molecules are the superoxide radical ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($OH^{\cdot}$) (Apel & Hirt, 2004; Munro & Treberg, 2017). A high ROS concentration is well known to exert oxidative stress, damage important macromolecules, (such as proteins, lipids and nucleic acids) and finally undermine cellular fitness (Dowling & Simmons, 2009; Munro & Treberg, 2017). Because redox reactions are tightly coupled with mitochondrial functioning, mitochondria themselves are often described as the primary source of cellular ROS, thus becoming a potential noxious environment for their own membranes and genome, finally leading to mitochondrial dysfunction (Shokolenko et al., 2009). Under the "mitochondrial oxidative stress theory of ageing" (Barja, 2014; Harman, 1972), it is predicted that the free radical attack will mostly affect the genome lying within the mitochondrion, in turn disrupting the OXPHOS activity. An impaired OXPHOS activity will then further amplify ROS dysregulation. This downward spiral of accumulating damage will finally drive the ageing process (Blier et al., 2017; Dowling & Simmons, 2009). Although potentially deleterious when found in excess, there is a now recognized signalling role of ROS in controlled concentrations. For example, ROS participate in the immune response, cell signalling and differentiation, and programmed apoptosis (Apel & Hirt, 2004; Dowling & Simmons, 2009; Munro & Treberg, 2017). Mitochondrial ROS generation also regulates mitophagy (Scherz-Shouval & Elazar, 2011), and mediates feedback signalling to the nucleus, modulating mitochondrial biogenesis as a compensatory mechanism to adjust OXPHOS yield (Moreno-Loshuertos et al., 2006). In order to mitigate or regulate ROS production, cells show a variety of antioxidant mechanisms. Examples of enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and peroxiredoxin. The AOX complex also adds to the repertoire of mitochondrial antioxidant defences. AOX is an alternative non-proton pumping oxidase that bypasses the "classic" cytochrome route (CIII plus CIV) by directly reducing O_2 with the electrons coming from the ubiquinol pool. During OXPHOS deficiency, AOX could act as an emergency "electron sink", reducing the excess reductive potential of ETS complexes, thus counteracting conditions that are known to enhance ROS formation (Abele, 2007; El-Khoury et al., 2014; Gueguen et al., 2003; McDonald et al., 2009; Munro et al., 2013; Parrino et al., 2000; Tschischka et al., 2000; Vanlerberghe, 2013; Venier et al., 2009).

A small but valuable genome, the mitochondrial DNA

Mitochondria have their own genome, the mitochondrial DNA (mtDNA), separated from the nuclear genome. Following the endosymbiont origin of mitochondria (Margulis, 1970), the evolution of mtDNA followed a progressive loss or transfer of genes to the nuclear genome. What remains of the mtDNA in animal species is a small double-stranded circular molecule of ~16.5 kb, present in several copies inside each mitochondrion. Overall, the mtDNA in animals contains 37 genes that encode 2 ribosomal RNAs (rRNAs), 22 transfer RNAs (tRNAs) and 13 peptides (Protein-Coding Genes or PCGs) subunits of the OXPHOS complexes (Boore, 1999). Exceptions however do exist (see (Breton et al., 2014)). The functional *repertoire* of the mtDNA now appears to also include additional genes, with functions ranging from protection to germline and sex determination (Angers et al., 2019; Breton et al., 2014; Breton et al., 2011; Guo et al., 2003; Milani et al., 2014b; Milani et al., 2015; Ouimet et al., 2020), as well as small noncoding RNAs, predicted to regulate nuclear genes (Passamonti et al., 2020; Pozzi & Dowling, 2019; Pozzi et al., 2017).

Even though still unknown, the reason why mitochondria retained some genes within the mtDNA could implicate the maintenance of a local control on respiratory metabolism by mitochondria (Lane, 2020). According to Allen (2015), the colocalization of gene and gene products within its original membrane-bound compartment allows direct regulatory control upon the expression of genes coding for respiratory complexes subunits following changes in redox state (see "CoRR" hypothesis; (Allen, 2015)). Nonetheless, most genes necessary for mitochondrial functioning are coded by the nuclear DNA (nDNA) and further imported into mitochondria. This also includes most of the subunits forming part of the respiratory machinery (figure 1.1) (Blier et al., 2001; Boore, 1999). The large protein complexes composing the ETS and ATP synthase are in fact chimeric units, composed by both mitochondrial- and nuclear-encoded subunits.

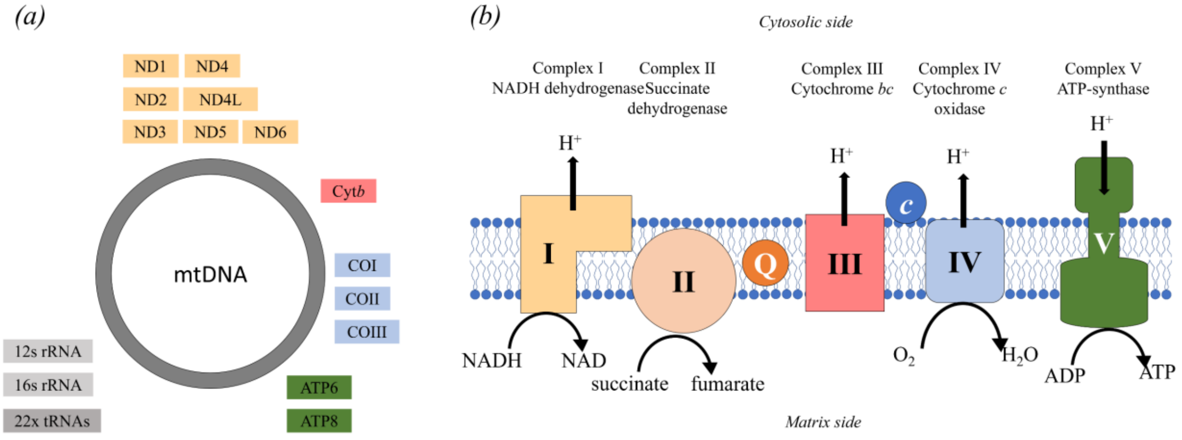


Figure 1.1. Schematic representation of the mitochondrial genome (mtDNA) and the electron transport system. *(a)* mitochondrial DNA molecule encoding for 13 peptides taking part in the OXPHOS machinery, two ribosomal RNAs

and 22 tRNAs; *(b)* electron transport system, whose enzymatic complexes are mosaic units, composed by both mitochondrial and nuclear encoded subunits.

Mitonuclear coevolution

The mosaic nature of respiratory complexes implies that subunits encoded by different genomes must directly interact and finely coordinate with each other. The two genomes are required to work harmoniously to fulfil the cell energy needs, and this inevitably rise the need of coevolution between them (i.e. mitonuclear coevolution). This universal selection for genomic match has been proposed to have played (and still play) a crucial role in the evolution of eukaryotes (Blier et al., 2001; Dowling et al., 2008; Hadjivasiliou et al., 2012; Havird et al., 2019; Hill et al., 2019; Lane, 2009, 2011; Wolff et al., 2014).

Despite the need of a proper coevolution, the two genomes evolve in different ways and at a different pace. Compared to the nuclear DNA (nDNA), the mtDNA is not mixed every generation by sexual reproduction but rather divides asexually. Furthermore, the mtDNA in animals has a mutation rate 10-50 times higher than its nuclear counterpart (Brown et al., 1979; Lane, 2009). Replication errors and oxidative stress are two potential mechanisms by which mtDNA mutations can proliferate (Aryaman et al., 2018; Rand, 2008). Contrary to the classic vision that mitochondrial genetic variation would be selectively neutral, accumulating studies have demonstrated that mitochondrial DNA variations exist and can have a pervasive effect on fitness, affecting mitochondrial functions (Bettinazzi et al., 2019b; Pichaud et al., 2012), longevity (Coskun et al., 2003; Niemi et al., 2003), fertility (James & Ballard, 2003; Montiel-Sosa et al., 2006; Nakada et al., 2006; Ruiz-Pesini et al., 2000), vulnerability to diseases (Ji et al., 2012; Taylor & Turnbull, 2005; Wallace, 1999) and adaptation to different thermal niches and diets (Camus et al., 2017a; Lajbner et al., 2018; Mishmar et al., 2003; Morales et al., 2018; Ruiz-Pesini et al., 2004). The mtDNA itself is now recognized to play an important role in the adaptive evolution of organisms. As a result of a the high mtDNA evolutionary rate, *de novo* mitonuclear combinations arise each generation and undergo selection for mitochondrial functioning. Given the penalty of failure for cellular fitness, directional selection would readily purge any deleterious combination. For example, severe mtDNA mutations have been found to be eliminated in the mammalian germline of mice (Fan et al., 2008; Stewart et al., 2008). At the organelle level, mitochondrial dynamics (i.e.

fission and fusion events) and selective mitophagy concurs in the elimination of poor performing organelles (Jin et al., 2010; Jin & Youle, 2012; Twig et al., 2008; Westermann, 2010). Even though purifying selection is accounted as the main force shaping mtDNA variation (reducing the genetic variation by getting rid of the most severe mutations) (Dowling et al., 2008; Li et al., 2010; Ruiz-Pesini et al., 2004; Stewart et al., 2008; Ye et al., 2014), some variants are transmitted across generations. On the one hand, mitochondrial mutations with a mild effect as well as neutral variations can escape selection (Alston et al., 2017; Hill et al., 2019). This gives ample opportunity to mtDNA polymorphisms to accumulate. On the other hand, a substantial fraction of mtDNA variation could be adaptive and undergo positive selection (Dowling et al., 2008; Hill et al., 2019; Klucnika & Ma, 2019; Lane, 2009; Wolff et al., 2014). During oocyte development, well performing mitochondria can segregate in a specific region, the Balbiani body (Bb). Eventually their genome is preferentially replicated, and transmitted to the future generation (Bilinski et al., 2017; Hill et al., 2014; Zhou et al., 2010). As functional mtDNA variations could easily affect the catalytic capacity of ETS enzymes, ATP-production efficiency, ROS formation and heat production (Pichaud et al., 2012), and enzymatic processes being temperature sensitive, the possible adaptive value of mtDNA evolution is assumed to mainly embrace thermal and dietary adaptation (Blier et al., 2001; Blier et al., 2014; Camus et al., 2017b).

Considering the intricate interactions between nuclear and mitochondrial encoded genes, any sequence change in the mtDNA, being it adaptive or not, might induce a coordinate response in the nuclear genome. Mitonuclear coevolution is predicted to promote intergenomic compatibility (Hill et al., 2019), and accumulating evidence supports the idea that change in the mitochondrial genome might trigger strong selective pressure for compensatory change in the nuclear genome (Barreto & Burton, 2013b; Barreto et al., 2018; Healy & Burton, 2020; Hill, 2020; Mishmar et al., 2006; Osada & Akashi, 2012). Overall, the rate of mitochondrial evolution provides a quick source of genetic variability that in the end drives the entire mitonuclear coevolution process and potentially foster evolutionary innovation (Blier et al., 2001; Dowling et al., 2008; Hill et al., 2019; Rand et al., 2004; Wolff et al., 2014). The evolution of the joint mitonuclear genotype is thus quite dynamic and, in absence of gene flow, populations can rapidly diverge in mitochondrial functions. Over time, different populations can become increasingly incompatible, and this can even isolate them reproductively, promoting speciation (Burton & Barreto, 2012; Gershoni et al., 2009; Lane, 2009; Wolff et al., 2014).

Cytonuclear incompatibility

Given the pivotal role of mitochondrial respiration for cell energy production, mitonuclear mismatch may hamper the structural and biochemical properties of respiratory complexes, causing respiratory deficiency and consequent fitness loss (Camus et al., 2020; Dowling et al., 2008; Hill et al., 2019; Lane, 2009, 2011; Latorre-Pellicer et al., 2016; Wolff et al., 2014). The deleterious consequences of mitonuclear mismatch has been revealed in many species, following either intra or interspecific crosses. These include yeast (Lee et al., 2008), various invertebrates (Burton et al., 2006; Demuth & Wade, 2007; Ellison et al., 2008; Niehuis et al., 2008; Rank et al., 2020; Sackton et al., 2003) and vertebrates (Bolnick et al., 2008; Chapdelaine et al., 2020). For example, in natural occurring cybrids (i.e. hybrids that possess the nuclear genome from one parental species and the mitochondrial genome from the other) between the redbelly and the fine dace (*Chrosomus eos* and C. *neogaeus*), the combined effect of mitonuclear combination and temperature variation was revealed to alter the activity of cytochrome *c* oxidase (encoded by both the mitochondrial and nuclear genome), while having no effect on the nuclear encoded citrate synthase (Chapdelaine et al., 2020). In the leaf beetle *Chrysomela aeneicollis*, natural introgression between populations characterized by distinct mitonuclear genotypes produced a fitness loss in mismatched individuals, further amplified by heat treatment. Individuals with matched mitonuclear genotype were fitter than mismatched ones for many key life-history traits, including fecundity, development and mating frequency in males (Rank et al., 2020). However, the most known example of intergenomic incompatibility comes from the experimental hybridization of isolated population of *Tigriopus californicus*, a small marine copepod. Burton and colleagues revealed that the mitonuclear mismatch deriving from laboratory crosses resulted in a severe F2 hybrid breakdown, typically characterized by lower mitochondrial ATP synthesis, reduced developmental rate, fecundity and viability, as well as increased oxidative stress (Barreto & Burton, 2013a, 2013b; Barreto et al., 2014; Barreto et al., 2018; Burton & Barreto, 2012; Burton et al., 2006; Ellison & Burton, 2006, 2008, 2010; Healy & Burton, 2020). Restoring of the original mitochondrial background re-established the fitness, confirming the disruption of mitonuclear interactions to be the cause of hybrid fitness breakdown.

An additional way to generate cytonuclear incompatibility is by mixing different mitochondria (i.e. heteroplasmy, a state where different mtDNA variants coexist). Indeed, the presence of different mitochondrial lineages with the same nuclear background can provoke deleterious effects on OXPHOS activity maintenance (Lane, 2011, 2012). Beyond heteroplasmic harmful mtDNA mutations, whose deleterious effect becomes apparent when their abundance exceeds a certain threshold (Stewart & Chinnery, 2015; Taylor & Turnbull, 2005; Wallace & Chalkia, 2013), heteroplasmy alone could also be unfavourable. Even two mtDNA types that separately work equally well with a certain nuclear genome, when coexisting in this nuclear background might cause disruption of the optimal dual mito-nuclear coadaptation (Lane, 2012). This has been documented in heteroplasmic mice, which suffered from reduced OXPHOS activity, lowered food intake, compromised respiration, accentuated stress response and cognitive impairment (Acton et al., 2007; Sharpley et al., 2012). The genetic instability and the consequent fitness penalty produced by uncontrolled heteroplasmy could potentially explain the advantage of a uniparental inheritance of cytoplasmic organelles (Christie et al., 2015; Hadjivasiliou et al., 2012; Lane, 2011, 2012). This is supported by a recent study on *Caenorhabditis elegans*, which showed that the delayed removal of the paternal mitochondria in this organism with strict maternal inheritance of mitochondria provokes an increased embryonic lethality in the resulting heteroplasmic animals (Zhou et al., 2016).

Mitochondrial inheritance

In metazoans, strict maternal inheritance (SMI) is the almost universal mechanism of mitochondrial transmission and several mechanisms ensuring SMI have been reported in the literature (Birky, 1995; Sato & Sato, 2017; Sato & Sato, 2013). Specifically, sperm mitochondria are eliminated in many different ways, i.e. either by segregation and further degradation during gametogenesis, by preventing them to enter the egg, or by post-fertilization mechanisms such as silencing or selective degradation (e.g. ubiquitination in mammals, depolarization and subsequent mitochondria degradation in *C. elegans*) (Birky, 2001; Sato & Sato, 2017; Sato & Sato, 2013; Sutovsky et al., 1999; Zhou et al., 2016). The evolutionary consequence of inheriting just one parental set of mitochondria is a strong reduction of mtDNA variability in the forming zygote, in other words, promoting homoplasmy (i.e. a condition in which all mitochondrial genomes are alike). It has been proposed that these different mechanisms ensuring SMI have arisen to avoid the spread of selfish cytoplasmic elements, limit mito-nuclear conflicts and optimize co-adaptation of mitochondrial and nuclear genes (Ballard & Whitlock, 2004; Christie et al., 2015; Hadjivasiliou et al., 2012; Havird et al., 2019).

Although having mitochondria and their genome transmitted by the mother could be advantageous in terms of genetic integrity, it also invokes a sex-specific selective sieve in the evolution of the mitochondrial genome. Conversely to oocyte derived mitochondria, sperm mitochondria (and their genome) are prevented from being passed to the future generation, and *de facto* constitute an evolutionary dead end. One downside of SMI is thus that the evolution of mtDNA is shaped by selection acting on females, and this could be deleterious for male fitness. Hypothetically, any new mitochondrial variant with sexually antagonistic effect, which is to say neutral or beneficial in its effect on females but harmful for males, can be retained within a population because selected in females. This proposed phenomenon is known as the “mother’s curse” (Gemmell et al., 2004). Potential support for the mother’s curse comes from the sexual asymmetry in the severity of certain mitochondrial diseases and from specific mitochondrial haplotypes with a pervasive effect on sperm motility and consequently male reproductive fitness, while being neutral in females (Camus et al., 2012; Frank & Hurst, 1996; Innocenti et al., 2011; Montiel-Sosa et al., 2006; Nakada et al., 2006; Ruiz-Pesini et al., 2000).

Doubly uniparental inheritance of mitochondria

The only stable exception to SMI is the doubly uniparental inheritance (DUI) of mitochondria (Breton et al., 2007; Passamonti & Ghiselli, 2009; Zouros, 2012). This unusual system has been so far reported in more than a hundred bivalve species (Gusman et al., 2016), and involves the concurrence of two different sex-linked mitochondrial lineages in the same species. The rule of uniparental inheritance is maintained as the two lineages are transmitted independently by the two sexes, which is to say, one lineage is transmitted by females through oocytes (F-type mtDNA), whereas the other by males through sperm (M-type mtDNA). After fertilization, the newly formed zygote starts by being heteroplasmic for both lineages. During development, sperm mitochondria are eliminated in future females, whereas they are maintained and actively segregated in the blastomere that will give rise to germ line cells in future males (figure 1.2). It is important to note that it is still unclear whether the link between gender and a specific mt lineage could be associative or causative (Breton et al., 2011). What we do know is the general pattern of mtDNA segregation.

Even though exceptions exist (e.g. (Obata et al., 2007)), adult females generally end up being homoplasmic for the maternally derived mitochondria (i.e. they only contain the F lineage in both germline and somatic), whereas males are heteroplasmic for both the maternally and the paternally acquired mitochondria. Specifically, male somatic cells are generally composed by only the F or both F- and M-type mtDNAs (heteroplasmic male somatic tissues), while sperm are homoplasmic for the only M-type mtDNA (Breton et al., 2007; Passamonti & Ghiselli, 2009; Zouros, 2012).

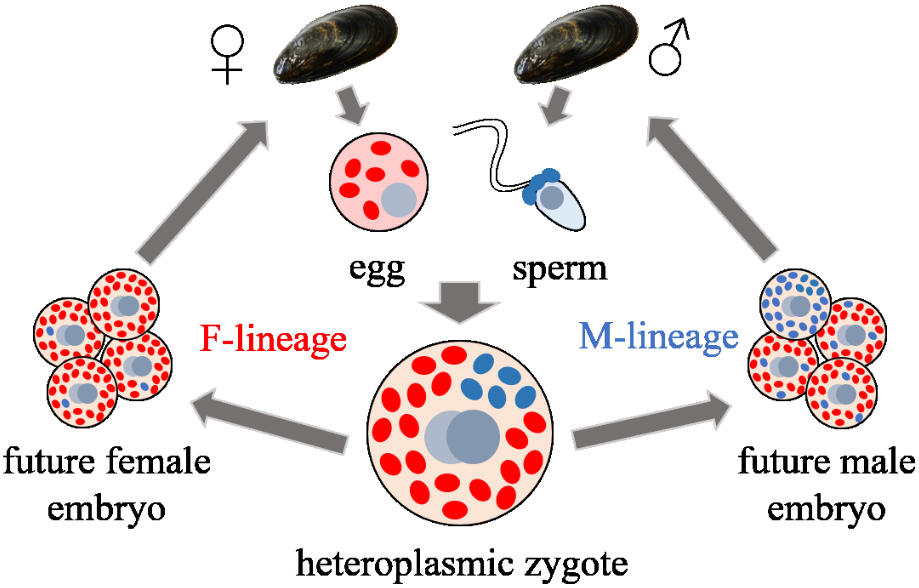


Figure 1.2. Schematic representation of the doubly uniparental inheritance (DUI) system of mitochondria transmission in bivalves. Two highly divergent and sex-specific mtDNA lineages compose the genetic landscape of these animals, on maternally derived (the F-type) and one paternally derived (the M-type). Even though leakage of the paternal mitochondrial DNA sometimes can happen, the general rule sees females homoplasmic for the F-type lineage in both oocytes and somatic tissues, while males are heteroplasmic. Sperm bear the only M-type lineage, while male soma present both F and M lineages in various proportions depending on tissue and species (Breton et al., 2007; Passamonti & Ghiselli, 2009; Zouros, 2012).

Heterogametes in these species generally bear only the correspondent sex-linked mitochondrial lineage (Ghiselli et al., 2010; Venetis et al., 2006), and this association has an intense effect on the evolution of the DUI mtDNA population. The two mtDNA types experience different selective pressure depending on the sex to which each of them is associated and this result in a separate evolution of the two mt lineages, which can reach 50% of DNA sequence divergence in some species and genes (Bettinazzi et al., 2016; Breton et al., 2007; Capt et al., 2020; Guerra et al., 2017; Passamonti & Ghiselli, 2009; Zouros, 2012). Besides their high sequence divergence, DUI-related haplotypes also present interesting uncommon features like extension of or insertion in the *cox2* gene, novel sex-specific open reading frames (ORFs), supernumerary genes and gene duplications. Some of these uncommon features have been suggested to be related to the functioning and the role of this unusual mechanism of mitochondrial inheritance (Bettinazzi et al., 2016; Breton et al., 2007; Breton et al., 2014; Capt et al., 2020; Passamonti & Ghiselli, 2009; Zouros, 2012). DUI mtDNAs also evolve faster than typical metazoan mtDNAs and, within them, the M-mtDNA has a higher rate of evolution than the F one (Breton et al., 2007; Passamonti & Ghiselli, 2009; Zouros, 2012). The faster evolution of both DUI mtDNAs is thought to be due to the relaxed selective constraints acting on DUI mtDNA lineages. Because of their unequal division of work, i.e. with a negligible role of the M-mtDNA in somatic tissues, sex-linked mtDNAs undergo different selective pressures. This reduction in the sum of total selection pressure on each lineage might explain their accelerated evolution compared to other animal mtDNAs (Hoeh et al., 1996). This unequal division of labour could also explain the fact that M-mtDNA evolves faster than the F-mtDNA, since it is (almost) only found within male gonads. In addition to this, the M-mtDNA is thought to have a higher intrinsic mutational rate than the F one due to a higher ROS-induced damaging, greater rates of mtDNA duplication during spermatogenesis, small effective number of mitochondria carried by sperm, and cyclic "bottleneck" events (Ghiselli et al., 2013).

Given the strict association between M-type mtDNA and sperm, some authors have hypothesized that the high amino acid divergence between the two DUI mtDNAs could link with functional adaptations related with sperm energy production, motility and viability (Breton et al., 2007; Burt & Trivers, 2006). So far, researches in this sense have been scarce and the results somehow counterintuitive. For example, the comparison between DUI-sperm carrying the "classical" male linage (M-type) and DUI-sperm carrying the female "masculinized" one (sometimes in *Mytilus* species the F-mtDNA invades male gonads, taking the place of the M-mtDNA) revealed lower performances (motility parameters) and lower maximal enzymatic capacity of ETS complexes in sperm carrying the M mitotype (Breton et al., 2009; Everett et al., 2004; Jha et al., 2008; Stewart et al., 2012). Although contrary to what expected if the establishment of a male-transmitted mtDNA lineage would increase male fitness through selection on sperm function, sperm motility is only one parameter to test. As the authors stated, the adaptive evolution of the M-type mtDNA might account for subtler metabolic and/or sperm functions (e.g. viability, longevity) (Breton et al., 2007; Breton et al., 2009; Everett et al., 2004; Jha et al., 2008). Whether functional phenotypic differences might associate with the two sex-linked mtDNA lineages in DUI species is still unknown. For example, we could expect different adaptations at the level of mitochondrial functionality and the more general cellular bioenergetics, or different performance and fertilization strategies adopted by male gametes in these species.

Because of its naturally heteroplasmic state, DUI constitutes a unique experimental system to study the mitonuclear coevolution of two mt genomes in a common nuclear background. It also offers an unparalleled occasion to scrutinise the phenotypic outcome of a separate male- and female-specific mtDNA evolution. Sperm mitochondria in DUI species are transmitted to sons, opening up an unprecedented opportunity for an animal mtDNA to escape the female-specific evolutionary constraints and respond to selection acting directly on males. An additional uniqueness is that the now transmitted paternal mitochondria must serve both as energy supplier for sperm motility as well as genetic template for the future generations of males. Knowing that sperm motility is energetically very demanding, and that aerobic metabolism is a potential source of oxidative stress, preserving the integrity of mtDNA information in sperm mitochondria could be very tricky. Overall, almost nothing is known about the evolutionary relevance of preserving two coexisting sex-linked mitochondrial lineages in bivalves, as well as how a faithful transmission of mtDNA is achieved in these species.

Objective and predictions

The general objective of my PhD project is to examine the linkage between mitochondrial genotype and phenotype. From an evolutionary point of view, the aim is to explore the adaptive value of sex-specific mtDNA variants, exploiting the unique opportunity given by the DUI system to evaluate the result of a male-specific evolution of the mitochondrial genome. Other complementary objectives are to examine how genetic integrity and a faithful transmission of sperm derived mtDNA might be achieved, and also to study the possible phenotypic consequences of heteroplasmy in somatic tissues of males.

Assumed that mitochondrial DNA variations are likely prone to influence mitochondrial functionality, the hypothesis to test is that different selective constraints acting on DUI mitochondria would have an extensive phenotypic repercussion on both mitochondrial and cellular metabolism, potentially promoting female and male-specific energetic adaptation. Hypothetically, having two highly divergent mitochondrial lineages characterized by different metabolisms and associated with different compartments (such as male and female gametes) could be advantageous to fulfil the different energetic demand between the two sexes (e.g. during gametogenesis or gonad development, for gamete activity and performance). Promoting a different metabolism between the two sex-linked mt lineages could also be the physiological answer allowing the maintenance of the DUI system by: i) minimizing the three genomes shared constraints regarding mito-nuclear coadaptation for energetic function and ii) dodging oxidative stress linked with OXPHOS activity (through the suppression, change or minimization of the respiratory activity). In the case of paternally derived mitochondria, any potential change in mitochondrial and cellular bioenergetics is expected to have a downstream effect on mitochondria preservation and transmission, as well as on sperm performance and reproductive fitness in general.

The experimental design involves the analysis of a total of seven bivalve species, three DUI and four SMI. To avoid revealing potential differences dictated by taxon rather than inheritance method, the selected species are phylogenetically distant, having a last common ancestor dated to the mid-Cambrian (~500 million years ago) (Gusman et al., 2016; Plazzi et al., 2016). Depending on the experiment, the DUI species examined are *Mytilus edulis* (Order Mytilida, Family Mytilidae) from Kensington (PE, Canada), *Ruditapes philippinarum* (Order Venerida, Family Veneridae) from Vancouver (BC, Canada) and *Arctica islandica* (Order Venerida, Family Arcticidae) from Perry (ME, USA). On the other hand, the SMI species are *Mya arenaria* (Order Myida, Family Myidae) and *Mercenaria mercenaria* (Order Venerida, Family Veneridae) from Barnstable (MA, USA), *Nuttallia obscurata* (Order Cardiida, Family Psammobiidae) from Vancouver (BC, Canada) and *Placopecten magellanicus* (Order Pectinida, Family Pectinidae) from both the Gulf of Maine (MA, USA) and Newport (QC, Canada). The three DUI species possibly represent independent origins of the DUI system. This is reflected by the fact that their sex-linked genomes (F- and M-type) cluster in a species-specific way rather than by their sex-specificity (Gusman et al., 2016; Plazzi & Passamonti, 2019; Plazzi et al., 2016). The within-species divergence between the F and M genomes ranges between 10-22 % in *M. edulis* (Breton et al., 2006; Stewart et al., 1995; Zouros, 2012), 6-8% in *A. islandica* (Gusman et al., 2016) and 16-32 % in *R. philippinarum* (Bettinazzi et al., 2016; Passamonti et al., 2003). In addition to be phylogenetically distant, each group (DUI and SMI) includes species that either burrow (i.e. the DUI, *A. islandica* and R. *philippinarum*, and the SMI *M. mercenaria*, *M. arenaria*, *N. obscurata*) or not (i.e. the DUI *M. edulis* and the SMI *P. magellanicus*), as well as both short and long lived species. The maximum reported longevity is of 15, 18 and 507 years respectively for the DUI *R. philippinarum, M. edulis* and *A. islandica*, and 6, 8, 28 and 106 years respectively for the SMI *N. obscurata*, *P. magellanicus*, *M. arenaria* and *M. mercenaria.* All the species analysed share a common reproduction strategy (i.e. gonochoric, broadcast spawning species), are suspension feeders and are collected in cold marine waters along both the Atlantic and Pacific North American coast (Borradaile, 1963; Dudas & Dower, 2006; Humphreys et al., 2007; Munro & Blier, 2012; Munro et al., 2013; Sukhotin et al., 2007).

The project integrates different state-of-the-art techniques in order to provide a most complete and exhaustive characterization of mitochondrial and cellular physiology. Analyses are carried on either gametes and somatic cells of female and male individuals. Overall, the project is divided in three linked chapters, each one focusing on different but complementary physiological aspects.

First study (chapter II): Mitochondrial functionality

The aim of the first study is an in-depth evaluation of mitochondrial functions through high-resolution respirometry, using a dedicated Oxygraph-2k (Oroboros Inc, Innsbruck, Austria). This technique allows the characterization of the real-time efficiency of substrate oxidation and cellular respiration, in turn the potential identification of functional divergence between a paternal and maternal mitochondrial phenotype. In other words, differences in mitochondrial functioning that could be further linked to the genetic divergence between sex-linked mt genomes. The mitochondrial phenotype is thoroughly characterized in female and male gametes and somatic cells of both DUI and SMI species. Previous evidence exists that oocytes and sperm in the DUI species *R. philippinarum* have active mitochondria (Milani & Ghiselli, 2015). Moreover, bioinformatic prediction and empirical evidence on cytochrome *c* activity suggest that the functioning of F- and M-type mitochondria might *de facto* differ (Breton et al., 2009; Skibinski et al., 2017). Therefore, the specific predictions for this chapter are that: i) bivalve gametes (DUI and SMI oocytes and sperm) would be able to perform OXPHOS, as well as that ii) difference in OXPHOS capacity and organization might be the result of a male-specific evolution of DUI M-type mtDNA.

Second study (chapter III): Gamete bioenergetics

The goal of the second study is to characterize the potential impact of bearing a sex-specific mitochondrial lineage upon the wider cellular energy and antioxidant metabolism. The activity of key enzymes linked with glycolysis, fermentation, fatty acid metabolism, tricarboxylic acid cycle, oxidative phosphorylation, as well as the antioxidant capacity is evaluated using a Mithras LB940 microplate reader (Berthold technologies, Germany). The extensive bioenergetic characterization is carried on oocytes and sperm of DUI and SMI bivalve species. Given the interconnection between the various bioenergetic pathways composing the energy metabolism, the expectation is that potential changes in OXPHOS stemming from the DUI male-specific evolution of MtDNA (Breton et al., 2009), might also underpin a reorganization of the general cellular bioenergetics. Finally, conversely from SMI species, the mitochondria present in both DUI gametes serve as a genetic template for the future generations. Therefore, a rational prediction sees DUI sperm enhancing their antioxidant capacity compared to SMI sperm.

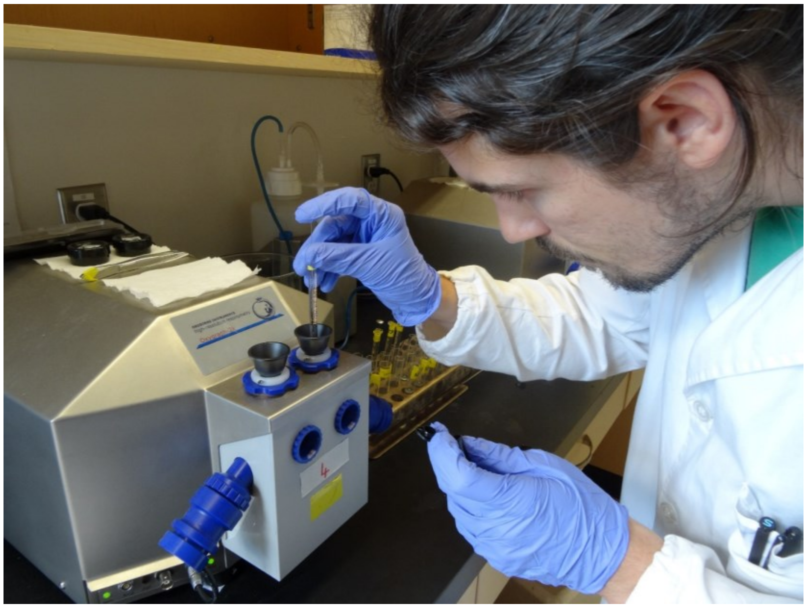
Third study (chapter IV): Sperm performance and reproductive fitness

The goal of the third study is to test whether a male specific evolution of the mt genome might impact sperm fitness traits and bioenergetics. Sperm motility traits of DUI and SMI species (whose sperm respectively bear a paternally and a maternally derived mitochondria) are characterized through a CEROS microscope combined with a computer-aided sperm analyser (CASA system) (Hamilton Thorne Inc, Beverly, USA). Potential differences in the bioenergetics sustaining spermatic functions are examined following the inhibition of the main pathways of energy production. Finally, the same analyses are conducted in presence/absence of oocytes, evaluating whether chemoattraction might impulse performance and bioenergetic changes in sperm, playing a role in the fertilization strategy of these species. Previous evidence exists that: i) sperm carrying M-type mitochondria swim slower than F-carrying ones in the DUI species *M. edulis* (Everett et al., 2004; Jha et al., 2008; Stewart et al., 2012), ii) SMI sperm of the species *Crassostrea gigas* exploit both OXPHOS and fermentation to sustain sperm motility (Boulais et al., 2019; Boulais et al., 2015), ii) egg-derived chemoattracts exert an effect upon M-type sperm performance in the DUI species *M. galloprovincialis* (Eads et al., 2016; Evans et al., 2012; Lymbery et al., 2017; Oliver & Evans, 2014). Therefore, the specific prediction is that SMI and DUI sperm would differ in their swimming performance, potentially also in their preferred pathway of energy production and in the response to oocyte detection.

CHAPTER II – METABOLIC REMODELLING ASSOCIATED WITH mtDNA: INSIGHTS INTO THE ADAPTIVE VALUE OF DOUBLY UNIPARENTAL INHERITANCE OF MITOCHONDRIA

Stefano Bettinazzi[a], Enrique Rodríguez[b], Liliana Milani[c], Pierre U. Blier[b], and Sophie Breton[a]

[a]Département de Sciences Biologiques, Université de Montréal, Montréal, QC H2V 2S9, Canada; [b]Département de Biologie, Université du Québec, Rimouski, QC G5L 3A1, Canada; [c]Dipartimento di Scienze Biologiche, Geologiche e Ambientali, Università di Bologna, Bologna 40126, Italia;



Published in *Proceedings of the Royal Society B* (2019) 286: 20182708

doi.org/10.1098/rspb.2018.2708

Abstract

Mitochondria produce energy through oxidative phosphorylation (OXPHOS), which depends on the expression of both nuclear and mitochondrial DNA (mtDNA). In metazoans, a striking exception from strictly maternal inheritance of mitochondria is the doubly uniparental inheritance (DUI). This unique system involves the maintenance of two highly-divergent mtDNAs (F- and M-type, 8-40% of nucleotide divergence) associated with gametes, and occasionally coexisting in somatic tissues. To address whether metabolic differences underlie this condition, we characterized the OXPHOS activity of oocytes, spermatozoa and gills of different species through respirometry. DUI species express different gender-linked mitochondrial phenotypes in gametes and partly in somatic tissues. The M-phenotype is specific to sperm and entails i) low coupled/uncoupled respiration rates, ii) a limitation by the phosphorylation system, iii) a null excess capacity of the final oxidases, supporting a strong control over the upstream complexes. To our knowledge, this is the first example of a phenotype resulting from direct selection on sperm mitochondria. This metabolic remodelling suggests an adaptive value of mtDNA variations, and we propose that bearing sex-linked mitochondria could assure the energetic requirements of different gametes, potentially linking male-energetic adaptation, mitotype preservation and inheritance, as well as resistance to both heteroplasmy and ageing.

1. Introduction

Mitochondria are the powerhouse of eukaryotic cells, providing energy through a mechanism known as oxidative phosphorylation (OXPHOS), involving different respiratory enzyme complexes in metazoans. Mitochondria possess their own genome, the mitochondrial DNA (mtDNA), that in animals, apart from some exceptions (Breton et al., 2014), encodes proteins that are all subunits of these complexes. The remaining subunits are encoded by the nuclear genome, making intergenomic co-evolution mandatory to preserve optimal mito-nuclear interactions and functioning of aerobic metabolism (Blier et al., 2001). As exemplified by hybridization events involving both interspecific and intraspecific crosses, the price of mito-nuclear mismatches is metabolism dysfunction and fitness loss (Barreto & Burton, 2013a). At the intraspecific level, mitochondrial genetic variations have been found to produce substantial phenotypic effects in both vertebrates and invertebrates. In humans for example, mtDNA variations affect longevity (Niemi et al., 2003), sperm motility (Ruiz-Pesini et al., 2000), thermal tolerance (Mishmar et al., 2003; Ruiz-Pesini et al., 2004) as well as susceptibility to diseases (Taylor & Turnbull, 2005). In *Drosophila*, mtDNA variations have been proven to impact mitochondrial functions and male fertility (Pichaud et al., 2012; Yee et al., 2013). The high mutation rate of the mtDNA in metazoans provides a fast source of variants on which natural selection can act (Lane, 2009), and emerging data suggest that organisms exploit the mitochondrial genetic system to fuel phenotypic variation and evolutionary innovation (Breton et al., 2014; Dowling et al., 2008; Gershoni et al., 2009; Wolff et al., 2014). For example, non-neutral mtDNA mutations can be functionally tested in the germ-line (Fan et al., 2008) and, if beneficial, they can be positively selected (Mishmar et al., 2003; Ruiz-Pesini et al., 2004), thus driving changes in nuclear genes and fuelling mito-nuclear co-evolution (Dowling et al., 2008; Lane, 2009, 2011). The mtDNA itself could be an important player in the adaptive evolution of organisms, potentially promoting speciation events (Dowling et al., 2008; Gershoni et al., 2009; Wolff et al., 2014).

In almost all multicellular eukaryotes, mitochondria are transmitted by one parental gamete, usually the maternal one (i.e. strict maternal inheritance - SMI) (Birky, 1995). The panoply of mechanisms ensuring SMI that have evolved independently in organisms is believed to limit heteroplasmy, i.e. the coexistence of different mitochondrial haplotypes in the same nuclear background, which has been shown to cause physiological dysfunction (Sharpley et al., 2012; Zhou et al., 2016). SMI thus prevents potential intergenomic conflicts (Lane, 2012; Radzvilavicius et al., 2017; Sharpley et al., 2012; Zhou et al., 2016). A plausible consequence of SMI, however, is that it puts severe antagonist sex-linked constraints on the evolution of mitochondria, e.g. mutations that are deleterious in males can reach high frequencies if they are advantageous or neutral in females, resulting in an adverse effect on sperm and male fitness (Mother's curse) (Frank & Hurst, 1996; Gemmell et al., 2004; Nakada et al., 2006; Ruiz-Pesini et al., 2000; Yee et al., 2013). Several evidences of paternal mitochondria leakage and consequent heteroplasmy have been reported in animals (Breton & Stewart, 2015), suggesting (i) a sexual conflict over the control of cytoplasmic inheritance (Radzvilavicius et al., 2017), and (ii) a sex-specific advantage associated with a sporadic but persistent paternal mtDNA leakage and segregation into separate somatic tissues (Burgstaller et al., 2014; Radzvilavicius et al., 2017). The most remarkable example pointing toward the adaptive evolution of paternal leakage and heteroplasmy is the enigmatic and unique case of doubly uniparental inheritance (DUI) of mitochondria (Breton et al., 2007; Passamonti &

Ghiselli, 2009; Zouros, 2012).

DUI is specific to some bivalve molluscs (Breton et al., 2007; Passamonti & Ghiselli, 2009; Zouros, 2012) and involves two sex-linked haplotypes (the F and the M-type) that coexist and are transmitted separately through eggs and sperm. Precisely, eggs contain the F-type mtDNA and sperm the M-type mtDNA, and both haplotypes can be extremely divergent, with up to 40% of nucleotide divergence (Breton et al., 2007). Eggs transmit their mitochondria to daughters and sons, and sperm only to sons, and females are usually homoplasmic for the F-type mtDNA whereas males possess the F-type mtDNA in their somatic tissues and the M-type mtDNA in their sperm (Breton et al., 2007; Passamonti & Ghiselli, 2009; Zouros, 2012). Some cases where both haplotypes have been detected in male and sometimes in female somatic tissues have, however, been reported (Breton et al., 2017). The naturally heteroplasmic DUI system represents a unique model to assess the adaptive value of mtDNA variations, and their potential evolutionary implications. It also represents an exclusive model to compare the mitochondrial phenotypes resulting from mtDNA selection for female- and male-related functions (sperm mitochondria in DUI species are not an evolutionary dead-end), and to measure the potential effects of heteroplasmy on somatic tissue bioenergetics.

The aim of the present study was to evaluate, for the first time, the mitochondrial functions associated with sex-linked mtDNAs in the DUI species *Arctica islandica* (Linnaeus, 1767; order Veneroida) and *Mytilus edulis* (Linnaeus, 1758; order Mytiloida), and to compare them with the mitochondrial functions of bivalves with SMI of mitochondria, i.e. *Placopecten magellanicus* (Gmelin, 1791; order Ostreoida) and *Mercenaria mercenaria* (Linnaeus, 1758; order Veneroida). Specifically, we analysed the mitochondrial phenotype of gametes and somatic cells in both female and male individuals of each species through high-resolution respirometry (Gnaiger, 2014), to identify functional divergences in mitochondrial activity and organization associated, in this case, with the divergent evolution of sex-linked mtDNA variants. The results are discussed in the context of the adaptive value of mtDNA, mtDNA preservation and inheritance, evolutionary meaning of the DUI system, ageing and heteroplasmy resistance through functional compensation between mt genomes.

2. Materials and methods

For each species, adult specimens were collected shortly before their spawning period and acclimated for four weeks in a 12 °C saltwater aquarium. Male and female somatic cells and gametes were prepared for respirometric analyses: gills were excised and permeabilized both mechanically and chemically as described elsewhere (Lemieux et al., 2017) and gametes were stripped and permeabilized following the protocol for high-resolution respirometry of permeabilized cells (Pesta & Gnaiger, 2012). Mitochondrial respiration was measured through high-resolution respirometry at 12 °C using an Oxygraph-2K (Oroboros Instruments, Austria) (Gnaiger, 2014), and flux through the electron transport system (ETS) and OXPHOS apparatus was assessed using a substrate-uncoupler-inhibitor titration protocol (figure 2.s1). Citrate synthase (CS) activity was determined through enzymatic assay (Breton et al., 2009) with a Mithras LB940 (Berthold technologies, Germany) and used as a marker of intracellular density of mitochondria. To document divergences in mitochondrial functions and not in aerobic capacity of cells/tissues, data were analysed as flux control ratios (FCRs), with oxygen fluxes normalized for an internal parameter, the maximal uncoupled respiratory rate (Gnaiger, 2014). This approach improves the possibilities of detecting differences dictated by mitochondrial organization that could further be associated to mitochondrial DNA divergences (Gnaiger, 2014). Statistical analyses were done with R software (R Core Team, 2016). Data were analysed in relation to three independent factors: species, sex and cell-type. In each species, differences associated with the factor sex were assessed using a two-tailed Student's *t* test for soma and gametes separately. The main effects of different combinations of two independent factors, as well as their interaction, were determined using a two-way ANOVA, followed by *a posteriori* Tukey's test. Significance was set at $p \leq 0.05$. Results are presented as means + 95% confidence interval bars (CIs). Detailed procedures and protocols are provided as *Supporting information*. An exhaustive list of the acronyms and abbreviations used is provided in table 2.s1. We used the terminology recently proposed by Lemieux et al. (Lemieux et al., 2017) and the MitoEAGLE working group (Gnaiger et al., 2019) which tried to harmonize the terminology on mitochondrial respiratory states and rates for a consistency of nomenclature to facilitate effective transdisciplinary communication.

3. Results and discussion

(a) OXPHOS coupling efficiency and ETS limitation

OXPHOS features in DUI versus SMI gametes are presented in figure 2.1, and in figure 2.s2 for somatic tissues. Figure 2.1*a* shows the OXPHOS coupling efficiency ($j_{\approx P}$), an indicator of both mitochondrial quality and coupling, that is calculated by expressing the respiration in the presence of NADH dehydrogenase (complex I or CI)-linked substrates (i.e. NADH-generating substrates N = pyruvate, malate and glutamate) in the absence of ADP (N_L or leak-state with N substrates combination and no ADP, State 2'), relative to the OXPHOS capacity following ADP addition (OXPHOS-state N_P, State 3). Our results indicate that the quality and the coupling capacity of mitochondria do not vary between eggs and sperm in any species (figure 2.1*a*). Figure 2.1*b,c*, respectively show the stimulatory effect of succinate dehydrogenase (complex II or CII) by its substrate succinate (S) and glycerophosphate dehydrogenase (GpDH) by its substrate glycerophosphate (Gp) on OXPHOS activity. Our results indicate no significant difference between gametes in OXPHOS stimulation with succinate or glycerophosphate, except in *M. edulis*, suggesting that this character may not be specific to DUI species. Given the increase in respiration following Gp addition, our results reveal the importance of the Gp-related metabolic pathway in some marine bivalves, possibly reflecting an energetic metabolism relying on both cytosolic and mitochondrial ATP-production and/or a tight regulation of lipid synthesis by direct control over Gp-content. This reliance on Gp could also have a significant impact on reactive oxygen species (ROS) production and management given that the GpDH complex is an important site for ROS production in the ETS (McDonald et al., 2017).

Figure 2.1*d* shows the apparent excess capacity of the ETS (j_{ExP}), an estimate of how close the maximal coupled respiration ($NPrcSGp_P$; i.e. respiration sustained by CI, CII, proline dehydrogenase (ProDH) and GpDH complexes, State 3) is to the maximal capacity of the system ($NPrcSGp_E$; i.e. with the addition of the uncoupler FCCP, State 3u), expressing the limitation acting on the OXPHOS itself (Gnaiger, 2014). Our results indicate different degrees of limitation, with DUI sperm characterized by a strongly limited OXPHOS relative to their maximum ETS potential, and these values greatly diverge from those of DUI oocytes (figure 2.1*d*). This reflects a strong functional divergence in OXPHOS limitation between gametes of DUI species, not found in SMI species (figures 2.1*d* and 2.s2*d*), highlighting the role of the phosphorylation system (ATP-synthase, adenosine nucleotide translocase and phosphate carrier) in controlling the OXPHOS activity in DUI sperm versus DUI oocytes. This is further confirmed by the quantitative analysis of both NPrcSGp$_P$ and NPrcSGp$_E$, expressed as pmol $O_2 \cdot s^{-1} \cdot$mU CS^{-1} (figures 2.1*e,f*), i.e. using the activity of citrate synthase (CS) as a standardizing factor for both parameters. In accordance with an OXPHOS limitation rather than an increase in the ETS potential, the max coupled respiration is found limited in DUI sperm compared to oocytes, whereas there are no differences between SMI gametes (figure 2.1*e*). Conversely, the difference in the max ETS capacity is not DUI-specific (figure 2.1*f*). The activity of citrate synthase does not differ among gametes in all species (figure 2.1*g*).

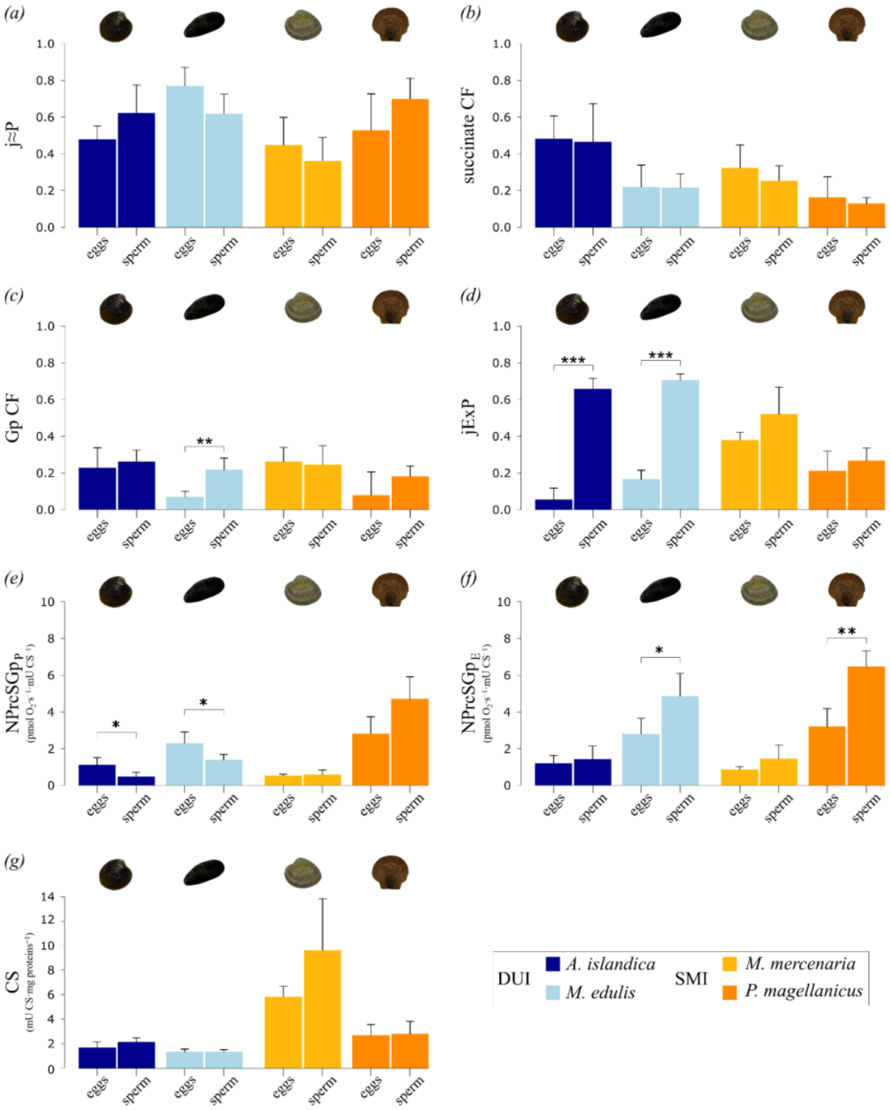


Figure 2.1. Respiratory factors comparison between oocytes and spermatozoa. DUI species: *A. islandica* (*n* = 10, 6), *M. edulis* (*n* = 5, 6). SMI species: *M. mercenaria* (*n* = 5, 6), *P. magellanicus* (*n* = 7, 9). *(a)* OXPHOS coupling efficiency. *(b)* Succinate control factor. *(c)* Glycerophosphate control factor. *(d)* Apparent excess capacity of the ETS. *(e)* Max OXPHOS capacity, coupled respiration sustained by CI-II-ProDH-GpDH complexes. *(f)* Max ETS capacity, uncoupled respiration sustained by CI-II-ProDH-GpDH complexes. *(g)* Citrate synthase activity. Values are presented as means + 95% CIs. Two-tailed Student's *t* test was performed independently for each parameter and each species. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$. Detailed summary is reported in tables 2.s2-2.s3.

(b) Intraspecific analyses

Flux control ratios (FCRs) comparisons between female and male gametes and gills are reported in figures 2.2 and 2.3, respectively. FCRs represent mitochondrial respiratory rates normalized for maximal ETS capacity ($\text{NPrcSGp}_{\text{E}}$) This measure allows the characterization of the relative capacity of the different mitochondrial complexes, which is dictated by mitochondrial properties and not by mitochondrial content or cell size. For both SMI species, the FCRs did not vary between gametes, except for respiration sustained by CI+ProDH+CII (NPrcS_{P}) in *P. magellanicus* (figures 2.2*a,b*). In sharp contrast, both DUI species were characterized by a strong functional divergence in OXPHOS capacity between eggs and sperm (figures 2.2*c,d*). FCRs in oocytes were higher than those in sperm for almost all of the parameters considered. These results are the logical corollary of the higher ETS/OXPHOS ratio observed for sperm in DUI species (figure 2.1*d*). As for gametes, male and female gills in SMI species showed the same OXPHOS organization and capacity (figures 2.3*a,b*). In DUI species, OXPHOS organization and capacity in gills differed according to sex only in *M. edulis*, with gills in males having lower FCRs than in females for respiration sustained by CI (N_{P}), CI-ProDH (NPr_{P}) and CI-ProDH-CII (NPrcS_{P}) (figure 2.3*c*). The divergence between *M. edulis* somatic tissue is reflected by j_{ExP} (figure 2.s2*d*).

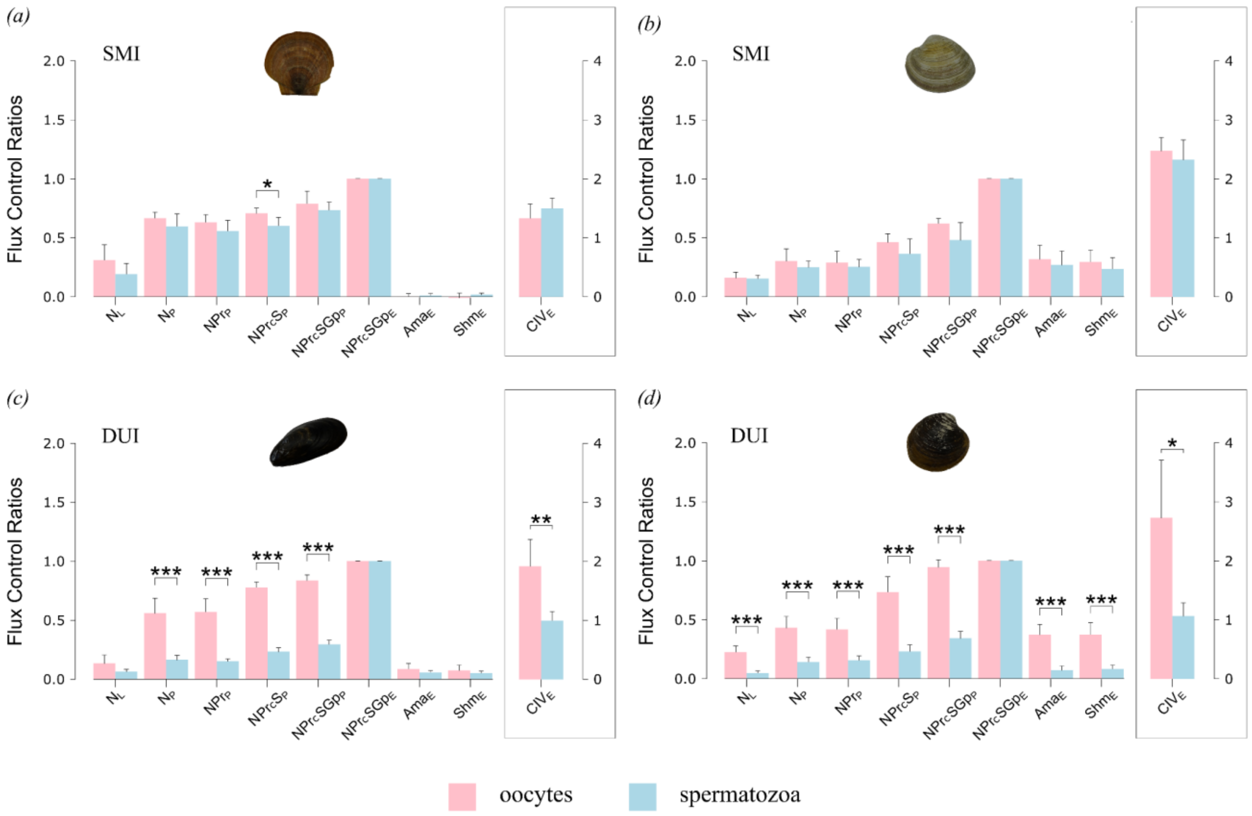


Figure 2.2. Flux control ratios comparison between oocytes and spermatozoa. *(a) P. magellanicus* (*n* = 7, 9). *(b) M. mercenaria* (*n* = 5, 6). *(c) M. edulis* (*n* = 5, 6). *(d) A. islandica* (*n* = 10, 6). Respiratory rates are normalized for the max ETS-capacity (NPrcSGp_{E}). Substrates combinations: N, NADH-generating substrates; c, cytochrome *c*; Pr, proline; S, succinate; Gp, glycerophosphate; Ama, antimycin A addition; Shm, SHAM addition; CIV, CIV activity in presence of ascorbate (As), TMPD (Tm), Ama and c. Respiratory states: $_{L}$, Leak-state; $_{P}$, OXPHOS-state (coupled respiration); $_{E}$, ETS-state (uncoupled respiration). Values are presented as means + 95% CIs. Two-tailed Student's *t* test was performed independently for each parameter and each species. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$. Detailed summary is reported in tables 2.s2-2.s4.

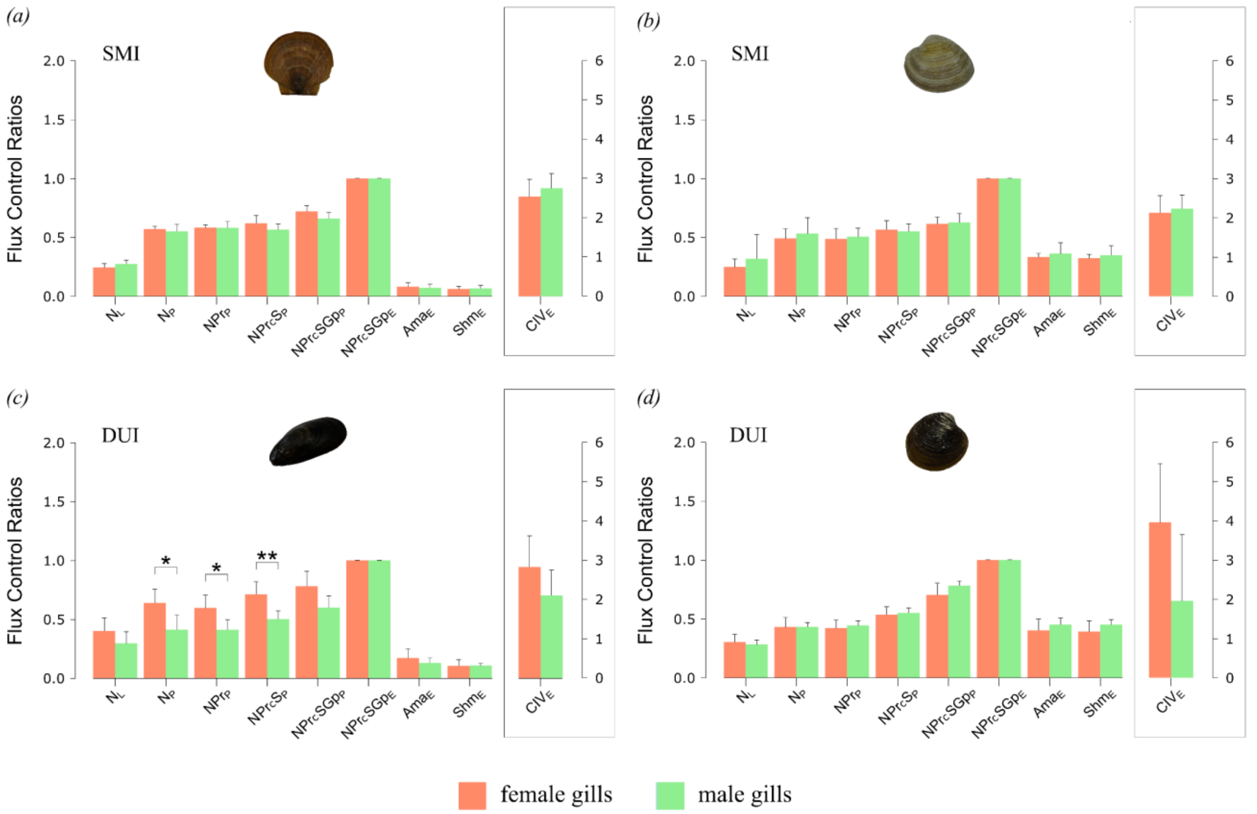


Figure 2.3. Flux control ratios comparison between female and male somatic cells. *(a) P. magellanicus* (*n* = 8, 5). *(b) M. mercenaria* (*n* = 5, 5). *(c) M. edulis* (*n* = 6, 6). *(d) A. islandica* (*n* = 5, 5). Respiratory rates are normalized for the max ETS-capacity (NPrcSGp_E). Values are presented as means + 95% CIs. Two-tailed Student's *t* test was performed independently for each parameter and each species. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$. For abbreviations refer to figure 2.2. Detailed summary is reported in tables 2.s2-2.s4.

The influence of factors sex and cell-type was also assessed on combined gametic and somatic groups (figure 2.s3). SMI species conserved an OXPHOS activity essentially unaffected by sex for both gills and gametes and presented only few differences driven by the cell-type (figures 2.s3*a,b*), potentially reflecting contrasting energetic regulations of gametic versus somatic cells. Again, in sharp contrast, DUI species showed respiratory parameters strongly affected by both factors (interaction effect in figures 2.s3*c,d*), pointing to the combination of maleness and gametes as the main cause of the divergence (see table 2.s4). DUI sperm diverged from both oocytes and gills at the OXPHOS level, and in the case of *M. edulis*, OXPHOS in male gills diverged from female gills, confirming the trend seen in figure 2.3*c*.

Altogether, our results reveal divergences in mitochondrial function between gametes (and partly gills) only in DUI species. As mentioned above, in these species, females are usually homoplasmic, whereas males possess sperm with paternal mitochondria and soma with maternal mitochondria (Breton et al., 2007). That said, some studies have also shown that both parental haplotypes can coexist and be expressed in somatic tissues, mostly in male individuals (Breton et al., 2017). In *M. edulis*, the genetic divergence between the two parental haplotypes reaches 10-22% (Breton et al., 2007; Passamonti & Ghiselli, 2009; Zouros, 2012), whereas it reaches 8% in *A. islandica* (Dégletagne et al., 2016). This divergence is reflected in the two highly different mitochondrial phenotypes observed in DUI species, i.e. one phenotype associated with the F-mtDNA and expressed in oocytes and somatic cells, and one associated with the M-mtDNA in sperm and characterized by lower FCRs, as a result of a strong limitation of the OXPHOS by the phosphorylation system. Lower FCRs, i.e. half-way between the "pure" eggs- and sperm-related phenotypes, were also observed in *Mytilus* male gills, which interestingly tested positive for the presence of M genome (figure 2.s4). In recent years, the vision of selective neutrality of mtDNA has been challenged, and our results add to the growing body of evidence showing that cytoplasmic genetic variation can influence fitness (Blier et al., 2001; Dowling et al., 2008; Mishmar et al., 2003; Ruiz-Pesini et al., 2004; Wolff et al., 2014). They are also in line with the Father's curse hypothesis (Breton et al., 2017), as DUI allows selection to act directly on the M-mtDNA, which can accumulate mutations that are beneficial or neutral in sperm, but potentially harmful when present and expressed in somatic tissues or in eggs. Since the OXPHOS capacity in heteroplasmic *Mytilus* male gills does not digress much from homoplasmic female gills, it is plausible that the amount of M-mtDNA does not reach the threshold required to produce a strong effect in male soma (Stewart & Chinnery, 2015). Additional analyses would be needed to confirm this idea.

(c) Interspecific comparisons: DUI versus SMI species

To question whether there is an interspecific correspondence of gamete-associated mitochondrial phenotypes, each parameter defining the OXPHOS activity was analysed separately within the DUI group (*M. edulis* and *A. islandica*) and the SMI group (*P. magellanicus* and *M. mercenaria*). The effects of factors "sex" and "species" were analysed, and the results are reported in figure 2.4 and table 2.s5. No interaction effect between the two factors was detected; however, DUI and SMI groups were respectively characterized by a strong main effect of sex and species, widespread among the parameters considered. In the DUI group, a main effect of sex was found for N_L, N_P, NPr_P, $NPrcS_P$, $NPrcSGp_P$ and cytochrome *c* oxidase (complex IV or CIV) activity (CIV_E). A main effect of factor "species" was only revealed for $NPrcSGp_P$. Conversely, the SMI group was characterized by a strong effect of factor "species" for N_P, NPr_P, $NPrcS_P$, $NPrcSGp_P$ and CIV_E. Sex only affected $NPrcS_P$. A PCA analysis (figure 2.s5) further confirmed the grouping of both DUI sperm together, in clear divergence from all the remnant mitochondrial phenotypes. In contrast to SMI species, for which the same mitochondrial phenotype is shared between sperm and eggs but differs between species, the two sex-linked DUI phenotypes show no interspecific divergences. This suggests that the "reorganization of mitochondrial respiration" observed in sperm of two distantly-related species (orders Mytiloida and Veneroida) could be an evolutionarily conserved character of DUI or reflect evolutionary convergence of male-specific adaptation.

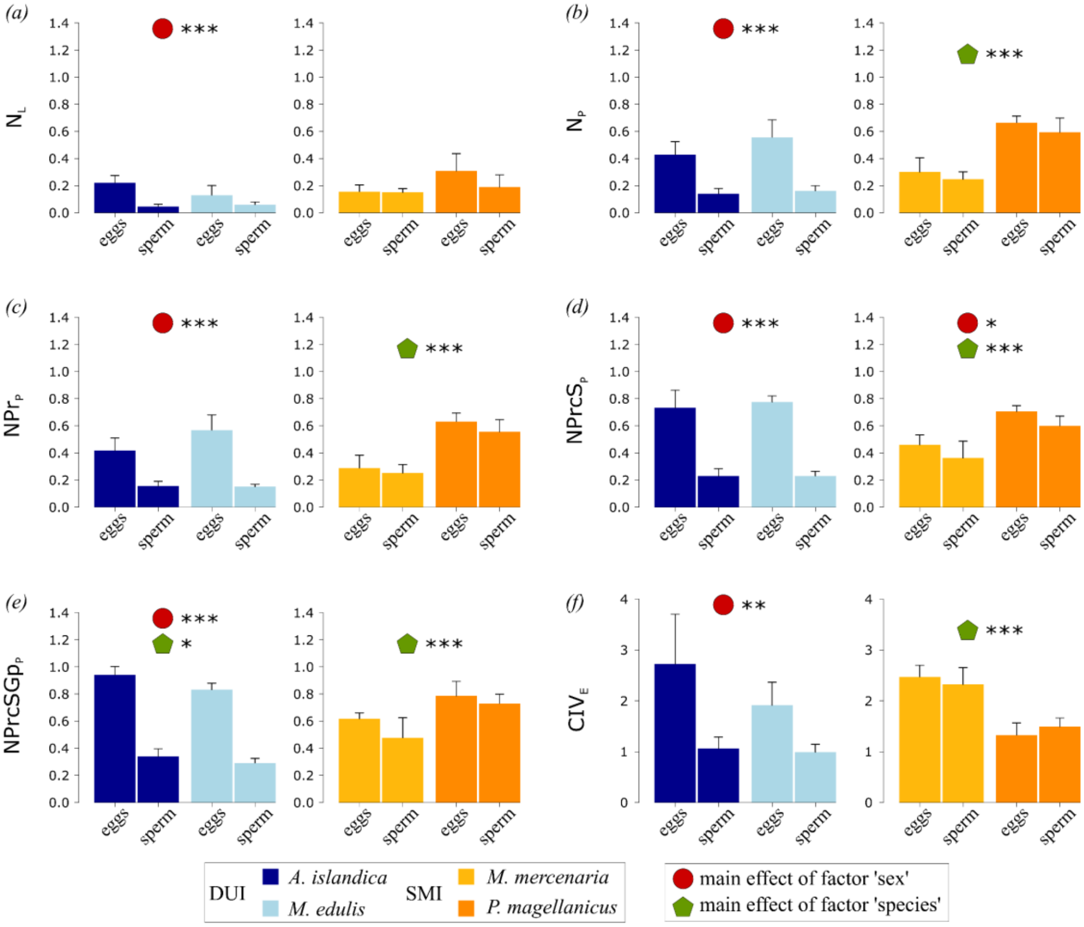


Figure 2.4. Interspecific comparison of gametes FCRs. *(a)* Leak respiration (N substrates and no ADP (D)). *(b)* Coupled respiration (N substrates and D). *(c)* Coupled respiration (NPr substrates). *(d)* Coupled respiration (NPrcS substrates). *(e)* Max coupled respiration (NPrcSGp substrates). *(f)* CIV activity. Values are presented as means + 95% CIs. Two-way ANOVA analysis was run separately for the DUI and the SMI species groups. DUI: *A. islandica* (n = 10, 6), *M. edulis* (n = 5, 6). SMI: *M. mercenaria* (n = 5, 6), *P. magellanicus* (n = 7, 9). Statistical differences are indicated as a circle (effect of "sex") and a pentagon (effect of "species"), with no interaction effect detected. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$. Detailed summary is reported in tables 2.s2-2.s5.

Could these changes in mitochondrial function seen in DUI sperm confer a selective advantage? Two nonexclusive hypotheses have been proposed to explain the retention and function of the M genome in bivalves with DUI: (i) it could increase the fitness of sperm and/or (ii) it could be involved in sex determination (Breton et al., 2007). The strong reorganization of mitochondrial respiration in DUI sperm corroborates the observations that F and M haplotypes are indeed under different selective pressures (Breton et al., 2007) and that natural selection acting directly on sperm may result in a modified mitochondrial metabolism. In previous studies of a particular *M. edulis* population where the F-mtDNA invaded the male route of inheritance, significant differences between sperm bearing F and M mitochondria were detected, with the former swimming faster (Jha et al., 2008) and having a higher CIV activity (Breton et al., 2009). Our results suggest that the reorganization of mitochondrial function in DUI sperm could affect male-specific functions (e.g. spermatogenesis, sperm motility, viability and fertility). According to (Eads et al., 2016), the optimal strategy for sperm in *Mytilus* might be to swim slowly and in tight circles in the absence of egg chemoattractant cues, but swim faster and straighter in their presence. It would be interesting to assess if typical "slower" sperm with the M-type mitochondria rely more on OXPHOS, until chemoattractant cues are detected and possibly cause a switch to a faster glycolytic ATP-production.

Intriguingly, a recent research has found that sperm success in *Mytilus* does not simply depend on which male or sperm is the "best" overall – instead, it depends on which male is the less genetically related, at the nuclear level, and most genetically related, at the F-type mitochondrial level, to the focal female, allowing at the same time for the enhancement of offspring heterozygosity, cytonuclear compatibility and reproductive fitness (Lymbery et al., 2017). However, this study did not look at M-type mtDNA, and whether it could somehow contribute to male success in DUI species remains to be fully resolved. The predominant physiological function of mitochondria is the generation of ATP by OXPHOS, but the mitochondrial reorganization observed in DUI sperm could be related to other aspects than sperm fitness. For example, the M-type genome have been hypothesized to be involved in sex determination in bivalves with DUI (Breton et al., 2011). This hypothesis arises from the sex-specific localization of the paternal mitochondria in embryos, which, together with the presence of sex-specific supernumerary mt genes is proposed to trigger the development of a certain sex (Breton et al., 2014; Breton et al., 2011). Even if the causative or associative relationship between DUI and sex is still an ongoing debate (Breton et al., 2011; Kenchington et al., 2009), in all cases, a mechanism that ensures the preservation and inheritance of sperm mitochondria in males is required. This mechanism could be based on mitochondrial performances. For example, the mitochondrial membrane potential ($\Delta\psi m$) is implicated in the binding of mitochondria to microtubules, thus potentially associated with the transport of healthy mitochondria in the germ-line (Milani, 2015). In *C. elegans*, loss of $\Delta\psi m$ precedes the degradation of paternal mitochondria shortly after fertilization (Zhou et al., 2016). Recent evidence suggests that DUI sperm mitochondria do exhibit a high $\Delta\psi m$ and “actively” segregate in the male germ line precursor blastomere (Milani & Ghiselli, 2015). A $\Delta\psi m$-dependent mechanism has thus been proposed to drive the observed sex-specific differences in mitochondrial transmission in DUI species, by which sperm mitochondria with high $\Delta\psi m$ would be segregated in the male germ-line precursor blastomere, and additional mechanisms would act to allow only germ cells containing spermatozoon-derived mitochondria to differentiate into male gametes (Milani, 2015).

According to our results, DUI sperm are characterized by low respiratory rates, likely as a consequence of the limitation by the phosphorylation system. A limited ATP-synthase activity has been found to result in a high $\Delta\psi m$, a slowed ETS activity with consequent high reducing potential stored in respiratory complexes, and an increased electron leakage and ROS production (Korshunov et al., 1997; Kucharczyk et al., 2009). The reorganization of DUI OXPHOS described here potentially represents an intriguing mechanism, combining energetic adaptation, preservation of paternal mitochondria and sex determination. Future analyses on the abovementioned traits are essential, since they can shed light on the mechanisms by which mitochondria are selected and inherited across generation in metazoans.

(d) Apparent excess capacity of cytochrome *c* oxidase

Figure 2.5 shows an apparent excess capacity of CIV (j_{ExCIV}), which was expressed as the percentage of activity exceeding the max capacity of the ETS. In the two SMI species, the CIV excess capacity reached 33-175% (*P. magellanicus*) and 112-147% (*M. mercenaria*) with no main effect of sex, but a strong effect of cell type only for *P. magellanicus* (figure 2.5). For DUI species, the CIV excess capacity of eggs, sperm, F- and M-gills was respectively 91%, 0%, 183%, 111% in *M. edulis*, and 173%, 6%, 296%, 95% in *A. islandica*, and j_{ExCIV} was strongly influenced by sex with a main effect of cell type also found in *M. edulis*. No interaction was observed between factors "sex" and "cell type" in all species.

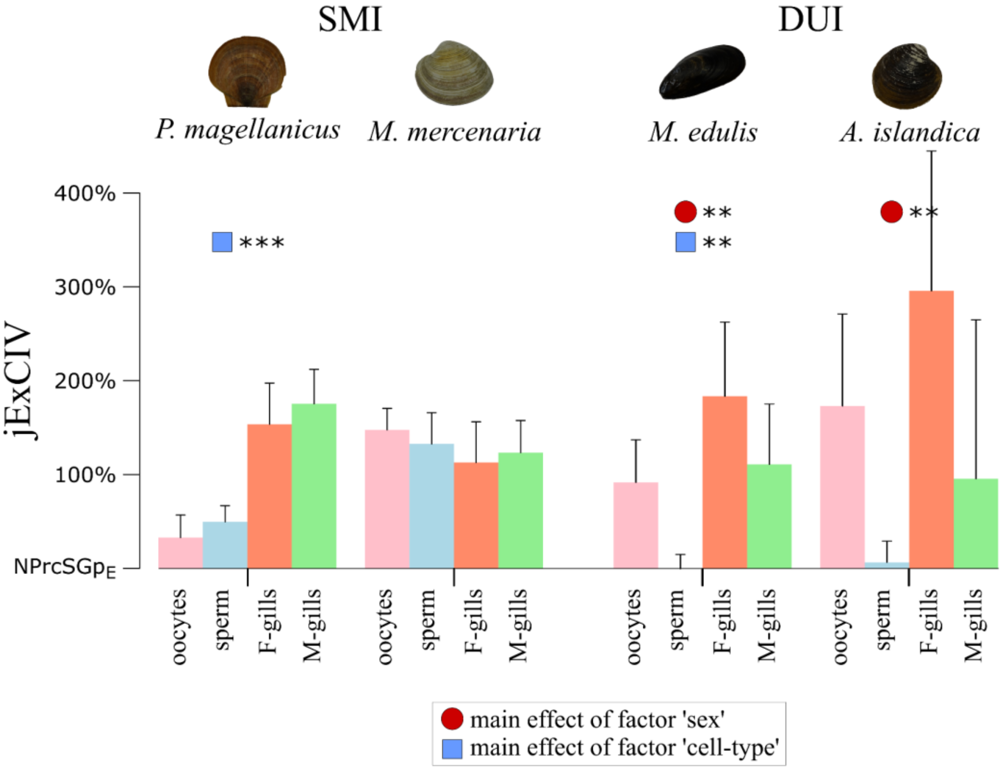


Figure 2.5. Apparent excess capacity of cytochrome *c* oxidase (j_{ExCIV}). j_{ExCIV} indicates the extent by which CIV activity exceeds the max ETS capacity ($NPrcSGp_E$). *P. magellanicus* (*n* = 7, 9, 8, 5); *M. mercenaria* (*n* = 5, 6, 5, 5); *M. edulis* (*n* = 5, 6, 6, 6); *A. islandica* (*n* = 10, 6, 5, 5). Values are presented as means ±+ 95% CIs. Two-way ANOVA analysis was performed independently for each species. Statistical differences are represented as a circle (effect of 'sex') and a square (effect of 'cell-type'), with no interaction effect detected. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$. Detailed summary is reported in tables 2.s2-2.s3.

Overall, our results indicate that contrary to SMI species, both DUI species showed a congruent trend in j_{ExCIV}, characterized by very high values associated with eggs and female soma, intermediate in male soma (in agreement with their heteroplasmic condition), and almost null excess capacity in sperm. An excess capacity of CIV has already been described in animals and is proposed to be functionally adaptive (Lemieux et al., 2017). This excess can enhance oxygen affinity (Gnaiger et al., 1998; Verkhovsky et al., 1996), regulate the redox state (Harrison et al., 2015) and preserve the oxidized state of upstream ETS complexes (Blier et al., 2017). Bivalves are often subject to wide changes in oxygen availability in the intertidal zone or in burrows (Munro et al., 2013), and the upregulation of CIV has been described during conditions where O_2 is scarce (Sussarellu et al., 2013). The maintenance of a high CIV excess capacity in bivalves could improve kinetic trapping of O_2 during hypoxic conditions and decrease the reducing charge stored in the upstream ETS enzymes and the consequent potential burst of ROS production during reoxygenation (Blier et al., 2017). The results presented here point to a radically different CIV threshold phenotype caused by divergent mitochondrial haplotypes in DUI species. The null j_{ExCIV} characterizing M mitochondria entails a tight respiratory control by CIV in DUI sperm, which might also be more sensitive to oxygen content in the medium. The high j_{ExCIV} values associated with DUI female soma and eggs directly links with a low control of respiration exerted by CIV, and with a high biochemical threshold. The control of ETS flux is here proposed to be under strong selective pressures to ensure proper metabolic regulation, at least in DUI species.

A high j_{ExCIV} could also mitigate the deleterious outcomes associated with both mutations accumulation and mtDNA heteroplasmy, given that higher defects in CIV activity could be sustained before impairing OXPHOS (Gnaiger et al., 1998; Mazat et al., 1997). In DUI species, male gills show intermediate CIV activity levels compared to the "pure" F-phenotype (eggs and F-gills) and the M-phenotype (sperm), but their overall respiratory activity does not significantly differ from their respective homoplasmic female counterpart (figure 2.3, figure 2.s3, figure 2.5). As a "functional complementation" between wild and mutant mtDNAs has already been observed (Beziat et al., 1997; Chomyn et al., 1992; Stewart & Chinnery, 2015), we posit that a "standard" respiratory activity in DUI male soma could be guaranteed by the F-mtDNA. The extreme j_{ExCIV} specific to the female phenotype could reflect the ability to sustain a potentially deleterious male one, a possible way by which heteroplasmy is dealt in DUI species.

Finally, the CIV excess capacity could lower ROS production by ensuring a sharp thermodynamic gradient (Blier et al., 2017). An age-associated decline in CIV activity and an increased ROS production is well documented and denotes CIV as a main target of respiratory dysfunction during ageing (Petrosillo et al., 2013; Ren et al., 2010). Both respiratory chain dysfunction and ROS production take part in the "death spiral" of increased oxidative stress that potentially leads to ageing (Balaban et al., 2005). One of our two DUI species, *A. islandica*, is the longest-living non-colonial metazoan recorded so far (maximum lifespan: 507 years) (Blier et al., 2017; Munro et al., 2013). Studies on this marine bivalve point to a lower H_2O_2 production compared to other short-lived species as a key adaptation for its increased lifespan (Munro et al., 2013), and an increment in the components upstream and downstream the principal ROS producing complexes has been proposed to be involved (Blier et al., 2017). The high excess capacity of CIV found in female *A. islandica* gills (figure 2.5; ≈300%) may partly explain the age-resistance of this extremely long-lived animal. Moreover, given that the excess capacity is far higher in females, the question arises whether slower ageing rate could be a F-haplotype related character. Conversely, the null CIV excess capacity specific of DUI sperm mitochondria fosters the need to characterize ROS production in DUI male gametes. In animals with SMI, the "division of labour" hypothesis postulates that sperm maximize energy production for motility by sacrificing mtDNA to OXPHOS and its mutagenic by-products, while oocytes repress OXPHOS (Allen, 1996). A potential overproduction of ROS in DUI sperm is intriguing, knowing that a viable mitochondrial genetic information has to be preserved in males since they also transmit their mtDNA. It is possible that DUI species have evolved specific mechanisms of ROS scavenging and/or mtDNA protection as ROS generation could be the price to pay to ensure high $\Delta\psi m$ and redox status of ETS for mtDNA selection and inheritance.

4. Conclusion

The adaptive value of mtDNA variation is still a conundrum. The metabolic consequences of carrying two divergent haplotypes, and how it affects mito-nuclear coevolution is even more intriguing. The DUI system is emerging as a useful model to test these questions, since this system is naturally heteroplasmic for a female- and a highly divergent male-derived mtDNA.

This study provides the first comparative analysis of mitochondrial OXPHOS activity and organization in gametes and somatic tissues of DUI versus SMI bivalve species. In contrast to SMI species, for which the single maternally-inherited haplotype expresses the same phenotype in eggs, sperm and gills, both DUI species share a reorganization of OXPHOS in sperm mitochondria. Specifically, eggs and female gills, homoplasmic for the F-type mtDNA, express a common "F-phenotype", whereas sperm and their M-type mitochondria express a "M-phenotype", which is characterized by low OXPHOS/ETS rates, a strong limitation by the phosphorylation system, and a high flux control of CIV over the upstream ETS complexes, with an almost null excess capacity of CIV.

The DUI system and its phylogenetic distribution restricted to bivalves is a peculiar phenomenon. In contrast to the possibility that this system could merely represent a tolerable non-lethal form of genetic load, our findings suggest a direct link between different mtDNA haplotypes and phenotypes in DUI species, providing an additional example of the extent by which mtDNA variations can influence mitochondrial bioenergetics. To our knowledge, our data represent the first description of a mitochondrial phenotype resulting from a male-driven evolution of mtDNA. They also potentially represent the first case of a mtDNA specifically adapted for male functions affecting the general OXPHOS activity in heteroplasmic cells. The CIV excess capacity exclusively observed in F-phenotype may provide a way to sustain changes in the ETS performance deriving from (i) the presence of a specialized M-phenotype, and (ii) the accumulations of age-related mutations (e.g. in *A. islandica*, the longest-lived metazoan found so far, the CIV excess capacity is particularly important).

Given that both distantly related DUI species share the same OXPHOS reorganization, we propose a convergent evolution of sex-linked mtDNAs for the DUI system. To further confirm this hypothesis, the analysis should be extended to other DUI species. This intriguing link between OXPHOS reorganization, DUI inheritance mechanism and sex determination definitely deserves further investigations.

Competing interests: We have no competing interests.

Author’s contributions: SBe carried out the lab work, data analysis, designed the experiment and drafted the manuscript; ER participated in both lab work and experiment design; LM conceived and coordinated the study; PUB coordinated and supervised the study; SBr conceived, coordinated and supervised the study. All authors gave final approval for publication.

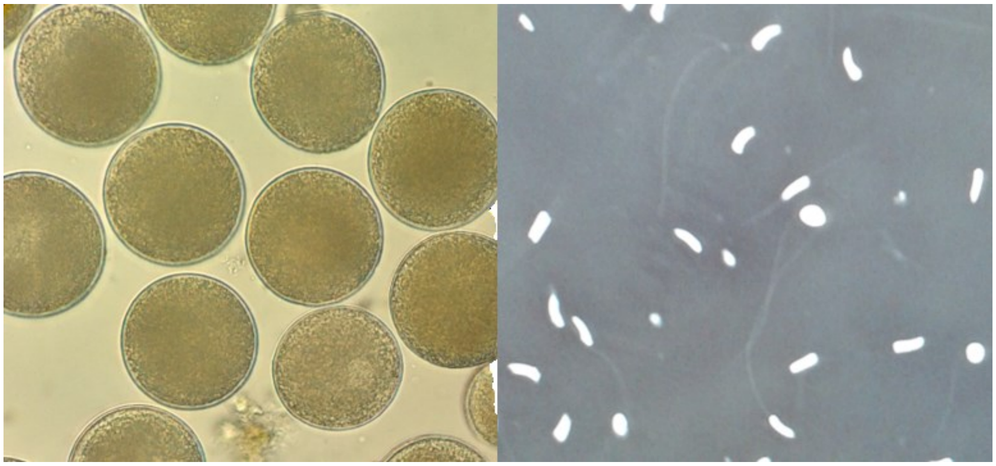
Funding: This work was supported by the Fonds de Recherche du Québec - nature et technologie (to SBr), the *Natural Sciences and Engineering Research Council* of Canada (to PUB), and the Italian Ministry of Education, University and Research MIUR-SIR Programme (to LM).

Acknowledgements: We thank F. Christen and F. Paquin for support and valuable comments.

CHAPTER III – BIOENERGETIC CONSEQUENCES OF SEX-SPECIFIC MITOCHONDRIAL DNA EVOLUTION

Stefano Bettinazzi[a], Liliana Milani[b], Pierre U Blier[c] and Sophie Breton[a]

[a]Département des sciences biologiques, Université de Montréal, Montréal, QC H2V 2S9, Canada; [b]Dipartimento di Scienze Biologiche, Geologiche e Ambientali, Università di Bologna, Bologna 40126, Italia; [c]Département de biologie, Université du Québec à Rimouski, Rimouski, QC G5L 3A1, Canada;



(in prep)

Keywords: mitochondria – DUI – energy metabolism – gametes

1. Introduction

Mitochondria are cellular organelles that play a fundamental role in cell bioenergetics, transducing energy from carburant to ATP through a mechanism known as oxidative phosphorylation (OXPHOS). Even though fundamental for cell bioenergetics, the OXPHOS mechanism also implies a potential cost for mitochondrial, and thus cellular and organismal fitness. OXPHOS is in fact susceptible to generate by-products of redox reaction such as reactive oxygen species (ROS), making mitochondria a major source of oxidative stress within the cell. Even though low concentration of ROS can serve an array of essential biological processes (e.g. immune response, cell signalling, programmed apoptosis, among other functions), an unbalanced ROS production makes mitochondria a potential corroding environment for their own membranes and genome (Dowling & Simmons, 2009; Munro & Treberg, 2017). Although most of the components of the respiratory complexes are encoded by the nuclear genome, part of the genetic information is retained in a short circular genome harboured within mitochondria, the mitochondrial DNA (mtDNA). As such, the mtDNA occupies a potentially hostile compartment in the cell, and the need arise to ensure the preservation and transmission of its genetic information to the future generations. Damage in the mtDNA can lead to mitochondrial dysfunction, compromise cellular fitness and life-history phenotypes, and contribute to the ageing process (Shokolenko et al., 2009; Sun et al., 2016). Since mitochondria and their genome are transmitted uniparentally by the mother in most animals (i.e. strict maternal inheritance, SMI) (Birky, 1995), the absence of recombination makes mtDNA even more vulnerable to the accumulation of harmful mutations throughout generations, a process known as Muller's ratchet (Lynch, 1996; Zhou et al., 2010). However, in spite of the mtDNA having the potential to accumulate deleterious mutations at a high rate, this phenomenon in animals appears to be surprisingly limited (Hill et al., 2014; Tworzydlo et al., 2020).

The germline must be protected from damage to ensure an accurate genetic transmission between generations. Germ cells appear to have significantly superior genome maintenance mechanisms compared to somatic cells (Monaghan & Metcalfe, 2019), and evidence suggests that mechanisms entailing both preservation and selection of healthy mitochondria might take place in the female germ line (Fan et al., 2008; Hill et al., 2014; Stewart et al., 2008). Two possible mechanisms of mitochondrial selection based on mitochondrial functional state have been proposed (Tworzydlo et al., 2020). On the one hand, selection might favour active mitochondria to discriminate the fittest mtDNA. In this context, a high inner membrane potential (Δψm) designates a functional and healthy mitochondrion, and in turn the likely integrity of its genome. Evidence exists in oocytes that highly active mitochondria characterized by high Δψm first cluster together in a transient complex named Balbiani body (Bb) localized near the nucleus (Fan et al., 2008; Hill et al., 2014; Zhou et al., 2010). The mtDNA variants of these selected mitochondria might then undergo enhanced selective replication and eventually disperse throughout the oocyte cytoplasm, ready to be passed to future generations (Hill et al., 2014; Tworzydlo et al., 2020). In parallel, a mechanism entailing both mitochondrial dynamics (fusion and fission events) and mitophagy cooperatively facilitates removal of defective mitochondria from the cell. This mechanism of intracellular mitochondrial quality-control likewise relies on Δψm variation to discriminate damaged organelles. Specifically, depolarized mitochondria appear to have less chance to re-fuse into the mitochondrial network following fission event. After segregation they are preferentially targeted to degradation by the cytosolic mitophagy machinery triggered by a depressed Δψm (Jin et al., 2010; Jin & Youle, 2012; Knorre, 2020; Sekine & Youle, 2018; Twig et al., 2008; Westermann, 2010; Youle & van der Bliek, 2012). On the other hand, selection might favour the transmission of functionally silenced mitochondria, with a high level of genetic and functional integrity. According to the "division of labour" hypothesis (Allen, 1996), the transmission of a viable mtDNA template across generation is ensured by anisogamy and gamete bioenergetic specialization. Small motile sperm, whose propulsion requires a constant supply of ATP, exploit mitochondrial respiration and sacrifice their genome to oxidative stress. Conversely, large immotile oocytes avoid mutational accumulation by repressing mitochondrial OXPHOS (Allen & de Paula, 2013). Strict maternal inheritance of mitochondria assures the transmission of oocyte-derived mitochondria, promoting the genetic integrity of mitochondrial (mt) components across generations. Even though evidences in some animal taxa support the presence of quiescent template mitochondria at least in some phases during oogenesis (de Paula et al., 2013a; de Paula et al., 2013b; Faron et al., 2015; Kogo et al., 2011), this hypothesis seems unlikely to represent the general rule (Ghiselli et al., 2018; Milani, 2015; Milani & Ghiselli, 2015; Monaghan & Metcalfe, 2019). Overall, selection could favour the transmission of either functionally active or silenced oocyte mitochondria, with the mechanism being specific to the animal lineage (Tworzydlo et al., 2020).

Strict maternal inheritance of mitochondria entails a hypothetical trade-off for cellular fitness. For one thing, SMI promotes homoplasmy (i.e. a state in which all mtDNAs are alike in an individual), proper mitonuclear interactions (Lane, 2011, 2012), as well as genetic integrity. For another, it introduces a sex-specific bias in mtDNA evolution. Sperm mitochondria are actively eliminated and that makes them an evolutionary dead end in the SMI system. As such, any evolutionary novelties linked with mtDNA evolution can only directly arise following selection for somatic or female functions. Hypothetically, this sex-specific selective sieve could have a deleterious effect upon sperm fitness and male fertility, as dysfunctional mt variants for sperm can be retained in the population if selected for female functions (i.e. Mother's curse) (Gemmell et al., 2004). Potential support comes from studies linking specific mitochondrial haplotypes with decreased sperm performance and male fertility (Montiel-Sosa et al., 2006; Nakada et al., 2006; Ruiz-Pesini et al., 2000). Nevertheless, exception to the general rule of SMI does exist, the most exceptional one being the doubly uniparental inheritance (DUI) of mitochondria (Breton et al., 2007; Passamonti & Ghiselli, 2009; Zouros, 2012). This peculiar system of mitochondria transmission has been reported in more than a hundred bivalve species (Gusman et al., 2016) and involves the existence in the same species of two sex-linked mitochondrial lineages, the F- and the M-mtDNA. Although both haplotypes sometimes coexist in somatic tissues (i.e. heteroplasmy), each lineage is strictly passed to the future generation by the only oocytes (the F-type) or sperm (the M-type). The association is tight and each mtDNA variant appears so far to constitute the genomic landscape of its respective gamete type (Ghiselli et al., 2010; Venetis et al., 2006). DUI thus represents a unique model to study mitonuclear coevolution among multiple genomes as well as the evolutionary relevance of sex-linked mitochondrial genome variation. DUI also provides an unparallel opportunity to test the assumptions stemming from both the "mother's curse" and the "division of labour" hypotheses. In fact, an important evolutionary novelty of DUI is that it represents a most unique opportunity for animal sperm mitochondria and their genome to dodge the female-specific evolutionary constraints and evolve adaptatively for male functions. Yet, the transmitted paternal mitochondrial genome also faces the unprecedented need to serve as a viable template for future generations.

Given their association with anisogamous gametes, the two sex-linked mtDNAs experience distinct sex-specific selective pressures, evolve separately, and show very high levels of nucleotidic divergence (up to 50%, depending on the considered gene and species) (Bettinazzi et al., 2016; Breton et al., 2007; Capt et al., 2020; Passamonti & Ghiselli, 2009; Zouros, 2012). Recent findings suggested a multiple origin of the DUI system in different bivalve taxa and linked it with episodes of intense selective pressure on specific mt-genes (Milani et al., 2014b; Plazzi & Passamonti, 2019). The highly divergent M-mt genome is functional, shows no sign of genetic decay, undergoes replication, transcription and translation (Breton et al., 2017; Breton et al., 2011; Capt et al., 2019; Ghiselli et al., 2018; Ghiselli et al., 2013; Guerra et al., 2016; Milani & Ghiselli, 2015; Milani et al., 2014a; Milani et al., 2015). It is thus conceivable that functional phenotypic differences might be associated with the two highly divergent DUI mitotypes. Different studies have shown that this is potentially the case, supporting the existence of a robust link between mitochondrial genotype variation and phenotype in DUI species, ranging from sperm performance to mitochondrial functionality. In striking contrast with sperm carrying maternally derived mitochondria (e.g. SMI sperm), selection on DUI sperm of the species *Mytilus edulis* and *Ruditapes philippinarum* appears to favour fitness traits such as lower speed and higher curvilinear trajectory (Bettinazzi et al., 2020; Everett et al., 2004; Jha et al., 2008), potentially increasing sperm endurance, survival and area covered in the open sea (Bettinazzi et al., 2020; Breton et al., 2007; Everett et al., 2004; Fitzpatrick et al., 2012; Jha et al., 2008; Levitan, 2000; Liu et al., 2011; Stewart et al., 2012). At the bioenergetic level, a strict OXPHOS-based mode of cellular bioenergetic fuels DUI sperm motility in absence of oocytes in *M. edulis* and *R. philippinarum* (Bettinazzi et al., 2020), and evidence in *Arctica islandica* and *M. edulis* suggests a strong reorganization of mitochondrial architecture (Bettinazzi et al., 2019b). Compared to female derived mitochondria, M-type sperm mitochondria display a limited OXPHOS activity compared to its maximum capacity set by the electron transport system (ETS), and a tight control by the phosphorylation system and cytochrome *c* oxidase upon the upstream respiratory complexes (Bettinazzi et al., 2019b; Breton et al., 2009). This architecture potentially depicts finely regulated M-type mitochondria, that function at a high reduction state of respiratory complexes and with the ability to preserve a high electrochemical gradient (Bettinazzi et al., 2020; Bettinazzi et al., 2019b; Milani & Ghiselli, 2015).

Although accumulating evidence suggests that the evolution of sex-specific mtDNA variants of DUI species might involve adaptation in mitochondrial functionality and sperm fitness, very little is known about the extent by which the DUI condition could affect the balance between the various catabolic pathways composing the wider gamete bioenergetics. The aim of the present study is to investigate the impact of carrying sex-specific mitochondrial variants upon bivalve gamete bioenergetics. We tested the cellular and mitochondrial metabolic capacity in oocytes and sperm of five species: *M. edulis* (Order Mytilida) and *R. philippinarum* (Order Venerida), DUI species bearing the female- and male-derived mitochondria in their oocytes and sperm, respectively; *Mercenaria mercenaria* (Order Venerida), *Mya arenaria* (Order Myida), and *Placopecten magellanicus* (Order Pectinida), SMI species whose gametes bear the solely female-derived mitochondria. We specifically evaluated the activities of key enzymes involved in different metabolic pathways, including pyruvate kinase (glycolytic pathway), lactate dehydrogenase (fermentation), carnitine palmitoyl transferase (fatty acid metabolism), citrate synthase and malate dehydrogenase (tricarboxylic acid cycle), NADH-dehydrogenase, coenzyme Q: cytochrome *c* oxidoreductase and cytochrome *c* oxidase (mitochondrial respiratory complex I, complex III and complex IV, respectively), as well as catalase (antioxidant defence). Assumed that variations in the mt-encoded components are likely to affect the functioning of respiratory complexes, a rational indication is that the evolution of M-mitochondria in DUI species would imply change in sperm bioenergetics, with a potential downstream impact on sperm performance, reproductive success and likely preservation of genomic integrity. The results stemming from this research represent the first in-depth characterization of DUI and SMI gamete bioenergetics. We provide clear evidence that a widespread reorganization of the energy metabolism characterized gametes of DUI species, supporting an evolutionary link between the retention of paternally derived mtDNA variants and male-specific energetic adaptation.

2. Materials and Methods

(a) Experimental animals. Adult bivalve specimens were obtained from culture farms or fish markets during their spawning period between June and September 2019. Prior to analysis, individuals were acclimated for four weeks in a 12°C recirculating seawater aquarium and fed *ad libitum* with a mix of microalgae. A total of five different species were tested: the DUI species *M. edulis* (Linnaeus, 1758) from Kensington (Prince Edward Island, Canada) and *R. philippinarum* (Adams & Reeve, 1850) from Vancouver (British Columbia, Canada), and the SMI species *M. mercenaria* (Linnaeus, 1758) and *M. arenaria* (Linnaeus, 1758) from Barnstable (MA, USA), and *P. magellanicus* (Gmelin, 1791) from the Gulf of Maine (MA, USA). To avoid potential taxon-driven bias in the results we selected distantly related species, i.e. with a last common ancestor dated ~510 Mya (mid-Cambrian). Also, the two DUI species investigated potentially represent independent origins of the DUI system (Plazzi & Passamonti, 2019; Plazzi et al., 2016). The sequence divergence between the DUI F- and M-lineages varies between 10-22% in *M. edulis* and 16-32% in *R. philippinarum* ((Bettinazzi et al., 2020; Bettinazzi et al., 2019b; Zouros, 2012) and reference therein).

(b) Gametes collection and preparation. Individual gonads were excised on ice and placed in a petri dish containing 2 ml of artificial sea water. Following sex and maturity determination through microscopic inspection of gonadal smears, gametes were stripped by performing incisions in the gonads. Mature sperm were let to actively swim out for 5 min, whereas oocytes were gently squeezed out of the gonad (Bettinazzi et al., 2020). Gamete samples were homogenized with a Polytron PT 1200 homogenizer (Polytron, Kinematica) in 3 x 15 s cycles separated by 30 s of resting on ice and then stored at −80°C prior to analysis of enzymatic activity.

(c) Quantification of enzymatic activity. Enzymatic activities were assessed at 25°C using a Mithras LB940 microplate reader (Berthold technologies, Germany) and data were analysed with the MikroWin 2010 software (Labsis Laborsysteme, Germany). All chemicals were purchased from Sigma-Aldrich (Oakville, Ontario, Canada). Enzymatic assays were performed in the following conditions:

Pyruvate kinase (PK) (EC 2.7.1.40): activity was determined in 50 mM imidazole-HCl buffer pH 7.4, 10 mM $MgCl_2$, 100 mM KCl, 5 mM ADP, 0.15 mM NADH, 5 mM phosphoenolpyruvate, 0.6 $U{\cdot}ml^{-1}$ LDH, following the oxidation of NADH at 340 nm ($\varepsilon = 6.22\ ml{\cdot}cm^{-1}{\cdot}\mu mol^{-1}$) for 4 min (Pelletier et al., 1994).

Lactate dehydrogenase (LDH) (EC 1.1.1.27): activity was measured in a reaction medium composed of 100 mM potassium phosphate buffer pH 7, 0.16 mM NADH, 0.4 mM pyruvate (omitted from the blank), 0.03 % triton X 100, recording the oxidation of NADH at 340 nm ($\varepsilon = 6.22\ ml{\cdot}cm^{-1}{\cdot}\mu mol^{-1}$) for 4 minutes (Thibault et al., 1997).

Carnitine palmitoyl transferase (CPT) (EC 2.3.1.21): capacity was assessed in 75 mM Tris-HCl buffer plus 5 mM EDTA pH 7, complemented with 0.25 mM DTNB, 0.035 mM palmitoyl CoA, 2 mM L-carnitine (omitted from the blank), following the reduction of DTNB at 405 nm ($\varepsilon = 13.6\ ml{\cdot}cm^{-1}{\cdot}\mu mol^{-1}$) for 4 minutes (Thibault et al., 1997).

Citrate synthase (CS) (EC 2.3.3.1): catalytic capacity was measured in a 100 mM imidazole-HCl buffer pH 8, containing 0.1 mM DTNB, 0.1 mM acetyl-CoA, 0.15 oxaloacetate (omitted from the blank), tracking the reduction of DTNB at 405 nm ($\varepsilon = 13.6$ ml·cm^{-1}·µmol^{-1}) for 4 minutes (Thibault et al., 1997).

Malate dehydrogenase (MDH) (EC 1.1.1.37): activity was determined in 100 mM potassium phosphate buffer pH 7.5 supplemented with 0.2 mM NADH and 0.5 mM oxaloacetate, following the oxidation of NADH at 340 nm ($\varepsilon = 6.22$ ml·cm^{-1}·µmol^{-1}) for 4 minutes (Bergmeyer, 1983).

Mitochondrial complex I + III (ETS) (EC 7.1.1.2 and 7.1.1.8): activity was measured in a reaction medium containing 100 mM imidazole-HCl buffer pH 8, 2 mM INT, 0.85 NADH, 0.03% (v/v) triton X 100, following the reduction of INT at 490 nm ($\varepsilon = 15.9$ ml·cm^{-1}·µmol^{-1}) for 6 minutes (Bergmeyer, 1983).

Cytochrome *c* oxidase (CCO) (EC 7.1.1.9): activity was assessed in 100 mM potassium phosphate buffer pH 8.0, 0.05% (v/v) tween-20, 0.03% (v/v) triton X 100, 1 mM ADP and 0.05 cytochrome *c*. Cytochrome *c* was reduced with the addition of 4.5 mM dithionite and the activity measured following the oxidation of cytochrome *c* at 550 nm ($\varepsilon = 19.1$ ml·cm^{-1}·µmol^{-1}) for 4 minutes. The specificity of the reaction was tested in presence of 0.33% (w/v) potassium ferricyanide (Thibault et al., 1997).

Catalase (CAT) (EC 1.11.1.6): catalytic capacity was quantified in 100 mM potassium phosphate buffer pH 7.5 complemented with 0.1% (v/v) triton X 100 and 60 mM H_2O_2, following the disappearance of H_2O_2 at 240 nm ($\varepsilon = 43.6$ ml·cm^{-1}·µmol^{-1}) for 1 min (Orr & Sohal, 1992).

Enzymatic activities were expressed as mU·mg^{-1} proteins, with U representing 1 µmol of substrate transformed to product per minute. Protein content (mg·ml^{-1}) was determined at 560 nm using the bicinchoninic acid method (Sigma BCA1-1 KT) with bovine serum albumin (BSA) as standard.

(d) Data analysis. Enzymatic activities (mU·mg^{-1} proteins) were measured for: n = 10, 10 *M. edulis* (oocytes and sperm respectively); n = 10, 10 *R. philippinarum*; n = 10, 6 *M. mercenaria*; n = 10, 10 *M. arenaria*; and n = 8, 8 *P. magellanicus*. To document qualitative difference in the equilibrium between bioenergetic pathways, the catalytic capacity of each enzyme was normalized for an internal parameter, and thus expressed as activity ratio. The normalization was either done for the activity of citrate synthase ('CS' in subscript, mU·mU CS^{-1}) or for the activity of cytochrome *c* oxidase ('CCO' in subscript, mU·mU CCO^{-1}). Furthermore, gamete energy metabolism of each species was resumed in a principal component analyses, which combined the enzymatic activity ratios normalized for CS (PCA_{CS}) (electronic supplementary material, figure 3.s1 and table 3.s1). The first principal component (PC1) accounted for 58.3% of the variability of the original parameters and provides a proxy of the general mitochondrial metabolism, as the parameters MDH, ETS and CCO heavily load on it, followed by PK and CPT. The second principal component (PC2) accounted for 18.3% of the total variability and mostly reflects the antioxidant capacity of gametes, as the parameter CAT mostly contributes to it, followed by the LDH representing the anaerobic metabolism. A detailed data summary is provided in the electronic supplementary table 3.s2. The software R was used for data and statistical analysis (R Core Team, 2016). The normality and homoscedasticity of data were verified using Shapiro and Levene's tests, respectively. When required, data were log transformed. The factors considered were: 'gametes' (two levels), 'species' (five levels) and 'inheritance' (two levels). Depending on the specific analysis, single or multiple factors were accounted. At the interspecific level, enzymatic activities were implemented in a linear mixed model which considered 'gametes' and 'inheritance' as fixed effect and controlled for the variability across species. The significance of the fixed effects and their possible interaction were determined through a Type III ANOVA, followed by a *post hoc* multi comparison with Holm adjustment. Intraspecific differences among gametes were determined separately for each enzyme activity using either two-tailed, Welch-Satterthwaite or permutational *t*-test. For all the analyses performed, statistical significance was set at $p \leq 0.05$. Results are graphically represented as means ± standard error of the mean (s.e.m.).

3. Results

The interspecific comparison of gamete enzymatic activities normalized for citrate synthase capacity ($mU \cdot mU\ CS^{-1}$) are reported in figure 3.1, supplementary figure 3.s2 and tables 3.s3-s4. Specifically, the interaction effect between gamete type (factor 'gametes' with two levels: eggs and sperm) and mitochondrial inheritance system (factor 'inheritance' with two levels: SMI and DUI) has been investigated for each enzyme separately. Results revealed a widespread interaction effect for all the enzymes examined (figure 3.1), indicating that the type of gamete and the inheritance system jointly influence the catalytic capacity of these enzymes. As revealed by the *post hoc* analysis, a substantial difference exists among the two groups of bivalves examined (figure 3.1, supplementary table 3.s3). In the three SMI species considered, sperm enzymatic activities were higher or equal, when expressed by CS activity, than the ones of eggs. In sharp contrast, a substantial reorganization of the energetic phenotype characterized the gametes of both distantly related DUI species, with lower enzymatic activity ratios in sperm compared to oocyte. This trend of "sperm energetic depression" was found to be widespread to all the different bioenergetic pathways analysed for DUI species (figure 3.1, supplementary figure 3.s2 and tables 3.s3-s4). Specifically, the trend was observed in the relative capacity of various enzymes with respect to CS, at the level of (i) glycolysis, measured as the activity of pyruvate kinase (figure 3.1*a*), (ii) anaerobic glycolysis, through the capacity of lactate dehydrogenase (figure 3.1*b*), (iii) fatty acid metabolism, through the enzyme carnitine palmitoyl transferase (figure 3.1*c*), (iv) tricarboxylic acid cycle, as the activity of malate dehydrogenase (figure 3.1*d*), and (v) electron transport and oxygen reduction, at the level of the respiratory complexes I + III and IV (figures 3.1*e,f*). Only the activity of the enzyme catalase (reflecting the antioxidant system capacity) relative to CS was higher in oocyte than in sperm in both DUI and SMI species (figure 3.1*g*).

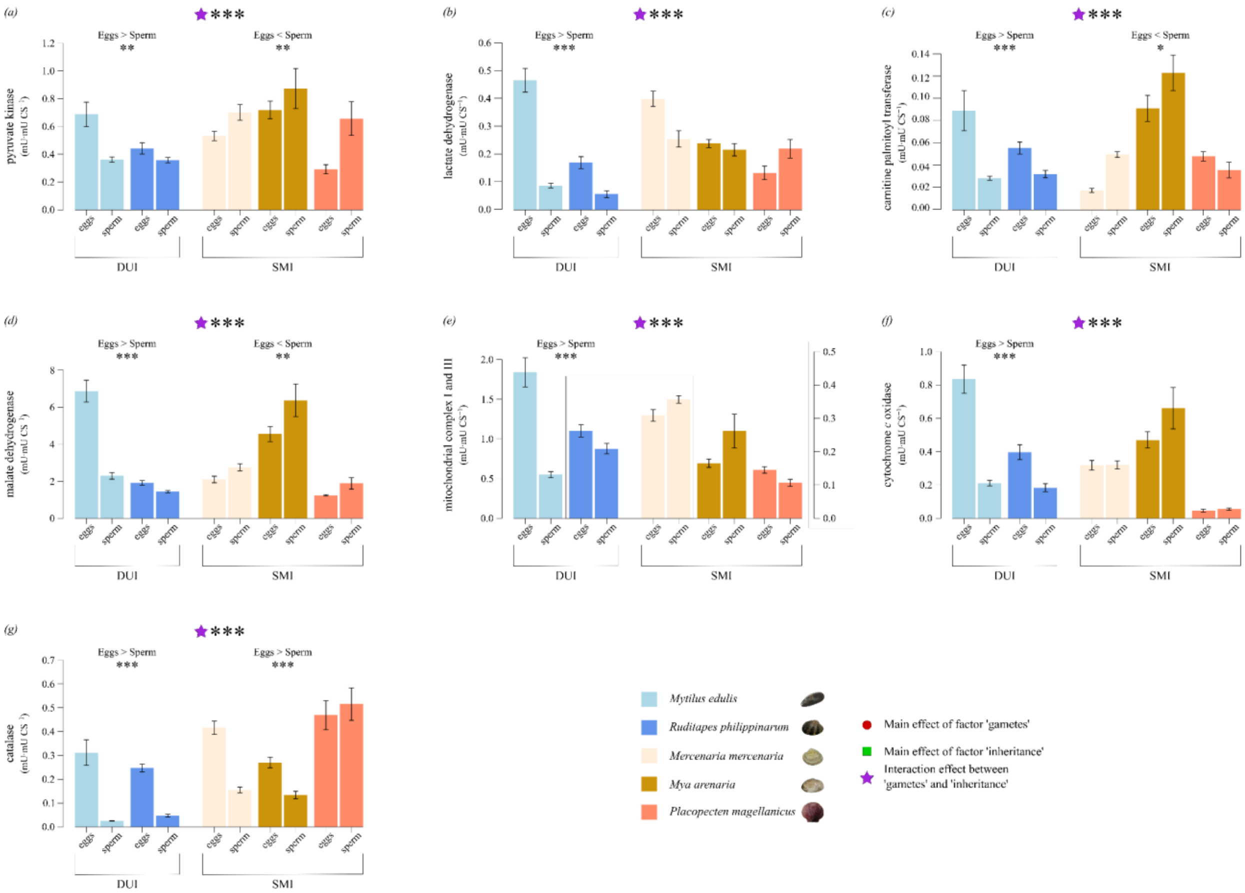


Figure 3.1. Interaction effect between gamete type (eggs, sperm) and mitochondrial inheritance system (SMI and DUI) on enzymatic activities normalized for citrate synthase capacity (mU·mU CS^{-1}). *(a)* Pyruvate kinase activity ratio. *(b)* Lactate dehydrogenase activity ratio. *(c)* Carnitine palmitoyl transferase activity ratio. *(d)* Malate dehydrogenase activity ratio. *(e)* Mitochondrial complex I and III activity ratio. *(f)* Cytochrome *c* oxidase activity ratio. *(g)* Catalase activity ratio. Data are presented as means ± s.e.m. The main effect of the two fixed factors 'gametes' and 'inheritance' are indicated with a circle and square respectively. Interaction effect is indicated with a star. $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$. DUI species: *M. edulis* (*n* = 10, 10), *R. philippinarum* (*n* = 10, 10). SMI species: *M. mercenaria* (*n* = 10, 6), *M. arenaria* (*n* = 10, 10), *P. magellanicus* (*n* = 8, 8). The parameters in boxes refers to the right ladder. Detailed summary is reported in electronic supplementary material, tables 3.s2 and 3.s3.

The divergence observed in DUI sperm bioenergetics was also reflected in the analysis of the principal components (figure 3.2, supplementary tables 3.s2-s3), either graphically (figure 3.2*a*), as DUI sperm clustered together and diverged from the other gametes, or by analysing each component alone (figure 3.2*b,c*). An interaction effect between the type of gamete and the inheritance mechanism was found for PC1, indicating that the difference between gametes is dependent on the transmission mechanism, i.e. that DUI-eggs differ from DUI sperm, whereas no difference was revealed for SMI gametes (figure 3.2*b,* supplementary table 3.s3). For PC2, only a main effect of gamete type was revealed, indicating that the existing difference among gametes is shared between SMI and DUI species (figure 3.2*c,* supplementary table 3.s3). Overall, the results of the principal component analysis reinforce the trend seen in figure 3.1 and figure 3.s2, i.e. the difference in gamete bioenergetics between SMI and DUI species is supported by PC1, which mostly reflects the gamete mitochondrial metabolism (MDH, ETS and CCO heavily load on it, partly reflecting the TCA and OXPHOS machinery) (supplementary figure 3.s1 and table 3.s1), whereas PC2, which mostly represents the antioxidant capacity (CAT heavily load on it) (supplementary figure 3.s1 and table 3.s1), corroborates what was already observed in figure 3.1*g*, i.e. that bivalve oocytes have higher antioxidant capacity than sperm, regardless of the species tested (DUI or SMI). In addition to be qualitatively evident when comparing the relative enzymatic activities over CS, the different trend in gamete bioenergetics between SMI and DUI species was also partially perceived quantitatively through the analysis of enzymatic activities normalized for protein content (electronic supplementary figure 3.s3, and table 3.s5). With the exception of PK and CS enzymes (figures 3.s3*a*,*d*), whose activities are higher in sperm than eggs in both DUI and SMI species, all the remnant enzymes showed a decreased activity in DUI sperm with respect to eggs when comparing with the activity balance of SMI gametes. Specifically, a diminished activity is reflected at the level of LDH and CAT (figures 3.s3*b*,*h*), where DUI sperm show a lower capacity compared to eggs, while SMI sperm and eggs showed no differences in activity, as well as at the level of CPT, MDH, ETS and CCO (figures 3.s3*c*,*e*,*f*,*g*), where DUI sperm activity matched the one of eggs, while SMI sperm had a higher capacity than eggs (table 3.s5).

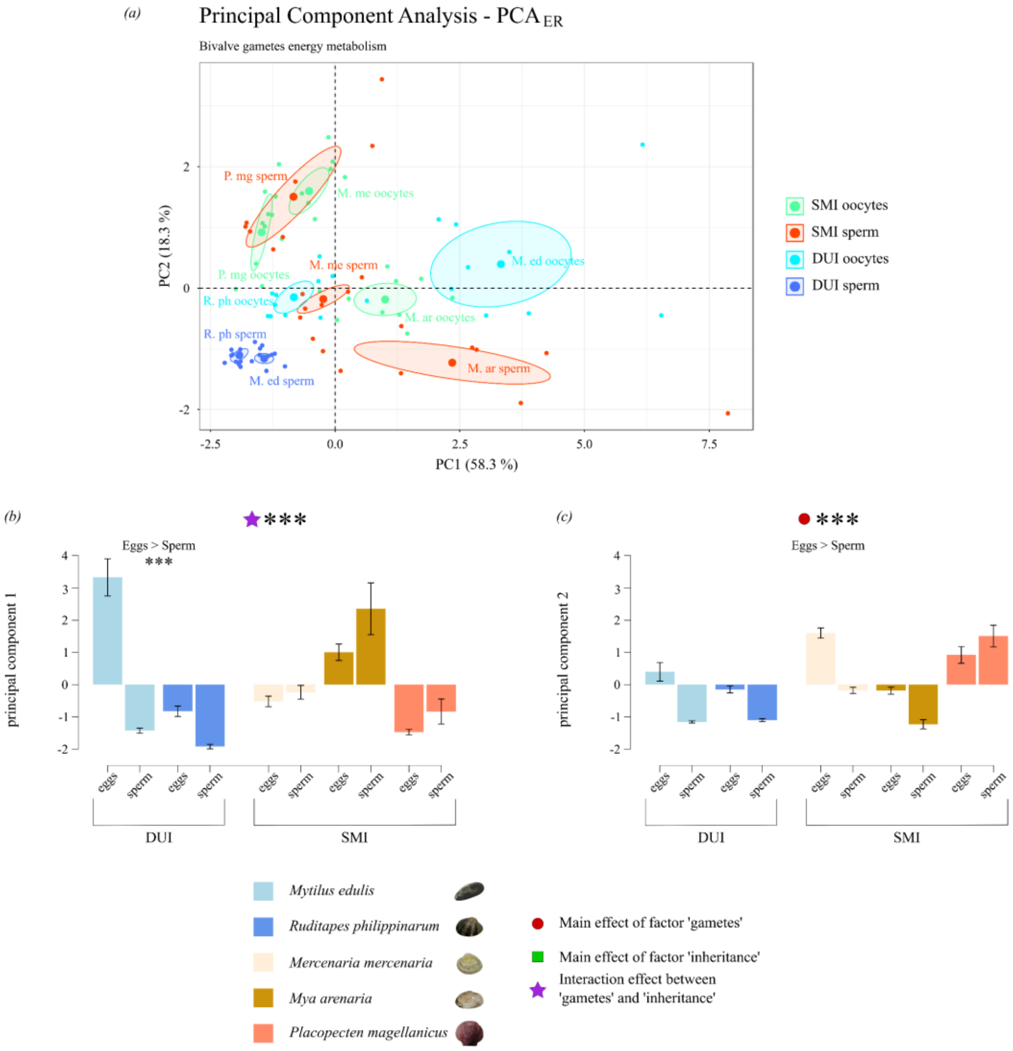


Figure 3.2. Principal component analysis (PCA$_{CS}$) based on the enzymatic activity ratios (mU·mU CS^{-1}) reported in figure 3.1, representing a proxy of the energy metabolism of bivalve species gametes. *(a)* PCA scatter plot with 95% confidence interval ellipses. Colours refer to different combinations of gamete type (oocytes, sperm) and inheritance mechanism (DUI and SMI). *(b)* First principal component of the PCA$_{CS}$. *(c)* Second principal component of the PCA$_{CS}$. Data are presented as means ± s.e.m. The main effect of the two fixed factors 'gametes' and 'inheritance' are indicated with a circle and square respectively. Interaction effect is indicated with a star. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$.

DUI species: *M. edulis* (M. ed, *n* = 10, 10), *R. philippinarum* (R. ph, *n* = 10, 10). SMI species: *M. mercenaria* (M. me, *n* = 10, 6), *M. arenaria* (M. ar, *n* = 10, 10), *P. magellanicus* (P. mg, *n* = 8, 8). Detailed summary is reported in electronic supplementary material, tables 3.s2 and 3.s3.

Differences between SMI and DUI gametes also exist in the pattern of regulation of metabolic pathways relative to mitochondrial respiration. Specifically, differences exist in the balance between the capacity of key enzymes of metabolite entrance in OXPHOS with the downstream maximal oxidative capacity of the electron transport chain (i.e. the activity of CCO) (figure 3.3, supplementary table 3.s6). In the three SMI species, both gamete types share the same metabolic regulation of the various energetic pathways in relation with the capacity of CCO, i.e. no difference exists between oocytes and sperm in the relative capacity of pyruvate kinase, citrate synthase, malate dehydrogenase, complex I + III with respect of the activity of cytochrome *c* oxidase (figure 3.3*a,b,c*). Similar results were also observed for the activity of LDH and CPT over CCO in *M. arenaria* and *P. magellanicus*, but not in *M. mercenaria*, and for CAT in *P. magellanicus*. Again, in striking contrast with the conserved balance among energy pathways in SMI gametes, DUI gametes showed a completely different trend. Specifically, DUI sperm are characterized by an excess capacity of the enzymes PK and CS with respect to the capacity of CCO in both *M. edulis* and *R. philippinarum*, as well as MDH and ETS in *R. philippinarum* (figure 3.3*d,e*), even though the trend is not significant for MDH and ETS in *M. edulis*. This DUI sperm-specific reorganization is consistent with the existence of a sperm-specific bottleneck in the catalytic efficiency of cytochrome *c* oxidase with respect to the upstream energetic pathways.

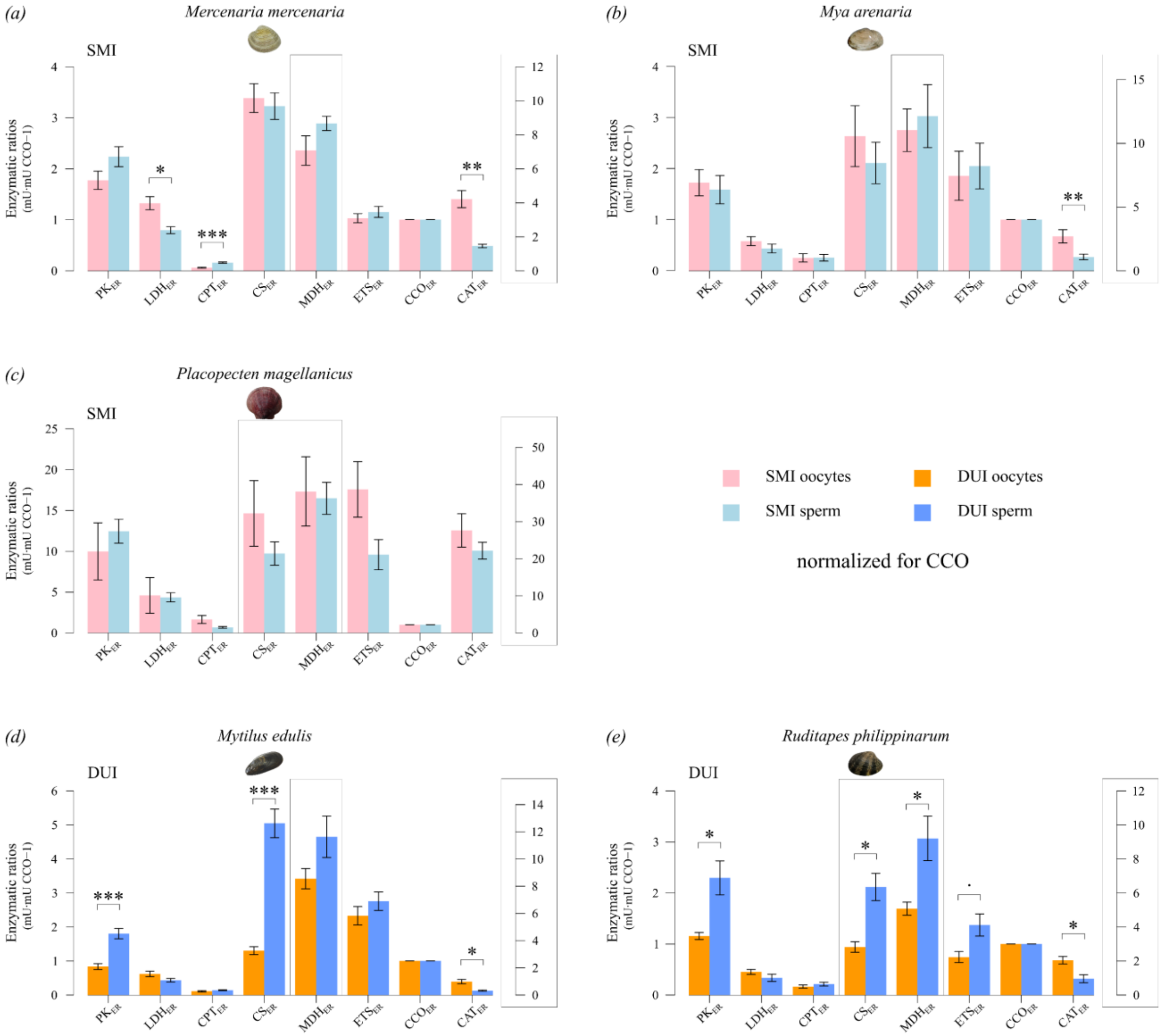


Figure 3.3. Intraspecific comparison between eggs and sperm enzymatic activities normalized for the capacity of cytochrome *c* oxidase (mU·mU CCO^{-1}). *(a) M. mercenaria* (*n* = 10, 6). *(b) M. arenaria* (*n* = 10, 10). *(c) P. magellanicus* (*n* = 8, 8). *(d) M. edulis* (*n* = 10, 10). *(e) R. philippinarum* (*n* = 10, 10). Enzymes: PK, pyruvate kinase; LDH, lactate dehydrogenase; CPT, carnitine palmitoyl transferase; CS, citrate synthase, MDH, malate dehydrogenase; ETS, electron transport chain; CCO, cytochrome *c* oxidase; CAT, catalase. Data are presented as means ± s.e.m. Two-tailed Student's *t* test was performed independently for each parameter and each species. ·$0.05 < p \leq 0.09$, *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$. *p*-values corrected with Holm adjustment for multiple testing. The parameters in boxes refer to the right ladder. Detailed summary is reported in electronic supplementary material, tables 3.s2 and 3.s6.

4. Discussion

Being the only exception to the universal rule of strict maternal inheritance of mitochondria in animals, the DUI system in bivalves provides a unique opportunity to study the extent by which carrying sex-specific mitochondrial variants could impact general bioenergetic specificity of different anisogamous gametes, and then establish if gamete bioenergetics might be associated to mitochondrial selection and preservation.

(a) In SMI species, gametes (bearing maternally inherited mitochondria) share a common catabolic organization and are characterized by sperm having higher enzymatic capacity than oocytes

Our findings on SMI gametes bioenergetics highlight a higher metabolic activity relative to both CS activity and protein content, in sperm than in eggs for most of the enzymes analysed (figures 3.1, 3.2, supplementary figures 3.s2, 3.s3 and tables 3.s3, 3.s4), potentially mirroring the different energetic needs linked with gamete specialization. Moreover, the analysis of catalase activity points over oocytes having a higher antioxidant capacity over CS activity than sperm (figure 3.1*g*). This result advocates for an improved capacity of oocytes to control ROS, potentially minimizing oxidative stress and any sort of related damage. To some extent, these contrasting bioenergetic phenotypes between sperm and eggs in SMI bivalve species support the "division of labour" hypothesis between anisogamous gametes bearing maternally inherited mitochondria, i.e. selection for mitochondrial quality and integrity will favour the transmission of oocyte organelles that are functionally silenced (Allen, 1996). Indeed, while the higher energy metabolism of SMI sperm might reflect their need to maximize their performance and fertilization success, which is in accordance with previous studies suggesting that ATP-dispendious motility traits such as high speed and straighter trajectory are indeed exploited by sperm of SMI bivalves (Bettinazzi et al., 2020), the maintenance of a lower metabolism together with an improved antioxidant capacity might reflect the advantage for SMI oocytes and their mitochondria (at least some of them) to preserve genetic integrity (Allen & de Paula, 2013; de Paula et al., 2013a; de Paula et al., 2013b). Evidence of oocyte mitochondria quiescence has been reported in both invertebrates and vertebrates, comprehending the jellyfish *Aurelia aurita*, the earthworm *Dendrobena veneta*, the fruitfly *Drosophila melanogaster*, the zebrafish *Danio rerio* and the frog *Xenopus laevis* (de Paula et al., 2013a; de Paula et al., 2013b; Faron et al., 2015; Kogo et al., 2011). However, it is important to note that bivalve oocytes are not completely quiescent. Even though the activity of key enzymes in the energy metabolism (normalized for both CS and protein content) in oocyte is generally lower compared to sperm, oocytes are bioenergetically active and do perform OXPHOS. This was also observed in a previous research involving the real time measurement of mitochondrial activity (Bettinazzi et al., 2019b). Mitochondrial activity in bivalve oocytes appears to vary with the gametogenic stage, with immature oocytes having less mitochondria with a lower Δψm compared to mature oocytes (Milani & Ghiselli, 2015), and studies on different taxa revealed that an increased in mitochondrial activity is essential for oocyte maturation, fertilization success and embryo development (Ge et al., 2012; Milani & Ghiselli, 2015; Ramalho-Santos et al., 2009; Van Blerkom, 2011). In light of this, it is possible that the lower (but not null) enzymatic activity in SMI oocytes compared to SMI sperm (relative to both CS and protein content) could either reflect the presence of oocytes in different maturation state (thus characterized by variable mitochondrial activity), or the presence of different subpopulations of mitochondria, one active (with energetic functions) and one bioenergetically dormant (with template function) (Allen & de Paula, 2013). Additional researches, such as the characterization of oocyte mitochondrial membrane potential through fluorescent methods, are necessary to confirm this.

The evolution of sperm mitochondrial bioenergetics in species with strict maternal inheritance of mitochondria is dictated by female-specific selective constraints in the evolution of mt components. Even though a difference in the magnitude of enzyme activities exists between sperm and eggs in SMI bivalve species (figure 3.1), our results reveal a common organization of the energetic phenotype shared among SMI gametes, implying a conserved balance between the capacity of upstream pathways (most notably glycolysis, TCA cycle and ETS) and the capacity of the final oxidase (CCO) (figure 3.3*a,b,c*). This result is also in line with precedent findings on mitochondrial OXPHOS activity and organization in the SMI species *M. mercenaria* and *P. magellanicus,* which revealed that the same mitochondrial phenotype is shared between both type of gametes and the soma (Bettinazzi et al., 2019b). Altogether, these results add to an accumulating body of evidence suggesting that, in SMI bivalves, the female-driven evolution of mtDNA is reflected in both oocytes and sperm which, despite having different metabolic activities, share a conserved mitochondrial energetic phenotype.

(b) In DUI species, sperm (bearing the paternally derived mitochondria) exhibit a general metabolic depression compared to DUI oocytes (bearing the maternally derived mitochondria), as well as a reorganization of the mitochondrial respiratory chain

According to the “division of labour” hypothesis, one could expect some sort of bioenergetic quiescence or a limited activity in both DUI sperm and oocytes, as predicted for gametes that transmit their mitochondria. Knowing the wide repertoire of catabolic modes that bivalves (and their sperm) are capable of (Bettinazzi et al., 2020; Boulais et al., 2019; Boulais et al., 2015; Muller et al., 2012), an ongoing debate exists on whether DUI sperm might exploit energy pathways alternative to aerobic respiration to altogether sustain their motility and reduce the oxidative stress on their mitochondria to be transmitted (Ghiselli et al., 2013; Milani & Ghiselli, 2015). Even though appealing, this does not appear to be the actual case. Previous studies in DUI species reported that both sperm and eggs mitochondria are transcriptionally active, show no difference in the conformation of mitochondrial cristae, generate electrochemical gradient and perform OXPHOS (Bettinazzi et al., 2020; Bettinazzi et al., 2019b; Milani & Ghiselli, 2015). Additionally, in the case of DUI sperm, a purely OXPHOS based mode of energy production appears to be exploited to sustain their motility (Bettinazzi et al., 2020). Our present findings based on the activity of respiratory complexes I, III and IV relative to both CS and protein content also confirm that male and female gamete mitochondria in DUI species are functionally active (figure 3.1*e*,*f*, supplementary figure 3.s3*f*,*g*). Furthermore, they do not indicate any upregulation of gatekeeper enzymes that could suggest the use of an alternative energy pathways to aerobiosis, e.g. compared to oocytes, the activity of lactate dehydrogenase over both CS activity and protein content in DUI sperm is lower (figure 3.1*b;* supplementary figure 3.s3*b*), and shows no increase in its relative contribution to the general sperm bioenergetics with respect to the capacity of mitochondrial CCO (figure 3.3). However, it is important to note that the enzymatic activities here reported reflect the maximum capacities and do not account of any modulation of metabolic pathways that may underlie *in vivo* physiological activity.

The DUI system provides the unprecedented opportunity for sperm mitochondria to evolve specifically for male functions, and a rational expectation is that DUI species could exploit this potential and exhibit sperm-specific bioenergetic adaptation (Breton et al., 2007). The evolutionary consequence of the DUI system is discernible at the bioenergetic level. In contrast to SMI species and in line with a divergent evolution of sex-linked mtDNAs, our findings reveal the existence of a specific DUI sperm bioenergetic phenotype, characterized by a restrained relative activity of key enzymes of glycolysis, fermentation, tricarboxylic acid cycle, fatty acid metabolism and OXPHOS over CS (used as a proxy of mitochondria content) (figures 3.1,2) partially perceived also relatively to protein content (supplementary figure 3.s3). At the level of mitochondrial functionality, accumulating evidence suggests that mitochondria bearing either the paternally or the maternally associated mtDNA differ in functional properties. A previous study showed that, compared to maternally-transmitted mitochondria in SMI and DUI species, M-type mitochondria present in sperm of DUI species (*M. edulis* and *A. islandica*) and in heteroplasmic male somatic tissues display a remodelled OXPHOS, characterized by a robust limitation in the activity of the electron transport system by the phosphorylation system and by a negligible spare capacity of cytochrome *c* oxidase with respect to the max ETS activity (Bettinazzi et al., 2019b). A catalytic depression of CCO activity was also detected for *M. edulis* M-type sperm when compared with "masculinized" sperm, carrying F-type mitochondria (Breton et al., 2009). In line with this previous evidence, our results reveal a stoichiometric rearrangement between upstream and downstream ETS complexes in DUI sperm compared to eggs in both *M. edulis* and *R. philippinarum*. This adjustment of gamete bioenergetics is not observed in the three SMI species tested and entails a limited CCO activity with respect to the upstream enzymes forming part of the ETS (complex I and III), as well as with gatekeeper enzymes of both glycolysis and TCA cycle (figure 3.3). A different control of mitochondrial respiration at the terminus of the respiratory chain (by both CCO and phosphorylation system) might be under selective pressures to ensure appropriate metabolic regulation of M-type mitochondria in DUI species. Altogether, this specific architecture could reflect sperm mitochondria evolution to cope with a high degree of reduction at the ETS, a potential increase in electron leakage and ROS flux and, interestingly, with the ability to preserve a high membrane potential (Bettinazzi et al., 2020; Bettinazzi et al., 2019b; Blier et al., 2017; Munro et al., 2013; Rodríguez et al., 2019).

(b1) Evolutionary significance of these changes

(b1.1) Preservation and Transmission

With the unprecedented need in DUI species to conserve the genetic integrity of paternally derived mitochondria, one could expect mechanisms in place to minimize oxidative stress and damage in

DUI sperm mitochondria. Our results rather suggest that the bioenergetic adaptation specific of DUI sperm does not necessarily lower the potential oxidative stress upon mitochondria. This is also further supported by the low antioxidant capacity (activity of the enzyme catalase relative to both CS and protein content) measured in sperm compared to oocytes (figure 3.1*g*; supplementary figure 3.s3*h*). In addition to catalase, other antioxidant enzymes participate in cellular ROS regulation, including superoxide dismutase, aconitase and glutathione peroxidase, among others (Munro & Treberg, 2017). Sperm themselves display a wide range of antioxidant mechanisms. For instance, human's seminal fluid has a high antioxidant capacity and a tenth of sperm proteins appear to be linked with antioxidant activity (Dowling & Simmons, 2009; Martínez-Heredia et al., 2006; Ramalho-Santos et al., 2009). In mice, sperm express a testes-specific isoform of cytochrome *c* with increased resistance to ROS-mediated damage and ability to catalyse their reduction (Liu et al., 2006). Although both metabolic reorganization and catalase activity suggest a higher oxidative stress in DUI sperm, future in-depth analyses on ROS flux and different antioxidant mechanisms are required. That said, the presence of ROS is not always deleterious *per se*. For example, mitochondrial ROS can act as signalling molecules, adjust OXPHOS by modulating mitochondrial biogenesis (Moreno-Loshuertos et al., 2006), control apoptosis and cell differentiation and regulate mitophagy ((Munro & Treberg, 2017) and reference therein). A mild oxidative stress also appears to be necessary to promote hyperactivation, capacitation and acrosome reaction in human sperm (Ramalho-Santos et al., 2009; Sanocka & Kurpisz, 2004). A putative high ROS production in DUI sperm can even be the price to pay for a slowed ETS and the maintenance of a high membrane potential, potentially reflecting a trade-off between paternal mitochondria preservation and transmission in DUI species (Bettinazzi et al., 2019b; Milani, 2015). The question thus remains open on how and whether DUI species prevent oxidative damage to M-type mitochondria in sperm and transmit undamaged paternal mitochondria to offspring.

The bioenergetic remodelling described here indicates that DUI sperm mitochondria are active and might maintain a high Δψm. This bioenergetic property was also suggested by previous respirometric and fluorometric analyses of DUI sperm mitochondria (Bettinazzi et al., 2020; Bettinazzi et al., 2019b; Milani & Ghiselli, 2015). As mentioned before, in contrast with the transmission of functionally silenced mitochondria, selection might favour the retention of highly active mitochondria instead, characterized by high mitochondrial membrane potential (Knorre, 2020; Tworzydlo et al., 2020). Indeed, the mitochondrial membrane potential is a trait strongly involved in mitochondrial selection, as this phenotype depicts both OXPHOS functionality and genome integrity. For example, evidence exists that mitochondria with high Δψm are preferentially attached to microtubules and transported to the Balbiani body, to undergo selective replication (Fan et al., 2008; Hill et al., 2014; Milani, 2015; Tworzydlo et al., 2020; Zhou et al., 2010). The mitophagy mechanisms itself relies on Δψm to selectively target dysfunctional mitochondria for degradation (Jin et al., 2010; Sekine & Youle, 2018; Twig et al., 2008; Westermann, 2010; Youle & van der Bliek, 2012). Recent findings indicate that the expression of genes related with the mitophagy process does not vary between gonads of F and M DUI individuals (Capt et al., 2019; Punzi et al., 2018), suggesting that the preservation of M mitochondria in DUI species might entail a mechanism other than a relaxation in the mitophagy process in male embryos. The suspected ability to maintain a high Δψm might determine the fate of a specific mitochondrion and may represent a way by which specific mtDNA variants could escape the quality control mechanism (Knorre, 2020). Overall, our (and previous) results support the intriguing hypothesis that, in DUI species, specific energetic adaptations of male mitochondria might confer the ability to evade degradation during fertilization, and thus play a key role in their own selection and transmission throughout generations (Bettinazzi et al., 2020; Bettinazzi et al., 2019b; Milani, 2015; Milani & Ghiselli, 2015).

(b1.2) Sperm performance

It has been hypothesized that one selective advantage favouring the retention of the paternally derived mitochondrial lineage in DUI species might involve adaptation for sperm and male fitness (Breton et al., 2007). In line with this hypothesis, DUI sperm of the species *M. edulis* and *R. philippinarum* (carrying paternally inherited mitochondria) were described to swim slower and in a more circular fashion than "classic" SMI sperm (carrying maternally inherited mitochondria) (Bettinazzi et al., 2020; Everett et al., 2004; Jha et al., 2008). Moreover, this DUI-specific motility phenotype appears to be completely dependent on the energy produced through OXPHOS (Bettinazzi et al., 2020). It is then possible that the herein described metabolic depression and OXPHOS reorganization in DUI sperm might link with a fertilization strategy which does not require the overexploitation of the energy metabolism. This is in line with a possible adaptation to sedentary life in sessile broadcast spawning marine organisms. Rather than an improved speed capacity, sperm motility traits such as slow speed and pronounced curved trajectories might better benefit male reproductive success by enhancing endurance, survival and area covered by sperm

(Bettinazzi et al., 2020; Boulais et al., 2019; Breton et al., 2007; Everett et al., 2004; Fitzpatrick et al., 2012; Jha et al., 2008; Levitan, 2000; Stewart et al., 2012). The present findings thus add to the growing body of evidence suggesting that selection on sperm mitochondria in DUI system might indeed foster the evolution of bioenergetic adaptations specific for male functions. Specifically, that selection on mt components of the OXPHOS produces changes in the OXPHOS mechanisms and organization that could altogether favour specific sperm performance traits, male reproductive fitness, as well as paternal mitochondria preservation and transmission.

5. Conclusion

Our study constitutes an unprecedented detailed analysis of the general energy metabolism of bivalve gametes, bearing either maternally or paternally derived mitochondria. We specifically investigated whether an adjustment of gamete bioenergetics could represent an evolutionary significant and conserved trait among DUI species. Our findings reveal a different organization of gamete bioenergetics among species with different mitochondrial inheritance system. On the one hand, SMI gametes (carrying maternally derived mitochondria) exhibit a bioenergetic pattern characterized by sperm having higher metabolic rates compared to oocytes but sharing a similar bioenergetic regulation. The only enzyme whose activity is biased towards the female gamete is catalase, reflecting a higher antioxidant potential. These results are partially in line with the prediction that, in species in which mitochondria are strictly maternally inherited, sperm would highly exploit their bioenergetic capacity for fertilization purposes, while oocytes would preserve genetic integrity by both lowering their energy metabolism and enhancing their antioxidant capacity.

On the other hand, DUI sperm (bearing paternally derived mitochondria) are characterized by a general metabolic depression compared to DUI oocytes (bearing maternally derived mitochondria). This is reflected at the level of the relative activity of all key enzymes involved in different metabolic pathways such as glycolysis, fermentation, fatty acid metabolism and mitochondrial respiration over the activity of citrate synthase, as well as partially when normalized for protein content. Additionally, paternal mitochondria in DUI sperm exhibit a remodelled OXPHOS dynamics, characterized by a tight control of cytochrome *c* oxidase upon the upstream respiratory complexes and energy pathways. This DUI-specific bioenergetic feature is in line with mitochondria evolved to function at a high reduction state of the ETS and maintain a high mitochondrial transmembrane potential. This in turn potentially reflects a high ROS flux and the ability of paternally derived mitochondria of DUI species to evade mitochondrial quality control mechanisms and be transmitted across generations. Finally, the bioenergetic reorganization in DUI sperm fits with a fertilization strategy that does not require the overexploitation of the energy metabolism and matches with previous evidence of a DUI-specific pattern of sperm performance. Our findings provide strong evidence that the existence of sex-linked mtDNAs in DUI species have an impact on the energy phenotype. The fact that the here described bioenergetic remodelling is shared among two distantly related DUI bivalves suggests a common evolutionary relevance of this peculiar system of mitochondria transmission in the light of energy adaptation.

Data accessibility: The datasets supporting this article have been uploaded as part of the supplementary material.

Author's contributions: SBe carried out the lab work, data analysis, designed the experiment and drafted the manuscript; LM and PUB supervised the study; SBr conceived, coordinated and supervised the study. All authors gave final approval for publication.

Competing interests: We have no competing interests.

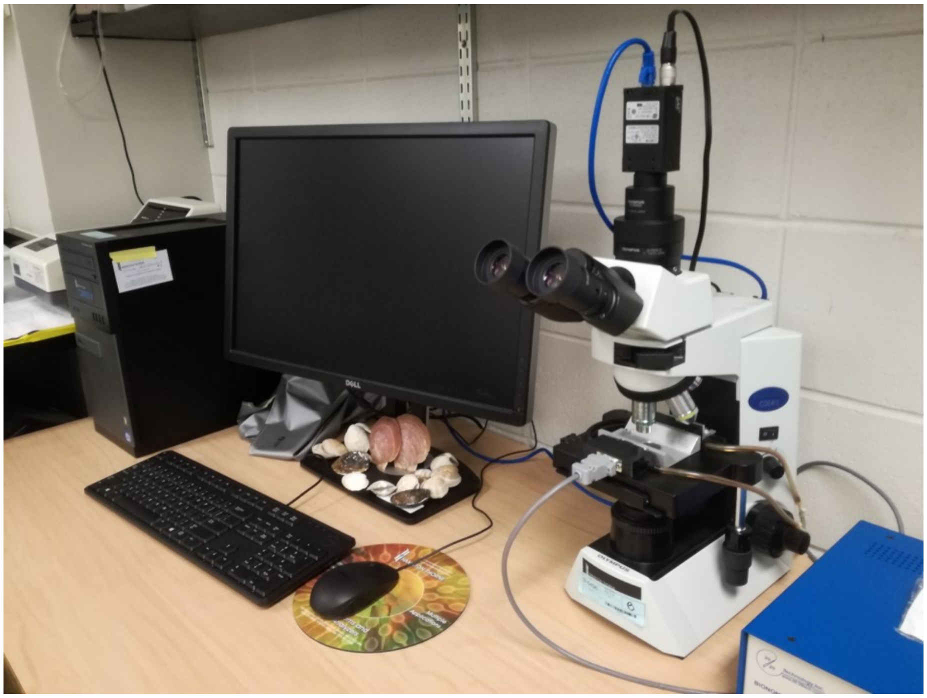
Funding: This work was supported by the Fonds de Recherche du Québec - nature et technologie (to SBr), the Natural Sciences and Engineering Research Council of Canada (to PUB), and the Italian Ministry of Education, University and Research MIUR-SIR Programme (to LM).

Acknowledgements: We thank A. Caron, F. Christen and E. Rodríguez for methodological support and valuable discussions.

CHAPTER IV – LINKING PATERNALLY INHERITED mtDNA VARIANTS AND SPERM PERFORMANCE

Stefano Bettinazzi[a], Sugahendni Nadarajah[ab], Andréanne Dalpé[a], Liliana Milani[c], Pierre U Blier[d] and Sophie Breton[a]

[a]Département des sciences biologiques, Université de Montréal, Montréal, QC H2V 2S9, Canada; [b]Département Sciences de l'Univers, Environnement, Ecologie, Sorbonne Université, 75005 Paris, France; [c]Dipartimento di Scienze Biologiche, Geologiche e Ambientali, Università di Bologna, Bologna 40126, Italia; [d]Département de biologie, Université du Québec à Rimouski, Rimouski, QC G5L 3A1, Canada;



Published in *Philosophical Transactions of the Royal Society B* (2020) 375:20190177

doi.org/10.1098/rstb.2019.0177

Abstract

Providing robust links between mitochondrial genotype and phenotype is of major importance given that mtDNA variants can affect reproductive success. Because of the strict maternal inheritance (SMI) of mitochondria in animals, haplotypes that negatively affect male fertility can become fixed in populations. This phenomenon is known as "mother's curse". Doubly uniparental inheritance (DUI) of mitochondria is a stable exception in bivalves, which entails two mtDNA lineages that evolve independently and are transmitted separately through oocytes and sperm. This makes the DUI mitochondrial lineages subject to different sex-specific selective sieves during mtDNA evolution, thus DUI is a unique model to evaluate how direct selection on sperm mitochondria could contribute to male reproductive fitness. In this study, we tested the impact of mtDNA variants on sperm performance and bioenergetics in DUI and SMI species. Analyses also involved measures of sperm performance following inhibition of main energy pathways and sperm response to oocyte presence. Compared to SMI, DUI sperm exhibited i) low speed and linearity ii) a strict OXPHOS-dependent strategy of energy production and iii) a partial metabolic shift towards fermentation following egg detection. Discussion embraces the adaptive value of mtDNA variation and suggests a link between male-energetic adaptation and paternal mitochondria preservation.

This article is part of the theme issue 'Linking the mitochondrial genotype to phenotype: a complex endeavour'.

1. Introduction

As accumulating evidence undermines the assumption of selective neutrality of mitochondrial DNA (mtDNA) variability, inferring links between mitochondrial genotype and phenotype becomes a major issue in evolutionary biology (Blier et al., 2001; Dowling et al., 2008). Non-neutral mtDNA variations can influence mitochondrial functionality (Bettinazzi et al., 2019b; Pichaud et al., 2012), longevity (Coskun et al., 2003; Dato et al., 2004; Niemi et al., 2003; Zhang et al., 2003), susceptibility to diseases (Taylor & Turnbull, 2005), adaptation to specific environments (Lajbner et al., 2018; Mishmar et al., 2003; Ruiz-Pesini et al., 2004) and could even drive speciation (Dowling et al., 2008; Gershoni et al., 2009; Lane, 2009). An added layer of complexity in the relationship between mtDNA evolution and fitness is the strict maternal inheritance (SMI) of mitochondria in most animal species (Birky, 1995). This sex-specific selective sieve in mtDNA evolution enables male-harming mutations with a bland repercussion on female fitness to persist and reach high frequencies in natural populations, a phenomenon known as "mother's curse" (Frank & Hurst, 1996; Gemmell et al., 2004; Innocenti et al., 2011). Evidence of this phenomenon comes, for example, from studies linking specific mtDNA haplotypes with decreased sperm motility and male fertility, while being of low impact on female reproduction (Montiel-Sosa et al., 2006; Nakada et al., 2006; Ruiz-Pesini et al., 2000).

A potential but uncommon compensatory mechanism resides in the paternal inheritance of mitochondria, the only stable example in animals being the doubly uniparental inheritance (DUI) of mitochondria in bivalve molluscs (Breton et al., 2007; Passamonti & Ghiselli, 2009; Zouros, 2012). The DUI system entails two sex-linked mtDNAs (the female or F-type and the male or M-type) transmitted separately through oocytes and sperm. These two mtDNA lineages evolve independently and remarkably exhibit from 8 to 40 % of DNA sequence divergence (Breton et al., 2007). Because the fidelity of gamete-specific transmission of the two mtDNAs is a basic requirement for explaining the evolutionary stability of DUI, this system does not represent a case of biparental inheritance of organelles (Breton et al., 2007; Passamonti & Ghiselli, 2009; Zouros, 2012). The oocytes carry the female-derived mitotype whereas sperm only bear the male-derived mitotype (Ghiselli et al., 2010; Venetis et al., 2006). In a few cases, the maternal mt lineage has been found to invade the male route and take the place of the paternal lineage. This has only been documented in *Mytilus* spp, a rare phenomenon named "masculinization" (Zouros, 2012). No evidence of masculinization events has been recorded in other DUI species (Breton et al., 2007; Ghiselli et al., 2010; Passamonti & Ghiselli, 2009; Zouros, 2012). As such, in all other DUI species a strict sex-specific mtDNA segregation in the germ line is the stable rule, with sperm carrying exclusively the M-type mitochondria (Ghiselli et al., 2010; Venetis et al., 2006).

The opportunity for natural selection to act directly on sperm mitochondria makes the DUI system an attractive model to evaluate the phenotype resulting from a male-specific evolution of mitochondria and thus the adaptive value of paternally-inherited mtDNA variants (Milani & Ghiselli, 2020). Furthermore, comparing the functions of male gametes carrying either male- or female-derived mitochondria (DUI vs SMI) brings an exceptional opportunity to test the effectiveness of the mother's curse hypothesis in bivalves. To date, DUI has been detected in more than 100 bivalve species and its distribution appears to be scattered (Gusman et al., 2016). Although a single origin of DUI near the origin of the modern class Bivalvia would represent the most parsimonious hypothesis, there is evidence for multiple independent origins of this peculiar system (Gusman et al., 2016; Plazzi & Passamonti, 2019; Zouros, 2012). This is reflected at the phylogenetic level, where F- and M-mitotypes of different species sometimes join according to their gender linkage, as seen in freshwater mussels, or they cluster together according to species relatedness, as seen in several marine species (Bettinazzi et al., 2016; Gusman et al., 2016; Plazzi & Passamonti, 2019; Plazzi et al., 2016). In a recent paper, the presence of selective signatures in the mitochondrial genomes of DUI species was investigated and few DUI-specific mutations were identified that gave support to the hypothesis of multiple independent origins (Plazzi & Passamonti, 2019). Interestingly, they documented episodes of acute directional selection associated with the origins of different DUI systems in six mt genes (i.e. *atp6*, *cox1*, *cox2*, *cox3*, *nad4L*, and *nad6*). As such, even in a scenario of multiple independent origins of the DUI system, a common increase in mutational events and selective pressure on specific mt genes appear to take place at the base of a DUI clade (Plazzi & Passamonti, 2019).

In accordance, a convergent phenotypic evolution has been suggested in the DUI marine clam, *Arctica islandica*, and marine mussel, *Mytilus edulis*, for which the mitochondrial phenotypes of the F- and M-type mitochondria have been recently characterized (Bettinazzi et al., 2019b). Compared to F-type mitochondria in eggs and gills, M-type mitochondria in sperm exhibit i) low respiratory activity compared to their maximum capacity (coupled oxidative phosphorylation rate/ uncoupled rate) because of a limitation by the phosphorylation system and ii) low excess capacity of cytochrome *c* oxidase (complex IV or CIV), which could link to a tight flux control of CIV over the upstream complexes. This energetic remodelling, that appears specific of DUI sperm even across distantly related DUI species, has been proposed to be involved in the preservation of the paternal mitochondrial lineage across generations, linking male-energetic adaptation with selection and inheritance of cytoplasmic organelle genomes (Bettinazzi et al., 2019b; Milani, 2015; Milani & Ghiselli, 2015).

Little is known about the extent to which the retention of a male-specific mitotype (and the expression of a rearranged mitochondrial phenotype) could affect sperm performance. For example, selection acting directly on male mitochondria has been proposed to lead to the evolution of genomes specifically adapted for sperm functions, fostering male reproductive success in DUI species (Breton et al., 2007; Burt & Trivers, 2006). So far, studies on *M. edulis* did not find any evidence that M-type mitochondria are linked to higher sperm swimming speed (Everett et al., 2004; Jha et al., 2008), suggesting that the adaptive value of DUI could embrace other sperm fitness traits, such as endurance, longevity, or response to either competing sperm or egg-derived chemical attractants (chemoattractants) (Breton et al., 2007; Everett et al., 2004; Stewart et al., 2012). Concerning ATP-production, knowing the flexible energetic metabolism of bivalve species (Muller et al., 2012) and the putative downregulation of both the oxidative phosphorylation (OXPHOS) and the swimming speed in sperm bearing M-type mitochondria (Bettinazzi et al., 2019b; Everett et al., 2004; Jha et al., 2008), the question arises whether DUI species would rely more on aerobic or glycolytic energy metabolism to sustain spermatic functions. Since DUI allows selection to act directly on male mt-encoded components, and keeping in mind the mother curse's effect in SMI systems, one prediction could be that the sperm of DUI species use OXPHOS while the sperm of SMI species might rely primarily on glycolysis. In other words, because mt genes are only or mainly involved in OXPHOS, the sperm of DUI species might rely more heavily on OXPHOS because selection can act more efficiently on their (mt) OXPHOS genes.

In animals, there is still controversy regarding the main energetic pathway of energy production in sperm, and the two processes are linked and non-mutually exclusive (du Plessis et al., 2015; Ferramosca & Zara, 2014; Moraes & Meyers, 2018; Ruiz-Pesini et al., 2007; Storey, 2008). Species strongly differ in the proportion of utilization of these two pathways (Boulais et al., 2015; Davila et al., 2016; du Plessis et al., 2015; Ferramosca & Zara, 2014; Miki et al., 2004; Moraes & Meyers, 2018; Nakada et al., 2006; Ruiz-Pesini et al., 2007; Storey, 2008; Tourmente et al., 2015). The balance between the aerobic and anaerobic capacity allows a flexible metabolic strategy to meet sperm energetic demand, which could vary depending on the surrounding environment and the presence of different substrates/chemicals (du Plessis et al., 2015; Moraes & Meyers, 2018; Ruiz-Pesini et al., 2007). For example, the sperm flagellar movement of the pacific oyster, *Crassostrea gigas*, passes from a phosphagen- and glycolytic-dependant metabolism to OXPHOS, when changing from the early to the long motility phase (Boulais et al., 2015). However, although the role played by OXPHOS has been confirmed in the sperm of various bivalve species (Bettinazzi et al., 2019b; Ghiselli et al., 2018; Milani & Ghiselli, 2015), there is still a lack of knowledge about the importance of the anaerobic metabolism. Moreover, although the presence of chemoattractants has been found to exert changes in sperm swimming behaviour and physiology in bivalves (Eisenbach & Giojalas, 2006; Evans et al., 2012; Lymbery et al., 2017; Oliver & Evans, 2014), whether egg-detection can influence sperm bioenergetics is still unknown. Beyond promoting gamete encounter, egg-derived chemoattractants also seem to mediate bivalves mate choice, as gametes could exploit these molecules to select for genetically compatible partners. This suggests a link between sperm chemotaxis and gamete-level sexual selection, increasing the role of gamete chemical signals in sessile marine invertebrates (Evans et al., 2012; Lymbery et al., 2017; Oliver & Evans, 2014). A change in steady-state speed following egg detection has been proposed for *Mytilus galloprovincialis* sperm. Specifically, mussel sperm would conserve energy by swimming slowly and in tight circles if eggs are absent in the water environment, but faster and straighter towards the more genetically compatible oocytes once detecting them (Eads et al., 2016). Whether the link between sperm chemotaxis and sexual selection at the gamete-level could be in some way related to DUI remains to be examined.

The goal of the present study was to test the impact of bearing paternal or maternal mitotypes upon bivalve sperm bioenergetics and performance. We aimed to infer: i) if bivalve species rely more on oxidative or glycolytic energy metabolism to sustain spermatic functions, ii) whether gamete chemoattraction may influence the metabolic pathways of spermatozoa and iii) whether a different energetic strategy may be the result of natural selection shaping the evolution of paternally-inherited mitochondria, thus reflecting male-specific energetic adaptation in DUI species. Sperm motility parameters were evaluated in five bivalve species. We compared sperm of the DUI species *Mytilus edulis* (Order Mytilida) and *Ruditapes philippinarum* (Order Venerida), bearing their male-specific mitochondria (i.e. the DUI M-type), with sperm of the SMI species *Mercenaria mercenaria* (Order Venerida), *Nuttallia obscurata* (Order: Cardiida), and *Placopecten magellanicus* (Order Pectinida), bearing their own species-specific and maternally-derived mitochondria (i.e. the SMI maternally-inherited type). To avoid potential taxon-driven bias in the results, the five bivalve species tested were selected to be distantly related. The strong evolutionary divergence between the mitochondrial lineages of these species is reflected in how their entire mt genomes cluster separately in a phylogenetic tree, with their last common ancestor being dated to the mid-Cambrian, ≈510 million years ago (Plazzi et al., 2016). Moreover, the DUI species used for this research likely represent two independent origins of DUI, as their sex-linked genomes (F- and M-type) cluster according to the species rather than by sex specificity (Gusman et al., 2016; Plazzi & Passamonti, 2019; Plazzi et al., 2016; Zouros, 2012). The nucleotidic divergence between the F and M genomes is gene-specific and ranges between 10-22 % in *M. edulis* (Bettinazzi et al., 2019b; Breton et al., 2006; Stewart et al., 1995; Zouros, 2012) and between 16-32 % in *R. philippinarum* (Bettinazzi et al., 2016; Passamonti et al., 2003).

The equilibrium between the aerobic and anaerobic metabolism to sustain sperm motility was assessed following the inhibition of the main pathways of energy production, and the potential change in this balance was assessed following the introduction of oocyte-derived chemoattractants. Our results are discussed in the light of the adaptive value of mtDNA variation, paternal inheritance of mtDNA, male-energetic adaptation and its evolutionary implications.

2. Materials and methods

(a) Animal collection. Adult bivalves were ordered from culture farms or bought in fish markets during their spawning period between June and August 2018, acclimated for four weeks in a 12 °C recirculating seawater aquarium and fed with a mix of microalgae. We tested five different broadcast spawning bivalve species: the DUI species *Mytilus edulis* (Linnaeus, 1758) from Kensington (Prince Edward Island, Canada) and *Ruditapes philippinarum* (Adams & Reeve, 1850) from Vancouver (British Columbia, Canada), as well as the SMI species *Mercenaria mercenaria* (Linnaeus, 1758) from Barnstable (Massachusetts, USA), *Nuttallia obscurata* (Reeve, 1857) from Vancouver (British Columbia, Canada) and *Placopecten magellanicus* (Gmelin, 1791) from Newport (Québec, Canada). Sex and maturity of individuals were assessed through microscopic examination of gonadal smears. The absence of masculinization in *M. edulis* sperm sample was tested by amplifying part of the M-mtDNA (654 bp) using the male-haplotype specific primers: MyEd-M-for (TACTGTTGGCACATACGAGAG) and MyEd-M-rev (TACTGTTGGCACATACGAGAG), designed on the complete *M. edulis* M-mtDNA (accession numbers AY823623.1). The specific primers were already tested on this species (Bettinazzi et al., 2019b). *M. edulis* oocytes (carrying the only F-mtDNA lineage) were tested to confirm the M-mtDNA specificity of the primers adopted. Results confirmed the presence of M-mtDNA in sperm and its absence in eggs.

(b) Gamete sample preparation. To test the effect of oocyte-derived chemoattractants on sperm motility, prior to experiments and for each species, one egg sample was collected, adjusted to 1:5 w/v with artificial seawater (ASW), homogenized (3 x 30 s at medium speed) using a PT 1200 homogenizer (Polytron, Kinematica), microfiltered and stored at −20 °C until use. Male gonads were excised and placed in a Petri dish containing 5 mL of ASW. Gametes were stripped by performing incisions in the gonads and allowing the motile mature sperm to actively swim out for 5 minutes. Total sperm count was determined by using a Petroff-Hausser counting chamber and the final concentration was corrected to $5 \cdot 10^6$ sperm·mL^{-1} by addition of ASW. Sperm suspensions were divided in 2 aliquots (475 µL each), one supplemented with 25 µL of ASW ('normal' group) and the other with 25 µL of species-specific egg-derived chemoattractants ('chemoattractants' group, 1:100 w/v). To assess the effect on sperm performance of metabolic inhibitors together with (or without) chemoattractants, each group was further divided into 5 aliquots (100 µL each): a) ASW ("control" group), and four treatments: b) 1 µM rotenone (Rot, inhibitor of mitochondrial respiratory complex I - NADH-dehydrogenase), c) 1 µM antimycin A (Ama, inhibitor of mitochondrial respiratory complex III – coenzyme Q: cytochrome *c* oxidoreductase), d) 5 µM oligomycin (Omy, inhibitor of mitochondrial ATP-synthase), e) 30 mM of sodium oxamate (Oxa, inhibitor of lactate dehydrogenase 4 (LDH4)). The effectiveness of these mitochondrial inhibitors to target specific mitochondrial complexes in bivalves and other animal models, as well as their optimal concentrations, have already been tested and verified through titration in previous studies (Bettinazzi et al., 2019a; Bettinazzi et al., 2019b; Munro et al., 2013; Tourmente et al., 2017). After inhibitor addition, sperm aliquots were incubated at 15 °C for 30 min prior to sperm motility parameters assessment (Tourmente et al., 2017). All chemicals were purchased from Sigma-Aldrich (Oakville, Ontario, Canada).

(c) Sperm performance parameters. After incubation, 10 µL of each sperm suspension was placed in a 20 µm deep microscopy chamber. A minimum of 500 sperm per treatment were analysed using a CEROS microscope (Hamilton Thorne Inc, Beverly, USA) with a 20x negative phase contrast objective. Recorded videos were manually verified to exclude drifting particles and drifting immotile sperm from the analysis. The following sperm motility parameters were estimated through a computer aided sperm analyser (CASA system): distance of average path (DAP, µm), straight-line distance (DSL, μm), curvilinear distance (DCL, μm), curvilinear velocity (VCL, $\mu m \cdot s^{-1}$), straight-line velocity (VSL, $\mu m \cdot s^{-1}$), average path velocity (VAP, $\mu m \cdot s^{-1}$), linearity (LIN = $VSL \cdot VCL^{-1}$), straightness (STR = $VSL \cdot VAP^{-1}$), wobble coefficient (WOB = $VAP \cdot VCL^{-1}$), amplitude of lateral head displacement (ALH, μm), and beat-cross frequency (BCF, Hz). For each sample, the value of each parameter represents the mean of all its individual sperm values. All these parameters describe various motility traits of male gametes, such as speed and linearity of the trajectory, and are widely employed to infer the reproductive fitness of individuals (Boulais et al., 2015; Davila et al., 2016; Eads et al., 2016; Everett et al., 2004; Fitzpatrick et al., 2012; Jha et al., 2008; Levitan, 2000; Liu et al., 2011; Miki et al., 2004; Oliver & Evans, 2014; Stewart et al., 2012; Tourmente et al., 2017; Tourmente et al., 2015).

(d) Data and statistical analysis. Sperm performances were measured for *n* = 11 *M. edulis*, *n* = 9 *R. philippinarum*, *n* = 9 *M. mercenaria*, *n* = 5 *N. obscurata* and *n* = 11 *P. magellanicus*. As sperm kinetic parameters have already been shown to be highly correlated (Tourmente et al., 2017), all parameters were combined and resumed by performing a principal component analysis (PCA) (figure 4.s1, table 4.s1). The first principal component PC1 accounted for 58% of the variability of the original parameters and reflects sperm velocity, as all the velocity parameters (VAP, VSL and VCL) heavily load on it. The second principal component (PC2) accounted for 21% of the variability and reflects the linearity of the path, due to the heavy load that LIN, WOB and STR have on it (figure 4.s1, table 4.s1). The assumptions of normality and homoscedasticity were verified using Shapiro and Levene's tests, respectively. Sperm motility parameters have been analysed in function of the factors: 'species' (five levels), 'treatment' (five levels) and presence of egg-derived chemoattractants (factor 'chemoattractants', two levels). Statistical analyses were performed considering single or multiple factors, depending on the biological question of interest. Interspecific differences in basal sperm motility (effect of factor 'species') in both absence or presence of egg chemical cues have been tested by means of one-way ANOVAs followed by a *post hoc* Tukey's multi comparison test (figures 4.1, 4.s2). The fixed effect of metabolic inhibition (factor 'treatment'), chemoattractants absence/presence (factor 'chemoattractants') and species (factor 'species') on sperm motility parameters were assessed either separately or combined through linear mixed effect models that controlled for by-subject variability and for the individual variability in the response to egg detection (figures 4.2, 4.3, 4.4, 4.s3). The significance of the fixed variables was determined by using a Type III ANOVA, followed by a *post hoc* pairwise comparison with holm correction for multiple testing. All the analyses and graphs have been made using R software (R Core Team, 2016). Statistical significance was set at $p \leq 0.05$. Results are presented as means ± standard error of the mean (s.e.m.).

3. Results and discussion

(a) Sperm carrying paternally inherited mitochondria exhibit low speed and accentuate curvilinear trajectory

The comparison of sperm motility parameters of DUI and SMI species is represented in figure 4.1 and figure 4.s2, respectively in absence or presence of egg-derived chemoattractants. Significant differences across species were detected for all the motility traits, in absence or presence of egg-derived chemoattractants. A detailed summary of the results is provided in table 4.s3. Among sperm velocity parameters, differences were reported for the average path velocity (VAP) (figures 4.1*a*, 4.s2*a*), straight-line velocity (VSL) (figures 4.1*b*, 4.s2*b*), curvilinear velocity (VCL) (figures 4.1*c*, 4.s2*c*) and are resumed in the first principal component (PC1) (figure 4.1*d*, $F = 41.92$, $p = 8.45e\text{-}14$; figure 4.s2*d*, $F = 32.18$, $p = 5.1e\text{-}12$), representing a proxy of the sperm velocity itself. Interspecific differences were also observed for all sperm trajectory parameters (LIN, WOB, STR, ALH, BFC, see table 4.s3), as resumed in PC2 (figure 4.1*e*, $F = 20.93$, $p = 2.25e\text{-}09$; figure 4.s2*e*, $F = 14.44$, $p = 2.2e\text{-}07$), which expresses the linearity of the path. This finding is corroborated in figure 4.s3, where a strong main effect of the factor ‘species’ is found widespread among all motility parameters (table 4.s4).

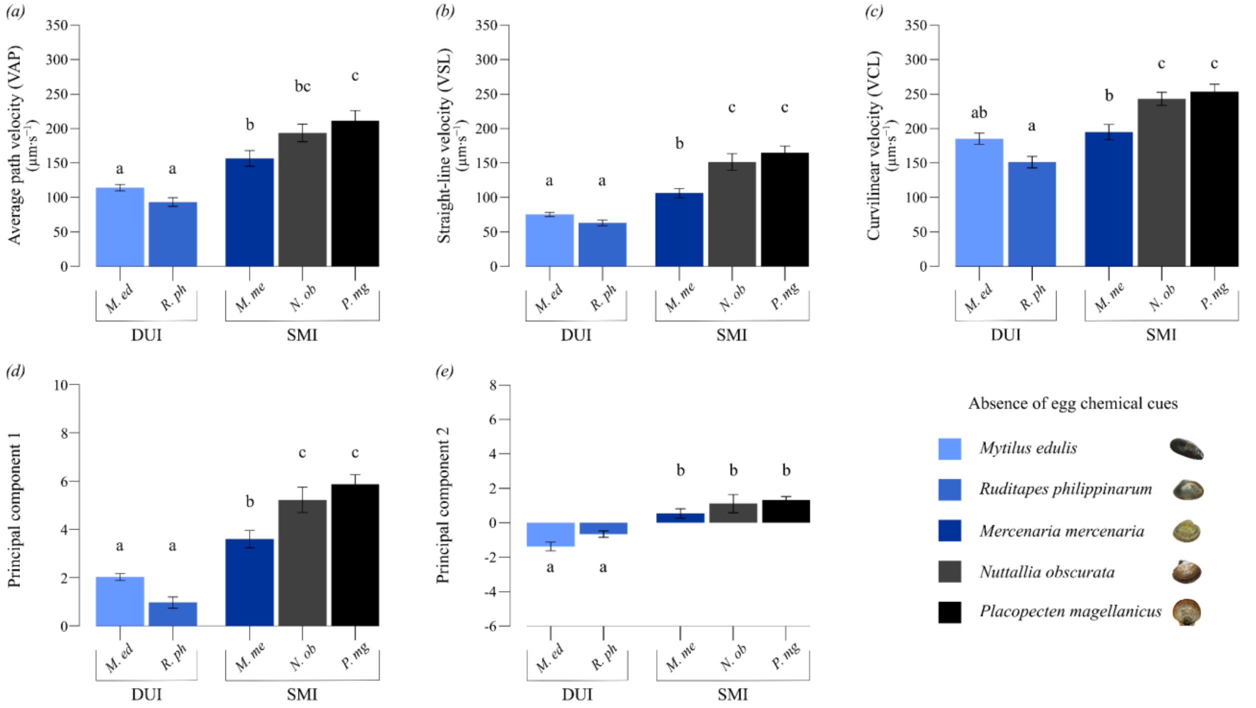


Figure 4.1. Basal sperm motility parameters in five bivalve species, DUI and SMI, without chemoattractants. *(a)* Average path velocity (µm·s^{-1}). *(b)* Straight-line velocity (µm·s^{-1}). *(c)* Curvilinear velocity (µm·s^{-1}). *(d)* First principal component of the PCA combining sperm velocity parameters. *(e)* Second principal component of the PCA. Data are presented as means ± s.e.m. Differences ($p \leq 0.05$) in a *post hoc* Tukey's test are indicated by different letters in subscript. DUI species: *M. edulis* (*M. ed*, $n = 11$), *R. philippinarum* (*R. ph*, $n = 9$). SMI species: *M. mercenaria* (*M. me*, $n = 9$), *N. obscurata* (*N. ob*, $n = 5$), *P. magellanicus* (*P. mg*, $n = 11$). Detailed summary is reported in electronic supplementary material, tables 4.s2 and 4.s3.

Interestingly, sperm of both DUI species (*M. edulis* and *R. philippinarum*) have a consistent lower speed (VAP, VSL, VCL and PC1) and a less linear path (LIN, WOB, STR and PC2) than sperm of the three SMI species (*M. mercenaria*, *N. obscurata* and *P. magellanicus*), regardless of the absence/presence of egg chemoattractants (figures 4.1, 4.s2, 4.s3). Egg-derived chemoattractants have been shown to exert an effect on sperm motility behaviour, specifically swimming speed and direction (Eads et al., 2016; Evans et al., 2012; Lymbery et al., 2017; Oliver & Evans, 2014). Contrary to our expectations, we did not detect any significant impact of egg presence on sperm velocity parameters (only a trend of increasing speed), and differences in velocity were explained by the only fixed factor 'species' (figure 4.s3, table 4.s4). Specifically, interspecific differences were detected for VAP (figure 4.s3*a*), VSL (figure 4.s3*b*), VCL (figure

4.s3*c*) and are resumed in PC1 (figure 4.s3*d*, $F = 53.22$, $p = 1.71e\text{-}15$). These results are consistent with a previous work on *M. edulis* in which no increase in sperm velocity parameters were observed under sperm competition and detection of oocytes (Stewart et al., 2012). Conversely, sperm trajectory was influenced by both factors 'species' and addition of 'chemoattractants' (figure 4.s3*e*, table 4.s4). Specifically, DUI and SMI sperm cluster separately based on a less linear trajectory of the former, while addition of chemoattractants produced a trend of decreased linearity in both groups.

In DUI species, the preservation of sex-linked mtDNAs in gametes has been proposed as a way to avoid sex-linked constraints of mitochondrial inheritance, and an opportunity for mitochondria to evolve adaptively for male and sperm fitness (Breton et al., 2007). Our results on bivalve sperm carrying either a female or a male-derived mitotype suggest that selection on sperm function might be acting differently in these groups, possibly due to DUI vs SMI system of organelle inheritance, favouring both low sperm speed and linearity in DUI species. This is congruent with previous studies in the species *M. edulis* that found sperm bearing the paternally-inherited mtDNA having equal or even lower speed than 'masculinized' sperm carrying the maternally-inherited mtDNA (Everett et al., 2004; Jha et al., 2008). The present findings thus provide additional evidence that the adaptive value of paternal mitochondria preservation in DUI species might embrace different sperm phenotypic traits than higher velocity or straightness, although it is still unclear whether the traits seen in DUI sperm increase or decrease sperm fitness (or are neutral) (Breton et al., 2007; Breton et al., 2009; Everett et al., 2004; Jha et al., 2008).

Swimming speed is just one sperm-fitness trait among many, and even a decreased velocity could represent an advantage depending on the fertilization strategy adopted. For instance, slower sperm with pronounced curved trajectories and a high angle change rate have already been associated with highest fertilization rates in *M. galloprovincialis* (Fitzpatrick et al., 2012; Liu et al., 2011). As a trade-off between sperm rapidity and endurance has already been demonstrated (Levitan, 2000), a slow sperm speed may reflect a strategy linked with energy preservation and/or swim endurance in the DUI species tested so far, shifting the selective pressure towards stamina rather than speed. Even in presence of eggs, selection may favour slow but constant-speed sperm that survive for a longer time and cover a larger distance due also to an increased oscillation around the average path, rather than faster sperm with a shorter lifespan and a straighter path. Based on the phylogenetic distance between the two DUI species pertaining to different Orders, i.e. Mytilida and Venerida, and likely representing two independent origins of DUI, the intriguing hypothesis that such sperm phenotype might reflect a shared DUI feature can be considered. We speculate that the fertilization success contributed to the evolution and preservation of the paternally-inherited and highly divergent M mtDNA lineage in DUI species. Also, the link between energy production limitation and ROS production should be considered, as a lower metabolic rate could reduce the oxidative stress and in turn preserve the integrity of the paternal mtDNA to be passed through generations. These hypotheses, however, remain to be tested.

(b) Sperm carrying paternally inherited mitochondria show a flexible metabolic strategy depending on the presence of egg-derived chemoattractants

The importance of aerobic and anaerobic pathways of energy production has been investigated through the addition of specific metabolic inhibitors and the results are reported in figure 4.2, and tables 4.s2, 4.s5. For all five species, the inhibition of the oxidative phosphorylation (i.e. through the separate addition of rotenone, antimycin A and oligomycin A, respectively inhibiting complex I, complex III and ATP synthase) strongly hampered all sperm velocity parameters analysed (VAP, VSL, VCL, PC1) (figures 4.2*a,b,c,d*; table 4.s5). By contrast, sperm trajectory parameters were only marginally affected by inhibitors and no congruent trend was detectable (figure 4.2*e*, table 4.s5). Our results thus suggest that, contrary to some other animal species including humans (Ferramosca & Zara, 2014; Moraes & Meyers, 2018; Storey, 2008), the energy production through the OXPHOS is mandatory to sustain sperm velocity in these bivalve species. The importance of the anaerobic pathway of energy production, assessed through the addition of sodium oxamate, an inhibitor of lactate dehydrogenase, revealed that lactic fermentation plays a different role in sperm bearing the paternally- or the maternally-inherited mitochondria. Indeed, contrary to sperm of SMI species (carrying the maternal mt lineage), for which the inhibition of lactate dehydrogenase impacted motility, sperm of DUI species (carrying the paternal mt lineage) remained unaffected (figure 4.2*a*,*b*,*c*,*d;* table 4.s5).

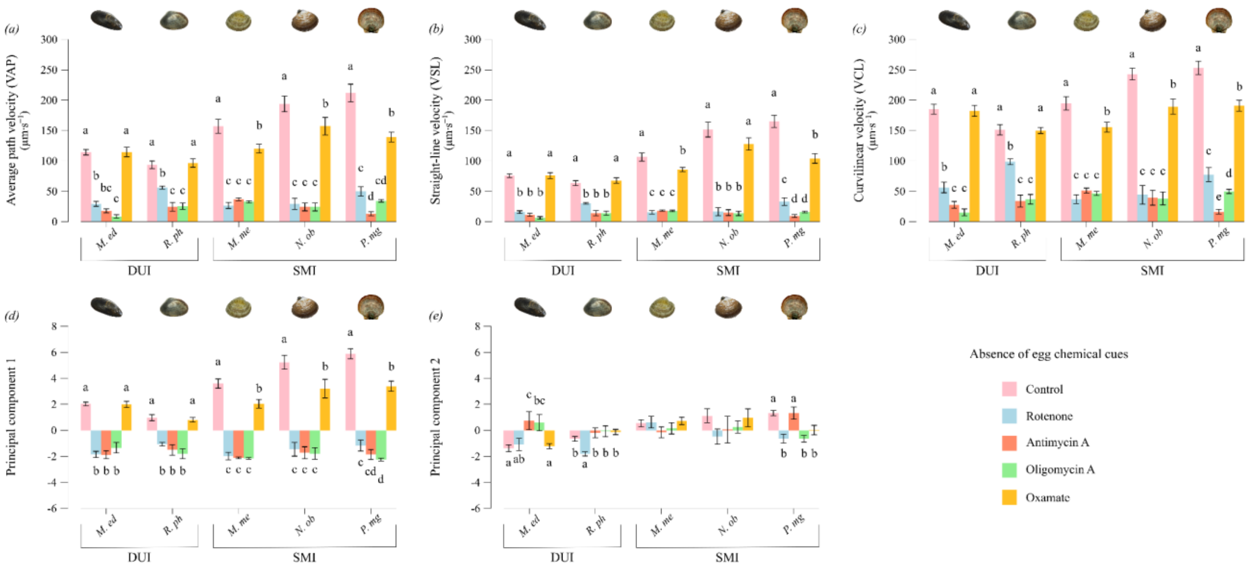


Figure 4.2. Effect of metabolic inhibitors on sperm motility parameters in five bivalve species, DUI and SMI, without chemoattractants. *(a)* Average path velocity ($\mu m \cdot s^{-1}$). *(b)* Straight-line velocity ($\mu m \cdot s^{-1}$). *(c)* Curvilinear velocity ($\mu m \cdot s^{-1}$). *(d)* First principal component of the PCA. *(e)* Second principal component of the PCA. Data are presented as means ± s.e.m. Statistical difference was set at $p \leq 0.05$. Difference among treatments are indicated by letters determined through a *post hoc* comparison adjusted using Holm's correction for multiple testing. For abbreviations refer to figure 4.1. Detailed summary is reported in electronic supplementary material, tables 4.s2 and 4.s5.

Marine bivalves exhibit a panoply of energy production strategies, including aerobic respiration, various cytosolic fermentation pathways (i.e. lactate and opine pathways) and even an oxygen-independent mitochondrial functioning through the malate-dismutation pathway (Dando et al., 1981; de Zwaan & Wijsman, 1976; Lee & Lee, 2011; Muller et al., 2012). A previous study on the pacific oyster *Crassostrea gigas* (SMI species) suggested that the ATP-dependent flagellar movement is sustained by both phosphagen and glycolytic metabolism during the early phase of movement, whereas oxidative phosphorylation would support sperm motility in the long motility phase (Boulais et al., 2015). Likewise, our results reveal that, in absence of oocytes, both fermentation and aerobic metabolism are important to sustain sperm motility in SMI species, but not in the two DUI species. Although the aerobic metabolism appears mandatory in both SMI and DUI species, a strictly OXPHOS-dependent strategy, or at least not dependent on lactic fermentation, could represent a DUI-specific and evolutionary conserved sperm metabolic rearrangement. Our results are congruent with the previous finding that, compared to maternally-transmitted mitochondria of either DUI or SMI species, male mitochondria in DUI species exhibit a reorganization of the oxidative phosphorylation system that may influence ATP production efficiency (Bettinazzi et al., 2019b; Breton et al., 2009). These variations entail differences in the catalytic capacity of various enzyme complexes (Breton et al., 2009) and the expression of a rearranged mitochondrial phenotype, characterized by a limitation of the aerobic metabolism by ATP-synthase and by a potential tight control of cytochrome *c* oxidase over the upstream respiratory enzymes (Bettinazzi et al., 2019b), strongly suggesting an evolutionary link between the OXPHOS mechanism and the DUI system itself. Taken all together, these results are somewhat in line with the prediction of the mother's curse hypothesis, i.e. that sperm of DUI species use OXPHOS (since mt-encoded components can be selected for sperm function) while sperm of SMI species (for which selection might be less efficient) might compensate reduced (or compromised) OXPHOS function with glycolysis. However, more species will have to be tested to clearly confirm the trend observed in the present study.

The equilibrium between the aerobic and anaerobic pathways was also investigated in presence of egg chemical cues, and results are reported in figure 4.3 and table 4.s6. In the three SMI species, addition of chemoattractants did not exert any change in the balance between the two pathways (i.e. both OXPHOS and lactic fermentation are required, with or without chemoattractants), whereas in DUI species, the presence of chemoattractants affected their proportion, i.e. both *M. edulis* and *R. philippinarum* sperm motility became sensitive to oxamate (for both average path and curvilinear velocities; figure 4.3*a*,*c*). No effect was detected for the straight-line velocity nor for the PC1 parameter (figure 4.3*b*,*d*), although for the latter a decreasing trend is detectable. For sperm trajectory no trend was detectable (figure 4.3*e*).

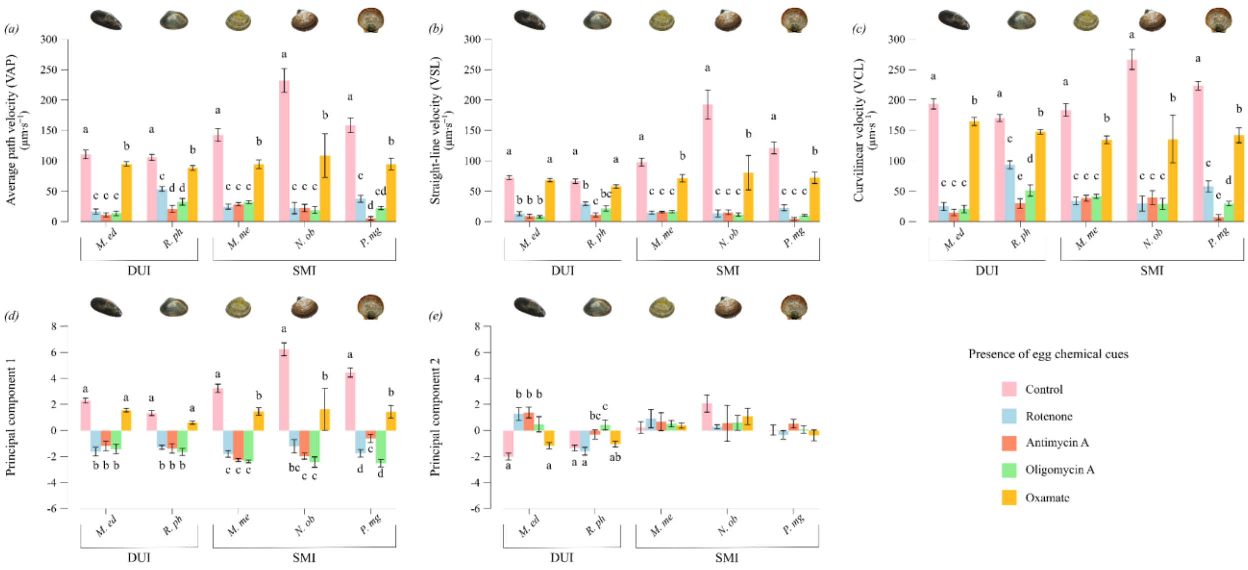


Figure 4.3. Effect of metabolic inhibitors on sperm motility parameters in five bivalve species, DUI and SMI, with chemoattractant. *(a)* Average path velocity (µm·s^{-1}). *(b)* Straight-line velocity (µm·s^{-1}). *(c)* Curvilinear velocity (µm·s^{-1}). *(d)* First principal component of the PCA. *(e)* Second principal component of the PCA. Data are presented as means ± s.e.m. Statistical difference was set at $p \leq 0.05$. Difference among treatments are indicated by letters determined through a *post hoc* comparison adjusted using Holm's correction for multiple testing. For abbreviations refer to figure 4.1. Detailed summary is reported in electronic supplementary material, tables 4.s2 and 4.s6.

Overall, the analysis of the energetic metabolism suggests that: i) both SMI and DUI species strongly rely on OXPHOS to sustain sperm motility; ii) for the SMI species analysed, both aerobic and anaerobic pathways of energy production appear to play a role in sustaining sperm motility, no matter the presence of female gamete compounds; and iii) only the DUI species show a flexible metabolic strategy depending on the presence of egg-derived chemoattractants. Specifically, *M. edulis* and *R. philippinarum* sperm appear to exclusively rely on OXPHOS activity after spawning but switch to a combined metabolic strategy in the presence of egg-derived compounds. This can also be seen in figure 4.4, where the interaction effect between LDH-inhibition (factor 'treatment') and presence of oocytes (factor 'chemoattractants') was investigated. For the three SMI species, no interaction effect is found for the velocity parameters, resumed in PC1 (figure 4.4). Sperm velocity was only affected by the addition of oxamate (i.e. *M. mercenaria* and *N. obscurata*) or, separately, by both oxamate and addition of chemoattractants (*P. magellanicus*). Conversely, for both DUI species, an interaction effect of glycolysis inhibition and chemoattractants addition was observed. The *post hoc* simple main effect analysis confirmed that the effect of glycolysis inhibition is dependent on egg presence and that this outcome does not derive solely from an increased speed after addition of chemoattractants nor a higher sensibility to lactic fermentation inhibition, but mainly by a combined influence of both (figure 4.4, table 4.s7).

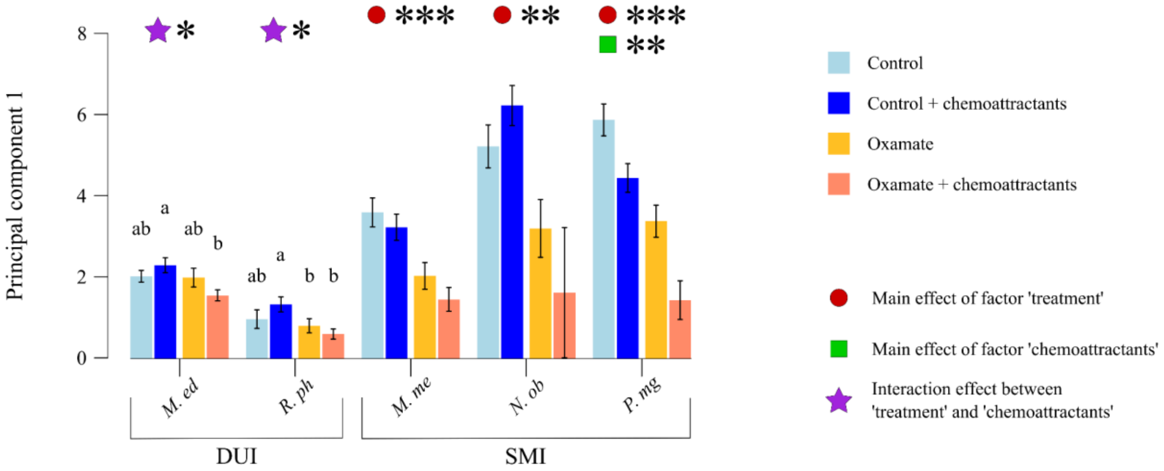


Figure 4.4. Interaction effect between glycolysis inhibition and addition of chemoattractants on the first principal component of the PCA, reflecting sperm velocity. Values are presented as means ± s.e.m. The main effect of the two fixed factors ‘treatment’ and ‘chemoattractants’ are indicated with a circle and square respectively. Interaction effect is indicated with a star. $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$. Letters indicate differences following a *post hoc* pairwise comparison. DUI, doubly uniparental inheritance; SMI, strict maternal inheritance. Species: *M. edulis* (*M. ed*, $n = 11$); *R. philippinarum* (*R. ph*, $n = 9$); *M. mercenaria* (*M. me*, $n = 9$); *N. obscurata* (*N. ob*, $n = 5$); *P. magellanicus* (*P. mg*, $n = 11$). Detailed summary is reported in electronic supplementary material, tables 4.s2 and 4.s7.

One possible explanation for the glycolytic switch relates to the ATP diffusion throughout sperm. While mitochondrial ATP diffusion from the mitochondrial midpiece would be slower and may not reach all areas, the colocalization of glycolytic enzymes close to the flagellum would make the switch to a more glycolytic-dependent energy production a good strategy to increase and sustain sperm swimming speed during sperm competition (Ferramosca & Zara, 2014; Moraes & Meyers, 2018). However, as our analyses did not reveal any significant increase in sperm velocity (figure 4.3, 4.4, 4.s3), the question arises on the purpose of such strategy in DUI species only in the presence of eggs.

Although it will be important to extend the analysis to other SMI and DUI species to confirm our finding, we propose that the detected metabolic shift in DUI sperm (passing from a completely OXPHOS dependent energy production strategy towards a combined aerobic and anaerobic strategy) could reflect (i) the importance of the lactate shuttle mechanism and (ii) a potential programmed increase in Δψm of sperm mitochondria, just before the fertilization event (preliminary analyses on Δψm support this hypothesis, figure 4.s4). In turn, this could potentially allow for paternal mitochondria to escape the classic strict maternal inheritance and be inherited across generations (Knorre, 2020). Lactate is erroneously seen as a merely waste product of anaerobic glycolysis, and increasing evidence points towards the aerobic and anaerobic metabolism to be well linked, with lactate produced under fully aerobic conditions and readily oxidized in mitochondria (i.e. lactate shuttle mechanism) (Brooks et al., 1999; Kane, 2014). This mechanism has already been proven to be important in sperm metabolism and is supported by a sperm-specific mitochondrial LDH isoform in mammals (Brooks et al., 1999; Ferramosca & Zara, 2014; Gallina et al., 1994; Moraes & Meyers, 2018; Passarella et al., 2008; Storey, 2008; Storey & Kayne, 1977). Lactate uptake and oxidation in the mitochondrial intermembrane space have been proposed to (i) favour the import of pyruvate into the matrix, where it participates in the tricarboxylic acid cycle and (ii) actively contribute to the mitochondrial electrochemical gradient by releasing protons in the proximity of the inner mitochondrial membrane (Brooks et al., 1999; Kane, 2014). The mitochondrial membrane potential (Δψm) designates active mitochondria and its role in the preservation of the DUI paternal mitochondria has already been proposed (Milani, 2015). Potential support comes from the direct observation of a high Δψm in sperm mitochondria of DUI species (Milani & Ghiselli, 2015), and from a metabolic remodelling specific of DUI male mitochondria in line with the maintenance of a high electrochemical gradient (Bettinazzi et al., 2019b). Our results based on two distantly related DUI species support this hypothesis.

4. Conclusion

Linking the mitochondrial genotype to the phenotype is a complex endeavour. Given the deleterious effect that the uniparental inheritance of mitochondria could have for male fertility, the DUI system reflects an unprecedent opportunity for mitochondria to evolve adaptively for male functions. Our results highlighted a significant divergence in sperm performance and partially in energy metabolism strategy between DUI and SMI species. The paternal mtDNAs of both DUI species associate with sperm swimming slower and in a more curvilinear trajectory compared to sperm of SMI species, carrying maternally inherited mitotypes. In DUI species, this fitness trait could be under selection for male functions (e.g. potentially increasing the fertilization success due to a higher endurance, longevity or distance covered by male gametes). The analysis of the energy metabolism revealed that, in absence of egg chemical cues, DUI sperm strictly rely on OXPHOS to sustain their motility, whereas sperm of SMI species combined both aerobic and anaerobic pathways of energy production, although still relying mostly on aerobic metabolism. Our results highlighted not only the importance of OXPHOS for bivalve sperm motility, but also revealed how its specific importance could vary between DUI and SMI species. These results are congruent with previous finding of a rearranged mitochondrial metabolism characterizing the male mitotype in DUI species and with the prediction that a male-driven selection of mt encoded components for sperm function could favour OXPHOS. Remarkably, the detection of egg-derived chemoattractants produced a partial metabolic shift in the DUI sperm we tested, implying a combined strategy of energy production, whereas it did not affect the energy pathway equilibrium in SMI sperm. However, even with an increased importance of lactic fermentation in the presence of eggs, the OXPHOS still remain mandatory to sustain sperm movement in these species and no increment in sperm swimming speed was detected. We thus propose a potential alternative role of this metabolic shift involving a programmed increase of the mitochondrial membrane potential in DUI species following egg detection, linking lactic oxidation pathway of ATP production with paternal mitochondria preservation at fertilization.

As sperm mitochondria in DUI species are not an evolutionary dead-end, the overmentioned rearranged phenotype can reflect the selective forces driving the evolution of sperm mitochondria in the absence of SMI. The authors herein propose that a metabolic remodelling is indeed associated with the existence and adaptive value of paternal mitochondria inheritance and that these male-specific energetic adaptations in DUI species could reflect selection for both fertilization success and male mitotype preservation. Even though additional species need to be tested to confirm the trend found in the present study, these results based on five distantly-related species of bivalves point in that direction, providing a clear reference for future experiment to confirm this trend. Further investigations are definitively necessary to test the intriguing hypothesis of a link between male-specific mtDNA variants, sperm energetic adaptation, paternal mitochondria preservation and inheritance.

Data accessibility: The datasets supporting this article have been uploaded as part of the supplementary material.

Author's contributions: SBe carried out the lab work, data analysis, designed the experiment and drafted the manuscript; SN and AD participated in the lab work; LM and PUB supervised the study; SBr conceived, coordinated and supervised the study. All authors gave final approval for publication.

Competing interests: We have no competing interests.

Funding: This work was supported by the Fonds de Recherche du Québec - nature et technologie (to SBr), the Natural Sciences and Engineering Research Council of Canada (to PUB), and the Italian Ministry of Education, University and Research MIUR-SIR Programme (to LM).

Acknowledgements: We thank the guest editors for the invitation to contribute to the special issue and we are grateful to five anonymous referees who greatly contributed to improving this manuscript by providing thoughtful and constructive comments.

CHAPTER V – GENERAL DISCUSSION

1. Bivalves as a model for mitochondrial biology

Despite mitochondrial biology being a constantly growing field of research, unanswered questions remain. For example, little is known about: i) the adaptive value of non-neutral mtDNA variations, ii) the extent by which a female-driven evolution of mtDNA might impact male reproductive fitness, iii) the potential phenotypic result of a male-driven evolution of the mtDNA, iv) the impact of heteroplasmy upon mitonuclear coadaptation and cell fitness, v) the fitness criteria underlying mitochondria selection and how a faithful transmission of mitochondrial genetic information might be achieved.

In this project, I explored these questions in a non-model group of organisms, the bivalves, taking advantage of the coexistence within this taxon of two mechanisms of uniparental mitochondria transmission. That said, without following any phylogenetic pattern, some bivalve species undergo a strict maternal inheritance of their mitochondria (SMI species, characterized by the presence of a solely maternally derived mtDNA lineage), whereas some undergo doubly uniparental inheritance of mitochondria (DUI species, in which two sex-specific mtDNA lineages coexist and associate with different gametes). As uncommon systems represent unique opportunities to unveil aspects that might be otherwise eclipsed, I specifically exploited the naturally heteroplasmic DUI system to investigate mitochondrial biology, preservation and inheritance, mitonuclear interactions, heteroplasmy and ageing. The results stemming from this PhD project provide a clear evidence of a robust link between the mitochondrial genotype and phenotype in SMI and DUI bivalve species. Specifically, I evaluated mitochondrial and cellular functions associated with sex-linked mtDNA variants in DUI species, compared homoplasmic with heteroplasmic tissues, and compared DUI and SMI species. My results revealed a clear divergence between the groups in all the phenotypic aspects considered. A divergence that likely reflects the different sex-specific selective pressures acting on their respective mitochondria.

2. Strict maternal inheritance, female driven mtDNA evolution and the "division of labour" between gametes

Mitochondria in SMI species are strictly passed through generations by the only mother, providing the opportunity to test the mitochondrial and bioenergetic phenotype resulting from a female-biased evolution of mtDNA and its expression in different compartments, such as oocyte, sperm and somatic tissues. In this section, I discuss the findings on the four distantly related SMI species analysed during this PhD project (*M. arenaria*, *M. mercenaria*, *N. obscurata* and *P. magellanicus*). Even though more species must be examined, the phenotypic congruence among species support the intriguing idea that these results might represent the overall general rule in SMI bivalves.

(a) Female-driven evolution of mtDNA

Current explanations on why mitochondria are almost always transmitted only by the mother include the avoidance of uncontrolled heteroplasmy (and its potential deleterious effect on cell and organismal fitness), limitation of potential mito-nuclear conflicts, promotion of an optimal dual co-adaptation between mitochondrial and nuclear genes and the preservation of mitochondrial genetic integrity (Acton et al., 2007; Allen, 1996; Christie et al., 2015; Hadjivasiliou et al., 2012; Havird et al., 2019; Lane, 2011, 2012; Sharpley et al., 2012; Zhou et al., 2016). However, there is also a potential downside. Although promoting homoplasmy might be advantageous, the selective elimination of sperm mitochondria excludes males from contributing to the evolution of the mtDNA. The result is a female driven evolution of mtDNA in SMI species, which could even result in the fixation of mt variants with antagonistic effect on male fitness (i.e. "mother's curse") (Camus et al., 2012; Frank & Hurst, 1996; Gemmell et al., 2004; Innocenti et al., 2011; Montiel-Sosa et al., 2006; Nakada et al., 2006; Ruiz-Pesini et al., 2000).

At the level of mitochondrial functionality, the SMI species tested during this PhD project exhibit a conserved OXPHOS organization between their own gametes and soma (chapter II) (Bettinazzi et al., 2019b). Specifically, no difference exists in the balance between the activity of different ETS complexes, intended as their relative contribution to the maximum capacity of the respiratory chain. Furthermore, maternally derived mitochondria show an almost null control of the OXPHOS activity exerted by both the phosphorylation system and cytochrome *c* oxidase (figures 2.2*a,b*, 2.3*a,b*, 2.5). Beyond the expression of a common mitochondrial phenotype, the conserved organization of SMI energy metabolism is also discernible at the level of the general gamete bioenergetics (chapter III) (Bettinazzi et al., i*n prep*). Oocytes and sperm share the same bioenergetic organization, intended as the relative contribution of the different energy pathways composing the wider energy metabolism (e.g. glycolysis, anaerobic glycolysis, fatty acid metabolism, tricarboxylic acid cycle, ETS), with respect to the downstream oxidative capacity of cytochrome *c* oxidase (figures 3.3*a,b,c*). From an evolutionary point of view, the shared bioenergetic phenotype in SMI heterogametes can be potentially traced back to the common evolutionary path of maternally derived mitochondria. As predicted by a female-driven evolution of mtDNA, results indicate that mitochondria present in sperm, oocyte and soma of SMI species are qualitatively the same. Therefore, both gametes and somatic cells exploit the same mitochondrial phenotype, even exhibit a common regulation of the general cellular bioenergetics. Although expected, sharing a common bioenergetic phenotype might not be ideal for heterogametes. Due to gamete specialization, oocyte and sperm likely differ in energetic needs and experience different sex-specific selective pressures on their mitochondria (Allen, 1996). In SMI species, however, the mitochondrially-encoded components of the ETS cannot experience direct selection in males. In the end, males exploit female derived mitochondria, whose phenotype might not be adapted for spermatic functions (or at least not as well adapted as the mitochondrial phenotype specific of DUI sperm). Following the “mother’s curse” hypothesis, this could potentially cause a reduction of sperm performance and male reproductive fitness (Gemmell et al., 2004). Even though speculative, it is possible that SMI sperm might need to compensate for a reduced or compromise OXPHOS activity that has been primarily tuned up for female-related functions. Potential support comes from the fact that SMI sperm (bearing maternally derived mitochondria) concurrently rely on both aerobic and anaerobic pathways to fuel their motility, whereas DUI sperm (whose mt encoded components of the OXPHOS evolved under male-specific pressure) wholly rely on OXPHOS to sustain their motility (figures 4.2, 4.3). Overall, our results based on the mitochondrial phenotype are altogether in line with an evolution of sperm mitochondrial bioenergetics in SMI species dictated by female-specific selective constraints, and somewhat with the predictions stemming from the “mother’s curse” hypothesis. However, more species will have to be tested to clearly confirm the trend observed.

(b) Gamete specialization and mtDNA preservation

Conversely to the inevitably deteriorating soma, the germline must serve as a genetic template for the future generations. The selection of viable mitochondria likely takes place in the female germline (Fan et al., 2008; Hill et al., 2014; Stewart et al., 2008) and appears to be linked with the mitochondrial functional state. On the one hand, mitochondria can be selected because highly active. This mechanisms is suggested to altogether exploit i) the active segregation of organelles characterized by a high Δψm into the so-called mitochondrial cloud (Balbiani body), where their mtDNA undergo selective replication (Hill et al., 2014; Tworzydlo et al., 2020; Zhou et al., 2010), and ii) the discrimination of dysfunctional mitochondrial units through fusion and fission events and subsequent elimination through mitophagy (Jin et al., 2010; Jin & Youle, 2012; Knorre, 2020; Sekine & Youle, 2018; Twig et al., 2008; Westermann, 2010; Youle & van der Bliek, 2012). On the other hand, given the link between OXPHOS activity and oxidative stress, functionally silenced mitochondria might preserve an undamaged genetic template and, for that reason, might be preferentially transmitted to the future generations. For example, in some species the transmission of undamaged mitochondria might be achieved by the "division of labour" between gametes (Allen, 1996; Allen & de Paula, 2013). Briefly, while sperm would take advantage of OXPHOS for motility, likely experiencing a higher oxidative stress on their mtDNA, eggs would preserve quiescent and undamaged subpopulations of mitochondria to be passed. The selective elimination of sperm mitochondria by strict maternal inheritance would then complete the circle, limiting the spread of potentially damaged mt genomes and genetic integrity. Even though this hypothesis is currently challenged by the lack of evidence on how "less active" mitochondria might be selected instead of the highly active ones (Ghiselli et al., 2018; Hill et al., 2014; Knorre, 2020; Milani, 2015; Milani & Ghiselli, 2015; Monaghan & Metcalfe, 2019; Tworzydlo et al., 2020), evidence in both invertebrates and vertebrates support the existence, at least in some species, of quiescent subpopulations of mitochondria in oocytes (de Paula et al., 2013a; de Paula et al., 2013b; Faron et al., 2015; Kogo et al., 2011).

In SMI bivalves, the mitochondrial phenotype and the general pattern of regulation of metabolic pathways do not vary between gametes (oocyte and sperm express a common bioenergetic phenotype), but differences exist in the magnitude of enzyme activities (chapter III) (Bettinazzi et al., *in prep*). Specifically, SMI sperm are characterized by a generally higher capacity of key enzymes of the energy metabolism compared to oocytes. This includes the activity (normalized for either the capacity of citrate synthase or the content of proteins) of pyruvate kinase (glycolysis), lactate dehydrogenase (anaerobic glycolysis), palmitoyl carnitine transferase (fatty acid metabolism), citrate synthase and malate dehydrogenase (tricarboxylic acid cycle), complexes

I, III and IV (electron transport system) (figures 3.1, 3.2, 3.s3). The contrasting bioenergetics between anisogamous gametes in SMI bivalves makes the results partially in line with the predictions stemming from the “division of labour” hypothesis. On the one hand, the higher metabolic capacity of SMI sperm might reflect the high need of ATP to sustain their motility, maximize their performance and likely increase the fertilization success. This suggestion is also supported by the results on sperm performance (chapter IV) (Bettinazzi et al., 2020), i.e. that SMI sperm concurrently exploit OXPHOS and anaerobic glycolysis to sustain a swimming behaviour characterized by ATP-dispendious traits such as high speed and straighter trajectory (figures 4.1, 4.2, 4.3, 4.4). On the other hand, the lower bioenergetic capacities of oocytes potentially reflect the need to preserve the genetic integrity of maternally transmitted mitochondria. Although downregulated when compared with sperm, oocyte mitochondria in these bivalve species are not inactive, which is to say, they do perform OXPHOS (figures 2.2, 2.5; 3.1, 3.s3). We have to keep in mind that the mitochondrial activity in oocytes may vary according to the maturation of gametes (Ge et al., 2012; Milani & Ghiselli, 2015; Ramalho-Santos et al., 2009; Van Blerkom, 2011) and that a putative “division of labour” might not necessarily involve the entire population of mitochondria within an egg (Allen & de Paula, 2013). According to that, a possible explanation for the low (but not null) bioenergetic capacity in oocytes might be the presence of a heterogeneous population of both oocytes and mitochondria, expressing a variable mitochondrial activity. That said, it is conceivable that: i) oocytes at different stages of maturation composed the samples analysed, ii) different subpopulations of mitochondria are present within each oocyte, one active and one quiescent, respectively fulfilling the energetic and template functions (Allen, 1996; Allen & de Paula, 2013). However, further experiments are needed to confirm these hypotheses. Finally, support to a “division of labour” in SMI gametes also comes from the analysis of the antioxidant capacity of gametes (figure 3.1*g*). In opposite trend to the activity of enzymes linked with the energy metabolism, the antioxidant enzyme catalase is found upregulated in oocytes compared to sperm. Even though the analysis of the antioxidant capacity should be extended to other enzymes (e.g. SOD, GPX), this result suggests that oocytes might possess and improved ability to regulate ROS and mitigate the potentially associated oxidative damage, in turn supporting the intriguing idea that bioenergetic specialization in SMI gametes might play a role in the transmission of undamaged mitochondrial template across generations.

Overall, our results on species characterized by a strict maternal inheritance of mitochondria suggest that: i) qualitatively, oocytes, sperm and somatic cells express of a common mitochondrial phenotype and pattern of regulation between metabolic pathways, ii) sperm exploit a combined aerobic/anaerobic form of energy production to swim in a fast and straight fashion, iii) quantitatively, sperm have a higher bioenergetic capacity than oocytes but a lower capacity to mitigate the oxidative stress (figure 5.1). Although speculative, I propose that these findings altogether might reflect the phenotypic results of a female-driven evolution of mtDNA upon gamete and soma bioenergetics, the different energetic needs of heterogametes and a potential mechanism of mitochondrial DNA preservation based on gamete bioenergetic specialization.

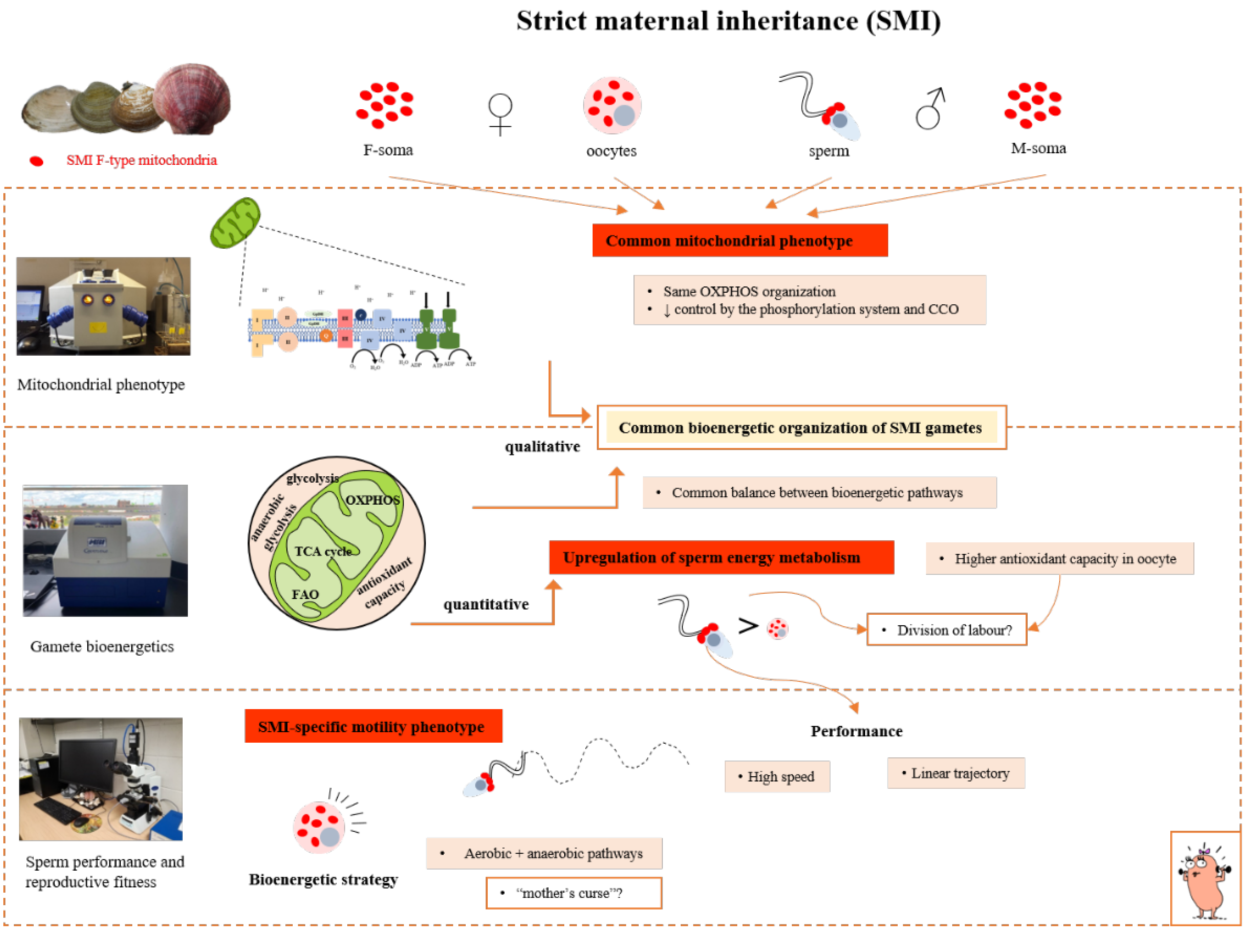


Figure 5.1. Schematic representation of the results on SMI species.

3. Doubly uniparental inheritance, insights into the adaptive value of a naturally heteroplasmic system

Conversely to SMI species, DUI species are characterized by the presence of two highly divergent and sex-linked mitochondrial lineages (the F- and the M-type), which are transmitted separately by heterogametes. Overall, female individuals are homoplasmic for the F-type mtDNA in both oocytes and somatic tissues, whereas male individuals are heteroplasmic. Sperm bear the only M-type mtDNA, while in male soma the two lineages can coexist (figure 1.2).

In line with the starting hypothesis that different selective pressure acting on DUI mitochondrial variants would promote female- and male-specific energetic adaptations, our results provide evidence that the divergent evolution of DUI mt genotypes links with extensive phenotypic variation at the level of i) mitochondrial functionality (chapter II and III), ii) cellular bioenergetics (chapter III) and iii) sperm performance (chapter IV). These phenotypic variations appear to be conserved among the three distantly related DUI species analysed (*M. edulis*, *A. islandica* and *R. philippinarum*), supporting the idea that convergent selective forces might drive the evolution of the DUI sex-linked mtDNA variants in different species (figure 5.2).

(a) Sex-specific mtDNA evolution and its impact upon gamete bioenergetics

In contrast to SMI species, the phenotypic consequence of DUI sex-specific mtDNA variations entails extensive qualitative and quantitative changes in mitochondrial and cellular bioenergetics (chapter II and III).

At the level of mitochondrial functionality and organization, maternally derived mitochondria (bearing the DUI F-type mtDNA) in oocyte and female homoplasmic soma display a common "female mt phenotype", whereas paternally derived mitochondria (bearing the DUI M-type mtDNA) exhibit a refashioned "male mt phenotype" in sperm. In accordance with their heteroplasmic condition, male somatic tissues bearing both mitochondrial lineages display a halfway mt phenotype between the two (figures 2.2, 2.3). Specifically, the OXPHOS remodelling characterizing M-type mitochondria entails i) a strong limitation of the ETS by the phosphorylation system (as indicated by the low coupled/uncoupled respiratory rates) and ii) a minimal spare capacity of cytochrome *c* oxidase with respect to the upstream complexes (indicated by the null excess capacity of CCO over the ETS) (figures 2.1, 2.2, 2.3, 2.5). The same stoichiometric reorganisation of electron transport components of M-type mitochondria (revealed through high resolution respirometry) is also supported by the ratios of enzyme activities over CCO (measured spectrophotometrically) (figure 3.3). Overall, for all three distantly related DUI species, compared to the F-type mitochondria in oocytes, the activity of cytochrome *c* oxidase in sperm M-type mitochondria is limiting with respect to the upstream respiratory complexes I and III of the ETS, as well as with respect to key enzymes of glycolysis and tricarboxylic acid cycle. This architecture potentially ensures an appropriate regulation of M-type mitochondria, providing extensive control upon the respiratory process at the terminus of the chain (i.e. at the level of both CCO and ATP-synthase complexes). In turn, these regulatory properties potentially depict mitochondria with a heightened sensibility to oxygen content in the medium (Gnaiger et al., 1998; Verkhovsky et al., 1996), and with the ability to cope with a high reducing state of their ETS complexes, which further links with higher ROS flux and the capacity to sustain a high membrane potential (Blier et al., 2017; Harrison et al., 2015; Korshunov et al., 1997; Kucharczyk et al., 2009).

The phenotypic consequence of carrying sex-specific mitochondrial variants is also perceived quantitatively at the level of the general gamete bioenergetics (chapter III) (Bettinazzi et al., *in prep*). In contrast to gametes of SMI species (whose sperm have a higher enzymatic capacity relatively to eggs), sperm of DUI species (carrying M-type mitochondria) display an adjustment of their bioenergetics towards a general metabolic depression when compared to oocytes (carrying F-type mitochondria) (figures 3.1, 3.s2, 3.s3). Even though a change in OXPHOS yield was somehow expected by the fact that the DUI sex-linked mitochondria qualitatively differ (i.e. DUI paternal mitochondria in sperm express a remodelled "male mitochondrial phenotype"), the depression of sperm bioenergetics not only involved the mitochondrial respiration (activity of respiratory complexes I + III and IV) (figure 3.1*e,f*), but also upstream bioenergetic pathways such as glycolysis (pyruvate kinase/CS activity) (figure 3.1*a*), anaerobic glycolysis (lactate dehydrogenase/CS activity) (figure 3.1*b*), fatty acid metabolism (carnitine palmitoyl transferase/CS activity) (figure 3.1*c*) and tricarboxylic acid cycle (malate dehydrogenase/CS activity) (figure 3.1*d*). For what concern the OXPHOS, a previous research also revealed a lower catalytic capacity of CCO in *M. edulis* sperm carrying the M-type mitochondria relative to sperm of the same species carrying the F-type mitochondria (i.e. following "masculinization" events) (Breton et al., 2009). Most interestingly, conversely to SMI sperm, which exploit both aerobic and anaerobic metabolism, DUI sperm motility is completely dependent on this remodelled OXPHOS (chapter IV) (Bettinazzi et al., 2020).

The DUI system represents an exclusive occasion for the mtDNA to break the classic evolutionary constraints and adapt separately for female and male functions. The two lineages entirely constitute the genetic landscape (mitochondrially speaking) of the respective gamete, and thus evolve under different (potentially antagonistic) sex-specific selective forces (Breton et al., 2007). Footprints of this divergent evolution can be found in the extreme divergence between the two mt lineages (ranging from 8 to 50% of nucleotide divergence depending on the species and the gene examined), as well as in specific DUI feature such as the presence of sex-specific additional genes (Bettinazzi et al., 2016; Breton et al., 2007; Breton et al., 2014; Breton et al., 2011; Capt et al., 2020; Passamonti & Ghiselli, 2009; Zouros, 2012). Whether the highly divergent male mitochondrial lineage in DUI species may simply represent a form of mutational load is a current debate (Ghiselli et al., 2018; Speijer, 2016). Specifically, it has been proposed that the presence of a potentially sub-functional mt genome might be tolerated by DUI sperm by relying on bioenergetic pathways other than mitochondrial respiration and/or relying on external forces (e.g the female incurrent syphon) to fertilize. However, accumulating evidence show that it could not be the case in bivalves. Even though highly divergent, the M-mt genome in DUI species appears to be functionally preserved. It replicates and its own genes are successfully transcribed and translated to proteins (Breton et al., 2017; Breton et al., 2011; Capt et al., 2019; Ghiselli et al., 2018; Ghiselli et al., 2013; Guerra et al., 2016; Milani & Ghiselli, 2015; Milani et al., 2014a; Milani et al., 2014b; Milani et al., 2015). Recent evidence on the DUI species *R. philippinarum* revealed well-formed cristae and a high mitochondrial membrane potential in mitochondria of both sperm and eggs (Milani & Ghiselli, 2015). As such, it is reasonable to think that the high amino acid divergence between the DUI mtDNA variants could produce change at the level of the energy phenotype, potentially fostering male- and female-specific bioenergetic adaptations (Breton et al., 2007; Burt & Trivers, 2006). A potential difference in the functioning of F and M-type DUI mitochondria was proposed by the comparison of the COX I protein in four DUI species through bioinformatic tools. Briefly, the authors found difference in the aminoacid properties of F and M-proteins with potential implication in ATP production, mitochondrial membrane potential and spermatic functions (Milani & Ghiselli, 2020; Skibinski et al., 2017). However, until now, no evidence existed that the high divergence between mtDNA lineages might really translate into difference at the level of mitochondrial physiology.

Our findings provide additional indication of the extent by which non neutral variations in mitochondrially encoded genes could affect the structural and biochemical properties of respiratory complexes, in the end the functioning of the OXPHOS machinery. In line with the prediction that different selective pressures on DUI mitochondria would have repercussion on mitochondrial functionality and potentially promote sex-specific energetic adaptation, our data link sex-specific mtDNA variation in DUI species with the expression of a remodelled mitochondrial phenotype in different gametes and somatic cells. Moreover, the metabolic remodelling specific of sperm mitochondria potentially represents a unique example of mitochondrial phenotype resulting from a male-driven evolution of mtDNA. Finally, the fact that distantly related DUI species (*A. islandica*, *M. edulis* and *R. philippinarum*) share a common reorganization of mitochondrial and cellular bioenergetics between eggs and sperm (as well as similar sperm performance), supports the intriguing idea of a convergent evolution of sex-linked mtDNAs for the DUI system.

(b) Adaptive value of a male-specific bioenergetic remodelling

The DUI-specific metabolic reorganization is shared among species that largely differ in terms of habitat, life-history traits and strategies. Due to the experimental design, all DUI species: i) pertain to different taxa, ii) reflect an independent origin of the DUI system, iii) do not share the same habitat, iv) have a longevity that range from few years to centuries. Specifically, M. edulis (Family Mytilidae) is an intertidal mussel that lives attached to the substrate, was collected in the Atlantic Ocean and has a MRL of ~18 years. R. philippinarum (Family Veneridae) is an intertidal burrowing clam, collected in the Pacific Ocean with a MRL of ~15 years. A. islandica (Family Arcticidae) is a subtidal burrowing clam from the Atlantic Ocean, with a MRL of ~507 years (Humphreys et al., 2007; Munro & Blier, 2012; Munro et al., 2013; Sukhotin et al., 2007). A rational indication is that any adaptive value of their shared metabolic remodelling might potentially link with shared traits among the three DUI species here analysed. These are, most notably, the reproductive strategy (i.e. gonochoric, broadcast-spawning sessile marine bivalves) and the ability to preserve sperm-derived mitochondria from destruction during fertilization.

(b1) Sperm performance and reproductive fitness

Given the strict association of M-type mitochondria with sperm in DUI species, a rational indication is that selection acting on this genome might foster male-specific bioenergetic adaptations with downstream impact upon sperm fitness (Breton et al., 2007; Breton et al., 2009; Burt & Trivers, 2006). In chapter IV, I explored the repercussion of bearing either a maternally or paternally derived mitochondria upon the sperm motility phenotype in the DUI species *M. edulis* and *R. philippinarum* and the SMI species *M. mercenaria*, *N. obscurata* and *P. magellanicus*. Intriguingly, beyond their particular bioenergetics, DUI sperm also exhibit a readapted motility phenotype. On the one hand motility in SMI sperm (carrying maternally inherited mitochondria), is sustained by both aerobic and anaerobic metabolism and is characterized by a fast and straight swim behaviour. On the other hand, DUI sperm (carrying paternally derived mitochondria) completely rely on the energy produced through OXPHOS to sustain their motility and are characterized by swimming at a lower speed and in a more circular fashion (figure 4.1). The striking difference in sperm performance suggests that different selective forces are acting in these two bivalve groups, potentially because of the different mitochondrial transmission mechanisms. Similar results were found in previous researches on *M. edulis*, where the authors compared the performance of sperm carrying either the M-type or the F-type mtDNA (i.e. intraspecific analysis, comparing “classic” vs “masculinized” sperm). Again, contrary to the expectation that bearing a paternally derived mitochondrial lineage would provide benefit in terms of speed, sperm with M-type mitochondria swim equally or even slower than “masculinized” sperm carrying the F-type (Everett et al., 2004; Jha et al., 2008; Stewart et al., 2012). Given the ATP-dependence of the flagellar movement (Moraes & Meyers, 2018), the DUI-specific sperm performance potentially links with a reproductive strategy that does not require the overexploitation of the energy metabolism. Interestingly, this is in line with the remodelled bioenergetics of DUI sperm (chapter II and III). Even though speculative, the here presented results suggest a link between a male-specific evolution of mtDNA, male-specific bioenergetic adaptation (OXPHOS reorganization and general metabolic depression of DUI sperm), sperm performance and potentially fertilization success. The question arises on whether such traits might be beneficial for male reproductive fitness.

Although one could expect a positive relationship between speed and fertilization rate, rapidity itself is just one of many key traits of sperm motility and fitness (Breton et al., 2007;

Everett et al., 2004; Jha et al., 2008). Depending on the fertilization strategy adopted, a decreased sperm speed might even be beneficial for sperm fitness in some organisms. Swimming slowly and in tight circles might underpin a strategy linked with energy preservation and increased gamete endurance. Enduring sperm that swim slowly and in tight circles would potentially cover a bigger area, increasing the chances to encounter female gametes and successfully reproduce in turbulent marine environment (Eads et al., 2016). For instance, evidence exists in the sea urchin *Lytechinus variegatus*, that sperm velocity and longevity trade off each other and influence fertilization (Levitan, 2000). In the DUI species *Mytilus galloprovincialis*, the highest fertilization rate is achieved by sperm swimming in a pronounced curvilinear fashion and with a high angle change rate (Fitzpatrick et al., 2012; Liu et al., 2011). Overall, in sessile broadcast spawning animals (such as bivalves), sperm fitness traits like slower speed and circular trajectory may indeed be beneficial for the reproductive success, altogether fostering endurance, longevity, fertilization rate and area covered by sperm.

Sperm chemoattraction is an important process that contributes to gamete encounter and thus fertilization success. Once detecting oocyte-derived chemical cues, the processes of chemotaxis and chemokinesis simultaneously cause a change in swimming direction and steady-state speed of sperm, finally promoting the accumulation of sperm around oocytes (Eisenbach & Giojalas, 2006; Riffell et al., 2004). In the DUI species *Mytilus galloprovincialis*, evidence suggests that sperm chemoattraction not only produce change in sperm swimming behaviour (i.e. sperm start swimming faster and straighter towards the oocytes) (Eads et al., 2016; Evans et al., 2012), but can even moderate mate choice (i.e. sperm preferentially swim towards the most genetically similar oocytes at the level of the mtDNA, but least similar at the nuclear level) (Lymbery et al., 2017; Oliver & Evans, 2014). However, whether bivalve sperm do change their performance after detecting eggs is still controversial. A previous research on *M. edulis* did not reveal any increase in sperm velocity following egg detection nor under sperm competition (Stewart et al., 2012). In line with that, the results of chapter IV (based on five different bivalve species, DUI and SMI) show no change in terms of speed and swimming trajectory following the addition of oocyte-derive chemoattractants. Although we revealed no change in sperm performance, what oocyte detection produces is a partial switch in DUI sperm bioenergetic strategy (figures 4.2, 4.3, 4.4). After detecting eggs, DUI sperm of *M. edulis* and *R. philippinarum* pass from a completely OXPHOS dependent strategy of energy production to a more mixed strategy, including (even though in minimal part) the activity of the lactate pathway.

One potential reason for the switch to a more glycolytic-dependent strategy of energy production is the need to suddenly increase swimming speed after egg-detection (sperm chemokinesis). While the ATP produced by OXPHOS must diffuse from the sperm midpiece (where the mitochondria are located in sperm) to the flagellum to fuel its beat, the colocalization of glycolytic enzymes with the dynein ATPases in the principle piece of the flagellum would support a rapid production, diffusion and consumption of ATP, in turn representing a good strategy to increase the swimming speed following egg-detection (du Plessis et al., 2015; Ferramosca & Zara, 2014; Moraes & Meyers, 2018). Although unclear in the species we tested, this is potentially the case for *M. galloprovincialis* (Evans et al., 2012). Another interesting possibility includes the increased importance of the lactate shuttle mechanism (Brooks et al., 1999). More than being a waste product of anaerobic glycolysis, increasing evidence suggests that lactate might be produced under fully aerobic condition, imported into the mitochondria and readily oxidized back into pyruvate. This process takes place in the intermembrane space and is proposed to contribute to the mitochondrial metabolism in two concurrent ways. First, lactate oxidation releases protons in the intermembrane space which contribute to establishing the mitochondrial electrochemical gradient. Concurrently, the resulting pyruvate is imported into the mitochondrial matrix where it participates in the tricarboxylic acid cycle after undergoing oxidative decarboxylation (Brooks et al., 1999; Kane, 2014). Interestingly, the process of lactate conversion to pyruvate within the mitochondria appears to be particularly important in sperm energy metabolism. For instance, exogenous lactate is efficiently used by stallion sperm mitochondria and, as its concentration increases so does mitochondrial respiration and sperm motility (Darr et al., 2016; Moraes & Meyers, 2018). Moreover, sperm-specific mitochondrial LDH isoforms are present in various mammals, including rabbits, equines and humans (e.g. LDH-X isoform in human sperm) (Brooks et al., 1999; Ferramosca & Zara, 2014; Gallina et al., 1994; Moraes & Meyers, 2018; Passarella et al., 2008; Storey, 2008; Storey & Kayne, 1977; Swegen et al., 2015).

It may be possible then that the bioenergetic switch in DUI sperm following oocyte-detection might altogether reflect the importance of the lactate shuttle in bivalve sperm metabolism and a peculiar “strategy” by which paternal mitochondria might increase their membrane potential just before fertilization. Accordingly, a concomitant increase in membrane potential after egg detection is supported by preliminary analysis on *M. edulis* (figure 4.s4), and specific properties of M-type mitochondria OXPHOS are also in line with the maintenance of a high electrochemical gradient (chapters II and III). Although speculative at this stage, the capacity to maintain a high mitochondrial membrane potential, even increasing it prior to fertilization, appears to be an important feature of M-type mitochondria, with a potential intriguing role in mitochondria preservation, selection and transmission.

(b2) Paternal mitochondria selection and transmission

The mechanism by which DUI sperm mitochondria are preserved and transmitted across generations is still unknown. Hypothetically, one mechanism by which paternally derived mitochondria might be preserved could be the relaxation of the mitophagy process in male individuals. Recent evidence, however, did not revealed any change in the expression of genes linked with mitophagy between M and F gonads of the DUI species *Utterbackia peninsularis* and *R. philippinarum* (Capt et al., 2019; Punzi et al., 2018). This suggests that other mechanisms should ensure the preservation and inheritance of sperm mitochondria, and these mechanisms could exploit mitochondrial performances.

In contrast with the idea that quiescent mitochondria might be preferentially inherited because genetically preserved (Allen, 1996; Allen & de Paula, 2013), selection may also favour the transmission of highly active (and thus functional) mitochondria (Tworzydlo et al., 2020). The $\Delta\psi m$ is a trait that indicates an active respiratory machinery, and several mechanisms involved in the process of mitochondrial selection rely on the electrochemical gradient to discriminate between functional and dysfunctional mitochondria. For example, the transmission of a functional subset of mitochondria to the female germline appears to rely on the active transport of mitochondria with high $\Delta\psi m$ to the Balbiani body, where they undergo selective replication (Fan et al., 2008; Hill et al., 2014; Milani, 2015; Tworzydlo et al., 2020; Zhou et al., 2010). Likewise, the process of elimination of dysfunctional mitochondria also exploit the $\Delta\psi m$. Evidence suggests that the intracellular mitochondrial quality control mechanism accounts on both mitochondrial dynamics (i.e. fusion and fission event) and mitophagy to: i) exclude depolarized mitochondria from the mitochondrial network (i.e. after fission, mitochondria with low $\Delta\psi m$ have less chance to refuse) and ii) selectively eliminate them (i.e. isolated and depolarized mitochondria are preferentially targeted by the mitophagy machinery) (Jin et al., 2010; Jin & Youle, 2012; Sekine & Youle, 2018; Twig et al., 2008; Westermann, 2010; Youle & van der Bliek, 2012). Because mitochondria selection highly relies on Δψm, hypothetically, any deleterious mutations in the mtDNA that translate in an increased Δψm might be selected, no matter if beneficial or not. This is the potential case of mutation affecting ATP-synthase and resulting in its partial inhibition (Knorre, 2020).

The ability to maintain a high Δψm might thus determine the fate of a specific mitochondrion and, even though speculative at this stage, accumulating evidence suggests that this could be the case in DUI species (Milani, 2015). The metabolic remodelling specific of DUI M-type mitochondria of *A. islandica*, *R. philippinarum* and *M. edulis* entails a limitation at the level of both cytochrome *c* oxidase and ATP-synthase, conferring the ability to DUI paternal mitochondria to preserve a high Δψm (chapters II and III) (Bettinazzi et al., 2019b); Bettinazzi et al., *in prep*). Moreover, sperm mitochondria of *R. philippinarum* and *M. edulis* are active, express a high membrane potential (Milani & Ghiselli, 2015), and even appear to have the ability to increase their Δψm following oocyte detection (chapter IV) (Bettinazzi et al., 2020). These evidences altogether foster the intriguing idea that maintaining a high Δψm might promote the preservation of paternal mitochondria in DUI species.

Overall, our findings support the idea that the DUI system may represent an elegant strategy for mitochondria and their genome to evolve adaptively for male-functions. Specifically, I propose that the adaptive value of the DUI system potentially involves the expression of male-specific bioenergetic adaptions with potential downstream repercussion on both sperm fitness and paternal mitochondria selfish transmission.

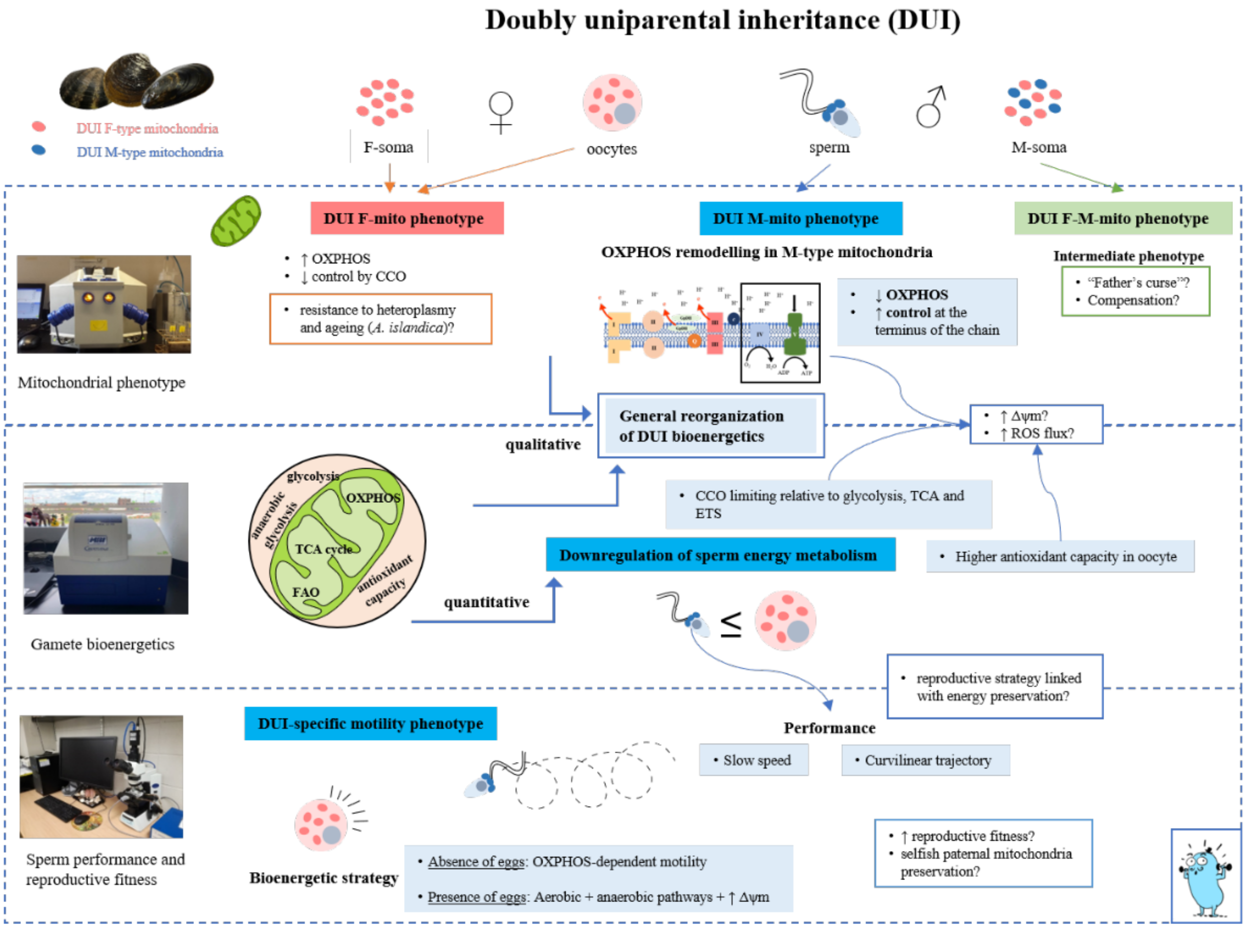


Figure 5.2. Schematic representation of the results on DUI species.

(c) DUI, the good and the bad

DUI sperm mitochondria are no more an evolutionary dead end and that provides the unprecedented opportunity for sperm mitochondria and their genome to undergo male-specific evolution. However, "all that glitters is not gold", and the coexistence of two mitochondrial lineages possibly entails a cost in terms of cell fitness. That said, it brings i) the need for sperm mitochondria to preserve their own genetic integrity and ii) the need of coevolution between three different genomes, the nuclear one and two highly divergent mitochondrial genomes (deal with heteroplasmy).

(c1) Genetic integrity

In the DUI system, both M- and F-type mtDNA lineages need to be preserved functional for the next generations, challenging the concept of 'division of labour' between gametes (Allen, 1996; Allen & de Paula, 2013; de Paula et al., 2013a; de Paula et al., 2013b). It is thus uncertain how (and if) DUI species might prevent oxidative stress on both mitochondrial lineages and transmit undamaged templates to offsprings.

Knowing the link between OXPHOS and ROS production, it has long been debated whether the persistence of a highly divergent paternal mitochondrial lineage in DUI species might be explained by the absence of mitochondrial activity in sperm (Ghiselli et al., 2018; Speijer, 2016). Bivalves are quite flexible in terms of energy metabolism, exploiting different energy pathways such as aerobic respiration, anaerobic respiration (i.e. malate-dismutation pathway in *M. edulis*), and anaerobic glycolysis through both lactate and opine pathways (Boulais et al., 2019; Boulais et al., 2015; Dando et al., 1981; de Zwaan & Wijsman, 1976; Donaghy et al., 2015; Lee & Lee, 2011; Muller et al., 2012). It has thus been proposed that DUI sperm could minimize oxidative stress by relying on pathways alternative to OXPHOS to sustain their motility (Ghiselli et al., 2018; Ghiselli et al., 2013; Milani & Ghiselli, 2015; Speijer, 2016). However, this does not appear to be the case. DUI sperm highly rely on their remodelled OXPHOS and, contrary to the expectations, the bioenergetic properties here described are in line with a high ROS flux, in turn with an increased risk of oxidative damage to sperm mitochondria.

An increased capacity of the respiratory system downstream the complexes that produce most ROS (e.g. an increased proportion of either CCO and ATP-synthase compared to complexes I and III) has been proposed to promote a sharp thermodynamic gradient, in turn reducing ROS flux and even promote longevity (Blier et al., 2017). Evidence also exists that CCO is one of the main targets for respiratory dysfunction during ageing and, while its activity declines, the oxidative stress increases (Petrosillo et al., 2013; Ren et al., 2010). For instance, the longest living animal recorded so far (determined lifespan: up to 507 years), the DUI species *A. islandica,* is characterized by i) increased peroxidation resistance in mitochondrial membranes and low H_2O_2 production in the soma when compared to other short-lived bivalve species (Munro & Blier, 2012; Munro et al., 2013) and ii) a huge surplus capacity of cytochrome *c* oxidase in female somatic cells.

This high excess capacity at the end of the respiratory chain is specific of DUI F-type mitochondria (figure 2.5) (Bettinazzi et al., 2019b). Conversely, the OXPHOS remodelling of M-type mitochondria implies i) a strong limitation of the ETS by means of ATP-synthase, and ii) a change in the stoichiometry of respiratory complexes towards a null excess capacity of CIV with respect to the upstream ETS complexes (figures 2.2, 2.5; 3.3) (Bettinazzi et al., 2019b); Bettinazzi et al., *in prep*). A limited activity of the ATP-synthase results in a lower proton influx to the matrix, thus in the maintenance of a high intermembrane potential. In turn, a slowed "proton cycle" exerts a negative feedback on ETS activity, with consequent high reducing state stored in its complexes, increased electron leakage and ROS production (Blier et al., 2017; Brand, 2000; Harrison et al., 2015; Korshunov et al., 1997; Kucharczyk et al., 2009; Turrens, 2003). Accordingly, dissipating the proton gradient through the action of uncoupling agents may help to minimize ROS production, oxidative damage and even the process of ageing (i.e. "uncoupling to survive" theory) (Brand, 2000). Overall, the architecture of M-type mitochondria indicates that DUI sperm might suffer an increased oxidative stress. Additional support comes from the low activity of the enzyme catalase (reflecting the antioxidant capacity) in sperm compared to oocytes (figure 3.1g) (Bettinazzi et al., *in prep*). However, catalase is just one antioxidant enzyme and the logical prediction is that sperm may display an array of antioxidant mechanism to defend themselves from ROS. In humans for example, one out of ten proteins in sperm have been found to be linked with antioxidant activity (Martínez-Heredia et al., 2006) and even the seminal fluid has a high antioxidant capacity (Dowling & Simmons, 2009). Interestingly, mice testes express a specific isoform of cytochrome *c* (T-Cc). Compared to the somatic counterpart (S-Cc), T-Cc catalyses the reduction of ROS three times faster and is even more resistant to ROS-mediated degradation (Liu et al., 2006). It would be interesting to investigate whether DUI sperm might have similar strategies to reduce the potential oxidative stress.

When left uncontrolled, excessive ROS production can have a deleterious effect upon sperm structure and function. Oxidative stress may result in lipid peroxidation, loss of $\Delta\psi m$, OXPHOS disruption, reduction of motility and apoptosis (Amaral et al., 2013; Moraes & Meyers, 2018; Sanocka & Kurpisz, 2004). However, as mentioned in the introduction, the presence of ROS is not always deleterious, and a mild oxidative stress may play an important role in sperm physiology. For example, the bio-positive effect of ROS on human ejaculates includes the induction of hyperactivation, capacitation and acrosome reaction (de Lamirande & Gagnon, 1993; de Lamirande et al., 1998; de Lamirande et al., 1997; Sanocka & Kurpisz, 2004).

Whether beneficial or not, counteracted or not, a high ROS flux in DUI sperm is just an indirect prediction based on OXPHOS reorganization and remains to be verified empirically. A high ROS production can even be the price to maintain a high membrane potential, potentially a side-effect of paternal mitochondria preservation in DUI species. Further investigations are thus needed to characterize ROS production, oxidative damage on macromolecules and antioxidant capacity in gametes of DUI species.

(c2) Implications for heteroplasmy

The main advantage for the almost universal uniparental inheritance of mitochondria in animals has been proposed to be the avoidance of the genetic instability and fitness loss deriving from uncontrolled heteroplasmy (Christie et al., 2015; Hadjivasiliou et al., 2012; Lane, 2011, 2012). As exemplified by evidence in both invertebrates and vertebrates, the presence of different mitochondrial DNAs within the same cell (i.e. heteroplasmy) has a potential deleterious effect upon cell fitness (Acton et al., 2007; Lane, 2012; Sharpley et al., 2012; Zhou et al., 2016). One potential reason for this is that mixing different mitochondria might generate cytonuclear incompatibility between the nuclear genes and two different set of mitochondrial genes (Hadjivasiliou et al., 2012). Disrupting the optimal dual mito-nuclear coadaptation might in turn impact OXPHOS functioning and organismal fitness (Lane, 2011, 2012). The selectively removal of paternally derived mitochondria carried by sperm is thus adaptive, as it promotes homoplasmy and mitonuclear match in the new-born. However, there are some exceptions like DUI. Although each mt lineage is strictly associated with a different gamete (M-type in sperm while F-type in oocytes), they sometimes coexist in somatic tissues of males (Breton et al., 2007; Passamonti & Ghiselli, 2009; Zouros, 2012). The question arises then on how the presence of two highly divergent mt lineages, even though potentially adaptive for gamete-specific functions, might be tolerated in the soma of DUI bivalves.

Some hypothetical and non-mutually exclusive mechanisms that could allow DUI species to manage this possibly harmful situation (deal with heteroplasmy) are: i) the alternative splicing of nuclear-encoded mitochondrial genes to produce isoforms that could efficiently interact with the two highly distinct haplotypes; ii) promoting nuclear heterozygosity and the presence of alleles specifically adapted to different mitotypes (Breton et al., 2017), and iii) silencing or downregulating the M genome in heteroplasmic somatic tissues.

Male-specific isoforms could alleviate the potential conflict in heteroplasmic cells and accommodate the high energy demand associated with sperm motility (Breton et al., 2007; Dowling et al., 2008; Dowling & Simmons, 2009). For instance, mice exhibit testes-specific isoforms of the nuclear-encoded cytochrome *c* (Hennig, 1975; Liu et al., 2006) and cytochrome *c* oxidase (subunit IVb) (Huttemann et al., 2003). Whether female- and male-specific nuclear isoforms exist in DUI species is still unknown. However, I argue that this might potentially be the case in the light of the results here presented (chapters II, III and IV). The mosaic nature of respiratory complexes makes strict mitonuclear coadaptation necessary to preserve mitochondrial functionality (Blier et al., 2001; Dowling et al., 2008; Hadjivasiliou et al., 2012; Havird et al., 2019; Hill et al., 2019; Lane, 2009, 2011; Wolff et al., 2014). That said, any variation in mitochondrially encoded genes (adaptive or not) potentially induces selective pressure for compensatory change in interacting nuclear genes (Barreto & Burton, 2013b; Barreto et al., 2018; Healy & Burton, 2020; Mishmar et al., 2006; Osada & Akashi, 2012). As the structural and biochemical properties of respiratory complexes are determined by mitonuclear interactions, it is thus conceivable that the phenotypic rearrangement between maternal and paternal DUI mitochondria revealed during this PhD might reflect sex-specific variation in OXPHOS genes at both the mitochondrial and nuclear levels.

Another possibility to deal with heteroplasmy is genomic imprinting, which leads to a pattern of nuclear gene expression that favour the alleles coadapted with the genes encoded by the specific mitochondrial lineage present in the cell (i.e. when the mtDNA is maternally inherited, selection might favour the expression of the maternal nuclear locus. Conversely, it would favour the expression of the paternal nuclear locus when the mtDNA is paternally inherited) (Wolf, 2009). Finally, DUI species could manage heteroplasmy by silencing or downregulating the M genome in heteroplasmic somatic tissues. However, this does not appear to be the case because M-mtDNA transcription in somatic tissues has been reported in both marine and freshwater DUI species (Breton et al., 2017; Milani et al., 2014a). Moreover, the respirometric analysis of *A. islandica* and *M. edulis* somatic cells (chapter II) suggests that heteroplasmy have an impact upon mitochondrial activity in these DUI species (figure 2.3, 2.5). Compared to female somatic cells of both species (homoplasmic for the F-type mtDNA), whose mitochondrial phenotype is the same as the one found in oocytes (also homoplasmic for the F-type mtDNA), male somatic cells (heteroplasmic for both the F- and the M-type mtDNA (figure 2.s4)), express a "half-way" mitochondrial phenotype between the "pure" oocyte- and sperm-related one. This is mostly evident in the species *M. edulis*, although *A. islandica* male somatic tissues have a similar trend. The transmission of M-type mtDNA in DUI entails that it may accumulates mutations that are beneficial for males, but not necessarily for female and somatic functions. As such, the decreased respiratory ratios in male somatic tissues not only reflect the potential impact of heteroplasmy upon mitochondrial bioenergetics, but also represents a possible case of "father's curse", in which a mtDNA specifically adapted for male affects the general bioenergetics of somatic cells (Breton et al., 2017). However, the mitochondrial phenotype of male heteroplasmic soma does not vary much from its counterpart in female homoplasmic soma. This could either be explained by an insufficient amount of M-mtDNA to produce a strong phenotypic effect in somatic tissues and/or by a functional complementation between genomes (Beziat et al., 1997; Chomyn et al., 1992; Stewart & Chinnery, 2015). In line with this possibility, the extreme excess capacity of cytochrome *c* oxidase specific of the female phenotype (figure 2.5) might potentially assure a sufficient mitochondrial activity in cells where the defective/specialized male one is present (Gnaiger et al., 1998; Mazat et al., 1997). Additional analyses are however required to confirm all these suggestions.

4. Future directions

During my PhD project, I combined powerful state-of-art techniques and technologies to investigate the physiology underlying mtDNA variation in bivalve species characterized by either strict maternal inheritance or doubly uniparental inheritance of mitochondria. The results are promising and suggest a link between mitochondrial genotype and several phenotypic aspects of DUI species. However, as we were only able to scratch the surface of it, future experiments are surely required. Potential future researches involve:

i) testing more distantly related bivalve species, both DUI and SMI, to confirm the results and interpretations on both gametes and somatic tissues;

ii) searching for the potential existence of sex-specific isoforms of nuclear encoded mitochondrial genes differentially expressed in female and male gonads and gametes of DUI species;

iii) investigating the capacity of enzymes and energy production pathways not yet or poorly envisioned. Examples of enzymes to be tested are hexokinase, pyruvate dehydrogenase, octopine dehydrogenase, alternative oxidase, hydroxy acyl CoA dehydrogenase, succinate dehydrogenase (Doucet-Beaupré et al., 2010; Hunter-Manseau et al., 2019; Muller et al., 2012; Thibault et al., 1997);

iv) verifying whether the DUI-specific sperm performance associate with an increased longevity, endurance and fertilization rate of male gametes in these species;

v) examining the potential presence of a subpopulation of quiescent mitochondria within oocytes of SMI and DUI bivalve species;

vi) characterizing the oxidative stress and antioxidant capacity in DUI gametes. It is still unknown how DUI species might transmit undamaged templates to offsprings, and our results unexpectedly pointed towards a potential higher oxidative stress on sperm mitochondria. An in-depth characterization of ROS regulation is thus necessary. A potential experiment would involve the use of high-resolution fluorespirometry (Gnaiger, 2014; Gnaiger et al., 2020) to simultaneously analyse the real-time oxygen consumption and ROS (H_2O_2) flux in DUI and SMI gametes, even at different temperatures of interest. H_2O_2 production can also be determined spectrophotometrically (e.g (Christen et al., 2018; Munro et al., 2013), together with the damage to macromolecules and the antioxidant capacity of various antioxidant enzymes. In addition to catalase, it would be worth it to determine the catalytic capacity of superoxide dismutase, aconitase and glutathione peroxidase;

vii) verifying the possible link between the maintenance of mitochondrial membrane potential and paternal mitochondria preservation and transmission in DUI species. A first experiment would consist to empirically verify the ability of M-type mitochondria in DUI sperm to maintain a high $\Delta\psi m$ and increase it following oocyte detection. This could be determined microscopically using two specific fluorescent dyes, one that stains the mitochondrial mass regardless of their activity and the other that is imported into the mitochondria based on the membrane potential (e.g. MitoSpy™ Green FM and MitoSpy™ Red CMXRos, BioLegend) (de Paula et al., 2013b; Milani & Ghiselli, 2015). In addition to confirm the proposed ability of DUI sperm mitochondria to sustain a high Δψm, it would be interesting to verify if the detection of oocytes (e.g. achieved by adding egg-derived chemical cues in the sperm solution (Evans et al., 2012; Lymbery et al., 2017; Oliver & Evans, 2014) determines an increased in the membrane potential of DUI sperm mitochondria, and whether this could differ in sperm of SMI species (whose mitochondria are eliminated during fertilization). If confirmed, a second experiment would be to determine the potential involvement of Δψm in the preservation of DUI paternally derived mitochondria during fertilization. This experiment would involve the use of specific uncoupling agents (e.g. FCCP (Gnaiger, 2014; Gnaiger et al., 2020)) in order to depolarize sperm mitochondria. The use of the fluorescent dyes would then allow us to i) confirm the effectiveness of sperm mitochondria depolarization (red dye) and ii) follow the real-time fate of paternally derived mitochondria following fertilization (both green and red dyes). For example, it would be possible to determine whether the stained and depolarized sperm mitochondria do segregate in the blastomere giving rise to the germline, as they usually do in male embryos. Finally, if a change in mitochondria selection do indeed happen during the embryo development, it would be interesting to determine any possible link with the determination of sex. However, two possible problems exist. The first is that we do not know in advance the sex of the embryos. However, in the case of depolarized mitochondria, we expect that sperm mitochondria should be destroyed after fertilization in both female and male individuals. The second one is that we know for sure that nullifying the Δψm has a severe impact on bivalve sperm motility and thus in their fertilization capacity (chapter IV). It would then be worth it to try low concentrations of uncoupling agents to produce a decrease in the Δψm but not a complete depolarization of sperm mitochondria, potentially maintaining a low (but sufficient) fertilization capacity in treated sperm.

5. Conclusion

During this PhD I investigated the extent by which non neutral variation in mitochondrial genes could affect the general phenotype and even be adaptive. The findings support a robust link between the mitochondrial genotype and phenotype, and a clear divergence between the two groups of bivalves analysed, characterized by either strict maternal inheritance (SMI) or doubly uniparental inheritance (DUI) of mitochondria. Concerning DUI, I provided evidence that the sex-specific evolution of mtDNA variants in DUI species results in the expression of different female and male bioenergetic phenotypes, and that this remodelling is conserved in distantly related bivalve species. Specifically, a male-specific evolution of M-type mitochondria results in: i) extensive qualitative change in the stoichiometry between ETS complexes, as well as in the balance between OXPHOS and the bioenergetic pathways upstream of it, ii) a general reorganization of gamete bioenergetics, with sperm completely relying on OXPHOS to sustain their motility, yet expressing a restrained maximal bioenergetic and antioxidant capacity compared to oocytes, iii) the expression of a DUI-specific sperm motility phenotype, characterized by slow speed and high curvilinear trajectory. Altogether, these findings suggest the sex-specific mtDNA variation in DUI species might be adaptive, involving the expression of male-specific bioenergetic adaptions with an intriguing downstream effect upon sperm reproductive fitness and, although speculative, on paternal mitochondria selection and transmission. Although potentially beneficial, the change in mitochondria functioning and the lower antioxidant capacity compared to oocytes suggest an increased risk of oxidative damage on sperm mitochondria. How these species could potentially manage to prevent oxidative damage on sperm mitochondria remains however still unresolved and deserves further investigation. Finally, the results suggest that the coexistence of both mitotypes has an impact onto the bioenergetics of male heteroplasmic cells, and that exclusive bioenergetic features of the female phenotype may potentially confer resistance to both heteroplasmy and ageing. The uncommon DUI system is a model of growing interest for addressing many aspects of mitochondrial and cellular biology, such as mitonuclear coevolution, mitochondria selection and inheritance, adaptive value of non-neutral mtDNA variations and their potential impact on male reproductive fitness and even sex-determination. Further investigations are surely needed to assess the exciting hypothesis of a link between the evolution of male-specific mtDNA variants, sperm energetic adaptation, paternal mitochondria preservation and inheritance.

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ANNEXES

Chapter II - Electronic supplementary material

(a) Supplementary materials and methods

Samples collection. Adult specimens of *Arctica islandica* (Linnaeus, 1767) were collected in June 2016 from Perry (Maine, USA), specimens of *Mercenaria mercenaria* (Linnaeus, 1758) were collected in June 2016 from Barnstable (Massachusetts, USA), specimens of *Mytilus edulis* (Linnaeus, 1758) were collected in July 2016 from Kensington (Prince Edward Island, Canada), and specimens of *Placopecten magellanicus* (Gmelin, 1791) were collected in July 2016 from Newport (Québec, Canada). Bivalves were shipped alive to the Université du Québec à Rimouski and acclimated for four weeks at 12 °C in a 120 L-aquarium with recirculating seawater. Individuals were fed twice a week with a mix of marine microalgae and feeding was stopped 48 hours prior to experiments. The sex was determined macroscopically through visual inspection of gonads and further confirmed microscopically after isolating and washing the gametes. Measures of OXPHOS-related parameters were conducted on gametes (mature eggs and motile sperm) and gills of female (F) and male (M) individuals. A total of 26 samples were analysed for *A. islandica* (eggs $n = 10$, sperm $n = 6$, F-gills $n = 5$ and M-gills $n = 5$), 23 samples for *M. edulis* (eggs $n = 5$, sperm $n = 6$, F-gills $n = 6$, M-gills $n = 6$), 21 samples for *M. mercenaria* (eggs $n = 5$, sperm $n = 6$, F-gills $n = 5$ and M-gills $n = 5$) and 29 samples for *P. magellanicus* (eggs $n = 7$, sperm $n = 9$, F-gills $n = 8$ and M-gills $n = 5$). Measurements were obtained in replicates for each biological sample.

Samples preparation. Animals were dissected on ice. Gills were excised and 40 ± 2 mg (wet-weight) of tissue per replicate was first rinsed with and then directly placed in 5 mL modified ice-cold relaxing buffer solution BIOPS [CaK_2EGTA (2.77 mM), K_2EGTA (7.23 mM), $MgCl_2 \cdot 6H_2O$ (6.56 mM), taurine (20 mM), Na_2phosphocreatine (15 mM), imidazole (20 mM), dithiothreitol (0.5 mM), MES hydrate (50 mM), Na_2ATP (5.77 mM), KCl (400 mM) at pH 7.10] (Pesta & Gnaiger, 2012). Tissues were mechanically permeabilized with fine tweezers and further chemically permeabilized with saponin (50 $\mu g.mL^{-1}$ BIOPS) following the procedures described elsewhere (Lemieux et al., 2017). Gametes were stripped from the excised gonads and washed out with salt-water. Eggs maturity and sperm activation/motility were determined microscopically under 40 x magnification. The density of eggs or sperm solution was measured, and a volume corresponding to 40 mg was used for respirometric analyses. The optimum saponin concentration for gametes was determined empirically following the protocol for permeabilization of initially intact cell suspension (Pesta & Gnaiger, 2012). Gamete samples were transferred into the respiration chamber of the Oxygraph-2k (Oroboros Instruments, Innsbruck, Austria) preloaded with 2 mL of the modified respiratory medium MiR05 [110 mM D-sucrose, 60 mM lactobionic acid, 20 mM taurine, 20 mM HEPES, 10 mM KH_2PO_4, 3 mM $MgCl_2$, 0.5 mM EGTA, BSA 1 $g \cdot L^{-1}$, 250 mM KCl] (Pesta & Gnaiger, 2012), and the detergent was immediately added to the respiratory chamber together with the substrates pyruvate (P), malate (M) and glutamate (G). For gills, pre-permeabilized samples were transferred into the respiration chamber preloaded with 2 mL of the respiratory medium MiR05 without further addition of detergent.

High-resolution respirometry. Respiration was measured at 12 °C with the Oxygraph-2k and the software DatLab V 5.2 (Oroboros Instruments, Innsbruck, Austria). For both gills and gametes, the same substrate-uncoupler-inhibitor titration (SUIT) protocol was performed (Gnaiger, 2014) (figure 2.s1). An exhaustive list of the acronyms and abbreviations used is provided in table 2.s1. A non-phosphorylating resting state (Leak-state, $_L$) fuelled through NADH dehydrogenase (complex I or CI) was achieved with the addition of NADH-linked substrates (N) (i.e. malate (M, 2 mM), glutamate (G, 24 mM) and pyruvate (P, 10 mM)) in absence of ADP (N_L). Addition of a saturating quantity of ADP (D, 5 mM) promoted oxidative phosphorylation (OXPHOS-state, $_P$) sustained by CI-related substrates (N_P). Addition of proline (Pr, 10 mM) stimulated the respiration (NPr_P) by promoting the activity of proline dehydrogenase (ProDH). Addition of cytochrome *c* (c, 10 μM) tested the outer mitochondrial membrane integrity by monitoring any additional increase in respiration indicative of disrupted outer membrane and endogenous loss of cytochrome *c*. Respiration fuelled by CI and succinate dehydrogenase (complex II or CII) was measured through the addition of the CII substrate succinate (S, 10 mM) ($NPrcS_P$), as well as glycerol-3-phosphate titration (Gp, 5 mM each step) assessed the contribution of glycerol-3-phosphate dehydrogenase (GpDH) on the OXPHOS ($NPrcSGp_P$). The maximal electron transport system (ETS) capacity was achieved by fully uncoupling mitochondria (ETS-state, $_E$) with stepwise titration of the protonophore carbonyl cyanide p-(trifluoromethoxy)-phenylhydrazone (FCCP, 0.25 μM each step) ($NPrcSGp_E$). Inhibition of coenzyme Q:cytochrome *c* oxidoreductase (complex III or CIII), alternative oxidase (AOX) and CI, respectively by antimycin A (Ama, 2.5 μM) (Ama_E), salicylhydroxamic acid - SHAM (Shm, 1 μM) (Shm_E), and rotenone (Rot, 1 μM) yielded AOX activity, as well as the residual oxygen consumption (ROX). Cytochrome *c* oxidase (complex IV or CIV) capacity (CIV_E) was determined by sequential addition of ascorbate (Asc, 2 mM) and TMPD (Tm, 0.5 mM) and the chemical background measured after addition of sodium azide (Azd, 57 mM) was subtracted. Mitochondrial respiration data were corrected for oxygen flux due to instrumental background at 12 °C (measured through dithionite titration in absence of sample), and for ROX (see above).

Citrate synthase activity. Chamber content was collected at the end of each experimental run and homogenized for 3 x 30 s with a PT 1200 homogenizer (Polytron, Kinematica) at maximum speed, and immediately stored at −80 °C for subsequent measurement of citrate synthase (CS) activity. CS activity was measured in triplicate using a Mithras LB940 microplate reader (Berthold technologies, Bad Wildbad, Germany), held at 25 °C, and data analysed with MikroWin 2010 V 5.15 software (Labsis Laborsysteme, Neunkirchen-Seelscheid, Germany). Specifically, homogenates (100 µL) were transferred to 100 mM imidazole-HCl pH.8, 0.1 mM 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), 0.1 mM acetyl-CoA and 0.15 mM oxaloacetate. The enzymatic activity was measured by following the increase in absorbance at a wavelength of 405 nm, and data were expressed in $mU{\cdot}mL^{-1}$, where U refers to 1 µmol of substrate transformed per minute (Breton et al., 2009).

Protein content. Samples protein concentration ($mg{\cdot}mL^{-1}$) was quantified using the bicinchoninic acid (BCA) assay kit (Sigma BCA1-1KT), using a bovine serum albumin (BSA) based standard curve. The absorbance was measured at a wavelength of 560 nm using a Mithras LB940 microplate reader.

Chemicals. All chemicals were obtained from Sigma-Aldrich (Oakville, Ontario, Canada) unless otherwise stated.

Data analysis. Mitochondrial respiration rates were expressed as mean respiration rates in pmol-O_2-consumed$\cdot s^{-1}\cdot$mU CS activity $^{-1}$ + 95% confidence interval bars (CIs). Additionally, qualitative parameters such as flux control ratios (FCRs) were obtained by normalizing the respiratory rates for an internal parameter, the maximal ETS capacity, achieved after FCCP-mediated uncoupling (Gnaiger, 2014). The maximal ETS capacity was sustained by convergent electron flow coming from CI, CII, ProDH and GpDH complexes ($NPrcSGp_E$). The CIV apparent excess capacity (j_{ExCIV}), which indicates the activity of cytochrome *c* oxidase exceeding the max ETS capacity, was expressed as $CIV_E/NPrcSGp_E - 1$. The relative changes in the oxygen flux rate (j) produced by the addition of a specific substrate (x) were expressed as flux control factors (FCFs) and calculated as $1-jx_1/jx_2$ (Gnaiger, 2014). The OXPHOS coupling efficiency ($j_{\approx P}$) indicates the capacity of the OXPHOS over the resting state and was calculated as $1-L/P$, with L and P respectively referring to Leak- and OXPHOS-state respiration sustained by NADH-linked substrates ($1-N_L/N_P$). The apparent excess capacity of the ETS (j_{ExP}) was calculated as $1-P/E$, with P and E referring respectively to coupled and uncoupled respiration, sustained by high convergent electron flux through CI-CII-ProDH-GpDH. It estimates how closed the maximal coupled respiration (OXPHOS) is to the maximal capacity of the system (uncoupled respiration) and, in other terms, it expresses the limitation acting on the OXPHOS itself (Gnaiger, 2014).

Statistical analysis. All statistical analyses were performed with R-studio software (R Core Team, 2016). The normality and homogeneity of data were verified using the Shapiro-Wilk test for the former and both Bartlett and Levene tests for the latter. Three independent factors were considered: "species" (four levels), "sex" (two levels) and "cell-type" (two levels). Depending on the specific biological question, statistical analyses were carried out considering single or multiple factors. For each species, the effect of the factor sex on gametic or somatic cells was determined using an unpaired two-tailed Student's *t* test. The main effects of different combinations of two independent factors, as well as their interaction, were determined using a two-way ANOVA, followed by a *post hoc* Tukey's multiple comparison test. A $p \leq 0.05$ was considered significant and differences are represented as * ($p \leq 0.05$), ** ($p \leq 0.01$) and *** ($p \leq 0.001$).

PCR amplification. Nucleic acid from gill samples was extracted with DNeasy Blood & Tissue Kit (Qiagen), examined via electrophoresis on a 1% agarose gel and quantified using a BioDrop µLITE spectrophotometer. The primers were designed based on the complete *M. edulis* mitochondrial genomes (accession numbers NC_006161.1 and AY823623.1) to selectively amplify part of the M-mtDNA (654 bp): MyEd-M-for (TACTGTTGGCACATACGAGAG) and MyEd-M-rev (ATAATTACTACTAACCATCTCATAA); and part of the F-mtDNA (505 bp): MyEd-F-for (GGGTTACCTTTTATGTAAATG) and MyEd-F-rev (ACAATCACTAAACCTTTCTTT). For *A. islandica*, primers were designed from partial *cytb* gene sequences (accession numbers AF202101.1 and AF202103.1) to amplify part of the M-mtDNA (318 bp): ArIs-M-for (CGCTGTACCTTATGTCGGCACAA) and ArIs-M-rev

(AACAAAATTTACAGGATCTAGGAA); and part of the F-mtDNA (115 bp): ArIs-F-for (GGTCCTTTTATTTTACTGGTT) and ArIs-F-rev (TATCTATGAAAAGGCAGGGC). The reaction volume was 50 μL, containing 5 μL of appropriately diluted DNA template, 5 μL of Taq Buffer (10x), 1 μL of dNTPs mix (10 mM), 2 μL of each primer (10 μM) and 0.25 μL of Taq DNA polymerase (5 U/μL) (Feldan). PCR reactions were carried on a TProfessional Basic Thermocycler with the following conditions: initial denaturation at 95 °C for 2 min, followed by 40 cycles of 95 °C for 20 s, 48-52 °C for 30 s and 72 °C for 40 s, followed by a final extension at 72 °C for 5 min. PCR products were examined on a 1% agarose gel with SYBR green dye (Life Technologies).

(b) Supplementary figures and tables

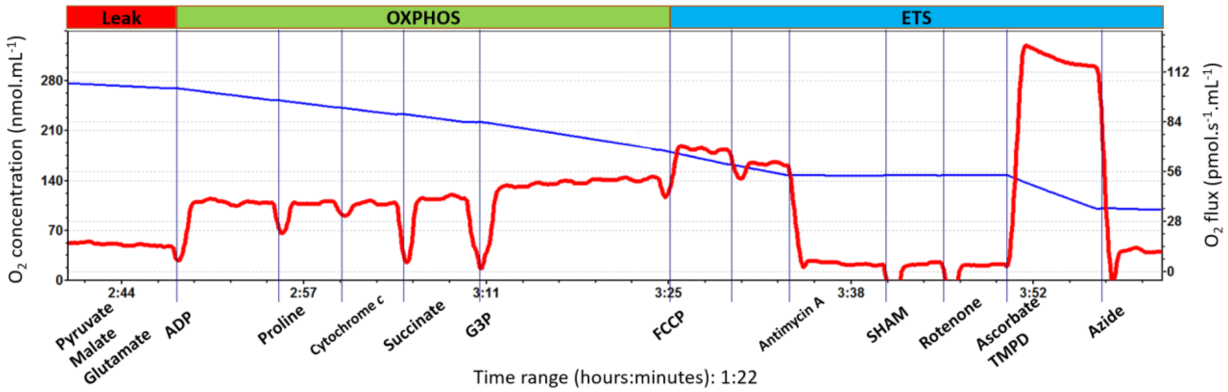

Figure 2.s1. SUIT protocol. Graph template representing the substrate-uncoupler-inhibitor-titration (SUIT) protocol adopted. The blue line indicates the oxygen concentration (nmol·mL^{-1}) whereas the red line shows the oxygen flux rate (pmol·s^{-1}·mL^{-1}). The addition of specific compounds is marked with a vertical line. The upper bar indicates the respiratory states: Leak-state, $_{L}$ (non-phosphorylating resting state in presence of N substrates and absence of ADP); OXPHOS-state, $_{P}$ (coupled respiration with different combination of substrates and presence of ADP); ETS-state, $_{E}$ (uncoupled respiration achieved after FCCP addition).

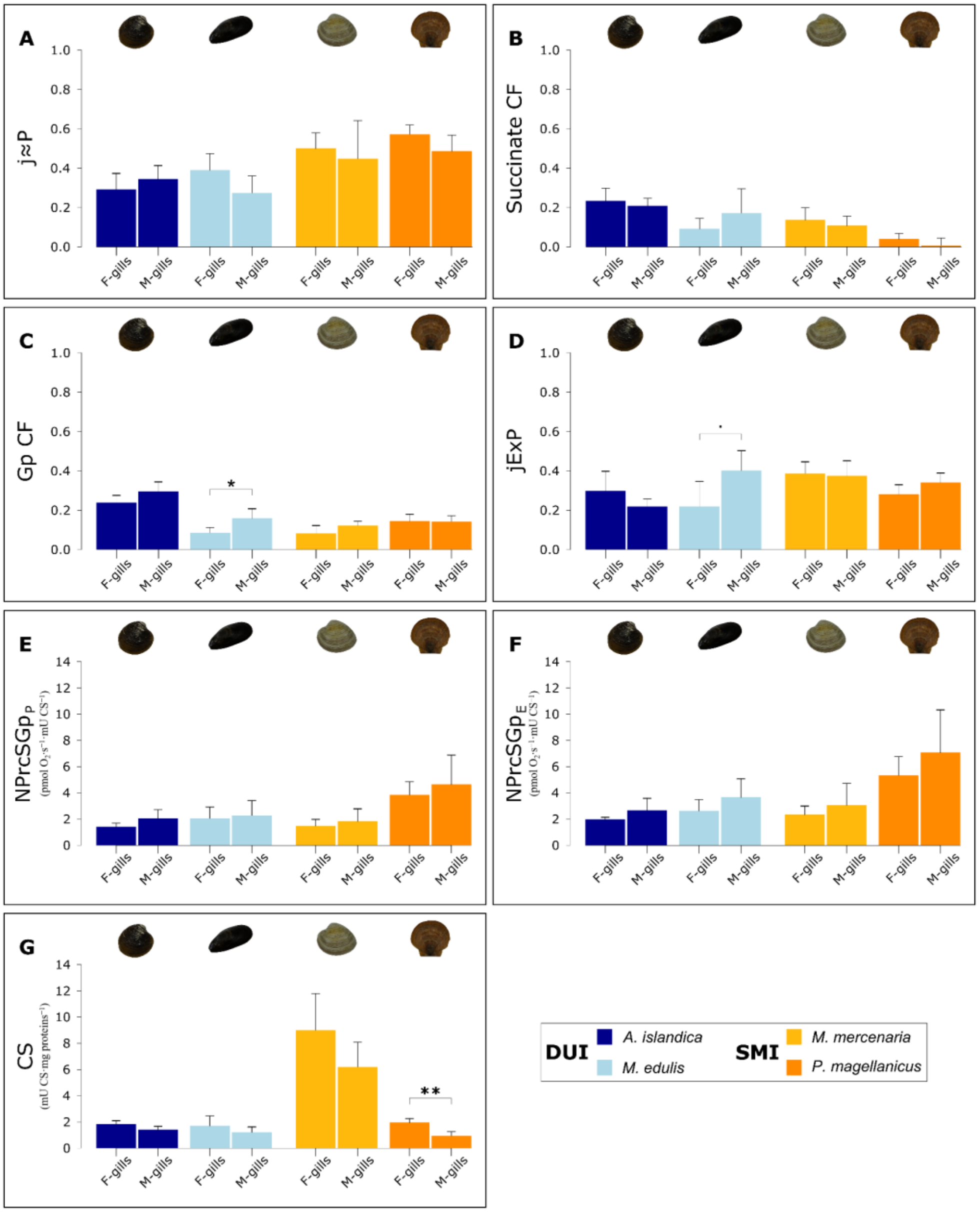

Figure 2.s2. Respiratory factors comparison between female and male gills. DUI species: *A. islandica* ($n = 5, 5$), *M. edulis* ($n = 6, 6$). SMI species: *M. mercenaria* ($n = 5, 5$), *P. magellanicus* ($n = 8, 5$). (A) OXPHOS coupling efficiency ($j_{\approx P}$), indicator of both mitochondrial quality and coupling. (B) Succinate control factor, indicating the respiratory stimulation after succinate addition. (C) Glycerophosphate control factor, indicating the respiratory stimulation after Gp addition. (D) Apparent excess capacity of the ETS (j_{ExP}). (E) Max coupled respiration sustained by CI-II-ProDH-GpDH complexes. (F) Max uncoupled respiration sustained by CI-II-ProDH-GpDH complexes. (G) Citrate synthase (CS) activity. Values are presented as means + 95% CIs. Two-tailed Student's *t* test (sex as independent factor) was performed independently for each parameter and each species. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$. Detailed summary is reported in tables 2.s2- 2.s3.

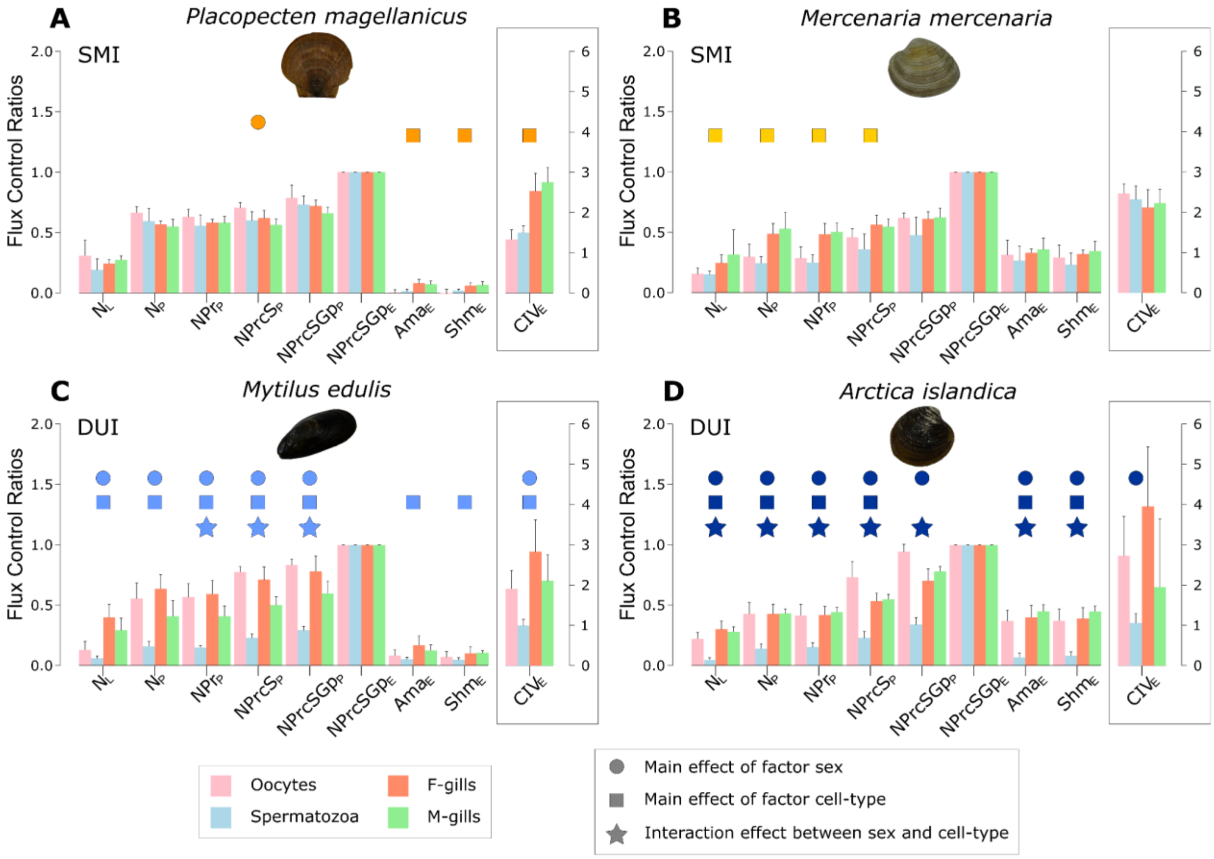

Figure 2.s3. Flux control ratios comparison between oocytes, spermatozoa, female (F) and male (M) gills of both DUI and SMI species. (A) *P. magellanicus* ($n = 7, 9, 8, 5$). (B) *M. mercenaria* ($n = 5, 6, 5, 5$). (C) *M. edulis* ($n = 5, 6, 6, 6$). (D) *A. islandica* ($n = 10, 6, 5, 5$). The parameters are normalized for the max ETS-capacity ($NPrcSGp_E$) and reflect the mitochondrial activity sustained by differ substrates, in different respiratory states. Values are presented as means + 95% CIs. Two-way ANOVA analysis (sex and cell-type as independent factors) was performed independently for each parameter and each species. Significance was set at $p \leq 0.05$ and results are represented as a circle (main effect of sex), square (main effect of cell-type) and star (interaction effect between factors sex and cell-type). For abbreviations, substrate combinations and respiratory states refer to table 2.s1, figures 2.2, 2.3. Detailed summary is reported in tables 2.s2- 2.s4.

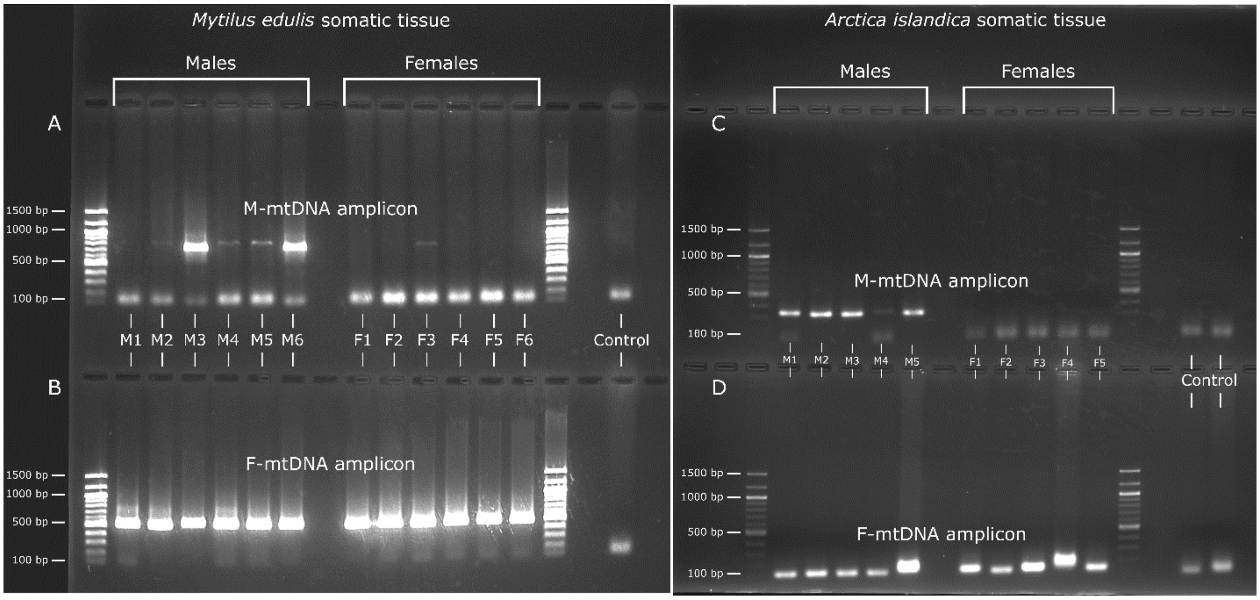

Figure 2.s4. Detection of M- and F-mtDNA. PCR amplifications of M-mtDNA and F-mtDNA partial sequences in *Mytilus edulis* and *Arctica islandica* gill samples. (A) Presence/absence of M-mtDNA in male (*n* = 6) and female (*n* = 6) individuals of *M. edulis*. (B) Presence/absence of F-mtDNA in male (*n* = 6) and female (*n* = 6) individuals of *M. edulis*. (C) Presence/absence of M-mtDNA in male (*n* = 5) and female (*n* = 5) individuals of *A. islandica*. (D) Presence/absence of F-mtDNA in male (*n* = 5) and female (*n* = 5) individuals of *A. islandica*. For *M. edulis*, five out of six males are heteroplasmic for both M- and F-mtDNA, whereas females, except individual F3 (see (Breton et al., 2017)), are homoplasmic for the only F-mtDNA. For *A. islandica*, all males are heteroplasmic for both M- and F-mtDNA, whereas all females are homoplasmic for the only F-mtDNA.

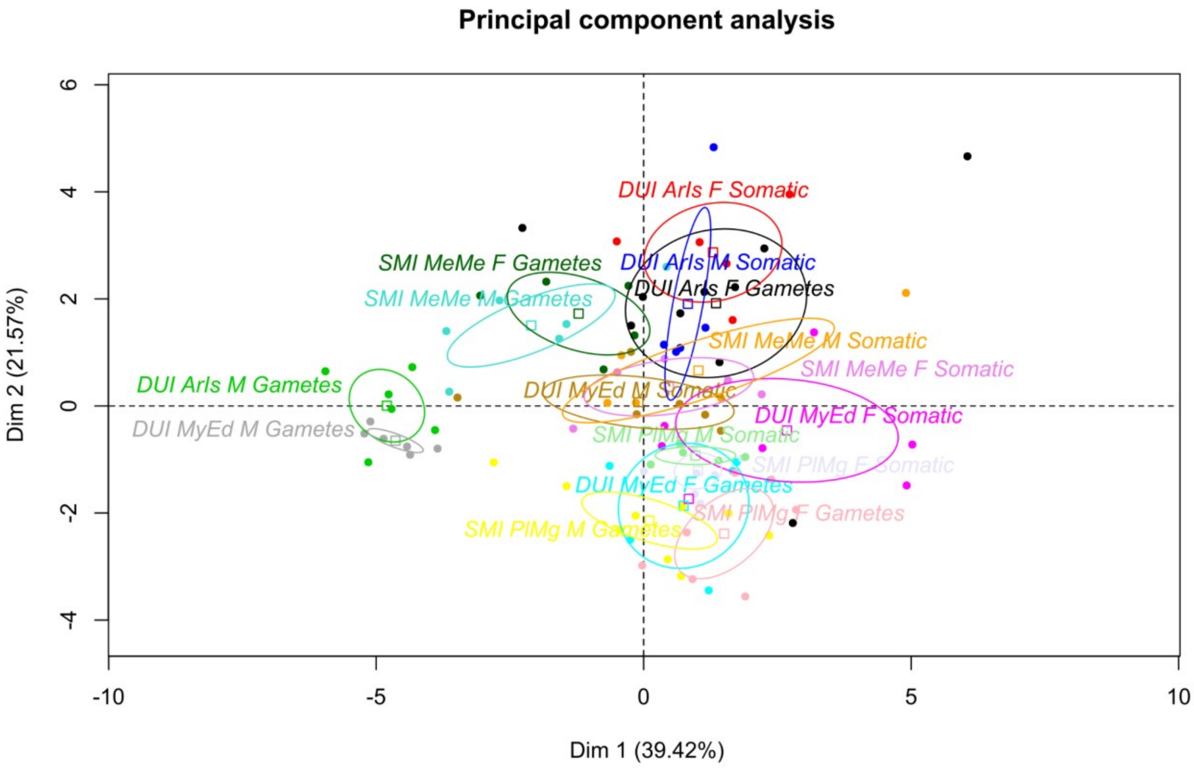

Figure 2.s5. PCA scatter plot with 95% confidence interval ellipses. Principal component analysis (PCA) based on the FCRs and FCFs reported in figures 2.1, 2.s2, 2.2, 2.3, 2.5, representing a proxy of the mitochondrial phenotypes specific of both DUI and SMI species. Colours refer to different combinations of species (ArIs, *A. islandica*; MyEd, *M. edulis*; MeMe, *M. mercenaria*; PlMg, *P. magellanicus*), cell-type (gametes; somatic cells) and sex (F, female; M, male).

Table 2.s1. List of acronyms and abbreviations.

Table S1: Acronyms and abbreviations	**Definition**	**Additional information**
Respiratory states		
LEAK-state, $_L$	mitochondrial respiratory State 4 or State 2' *see ref (9, 36, 52, 53)	Non-phosphorylating resting state. Substrates (N) with no ADP
OXPHOS-state, $_P$	mitochondrial respiratory State 3	Coupled respiration. Substrates with ADP
ETS-state, $_E$	mitochondrial respiratory State 3u	Uncoupled respiration. Substrates with ADP and protonophore
ROX	Residual oxygen consumption	ETS inhibited
Substrates, uncoupler and inhibitors		
P	Pyruvate	Substrate for NADH dehydrogenase (complex I)
M	Malate	Substrate for NADH dehydrogenase (complex I)
G	Glutamate	Substrate for NADH dehydrogenase (complex I)
N	NADH-linked substrates	Combination of pyruvate, malate and glutamate (PMG)
ADP	Adenosine diphosphate	
Pr	Proline	Substrate for proline dehydrogenase (ProDH)
c	Cytochrome *c*	Heme protein component of the electron transport system
S	Succinate	Substrate for succinate dehydrogenase (complex II)
Gp	Glycerophosphate	Substrate for glycerophosphate dehydrogenase (GpDH)
FCCP	Carbonyl cyanide p-(trifluoromethoxy)-phenylhydrazone	Protonophore, mitochondrial uncoupler
Ama	Antimycin A	Inhibitor of coenzyme Q:cytochrome *c* oxidoreductase (complex III)
Shm	Salicylhydroxamic acid - SHAM	Inhibitor of alternative oxidase (AOX)
Rot	Rotenone	Inhibitor of NADH dehydrogenase (complex I)
Asc	Ascorbate	Tm reducer
Tm	N,N,N',N'-Tetramethyl-p-phenylenediamine dihydrochloride - TMPD	Cytochrome *c* reducer
Azd	Sodium Azide	Inhibitor of cytochrome *c* oxidase (complex IV)
Measured parameters		
N_L	Leak-state, $_L$ with N substrates combination, no ADP	
N_P	OXPHOS-state, $_P$ with N substrates combination	
NPr_P	OXPHOS-state, $_P$ with NPr substrates combination	
$NPrc_P$	OXPHOS-state, $_P$ with NPrc substrates combination	
$NPrcS_P$	OXPHOS-state, $_P$ with NPrcS substrates combination	
$NPrcSGp_P$	OXPHOS-state, $_P$ with NPrcSGp substrates combination	
$NPrcSGp_E$	ETS-state, $_E$ with NPrcSGp substrates combination	
Ama_E	Residual mitochondrial respiration after inhibition of complex III	
Shm_E	Residual mitochondrial respiration after inhibition of complex III and AOX	
CIV_E	ETS-state, $_E$ complex IV standalone capacity	Ascorbate + TMPD as electron donors
FCRs	Flux control ratios; Formula = j/z	Oxygen fluxes (j) normalized for a common maximum oxygen flux (z)
FCFs	Flux control factors; Formula = 1−(jx1⁄jx2)	Changes in the oxygen flux rate (j) produced by substrate (x) addition
Succinate CF	Succinate control factor	Changes in the oxygen flux rate following S addition
Gp CF	Glycerophosphate control factor	Changes in the oxygen flux rate following Gp addition
$j_{\approx P}$	OXPHOS coupling efficiency; Formula = 1−(State 2'⁄State 3)	State 2' (N substrates only), State 3 (N substrates with ADP)
j_{ExP}	ETS apparent excess capacity; Formula = 1−(State 3⁄State 3u)	State 3 and State 3u (NPrcSGp substrates)
j_{ExCIV}	Apparent excess capacity of cytochrome *c* oxidase; Formula = (CIV_E/$NPrcSGp_E$) – 1	Activity of CIV exceeding the max State 3u capacity

Table 2.s2. Data summary table. Absolute respiratory rates (pmol $O_2 \cdot s^{-1} \cdot mU\ CS^{-1}$), citrate synthase (CS) activities (mU·mg proteins^{-1}), flux control ratios (FCRs) and flux control factors (FCFs) measured for male and female gametes and somatic cells of the bivalve species *A. islandica*, *M. edulis*, *M. mercenaria* and *P. magellanicus*.

Respiratory rates (pmol $O_2 \cdot s^{-1} \cdot mU\ CS^{-1}$)									mU·mg Proteins^{-1}	FCRs									FCFs					Table S2			
N_L	N_P	NPr_P	$NPrcS_P$	$NPrcSGp_P$	$NPrcSGp_E$	Ama_E	Shm_E	CIV_E	CS	N_L	N_P	NPr_P	$NPrcS_P$	$NPrcSGp_P$	$NPrcSGp_E$	Ama_E	Shm_E	CIV_E	$j_{\approx P}$	S CF	Gp CF	j_{ExP}	j_{ExCIV}	Inheritance	Species	Sex	Cell-Type
0.184	0.362	0.34	0.552	0.809	0.839	0.247	0.251	2.108	2.995	0.208	0.43	0.397	0.649	0.96	1	0.284	0.288	2.576	0.521	0.599	0.326	0.04	1.576	DUI	ArIs	F	Gametes
0.232	0.454	0.436	0.693	0.875	0.933	0.27	0.328	1.811	2.57	0.247	0.485	0.467	0.742	0.938	1	0.289	0.351	1.94	0.493	0.382	0.21	0.062	0.94	DUI	ArIs	F	Gametes
0.106	0.19	0.215	0.451	0.629	0.679	0.232	0.257	1.534	2.174	0.146	0.26	0.307	0.654	0.929	1	0.316	0.355	2.36	0.433	0.45	0.295	0.071	1.36	DUI	ArIs	F	Gametes
0.177	0.331	0.264	0.423	0.853	0.839	0.208	0.138	1.809	2.087	0.199	0.376	0.299	0.49	1.016	1	0.234	0.146	2.157	0.517	0.496	0.518	-0.016	1.157	DUI	ArIs	F	Gametes
0.675	1.846	1.902	2.356	2.466	2.546	0.472	0.428	2.541	1.976	0.267	0.727	0.749	0.927	0.97	1	0.186	0.168	1	0.64	0.213	0.045	0.03	0	DUI	ArIs	F	Gametes
1.233	1.485	1.335	1.707	1.936	2.236	1.133	1.234	3.06	1.514	0.378	0.474	0.417	0.663	0.886	1	0.443	0.468	1.765	0.293	0.478	0.244	0.114	0.765	DUI	ArIs	F	Gametes
0.162	0.234	0.21	0.515	0.936	1.188	0.406	0.419	1.797	1.128	0.138	0.197	0.176	0.43	0.787	1	0.345	0.353	1.513	0.305	0.966	0.456	0.213	0.513	DUI	ArIs	F	Gametes
0.212	0.37	0.276	0.702	0.706	0.612	0.393	0.409	3.904	1.017	0.344	0.601	0.449	1.147	1.152	1	0.641	0.669	6.413	0.425	0.34	0.003	-0.152	5.413	DUI	ArIs	F	Gametes
0.249	0.615	0.754	1.243	1.384	1.453	0.843	0.847	6.048	0.611	0.157	0.393	0.497	0.843	0.961	1	0.546	0.555	4.054	0.607	0.405	0.12	0.039	3.054	DUI	ArIs	F	Gametes
0.102	0.234	0.28	0.533	0.569	0.661	0.296	0.259	2.237	0.929	0.157	0.356	0.415	0.791	0.843	1	0.428	0.378	3.529	0.561	0.507	0.06	0.157	2.529	DUI	ArIs	F	Gametes
0.051	0.079	0.09	0.161	0.244	0.597	0.067	0.057	0.465	1.679	0.087	0.138	0.153	0.276	0.397	1	0.11	0.093	0.842	0.328	0.701	0.19	0.603	-0.158	DUI	ArIs	M	Gametes
0.025	0.079	0.076	0.066	0.175	0.65	0.02	0.04	0.749	2.328	0.038	0.121	0.117	0.102	0.262	1	0.029	0.058	1.116	0.65	0.622	0.325	0.738	0.116	DUI	ArIs	M	Gametes
0.068	0.364	0.362	0.442	0.675	1.534	0.211	0.246	1.618	2.813	0.044	0.237	0.236	0.289	0.441	1	0.138	0.16	1.047	0.814	0.192	0.345	0.559	0.047	DUI	ArIs	M	Gametes
0.052	0.112	0.105	0.226	0.267	0.945	0.026	0.068	1.402	1.915	0.056	0.118	0.108	0.238	0.285	1	0.03	0.072	1.524	0.523	0.49	0.152	0.715	0.524	DUI	ArIs	M	Gametes
0.071	0.179	0.267	0.444	0.646	1.784	0.088	0.082	2.085	1.899	0.039	0.104	0.161	0.254	0.369	1	0.052	0.047	1.157	0.578	0.352	0.313	0.631	0.157	DUI	ArIs	M	Gametes
0.059	0.387	0.442	0.657	0.867	2.965	0.186	0.191	2.037	2.337	0.021	0.131	0.154	0.226	0.295	1	0.063	0.064	0.709	0.84	0.23	0.237	0.705	-0.291	DUI	ArIs	M	Gametes
0.64	1.089	1.071	1.363	1.847	2.083	1.197	1.177	2.328	1.984	0.303	0.516	0.502	0.646	0.885	1	0.575	0.566	1.137	0.413	0.158	0.271	0.115	0.137	DUI	ArIs	F	Somatic
0.507	0.777	0.754	1.021	1.321	1.887	0.702	0.669	8.115	2.034	0.263	0.41	0.395	0.523	0.699	1	0.379	0.365	4.496	0.36	0.262	0.252	0.301	3.496	DUI	ArIs	F	Somatic
0.353	0.467	0.497	0.729	0.968	1.734	0.498	0.511	7.065	1.534	0.205	0.283	0.299	0.434	0.572	1	0.297	0.304	4.181	0.252	0.336	0.244	0.428	3.181	DUI	ArIs	F	Somatic
0.781	0.991	0.995	1.156	1.515	2.158	0.697	0.714	8.6	2.124	0.342	0.442	0.444	0.519	0.694	1	0.323	0.329	4.233	0.239	0.166	0.255	0.306	3.233	DUI	ArIs	F	Somatic
0.822	1.019	0.956	1.137	1.359	2.042	0.892	0.808	11.721	1.458	0.402	0.498	0.467	0.556	0.664	1	0.436	0.395	5.736	0.19	0.237	0.164	0.336	4.736	DUI	ArIs	F	Somatic
0.49	0.701	0.7	0.905	1.391	1.889	1.022	0.968	9.843	1.287	0.257	0.366	0.365	0.473	0.737	1	0.541	0.51	5.329	0.291	0.253	0.357	0.263	4.329	DUI	ArIs	M	Somatic
0.378	0.671	0.716	0.913	1.329	1.586	0.701	0.697	1.552	1.655	0.234	0.423	0.456	0.575	0.842	1	0.445	0.442	0.975	0.448	0.231	0.316	0.158	-0.025	DUI	ArIs	M	Somatic
0.791	1.129	1.126	1.37	1.887	2.33	1.107	1.12	2.113	1.319	0.343	0.473	0.469	0.572	0.809	1	0.483	0.485	0.929	0.268	0.152	0.293	0.191	-0.071	DUI	ArIs	M	Somatic
0.933	1.59	1.692	2.117	2.673	3.611	1.362	1.376	6.471	1.737	0.26	0.448	0.477	0.59	0.747	1	0.379	0.383	1.808	0.405	0.229	0.208	0.253	0.808	DUI	ArIs	M	Somatic
1.21	1.753	1.756	2.076	2.992	3.91	1.55	1.624	3.007	1.047	0.315	0.455	0.453	0.547	0.78	1	0.409	0.433	0.74	0.308	0.167	0.299	0.22	-0.26	DUI	ArIs	M	Somatic
0.192	0.227	0.2	0.355	0.545	0.97	0.205	0.185	2.563	4.381	0.198	0.234	0.207	0.366	0.562	1	0.211	0.19	2.642	0.154	0.398	0.349	0.438	1.642	SMI	MeMe	F	Gametes
0.074	0.133	0.14	0.377	0.589	1.004	0.204	0.222	2.298	7.021	0.074	0.133	0.14	0.376	0.587	1	0.204	0.221	2.289	0.445	0.532	0.36	0.413	1.289	SMI	MeMe	F	Gametes
0.214	0.425	0.338	0.509	0.637	0.987	0.408	0.337	2.22	5.946	0.217	0.431	0.343	0.516	0.645	1	0.413	0.342	2.249	0.497	0.183	0.2	0.355	1.249	SMI	MeMe	F	Gametes
0.098	0.227	0.252	0.361	0.468	0.681	0.168	0.167	1.6	6.201	0.144	0.333	0.37	0.531	0.687	1	0.247	0.246	2.348	0.569	0.217	0.228	0.313	1.348	SMI	MeMe	F	Gametes
0.099	0.231	0.236	0.317	0.382	0.616	0.313	0.293	1.756	5.77	0.161	0.376	0.383	0.515	0.62	1	0.508	0.476	2.851	0.572	0.292	0.169	0.38	1.851	SMI	MeMe	F	Gametes
0.255	0.377	0.378	0.488	0.873	2.261	0.341	0.328	4.606	16.936	0.112	0.166	0.166	0.215	0.386	1	0.15	0.145	2.042	0.327	0.17	0.446	0.614	1.042	SMI	MeMe	M	Gametes
0.45	0.494	0.463	0.715	1.07	2.417	0.483	0.45	4.69	15.629	0.186	0.204	0.192	0.296	0.443	1	0.2	0.186	1.94	0.088	0.365	0.332	0.557	0.94	SMI	MeMe	M	Gametes
0.255	0.417	0.418	0.512	0.596	2.161	0.231	0.206	4.068	6.04	0.118	0.193	0.194	0.237	0.276	1	0.107	0.095	1.883	0.389	0.193	0.142	0.724	0.883	SMI	MeMe	M	Gametes
0.078	0.142	0.157	0.28	0.355	0.437	0.224	0.184	1.227	5.909	0.178	0.325	0.358	0.64	0.812	1	0.512	0.421	2.807	0.454	0.405	0.212	0.188	1.807	SMI	MeMe	M	Gametes
0.141	0.219	0.222	0.29	0.317	0.762	0.228	0.183	1.97	8.23	0.185	0.288	0.291	0.38	0.416	1	0.299	0.24	2.583	0.356	0.198	0.087	0.584	1.583	SMI	MeMe	M	Gametes
0.085	0.193	0.193	0.254	0.337	0.621	0.21	0.198	1.677	5.253	0.137	0.311	0.312	0.409	0.543	1	0.339	0.318	2.702	0.56	0.194	0.247	0.457	1.702	SMI	MeMe	M	Gametes
0.277	0.514	0.491	0.631	0.705	1.242	0.412	0.362	2.29	7.594	0.223	0.414	0.395	0.508	0.567	1	0.332	0.292	1.844	0.461	0.248	0.104	0.433	0.844	SMI	MeMe	F	Somatic
0.264	0.777	0.791	0.928	1.046	1.971	0.547	0.534	2.855	14.571	0.134	0.394	0.401	0.471	0.531	1	0.278	0.271	1.449	0.66	0.071	0.112	0.469	0.449	SMI	MeMe	F	Somatic
0.897	1.655	1.652	1.864	1.994	2.942	1.05	1.039	7.044	7.182	0.305	0.562	0.562	0.634	0.678	1	0.357	0.353	2.394	0.458	0.125	0.065	0.322	1.394	SMI	MeMe	F	Somatic
0.908	1.734	1.739	1.902	1.919	2.806	0.961	0.953	7.644	8.572	0.324	0.618	0.62	0.678	0.684	1	0.342	0.34	2.724	0.476	0.081	0.009	0.316	1.724	SMI	MeMe	F	Somatic
0.721	1.285	1.282	1.509	1.707	2.792	1.005	0.997	6.225	7.102	0.258	0.46	0.459	0.541	0.611	1	0.36	0.357	2.229	0.439	0.153	0.116	0.389	1.229	SMI	MeMe	F	Somatic
0.83	0.908	0.709	0.76	0.897	1.167	0.628	0.576	3.089	9.616	0.711	0.778	0.608	0.651	0.769	1	0.538	0.494	2.647	0.087	0.039	0.153	0.231	1.647	SMI	MeMe	M	Somatic
0.299	0.877	0.879	0.96	1.107	1.963	0.526	0.512	4.541	6.552	0.152	0.447	0.448	0.489	0.564	1	0.268	0.261	2.314	0.659	0.144	0.132	0.436	1.314	SMI	MeMe	M	Somatic
1.255	2.731	2.91	3.208	3.605	6.116	1.74	1.739	12.428	4.035	0.205	0.446	0.476	0.524	0.589	1	0.284	0.284	2.032	0.541	0.099	0.11	0.411	1.032	SMI	MeMe	M	Somatic
0.639	1.395	1.409	1.663	1.897	3.385	1.174	1.15	8.48	5.89	0.189	0.412	0.416	0.491	0.56	1	0.347	0.34	2.505	0.542	0.178	0.123	0.44	1.505	SMI	MeMe	M	Somatic
0.903	1.525	1.525	1.565	1.711	2.633	0.969	0.948	4.385	4.896	0.343	0.579	0.579	0.594	0.65	1	0.368	0.36	1.666	0.408	0.074	0.086	0.35	0.666	SMI	MeMe	M	Somatic
0.705	1.73	1.512	2.195	2.322	2.735	0.378	0.401	4.844	0.943	0.258	0.633	0.553	0.803	0.849	1	0.138	0.147	1.771	0.592	0.3	0.055	0.151	0.771	DUI	MyEd	F	Gametes
0.422	2.73	2.759	2.83	2.94	3.69	0.088	0.063	5.804	1.557	0.114	0.74	0.748	0.767	0.797	1	0.024	0.017	1.573	0.846	-0.007	0.037	0.203	0.573	DUI	MyEd	F	Gametes
0.092	0.539	0.609	1.13	1.291	1.547	0.139	0.092	3.1	1.462	0.059	0.349	0.394	0.731	0.835	1	0.09	0.059	2.005	0.83	0.343	0.125	0.165	1.005	DUI	MyEd	F	Gametes
0.353	1.237	1.348	1.884	2.031	2.217	0.302	0.23	6.135	1.291	0.159	0.558	0.608	0.85	0.916	1	0.136	0.104	2.767	0.715	0.261	0.072	0.084	1.767	DUI	MyEd	F	Gametes
0.259	1.93	2.052	2.779	2.932	3.797	0.146	0.133	5.573	1.541	0.068	0.508	0.54	0.732	0.772	1	0.039	0.035	1.468	0.866	0.202	0.052	0.228	0.468	DUI	MyEd	F	Gametes
0.332	0.941	0.906	1.319	1.677	5.099	0.263	0.141	4.919	1.351	0.065	0.185	0.178	0.259	0.329	1	0.052	0.028	0.965	0.648	0.19	0.213	0.671	-0.035	DUI	MyEd	M	Gametes
0.268	0.672	0.477	0.804	0.947	2.873	0.237	0.206	3.16	1.048	0.093	0.234	0.166	0.28	0.329	1	0.082	0.072	1.1	0.601	0.138	0.151	0.671	0.1	DUI	MyEd	M	Gametes
0.557	1.125	1.161	1.336	1.862	7.475	0.284	0.299	6.278	1.44	0.075	0.151	0.155	0.179	0.249	1	0.038	0.04	0.84	0.505	0.107	0.283	0.751	-0.16	DUI	MyEd	M	Gametes
0.084	0.488	0.615	1.099	1.555	4.692	0.362	0.361	3.987	1.605	0.018	0.104	0.131	0.234	0.331	1	0.077	0.077	0.85	0.828	0.346	0.294	0.669	-0.15	DUI	MyEd	M	Gametes
0.331	0.605	0.608	0.912	1.238	5.131	0.19	0.172	4.563	1.528	0.064	0.118	0.119	0.178	0.241	1	0.037	0.034	0.889	0.453	0.222	0.263	0.759	-0.111	DUI	MyEd	M	Gametes
0.225	0.697	0.611	0.992	1.096	3.876	0.164	0.176	5.176	1.306	0.058	0.18	0.158	0.256	0.283	1	0.042	0.046	1.335	0.677	0.302	0.095	0.717	0.335	DUI	MyEd	M	Gametes
2.161	3.168	3.104	3.831	4.107	4.07	0.06	-0.03	11.89	1.188	0.52	0.772	0.756	0.933	1.004	1	0.012	-0.011	2.924	0.342	0.114	0.073	-0.004	1.924	DUI	MyEd	F	Somatic
1.198	1.868	1.653	2.149	2.275	3.827	0.604	0.426	6.115	1.285	0.318	0.495	0.437	0.567	0.599	1	0.155	0.111	1.602	0.36	0.113	0.059	0.401	0.602	DUI	MyEd	F	Somatic
0.657	0.979	0.891	1.203	1.368	1.682	0.516	0.242	7.525	1.588	0.387	0.579	0.518	0.716	0.819	1	0.306	0.132	4.549	0.332	0.092	0.125	0.181	3.549	DUI	MyEd	F	Somatic
0.992	1.623	1.496	1.66	1.802	2.465	0.414	0.378	5.183	3.566	0.399	0.667	0.589	0.674	0.722	1	0.158	0.14	2.216	0.4	0.119	0.065	0.278	1.216	DUI	MyEd	F	Somatic
1.046	1.492	1.347	1.394	1.581	1.767	0.366	0.135	5.16	1.574	0.581	0.834	0.766	0.783	0.908	1	0.215	0.074	2.97	0.306	-0.041	0.128	0.092	1.97	DUI	MyEd	F	Somatic
0.381	0.938	0.977	1.158	1.229	1.926	0.343	0.341	5.274	1.065	0.198	0.487	0.507	0.601	0.638	1	0.178	0.177	2.738	0.594	0.145	0.057	0.362	1.738	DUI	MyEd	F	Somatic
2.729	4.14	3.858	4.252	4.986	7.089	1.384	0.916	12.391	0.536	0.377	0.57	0.523	0.598	0.709	1	0.195	0.108	1.93	0.338	0.079	0.156	0.291	0.93	DUI	MyEd	M	Somatic
1.32	1.804	1.66	1.885	2.294	3.342	0.402	0.46	6.27	1.64	0.395	0.535	0.492	0.562	0.687	1	0.122	0.137	1.896	0.251	0.157	0.181	0.313	0.896	DUI	MyEd	M	Somatic
1.354	1.538	1.494	1.774	2.219	3.396	0.417	0.342	5.622	1.932	0.399	0.453	0.44	0.523	0.655	1	0.123	0.101	1.661	0.119	0.138	0.2	0.345	0.661	DUI	MyEd	M	Somatic
0.632	0.833	0.842	1.117	1.256	2.022	0.085	0.226	6.563	0.915	0.27	0.355	0.366	0.508	0.578	1	0.029	0.106	3.336	0.218	0.353	0.151	0.422	2.336	DUI	MyEd	M	Somatic
0.285	0.417	0.752	1.122	1.192	3.245	0.511	0.379	3.536	1.141	0.086	0.13	0.231	0.344	0.363	1	0.16	0.118	1.086	0.276	0.341	0.042	0.637	0.086	DUI	MyEd	M	Somatic
0.687	1.219	1.193	1.348	1.719	2.851	0.402	0.2	7.807	1.106	0.241	0.428	0.418	0.473	0.604	1	0.141	0.07	2.74	0.437	-0.043	0.214	0.396	1.74	DUI	MyEd	M	Somatic
0.385	2.245	2.288	2.346	3.089	3.235	0.09	0.033	2.811	2.687	0.113	0.686	0.702	0.721	0.955	1	0.032	0.012	0.842	0.84	0.012	0.244	0.045	-0.158	SMI	PlMg	F	Gametes
0.935	3.415	3.359	3.615	3.942	4.404	-0.042	-0.079	4.997	2.136	0.205	0.766	0.754	0.822	0.889	1	-0.008	-0.02	1.113	0.737	0.055	0.072	0.111	0.113	SMI	PlMg	F	Gametes
0.115	0.678	0.584	0.65	0.516	0.995	0.012	0.073	1.894	1.267	0.115	0.684	0.587	0.654	0.519	1	0.012	0.075	1.905	0.827	0.233	-0.263	0.481	0.905	SMI	PlMg	F	Gametes
0.694	1.015	0.94	1.171	1.336	1.782	0.084	-0.007	2.325	1.86	0.428	0.617	0.547	0.678	0.764	1	0.061	0.013	1.358	0.314	0.288	0.115	0.236	0.358	SMI	PlMg	F	Gametes
0.618	1.179	1.122	1.501	1.5	2.169	-0.072	-0.187	3.013	1.632	0.302	0.558	0.529	0.709	0.708	1	-0.039	-0.093	1.424	0.476	0.234	-0.001	0.292	0.424	SMI	PlMg	F	Gametes
1.695	2.21	2.181	2.074	2.552	3.189	-0.041	0.016	4.114	3.993	0.531	0.693	0.684	0.65	0.8	1	-0.013	0.005	1.29	0.23	-0.039	0.187	0.2	0.29	SMI	PlMg	F	Gametes
0.941	1.289	1.21	1.398	1.735	1.972	-0.033	-0.078	2.711	2.075	0.478	0.654	0.614	0.709	0.879	1	-0.017	-0.039	1.376	0.27	0.359	0.191	0.121	0.376	SMI	PlMg	F	Gametes
0.792	2.296	2.294	2.61	3.276	5.587	0.002	0.003	9.59	4.374	0.147	0.411	0.41	0.467	0.585	1	0	0	1.746	0.642	0.101	0.202	0.415	0.746	SMI	PlMg	M	Gametes
0.193	1.686	1.665	2.265	3.326	5.74	0.043	0.031	11.269	2.214	0.039	0.306	0.297	0.406	0.586	1	0.01	0.004	1.971	0.876	0.215	0.309	0.414	0.971	SMI	PlMg	M	Gametes
1.007	3.851	3.71	4.013	5.248	6.885	0.027	0.037	11.813	2.671	0.143	0.562	0.539	0.583	0.765	1	0.004	0.006	1.722	0.743	0.086	0.238	0.235	0.722	SMI	PlMg	M	Gametes
0.475	3.322	3.344	3.876	5.392	6.206	-0.03	0.056	8.259	3.512	0.062	0.528	0.544	0.63	0.859	1	-0.001	0.013	1.466	0.885	0.153	0.265	0.141	0.466	SMI	PlMg	M	Gametes
0.596	3.466	3.28	3.785	4.263	5.305	0.005	0.139	7.536	1.384	0.112	0.658	0.62	0.716	0.805	1	0	0.025	1.429	0.828	0.124	0.111	0.195	0.429	SMI	PlMg	M	Gametes
0.996	5.715	4.876	5.865	6.363	7.938	-0.115	0.176	10.169	1.327	0.116	0.727	0.632	0.749	0.815	1	-0.017	0.02	1.327	0.839	0.132	0.081	0.185	0.327	SMI	PlMg	M	Gametes
2.138	4.253	4.003	3.472	4.326	5.341	0.059	0.04	6.68	1.252	0.41	0.806	0.759	0.653	0.809	1	0.012	0.008	1.24	0.501	0.134	0.192	0.191	0.24	SMI	PlMg	M	Gametes
0.971	1.994	1.697	1.826	1.939	3.06	0.167	0.143	4.297	0.901	0.318	0.651	0.554	0.596	0.633	1	0.053	0.046	1.4	0.511	0.178	0.056	0.367	0.4	SMI	PlMg	M	Gametes
0.981	1.852	1.716	1.594	1.938	2.634	0.166	0.147	3.083	0.637	0.374	0.707	0.652	0.606	0.739	1	0.063	0.056	1.173	0.471	0.053	0.177	0.261	0.173	SMI	PlMg	M	Gametes
0.992	2.187	2.178	2.2	2.563	3.853	0.296	0.272	12.469	5.302	0.252	0.568	0.565	0.572	0.667	1	0.08	0.072	3.251	0.556	0.031	0.142	0.333	2.251	SMI	PlMg	F	Somatic
1.352	3.142	3.062	2.943	3.409	5.37	0.299	0.227	18.453	2.012	0.252	0.585	0.57	0.548	0.633	1	0.057	0.044	3.425	0.569	0.02	0.134	0.367	2.425	SMI	PlMg	F	Somatic
0.661	2.139	2.296	3.182	3.29	3.827	0.696	0.495	11.14	2.378	0.173	0.555	0.598	0.83	0.86	1	0.184	0.131	2.883	0.685	0.127	0.034	0.14	1.883	SMI	PlMg	F	Somatic
2.297	5.007	5.363	5.323	6.512	8.761	0.289	0.267	17.607	1.243	0.263	0.57	0.611	0.606	0.742	1	0.033	0.03	2.014	0.533	0.015	0.184	0.258	1.014	SMI	PlMg	F	Somatic
1.544	3.416	3.542	3.703	4.629	6.969	0.635	0.477	13.259	1.619	0.222	0.49	0.509	0.532	0.665	1	0.091	0.068	1.905	0.548	-0.012	0.2	0.335	0.905	SMI	PlMg	F	Somatic
1.173	2.155	2.038	2.136	2.5	3.524	0.342	0.217	6.895	2.192	0.332	0.612	0.58	0.608	0.711	1	0.098	0.062	1.951	0.454	0.041	0.146	0.289	0.951	SMI	PlMg	F	Somatic
0.852	2.339	2.396	2.474	3.012	4.029	0.2	0.174	11.399	2.263	0.212	0.582	0.595	0.615	0.749	1	0.05	0.044	2.848	0.636	0.032	0.179	0.251	1.848	SMI	PlMg	F	Somatic
1.182	2.894	3.096	3.157	3.613	4.909	0.269	0.192	9.751	1.964	0.244	0.592	0.633	0.647	0.738	1	0.054	0.039	2.007	0.588	0.054	0.124	0.262	1.007	SMI	PlMg	F	Somatic
1.853	4.399	4.623	4.45	5.26	7.461	0.487	0.423	24.287	0.911	0.25	0.595	0.625	0.6	0.709	1	0.069	0.06	3.257	0.579	-0.043	0.154	0.291	2.257	SMI	PlMg	M	Somatic
1.99	4.087	4.178	4.037	4.507	6.21	0.693	0.706	17.248	1.929	0.314	0.643	0.657	0.638	0.711	1	0.117	0.113	2.819	0.512	0.043	0.102	0.289	1.819	SMI	PlMg	M	Somatic
2.666	6.138	6.706	6.413	7.606	11.612	0.923	0.873	35.147	0.605	0.226	0.532	0.58	0.555	0.658	1	0.079	0.075	3.032	0.559	-0.028	0.157	0.342	2.032	SMI	PlMg	M	Somatic
1.333	2.305	2.507	2.434	2.813	4.744	0.107	0.141	10.724	1.365	0.282	0.48	0.518	0.502	0.576	1	0.023	0.028	2.273	0.405	0.049	0.112	0.424	1.273	SMI	PlMg	M	Somatic
1.372	2.216	2.311	2.34	2.864	4.447	0.33	0.251	10.684	0.914	0.304	0.506	0.528	0.532	0.65	1	0.073	0.058	2.385	0.368	0.013	0.183	0.35	1.385	SMI	PlMg	M	Somatic

Table 2.s3. Statistic tests summary table for figures 2.1, 2.s2, 2.5. Students *t* test summary (:sex, main effect of factor sex) on gametes and somatic cells separately. Two-way ANOVA summary (:sex, main effect of factor sex; :cell-type, main effect of cell type; :sex:cell-type, interaction effect between factors sex and cell-type), followed by Tukey *post hoc* test. Sex: F, female; M, male; Cell-type: gametes; somatic cells. Species: *A. islandica*; *M. edulis*; *M. mercenaria*; *P. magellanicus*. Parameters: $j_{\approx P}$, OXPHOS coupling efficiency; Succinate CF, succinate control factor; Gp CF, glycerophosphate control factor; j_{ExP}, apparent excess capacity of the ETS; j_{ExCIV}, apparent excess capacity of CIV; $NPrcSGp_P$, max coupled respiration (pmol $O_2 \cdot s^{-1} \cdot mU\ CS^{-1}$); $NPrcSGp_E$, max uncoupled respiration (pmol $O_2 \cdot s^{-1} \cdot mU\ CS^{-1}$); CS, citrate synthase activity.

Table S3	$j_{\approx P}$	Succinate CF	Gp CF	j_{ExP}	j_{ExCIV}	$NPrcSGp_P$	$NPrcSGp_E$	CS
Arctica islandica								
Student *t* test								
gametes, :sex	t_{14}=-1.87, p=0.0823	t_{14}=0.50, p=0.62	t_{14}=-0.427, p=0.676	t_{14}=-12.95, p=3.49e-09 ***	t_{14}=2.52, p=0.014 *	t_{14}=2.32, p=0.028 *	t_{14}=-0.54, p=0.59	t_{14}=-1.32, p=0.20
somatic, :sex	t_{8}=-0.97, p=0.357	t_{8}=0.66, p=0.523	t_{8}=-1.86, p=0.099	t_{8}=1.46, p=0.181	t_{8}=1.74, p=0.12	t_{8}=-3.3, p=0.11	t_{8}=-1.45, p=0.184	t_{8}=2.23, p=0.056
two-way ANOVA								
:sex	$F_{1,22}$=2.39, p=0.135	$F_{1,22}$=0.33, p=0.58	$F_{1,22}$=1.79, p=0.194	$F_{1,22}$=83.45, p=6.1e-09 ***	$F_{1,22}$=8.99, p=6.6e-03 **	$F_{1,22}$=0.006, p=0.94	$F_{1,22}$=3, p=0.09	$F_{1,22}$=0.33, p=0.56
:cell-type	$F_{1,22}$=19.18, p=2.4e-04 **	$F_{1,22}$=12.39, p=1.1e-03 **	$F_{1,22}$=0.956, p=0.339	$F_{1,22}$=3.35, p=0.08	$F_{1,22}$=2.99, p=0.09	$F_{1,22}$=15, p=7.8e-04 ***	$F_{1,22}$=10.4, p=3.8e-03 **	$F_{1,22}$=0.47, p=0.49
:sex:cell-type	$F_{1,22}$=0.73, p=0.40	$F_{1,22}$=0.039, p=0.85	$F_{1,22}$=0.30, p=0.587	$F_{1,22}$=88.8, p=3.52e-09 ***	$F_{1,22}$=0.0754, p=0.7839	$F_{1,22}$=8.31, p=8.6e-03 **	$F_{1,22}$=0.57, p=0.45	$F_{1,22}$=4.18, p=0.052
Tukey comparison								
M:gametes-F:gametes				0e-07 ***		0.14	0.94	
F:somatic-F:gametes				3.1e-04 ***		0.78	0.26	
M:somatic-F:gametes				0.015 *		0.023 *	9.4e-03 **	
F:somatic-M:gametes				5.2e-06 ***		0.049 *	0.61	
M:somatic-M:gametes				2e-07 ***		5.3e-04 ***	0.055	
M:somatic-F:somatic				0.49		0.26	0.49	
Mytilus edulis								
Student *t* test								
gametes, :sex	t_{9}=1.98, p=0.0779	t_{9}=0.035, p=0.972	$t_{1,9}$=-3.85, p=3.88e-03 **	$t_{1,9}$=-18.48, p=1.81e-08 ***	t_{9}=4.06, p=2.81e-03 **	t_{9}=2.82, p=0.019 *	t_{9}=-2.58, p=0.029 *	t_{9}=-0.15, p=0.88
somatic, :sex	t_{10}=1.88, p=0.0886	t_{10}=-1.17, p=0.266	t_{10}=-2.54, p=0.0291 *	t_{10}=-2.21, p=0.051	t_{10}=1.39, p=0.193	t_{10}=-0.3, p=0.71	t_{10}=-1.23, p=0.26	t_{10}=1.15, p=0.31
two-way ANOVA								
:sex	$F_{1,19}$=5.77, p=0.0266 *	$F_{1,19}$=0.85, p=0.368	$F_{1,19}$=21.61, p=1.75e-04 ***	$F_{1,19}$=62.58, p=1.98e-07 ***	$F_{1,19}$=8.699, p=8e-03 **	$F_{1,19}$=0.71, p=0.42	$F_{1,19}$=7, p=0.015 *	$F_{1,19}$=1, p=0.37
:cell-type	$F_{1,19}$=56, p=4.45e-07 ***	$F_{1,19}$=3.12, p=0.09	$F_{1,19}$=0.97, p=0.33	$F_{1,19}$=8.86, p=7.7e-03 **	$F_{1,19}$=12.064, p=2.5e-03 **	$F_{1,19}$=0.61, p=0.45	$F_{1,19}$=1.5, p=0.23	$F_{1,19}$=0.15, p=0.78
:sex:cell-type	$F_{1,19}$=0.132, p=0.72	$F_{1,19}$=0.72, p=0.40	$F_{1,19}$=2.55, p=0.12	$F_{1,19}$=15.45, p=8.97e-04 ***	$F_{1,19}$=0.110, p=0.743	$F_{1,19}$=1.89, p=0.19	$F_{1,19}$=0.77, p=0.38	$F_{1,19}$=1.21, p=0.33
Tukey comparison								
M:gametes-F:gametes				6e-07 ***				
F:somatic-F:gametes				0.85				
M:somatic-F:gametes				0.01 *				
F:somatic-M:gametes				1.4e-06 ***				
M:somatic-M:gametes				5.6e-04 ***				
M:somatic-F:somatic				0.04 *				
Mercenaria mercenaria								
Student *t* test								
gametes, :sex	t_{9}=0.85, p=0.414	t_{9}=0.95, p=0.367	t_{9}=0.24, p=0.812	t_{9}=-1.64, p=0.134	t_{9}=0.697, p=0.503	t_{9}=-0.45, p=0.66	t_{9}=-0.92, p=0.37	t_{9}=-1.58, p=0.147
somatic, :sex	t_{8}=0.483, p=0.641	t_{8}=0.71, p=0.496	t_{8}=-1.71, p=0.125	t_{8}=0.25, p=0.809	t_{8}=-0.371, p=0.72	t_{8}=-0.68, p=0.51	t_{8}=-0.77, p=0.46	t_{8}=1.64, p=0.14
two-way ANOVA								
:sex	$F_{1,17}$=0.88, p=0.35	$F_{1,17}$=0.98, p=0.33	$F_{1,17}$=0.20, p=0.65	$F_{1,17}$=1.6, p=0.22	$F_{1,17}$=0.011, p=0.918	$F_{1,17}$=0.66, p=0.45	$F_{1,17}$=1.39, p=0.25	$F_{1,17}$=0.11, p=0.76
:cell-type	$F_{1,17}$=0.87, p=0.36	$F_{1,17}$=14.95, p=1.2e-03 **	$F_{1,17}$=15.95, p=9.4e-004 ***	$F_{1,17}$=1.93, p=0.18	$F_{1,17}$=1.509, p=0.236	$F_{1,17}$=16.78, p=2e-04 ***	$F_{1,17}$=10.26, p=5.2e-03	$F_{1,17}$=0.01, p=0.92
:sex:cell-type	$F_{1,17}$=0.05, p=0.82	$F_{1,17}$=0.23, p=0.63	$F_{1,17}$=0.55, p=0.46	$F_{1,17}$=2.27, p=0.15	$F_{1,17}$=0.527, p=0.478	$F_{1,17}$=0.31, p=0.61	$F_{1,17}$=0.013, p=0.91	$F_{1,17}$=4.86, p=0.0425 *
Tukey comparison								
M:gametes-F:gametes								0.29
F:somatic-F:gametes								0.48
M:somatic-F:gametes								0.99
F:somatic-M:gametes								0.98
M:somatic-M:gametes								0.36
M:somatic-F:somatic								0.57
Placopecten magellanicus								
Student *t* test								
gametes, :sex	t_{14}=-1.56, p=0.14	t_{14}=0.60, p=0.55	t_{14}=-1.58, p=0.136	t_{14}=-0.88, p=0.392	t_{14}=-1.14, p=0.272	t_{7}=-2.35, p=0.051	t_{7}=-4.99, p=1.5e-03 **	t_{7}=-0.13, p=0.89
somatic, :sex	t_{11}=1.92, p=0.08	t_{11}=1.37, p=0.197	t_{11}=0.05, p=0.961	t_{11}=-1.60, p=0.137	t_{11}=-0.67, p=0.512	t_{9}=-0.72, p=0.48	t_{9}=-1.11, p=0.29	t_{9}=4.3, p=1.9e-03 **
two-way ANOVA								
:sex	$F_{1,25}$=0.42, p=0.51	$F_{1,25}$=1, p=0.32	$F_{1,25}$=1.74, p=0.20	$F_{1,25}$=1.39, p=0.24	$F_{1,25}$=1.36, p=0.24	$F_{1,16}$=3.14, p=0.095	$F_{1,16}$=6, p=0.0254 *	$F_{1,16}$=0.52, p=0.48
:cell-type	$F_{1,25}$=1.72, p=0.19	$F_{1,25}$=15.1, p=5e-04 ***	$F_{1,25}$=0.1, p=0.77	$F_{1,25}$=3.2, p=0.085	$F_{1,25}$=55.74, p=1e-04 ***	$F_{1,16}$=0.53, p=0.4748	$F_{1,16}$=2.5, p=0.13	$F_{1,16}$=13.74, p=1.9e-03 **
:sex:cell-type	$F_{1,25}$=3.9, p=0.06	$F_{1,25}$=0, p=0.99	$F_{1,25}$=1.8, p=0.19	$F_{1,25}$=0.004, p=0.95	$F_{1,25}$=0.02, p=0.87	$F_{1,16}$=0.64, p=0.4357	$F_{1,16}$=0.73, p=0.4	$F_{1,16}$=2.51, p=0.13

Table 2.s4. Statistic tests summary table for figures 2.2- 2.3- 2.s3. Students *t* test summary (:sex, main effect of factor sex) on gametes and somatic cells separately. Two-way ANOVA summary (:sex, main effect of factor sex; :cell-type, main effect of cell type; :sex:cell-type, interaction effect between factors sex and cell-type), followed by Tukey *post hoc* test. Sex: F, female; M, male; Cell-type: gametes; somatic cells. Species: *A. islandica*; *M. edulis*; *M. mercenaria*; *P. magellanicus*. Substrates combinations: N, CI-linked substrates pyruvate (P), malate (M) and glutamate (G); c, cytochrome *c*; Pr, proline; S, succinate; Gp, glycerophosphate; Ama, antimycin A addition; Shm, SHAM addition; CIV, CIV activity in presence of ascorbate (As), TMPD (Tm), antimycin A (Ama) and cytochrome *c* (c). Respiratory states: $_{L}$, Leak-state (non-phosphorylating resting state); $_{P}$, OXPHOS-state (coupled respiration); $_{E}$, ETS-state (uncoupled respiration).

Table S4	N_L	N_P	NPr_P	$NPrcS_P$	$NPrcSGp_P$	Ama_E	Shm_E	CIV_E
Arctica islandica								
Student *t* test								
gametes, :sex	t_{14}=7.59, p=2.5e-06 ***	t_{14}=4.38, p=6.19e-04 ***	t_{14}=4.09, p=7e-04 ***	t_{14}=5.64, p=6.04e-05 ***	t_{14}=12.95, p=3.49e-09 ***	t_{14}=4.95, p=2.11e-04 ***	t_{14}=4.3, p=7.33e-04 ***	t_{14}=2.52, p=0.012 *
somatic, :sex	t_8=0.54, p=0.604	t_8=-0.068, p=0.947	t_8=-0.55, p=0.595	t_8=-0.39, p=0.703	t_8=-1.46, p=0.181	t_8=-0.86, p=0.415	t_8=-1.15, p=0.283	t_8=1.74, p=0.12
two-way ANOVA								
:sex	$F_{1,22}$=13.30, p=1.42e-03 **	$F_{1,22}$=12.67, p=1.76e-03 **	$F_{1,22}$=7.64, p=9e-03 **	$F_{1,22}$=26..47, p=1e-04 ***	$F_{1,22}$=83.45, p=6.10e-09 ***	$F_{1,22}$=10.30, p=4e-03 **	$F_{1,22}$=6.07, p=0.0236 *	$F_{1,22}$=8.99, p=7.2e-03 **
:cell-type	$F_{1,22}$=30.33, p=1.56e-05 ***	$F_{1,22}$=8.54, p=7.89e-03 **	$F_{1,22}$=11.46, p=2.2e-03 **	$F_{1,22}$=8.23, p=7.1e-0.3 **	$F_{1,22}$=3.354, p=0.08	$F_{1,22}$=18.32, p=3e-04 ***	$F_{1,22}$=16.87, p=9e-04 ***	$F_{1,22}$=2.99, p=0.1
:sex:cell-type	$F_{1,22}$=8.17, p=9.13e-03 **	$F_{1,22}$=10.47, p=3.8e-03 **	$F_{1,22}$=10.79, p=2.6e-03 **	$F_{1,22}$=29.78, p=1e-04 ***	$F_{1,22}$=88.80, p=3.52e-09 ***	$F_{1,22}$=15.64, p=6.7e-04 ***	$F_{1,22}$=13.79, p=1.3e-03 **	$F_{1,22}$=0.0754, p=0.7839
Tukey comparison								
M:gametes-F:gametes	2.0e-04 ***	2.48e-04 ***	4.8e-04 ***	4.3e-06 ***	0e-07 ***	1.17e-04 ***	3.7e-04 ***	
F:somatic-F:gametes	0.1638	1	0.99	0.082	3.1e-04 ***	0.953	0.99	
M:somatic-F:gametes	0.4069	0.99	0.96	0.12	0.015 *	0.543	0.618	
F:somatic-M:gametes	1.25e-05 ***	1.48e-03 **	2.2e-03 **	9.5e-03 **	5.2e-06 ***	2.51e-04 ***	1.1e-03 **	
M:somatic-M:gametes	4.2e-05 ***	1.3e-03 **	9.5e-04 ***	6.2e-03 **	2e-07 ***	4.3e-05 ***	1.4e-04 ***	
M:somatic-F:somatic	0.9573	0.99	0.98	0.99	0.498	0.88	0.85	
Mytilus edulis								
Student *t* test								
gametes, :sex	t_9=2.01, p=0.0752	t_9=6.31, p=1.39e-04 ***	t_9=7.95, p=2.32e-05 ***	t_9=19.31, p=1.23e-08 ***	t_9=18.48, p=1.81e-08 ***	t_9=1.30, p=0.223	t_9=0.998, p=0.344	t_9=4.06, p=2.81e-03 **
somatic, :sex	t_{10}=1.40, p=0.189	t_{10}=2.60, p=0.0264 *	t_{10}=2.61, p=0.0258 *	t_{10}=3.22, p=9e-03 **	t_{10}=2.21, p=0.051	t_{10}=0.93, p=0.373	t_{10}=-0.10, p=0.92	t_{10}=1.39, p=0.193
two-way ANOVA								
:sex	$F_{1,19}$=5.46, p=0.0305 *	$F_{1,19}$=33.32, p=1.46e-05 ***	$F_{1,19}$=46.02, p=1.77e-06 ***	$F_{1,19}$=103.96, p=3.84e-09 ***	$F_{1,19}$=62.58, p=1.98e-07 ***	$F_{1,19}$=2.36, p=0.14	$F_{1,19}$=0.392, p=0.538	$F_{1,19}$=8.699, p=8.23e-03 **
:cell-type	$F_{1,19}$=34.08, p=1.27e-05 ***	$F_{1,19}$=9.65, p=5.81e-03 **	$F_{1,19}$=11.24, p=3.34e-03 **	$F_{1,19}$=9.12, p=7e-03 **	$F_{1,19}$=8.86, p=7.7e-03 **	$F_{1,19}$=9.13, p=7e-03 **	$F_{1,19}$=5.947, p=0.0247 *	$F_{1,19}$=12.064, p=2.5e-03 **
:sex:cell-type	$F_{1,19}$=0.18, p=0.67	$F_{1,19}$=2.37, p=0.14	$F_{1,19}$=6.85, p=0.0169 *	$F_{1,19}$=20.53, p=2.2e-04 ***	$F_{1,19}$=15.45, p=8.9e-04 ***	$F_{1,19}$=0.049, p=0.82	$F_{1,19}$=0.497, p=0.48	$F_{1,19}$=0.110, p=0.743
Tukey comparison								
M:gametes-F:gametes			1.8e-05 ***	0e-07 ***	6e-07 ***			
F:somatic-F:gametes			0.97	0.63	0.85			
M:somatic-F:gametes			0.1	3e-04 ***	0.01 *			
F:somatic-M:gametes			4e-06 ***	1e-07 ***	1.4e-06 ***			
M:somatic-M:gametes			2.3e-03 **	2.1e-04 ***	5.6e-04 ***			
M:somatic-F:somatic			0.034 *	2.8e-03 **	0.041 *			
Mercenaria mercenaria								
Student *t* test								
gametes, :sex	t_9=0.21, p=0.837	t_9=0.93, p=0.373	t_9=0.64, p=0.536	t_9=1.25, p=0.24	t_9=1.64, p=0.134	t_9=0.56, p=0.589	t_9=0.849, p=0.418	t_9=0.697, p=0.503
somatic, :sex	t_8=-0.65, p=0.67	t_8=-0.54, p=0.608	t_8=-0.30, p=0.766	t_8=0.32, p=0.756	t_8=-0.25, p=0.809	t_8=-0.54, p=0.601	t_8=-0.57, p=0.584	t_8=-0.371, p=0.72
two-way ANOVA								
:sex	$F_{1,17}$=0.375, p=0.665	$F_{1,17}$=0.141, p=0.711	$F_{1,17}$=0.05, p=0.822	$F_{1,17}$=1.92, p=0.183	$F_{1,17}$=1.60, p=0.224	$F_{1,17}$=0.084, p=0.776	$F_{1,17}$=0.283, p=0.601	$F_{1,17}$=0.011, p=0.918
:cell-type	$F_{1,17}$=5.8, p=5.7e-03 **	$F_{1,17}$=24.32, p=1.29e-04 ***	$F_{1,17}$=31.15, p=2e-04 ***	$F_{1,17}$=9.84, p=6e-03 **	$F_{1,17}$=1.93, p=0.185	$F_{1,17}$=1.224, p=0.284	$F_{1,17}$=2.842, p=0.11	$F_{1,17}$=1.509, p=0.236
:sex:cell-type	$F_{1,17}$=0.52, p=0.58	$F_{1,17}$=0.985, p=0.334	$F_{1,17}$=0.447, p=0.504	$F_{1,17}$=0.748, p=0.399	$F_{1,17}$=2.27, p=0.1509	$F_{1,17}$=0.545, p=0.471	$F_{1,17}$=0.995, p=0.33	$F_{1,17}$=0.527, p=0.478
Placopecten magellanicus								
Student *t* test								
gametes, :sex	t_{14}=1.53, p=0.148	t_{14}=1.08, p=0.295	t_{14}=1.26, p=0.227	t_{14}=2.3, p=0.0368 *	t_{14}=0.88, p=0.392	t_{14}=-0.64, p=0.53	t_{14}=-1.42, p=0.177	t_{14}=-1.14, p=0.272
somatic, :sex	t_{11}=-1.29, p=0.222	t_{11}=0.64, p=0.532	t_{11}=0.036, p=0.971	t_{11}=1.17, p=0.28	t_{11}=1.60, p=0.137	t_{11}=0.35, p=0.73	t_{11}=-0.31, p=0.762	t_{11}=-0.67, p=0.512
two-way ANOVA								
:sex	$F_{1,25}$=2.63, p=0.117	$F_{1,25}$=1.264, p=0.272	$F_{1,25}$=1.322, p=0.261	$F_{1,25}$=5.00, p=0.0344 *	$F_{1,25}$=1.399, p=0.248	$F_{1,25}$=0.0015, p=0.969	$F_{1,25}$=0.305, p=0.585	$F_{1,25}$=0.007, p=0.933
:cell-type	$F_{1,25}$=2.4, p=0.134	$F_{1,25}$=2.00, p=0.169	$F_{1,25}$=0.166, p=0.687	$F_{1,25}$=3.642, p=0.0679	$F_{1,25}$=3.206, p=0.085	$F_{1,25}$=24.36, p=1e-04 ***	$F_{1,25}$=19.46, p=1.71e-04 ***	$F_{1,25}$=60.66, p=3.81e-08 ***
:sex:cell-type	$F_{1,25}$=2.108, p=0.159	$F_{1,25}$=0.567, p=0.459	$F_{1,25}$=1.080, p=0.309	$F_{1,25}$=0.604, p=0.444	$F_{1,25}$=0.004, p=0.95	$F_{1,25}$=0.444, p=0.511	$F_{1,25}$=0.631, p=0.434	$F_{1,25}$=0.036, p=0.85

Table 2.s5. Statistic tests summary table for figure 2.4. Two-way ANOVA summary (:sex, main effect of factor sex; :species, main effect of species; :sex:species, interaction effect between factors sex and species), followed by Tukey *post hoc* test. Sex: F, female; M, male; Species: *A. islandica*; *M. edulis*; *M. mercenaria*; *P. magellanicus*. Substrates combinations: N, CI-linked substrates pyruvate (P), malate (M) and glutamate (G); c, cytochrome *c*; Pr, proline; S, succinate; Gp, glycerophosphate; Ama, antimycin A addition; Shm, SHAM addition; CIV, CIV activity in presence of ascorbate (As), TMPD (Tm), antimycin A (Ama) and cytochrome *c* (c). Respiratory states: $_{L}$, Leak-state (non-phosphorylating resting state); $_{P}$, OXPHOS-state (coupled respiration); $_{E}$, ETS-state (uncoupled respiration).

Table S5	**N_L**	**N_P**	**NPr_P**	**$NPrcS_P$**	**$NPrcSGp_P$**	**Ama_E**	**Shm_E**	**CIV_E**
DUI gametes: *Arctica islandica + Mytilus edulis*								
two-way ANOVA								
:sex	$F_{1,23}$=22.89, p=1e-04 ***	$F_{1,23}$=48.82, p=4.03e-07 ***	$F_{1,23}$=59.39, p=1e-04 ***	$F_{1,23}$=122.45, p=1e-04 ***	$F_{1,23}$=396, p=5.38e-16 ***	$F_{1,23}$=39.45, p=2.08e-06 ***	$F_{1,23}$=14.13 p=7e-04 ***	$F_{1,23}$=10.05, p=1e-03 **
:species	$F_{1,23}$=2.291, p=0.1484	$F_{1,23}$=2.65, p=0.117	$F_{1,23}$=2.797, p=0.105	$F_{1,23}$=0.34, p=0.56	$F_{1,23}$=7.03, p=0.0142 *	$F_{1,23}$=18.69, p=2.52e-04 ***	$F_{1,23}$=15.99, p=6e-04 ***	$F_{1,23}$=1.17, p=0.313
:sex:species	$F_{1,23}$=4.324, p=0.0501	$F_{1,23}$=1.293, p=0.267	$F_{1,23}$=3.10, p=0.09	$F_{1,23}$=0.068, p=0.79	$F_{1,23}$=1.07, p=0.3115	$F_{1,23}$=12.27, p=1.9e-03 **	$F_{1,23}$=10.26, p=3.5e-03 **	$F_{1,23}$=0.8353, p=0.3995
Tukey comparison								
M:ArIs-F:ArIs						8.3e-06 ***	1e-04 ***	
F:MyEd-F:ArIs						6.7e-05 ***	1.4e-04 ***	
M:MyEd-F:ArIs						2.7e-06 ***	2.4e-05 ***	
F:MyEd-M:ArIs						0.9659	0.99	
M:MyEd-M:ArIs						0.9719	0.94	
M:MyEd-F:MyEd						0.8154	0.98	
SMI gametes: *Mercenaria mercenaria + Placopecten magellanicus*								
two-way ANOVA								
:sex	$F_{1,23}$=2.00, p=0.17	$F_{1,23}$=1.63, p=0.214	$F_{1,23}$=1.62, p=0.215	$F_{1,23}$=5.62, p=0.0264 *	$F_{1,23}$=2.91, p=0.1	$F_{1,16}$=2.96, p=0.1	$F_{1,20}$=2.82, p=0.109	$F_{1,23}$=0.03, p=0.864
:species	$F_{1,23}$=1.05, p=0.316	$F_{1,23}$=60.82, p=6.62e-08 ***	$F_{1,23}$=57.42, p=1.07e-07 ***	$F_{1,23}$=33.09, p=7.36e-06 ***	$F_{1,23}$=17.61, p=3.45e-04 ***	$F_{1,16}$=23.90, p=1.64e-04 ***	$F_{1,20}$=35.38, p=8.12e-06 ***	$F_{1,23}$=61.03, p=6.44e-08 ***
:sex:species	$F_{1,23}$=1.45, p=0.24	$F_{1,23}$=0.034, p=0.855	$F_{1,23}$=0.20, p=0.658	$F_{1,23}$=0.008, p=0.929	$F_{1,23}$=0.695, p=0.413	$F_{1,16}$=0.749, p=0.399	$F_{1,20}$=0.042, p=0.84	$F_{1,23}$=1.61, p=0.217

Chapter III - Electronic supplementary material

(a) Supporting figures

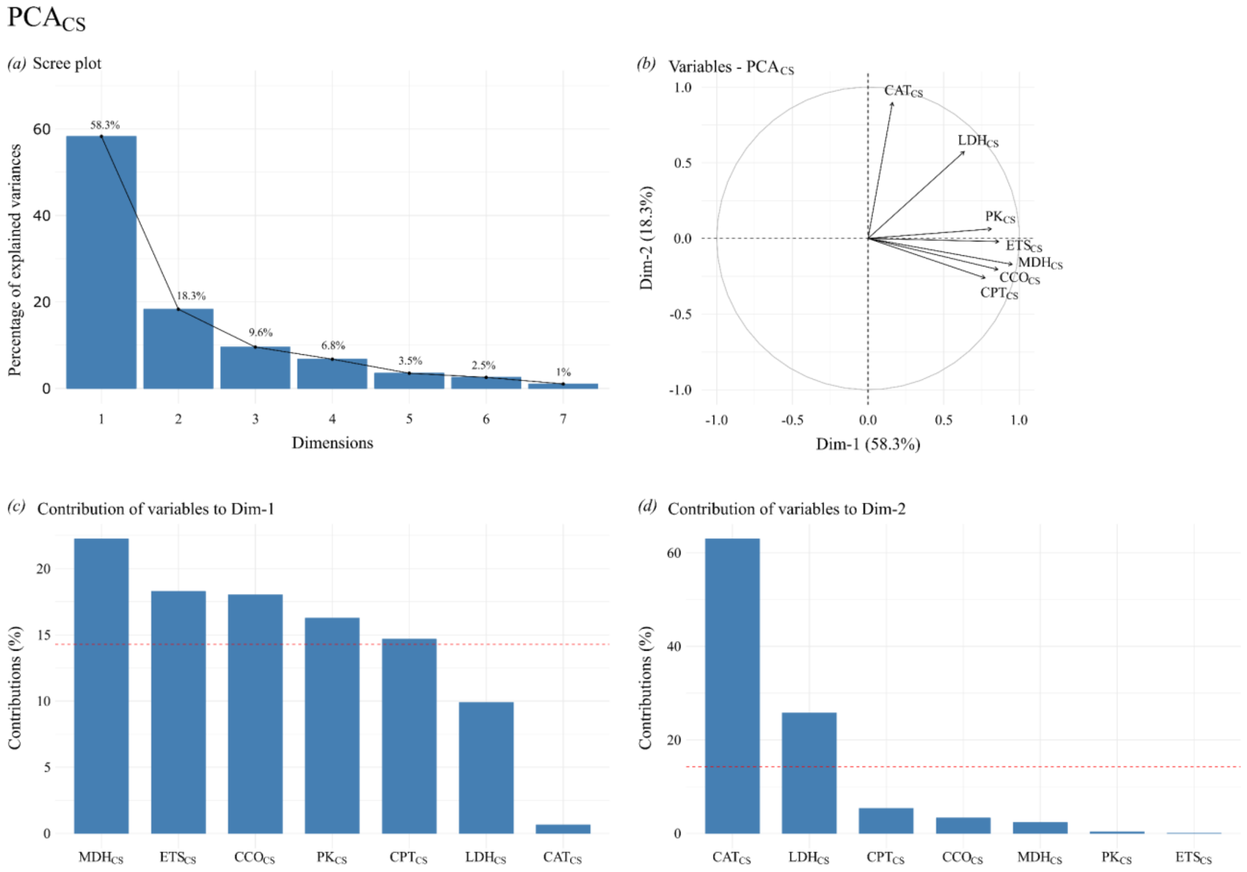

Figure 3.s1. PCA$_{CS}$ summary. *(a)* Percentage of explained variance of each principal component. *(b)* Variable correlation plots. *(c)* Contribution of variables to the first principal component (PC1). *(d)* Contribution of variables to the second principal component (PC2). Parameters: PK, pyruvate kinase; LDH, lactate dehydrogenase; CPT, carnitine palmitoyl transferase; CS, citrate synthase, MDH, malate dehydrogenase; ETS, electron transport chain; CCO, cytochrome *c* oxidase; CAT, catalase. Additional information in tables 3.s1 and 3.s2.

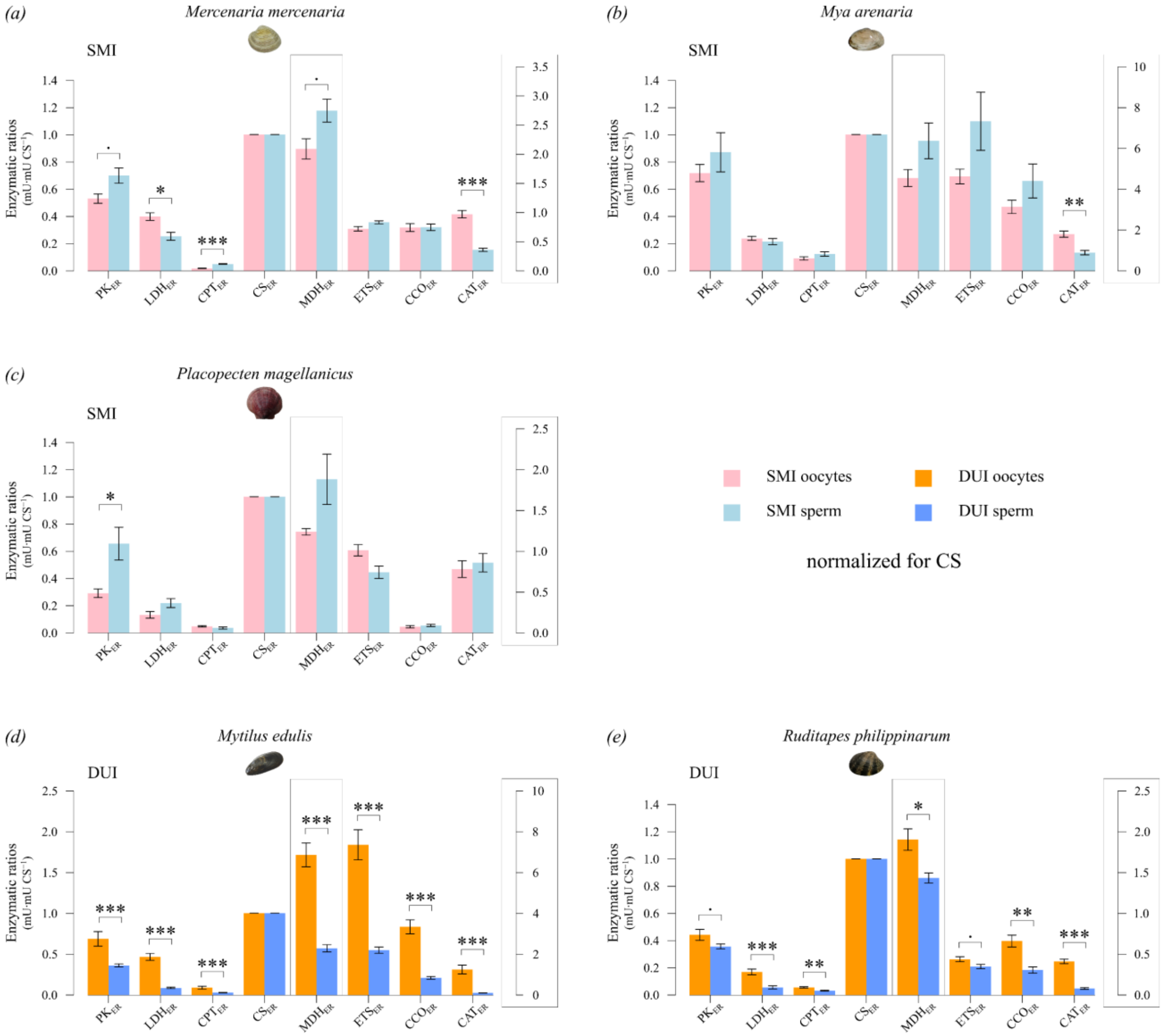

Figure 3.s2. Intraspecific comparison between eggs and sperm enzymatic activity ratios (mU·mU CS^{-1}). *(a) M. mercenaria* (n = 10, 6). *(b) M. arenaria* (n = 10, 10). *(c) P. magellanicus* (n = 8, 8). *(d) M. edulis* (n = 10, 10). *(e) R. philippinarum* (n = 10, 10). Enzymes: PK, pyruvate kinase; LDH, lactate dehydrogenase; CPT, carnitine palmitoyl transferase; CS, citrate synthase, MDH, malate dehydrogenase; ETS, electron transport chain; CCO, cytochrome *c* oxidase; CAT, catalase. Data are presented as means ± s.e.m. Two-tailed Student's *t* test was performed independently for each parameter and each species. $\cdot 0.05 < p \leq 0.09$, $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$. *p*-values corrected with Holm adjustment for multiple testing. The parameters in boxes refer to the right ladder. Detailed summary is reported in electronic supplementary material, tables 3.s2 and 3.s4.

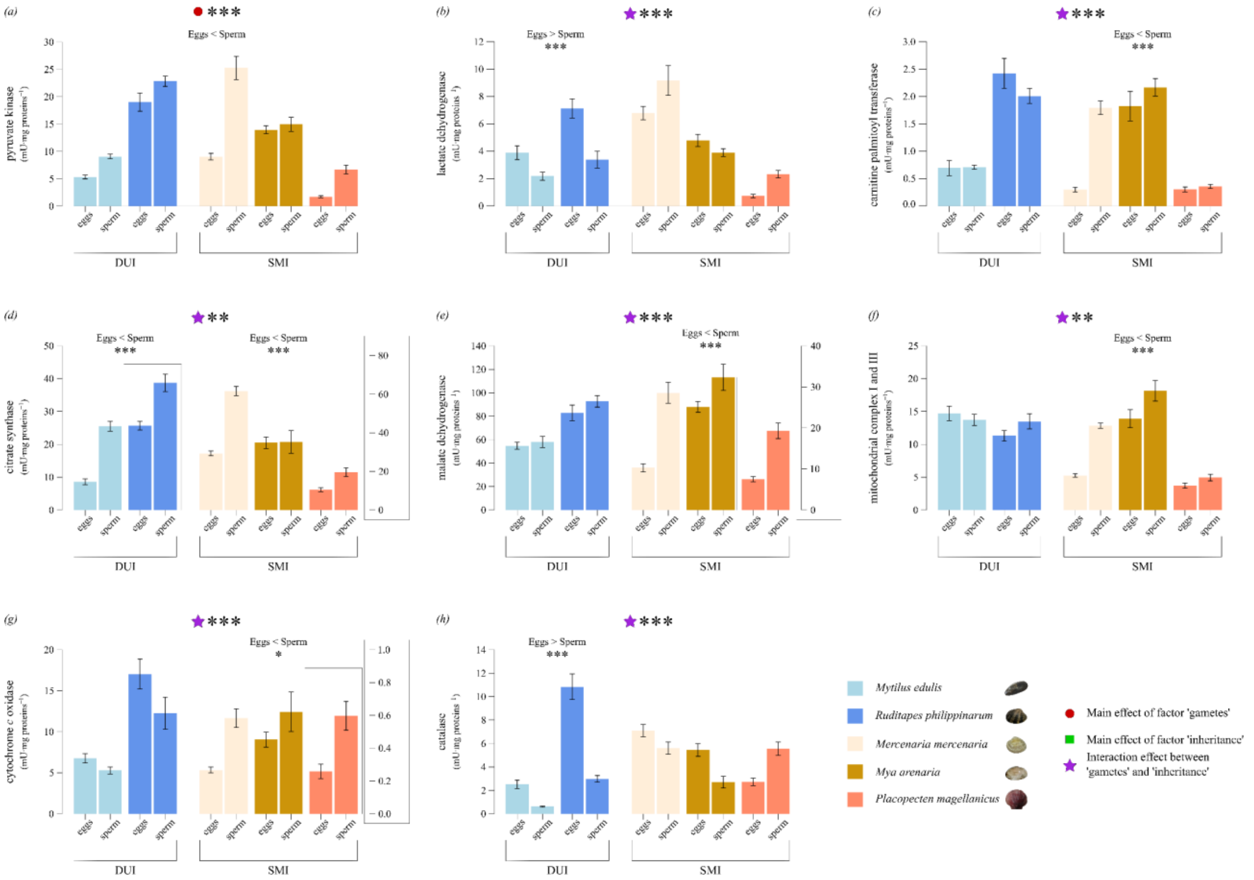

Figure 3.s3. Interaction effect between gamete type (eggs, sperm) and mitochondrial inheritance system (SMI and DUI) on enzymatic activities (mU·mg proteins^{-1}). *(a)* Pyruvate kinase activity. *(b)* Lactate dehydrogenase activity. *(c)* Carnitine palmitoyl transferase activity. *(d)* Malate dehydrogenase activity. *(e)* Mitochondrial complex I and III activity. *(f)* Cytochrome *c* oxidase activity. *(g)* Catalase activity. Data are presented as means ± s.e.m. The main effect of the two fixed factors 'gametes' and 'inheritance' are indicated with a circle and square respectively. Interaction effect is indicated with a star. **p* ≤ 0.05, ***p* ≤ 0.01, ****p* ≤ 0.001. DUI species: *M. edulis* (*n* = 10, 10), *R. philippinarum* (*n* = 10, 10). SMI species: *M. mercenaria* (*n* = 10, 6), *M. arenaria* (*n* = 10, 10), *P. magellanicus* (*n* = 8, 8). Detailed summary is reported in tables 3.s2 and 3.s5.

(b) Supporting tables

Table 3.s1. PCA summary. Contribution and correlation of the variables with principal components. PCA_{CS} refers to a principal component analysis implemented with enzymatic ratios (mU·mU CS^{-1}). The contributions of variables in accounting for the variability in a given principal component are expressed in percentage. Significant correlation coefficients ($p \leq 0.05$) are shown in bold.

Table s1						
PCA_{CS}		**$PC1_{CS}$**			**$PC2_{CS}$**	
Variable		Contribution	Correlation		Contribution	Correlation
PK_{CS} (mU·mg CS^{-1})		16.26	**0.81**		0.31	0.06
LDH_{CS} (mU·mg CS^{-1})		9.90	**0.64**		25.75	**0.57**
CPT_{CS} (mU·mg CS^{-1})		14.67	**0.77**		5.35	**-0.26**
MDH_{CS} (mU·mg CS^{-1})		22.24	**0.95**		2.30	-0.17
ETS_{CS} (mU·mg CS^{-1})		18.28	**0.86**		0.03	-0.02
CCO_{CS} (mU·mg CS^{-1})		18.03	**0.86**		3.30	**-0.21**
CAT_{CS} (mU·mg CS^{-1})		0.63	0.16		62.95	**0.90**

Table 3.s2. Data summary table. Data are reported as enzymatic activity (mU·mg $proteins^{-1}$) as well as enzymatic ratios, either normalized for citrate synthase ('CS' in subscript, mU·mU CS^{-1}) or cytochrome *c* oxidase ('CCO' in subscript, mU·mU CCO^{-1}). Enzymes: PK, pyruvate kinase; LDH, lactate dehydrogenase; CPT, carnitine palmitoyl transferase; CS, citrate synthase, MDH, malate dehydrogenase; ETS, electron transport chain; CCO, cytochrome *c* oxidase; CAT, catalase; PC1, principal component 1; PC2, principal component 2. Gametes: Oocytes; Sperm. Species: *Mytilus edulis* (*n* = 10, 10); *Ruditapes philippinarum* (*n* = 10, 10); *Mercenaria mercenaria* (*n* = 10, 6); *Mya arenaria* (*n* = 10, 10); *Placopecten magellanicus* (*n* = 8, 8). Inheritance: DUI, doubly uniparental inheritance; SMI, strict maternal inheritance.

Table s2			Enzymatic activity (mU·mg proteins^{-1})								Enzymatic ratios (mU·mU CS^{-1})								PCA$_{CS}$		Enzymatic ratios (mU·mU CCO^{-1})							
Species	**Gametes**	**Inheritance**	**PK**	**LDH**	**CPT**	**CS**	**MDH**	**ETS**	**CCO**	**CAT**	**PK$_{CS}$**	**LDH$_{CS}$**	**CPT$_{CS}$**	**CS$_{CS}$**	**MDH$_{CS}$**	**ETS$_{CS}$**	**CCO$_{CS}$**	**CAT$_{CS}$**	**PC1$_{CS}$**	**PC2$_{CS}$**	**PK$_{CCO}$**	**LDH$_{CCO}$**	**CPT$_{CCO}$**	**CS$_{CCO}$**	**MDH$_{CCO}$**	**ETS$_{CCO}$**	**CCO$_{CCO}$**	**CAT$_{CCO}$**
M. edulis	Oocytes	DUI	5.38	6.20	0.50	10.64	56.61	13.62	6.57	2.12	0.51	0.58	0.05	1.00	5.32	1.28	0.62	0.20	2.08	1.13	0.82	1.14	0.08	1.62	8.62	2.07	1.00	0.32
M. edulis	Oocytes	DUI	5.92	6.75	0.59	11.79	70.05	17.42	9.51	3.86	0.50	0.57	0.05	1.00	5.94	1.48	0.81	0.33	2.42	1.05	0.62	0.71	0.06	1.24	7.36	1.83	1.00	0.41
M. edulis	Oocytes	DUI	7.15	4.71	0.62	11.77	68.06	20.85	9.13	2.45	0.61	0.40	0.05	1.00	5.78	1.77	0.78	0.21	2.33	-0.01	0.78	0.52	0.07	1.29	7.46	2.29	1.00	0.27
M. edulis	Oocytes	DUI	5.29	4.11	0.93	5.90	45.44	19.13	5.17	4.18	0.90	0.70	0.16	1.00	7.71	3.24	0.88	0.71	6.16	2.36	1.02	0.79	0.18	1.14	8.78	3.70	1.00	0.81
M. edulis	Oocytes	DUI	4.17	3.99	0.36	7.66	48.25	13.12	6.76	1.64	0.54	0.52	0.05	1.00	6.30	1.71	0.88	0.21	2.67	0.34	0.62	0.59	0.05	1.13	7.13	1.94	1.00	0.24
M. edulis	Oocytes	DUI	3.98	3.43	0.59	11.81	51.19	14.53	6.35	2.34	0.34	0.29	0.05	1.00	4.33	1.23	0.54	0.20	0.64	-0.21	0.63	0.54	0.09	1.86	8.07	2.29	1.00	0.37
M. edulis	Oocytes	DUI	4.06	2.93	0.45	6.49	45.18	11.27	6.07	0.82	0.62	0.45	0.07	1.00	6.96	1.74	0.94	0.13	3.03	-0.45	0.67	0.48	0.07	1.07	7.44	1.86	1.00	0.13
M. edulis	Oocytes	DUI	6.14	2.25	1.85	8.95	62.74	14.63	7.81	4.08	0.69	0.25	0.21	1.00	7.01	1.64	0.87	0.46	3.88	-0.42	0.79	0.29	0.24	1.15	8.03	1.87	1.00	0.52
M. edulis	Oocytes	DUI	6.33	2.05	0.65	4.74	52.07	9.85	7.01	1.59	1.34	0.43	0.14	1.00	10.98	2.08	1.48	0.34	6.54	-0.45	0.90	0.29	0.09	0.68	7.43	1.41	1.00	0.23
M. edulis	Oocytes	DUI	4.65	2.57	0.41	5.65	46.72	12.47	3.10	1.88	0.82	0.45	0.07	1.00	8.27	2.21	0.55	0.33	3.49	0.59	1.50	0.83	0.13	1.82	15.05	4.02	1.00	0.61
M. edulis	Sperm	DUI	11.08	4.53	0.77	33.24	72.50	15.20	6.79	0.47	0.33	0.14	0.02	1.00	2.18	0.46	0.20	0.01	-1.51	-0.99	1.63	0.67	0.11	4.90	10.69	2.24	1.00	0.07
M. edulis	Sperm	DUI	9.72	2.56	0.58	26.99	64.80	15.94	5.21	0.43	0.36	0.09	0.02	1.00	2.40	0.59	0.19	0.02	-1.44	-1.13	1.87	0.49	0.11	5.18	12.44	3.06	1.00	0.08
M. edulis	Sperm	DUI	9.00	2.53	0.60	22.52	46.84	14.96	5.88	0.52	0.40	0.11	0.03	1.00	2.08	0.66	0.26	0.02	-1.21	-1.08	1.53	0.43	0.10	3.83	7.97	2.55	1.00	0.09
M. edulis	Sperm	DUI	8.90	1.43	0.80	32.14	47.61	17.49	5.19	0.83	0.28	0.04	0.02	1.00	1.48	0.54	0.16	0.03	-1.90	-1.21	1.71	0.28	0.15	6.19	9.17	3.37	1.00	0.16
M. edulis	Sperm	DUI	8.73	1.37	0.89	25.08	61.66	9.83	7.83	0.69	0.35	0.05	0.04	1.00	2.46	0.39	0.31	0.03	-1.38	-1.36	1.11	0.17	0.11	3.20	7.87	1.26	1.00	0.09
M. edulis	Sperm	DUI	8.18	1.74	0.66	26.43	54.89	11.88	5.84	0.68	0.31	0.07	0.02	1.00	2.08	0.45	0.22	0.03	-1.67	-1.20	1.40	0.30	0.11	4.53	9.40	2.03	1.00	0.12
M. edulis	Sperm	DUI	6.93	1.81	0.63	17.75	29.37	12.07	4.20	0.50	0.39	0.10	0.04	1.00	1.65	0.68	0.24	0.03	-1.27	-1.10	1.65	0.43	0.15	4.22	6.99	2.87	1.00	0.12
M. edulis	Sperm	DUI	7.74	1.93	0.55	22.07	49.09	16.84	4.08	0.56	0.35	0.09	0.03	1.00	2.22	0.76	0.18	0.03	-1.35	-1.12	1.90	0.47	0.14	5.41	12.03	4.13	1.00	0.14
M. edulis	Sperm	DUI	10.89	1.66	0.84	22.09	76.91	10.29	4.44	0.55	0.49	0.07	0.04	1.00	3.48	0.47	0.20	0.03	-1.01	-1.29	2.45	0.37	0.19	4.98	17.32	2.32	1.00	0.12
M. edulis	Sperm	DUI	9.20	2.29	0.76	26.58	74.49	12.45	3.33	0.90	0.35	0.09	0.03	1.00	2.80	0.47	0.13	0.03	-1.51	-1.10	2.76	0.69	0.23	7.97	22.35	3.73	1.00	0.27
R. philippinarum	Oocytes	DUI	16.16	10.86	2.25	32.44	76.27	11.54	14.39	7.28	0.50	0.33	0.07	1.00	2.35	0.36	0.44	0.22	-0.05	0.20	1.12	0.75	0.16	2.25	5.30	0.80	1.00	0.51
R. philippinarum	Oocytes	DUI	30.09	10.95	2.68	52.24	129.26	15.62	23.33	19.74	0.58	0.21	0.05	1.00	2.47	0.30	0.45	0.38	-0.31	0.52	1.29	0.47	0.12	2.24	5.54	0.67	1.00	0.85
R. philippinarum	Oocytes	DUI	20.85	6.71	1.55	44.51	81.10	8.12	14.78	10.30	0.47	0.15	0.03	1.00	1.82	0.18	0.33	0.23	-1.18	-0.11	1.41	0.45	0.11	3.01	5.49	0.55	1.00	0.70
R. philippinarum	Oocytes	DUI	23.90	7.12	1.28	35.11	78.16	8.55	18.37	9.48	0.68	0.20	0.04	1.00	2.23	0.24	0.52	0.27	-0.33	0.11	1.30	0.39	0.07	1.91	4.26	0.47	1.00	0.52
R. philippinarum	Oocytes	DUI	14.84	6.68	3.80	49.98	84.59	14.67	10.71	12.42	0.30	0.13	0.08	1.00	1.69	0.29	0.21	0.25	-1.20	-0.27	1.39	0.62	0.36	4.67	7.90	1.37	1.00	1.16
R. philippinarum	Oocytes	DUI	21.47	5.47	2.44	42.16	92.83	11.85	29.06	10.34	0.51	0.13	0.06	1.00	2.20	0.28	0.69	0.25	-0.29	-0.49	0.74	0.19	0.08	1.45	3.19	0.41	1.00	0.36
R. philippinarum	Oocytes	DUI	17.60	5.19	2.61	49.99	80.16	9.40	16.47	10.52	0.35	0.10	0.05	1.00	1.60	0.19	0.33	0.21	-1.35	-0.46	1.07	0.32	0.16	3.04	4.87	0.57	1.00	0.64
R. philippinarum	Oocytes	DUI	18.25	8.10	3.35	50.12	86.07	10.12	18.44	9.64	0.36	0.16	0.07	1.00	1.72	0.20	0.37	0.19	-1.00	-0.45	0.99	0.44	0.18	2.72	4.67	0.55	1.00	0.52
R. philippinarum	Oocytes	DUI	14.84	5.64	1.26	40.59	73.79	10.50	15.15	10.27	0.37	0.14	0.03	1.00	1.82	0.26	0.37	0.25	-1.26	-0.09	0.98	0.37	0.08	2.68	4.87	0.69	1.00	0.68
R. philippinarum	Oocytes	DUI	11.82	4.72	3.02	39.05	43.25	12.46	9.37	8.20	0.30	0.12	0.08	1.00	1.11	0.32	0.24	0.21	-1.29	-0.46	1.26	0.50	0.32	4.17	4.62	1.33	1.00	0.88
R. philippinarum	Sperm	DUI	21.73	7.50	1.25	55.53	100.93	9.63	15.10	2.20	0.39	0.14	0.02	1.00	1.82	0.17	0.27	0.04	-1.61	-0.89	1.44	0.50	0.08	3.68	6.68	0.64	1.00	0.15
R. philippinarum	Sperm	DUI	20.34	4.52	2.03	41.70	61.94	11.60	6.08	2.22	0.49	0.11	0.05	1.00	1.49	0.28	0.15	0.05	-1.46	-0.94	3.34	0.74	0.33	6.85	10.18	1.91	1.00	0.36
R. philippinarum	Sperm	DUI	19.19	1.56	2.25	51.62	83.94	11.26	5.14	4.90	0.37	0.03	0.04	1.00	1.63	0.22	0.10	0.09	-1.90	-1.01	3.73	0.30	0.44	10.04	16.33	2.19	1.00	0.95
R. philippinarum	Sperm	DUI	25.82	3.51	2.09	72.01	101.51	17.26	7.61	2.87	0.36	0.05	0.03	1.00	1.41	0.24	0.11	0.04	-2.05	-1.09	3.39	0.46	0.27	9.46	13.33	2.27	1.00	0.38
R. philippinarum	Sperm	DUI	25.60	4.28	1.84	70.94	103.61	13.27	7.54	3.22	0.36	0.06	0.03	1.00	1.46	0.19	0.11	0.05	-2.08	-1.01	3.40	0.57	0.24	9.41	13.75	1.76	1.00	0.43
R. philippinarum	Sperm	DUI	24.05	1.16	2.87	70.86	105.85	21.29	11.79	3.09	0.34	0.02	0.04	1.00	1.49	0.30	0.17	0.04	-1.89	-1.30	2.04	0.10	0.24	6.01	8.98	1.81	1.00	0.26
R. philippinarum	Sperm	DUI	24.88	4.60	1.79	87.33	100.77	14.08	24.50	4.03	0.28	0.05	0.02	1.00	1.15	0.16	0.28	0.05	-2.08	-1.11	1.02	0.19	0.07	3.56	4.11	0.57	1.00	0.16
R. philippinarum	Sperm	DUI	26.30	2.36	1.74	85.39	109.56	14.92	15.75	2.09	0.31	0.03	0.02	1.00	1.28	0.17	0.18	0.02	-2.21	-1.23	1.67	0.15	0.11	5.42	6.96	0.95	1.00	0.13
R. philippinarum	Sperm	DUI	17.96	2.89	2.40	59.48	76.68	11.54	10.72	2.20	0.30	0.05	0.04	1.00	1.29	0.19	0.18	0.04	-1.99	-1.21	1.68	0.27	0.22	5.55	7.16	1.08	1.00	0.21
R. philippinarum	Sperm	DUI	22.17	1.53	1.84	62.65	81.20	9.67	18.04	2.89	0.35	0.02	0.03	1.00	1.30	0.15	0.29	0.05	-1.93	-1.25	1.23	0.08	0.10	3.47	4.50	0.54	1.00	0.16
M. mercenaria	Oocytes	SMI	11.92	8.54	0.47	20.33	47.86	6.16	5.60	5.91	0.59	0.42	0.02	1.00	2.35	0.30	0.28	0.29	-0.41	1.14	2.13	1.53	0.08	3.63	8.55	1.10	1.00	1.06
M. mercenaria	Oocytes	SMI	11.76	8.74	0.47	18.52	46.46	6.02	3.98	10.42	0.63	0.47	0.03	1.00	2.51	0.32	0.21	0.56	-0.13	2.48	2.95	2.20	0.12	4.65	11.67	1.51	1.00	2.62
M. mercenaria	Oocytes	SMI	10.51	7.74	0.10	16.24	50.99	5.62	4.53	6.97	0.65	0.48	0.01	1.00	3.14	0.35	0.28	0.43	-0.09	1.96	2.32	1.71	0.02	3.58	11.25	1.24	1.00	1.54
M. mercenaria	Oocytes	SMI	8.93	6.33	0.24	13.74	27.58	4.54	5.67	6.69	0.65	0.46	0.02	1.00	2.01	0.33	0.41	0.49	-0.05	2.08	1.57	1.12	0.04	2.42	4.86	0.80	1.00	1.18
M. mercenaria	Oocytes	SMI	9.76	8.53	0.40	15.80	36.26	4.38	7.41	6.10	0.62	0.54	0.03	1.00	2.30	0.28	0.47	0.39	0.20	1.83	1.32	1.15	0.05	2.13	4.89	0.59	1.00	0.82
M. mercenaria	Oocytes	SMI	8.93	7.09	0.37	19.45	45.67	4.73	6.11	8.60	0.46	0.36	0.02	1.00	2.35	0.24	0.31	0.44	-0.67	1.56	1.46	1.16	0.06	3.18	7.47	0.77	1.00	1.41
M. mercenaria	Oocytes	SMI	7.17	5.80	0.29	17.43	28.20	5.93	4.34	4.68	0.41	0.33	0.02	1.00	1.62	0.34	0.25	0.27	-1.07	0.81	1.65	1.34	0.07	4.02	6.50	1.37	1.00	1.08
M. mercenaria	Oocytes	SMI	7.19	5.76	0.18	18.91	24.65	5.37	5.61	8.54	0.38	0.30	0.01	1.00	1.30	0.28	0.30	0.45	-1.19	1.51	1.28	1.03	0.03	3.37	4.39	0.96	1.00	1.52
M. mercenaria	Oocytes	SMI	7.21	4.79	0.28	17.31	26.86	3.88	3.81	6.94	0.42	0.28	0.02	1.00	1.55	0.22	0.22	0.40	-1.27	1.21	1.89	1.26	0.07	4.55	7.05	1.02	1.00	1.82
M. mercenaria	Oocytes	SMI	6.93	4.62	0.20	13.88	24.47	5.67	6.08	6.01	0.50	0.33	0.01	1.00	1.76	0.41	0.44	0.43	-0.54	1.40	1.14	0.76	0.03	2.28	4.02	0.93	1.00	0.99
M. mercenaria	Sperm	SMI	33.71	12.61	2.21	39.40	120.91	13.99	13.02	5.38	0.86	0.32	0.06	1.00	3.07	0.36	0.33	0.14	0.26	-0.06	2.59	0.97	0.17	3.03	9.29	1.07	1.00	0.41
M. mercenaria	Sperm	SMI	29.25	11.98	1.76	32.87	110.75	12.93	12.17	5.36	0.89	0.36	0.05	1.00	3.37	0.39	0.37	0.16	0.54	0.18	2.40	0.98	0.14	2.70	9.10	1.06	1.00	0.44
M. mercenaria	Sperm	SMI	24.27	9.49	2.11	40.78	124.84	13.93	15.38	6.87	0.60	0.23	0.05	1.00	3.06	0.34	0.38	0.17	-0.27	-0.28	1.58	0.62	0.14	2.65	8.12	0.91	1.00	0.45
M. mercenaria	Sperm	SMI	21.58	6.60	1.61	34.11	73.68	12.48	8.81	3.83	0.63	0.19	0.05	1.00	2.16	0.37	0.26	0.11	-0.70	-0.48	2.45	0.75	0.18	3.87	8.37	1.42	1.00	0.44
M. mercenaria	Sperm	SMI	21.57	6.64	1.65	33.26	79.97	12.22	7.99	4.84	0.65	0.20	0.05	1.00	2.40	0.37	0.24	0.15	-0.60	-0.34	2.70	0.83	0.21	4.16	10.01	1.53	1.00	0.61
M. mercenaria	Sperm	SMI	21.03	7.71	1.43	36.64	88.36	11.48	12.48	7.26	0.57	0.21	0.04	1.00	2.41	0.31	0.34	0.20	-0.65	-0.10	1.68	0.62	0.11	2.94	7.08	0.92	1.00	0.58
M. arenaria	Oocytes	SMI	10.07	3.14	1.89	13.79	59.80	8.72	7.92	2.94	0.73	0.23	0.14	1.00	4.34	0.63	0.57	0.21	1.45	-0.75	1.27	0.40	0.24	1.74	7.55	1.10	1.00	0.37
M. arenaria	Oocytes	SMI	16.04	7.07	1.72	21.75	80.29	11.50	13.40	6.92	0.74	0.33	0.08	1.00	3.69	0.53	0.62	0.32	1.05	0.36	1.20	0.53	0.13	1.62	5.99	0.86	1.00	0.52
M. arenaria	Oocytes	SMI	14.57	7.25	1.35	28.70	89.54	14.72	9.41	5.73	0.51	0.25	0.05	1.00	3.12	0.51	0.33	0.20	-0.31	-0.05	1.55	0.77	0.14	3.05	9.52	1.56	1.00	0.61
M. arenaria	Oocytes	SMI	14.99	4.94	3.71	30.33	91.31	23.41	3.87	6.86	0.49	0.16	0.12	1.00	3.01	0.77	0.13	0.23	0.04	-0.53	3.87	1.28	0.96	7.84	23.60	6.05	1.00	1.77
M. arenaria	Oocytes	SMI	12.12	4.22	2.62	18.97	93.16	19.07	9.84	8.36	0.64	0.22	0.14	1.00	4.91	1.01	0.52	0.44	1.73	0.15	1.23	0.43	0.27	1.93	9.47	1.94	1.00	0.85
M. arenaria	Oocytes	SMI	11.90	3.12	2.07	16.10	72.41	10.58	7.94	4.74	0.74	0.19	0.13	1.00	4.50	0.66	0.49	0.29	1.29	-0.44	1.50	0.39	0.26	2.03	9.12	1.33	1.00	0.60
M. arenaria	Oocytes	SMI	14.32	5.00	1.51	23.74	81.36	10.89	13.45	6.17	0.60	0.21	0.06	1.00	3.43	0.46	0.57	0.26	0.27	-0.17	1.07	0.37	0.11	1.77	6.05	0.81	1.00	0.46
M. arenaria	Oocytes	SMI	13.47	4.52	1.64	19.55	104.30	13.79	7.99	4.30	0.69	0.23	0.08	1.00	5.34	0.71	0.41	0.22	0.95	-0.40	1.69	0.57	0.20	2.45	13.05	1.73	1.00	0.54
M. arenaria	Oocytes	SMI	13.74	4.54	0.78	16.51	105.51	12.70	6.91	4.50	0.83	0.27	0.05	1.00	6.39	0.77	0.42	0.27	1.22	0.12	1.99	0.66	0.11	2.39	15.28	1.84	1.00	0.65
M. arenaria	Oocytes	SMI	18.08	4.11	0.96	15.03	100.89	13.27	9.58	3.74	1.20	0.27	0.06	1.00	6.71	0.88	0.64	0.25	2.35	-0.16	1.89	0.43	0.10	1.57	10.53	1.39	1.00	0.39
M. arenaria	Sperm	SMI	21.42	4.66	1.86	16.28	117.25	15.83	6.60	1.26	1.32	0.29	0.11	1.00	7.20	0.97	0.41	0.08	2.75	-0.98	3.24	0.71	0.28	2.47	17.76	2.40	1.00	0.19
M. arenaria	Sperm	SMI	14.83	4.34	2.35	19.03	67.66	21.84	7.11	3.51	0.78	0.23	0.12	1.00	3.56	1.15	0.37	0.18	1.33	-0.63	2.09	0.61	0.33	2.68	9.52	3.07	1.00	0.49
M. arenaria	Sperm	SMI	18.03	4.52	1.96	16.72	169.67	19.32	18.78	3.94	1.08	0.27	0.12	1.00	10.15	1.16	1.12	0.24	4.24	-1.07	0.96	0.24	0.10	0.89	9.03	1.03	1.00	0.21
M. arenaria	Sperm	SMI	17.81	2.89	2.23	9.89	106.06	27.19	13.36	1.39	1.80	0.29	0.23	1.00	10.73	2.75	1.35	0.14	7.88	-2.06	1.33	0.22	0.17	0.74	7.94	2.04	1.00	0.10
M. arenaria	Sperm	SMI	13.40	4.11	1.96	13.78	104.74	16.74	5.89	1.67	0.97	0.30	0.14	1.00	7.60	1.21	0.43	0.12	2.84	-1.01	2.28	0.70	0.33	2.34	17.79	2.84	1.00	0.28
M. arenaria	Sperm	SMI	7.94	3.01	2.59	16.21	99.07	17.23	3.27	1.76	0.49	0.19	0.16	1.00	6.11	1.06	0.20	0.11	1.32	-1.40	2.43	0.92	0.79	4.96	30.32	5.27	1.00	0.54
M. arenaria	Sperm	SMI	11.46	2.09	1.77	11.69	88.30	15.51	12.85	1.40	0.98	0.18	0.15	1.00	7.55	1.33	1.10	0.12	3.72	-1.89	0.89	0.16	0.14	0.91	6.87	1.21	1.00	0.11
M. arenaria	Sperm	SMI	14.87	4.00	2.50	33.58	121.06	13.01	11.18	5.58	0.44	0.12	0.07	1.00	3.61	0.39	0.33	0.17	-0.44	-0.83	1.33	0.36	0.22	3.00	10.82	1.16	1.00	0.50
M. arenaria	Sperm	SMI	18.58	4.89	3.13	44.12	174.49	23.82	29.34	4.38	0.42	0.11	0.07	1.00	3.95	0.54	0.67	0.10	0.11	-1.36	0.63	0.17	0.11	1.50	5.95	0.81	1.00	0.15
M. arenaria	Sperm	SMI	10.86	4.49	1.33	25.57	80.78	10.82	15.74	2.02	0.42	0.18	0.05	1.00	3.16	0.42	0.62	0.08	-0.24	-1.04	0.69	0.29	0.08	1.62	5.13	0.69	1.00	0.13
P. magellanicus	Oocytes	SMI	2.29	1.37	0.32	6.17	6.78	2.60	0.07	1.10	0.37	0.22	0.05	1.00	1.10	0.42	0.01	0.18	-1.46	0.03	32.93	19.71	4.67	88.71	97.59	37.43	1.00	15.85
P. magellanicus	Oocytes	SMI	1.08	0.72	0.42	7.07	8.56	5.00	0.35	2.77	0.15	0.10	0.06	1.00	1.21	0.71	0.05	0.39	-1.58	0.40	3.05	2.03	1.19	19.93	24.14	14.09	1.00	7.82
P. magellanicus	Oocytes	SMI	1.25	0.97	0.13	4.38	5.74	2.50	0.27	2.36	0.29	0.22	0.03	1.00	1.31	0.57	0.06	0.54	-1.41	1.59	4.71	3.64	0.51	16.51	21.62	9.40	1.00	8.87
P. magellanicus	Oocytes	SMI	1.54	0.42	0.48	7.40	9.74	5.41	0.23	4.37	0.21	0.06	0.07	1.00	1.32	0.73	0.03	0.59	-1.46	1.07	6.70	1.81	2.09	32.18	42.33	23.54	1.00	19.01
P. magellanicus	Oocytes	SMI	2.44	0.44	0.42	9.64	9.96	4.47	0.22	2.95	0.25	0.05	0.04	1.00	1.03	0.46	0.02	0.31	-1.99	-0.02	10.85	1.96	1.89	42.86	44.28	19.89	1.00	13.09
P. magellanicus	Oocytes	SMI	1.42	0.90	0.18	5.07	6.69	3.54	0.46	2.51	0.28	0.18	0.04	1.00	1.32	0.70	0.09	0.50	-1.34	1.22	3.09	1.97	0.39	11.07	14.60	7.73	1.00	5.49
P. magellanicus	Oocytes	SMI	1.72	0.52	0.21	3.98	5.17	2.32	0.14	2.93	0.43	0.13	0.05	1.00	1.30	0.58	0.03	0.74	-1.13	2.04	12.60	3.85	1.58	29.22	37.94	17.04	1.00	21.48
P. magellanicus	Oocytes	SMI	1.89	0.54	0.23	5.47	7.15	3.69	0.32	2.80	0.35	0.10	0.04	1.00	1.31	0.67	0.06	0.51	-1.41	1.02	5.84	1.67	0.71	16.88	22.09	11.39	1.00	8.64
P. magellanicus	Sperm	SMI	11.42	3.70	0.46	9.73	31.29	4.91	0.84	5.83	1.17	0.38	0.05	1.00	3.22	0.50	0.09	0.60	0.75	2.34	13.67	4.42	0.55	11.64	37.45	5.87	1.00	6.98
P. magellanicus	Sperm	SMI	7.34	2.09	0.51	6.25	19.83	2.10	0.48	5.85	1.17	0.33	0.08	1.00	3.17	0.34	0.08	0.94	0.94	3.44	15.36	4.37	1.06	13.10	41.53	4.40	1.00	12.26
P. magellanicus	Sperm	SMI	8.08	2.57	0.31	11.06	20.44	4.46	0.55	6.07	0.73	0.23	0.03	1.00	1.85	0.40	0.05	0.55	-0.80	1.75	14.58	4.63	0.56	19.96	36.87	8.04	1.00	10.95
P. magellanicus	Sperm	SMI	4.71	1.27	0.30	12.20	14.59	5.29	0.87	5.64	0.39	0.10	0.02	1.00	1.20	0.43	0.07	0.46	-1.70	0.93	5.44	1.46	0.35	14.09	16.85	6.11	1.00	6.52
P. magellanicus	Sperm	SMI	6.10	2.63	0.46	17.81	18.88	6.48	0.93	8.17	0.34	0.15	0.03	1.00	1.06	0.36	0.05	0.46	-1.77	1.08	6.60	2.84	0.49	19.24	20.40	7.01	1.00	8.83
P. magellanicus	Sperm	SMI	6.07	2.80	0.30	15.29	16.26	4.24	0.42	6.13	0.40	0.18	0.02	1.00	1.06	0.28	0.03	0.40	-1.80	1.01	14.53	6.69	0.73	36.58	38.91	10.15	1.00	14.67
P. magellanicus	Sperm	SMI	4.41	1.44	0.30	8.13	14.19	5.03	0.26	3.21	0.54	0.18	0.04	1.00	1.74	0.62	0.03	0.39	-1.05	0.84	16.84	5.49	1.16	31.03	54.13	19.18	1.00	12.25
P. magellanicus	Sperm	SMI	5.39	2.09	0.24	10.88	18.85	6.81	0.43	3.46	0.50	0.19	0.02	1.00	1.73	0.63	0.04	0.32	-1.24	0.64	12.51	4.85	0.55	25.24	43.73	15.80	1.00	8.02

Table 3.s3. Interaction effect between gamete type (factor 'gametes', two levels, oocytes and sperm) and mitochondrial inheritance system (factor 'inheritance', two levels, SMI and DUI) on enzymatic activity ratios ('CS' in subscript, mU·mU CS^{-1}) in five bivalve species. Values are presented as means ± s.e.m. The main effect of the two fixed factors 'gametes' and 'inheritance', as well as their interaction, was assessed for each parameter separately through a linear mixed effect model which accounted for the by-species variability in gamete energy metabolism. Simple main effects were determined through a *post hoc* pairwise comparison, with *p*-values adjusted using Holm's correction for multiple testing. Significant differences ($p \leq 0.05$) are shown in bold. '*:gametes*', main effect of factor 'gametes'; '*:inheritance*', main effect of factor 'inheritance'; '*:gametes :inheritance*', interaction effect between factor 'gametes' and factor 'inheritance'. For parameter abbreviations refer to table 3.s2.

Table s3			**Enzymatic ratios (mU·mU CS^{-1})**							**PCA$_{CS}$**		**Supplementary ratios (mU·mU^{-1})**	
Species	**Inheritance**	**Gametes**	**PK$_{CS}$**	**LDH$_{CS}$**	**CPT$_{CS}$**	**MDH$_{CS}$**	**ETS$_{CS}$**	**CCO$_{CS}$**	**CAT$_{CS}$**	**PC1$_{CS}$**	**PC2$_{CS}$**	**CS·MDH^{-1}**	**ETS·CCO^{-1}**
M. edulis	DUI	Oocytes	0.69 ± 0.09	0.47 ± 0.04	0.09 ± 0.02	6.86 ± 0.59	1.84 ± 0.18	0.83 ± 0.09	0.31 ± 0.05	3.32 ± 0.58	0.39 ± 0.29	0.15 ± 0.01	2.33 ± 0.27
		Sperm	0.36 ± 0.02	0.09 ± 0.01	0.03 ± 0	2.28 ± 0.18	0.55 ± 0.04	0.21 ± 0.02	0.02 ± 0	-1.42 ± 0.08	-1.16 ± 0.03	0.46 ± 0.04	2.76 ± 0.27
R. philippinarum	DUI	Oocytes	0.44 ± 0.04	0.17 ± 0.02	0.06 ± 0.01	1.9 ± 0.13	0.26 ± 0.02	0.4 ± 0.04	0.25 ± 0.02	-0.83 ± 0.16	-0.15 ± 0.11	0.55 ± 0.05	0.74 ± 0.11
		Sperm	0.36 ± 0.02	0.06 ± 0.01	0.03 ± 0	1.43 ± 0.06	0.21 ± 0.02	0.18 ± 0.02	0.05 ± 0.01	-1.92 ± 0.07	-1.1 ± 0.04	0.71 ± 0.03	1.37 ± 0.22
M. mercenaria	SMI	Oocytes	0.53 ± 0.03	0.4 ± 0.03	0.02 ± 0	2.09 ± 0.17	0.31 ± 0.02	0.32 ± 0.03	0.42 ± 0.03	-0.52 ± 0.16	1.6 ± 0.16	0.51 ± 0.04	1.03 ± 0.09
		Sperm	0.7 ± 0.06	0.25 ± 0.03	0.05 ± 0	2.75 ± 0.2	0.36 ± 0.01	0.32 ± 0.02	0.15 ± 0.01	-0.24 ± 0.21	-0.18 ± 0.1	0.37 ± 0.03	1.15 ± 0.11
M. arenaria	SMI	Oocytes	0.72 ± 0.06	0.24 ± 0.01	0.09 ± 0.01	4.54 ± 0.41	0.69 ± 0.05	0.47 ± 0.05	0.27 ± 0.02	1 ± 0.26	-0.19 ± 0.11	0.24 ± 0.02	1.86 ± 0.48
		Sperm	0.87 ± 0.14	0.21 ± 0.02	0.12 ± 0.02	6.36 ± 0.87	1.1 ± 0.21	0.66 ± 0.12	0.13 ± 0.02	2.35 ± 0.8	-1.23 ± 0.14	0.19 ± 0.03	2.05 ± 0.45
P. magellanicus	SMI	Oocytes	0.29 ± 0.03	0.13 ± 0.02	0.05 ± 0	1.24 ± 0.04	0.61 ± 0.04	0.05 ± 0.01	0.47 ± 0.06	-1.47 ± 0.09	0.92 ± 0.26	0.81 ± 0.03	17.57 ± 3.41
		Sperm	0.66 ± 0.12	0.22 ± 0.03	0.04 ± 0.01	1.88 ± 0.31	0.45 ± 0.05	0.05 ± 0.01	0.51 ± 0.07	-0.83 ± 0.39	1.51 ± 0.34	0.63 ± 0.09	9.57 ± 1.86
	ANOVA	*:gametes*	$F_{1,85}$=0.05, P=0.82	**$F_{1,85}$=68.18, P=1.70e-12*** **	**$F_{1,85}$=5.45, P=0.021* **	**$F_{1,85}$=8.29, P=0.005** **	**$F_{1,85}$=18.79, P=3.98e-05*** **	**$F_{1,85}$=24, P=4.49e-06*** **	**$F_{1,85}$=255.69, P=<2.2e-16*** **	**$F_{1,85}$=14.52, P=0.00026*** **	**$F_{1,85}$=51.83, P=2.23e-10*** **	**$F_{1,85}$=4.91, P=0.029* **	$F_{1,85}$=1.46, P=0.23
		:inheritance	$F_{1,2.9}$=1.28, P=0.34	$F_{1,2.9}$=1.77, P=0.27	$F_{1,2.9}$=0.015, P=0.9	$F_{1,3}$=0.0035, P=0.95	$F_{1,3}$=0.014, P=0.91	$F_{1,2.9}$=0.28, P=0.63	$F_{1,3}$=9.5, P=0.054	$F_{1,3}$=0.042, P=0.84	$F_{1,3}$=1.54, P=0.3	$F_{1,2.9}$=0.0021, P=0.96	$F_{1,2.9}$=0.33, P=0.6
		:gametes :inheritance	**$F_{1,85}$=26.98, P=1.38e-06*** **	**$F_{1,85}$=65.40, P=3.76e-12*** **	**$F_{1,85}$=33.82, P=1.03e-07*** **	**$F_{1,85}$=55.44, P=7.25e-11*** **	**$F_{1,85}$=28.65, P=7.23e-07*** **	**$F_{1,85}$=49.9, P=4.15e-10*** **	**$F_{1,85}$=87.84, P=9.45e-15*** **	**$F_{1,85}$=45.62, P=1.67e-09*** **	$F_{1,85}$=3.28, P=0.073	**$F_{1,85}$=45.19, P=1.93e-09*** **	**$F_{1,85}$=5.72, P=0.018* **
	Multicomparison	***sperm DUI - eggs DUI***	**P=0.0014** **	**P<2e-16*** **	**P=3.13e-07*** **	**P=3.2e-11*** **	**P=5.88e-10*** **	**P=7.99e-15*** **	**P<2e-16*** **	**P=1.03e-11*** **		**P=1.43e-08*** **	P=0.09
		eggs SMI - eggs DUI	P=0.74	P=1	P=1	P=1	P=1	P=0.95	P=0.69	P=0.79		P=1	P=1
		sperm SMI - eggs DUI	P=0.74	P=1	P=1	P=1	P=1	P=0.97	P=0.69	P=0.79		P=1	P=1
		eggs SMI - sperm DUI	P=0.74	**P=0.011* **	P=1	P=1	P=1	P=1	**P=2.88e-15*** **	P=0.79		P=1	P=1
		sperm SMI - sperm DUI	**P=0.039* **	**P=0.011* **	P=0.95	P=1	P=1	P=1	**P=1.61e-06*** **	P=0.51		P=1	P=1
		sperm SMI - eggs SMI	**P=0.0011** **	P=1	**P=0.04* **	**P=0.0028** **	P=1	P=0.51	**P=1.79e-06*** **	P=0.13		**P=0.0034** **	P=1

Table 3.s4. Intraspecific comparison between oocyte and sperm enzymatic activity ratios (mU·mU CS^{-1}). Values are presented as means ± s.e.m. The main effect of the fixed factor 'gametes' was assessed for each parameter and each species separately through a Students *t* test. Significant differences ($p \leq 0.05$) are shown in bold. *p*-values corrected with Holm adjustment for multiple testing. '*:gametes*', main effect of factor 'gametes'. For parameter abbreviations refer to table 3.s2.

Table s4			**Enzymatic ratios (mU·mU CS^{-1})**							
Species	**Inheritance**	**Gamete**	**PK_{CS}**	**LDH_{CS}**	**CPT_{CS}**	**CS_{CS}**	**MDH_{CS}**	**ETS_{CS}**	**CCO_{CS}**	**CAT_{CS}**
M. edulis	DUI	Oocyte	0.69 ± 0.09	0.47 ± 0.04	0.09 ± 0.018	1 ± 0	6.86 ± 0.59	1.84 ± 0.18	0.83 ± 0.09	0.31 ± 0.05
		Sperm	0.36 ± 0.02	0.09 ± 0.01	0.03 ± 0.002	1 ± 0	2.28 ± 0.18	0.55 ± 0.04	0.21 ± 0.02	0.02 ± 0
	Student *t* test	*:gametes*	**$t_{1,18}$=3.6, P=0.002****	**$t_{1,9.7}$=8.7, P=6.63e-06*****	**$t_{1,18}$=3.33, P< 0.0001*****		**$t_{1,18}$=7.47, P< 0.0001*****	**$t_{1,18}$=6.85, P<0.0001*****	**$t_{1,18}$=11, P=1.85e-09*****	**$t_{1,18}$=13.79, P=5.2e-11*****
		p adjusted	**2e-03****	**1.32e-05*****	**<5e-06*****		**<5e-06*****	**<5e-06*****	**1.11e-08*****	**3.64e-10*****
R. philippinarum	DUI	Oocyte	0.44 ± 0.04	0.17 ± 0.02	0.06 ± 0.005	1 ± 0	1.9 ± 0.13	0.26 ± 0.02	0.4 ± 0.04	0.25 ± 0.02
		Sperm	0.36 ± 0.02	0.06 ± 0.01	0.03 ± 0.003	1 ± 0	1.43 ± 0.06	0.21 ± 0.02	0.18 ± 0.02	0.05 ± 0.01
	Student *t* test	*:gametes*	$t_{1,12.5}$=1.95, P=0.07·	**$t_{1,18}$=4.62, P=0.0001*****	**$t_{1,18}$=3.67, P=0.0017****		**$t_{1,18}$=3.26, P=0.0043****	**$t_{1,18}$=2.23, P=0.038***	**$t_{1,18}$=4.27, P=0.00045*****	**$t_{1,18}$=11.46, P=0.0001*****
		p adjusted	0.076·	**0.0007*****	**0.0068****		**0.013***	0.076·	**0.0022****	**0.0007*****
M. mercenaria	SMI	Oocyte	0.53 ± 0.03	0.4 ± 0.03	0.02 ± 0.002	1 ± 0	2.09 ± 0.17	0.31 ± 0.02	0.32 ± 0.03	0.42 ± 0.03
		Sperm	0.7 ± 0.06	0.25 ± 0.03	0.05 ± 0.002	1 ± 0	2.75 ± 0.2	0.36 ± 0.01	0.32 ± 0.02	0.15 ± 0.01
	Student *t* test	*:gametes*	**$t_{1,14}$=-2.74, P=0.016***	**$t_{1,14}$=3.4, P=0.0042****	**$t_{1,14}$=-10, P=8.41e-08*****		**$t_{1,14}$=-2.42, P=0.03***	$t_{1,14}$=-2, P=0.06·	$t_{1,14}$=-0.06, P=0.95	**$t_{1,14}$=7, P=5.65e-06*****
		p adjusted	0.064·	**0.021***	**5.88e-07*****		0.09·	0.12	0.95	**3.39e-05*****
M. arenaria	SMI	Oocyte	0.72 ± 0.06	0.24 ± 0.01	0.09 ± 0.012	1 ± 0	4.54 ± 0.41	0.69 ± 0.05	0.47 ± 0.05	0.27 ± 0.02
		Sperm	0.87 ± 0.14	0.21 ± 0.02	0.12 ± 0.016	1 ± 0	6.36 ± 0.87	1.1 ± 0.21	0.66 ± 0.12	0.13 ± 0.02
	Student *t* test	*:gametes*	$t_{1,12.4}$=-0.97, P=0.35	$t_{1,18}$=0.85, P=0.4	$t_{1,18}$=-1.61, P=0.12		$t_{1,12.8}$=-1.88, P=0.082·	$t_{1,18}$=-1.84, P=0.054·	$t_{1,11.7}$=-1.42, P=0.18	**$t_{1,18}$=4.97, P=0.0002*****
		p adjusted	0.7	0.7	0.48		0.41	0.32	0.54	**0.0014****
P. magellanicus	SMI	Oocyte	0.29 ± 0.03	0.13 ± 0.02	0.05 ± 0.004	1 ± 0	1.24 ± 0.04	0.61 ± 0.04	0.05 ± 0.01	0.47 ± 0.06
		Sperm	0.66 ± 0.12	0.22 ± 0.03	0.04 ± 0.007	1 ± 0	1.88 ± 0.31	0.45 ± 0.05	0.05 ± 0.01	0.51 ± 0.07
	Student *t* test	*:gametes*	**$t_{1,14}$=-2.9, P=0.0024****	$t_{1,14}$=-2.1, P=0.053·	$t_{1,14}$=1.48, P=0.15		**$t_{1,14}$=-2, P=0.03***	**$t_{1,14}$=2.63, P=0.02***	$t_{1,14}$=-0.79, P=0.44	$t_{1,14}$=-0.5, P=0.61
		p adjusted	**0.0168***	0.21	0.45		0.15	0.12	0.88	0.88

Table 3.s5. Interaction effect between gamete type (factor 'gametes', two levels, oocytes and sperm) and mitochondrial inheritance system (factor 'inheritance', two levels, SMI and DUI) on enzymatic activity (mU·mg proteins^{-1}) in five bivalve species. Values are presented as means ± s.e.m. The main effect of the two fixed factors 'gametes' and 'inheritance', as well as their interaction, was assessed for each parameter separately through a linear mixed effect model which accounted for the by-species variability in gamete energy metabolism. Simple main effects were determined through a *post hoc* pairwise comparison, with *p*-values adjusted using Holm's correction for multiple testing. Significant differences ($p \leq 0.05$) are shown in bold. '*:gametes*', main effect of factor 'gametes'; '*:inheritance*', main effect of factor 'inheritance'; '*:gametes :inheritance*', interaction effect between factor 'gametes' and factor 'inheritance'. For parameter abbreviations refer to table 3.s2.

Table s5			**Enzymatic activity (mU·mg proteins^{-1})**							
Species	**Inheritance**	**Gamete**	**PK**	**LDH**	**CPT**	**CS**	**MDH**	**ETS**	**CCO**	**CAT**
M. edulis	DUI	Oocytes	5.31 ± 0.34	3.9 ± 0.51	0.7 ± 0.14	8.54 ± 0.89	54.63 ± 2.95	14.69 ± 1.1	6.75 ± 0.59	2.5 ± 0.37
		Sperm	9.04 ± 0.41	2.19 ± 0.29	0.71 ± 0.04	25.49 ± 1.49	57.82 ± 4.76	13.7 ± 0.86	5.28 ± 0.43	0.61 ± 0.05
R. philippinarum	DUI	Oocytes	18.98 ± 1.68	7.15 ± 0.7	2.43 ± 0.27	43.62 ± 2.18	82.55 ± 6.66	11.28 ± 0.78	17.01 ± 1.83	10.82 ± 1.08
		Sperm	22.8 ± 0.94	3.39 ± 0.61	2.01 ± 0.14	65.75 ± 4.56	92.6 ± 4.94	13.45 ± 1.15	12.23 ± 1.94	2.97 ± 0.29
M. mercenaria	SMI	Oocytes	9.03 ± 0.61	6.8 ± 0.49	0.3 ± 0.04	17.16 ± 0.71	35.9 ± 3.41	5.23 ± 0.25	5.31 ± 0.36	7.09 ± 0.53
		Sperm	25.24 ± 2.11	9.17 ± 1.08	1.8 ± 0.12	36.18 ± 1.36	99.75 ± 8.94	12.84 ± 0.4	11.64 ± 1.13	5.59 ± 0.52
M. arenaria	SMI	Oocytes	13.93 ± 0.71	4.79 ± 0.44	1.82 ± 0.27	20.45 ± 1.79	87.86 ± 4.62	13.87 ± 1.38	9.03 ± 0.91	5.43 ± 0.53
		Sperm	14.92 ± 1.3	3.9 ± 0.29	2.17 ± 0.16	20.69 ± 3.4	112.91 ± 11.1	18.13 ± 1.58	12.41 ± 2.42	2.69 ± 0.48
P. magellanicus	SMI	Oocytes	1.7 ± 0.17	0.74 ± 0.12	0.3 ± 0.05	6.15 ± 0.66	7.47 ± 0.63	3.69 ± 0.42	0.26 ± 0.04	2.72 ± 0.32
		Sperm	6.69 ± 0.8	2.32 ± 0.28	0.36 ± 0.03	11.42 ± 1.32	19.29 ± 1.9	4.92 ± 0.51	0.6 ± 0.09	5.55 ± 0.56
	ANOVA	*:gametes*	**$F_{1,85}$=41, P=7.84e-09*** **	**$F_{1,85}$=7.37, P=0.008** **	$F_{1,85}$=2.94, P=0.09	**$F_{1,85}$=68, P=1.80e-12*** **	**$F_{1,85}$=22.91, P=7.07e-06*** **	**$F_{1,85}$=12.83, P=0.00056*** **	$F_{1,85}$=0.0085, P=0.92	**$F_{1,85}$=65.64, P=3.52e-12*** **
		:inheritance	$F_{1,3}$=0.12, P=0.74	$F_{1,3}$=0.026, P=0.88	$F_{1,3}$=0.19, P=0.69	$F_{1,3}$=0.79, P=0.43	$F_{1,3}$=0.13, P=0.74	$F_{1,3}$=0.66, P=0.47	$F_{1,3}$=0.57, P=0.5	$F_{1,3}$=0.74, P=0.45
		:gametes :inheritance	$F_{1,85}$=3.21, P=0.07	**$F_{1,85}$=26.10, P=1.95e-06*** **	**$F_{1,85}$=12.15, P=0.00077*** **	**$F_{1,85}$=7.27, P=0.008** **	**$F_{1,85}$=9.98, P=0.0021** **	**$F_{1,85}$=7.4, P=0.0078** **	**$F_{1,85}$=13.59, P=0.00039*** **	**$F_{1,85}$=40.85, P=8.41e-09*** **
	Multi comparison	***sperm DUI - eggs DUI***		**P=1.04e-06*** **	P=1	**P=1.62e-12*** **	P=1	P=1	P=0.081	**P=<2e-16*** **
		eggs SMI - eggs DUI		P=1	P=1	P=1	P=1	P=0.89	P=0.68	P=1
		sperm SMI - eggs DUI		P=1	P=1	P=1	P=1	P=1	P=1	P=1
		eggs SMI - sperm DUI		P=1	P=1	P=0.3	P=1	P=0.89	P=1	P=0.22
		sperm SMI - sperm DUI		P=1	P=1	P=0.72	P=1	P=1	P=1	P=0.31
		sperm SMI - eggs SMI		P=0.35	**P=0.00052*** **	**P=0.000142*** **	**P=1.24e-08*** **	**P=1.19e-05*** **	**P=0.026* **	P=0.59

Table 3.s6. Intraspecific comparison between oocyte and sperm enzymatic activity ratios (mU·mU CCO^{-1}). Values are presented as means ± s.e.m. The main effect of the fixed factor 'gametes' was assessed for each parameter and each species separately through a Students *t* test. Significant differences ($p \leq 0.05$) are shown in bold. *p*-values corrected with Holm adjustment for multiple testing. '*:gametes*', main effect of factor 'gametes'. For parameter abbreviations refer to table 3.s2.

Table s6			**Enzymatic ratios (mU·mU CCO^{-1})**							
Species	**Inheritance**	**Gamete**	**PK$_{CCO}$**	**LDH$_{CCO}$**	**CPT$_{CCO}$**	**CS$_{CCO}$**	**MDH$_{CCO}$**	**ETS$_{CCO}$**	**CCO$_{CCO}$**	**CAT$_{CCO}$**
M. edulis	DUI	Oocyte	0.83 ± 0.08	0.62 ± 0.08	0.11 ± 0.02	1.3 ± 0.12	8.54 ± 0.74	2.33 ± 0.27	1 ± 0	0.39 ± 0.06
		Sperm	1.8 ± 0.15	0.43 ± 0.05	0.14 ± 0.01	5.04 ± 0.42	11.62 ± 1.52	2.76 ± 0.27	1 ± 0	0.13 ± 0.02
	Student *t* test	*:gametes*	**$t_{1,18}$=-5.5, P=1e-04*****	$t_{1,18}$=1.93, P=0.068·	$t_{1,18}$=-1.49, P=0.15	**$t_{1,18}$=-8.58, P=0.0001*****	$t_{1,18}$=-1.81, P=0.08·	$t_{1,18}$=-1.12, P=0.27		**$t_{1,18}$=10.4, P=0.0024****
		p adjusted	**0.0007*****	0.272	0.3	**0.0007*****	0.272	0.3		**0.012***
R. philippinarum	DUI	Oocyte	1.15 ± 0.07	0.45 ± 0.05	0.16 ± 0.03	2.81 ± 0.31	5.07 ± 0.38	0.74 ± 0.11	1 ± 0	0.68 ± 0.07
		Sperm	2.29 ± 0.33	0.34 ± 0.07	0.21 ± 0.04	6.35 ± 0.8	9.2 ± 1.3	1.37 ± 0.22	1 ± 0	0.32 ± 0.08
	Student *t* test	*:gametes*	**$t_{1,9.7=}$-3.35, P=0.007****	$t_{1,18}$=1.33, P=0.20	$t_{1,18}$=-0.99, P=0.33	**$t_{1,11.6}$=-4.11, P=0.0015****	**$t_{1,10}$=-3, P=0.011***	**$t_{1,13}$=-2.6, P=0.02***		**$t_{1,18}$=3.35, P=0.0043****
		p adjusted	**0.035***	0.4	0.4	**0.0105***	**0.044***	0.06·		**0.0258***
M. mercenaria	SMI	Oocyte	1.77 ± 0.18	1.32 ± 0.13	0.06 ± 0.01	3.38 ± 0.28	7.07 ± 0.87	1.03 ± 0.09	1 ± 0	1.4 ± 0.17
		Sperm	2.23 ± 0.2	0.79 ± 0.07	0.16 ± 0.01	3.23 ± 0.26	8.66 ± 0.42	1.15 ± 0.11	1 ± 0	0.49 ± 0.03
	Student *t* test	*:gametes*	$t_{1,14}$=-1.67, P=0.11	**$t_{1,14}$=3, P=0.008****	**$t_{1,14}$=-6.39, P=1.66e-05*****	$t_{1,14}$=0.37, P=0.71	$t_{1,14}$=-1.35, P=0.19	$t_{1,14}$=-0.86, P=0.4		**$t_{1,14}$=4.19, P=6e-04*****
		p adjusted	0.44	**0.04***	**0.000112*****	0.8	0.57	0.8		**0.0036****
M. arenaria	SMI	Oocyte	1.72 ± 0.26	0.58 ± 0.09	0.25 ± 0.08	2.64 ± 0.6	11.02 ± 1.67	1.86 ± 0.48	1 ± 0	0.68 ± 0.13
		Sperm	1.59 ± 0.28	0.44 ± 0.09	0.26 ± 0.07	2.11 ± 0.41	12.11 ± 2.46	2.05 ± 0.45	1 ± 0	0.27 ± 0.06
	Student *t* test	*:gametes*	$t_{1,18}$=0.36, P=0.72	$t_{1,18}$=1.18, P=0.26	$t_{1,18}$=-0.03, P=0.96	$t_{1,18}$=0.73, P=0.53	$t_{1,18}$=-0.36, P=0.72	$t_{1,18}$=-0.29, P=0.75		**$t_{1,18}$=2.87, P=9e-04*****
		p adjusted	1	1	1	1	1	1		**0.0063****
P. magellanicus	SMI	Oocyte	9.97 ± 3.5	4.58 ± 2.18	1.63 ± 0.49	32.17 ± 8.86	38.07 ± 9.33	17.57 ± 3.41	1 ± 0	12.53 ± 2.04
		Sperm	12.44 ± 1.47	4.35 ± 0.56	0.68 ± 0.1	21.36 ± 3.17	36.23 ± 4.31	9.57 ± 1.86	1 ± 0	10.06 ± 1.03
	Student *t* test	*:gametes*	$t_{1,14}$=-0.65, P=0.57	$t_{1,14}$=0.1, P=0.99	**$t_{1,14}$=1.9, P=0.038***	$t_{1,14}$=1.14, P=0.3	$t_{1,14}$=0.18, P=0.88	$t_{1,14}$=2.06, P=0.058·		$t_{1,14}$=1.08, P=0.29
		p adjusted	1	1	0.26	1	1	0.348		1

Chapter IV - Electronic supplementary material

(a) Supporting figures

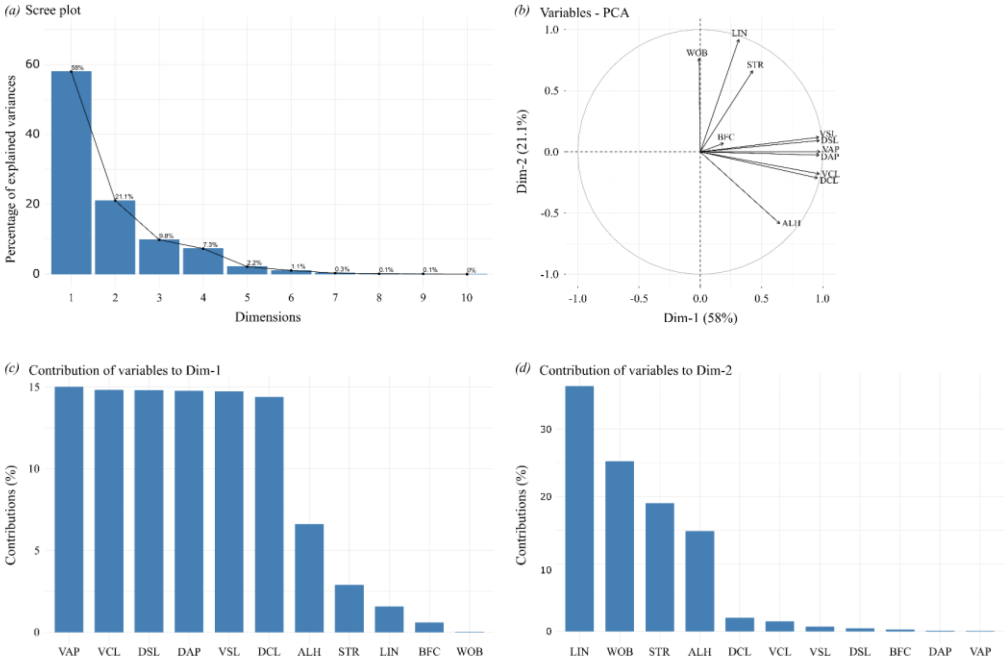

Figure 4.s1. PCA summary. *(a)* Percentage of explained variance of each principal component. *(b)* Variable correlation plots. *(c)* Contribution of variables to the first principal component (PC1). *(d)* Contribution of variables to the second principal component (PC2). Sperm motility parameters: DAP, average path distance (µm); DSL, straight-line distance (µm); DCL, curvilinear distance (µm); VAP, average path velocity ($\mu m \cdot s^{-1}$); VSL, straight-line velocity ($\mu m \cdot s^{-1}$); VCL, curvilinear velocity ($\mu m \cdot s^{-1}$); STR, straightness (VSL/VAP); LIN, linearity (VSL/VCL); ALH, amplitude of lateral head displacement (µm); BFC, beat-cross frequency (Hz); WOB, wobble coefficient (VAP/VCL). Additional information in table 4.s1.

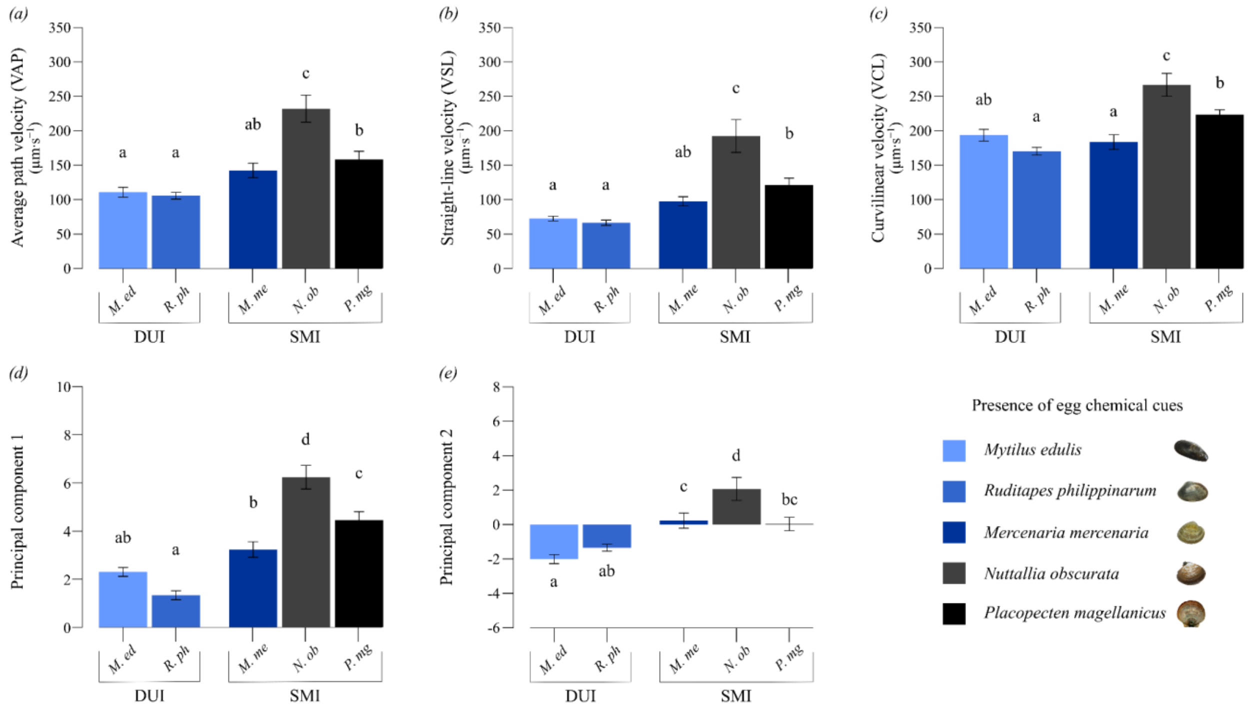

Figure 4.s2. Basal sperm motility parameters in five bivalve species, DUI and SMI, with presence of chemoattractants. *(a)* Average path velocity (µm·s^{-1}). *(b)* Straight-line velocity (µm·s^{-1}). *(c)* Curvilinear velocity (µm·s^{-1}). *(d)* First principal component of the PCA combining sperm velocity parameters. *(e)* Second principal component of the PCA. Data are presented as means ± s.e.m. Differences ($p \leq 0.05$) in a *post hoc* Tukey's test are indicated by different letters. DUI species: *M. edulis* (*M. ed*, *n* = 11), *R. philippinarum* (*R. ph*, *n* = 9). SMI species: *M. mercenaria* (*M. me*, *n* = 9), *N. obscurata* (*N. ob*, *n* = 5), *P. magellanicus* (*P. mg*, *n* = 11). Detailed summary is reported in electronic supplementary material, tables 4.s2 and 4.s3.

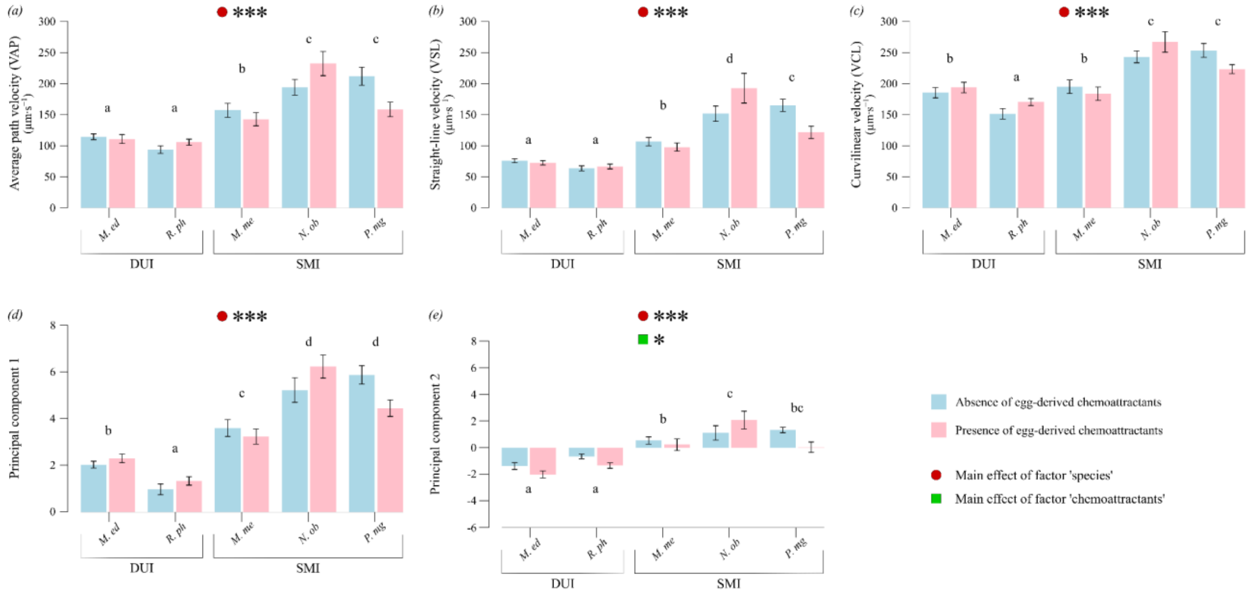

Figure 4.s3. Sperm motility parameters comparison among five bivalve species, DUI and SMI, with and without chemoattractants. *(a)* Average path velocity ($\mu m \cdot s^{-1}$). *(b)* Straight-line velocity ($\mu m \cdot s^{-1}$). *(c)* Curvilinear velocity ($\mu m \cdot s^{-1}$). *(d)* First principal component of the PCA combining sperm velocity parameters. *(e)* Second principal component of the PCA. Values are presented as means ± s.e.m. A linear mixed model was implemented for each parameter separately. The main effect of the two fixed factors 'species' and 'chemoattractants' are indicated with a circle and square respectively. $^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$. Differences among species are indicated by letters. DUI, doubly uniparental inheritance; SMI, strict maternal inheritance. Species: *M. edulis* (*M. ed*, *n* = 11); *R. philippinarum* (*R. ph*, *n* = 9); *M. mercenaria* (*M. me*, *n* = 9); *N. obscurata* (*N. ob*, *n* = 5); *P. magellanicus* (*P. mg*, *n* = 11). Detailed summary is reported in electronic supplementary material, tables 4.s2 and 4.s4.

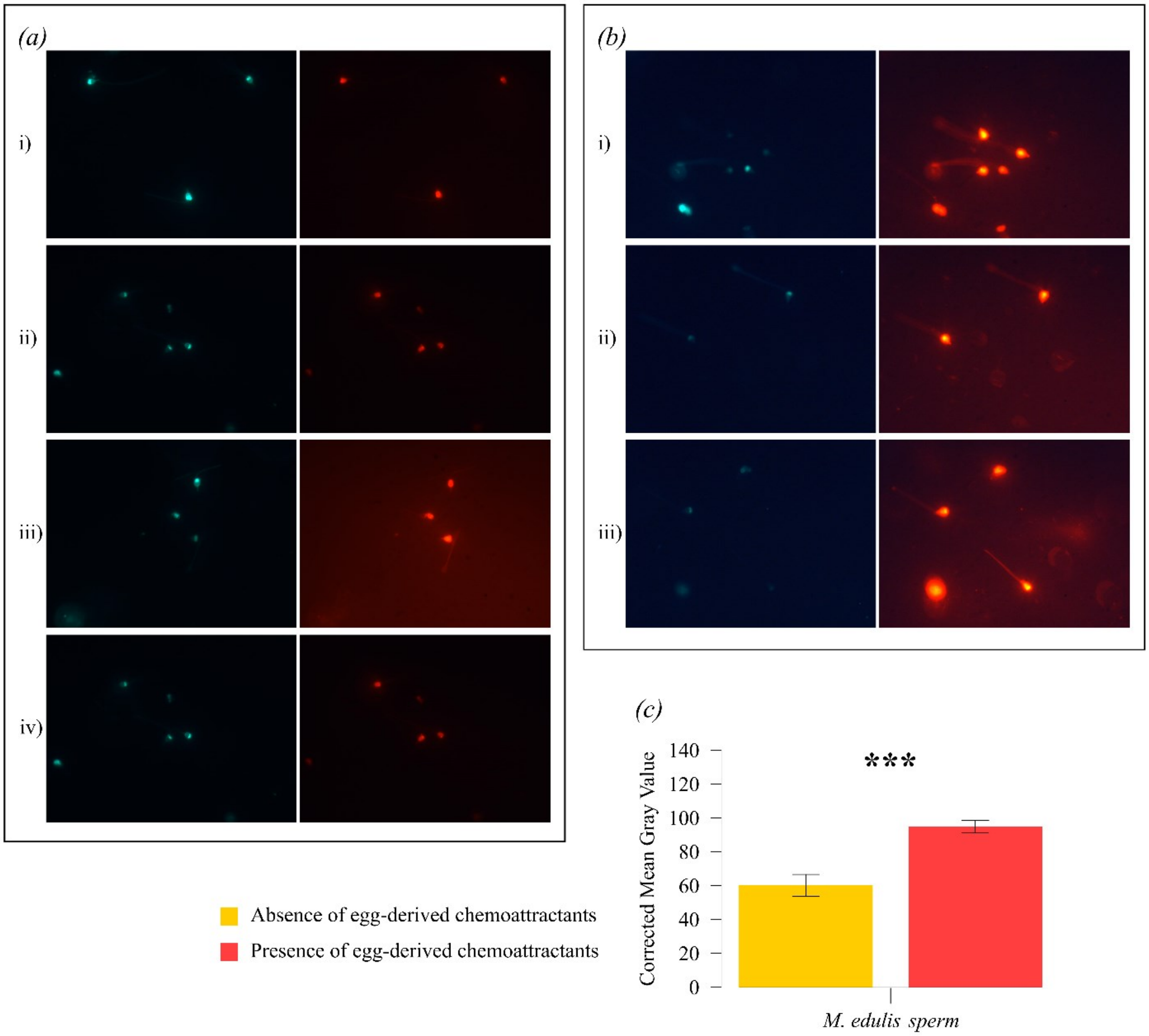

Figure 4.s4. Membrane potential of *Mytilus edulis* (DUI) sperm mitochondria following the addition of egg-derived chemoattractants. The fluorescent dyes MitoSpy™ Green FM (400 nM, excitation/emission 490/516 nm) and MitoSpy™ Red CMXRos (500 nM; excitation/emission 577/598 nm) (BioLegend Inc, San Diego, California) were used to localize sperm mitochondria (green stain) and quantify their membrane potential (red stain), respectively. *(a)* Absence of egg-derived chemoattractants. *(b)* Presence of egg-derived chemoattractants. *(c)* Quantification and comparison of sperm mitochondria membrane potential without (n = 15 spermatozoa) and with egg-derived chemoattractants (n = 15 spermatozoa). Fluorescence intensity has been quantified as mean grey value per pixel and corrected for the relative background fluorescence. Values are presented as means ± s.e.m. The effect of oocytes detection has been tested through a paired t test. $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$.

(b) Supporting tables

Table 4.s1. PCA summary. Contribution and correlation of the variables with principal components. The contributions of variables in accounting for the variability in a given principal component are expressed in percentage. Significant correlation coefficients ($p \leq 0.05$) are shown in bold.

Table s1	**PC1**		**PC2**	
Variable	Contribution	Correlation	Contribution	Correlation
DAP	14.74	**0.97**	0.03	-0.03
DSL	14.80	**0.97**	0.37	**0.09**
DCL	14.37	**0.96**	1.97	**-0.21**
VAP	14.99	**0.98**	0.00	0.00
VSL	14.71	**0.97**	0.62	**0.12**
VCL	14.80	**0.97**	1.40	**-0.18**
STR	2.87	**0.43**	18.97	**0.66**
LIN	1.55	**0.31**	36.37	**0.92**
ALH	6.60	**0.65**	14.84	**-0.59**
BFC	0.56	**0.19**	0.21	0.07
WOB	0.00	-0.01	25.22	**0.76**

Table 4.s2. Data summary table. Sperm motility parameters measured. Inheritance: DUI, doubly uniparental inheritance; SMI, strict maternal inheritance. Species: MyEd, *M. edulis* (n = 11); RuPh, *R. philippinarum* (n = 9); MeMe, *M. mercenaria* (n = 9); NuOb, *N. obscurata* (n = 5); PlMg, *P. magellanicus* (n = 11). Sperm motility parameters: DAP, average path distance (µm); DSL, straight-line distance (µm); DCL, curvilinear distance (µm); VAP, average path velocity ($\mu m \cdot s^{-1}$); VSL, straight-line velocity ($\mu m \cdot s^{-1}$); VCL, curvilinear velocity ($\mu m \cdot s^{-1}$); STR, straightness (VSL/VAP); LIN, linearity (VSL/VCL); ALH, amplitude of lateral head displacement (µm); BFC, beat-cross frequency (Hz); WOB, wobble coefficient (VAP/VCL); PC1, principal component 1; PC2, principal component 2.

Table 4.s2

DAP	DSL	DCL	VAP	VSL	VCL	STR	LIN	ALH	BFC	WOB	PC1	PC2	Inheritance	Species	Treatment	Chem	ID
52.90	32.39	93.14	107.70	68.10	183.81	63.99	38.74	10.58	23.11	59.83	1.65	-1.77	DUI	MyEd	Ctrl	N	MyEd_1
9.70	6.73	14.42	21.49	14.99	32.81	73.17	51.80	2.56	29.28	69.60	-2.42	1.15	DUI	MyEd	Rot	N	MyEd_1
9.38	6.93	13.37	19.98	15.04	28.95	72.33	55.30	1.96	33.31	71.78	-2.45	1.51	DUI	MyEd	Ama	N	MyEd_1
7.15	3.92	14.26	15.54	9.66	31.20	55.47	32.68	2.17	45.75	52.27	-2.87	-1.08	DUI	MyEd	Omy	N	MyEd_1
58.69	37.96	100.53	129.96	87.97	214.06	68.65	44.32	9.41	30.10	63.30	2.46	-1.01	DUI	MyEd	Oxa	N	MyEd_1
104.38	40.19	140.71	165.54	76.38	225.83	45.19	32.86	8.64	28.01	74.01	3.19	-1.83	DUI	MyEd	Ctrl	ch	MyEd_1
10.77	8.31	14.75	14.65	11.30	20.05	79.85	64.59	1.51	25.37	78.67	-2.46	2.57	DUI	MyEd	Rot	ch	MyEd_1
9.47	7.79	11.82	13.69	11.30	17.05	87.03	68.06	0.79	29.55	78.53	-2.44	3.08	DUI	MyEd	Ama	ch	MyEd_1
8.67	7.32	10.49	13.33	11.37	15.96	87.64	72.22	2.01	28.02	82.14	-2.33	3.32	DUI	MyEd	Omy	ch	MyEd_1
54.19	30.52	95.23	106.95	64.99	187.67	66.61	40.11	11.54	23.75	59.46	1.78	-1.79	DUI	MyEd	Oxa	ch	MyEd_1
65.31	46.66	92.66	108.46	79.79	152.52	77.07	53.05	8.68	30.48	69.16	2.18	0.21	DUI	MyEd	Ctrl	N	MyEd_2

8.11	4.39	15.09	22.54	13.76	42.30	55.90	38.17	3.57	40.30	59.17	-2.57	-0.68	DUI	MyEd	Rot	N	MyEd_2
8.25	6.19	13.35	27.14	21.25	43.69	72.35	51.22	3.16	34.04	65.37	-2.22	0.82	DUI	MyEd	Ama	N	MyEd_2
8.95	5.66	18.22	24.88	16.88	52.88	61.77	36.46	4.86	46.40	52.16	-2.17	-1.10	DUI	MyEd	Omy	N	MyEd_2
52.59	32.82	77.62	109.51	71.61	155.20	70.46	47.02	10.35	26.12	67.04	1.55	-0.64	DUI	MyEd	Oxa	N	MyEd_2
67.14	45.80	116.62	116.47	80.54	200.60	71.20	39.96	12.00	26.51	57.23	2.81	-1.85	DUI	MyEd	Ctrl	ch	MyEd_2
9.10	6.55	12.32	12.38	8.91	16.76	76.84	60.43	1.51	27.07	76.71	-2.65	2.19	DUI	MyEd	Rot	ch	MyEd_2
6.39	5.75	8.10	8.97	8.08	11.38	89.50	69.87	0.72	27.32	77.70	-2.62	3.21	DUI	MyEd	Ama	ch	MyEd_2
6.60	6.02	9.10	8.98	8.19	12.37	91.22	66.39	0.61	31.31	72.72	-2.58	2.87	DUI	MyEd	Omy	ch	MyEd_2
41.76	31.67	68.75	105.76	82.55	167.53	80.01	53.34	8.82	29.64	65.61	1.52	0.14	DUI	MyEd	Oxa	ch	MyEd_2
50.41	37.41	94.46	113.40	83.41	195.51	73.92	40.84	9.32	26.42	55.22	2.01	-1.39	DUI	MyEd	Ctrl	N	MyEd_3
14.55	6.85	21.96	20.82	9.53	32.31	63.24	49.35	2.68	33.05	72.90	-2.44	0.86	DUI	MyEd	Rot	N	MyEd_3
9.27	7.50	11.27	12.61	10.20	15.33	83.76	68.27	0.84	23.69	81.90	-2.57	3.13	DUI	MyEd	Ama	N	MyEd_3
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	DUI	MyEd	Omy	N	MyEd_3
44.29	31.90	81.30	85.67	63.58	153.32	74.41	42.96	8.75	30.71	57.30	1.13	-1.00	DUI	MyEd	Oxa	N	MyEd_3
49.63	31.93	91.12	96.06	66.14	169.67	74.04	37.78	11.05	21.54	53.58	1.54	-1.87	DUI	MyEd	Ctrl	ch	MyEd_3
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	DUI	MyEd	Rot	ch	MyEd_3
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	DUI	MyEd	Ama	ch	MyEd_3
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	DUI	MyEd	Omy	ch	MyEd_3
49.18	32.12	92.18	89.81	59.87	162.63	70.49	38.73	9.90	22.42	55.43	1.28	-1.69	DUI	MyEd	Oxa	ch	MyEd_3
68.25	39.71	100.57	125.83	79.64	182.85	64.65	43.54	8.55	29.31	68.01	2.20	-0.78	DUI	MyEd	Ctrl	N	MyEd_4
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	DUI	MyEd	Rot	N	MyEd_4
7.21	6.57	8.83	12.08	11.14	14.43	90.96	73.76	1.11	30.06	80.85	-2.41	3.57	DUI	MyEd	Ama	N	MyEd_4
4.87	4.18	6.77	6.62	5.69	9.20	85.85	61.79	0.49	28.49	71.98	-2.86	2.45	DUI	MyEd	Omy	N	MyEd_4
52.41	36.49	111.77	82.47	56.41	173.07	68.42	32.86	11.45	18.38	48.18	1.58	-2.72	DUI	MyEd	Oxa	N	MyEd_4
59.00	39.34	115.72	94.26	63.18	182.44	68.19	34.77	10.43	29.28	51.29	1.98	-2.29	DUI	MyEd	Ctrl	ch	MyEd_4
10.25	8.05	13.59	19.58	14.42	28.75	81.59	64.70	1.85	22.44	78.32	-2.35	2.54	DUI	MyEd	Rot	ch	MyEd_4
12.90	6.41	16.15	27.65	18.69	33.34	74.81	60.04	2.85	43.11	80.13	-2.03	2.18	DUI	MyEd	Ama	ch	MyEd_4
6.13	4.95	8.44	15.60	10.60	24.80	79.00	62.68	2.13	23.42	74.94	-2.60	2.17	DUI	MyEd	Omy	ch	MyEd_4
47.13	32.01	93.51	85.84	58.22	164.12	68.89	36.18	9.47	29.59	52.27	1.22	-1.93	DUI	MyEd	Oxa	ch	MyEd_4
50.74	29.47	91.10	117.82	72.55	206.85	63.87	37.37	11.04	25.88	58.24	1.84	-2.00	DUI	MyEd	Ctrl	N	MyEd_5
21.86	9.21	41.87	44.09	22.98	78.03	49.83	34.07	5.52	26.58	61.87	-1.70	-1.39	DUI	MyEd	Rot	N	MyEd_5
19.03	5.39	26.48	26.04	7.38	36.33	37.47	29.61	3.07	26.59	76.33	-2.86	-0.82	DUI	MyEd	Ama	N	MyEd_5
15.06	14.88	15.99	20.48	20.24	21.75	98.82	93.07	0.78	25.05	94.18	-1.71	5.43	DUI	MyEd	Omy	N	MyEd_5
52.16	34.31	89.69	116.32	78.52	194.98	68.12	40.26	10.33	28.35	59.31	1.94	-1.51	DUI	MyEd	Oxa	N	MyEd_5
49.05	26.85	104.62	97.32	55.98	200.47	57.56	29.86	11.75	28.58	49.65	1.52	-3.15	DUI	MyEd	Ctrl	ch	MyEd_5
17.45	7.54	27.27	28.64	14.28	42.95	52.13	37.86	4.29	24.85	68.49	-2.37	-0.55	DUI	MyEd	Rot	ch	MyEd_5
16.13	6.47	17.74	21.94	8.80	24.13	64.02	53.20	0.90	27.88	86.80	-2.68	2.03	DUI	MyEd	Ama	ch	MyEd_5
12.26	7.59	17.90	29.85	23.22	38.96	84.21	68.64	5.65	30.57	78.59	-1.59	2.34	DUI	MyEd	Omy	ch	MyEd_5
45.44	28.99	85.97	95.69	62.42	166.92	66.64	39.25	12.70	20.72	57.78	1.40	-2.03	DUI	MyEd	Oxa	ch	MyEd_5
63.63	38.92	126.57	107.87	67.45	208.17	62.60	33.96	14.17	21.78	53.40	2.59	-3.01	DUI	MyEd	Ctrl	N	MyEd_6
22.83	11.47	47.05	41.23	23.35	82.39	49.52	28.60	5.98	20.17	57.09	-1.67	-2.03	DUI	MyEd	Rot	N	MyEd_6
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	DUI	MyEd	Ama	N	MyEd_6
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	DUI	MyEd	Omy	N	MyEd_6
73.75	46.98	102.09	174.15	113.41	245.40	66.52	47.26	11.42	21.56	70.45	3.62	-0.88	DUI	MyEd	Oxa	N	MyEd_6
59.67	45.92	105.28	90.31	65.98	158.85	73.83	41.42	10.83	17.32	56.72	1.92	-1.54	DUI	MyEd	Ctrl	ch	MyEd_6

12.87	7.69	32.87	30.56	23.07	58.65	68.34	51.19	6.08	29.21	61.73	-1.66	-0.01	DUI	MyEd	Rot	ch	MyEd_6
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	DUI	MyEd	Ama	ch	MyEd_6
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	DUI	MyEd	Omy	ch	MyEd_6
35.47	28.68	64.93	65.91	58.25	112.47	91.13	57.45	10.48	10.98	62.16	0.70	0.22	DUI	MyEd	Oxa	ch	MyEd_6
57.79	43.28	98.26	91.93	67.63	153.89	74.99	44.38	9.87	26.79	59.14	1.83	-1.02	DUI	MyEd	Ctrl	N	MyEd_7
28.84	12.73	58.46	41.91	19.04	87.20	40.72	20.21	8.75	23.62	51.28	-1.37	-3.41	DUI	MyEd	Rot	N	MyEd_7
12.52	3.59	25.64	20.76	5.06	43.73	39.98	27.39	7.78	31.41	56.16	-2.51	-2.52	DUI	MyEd	Ama	N	MyEd_7
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	DUI	MyEd	Omy	N	MyEd_7
73.82	41.82	105.78	121.23	70.94	181.44	65.56	41.96	9.77	30.86	65.26	2.36	-1.15	DUI	MyEd	Oxa	N	MyEd_7
60.41	45.67	108.31	91.14	68.28	163.91	76.03	42.79	10.22	28.49	56.15	2.10	-1.30	DUI	MyEd	Ctrl	ch	MyEd_7
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	DUI	MyEd	Rot	ch	MyEd_7
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	DUI	MyEd	Ama	ch	MyEd_7
18.28	5.99	27.53	24.86	8.14	37.44	30.89	20.67	6.13	67.48	66.06	-2.32	-2.14	DUI	MyEd	Omy	ch	MyEd_7
51.26	38.47	86.03	82.36	62.57	138.36	77.76	45.10	8.69	32.97	58.23	1.35	-0.70	DUI	MyEd	Oxa	ch	MyEd_7
53.18	32.45	94.51	141.84	93.53	234.81	66.30	39.34	11.50	27.57	58.69	2.57	-1.87	DUI	MyEd	Ctrl	N	MyEd_8
28.61	16.32	53.95	38.95	22.21	73.48	49.38	28.35	6.72	35.44	53.28	-1.30	-2.26	DUI	MyEd	Rot	N	MyEd_8
18.85	8.40	30.12	25.88	11.59	41.41	43.11	28.59	3.98	28.57	65.50	-2.55	-1.37	DUI	MyEd	Ama	N	MyEd_8
9.35	3.41	23.27	14.49	4.68	37.38	48.30	38.33	7.08	37.40	55.78	-2.46	-1.61	DUI	MyEd	Omy	N	MyEd_8
58.68	37.35	90.84	129.07	86.40	198.23	69.07	44.50	11.82	23.62	64.23	2.41	-1.24	DUI	MyEd	Oxa	N	MyEd_8
62.54	46.53	116.06	114.16	84.84	203.90	73.13	40.92	11.22	31.20	55.01	2.81	-1.72	DUI	MyEd	Ctrl	ch	MyEd_8
5.96	5.14	7.76	8.10	6.99	10.55	86.26	66.26	0.53	21.37	76.81	-2.81	2.88	DUI	MyEd	Rot	ch	MyEd_8
13.82	11.10	17.87	19.18	15.44	24.73	79.29	61.34	1.07	27.37	76.71	-2.30	2.36	DUI	MyEd	Ama	ch	MyEd_8
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	DUI	MyEd	Omy	ch	MyEd_8
57.83	41.23	97.56	107.80	78.52	177.48	75.14	44.19	10.65	26.66	59.21	2.19	-1.15	DUI	MyEd	Oxa	ch	MyEd_8
73.72	38.79	112.13	119.87	65.66	178.06	58.54	38.17	11.65	25.60	66.11	2.29	-1.82	DUI	MyEd	Ctrl	N	MyEd_9
14.40	5.27	30.12	31.81	10.15	63.46	35.97	19.53	5.01	22.54	51.11	-2.65	-3.00	DUI	MyEd	Rot	N	MyEd_9
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	DUI	MyEd	Ama	N	MyEd_9
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	DUI	MyEd	Omy	N	MyEd_9
76.83	42.85	118.27	116.22	66.37	177.86	61.81	39.79	10.63	32.55	64.69	2.47	-1.55	DUI	MyEd	Oxa	N	MyEd_9
69.61	37.97	124.46	139.86	84.09	254.48	57.70	31.89	13.55	25.55	55.00	3.16	-3.12	DUI	MyEd	Ctrl	ch	MyEd_9
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	DUI	MyEd	Rot	ch	MyEd_9
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	DUI	MyEd	Ama	ch	MyEd_9
19.53	7.47	28.74	26.55	10.15	39.09	38.23	25.97	7.08	40.39	67.94	-2.28	-1.86	DUI	MyEd	Omy	ch	MyEd_9
42.64	30.16	85.49	95.84	69.31	179.16	72.80	39.98	10.36	30.58	54.14	1.48	-1.62	DUI	MyEd	Oxa	ch	MyEd_9
58.95	38.14	88.01	129.92	89.74	192.56	68.48	45.84	9.62	25.45	65.99	2.23	-0.77	DUI	MyEd	Ctrl	N	MyEd_10
15.42	7.86	30.17	34.01	20.33	90.62	53.82	28.35	6.13	27.15	59.90	-1.89	-1.71	DUI	MyEd	Rot	N	MyEd_10
7.88	7.83	8.30	26.20	26.04	27.60	99.42	94.36	6.08	36.57	94.91	-1.27	4.92	DUI	MyEd	Ama	N	MyEd_10
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	DUI	MyEd	Omy	N	MyEd_10
45.55	33.97	71.18	100.39	77.26	152.75	76.79	49.89	8.58	31.20	65.00	1.39	-0.10	DUI	MyEd	Oxa	N	MyEd_10
59.31	45.32	94.30	115.12	89.36	179.79	76.79	48.63	8.62	31.60	63.08	2.34	-0.35	DUI	MyEd	Ctrl	ch	MyEd_10
7.83	7.42	8.46	36.02	34.14	38.93	94.79	87.70	6.24	9.21	92.52	-1.46	4.15	DUI	MyEd	Rot	ch	MyEd_10
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	DUI	MyEd	Ama	ch	MyEd_10
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	DUI	MyEd	Omy	ch	MyEd_10
49.55	39.52	85.56	102.53	82.29	172.63	79.42	47.33	8.84	30.58	58.81	1.87	-0.57	DUI	MyEd	Oxa	ch	MyEd_10

42.73	28.41	74.02	90.00	62.00	144.55	68.91	44.80	9.84	19.05	63.83	0.88	-0.93	DUI	MyEd	Ctrl	N	MyEd_11
15.51	8.77	23.76	22.69	13.48	34.15	61.97	46.31	2.66	27.00	69.52	-2.42	0.47	DUI	MyEd	Rot	N	MyEd_11
14.03	9.02	33.14	22.21	14.36	51.28	63.81	40.07	6.28	36.93	53.40	-1.86	-1.09	DUI	MyEd	Ama	N	MyEd_11
5.95	5.09	8.54	8.32	7.04	11.95	87.47	61.44	0.66	27.02	70.20	-2.75	2.36	DUI	MyEd	Omy	N	MyEd_11
40.00	25.49	72.41	92.67	59.83	156.84	65.98	40.94	11.91	19.10	61.43	0.96	-1.63	DUI	MyEd	Oxa	N	MyEd_11
53.02	34.25	105.70	94.66	60.22	187.47	65.03	32.84	14.25	16.30	50.54	1.88	-3.09	DUI	MyEd	Ctrl	ch	MyEd_11
12.00	5.87	18.73	30.12	26.69	56.38	67.40	41.32	3.39	30.87	65.48	-2.15	0.12	DUI	MyEd	Rot	ch	MyEd_11
6.82	7.65	12.49	26.89	33.56	53.15	117.45	67.46	6.46	31.40	59.15	-1.09	2.22	DUI	MyEd	Ama	ch	MyEd_11
15.44	6.44	30.42	28.43	11.78	56.39	41.54	30.43	5.25	67.03	62.05	-2.02	-1.48	DUI	MyEd	Omy	ch	MyEd_11
62.32	43.69	116.61	99.05	70.24	181.31	71.87	40.57	11.09	19.84	55.85	2.26	-1.78	DUI	MyEd	Oxa	ch	MyEd_11
48.56	29.31	68.36	92.39	62.15	127.67	71.82	52.87	6.59	25.19	72.67	0.73	0.53	DUI	RuPh	Ctrl	N	RuPh_1
27.60	15.63	49.23	50.10	29.18	86.83	57.21	36.63	7.11	23.09	61.38	-1.08	-1.32	DUI	RuPh	Rot	N	RuPh_1
12.42	6.34	20.50	38.07	31.15	57.76	61.38	39.19	4.14	21.70	62.35	-2.14	-0.49	DUI	RuPh	Ama	N	RuPh_1
16.87	9.12	28.81	32.62	18.80	59.60	58.11	34.94	6.07	39.30	61.94	-1.82	-1.02	DUI	RuPh	Omy	N	RuPh_1
45.46	23.21	57.65	101.42	58.86	127.81	62.71	50.75	5.82	21.13	80.15	0.27	0.63	DUI	RuPh	Oxa	N	RuPh_1
57.34	39.05	86.07	114.22	76.55	164.59	68.22	46.20	9.19	24.90	68.55	1.78	-0.54	DUI	RuPh	Ctrl	ch	RuPh_1
17.02	6.75	28.58	39.86	25.43	61.26	58.43	41.87	4.52	21.08	67.50	-2.00	-0.29	DUI	RuPh	Rot	ch	RuPh_1
20.84	6.32	25.74	33.23	12.14	40.71	44.41	37.32	5.87	33.17	83.49	-2.24	-0.19	DUI	RuPh	Ama	ch	RuPh_1
16.44	9.65	22.91	59.13	44.32	83.61	75.57	57.08	5.80	17.59	75.88	-1.15	1.24	DUI	RuPh	Omy	ch	RuPh_1
26.67	18.73	59.18	76.34	56.95	135.99	75.74	45.96	7.33	24.85	58.97	0.11	-0.50	DUI	RuPh	Oxa	ch	RuPh_1
66.85	41.23	102.25	123.21	81.75	188.13	66.97	43.75	8.24	28.90	65.73	2.25	-0.78	DUI	RuPh	Ctrl	N	RuPh_2
33.49	14.77	67.19	64.09	32.16	119.85	51.54	30.68	8.64	21.77	57.88	-0.54	-2.29	DUI	RuPh	Rot	N	RuPh_2
11.87	7.81	12.97	32.30	21.26	35.28	65.81	60.24	2.76	35.36	91.54	-2.18	2.49	DUI	RuPh	Ama	N	RuPh_2
19.34	7.59	24.75	37.76	18.07	46.76	45.68	38.88	2.78	17.93	81.21	-2.52	0.13	DUI	RuPh	Omy	N	RuPh_2
53.97	33.78	72.93	125.13	91.50	166.46	72.07	54.46	7.46	28.99	75.06	1.75	0.62	DUI	RuPh	Oxa	N	RuPh_2
63.28	37.42	97.45	125.56	82.25	188.57	64.45	41.99	9.67	22.09	64.95	2.13	-1.21	DUI	RuPh	Ctrl	ch	RuPh_2
24.36	10.08	44.68	33.71	14.08	61.57	46.84	29.42	5.32	21.32	61.20	-2.02	-1.75	DUI	RuPh	Rot	ch	RuPh_2
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	DUI	RuPh	Ama	ch	RuPh_2
14.21	6.99	20.48	28.05	18.80	38.60	67.33	53.13	3.31	22.03	76.13	-2.25	1.20	DUI	RuPh	Omy	ch	RuPh_2
39.38	25.90	70.95	92.12	65.01	167.25	71.13	37.74	9.71	20.22	54.20	0.89	-1.70	DUI	RuPh	Oxa	ch	RuPh_2
55.46	30.89	78.15	106.11	67.22	149.50	65.02	45.30	5.98	30.05	70.90	1.03	-0.09	DUI	RuPh	Ctrl	N	RuPh_3
28.45	11.99	49.90	49.35	23.56	86.04	45.56	26.85	8.19	16.10	60.39	-1.39	-2.40	DUI	RuPh	Rot	N	RuPh_3
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	DUI	RuPh	Ama	N	RuPh_3
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	DUI	RuPh	Omy	N	RuPh_3
37.03	24.87	56.16	97.94	68.31	142.63	73.10	51.30	6.63	21.32	69.28	0.53	0.31	DUI	RuPh	Oxa	N	RuPh_3
56.78	32.33	106.49	84.69	49.47	165.93	59.22	31.77	7.58	36.74	53.87	1.15	-2.13	DUI	RuPh	Ctrl	ch	RuPh_3
29.86	13.24	54.77	62.21	31.90	102.75	50.65	34.56	7.49	17.00	64.45	-0.97	-1.60	DUI	RuPh	Rot	ch	RuPh_3
13.41	9.55	32.60	18.24	12.99	44.33	71.22	29.30	10.91	41.32	41.14	-1.49	-2.59	DUI	RuPh	Ama	ch	RuPh_3
20.40	8.47	28.81	30.90	12.79	45.95	41.46	31.09	3.68	28.14	74.29	-2.50	-0.81	DUI	RuPh	Omy	ch	RuPh_3
41.50	21.59	81.33	70.35	40.58	135.49	57.29	31.03	7.39	33.66	53.02	0.10	-2.16	DUI	RuPh	Oxa	ch	RuPh_3
43.65	28.20	82.96	81.70	53.73	150.20	69.82	39.82	7.41	27.56	57.11	0.71	-1.17	DUI	RuPh	Ctrl	N	RuPh_4
24.01	11.12	44.92	48.48	24.68	82.70	50.86	33.49	5.76	26.98	61.29	-1.51	-1.46	DUI	RuPh	Rot	N	RuPh_4
20.21	6.26	24.67	40.72	13.21	49.25	30.72	25.27	5.51	12.25	81.85	-2.67	-1.36	DUI	RuPh	Ama	N	RuPh_4
15.51	7.42	21.49	31.31	18.73	41.02	53.68	45.72	4.90	33.84	77.08	-2.16	0.32	DUI	RuPh	Omy	N	RuPh_4
36.79	29.44	89.75	59.13	48.48	137.25	79.81	37.19	5.27	42.19	44.95	0.49	-1.25	DUI	RuPh	Oxa	N	RuPh_4

47.83	33.08	90.70	107.09	74.29	196.88	69.72	37.55	8.86	35.57	55.18	1.72	-1.58	DUI	RuPh	Ctrl	ch	RuPh_4
18.51	9.00	44.59	43.76	25.60	90.26	59.24	32.59	5.34	28.64	51.58	-1.52	-1.70	DUI	RuPh	Rot	ch	RuPh_4
14.28	8.17	17.44	23.47	13.32	28.66	62.40	51.63	2.46	22.28	81.93	-2.55	1.41	DUI	RuPh	Ama	ch	RuPh_4
16.09	7.08	23.74	26.70	13.28	38.99	51.23	39.24	4.04	33.38	73.12	-2.40	-0.17	DUI	RuPh	Omy	ch	RuPh_4
43.24	27.75	78.03	78.05	51.33	141.36	67.61	39.55	6.06	33.11	58.28	0.46	-0.95	DUI	RuPh	Oxa	ch	RuPh_4
53.50	34.05	91.33	117.77	79.61	189.54	68.39	43.17	8.51	28.59	61.85	1.83	-0.98	DUI	RuPh	Ctrl	N	RuPh_5
36.09	18.53	60.89	61.36	32.69	101.64	54.54	32.24	5.86	26.00	59.37	-0.77	-1.59	DUI	RuPh	Rot	N	RuPh_5
21.56	7.40	32.59	49.12	21.10	69.34	41.68	30.86	6.02	19.72	70.87	-2.01	-1.40	DUI	RuPh	Ama	N	RuPh_5
13.76	8.25	23.82	37.91	25.40	65.66	63.33	41.75	3.98	17.20	65.34	-2.08	-0.19	DUI	RuPh	Omy	N	RuPh_5
59.98	29.61	79.42	115.30	64.58	157.42	60.78	44.37	6.80	23.12	74.52	1.11	-0.25	DUI	RuPh	Oxa	N	RuPh_5
62.36	32.05	97.53	121.42	71.31	180.83	59.86	41.91	8.74	24.58	68.01	1.74	-1.08	DUI	RuPh	Ctrl	ch	RuPh_5
29.65	10.07	61.00	60.25	24.03	116.70	43.88	22.69	6.75	31.87	51.08	-1.08	-2.95	DUI	RuPh	Rot	ch	RuPh_5
17.23	7.42	26.88	31.26	15.89	47.22	48.23	35.14	4.16	25.86	70.19	-2.39	-0.69	DUI	RuPh	Ama	ch	RuPh_5
17.98	9.25	31.44	45.66	28.79	80.43	56.13	32.57	6.74	19.53	58.73	-1.67	-1.59	DUI	RuPh	Omy	ch	RuPh_5
48.53	31.05	72.16	99.04	66.76	147.77	70.94	49.09	7.04	25.22	69.25	0.98	0.05	DUI	RuPh	Oxa	ch	RuPh_5
42.13	29.07	76.52	94.85	69.32	165.10	72.72	42.51	8.69	25.30	57.96	1.11	-1.06	DUI	RuPh	Ctrl	N	RuPh_6
23.77	11.82	45.41	53.29	29.14	95.28	52.86	31.24	6.73	20.88	58.27	-1.32	-1.84	DUI	RuPh	Rot	N	RuPh_6
21.10	10.78	33.00	45.70	31.52	69.99	64.84	42.79	6.25	20.06	69.79	-1.46	-0.17	DUI	RuPh	Ama	N	RuPh_6
10.28	9.51	14.51	28.56	26.92	38.70	88.85	66.74	3.78	34.13	74.50	-1.69	2.48	DUI	RuPh	Omy	N	RuPh_6
40.88	29.82	71.25	95.18	73.67	155.51	77.91	47.24	7.99	26.90	60.93	1.10	-0.37	DUI	RuPh	Oxa	N	RuPh_6
54.35	31.98	83.38	105.95	69.43	157.96	67.38	44.82	6.97	29.94	67.00	1.27	-0.40	DUI	RuPh	Ctrl	ch	RuPh_6
25.82	13.09	49.92	51.72	27.74	94.82	51.99	29.72	7.20	21.80	56.06	-1.23	-2.13	DUI	RuPh	Rot	ch	RuPh_6
22.25	8.61	30.38	36.41	15.97	48.79	45.99	36.08	5.33	18.73	76.29	-2.22	-0.61	DUI	RuPh	Ama	ch	RuPh_6
9.36	6.81	13.15	27.96	22.94	37.78	74.98	59.24	4.59	35.90	77.72	-1.95	1.76	DUI	RuPh	Omy	ch	RuPh_6
37.78	21.36	60.86	90.04	55.24	140.95	64.06	40.10	7.99	24.74	61.73	0.28	-1.11	DUI	RuPh	Oxa	ch	RuPh_6
32.17	20.08	57.59	73.90	51.76	126.75	80.09	42.58	8.37	20.38	57.35	0.14	-0.77	DUI	RuPh	Ctrl	N	RuPh_7
21.41	11.10	37.71	51.55	28.87	84.78	54.81	35.48	5.67	21.74	64.14	-1.53	-1.08	DUI	RuPh	Rot	N	RuPh_7
4.81	2.42	9.64	11.43	6.64	21.08	54.82	33.24	1.93	50.48	55.88	-3.05	-0.79	DUI	RuPh	Ama	N	RuPh_7
9.50	4.99	14.72	20.76	11.64	31.80	57.00	38.54	2.56	47.23	65.18	-2.63	-0.12	DUI	RuPh	Omy	N	RuPh_7
43.40	27.64	68.71	114.29	78.12	172.24	70.52	47.35	8.51	22.87	67.09	1.25	-0.39	DUI	RuPh	Oxa	N	RuPh_7
50.72	24.34	88.96	107.93	59.58	174.67	52.58	32.38	9.49	25.18	60.17	1.04	-2.25	DUI	RuPh	Ctrl	ch	RuPh_7
28.27	15.68	51.61	59.60	35.49	103.42	53.93	32.61	6.68	19.15	59.44	-0.99	-1.70	DUI	RuPh	Rot	ch	RuPh_7
16.90	6.72	25.01	43.41	27.95	57.12	54.40	46.08	7.77	35.82	74.90	-1.58	-0.15	DUI	RuPh	Ama	ch	RuPh_7
11.13	5.55	14.01	40.04	21.94	46.24	69.22	56.26	4.19	33.81	81.04	-1.98	1.63	DUI	RuPh	Omy	ch	RuPh_7
45.93	26.00	73.56	108.44	69.58	161.05	67.69	43.92	7.63	24.45	65.94	1.00	-0.59	DUI	RuPh	Oxa	ch	RuPh_7
45.59	29.73	76.79	79.24	54.01	130.59	69.55	42.47	5.46	33.34	61.06	0.48	-0.49	DUI	RuPh	Ctrl	N	RuPh_8
32.05	16.70	57.90	69.06	36.52	113.66	49.95	31.55	8.42	16.39	64.06	-0.66	-1.92	DUI	RuPh	Rot	N	RuPh_8
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	DUI	RuPh	Ama	N	RuPh_8
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	DUI	RuPh	Omy	N	RuPh_8
28.55	25.81	56.66	82.39	72.40	154.10	86.98	47.64	5.25	33.94	54.78	0.57	0.06	DUI	RuPh	Oxa	N	RuPh_8
39.49	22.93	70.73	96.80	62.27	157.36	61.66	39.02	9.09	17.35	62.10	0.61	-1.45	DUI	RuPh	Ctrl	ch	RuPh_8
20.72	11.46	32.07	64.95	44.75	96.56	62.61	46.36	7.19	12.10	71.56	-1.05	-0.17	DUI	RuPh	Rot	ch	RuPh_8
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	DUI	RuPh	Ama	ch	RuPh_8
9.01	8.63	19.84	32.71	30.99	86.72	95.97	53.80	7.76	39.03	55.65	-0.97	0.51	DUI	RuPh	Omy	ch	RuPh_8

42.92	26.15	78.25	88.73	56.31	154.28	68.18	38.73	8.11	23.46	57.06	0.70	-1.38	DUI	RuPh	Oxa	ch	RuPh_8
42.57	27.95	80.18	71.63	48.90	131.09	70.20	39.61	6.77	29.08	56.15	0.41	-1.09	DUI	RuPh	Ctrl	N	RuPh_9
28.96	14.86	61.93	56.61	31.05	114.94	53.91	28.80	7.50	26.44	51.80	-0.78	-2.42	DUI	RuPh	Rot	N	RuPh_9
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	DUI	RuPh	Ama	N	RuPh_9
13.71	2.19	16.96	37.30	5.96	46.14	15.98	12.92	4.71	13.60	80.84	-3.36	-2.33	DUI	RuPh	Omy	N	RuPh_9
37.12	23.63	63.38	76.41	51.38	135.03	71.63	42.92	5.89	30.75	59.46	0.17	-0.54	DUI	RuPh	Oxa	N	RuPh_9
45.98	26.08	79.44	84.94	50.90	143.60	62.58	36.90	7.59	26.99	59.38	0.52	-1.43	DUI	RuPh	Ctrl	ch	RuPh_9
32.15	15.45	60.67	62.37	32.99	111.01	52.13	30.30	7.23	26.20	56.39	-0.74	-2.11	DUI	RuPh	Rot	ch	RuPh_9
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	DUI	RuPh	Ama	ch	RuPh_9
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	DUI	RuPh	Omy	ch	RuPh_9
50.30	31.61	80.60	90.05	55.82	142.06	65.07	40.52	8.73	23.14	63.27	0.88	-1.13	DUI	RuPh	Oxa	ch	RuPh_9
119.39	57.49	142.31	191.00	104.18	227.17	57.96	46.58	11.02	21.57	81.68	4.56	-0.66	SMI	MeMe	Ctrl	N	MeMe_1
15.38	8.17	18.43	20.91	11.11	25.06	56.35	47.05	1.54	21.82	81.86	-2.79	1.12	SMI	MeMe	Rot	N	MeMe_1
14.38	8.03	20.87	19.56	10.93	28.38	76.44	56.22	2.41	20.38	72.94	-2.37	1.57	SMI	MeMe	Ama	N	MeMe_1
22.84	10.79	35.81	36.57	18.48	55.62	52.36	37.28	5.08	17.47	69.39	-2.00	-0.71	SMI	MeMe	Omy	N	MeMe_1
65.57	43.03	87.71	108.98	75.39	146.02	73.94	52.79	8.39	19.81	72.61	1.83	0.26	SMI	MeMe	Oxa	N	MeMe_1
60.40	41.54	93.47	112.95	71.50	168.70	68.52	45.56	15.87	16.86	65.99	2.42	-1.68	SMI	MeMe	Ctrl	ch	MeMe_1
19.20	18.15	21.70	26.12	24.68	29.52	94.77	83.40	1.19	30.37	88.03	-1.48	4.49	SMI	MeMe	Rot	ch	MeMe_1
16.07	14.10	17.98	21.86	19.17	24.45	86.94	77.71	0.82	27.95	89.20	-1.94	4.07	SMI	MeMe	Ama	ch	MeMe_1
16.16	8.47	20.31	23.05	12.44	29.03	63.28	51.59	2.56	19.27	80.74	-2.51	1.33	SMI	MeMe	Omy	ch	MeMe_1
50.08	35.23	83.94	68.11	47.91	114.15	73.78	48.72	10.25	15.54	64.49	0.85	-0.63	SMI	MeMe	Oxa	ch	MeMe_1
99.81	55.78	122.49	190.75	122.00	230.44	65.51	52.04	8.49	27.52	79.62	4.24	0.20	SMI	MeMe	Ctrl	N	MeMe_2
24.45	12.57	36.02	35.86	18.68	52.40	57.03	41.88	5.06	17.37	72.02	-1.87	-0.20	SMI	MeMe	Rot	N	MeMe_2
28.31	10.77	40.85	43.70	18.57	61.77	42.22	30.16	6.36	14.20	71.14	-1.91	-1.50	SMI	MeMe	Ama	N	MeMe_2
22.00	8.67	29.23	33.34	14.47	43.66	45.38	35.85	3.73	19.61	77.66	-2.44	-0.34	SMI	MeMe	Omy	N	MeMe_2
80.76	46.05	106.89	142.66	90.39	182.05	68.13	49.98	9.20	24.83	73.76	2.85	-0.17	SMI	MeMe	Oxa	N	MeMe_2
99.23	65.37	120.29	175.75	124.16	210.83	72.48	58.30	7.12	30.61	80.67	4.24	1.04	SMI	MeMe	Ctrl	ch	MeMe_2
22.42	11.20	31.09	32.42	16.63	44.55	58.98	48.03	4.18	17.15	78.40	-2.05	0.62	SMI	MeMe	Rot	ch	MeMe_2
18.87	9.56	23.67	28.14	14.77	34.94	54.79	43.79	2.82	20.61	79.93	-2.48	0.61	SMI	MeMe	Ama	ch	MeMe_2
18.25	8.75	23.34	28.96	15.36	36.50	55.39	44.94	2.79	21.31	80.01	-2.46	0.70	SMI	MeMe	Omy	ch	MeMe_2
75.48	52.40	98.72	129.54	94.81	166.27	74.51	54.88	6.67	29.23	73.56	2.62	0.70	SMI	MeMe	Oxa	ch	MeMe_2
107.74	73.59	129.57	162.82	114.33	194.71	72.28	58.72	6.91	28.13	82.16	4.28	1.14	SMI	MeMe	Ctrl	N	MeMe_3
5.06	4.08	7.59	6.88	5.54	10.32	80.58	53.75	0.72	12.52	66.70	-3.12	1.50	SMI	MeMe	Rot	N	MeMe_3
28.66	11.09	44.90	45.11	18.97	69.28	40.55	26.76	6.96	18.14	65.95	-1.77	-2.06	SMI	MeMe	Ama	N	MeMe_3
21.28	9.86	35.23	28.94	13.41	47.91	49.92	33.09	4.69	20.25	66.08	-2.26	-1.09	SMI	MeMe	Omy	N	MeMe_3
68.24	42.81	80.20	131.84	95.80	158.30	88.23	59.76	7.76	18.08	78.79	2.36	1.48	SMI	MeMe	Oxa	N	MeMe_3
110.70	61.61	138.81	163.71	94.20	204.59	61.56	46.26	12.09	17.78	77.58	4.19	-0.90	SMI	MeMe	Ctrl	ch	MeMe_3
16.17	8.64	27.19	23.39	12.55	39.85	54.59	32.36	8.60	43.72	62.16	-1.88	-1.52	SMI	MeMe	Rot	ch	MeMe_3
18.90	9.36	32.12	38.01	21.15	60.85	53.41	38.84	7.23	22.20	65.74	-1.78	-1.05	SMI	MeMe	Ama	ch	MeMe_3
19.41	8.93	27.25	31.64	14.05	45.84	49.21	38.34	3.78	25.68	73.92	-2.35	-0.26	SMI	MeMe	Omy	ch	MeMe_3
50.97	31.89	72.48	91.66	61.04	129.35	71.25	51.03	7.39	23.54	70.69	0.87	0.19	SMI	MeMe	Oxa	ch	MeMe_3
93.46	65.23	113.99	168.14	124.17	204.33	74.69	60.42	6.92	31.52	80.81	4.06	1.29	SMI	MeMe	Ctrl	N	MeMe_4
23.26	11.03	33.11	37.12	19.38	51.37	53.07	42.14	4.73	16.18	74.84	-2.02	-0.12	SMI	MeMe	Rot	N	MeMe_4
24.34	9.55	34.17	36.34	15.20	51.18	44.62	33.78	4.94	15.33	75.14	-2.24	-0.81	SMI	MeMe	Ama	N	MeMe_4

22.50	10.81	35.69	40.28	20.48	61.18	48.93	35.19	5.06	18.84	72.99	-1.99	-0.72	SMI	MeMe	Omy	N	MeMe_4
74.77	54.57	98.25	130.51	99.09	170.65	76.35	56.68	6.96	30.89	73.40	2.80	0.81	SMI	MeMe	Oxa	N	MeMe_4
91.44	69.88	112.48	162.87	129.77	200.13	80.18	64.41	6.60	32.39	80.26	4.17	1.70	SMI	MeMe	Ctrl	ch	MeMe_4
14.77	6.74	18.98	25.06	13.36	31.31	54.86	45.06	3.09	22.49	80.46	-2.62	0.69	SMI	MeMe	Rot	ch	MeMe_4
17.48	8.30	22.12	27.27	12.86	34.54	49.47	39.62	2.75	18.91	79.42	-2.68	0.22	SMI	MeMe	Ama	ch	MeMe_4
20.49	9.47	28.53	31.60	15.42	44.76	49.05	37.85	3.80	19.01	76.96	-2.38	-0.18	SMI	MeMe	Omy	ch	MeMe_4
65.93	49.81	92.67	102.31	79.33	144.85	79.46	54.81	6.79	28.96	68.98	2.02	0.62	SMI	MeMe	Oxa	ch	MeMe_4
76.43	43.39	103.38	154.38	101.56	204.40	67.64	49.17	9.01	28.39	73.07	3.02	-0.22	SMI	MeMe	Ctrl	N	MeMe_5
24.79	10.79	38.66	43.52	20.86	65.12	47.07	32.15	5.88	15.45	67.15	-1.89	-1.38	SMI	MeMe	Rot	N	MeMe_5
18.01	9.79	21.57	34.04	21.47	40.03	59.95	51.69	2.98	19.93	84.83	-2.23	1.39	SMI	MeMe	Ama	N	MeMe_5
20.08	8.69	27.79	30.82	13.38	42.59	46.96	36.37	4.35	21.99	75.79	-2.40	-0.43	SMI	MeMe	Omy	N	MeMe_5
76.20	46.25	100.56	143.27	94.78	184.66	70.41	52.39	9.04	25.74	74.75	2.84	0.12	SMI	MeMe	Oxa	N	MeMe_5
73.72	48.11	100.44	132.98	93.50	177.76	73.19	52.04	7.25	31.42	71.60	2.65	0.32	SMI	MeMe	Ctrl	ch	MeMe_5
24.76	9.66	37.74	42.95	18.88	63.49	43.07	30.15	5.73	16.30	69.10	-2.02	-1.47	SMI	MeMe	Rot	ch	MeMe_5
7.17	6.11	8.99	9.75	8.31	12.22	85.21	67.98	0.58	16.70	79.78	-2.77	3.05	SMI	MeMe	Ama	ch	MeMe_5
21.91	10.95	27.53	37.11	20.14	45.48	57.46	48.82	3.93	16.47	82.77	-2.07	0.89	SMI	MeMe	Omy	ch	MeMe_5
43.97	31.16	68.98	79.04	58.23	122.09	73.86	48.24	6.49	29.97	63.82	0.57	-0.04	SMI	MeMe	Oxa	ch	MeMe_5
57.05	41.64	84.47	88.39	64.88	130.01	76.06	50.81	6.77	25.40	66.56	1.26	0.19	SMI	MeMe	Ctrl	N	MeMe_6
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	SMI	MeMe	Rot	N	MeMe_6
18.31	8.46	24.02	33.64	17.48	44.79	53.19	43.03	3.79	21.58	78.98	-2.31	0.34	SMI	MeMe	Ama	N	MeMe_6
15.10	7.69	20.37	27.62	16.78	36.12	62.92	49.13	3.11	18.57	77.28	-2.41	0.94	SMI	MeMe	Omy	N	MeMe_6
53.91	39.62	76.91	97.55	73.84	135.05	77.35	53.97	7.41	23.61	69.65	1.37	0.48	SMI	MeMe	Oxa	N	MeMe_6
99.77	40.27	130.08	185.32	95.13	232.37	56.25	44.68	16.09	14.20	78.30	4.15	-1.72	SMI	MeMe	Ctrl	ch	MeMe_6
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	SMI	MeMe	Rot	ch	MeMe_6
17.03	8.38	21.40	29.06	15.60	35.32	55.18	46.42	3.45	21.04	82.88	-2.44	0.83	SMI	MeMe	Ama	ch	MeMe_6
20.58	9.90	29.18	34.79	18.12	49.21	54.12	42.11	4.92	14.46	74.38	-2.14	-0.13	SMI	MeMe	Omy	ch	MeMe_6
39.25	30.03	65.32	66.50	50.86	109.47	77.34	48.04	7.65	23.31	61.52	0.31	-0.24	SMI	MeMe	Oxa	ch	MeMe_6
110.05	62.56	133.71	171.98	103.60	207.39	66.46	51.27	8.67	23.50	78.60	4.16	0.07	SMI	MeMe	Ctrl	N	MeMe_7
15.58	10.87	18.23	22.75	16.27	26.58	74.58	61.86	1.65	27.77	83.16	-2.24	2.49	SMI	MeMe	Rot	N	MeMe_7
22.40	11.34	29.43	39.82	21.17	50.97	57.35	47.95	4.29	16.87	80.94	-1.96	0.69	SMI	MeMe	Ama	N	MeMe_7
24.60	13.68	35.93	33.45	18.61	48.86	52.02	37.08	3.81	19.05	74.12	-2.09	-0.29	SMI	MeMe	Omy	N	MeMe_7
87.62	47.16	110.57	140.64	80.90	174.41	71.55	52.69	10.46	20.47	75.37	2.99	-0.06	SMI	MeMe	Oxa	N	MeMe_7
76.28	56.73	97.08	123.97	93.13	160.43	78.66	59.30	7.32	24.58	75.17	2.71	1.03	SMI	MeMe	Ctrl	ch	MeMe_7
10.63	9.17	13.53	23.94	20.94	30.37	86.61	68.29	7.37	42.34	78.70	-1.44	2.28	SMI	MeMe	Rot	ch	MeMe_7
16.21	9.40	22.11	30.89	17.82	41.16	59.88	48.52	4.47	29.24	78.11	-2.11	0.73	SMI	MeMe	Ama	ch	MeMe_7
15.88	6.81	18.38	21.59	9.26	24.99	56.43	46.13	1.03	20.78	85.11	-2.88	1.31	SMI	MeMe	Omy	ch	MeMe_7
58.27	46.13	79.82	92.05	73.43	124.96	83.05	59.60	5.97	26.26	71.54	1.49	1.25	SMI	MeMe	Oxa	ch	MeMe_7
76.16	59.07	100.05	116.24	91.38	151.82	79.51	59.51	7.20	27.78	74.52	2.69	1.08	SMI	MeMe	Ctrl	N	MeMe_8
18.45	13.75	23.68	36.55	28.72	53.59	76.30	65.22	3.08	28.95	82.61	-1.55	2.46	SMI	MeMe	Rot	N	MeMe_8
21.90	9.14	29.92	37.71	18.06	50.40	49.11	38.93	4.46	18.56	76.55	-2.19	-0.25	SMI	MeMe	Ama	N	MeMe_8
14.60	11.56	19.22	22.28	16.91	29.51	84.03	70.81	1.97	26.32	81.23	-1.99	3.05	SMI	MeMe	Omy	N	MeMe_8
48.37	37.69	68.56	86.65	70.17	118.87	81.55	58.91	6.67	22.64	71.53	1.01	1.08	SMI	MeMe	Oxa	N	MeMe_8
60.83	50.72	85.87	89.39	73.34	126.26	81.45	58.33	6.60	26.22	70.53	1.67	0.99	SMI	MeMe	Ctrl	ch	MeMe_8
8.87	8.24	11.31	12.07	11.20	15.38	92.84	72.86	1.41	24.55	78.48	-2.34	3.38	SMI	MeMe	Rot	ch	MeMe_8
23.33	6.50	37.11	35.72	11.04	57.81	33.25	18.93	8.85	28.02	61.90	-2.08	-3.04	SMI	MeMe	Ama	ch	MeMe_8

23.07	8.21	28.16	31.38	11.17	38.30	39.36	33.81	2.86	23.40	82.96	-2.65	-0.20	SMI	MeMe	Omy	ch	MeMe_8
57.41	43.34	81.36	98.30	76.21	137.36	78.43	54.23	7.74	21.79	68.85	1.59	0.42	SMI	MeMe	Oxa	ch	MeMe_8
89.26	66.17	107.81	166.51	130.50	200.32	79.59	65.41	6.73	30.73	81.93	4.06	1.80	SMI	MeMe	Ctrl	N	MeMe_9
20.36	8.48	28.38	34.02	15.74	46.23	46.95	37.56	3.87	20.37	76.48	-2.38	-0.28	SMI	MeMe	Rot	N	MeMe_9
21.65	9.09	33.87	39.13	19.65	60.40	49.16	36.69	5.26	21.55	69.20	-2.00	-0.85	SMI	MeMe	Ama	N	MeMe_9
19.00	9.79	26.09	38.87	24.23	50.03	60.17	50.12	4.41	21.74	80.48	-1.93	0.89	SMI	MeMe	Omy	N	MeMe_9
19.16	17.43	26.10	96.86	89.69	126.83	92.44	69.48	4.69	20.63	74.88	0.20	2.48	SMI	MeMe	Oxa	N	MeMe_9
75.70	57.08	95.07	133.21	101.87	168.54	76.86	61.12	7.00	27.40	78.06	2.87	1.28	SMI	MeMe	Ctrl	ch	MeMe_9
18.02	7.57	26.04	35.07	15.58	50.83	47.53	36.64	4.04	22.52	75.53	-2.39	-0.37	SMI	MeMe	Rot	ch	MeMe_9
21.32	9.31	28.47	33.08	16.71	42.84	58.66	47.12	3.46	22.77	76.74	-2.15	0.62	SMI	MeMe	Ama	ch	MeMe_9
15.80	8.01	20.21	46.70	30.04	56.22	60.88	51.88	3.81	19.49	82.89	-1.97	1.20	SMI	MeMe	Omy	ch	MeMe_9
73.44	58.94	97.21	120.78	98.24	159.68	80.93	59.27	6.47	30.57	72.69	2.74	1.14	SMI	MeMe	Oxa	ch	MeMe_9
130.02	86.00	156.31	226.13	158.58	276.30	70.02	55.68	8.47	33.70	78.83	6.45	0.45	SMI	NuOb	Ctrl	N	NuOb_1
15.61	8.42	19.70	21.23	11.45	26.79	58.25	48.24	2.97	17.63	80.77	-2.63	0.96	SMI	NuOb	Rot	N	NuOb_1
15.30	11.91	17.52	20.81	16.19	23.83	77.82	67.95	1.55	24.22	87.32	-2.21	3.10	SMI	NuOb	Ama	N	NuOb_1
13.13	6.86	18.66	36.59	13.16	58.50	59.33	44.67	4.82	24.39	71.03	-2.19	0.06	SMI	NuOb	Omy	N	NuOb_1
36.57	23.79	63.86	110.67	91.71	151.06	47.11	32.16	5.51	29.74	60.16	0.40	-1.68	SMI	NuOb	Oxa	N	NuOb_1
78.09	75.58	81.62	292.04	282.64	305.26	96.78	92.59	5.93	33.66	95.67	7.05	4.69	SMI	NuOb	Ctrl	ch	NuOb_1
14.89	7.82	23.95	38.99	27.91	53.59	65.38	55.13	6.10	31.75	74.97	-1.62	0.84	SMI	NuOb	Rot	ch	NuOb_1
13.80	8.19	23.28	28.48	16.89	48.03	59.32	35.17	5.70	41.27	59.29	-2.04	-1.01	SMI	NuOb	Ama	ch	NuOb_1
23.89	9.70	41.93	37.89	18.37	62.92	59.46	49.78	10.69	45.33	71.28	-0.92	-0.42	SMI	NuOb	Omy	ch	NuOb_1
13.99	12.07	18.54	19.03	16.42	25.21	86.27	65.13	2.68	14.25	75.50	-2.13	2.40	SMI	NuOb	Oxa	ch	NuOb_1
97.19	69.23	126.61	172.88	124.16	225.29	68.99	52.19	8.12	28.49	72.56	4.37	0.04	SMI	NuOb	Ctrl	N	NuOb_2
20.54	5.91	32.11	33.22	9.56	51.92	28.77	18.40	5.59	51.75	63.98	-2.35	-2.49	SMI	NuOb	Rot	N	NuOb_2
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	SMI	NuOb	Ama	N	NuOb_2
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	SMI	NuOb	Omy	N	NuOb_2
74.16	60.08	88.41	164.64	138.72	194.23	81.58	66.97	7.15	27.72	80.47	3.65	1.87	SMI	NuOb	Oxa	N	NuOb_2
119.68	85.18	139.58	214.53	164.76	245.88	78.26	64.57	7.95	31.36	82.82	6.06	1.51	SMI	NuOb	Ctrl	ch	NuOb_2
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	SMI	NuOb	Rot	ch	NuOb_2
12.80	8.07	17.78	41.70	29.32	75.98	77.40	55.31	5.39	46.10	73.10	-1.32	1.29	SMI	NuOb	Ama	ch	NuOb_2
11.26	6.66	14.79	16.97	10.49	22.23	69.75	55.70	1.36	37.74	78.16	-2.56	1.89	SMI	NuOb	Omy	ch	NuOb_2
69.63	54.62	97.48	108.60	79.25	161.16	66.90	53.31	10.44	26.93	69.83	2.47	-0.33	SMI	NuOb	Oxa	ch	NuOb_2
74.79	54.98	108.22	157.68	123.62	222.59	74.49	54.72	7.08	39.38	69.52	3.64	0.42	SMI	NuOb	Ctrl	N	NuOb_3
19.06	11.73	29.10	32.95	21.36	49.50	63.40	44.75	4.39	30.66	68.08	-1.86	0.13	SMI	NuOb	Rot	N	NuOb_3
16.51	6.79	25.77	31.01	12.43	46.73	46.21	30.85	4.37	19.14	65.92	-2.57	-1.25	SMI	NuOb	Ama	N	NuOb_3
17.70	9.99	32.96	29.42	16.48	52.22	55.00	32.67	4.72	27.11	58.82	-2.14	-1.28	SMI	NuOb	Omy	N	NuOb_3
71.07	53.24	85.10	176.67	145.90	204.60	78.15	64.07	7.22	27.18	79.16	3.60	1.54	SMI	NuOb	Oxa	N	NuOb_3
86.96	69.31	108.82	172.91	145.36	213.96	83.72	65.77	6.37	36.35	77.58	4.43	1.81	SMI	NuOb	Ctrl	ch	NuOb_3
13.02	7.01	19.87	28.80	16.18	42.64	63.91	46.77	4.96	22.88	69.82	-2.22	0.24	SMI	NuOb	Rot	ch	NuOb_3
14.72	7.05	32.24	20.01	9.59	43.85	47.92	21.87	14.13	40.36	45.65	-1.62	-3.88	SMI	NuOb	Ama	ch	NuOb_3
12.70	6.79	16.90	25.42	16.72	32.32	64.06	50.27	3.38	16.71	77.49	-2.50	1.01	SMI	NuOb	Omy	ch	NuOb_3
97.34	73.76	108.71	188.89	149.67	208.93	75.39	66.84	6.88	25.59	87.71	4.60	2.02	SMI	NuOb	Oxa	ch	NuOb_3
107.35	89.64	123.55	215.01	187.67	244.10	87.44	75.52	5.98	34.80	86.17	6.10	2.90	SMI	NuOb	Ctrl	N	NuOb_4
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	SMI	NuOb	Rot	N	NuOb_4

17.39	7.62	32.73	33.57	14.72	63.19	43.83	23.29	7.20	9.65	53.13	-2.29	-2.84	SMI	NuOb	Ama	N	NuOb_4
10.20	5.86	13.47	21.45	16.13	29.60	74.19	53.41	2.93	17.18	74.44	-2.51	1.39	SMI	NuOb	Omy	N	NuOb_4
93.20	78.76	116.09	139.30	118.54	172.75	86.19	67.31	5.30	34.70	77.33	3.98	2.10	SMI	NuOb	Oxa	N	NuOb_4
123.36	87.13	143.47	232.29	175.02	273.41	75.34	62.35	8.23	25.80	82.33	6.46	1.18	SMI	NuOb	Ctrl	ch	NuOb_4
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	SMI	NuOb	Rot	ch	NuOb_4
7.46	6.56	10.96	10.14	8.93	14.90	88.06	60.46	0.72	36.60	68.50	-2.54	2.28	SMI	NuOb	Ama	ch	NuOb_4
3.88	3.37	6.30	5.28	4.58	8.56	86.77	53.47	0.44	30.61	61.63	-2.96	1.57	SMI	NuOb	Omy	ch	NuOb_4
16.77	7.55	28.58	37.11	18.15	62.07	55.72	37.14	3.97	26.33	67.10	-2.14	-0.52	SMI	NuOb	Oxa	ch	NuOb_4
103.92	82.68	130.47	196.19	162.56	244.84	82.44	66.25	6.66	33.07	79.33	5.55	1.76	SMI	NuOb	Ctrl	N	NuOb_5
41.35	27.95	66.59	57.76	38.71	92.45	52.75	35.95	7.60	14.09	73.10	-0.46	-1.02	SMI	NuOb	Rot	N	NuOb_5
9.85	8.29	17.27	34.58	29.97	61.82	86.97	58.39	5.44	21.30	66.62	-1.57	1.27	SMI	NuOb	Ama	N	NuOb_5
12.90	8.21	19.89	32.38	20.74	49.01	72.90	52.25	2.71	26.89	70.08	-2.10	1.12	SMI	NuOb	Omy	N	NuOb_5
94.22	61.10	112.51	193.22	143.44	222.97	68.68	59.34	7.75	26.73	81.46	4.37	0.94	SMI	NuOb	Oxa	N	NuOb_5
121.79	89.19	145.47	247.50	193.21	294.14	79.36	64.51	9.76	31.71	80.86	7.14	1.16	SMI	NuOb	Ctrl	ch	NuOb_5
15.55	6.42	20.33	41.75	22.06	51.61	53.33	44.26	4.37	15.30	79.56	-2.28	0.32	SMI	NuOb	Rot	ch	NuOb_5
7.86	7.55	9.62	10.69	10.27	13.09	96.12	78.49	0.52	27.83	81.66	-2.36	4.06	SMI	NuOb	Ama	ch	NuOb_5
4.22	2.48	9.45	8.77	6.02	18.79	58.62	31.00	1.49	40.80	49.94	-3.21	-1.09	SMI	NuOb	Omy	ch	NuOb_5
118.47	84.95	140.39	186.94	137.38	219.86	77.68	64.51	5.39	36.42	82.36	5.29	1.83	SMI	NuOb	Oxa	ch	NuOb_5
70.18	57.55	116.18	106.40	87.95	173.42	79.58	47.85	7.13	30.60	58.36	2.68	-0.39	SMI	PlMg	Ctrl	N	PlMg_1
16.66	7.87	27.73	24.24	11.91	39.70	48.01	31.46	3.19	24.55	65.00	-2.65	-1.02	SMI	PlMg	Rot	N	PlMg_1
2.58	1.61	6.19	3.51	2.19	8.42	62.45	26.08	0.47	40.36	41.76	-3.50	-1.48	SMI	PlMg	Ama	N	PlMg_1
19.43	6.17	26.70	26.42	8.39	36.31	38.76	27.56	3.07	18.73	73.41	-2.91	-1.07	SMI	PlMg	Omy	N	PlMg_1
47.28	23.05	80.49	81.35	41.72	131.62	54.79	38.07	12.26	15.05	65.82	0.54	-1.99	SMI	PlMg	Oxa	N	PlMg_1
75.11	64.62	119.07	116.76	101.58	180.85	85.15	54.64	7.05	33.78	62.86	3.28	0.39	SMI	PlMg	Ctrl	ch	PlMg_1
15.32	9.78	26.78	21.41	13.71	37.10	71.89	47.73	2.97	18.88	64.88	-2.29	0.50	SMI	PlMg	Rot	ch	PlMg_1
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	SMI	PlMg	Ama	ch	PlMg_1
13.78	10.65	15.77	22.43	17.39	25.33	73.15	61.98	1.54	22.39	83.34	-2.37	2.46	SMI	PlMg	Omy	ch	PlMg_1
64.89	47.78	108.02	101.34	76.52	160.69	69.69	40.92	7.46	24.75	57.12	1.94	-1.17	SMI	PlMg	Oxa	ch	PlMg_1
128.30	97.52	156.11	198.39	153.09	244.60	77.55	61.33	6.83	33.79	78.54	6.21	1.23	SMI	PlMg	Ctrl	N	PlMg_2
15.67	8.96	20.29	22.15	12.74	28.65	62.09	49.08	3.53	21.44	78.73	-2.44	0.96	SMI	PlMg	Rot	N	PlMg_2
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	SMI	PlMg	Ama	N	PlMg_2
22.05	10.21	33.79	29.99	13.88	45.95	42.35	26.99	4.33	11.22	65.79	-2.53	-1.62	SMI	PlMg	Omy	N	PlMg_2
81.59	47.36	118.04	125.87	77.33	193.57	63.82	38.04	10.35	17.30	62.91	2.69	-1.71	SMI	PlMg	Oxa	N	PlMg_2
93.99	78.21	133.46	147.05	124.27	210.58	83.57	60.67	6.68	34.04	72.32	4.48	1.13	SMI	PlMg	Ctrl	ch	PlMg_2
21.47	13.98	26.94	29.20	19.01	36.64	70.32	56.13	2.44	21.55	79.89	-2.00	1.74	SMI	PlMg	Rot	ch	PlMg_2
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	SMI	PlMg	Ama	ch	PlMg_2
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	SMI	PlMg	Omy	ch	PlMg_2
39.58	18.97	72.22	53.83	25.80	98.22	39.45	25.19	7.76	16.70	73.52	-0.92	-2.01	SMI	PlMg	Oxa	ch	PlMg_2
92.75	81.50	132.67	146.53	130.22	208.21	88.87	60.98	6.40	33.68	68.12	4.59	1.16	SMI	PlMg	Ctrl	N	PlMg_3
13.98	1.42	17.99	19.01	1.93	24.47	14.40	10.42	1.57	45.13	76.38	-3.65	-2.09	SMI	PlMg	Rot	N	PlMg_3
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	SMI	PlMg	Ama	N	PlMg_3
28.75	8.49	47.34	39.09	11.55	64.38	38.12	24.80	7.18	18.60	68.03	-1.95	-2.17	SMI	PlMg	Omy	N	PlMg_3
82.56	60.40	139.10	132.04	97.84	220.66	74.15	44.36	11.72	23.65	58.86	3.87	-1.50	SMI	PlMg	Oxa	N	PlMg_3
106.85	76.56	150.96	153.65	111.44	216.29	71.42	48.28	7.78	32.06	65.90	4.56	-0.43	SMI	PlMg	Ctrl	ch	PlMg_3
16.47	8.43	20.56	22.39	11.46	27.96	52.84	44.44	3.63	15.68	81.11	-2.66	0.52	SMI	PlMg	Rot	ch	PlMg_3

NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	SMI	PlMg	Ama	ch	PlMg_3
13.45	1.15	14.88	18.29	1.57	20.24	8.57	7.75	0.61	35.62	90.39	-4.00	-1.58	SMI	PlMg	Omy	ch	PlMg_3
33.19	14.44	47.45	48.63	22.18	68.31	47.50	35.23	6.37	18.32	74.27	-1.47	-0.92	SMI	PlMg	Oxa	ch	PlMg_3
106.67	73.85	118.48	221.26	169.91	242.13	76.71	68.65	8.88	21.57	88.93	5.58	1.86	SMI	PlMg	Ctrl	N	PlMg_4
17.54	10.14	22.38	31.34	20.94	39.29	67.44	55.06	3.30	20.88	80.32	-2.09	1.51	SMI	PlMg	Rot	N	PlMg_4
12.93	9.73	15.03	26.96	21.63	30.61	77.58	66.87	2.67	25.02	85.94	-2.08	2.83	SMI	PlMg	Ama	N	PlMg_4
17.41	8.34	21.74	26.31	12.52	32.36	54.86	43.76	2.85	24.31	79.42	-2.54	0.61	SMI	PlMg	Omy	N	PlMg_4
55.48	45.94	72.38	123.35	105.50	157.56	68.99	50.85	5.74	33.99	71.67	1.86	0.51	SMI	PlMg	Oxa	N	PlMg_4
128.21	87.63	164.30	185.57	130.34	235.27	71.20	52.83	6.21	39.73	74.45	5.62	0.45	SMI	PlMg	Ctrl	ch	PlMg_4
16.45	7.52	21.63	22.46	10.28	29.55	48.87	37.69	3.03	24.09	76.77	-2.75	-0.03	SMI	PlMg	Rot	ch	PlMg_4
11.41	7.13	18.02	27.05	19.59	40.45	66.88	49.96	6.01	35.65	69.03	-1.96	0.39	SMI	PlMg	Ama	ch	PlMg_4
15.41	7.16	20.15	23.62	11.78	30.45	52.02	39.99	3.12	20.22	76.52	-2.73	0.14	SMI	PlMg	Omy	ch	PlMg_4
53.12	45.04	62.21	83.65	71.75	96.92	77.32	63.74	4.06	29.22	80.32	0.84	2.10	SMI	PlMg	Oxa	ch	PlMg_4
143.53	94.96	161.06	257.39	183.30	280.60	72.24	64.82	8.24	21.12	89.95	7.25	1.52	SMI	PlMg	Ctrl	N	PlMg_5
27.68	20.81	45.23	48.07	35.08	74.76	67.15	43.08	4.74	26.25	64.78	-1.05	-0.13	SMI	PlMg	Rot	N	PlMg_5
8.27	6.92	10.23	11.25	9.41	13.91	82.34	66.58	0.72	25.38	80.95	-2.65	2.98	SMI	PlMg	Ama	N	PlMg_5
21.94	8.04	35.11	31.75	12.68	49.95	40.42	27.95	4.87	20.73	69.64	-2.41	-1.47	SMI	PlMg	Omy	N	PlMg_5
117.00	75.09	157.45	177.97	118.64	236.17	68.27	51.11	6.46	35.23	75.45	4.97	0.25	SMI	PlMg	Oxa	N	PlMg_5
67.60	44.53	129.50	102.40	67.06	195.70	68.74	36.41	9.34	32.14	52.45	2.43	-2.02	SMI	PlMg	Ctrl	ch	PlMg_5
14.35	5.87	18.37	19.95	8.37	25.47	47.32	37.40	2.57	25.02	78.34	-2.95	0.07	SMI	PlMg	Rot	ch	PlMg_5
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	SMI	PlMg	Ama	ch	PlMg_5
14.37	6.55	19.10	19.91	9.16	26.37	53.02	39.72	2.62	24.60	74.20	-2.82	0.13	SMI	PlMg	Omy	ch	PlMg_5
53.11	47.40	79.58	89.27	81.30	129.56	81.71	57.03	3.80	35.63	68.09	1.38	1.27	SMI	PlMg	Oxa	ch	PlMg_5
116.62	99.67	146.40	200.57	174.86	250.16	87.70	68.56	6.15	40.76	78.27	6.44	2.08	SMI	PlMg	Ctrl	N	PlMg_6
40.84	27.92	74.40	64.07	43.64	113.20	65.87	38.11	9.77	15.43	57.61	0.20	-1.66	SMI	PlMg	Rot	N	PlMg_6
17.24	13.14	20.46	25.45	19.32	30.11	76.25	66.34	2.87	12.82	86.46	-2.07	2.67	SMI	PlMg	Ama	N	PlMg_6
21.55	10.28	29.28	31.40	15.44	42.33	48.39	38.03	4.09	17.66	77.39	-2.36	-0.21	SMI	PlMg	Omy	N	PlMg_6
87.82	76.48	123.75	132.47	115.59	185.12	80.93	56.62	6.20	34.26	66.83	3.85	0.70	SMI	PlMg	Oxa	N	PlMg_6
91.30	71.20	136.08	147.71	116.60	220.49	79.04	50.89	8.63	30.66	63.92	4.33	-0.25	SMI	PlMg	Ctrl	ch	PlMg_6
29.98	16.58	54.01	45.49	25.25	79.43	51.76	31.99	8.34	15.51	63.39	-1.17	-1.82	SMI	PlMg	Rot	ch	PlMg_6
12.53	10.89	16.66	17.04	14.81	22.66	85.90	64.22	2.68	14.06	74.49	-2.25	2.29	SMI	PlMg	Ama	ch	PlMg_6
20.55	9.84	31.15	28.74	13.76	44.12	49.23	37.05	4.10	20.99	74.30	-2.35	-0.39	SMI	PlMg	Omy	ch	PlMg_6
47.95	34.81	86.51	72.97	51.49	126.97	69.23	39.51	10.01	17.66	57.53	0.83	-1.55	SMI	PlMg	Oxa	ch	PlMg_6
111.67	77.78	133.37	209.54	158.62	247.00	76.41	61.33	7.31	31.81	80.44	5.59	1.26	SMI	PlMg	Ctrl	N	PlMg_7
32.59	20.49	64.95	55.89	36.23	102.86	59.04	37.05	7.43	21.44	62.76	-0.55	-1.28	SMI	PlMg	Rot	N	PlMg_7
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	SMI	PlMg	Ama	N	PlMg_7
22.21	10.16	33.91	39.10	21.18	55.46	55.42	44.49	4.79	16.95	74.80	-1.92	0.05	SMI	PlMg	Omy	N	PlMg_7
98.55	68.47	123.99	153.46	110.68	194.60	73.80	54.47	5.96	32.44	74.89	3.85	0.77	SMI	PlMg	Oxa	N	PlMg_7
65.63	45.77	136.54	103.71	74.13	209.88	72.07	35.06	11.48	21.39	48.61	2.75	-2.57	SMI	PlMg	Ctrl	ch	PlMg_7
32.02	14.90	58.68	48.93	23.15	87.97	49.50	32.32	8.38	19.30	62.69	-1.06	-1.94	SMI	PlMg	Rot	ch	PlMg_7
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	SMI	PlMg	Ama	ch	PlMg_7
18.49	6.81	22.86	26.73	9.57	33.14	49.26	41.20	3.65	17.37	81.54	-2.65	0.26	SMI	PlMg	Omy	ch	PlMg_7
59.54	38.10	104.87	98.10	67.85	172.52	71.72	38.78	10.31	22.61	55.53	1.88	-1.73	SMI	PlMg	Oxa	ch	PlMg_7
125.68	93.82	144.31	247.57	198.04	282.69	80.54	68.45	7.56	33.89	85.23	7.12	1.96	SMI	PlMg	Ctrl	N	PlMg_8

33.66	23.13	58.28	55.73	39.00	93.92	61.34	38.38	6.92	25.66	62.49	-0.54	-1.02	SMI	PlMg	Rot	N	PlMg_8
13.96	9.86	18.61	18.98	13.41	25.30	69.06	52.67	1.79	24.63	77.70	-2.50	1.56	SMI	PlMg	Ama	N	PlMg_8
23.95	9.99	37.88	39.86	19.30	60.02	50.38	36.94	5.92	17.72	71.37	-1.88	-0.81	SMI	PlMg	Omy	N	PlMg_8
102.32	79.44	138.18	152.14	119.04	209.70	76.36	53.35	8.22	34.45	69.12	4.62	0.17	SMI	PlMg	Oxa	N	PlMg_8
110.07	85.43	141.43	199.15	161.73	251.66	80.79	61.20	7.37	36.43	75.25	5.86	1.13	SMI	PlMg	Ctrl	ch	PlMg_8
21.43	10.81	34.72	32.96	17.61	52.41	49.01	33.59	5.30	20.35	69.33	-2.11	-1.00	SMI	PlMg	Rot	ch	PlMg_8
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	SMI	PlMg	Ama	ch	PlMg_8
16.15	6.89	21.01	22.14	9.45	28.84	48.41	38.01	2.75	21.75	78.31	-2.83	0.08	SMI	PlMg	Omy	ch	PlMg_8
62.33	48.86	101.14	108.60	85.98	172.13	73.93	45.94	8.12	30.78	61.16	2.28	-0.61	SMI	PlMg	Oxa	ch	PlMg_8
110.58	75.80	127.76	259.72	202.36	296.00	78.25	66.63	9.30	27.29	85.01	6.69	1.53	SMI	PlMg	Ctrl	N	PlMg_9
30.01	18.41	51.13	49.97	30.57	82.99	57.09	38.56	6.60	17.79	68.28	-1.07	-0.83	SMI	PlMg	Rot	N	PlMg_9
17.18	9.33	22.66	25.67	13.98	34.21	58.70	45.95	3.36	21.11	77.90	-2.42	0.67	SMI	PlMg	Ama	N	PlMg_9
19.25	8.90	27.82	32.28	16.83	45.55	51.83	40.40	4.03	17.83	75.31	-2.33	-0.09	SMI	PlMg	Omy	N	PlMg_9
87.74	70.23	106.83	171.99	144.26	204.92	80.88	63.51	5.98	34.28	76.90	4.26	1.64	SMI	PlMg	Oxa	N	PlMg_9
100.19	70.28	120.77	221.68	172.76	261.78	77.58	63.75	8.09	29.98	81.66	5.57	1.39	SMI	PlMg	Ctrl	ch	PlMg_9
22.27	12.44	38.41	38.65	23.52	63.74	57.63	39.45	5.95	17.71	66.66	-1.69	-0.71	SMI	PlMg	Rot	ch	PlMg_9
9.28	7.53	11.10	12.62	10.24	15.10	80.98	67.74	0.69	26.30	83.06	-2.60	3.11	SMI	PlMg	Ama	ch	PlMg_9
15.59	7.25	20.28	21.94	10.47	28.46	52.44	42.14	2.61	20.61	78.83	-2.77	0.44	SMI	PlMg	Omy	ch	PlMg_9
83.67	63.29	108.46	155.79	124.76	196.99	76.13	53.90	6.37	35.30	69.24	3.67	0.55	SMI	PlMg	Oxa	ch	PlMg_9
105.69	71.69	124.78	237.27	180.67	277.37	76.32	63.39	8.98	27.76	82.90	6.00	1.24	SMI	PlMg	Ctrl	N	PlMg_10
40.16	28.12	68.62	70.62	50.25	114.53	62.94	37.62	8.67	17.81	59.00	0.13	-1.51	SMI	PlMg	Rot	N	PlMg_10
9.32	8.09	11.85	12.68	11.01	16.11	86.25	65.85	0.89	25.62	76.21	-2.51	2.80	SMI	PlMg	Ama	N	PlMg_10
23.90	10.68	33.43	49.42	26.84	67.81	52.95	41.03	5.04	20.02	75.81	-1.72	-0.16	SMI	PlMg	Omy	N	PlMg_10
71.51	57.83	100.62	123.70	102.99	169.72	78.63	53.54	5.70	33.22	65.49	2.72	0.52	SMI	PlMg	Oxa	N	PlMg_10
95.64	73.67	125.96	176.86	142.70	229.45	81.56	59.32	7.02	35.41	72.84	4.83	1.01	SMI	PlMg	Ctrl	ch	PlMg_10
28.10	17.36	48.06	45.43	28.74	76.09	59.15	38.10	7.11	16.99	63.92	-1.17	-1.07	SMI	PlMg	Rot	ch	PlMg_10
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	SMI	PlMg	Ama	ch	PlMg_10
17.74	7.43	25.63	33.45	16.30	50.19	47.81	36.12	3.86	21.71	74.77	-2.43	-0.40	SMI	PlMg	Omy	ch	PlMg_10
58.51	46.91	101.83	90.42	72.93	154.14	76.61	44.20	8.93	24.07	56.44	1.88	-1.01	SMI	PlMg	Oxa	ch	PlMg_10
124.74	80.57	144.74	242.53	174.01	280.26	73.11	61.33	8.56	26.93	84.22	6.48	1.09	SMI	PlMg	Ctrl	N	PlMg_11
53.31	34.58	70.51	105.07	76.93	135.02	64.05	45.33	4.91	34.41	69.93	0.93	0.08	SMI	PlMg	Rot	N	PlMg_11
8.54	5.81	10.25	14.51	10.60	17.08	74.95	62.85	1.05	18.65	83.63	-2.78	2.63	SMI	PlMg	Ama	N	PlMg_11
20.71	10.15	32.13	28.84	14.34	44.89	54.60	39.69	4.24	21.27	72.59	-2.21	-0.20	SMI	PlMg	Omy	N	PlMg_11
101.86	70.47	126.72	154.39	108.23	192.43	73.00	55.92	5.81	35.97	76.09	3.96	0.91	SMI	PlMg	Oxa	N	PlMg_11
112.66	75.24	148.67	184.08	130.03	239.86	73.49	52.16	7.91	32.88	71.72	5.17	0.10	SMI	PlMg	Ctrl	ch	PlMg_11
48.84	36.62	71.00	83.47	66.04	116.59	63.19	43.11	4.30	33.01	67.15	0.47	-0.10	SMI	PlMg	Rot	ch	PlMg_11
NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.00	0.00	SMI	PlMg	Ama	ch	PlMg_11
18.44	7.79	26.96	25.48	10.98	37.09	48.57	35.33	3.13	23.19	73.84	-2.62	-0.35	SMI	PlMg	Omy	ch	PlMg_11
83.46	65.37	114.99	134.98	108.34	183.11	81.02	57.02	6.10	35.20	70.04	3.46	0.90	SMI	PlMg	Oxa	ch	PlMg_11

Table 4.s3. Sperm motility parameters comparison between five bivalve species, both DUI and SMI, with and without chemoattractants. Values are presented as means ± s.e.m. The effect of the factor ‘species’ (*F* and *p*) was assessed separately for each parameter and presence/absence of egg chemical cues by means of a one way ANOVA, followed by a Tukey's multi comparison test (result indicates by letters in superscript). Significant differences ($p \leq 0.05$) are shown in bold. Inheritance: For abbreviations refer to table 4.s2.

Tables s3	Inheritance	Species	DAP	DSL	DCL	VAP	VSL	VCL	STR	LIN	ALH	BFC	WOB	PC1	PC2
without chemoattractants	DUI	MyEd	57.96 ± 2.75^{a}	36.88 ± 1.71^{a}	96.86 ± 4.05^{ab}	114.06 ± 4.66^{a}	75.41 ± 3.16^{a}	184.87 ± 8.19^{ab}	67.57 ± 1.74^{a}	41.82 ± 1.57^{a}	10.44 ± 0.49^{a}	25.59 ± 0.99^{a}	61.6 ± 1.59^{a}	2.02 ± 0.14^{a}	-1.38 ± 0.26^{a}
	DUI	RuPh	47.83 ± 3.29^{a}	30.06 ± 1.87^{a}	79.35 ± 4.24^{a}	93.42 ± 6.28^{a}	63.16 ± 4.04^{a}	150.95 ± 8.34^{a}	70.51 ± 1.43^{a}	43.56 ± 1.31^{a}	7.34 ± 0.4^{b}	27.6 ± 1.22^{ab}	62.31 ± 2.05^{a}	0.97 ± 0.23^{a}	-0.66 ± 0.19^{a}
	SMI	MeMe	92.15 ± 6.57^{b}	58.32 ± 3.47^{b}	115.31 ± 6.13^{bc}	156.69 ± 11.27^{b}	106.29 ± 6.65^{b}	194.51 ± 11.01^{b}	71.08 ± 2.41^{a}	54.88 ± 2.1^{b}	7.97 ± 0.49^{b}	27.17 ± 1.07^{a}	77.66 ± 1.76^{b}	3.59 ± 0.36^{b}	0.54 ± 0.27^{b}
	SMI	NuOb	102.65 ± 8.88^{b}	76.51 ± 6.39^{c}	129.03 ± 7.79^{c}	193.58 ± 12.73^{bc}	151.32 ± 12.26^{c}	242.62 ± 9.6^{c}	76.68 ± 3.59^{ab}	60.87 ± 4.38^{bc}	7.26 ± 0.46^{b}	33.89 ± 1.74^{b}	77.28 ± 2.9^{b}	5.22 ± 0.53^{c}	1.11 ± 0.53^{b}
	SMI	PlMg	112.4 ± 5.89^{b}	82.25 ± 3.92^{c}	136.9 ± 4.46^{c}	211.56 ± 14.47^{c}	164.82 ± 9.82^{c}	252.95 ± 11.04^{c}	78.84 ± 1.59^{b}	63.03 ± 1.79^{c}	7.76 ± 0.33^{b}	29.93 ± 1.74^{ab}	80 ± 2.82^{b}	5.87 ± 0.39^{c}	1.32 ± 0.2^{b}
		F	(4,40) 29.69	(4,40) 50.13	(4,40) 22.11	(4,40) 23.27	(4,40) 39.13	(4,40) 17.58	(4,40) 5.93	(4,40) 24.79	$(4,40)\ 9.96^{b}$	(4,40) 4.07	$(4,40)\ 16.98^{b}$	(4,40) 41.92	(4,40) 20.93
		P	**1.69E-11**	**4.62E-15**	**1.08E-09**	**5.42E-10**	**2.51E-13**	**2.11E-08**	**7.50E-04**	**2.26E-10**	**1.44E-05**	**7.20E-03**	**3.23E-08**	**8.45E-14**	**2.25E-09**
with chemoattractants	DUI	MyEd	63.07 ± 4.57^{a}	39.98 ± 2.02^{a}	111.17 ± 4.18^{b}	110.45 ± 7.16^{a}	72.27 ± 3.39^{a}	193.4 ± 8.51^{ab}	67.15 ± 2.99^{ab}	37.61 ± 1.72^{a}	11.14 ± 0.53^{a}	25.85 ± 1.58^{a}	56.57 ± 2.07^{a}	2.3 ± 0.18^{ab}	-2.01 ± 0.26^{a}
	DUI	RuPh	53.13 ± 2.61^{a}	31.03 ± 1.86^{a}	88.97 ± 3.57^{a}	105.4 ± 4.82^{a}	66.23 ± 3.8^{a}	170.04 ± 5.6^{a}	62.85 ± 1.78^{a}	39.17 ± 1.69^{a}	8.58 ± 0.32^{ab}	27.04 ± 2.07^{ab}	62.13 ± 1.81^{ab}	1.33 ± 0.18^{a}	-1.34 ± 0.21^{ab}
	SMI	MeMe	83.12 ± 5.97^{ab}	54.59 ± 3.42^{b}	108.18 ± 6.07^{ab}	142.24 ± 10.55^{ab}	97.4 ± 6.54^{ab}	183.29 ± 10.63^{a}	72.13 ± 2.87^{bc}	54.44 ± 2.48^{b}	9.55 ± 1.34^{ab}	24.61 ± 2.26^{a}	75.35 ± 1.66^{cd}	3.23 ± 0.32^{b}	0.23 ± 0.44^{c}
	SMI	NuOb	105.98 ± 9.69^{b}	81.28 ± 3.79^{c}	123.79 ± 12.47^{bc}	231.85 ± 19.55^{c}	192.2 ± 23.9^{c}	266.53 ± 16.58^{c}	82.69 ± 3.77^{c}	69.96 ± 5.68^{c}	7.65 ± 0.69^{b}	31.78 ± 1.74^{ab}	83.85 ± 3.09^{d}	6.23 ± 0.49^{d}	2.07 ± 0.66^{d}
	SMI	PlMg	95.2 ± 5.9^{b}	70.29 ± 4.23^{c}	136.98 ± 4.13^{c}	158.06 ± 11.88^{b}	121.15 ± 9.81^{b}	222.89 ± 7.2^{b}	76.78 ± 1.7^{c}	52.29 ± 2.87^{b}	7.96 ± 0.44^{b}	32.59 ± 1.39^{b}	67.45 ± 3.01^{bc}	4.44 ± 0.35^{c}	0.03 ± 0.39^{bc}
		F	(4,40) 13.93	(4,40) 35.65	(4,40) 11.75	(4,40) 17.18	(4,40) 24.14	(4,40) 13.11	(4,40) 7.53	(4,40) 19.27	(4,40) 3.77	(4,40) 3.79	(4,40) 16.49	(4,40) 32.18	(4,40) 14.44
		P	**3.32E-07**	**1.07E-12**	**2.09E-06**	**2.80E-08**	**3.26E-10**	**6.48E-07**	**1.20E-04**	**6.64E-09**	**1.00E-02**	**1.00E-02**	**4.62E-08**	**5.10E-12**	**2.20E-07**

Table 4.s4. Sperm motility parameters comparison between five bivalve species, both DUI and SMI, with and without chemoattractants. Values are presented as means ± s.e.m. The main effect of the two fixed factors ‘species’ and ‘chemoattractants’, was assessed for each parameter separately through a liner mixed effect model which took into account the by-subject variability. Differences among species are indicated by letters in superscript and were determined through a *post hoc* pairwise comparison, with *p*-values adjusted using Holm's correction for multiple testing. Significant differences ($p \leq 0.05$) are shown in bold. Inheritance: DUI, doubly uniparental inheritance; SMI, strict maternal inheritance. Species: MyEd, *M. edulis* (n = 11); RuPh, *R. philippinarum* (n = 9); MeMe, *M. mercenaria* (n = 9); NuOb, *N. obscurata* (n = 5); PlMg, *P. magellanicus* (n = 11). ‘Control-N’, basal sperm motility without chemoattractants; ‘Control-ch’, basal sperm motility with chemoattractants. ‘:species’, main effect of factor ‘species'; ‘:chem’, main effect of factor 'chemoattractants'. For abbreviations refer to table 4.s2.

Table s4															
Species	**Inheritance**	**Treatment**	**DAP**	**DSL**	**DCL**	**VAP**	**VSL**	**VCL**	**STR**	**LIN**	**ALH**	**BFC**	**WOB**	**PC1**	**PC2**
MyEd	DUI	Control-N	57.96 ± 2.75^{a}	36.88 ± 1.71^{a}	96.86 ± 4.05^{b}	114.06 ± 4.66^{a}	75.41 ± 3.16^{a}	184.87 ± 8.19^{b}	67.57 ± 1.74^{a}	41.82 ± 1.57^{a}	10.44 ± 0.49^{b}	25.59 ± 0.99^{a}	61.6 ± 1.59^{a}	2.02 ± 0.14^{b}	-1.38 ± 0.26^{a}
		Control-ch	63.07 ± 4.57^{a}	39.98 ± 2.02^{a}	111.17 ± 4.18^{b}	110.45 ± 7.16^{a}	72.27 ± 3.39^{a}	193.4 ± 8.51^{b}	67.15 ± 2.99^{a}	37.61 ± 1.72^{a}	11.14 ± 0.53^{b}	25.85 ± 1.58^{a}	56.57 ± 2.07^{a}	2.3 ± 0.18^{b}	-2.01 ± 0.26^{a}
RuPh	DUI	Control-N	47.83 ± 3.29^{a}	30.06 ± 1.87^{a}	79.35 ± 4.24^{a}	93.42 ± 6.28^{a}	63.16 ± 4.04^{a}	150.95 ± 8.34^{a}	70.51 ± 1.43^{a}	43.56 ± 1.31^{a}	7.34 ± 0.4^{a}	27.6 ± 1.22^{ab}	62.31 ± 2.05^{a}	0.97 ± 0.23^{a}	-0.66 ± 0.19^{a}
		Control-ch	53.13 ± 2.61^{a}	31.03 ± 1.86^{a}	88.97 ± 3.57^{a}	105.4 ± 4.82^{a}	66.23 ± 3.8^{a}	170.04 ± 5.6^{a}	62.85 ± 1.78^{a}	39.17 ± 1.69^{a}	8.58 ± 0.32^{a}	27.04 ± 2.07^{ab}	62.13 ± 1.81^{a}	1.33 ± 0.18^{a}	-1.34 ± 0.21^{a}
MeMe	SMI	Control-N	92.15 ± 6.57^{b}	58.32 ± 3.47^{b}	115.31 ± 6.13^{bc}	156.69 ± 11.27^{b}	106.29 ± 6.65^{b}	194.51 ± 11.01^{b}	71.08 ± 2.41^{a}	54.88 ± 2.1^{b}	7.97 ± 0.49^{a}	27.17 ± 1.07^{a}	77.66 ± 1.76^{b}	3.59 ± 0.36^{c}	0.54 ± 0.27^{b}
		Control-ch	83.12 ± 5.97^{b}	54.59 ± 3.42^{b}	108.18 ± 6.07^{bc}	142.24 ± 10.55^{b}	97.4 ± 6.54^{b}	183.29 ± 10.63^{b}	72.13 ± 2.87^{a}	54.44 ± 2.48^{b}	9.55 ± 1.34^{a}	24.61 ± 2.26^{a}	75.35 ± 1.66^{b}	3.23 ± 0.32^{c}	0.23 ± 0.44^{b}
NuOb	SMI	Control-N	102.65 ± 8.88^{c}	76.51 ± 6.39^{c}	129.03 ± 7.79^{cd}	193.58 ± 12.73^{c}	151.32 ± 12.26^{d}	242.62 ± 9.6^{c}	76.68 ± 3.59^{b}	60.87 ± 4.38^{c}	7.26 ± 0.46^{a}	33.89 ± 1.74^{c}	77.28 ± 2.9^{b}	5.22 ± 0.53^{d}	1.11 ± 0.53^{c}
		Control-ch	105.98 ± 9.69^{c}	81.28 ± 3.79^{c}	123.79 ± 12.47^{cd}	231.85 ± 19.55^{c}	192.2 ± 23.9^{d}	266.53 ± 16.58^{c}	82.69 ± 3.77^{b}	69.96 ± 5.68^{c}	7.65 ± 0.69^{a}	31.78 ± 1.74^{c}	83.85 ± 3.09^{b}	6.23 ± 0.49^{d}	2.07 ± 0.66^{c}
PlMg	SMI	Control-N	112.4 ± 5.89^{c}	82.25 ± 3.92^{c}	136.9 ± 4.46^{d}	211.56 ± 14.47^{c}	164.82 ± 9.82^{c}	252.95 ± 11.04^{c}	78.84 ± 1.59^{b}	63.03 ± 1.79^{b}	7.76 ± 0.33^{a}	29.93 ± 1.74^{bc}	80 ± 2.82^{b}	5.87 ± 0.39^{d}	1.32 ± 0.2^{bc}
		Control-ch	95.2 ± 5.9^{c}	70.29 ± 4.23^{c}	136.98 ± 4.13^{d}	158.06 ± 11.88^{c}	121.15 ± 9.81^{c}	222.89 ± 7.2^{c}	76.78 ± 1.7^{b}	52.29 ± 2.87^{b}	7.96 ± 0.44^{a}	32.59 ± 1.39^{bc}	67.45 ± 3.01^{b}	4.44 ± 0.35^{d}	0.03 ± 0.39^{bc}
		:species	**$F_{4,84}$=39.26, P<2.16e-16*****	**$F_{4,40}$=63.23, P<2.16e-16*****	**$F_{4,84}$=30.72, P=9.37e-16*****	**$F_{4,40}$=26.37, P=9.42e-11*****	**$F_{4,40}$=38.64, P=3e-13*****	**$F_{4,40}$=21.75, P=1.35e-09*****	**$F_{4,40}$=10.9, P=4.52e-06*****	**$F_{4,84}$=34.4, P<2e-16*****	**$F_{4,40}$=9.58, P=1.56e-05*****	**$F_{4,40}$=6, P=6.5e-04*****	**$F_{4,84}$=26, P=4.88e-14*****	**$F_{4,40}$=53.22, P=1.71e-15*****	**$F_{4,40}$=28.8, P=2.65e-11*****
		:chem	$F_{1,84}$=0.9, P=0.34	$F_{1,44}$=1.36, P=0.24	$F_{1,84}$=1, P=0.3	$F_{1,44}$=2.34, P=0.13	$F_{1,44}$=2.36, P=0.13	$F_{1,44}$=0.033, P=0.85	$F_{1,44}$=0.76, P=0.38	**$F_{1,84}$=5.23, P=0.024***	**$F_{1,44}$=5.14, P=0.028***	$F_{1,44}$=0.02, P=0.88	**$F_{1,84}$=6.62, P=0.012***	$F_{1,44}$=0.77, P=0.38	**$F_{1,44}$=7.13, P=0.0106***

Table 4.s5. Effect of metabolic inhibitors on sperm motility parameters in five bivalve species, both DUI and SMI, without chemoattractants. Values are presented as means ± s.e.m. The effect of the factor 'treatment' (*F* and *p*) was assessed for each species and each parameter separately through a liner mixed effect model, which took into account the by-subject variability. Difference among treatments (indicated by letters in superscript) were determined through a *post hoc* pairwise comparison, with *p*-values adjusted using Holm's correction for multiple testing. Significant differences ($p \leq 0.05$) are shown in bold. For abbreviations refer to table 4.s2.

Table s5

Species	**Inheritance**	**Treatment**	**DAP**	**DSL**	**DCL**	**VAP**	**VSL**	**VCL**	**STR**	**LIN**	**ALH**	**BFC**	**WOB**	**PC1**	**PC2**
MyEd	DUI	Control	57.96 ± 2.75^{a}	36.88 ± 1.71^{a}	96.86 ± 4.05^{a}	114.06 ± 4.66^{a}	75.41 ± 3.16^{a}	184.87 ± 8.19^{a}	67.57 ± 1.74^{a}	41.82 ± 1.57	10.44 ± 0.49^{a}	25.59 ± 0.99	61.6 ± 1.59	2.02 ± 0.14^{a}	−1.38 ± 0.26^{a}
		Rotenone	16.35 ± 2.64^{b}	8.15 ± 1.32^{b}	30.62 ± 5.46^{b}	29.05 ± 3.96^{b}	15.44 ± 2.15^{b}	56.07 ± 8.85^{b}	48.5 ± 5.77^{a}	31.34 ± 4.56	4.51 ± 0.74^{b}	25.92 ± 3.14	55.07 ± 5.95	−1.86 ± 0.24^{b}	−1.09 ± 0.47^{ab}
		Antimycin A	9.67 ± 1.9^{bc}	5.58 ± 0.94^{b}	15.5 ± 3.51^{c}	17.54 ± 3.04^{bc}	11.1 ± 2.43^{b}	27.52 ± 5.39^{c}	54.84 ± 10.22^{a}	42.6 ± 8.93	3.11 ± 0.8^{bc}	25.56 ± 4	58.75 ± 9.46	−1.88 ± 0.31^{b}	0.74 ± 0.7^{c}
		Oligomycin	4.67 ± 1.55^{c}	3.38 ± 1.34^{b}	7.91 ± 2.63^{c}	8.21 ± 2.8^{c}	5.84 ± 2.17^{b}	14.94 ± 5.57^{c}	39.79 ± 12.28^{a}	29.43 ± 9.81	1.46 ± 0.72^{c}	19.1 ± 5.88	36.05 ± 10.97	−1.35 ± 0.4^{b}	0.59 ± 0.62^{bc}
		Oxamate	57.16 ± 3.82^{a}	36.54 ± 1.78^{a}	92.86 ± 4.85^{a}	114.33 ± 7.76^{a}	75.66 ± 4.86^{a}	182.1 ± 8.85^{a}	68.71 ± 1.25^{a}	42.89 ± 1.39	10.4 ± 0.36^{a}	26.6 ± 1.55	62.38 ± 1.78	1.99 ± 0.23^{a}	−1.22 ± 0.2^{a}
		F	(4,40) 99.35	(4,50) 138.29	(4,40) 108.23	(4,40) 132.67	(4,40) 144.32	(4,40) 138.46	(4,40) 2.85	(4,40) 1.14	(4,40) 47.36	(4,40) 0.87	(4,40) 2.41	(4,40) 67.46	(4,40) 4.87
		P	**< 2.2E-16**	**< 2.2E-16**		**< 2.2E-16**	**< 2.2E-16**	**< 2.2E-16**	**0.035**	0.349	**1.17E-14**	0.48	0.06	**< 2.2E-16**	**2.60E-03**
RuPh	DUI	Control	47.83 ± 3.29^{a}	30.06 ± 1.87^{a}	79.35 ± 4.24^{a}	93.42 ± 6.28^{a}	63.16 ± 4.04^{a}	150.95 ± 8.34^{a}	70.51 ± 1.43^{a}	43.56 ± 1.31^{ab}	7.34 ± 0.4^{a}	27.6 ± 1.22	62.31 ± 2.05	0.97 ± 0.23^{a}	−0.66 ± 0.19^{b}
		Rotenone	28.43 ± 1.62^{b}	14.06 ± 0.89^{b}	52.79 ± 3.22^{c}	55.99 ± 2.44^{b}	29.76 ± 1.33^{b}	98.41 ± 4.87^{b}	52.36 ± 1.13^{ab}	31.88 ± 1.02^{abc}	7.1 ± 0.39^{a}	22.15 ± 1.34	59.84 ± 1.25	−1.07 ± 0.13^{b}	−1.81 ± 0.16^{a}
		Antimycin A	10.22 ± 3.11^{c}	4.56 ± 1.35^{c}	14.82 ± 4.51^{d}	24.15 ± 7.01^{c}	13.88 ± 4.33^{b}	33.63 ± 9.84^{c}	35.47 ± 9.62^{b}	25.73 ± 7.2^{c}	2.96 ± 0.88^{b}	17.73 ± 5.75	48.03 ± 12.48	−1.5 ± 0.4^{b}	−0.19 ± 0.38^{b}
		Oligomycin	11 ± 2.31^{c}	5.45 ± 1.27^{c}	16.12 ± 3.43^{d}	25.14 ± 5.08^{c}	13.95 ± 3.38^{b}	36.63 ± 7.72^{c}	42.51 ± 10.2^{b}	31.05 ± 7.45^{bc}	3.2 ± 0.7^{b}	22.58 ± 5.65	56.23 ± 10.88	−1.81 ± 0.38^{b}	−0.08 ± 0.42^{b}
		Oxamate	42.58 ± 3.2^{a}	27.53 ± 1.16^{a}	68.43 ± 3.79^{b}	96.35 ± 6.99^{a}	67.48 ± 4.5^{a}	149.83 ± 5^{a}	72.83 ± 2.71^{a}	47.02 ± 1.7^{a}	6.62 ± 0.39^{a}	27.91 ± 2.32	65.14 ± 3.72	0.8 ± 0.17^{a}	−0.13 ± 0.2^{b}
		F	(4,32) 61.60	(4,32) 125.12	(4,32) 88.08	(4,32) 51.64	(4,32) 74.39	(4, 32) 81.42	(4,32) 8.49	(4,32) 4.78	(4,32) 15.53	(4,32) 1.34	(4,32) 0.88	(4,32) 23.14	(4,32) 6.44
		P	**1.40E-14**	**< 2.2E-16**	**< 2.2E-16**	**1.63E-13**	**9.62E-16**	**2.62E-16**	**8.64E-05**	**3.80E-03**	**3.66E-07**	0.27	0.48	**4.63E-09**	**6.40E-04**
MeMe	SMI	Control	92.15 ± 6.57^{a}	58.32 ± 3.47^{a}	115.31 ± 6.13^{a}	156.69 ± 11.27^{a}	106.29 ± 6.65^{a}	194.51 ± 11.01^{a}	71.08 ± 2.41^{ab}	54.88 ± 2.1^{ab}	7.97 ± 0.49^{a}	27.17 ± 1.07^{a}	77.66 ± 1.76	3.59 ± 0.36^{a}	0.54 ± 0.27
		Rotenone	16.37 ± 2.89^{c}	8.86 ± 1.45^{c}	22.68 ± 4.34^{c}	26.4 ± 4.97^{c}	15.14 ± 2.86^{c}	36.74 ± 7.38^{c}	54.66 ± 8.04^{bc}	42.4 ± 6.43^{ab}	2.95 ± 0.69^{b}	17.83 ± 2.89^{b}	67.2 ± 8.65	−1.98 ± 0.3^{c}	0.62 ± 0.45
		Antimycin A	22 ± 1.57^{c}	9.7 ± 0.39^{c}	31.07 ± 2.77^{c}	36.56 ± 2.49^{c}	17.94 ± 1.08^{c}	50.8 ± 4.1^{c}	52.51 ± 3.7^{c}	40.58 ± 3.32^{b}	4.61 ± 0.49^{b}	18.5 ± 0.88^{b}	75.07 ± 1.99	−2.11 ± 0.07^{c}	−0.16 ± 0.42
		Oligomycin	20.22 ± 1.15^{c}	10.17 ± 0.6^{c}	29.48 ± 2.22^{c}	32.46 ± 1.91^{c}	17.42 ± 1.18^{c}	46.16 ± 3.2^{c}	55.85 ± 4.02^{bc}	42.77 ± 4.05^{ab}	4.02 ± 0.33^{b}	20.43 ± 0.88^{b}	75 ± 1.66	−2.17 ± 0.07^{c}	0.14 ± 0.43
		Oxamate	63.84 ± 6.95^{b}	41.62 ± 3.43^{b}	83.97 ± 8.64^{b}	119.88 ± 7.46^{b}	85.56 ± 3.56^{b}	155.2 ± 8.18^{b}	77.77 ± 2.74^{a}	56.29 ± 1.97^{a}	7.84 ± 0.56^{a}	22.97 ± 1.29^{ab}	73.86 ± 0.86	2.03 ± 0.33^{b}	0.72 ± 0.28
		F	(4,32) 67.76	(4,32) 105.66	(4,32) 71.78	(4,32) 108.33	(4,32) 179.89	(4,32) 132.12	(4,40) 5.93	(4,32) 3.87	(4,32) 19.63	(4,40) 5.69	(4,32) 0.94	(4,40) 112.92	(4,32) 1
		P	**3.65E-15**	**< 2.2E-16**	**1.60E-15**	**< 2.2E-16**	**< 2.2E-16**	**< 2.2E-16**	**7.50E-04**	**0.011**	**3.00E-08**	**9.99E-04**	0.45	**< 2.2E-16**	0.42
NuOb	SMI	Control	102.65 ± 8.88^{a}	76.51 ± 6.39^{a}	129.03 ± 7.79^{a}	193.58 ± 12.73^{a}	151.32 ± 12.26^{a}	242.62 ± 9.6^{a}	76.68 ± 3.59	60.87 ± 4.38	7.26 ± 0.46^{a}	33.89 ± 1.74	77.28 ± 2.9	5.22 ± 0.53^{a}	1.11 ± 0.53
		Rotenone	19.31 ± 6.61^{c}	10.8 ± 4.7^{c}	29.5 ± 10.84^{c}	29.03 ± 9.39^{c}	16.22 ± 6.57^{b}	44.13 ± 15.28^{c}	40.63 ± 11.76	29.47 ± 9	4.11 ± 1.28^{ab}	22.83 ± 8.72	57.19 ± 14.57	−1.46 ± 0.52^{c}	−0.48 ± 0.59
		Antimycin A	11.81 ± 3.23^{c}	6.92 ± 1.94^{c}	18.66 ± 5.48^{c}	23.99 ± 6.48^{c}	14.66 ± 4.78^{b}	39.11 ± 12.08^{c}	50.97 ± 15.31	36.1 ± 12.26	3.71 ± 1.3^{ab}	14.86 ± 4.45	54.6 ± 14.71	−1.73 ± 0.46^{c}	0.06 ± 1.02
		Oligomycin	10.79 ± 2.95^{c}	6.18 ± 1.69^{c}	17 ± 5.33^{c}	23.97 ± 6.48^{c}	13.3 ± 3.54^{b}	37.87 ± 10.63^{c}	52.28 ± 13.59	36.6 ± 9.87	3.04 ± 0.88^{b}	19.11 ± 5.11	54.87 ± 13.97	−1.79 ± 0.45^{c}	0.26 ± 0.47
		Oxamate	73.84 ± 10.46^{b}	55.39 ± 8.96^{b}	93.19 ± 9.61^{b}	156.9 ± 14.52^{b}	127.66 ± 10.2^{a}	189.12 ± 12.51^{b}	72.34 ± 6.93	57.97 ± 6.61	6.59 ± 0.49^{ab}	29.21 ± 1.46	75.72 ± 3.95	3.2 ± 0.71^{b}	0.95 ± 0.69
		F	(4,20) 35.23	(4,20) 35.63	(4,20) 39.24	(4,16) 63.59	(4,16) 78.21	(4,16) 75.57	(4,16) 2.18	(4,16) 2.8	(4,20) 3.81	(4,20) 2.29	(4,16) 1.13	(4,16) 38.34	(4,20) 0.91
		P	**8.56E-09**	**7.76E-09**	**3.35E-09**	**1.28E-09**	**2.70E-10**	**3.50E-10**	0.11	0.06	**0.018**	0.09	0.37	**5.22E-08**	0.47
PlMg	SMI	Control	112.4 ± 5.89^{a}	82.25 ± 3.92^{a}	136.9 ± 4.46^{a}	211.56 ± 14.47^{a}	164.82 ± 9.82^{a}	252.95 ± 11.04^{a}	78.84 ± 1.59^{a}	63.03 ± 1.79^{a}	7.76 ± 0.33^{a}	29.93 ± 1.74^{a}	80 ± 2.82^{a}	5.87 ± 0.39^{a}	1.32 ± 0.2^{a}
		Rotenone	29.28 ± 3.79^{c}	18.35 ± 3.07^{c}	47.41 ± 6.59^{c}	49.65 ± 7.67^{c}	32.66 ± 6.3^{c}	77.22 ± 11.67^{c}	57.22 ± 4.59^{bc}	38.56 ± 3.43^{bc}	5.51 ± 0.77^{b}	24.62 ± 2.58^{ab}	67.75 ± 2.35^{ab}	−1.16 ± 0.42^{c}	−0.64 ± 0.34^{b}
		Antimycin A	8.18 ± 2.03^{d}	5.86 ± 1.42^{d}	10.48 ± 2.49^{e}	12.64 ± 3.23^{d}	9.23 ± 2.35^{d}	15.98 ± 3.89^{e}	53.42 ± 10.62^{bc}	41.2 ± 8.78^{bc}	1.26 ± 0.37^{c}	17.6 ± 3.95^{b}	55.5 ± 11.35^{b}	−1.86 ± 0.38^{cd}	1.33 ± 0.47^{a}
		Oligomycin	21.92 ± 0.9^{c}	9.22 ± 0.41^{d}	32.65 ± 2^{d}	34.04 ± 2.13^{cd}	15.72 ± 1.55^{d}	49.55 ± 3.4^{d}	48.01 ± 2.05^{c}	35.6 ± 2.21^{c}	4.58 ± 0.37^{b}	18.64 ± 0.98^{b}	73.05 ± 1.24^{ab}	−2.25 ± 0.11^{d}	−0.65 ± 0.26^{b}
		Oxamate	84.88 ± 6.25^{b}	61.34 ± 5.11^{b}	117.05 ± 7.66^{b}	138.98 ± 8.14^{b}	103.8 ± 7.91^{b}	190.55 ± 8.85^{b}	72.15 ± 2.37^{ab}	50.89 ± 2.37^{ab}	7.67 ± 0.77^{a}	29.99 ± 2.29^{a}	69.46 ± 1.81^{ab}	3.38 ± 0.39^{b}	0.02 ± 0.36^{b}
		F	(4,40) 158.38	(4,40) 167.67	(4,40) 149.53	(4,40) 151.78	(4,40) 189.51	(4,40) 194.35	(4,40) 6.62	(4,40) 8.42	(4,50) 22.7	(4,50) 5.59	(4,40) 3.02	(4,40) 136.51	(4,40) 13.63
		P	**< 2.2E-16**	**< 2.2E-16**	**< 2.2E-16**	**< 2.2E-16**	**< 2.2E-16**	**< 2.2E-16**	**3.40E-04**	**4.96E-05**	**9.83E-11**	**8.30E-04**	**0.028**	**< 2.2E-16**	**4.23E-07**

Table 4.s6. Effect of metabolic inhibitors on sperm motility parameters in five bivalve species, both DUI and SMI, with chemoattractants. Values are presented as means ± s.e.m. The effect of the factor 'treatment' (*F* and *p*) was assessed for each species and each parameter separately through a liner mixed effect model, which took into account the by-subject variability. Difference among treatments (indicated by letters in superscript) were determined through a *post hoc* pairwise comparison, with *p*-values adjusted using Holm's correction for multiple testing. Significant differences ($p \leq 0.05$) are shown in bold. For abbreviations refer to table 4.s2.

Table s6

Species	Inheritance	Treatment	DAP	DSL	DCL	VAP	VSL	VCL	STR	LIN	ALH	BFC	WOB	PC1	PC2
MyEd	DUI	Control	63.07 ± 4.57^{a}	39.98 ± 2.02^{a}	111.17 ± 4.18^{a}	110.45 ± 7.16^{a}	72.27 ± 3.39^{a}	193.4 ± 8.51^{a}	67.15 ± 2.99	37.61 ± 1.72	11.14 ± 0.53^{a}	25.85 ± 1.58	56.57 ± 2.07	2.3 ± 0.18^{a}	−2.01 ± 0.26^{a}
		Rotenone	7.84 ± 1.76^{c}	5.14 ± 1.03^{c}	12.34 ± 3.28^{c}	16.37 ± 4.08^{c}	12.71 ± 3.44^{b}	24.82 ± 6.62^{c}	55.2 ± 11.19	43.1 ± 9.26	2.31 ± 0.71^{b}	17.31 ± 3.75	54.43 ± 10.82	−1.63 ± 0.34^{b}	1.26 ± 0.49^{b}
		Antimycin A	5.96 ± 1.92^{c}	4.11 ± 1.25^{c}	7.65 ± 2.36^{c}	10.76 ± 3.48^{c}	8.72 ± 3.23^{b}	14.89 ± 5.32^{c}	46.55 ± 13.99	34.54 ± 10.06	1.16 ± 0.59^{b}	16.97 ± 5.07	41.73 ± 12.21	−1.2 ± 0.37^{b}	1.37 ± 0.41^{b}
		Oligomycin	7.9 ± 2.28^{c}	4.16 ± 1.02^{c}	12.06 ± 3.69^{c}	13.42 ± 3.75^{c}	7.59 ± 2.18^{b}	20.46 ± 6.07^{c}	41.16 ± 11.62	31.55 ± 9.22	2.62 ± 0.86^{b}	26.2 ± 7.61	45.86 ± 11.09	−1.43 ± 0.35^{b}	0.47 ± 0.58^{b}
		Oxamate	48.8 ± 2.3^{b}	34.28 ± 1.62^{b}	88.35 ± 4.19^{b}	94.32 ± 3.82^{b}	68.11 ± 2.8^{a}	164.57 ± 6.52^{b}	74.61 ± 2.18	43.84 ± 1.99	10.23 ± 0.38^{a}	25.25 ± 1.95	58.09 ± 1.12	1.55 ± 0.14^{a}	−1.17 ± 0.25^{a}
		F	(4,40) 104.52	(4,40) 163.75	(4,40) 214.96	(4,40) 121.68	(4,40) 127.77	(4,40) 217.79	(4,40) 2.46	(4,40) 0.63	(4,40) 66.42	(4,40) 1.19	(4,40) 0.81	(4,40) 45.95	(4,40) 17.14
		P	**< 2.2E-16**	**< 2.2E-16**	**< 2.2E-16**	**< 2.2E-16**	**< 2.2E-16**	**< 2.2E-16**	0.06	0.64	**< 2.2E-16**	0.32	0.52	**1.92E-14**	**2.88E-08**
RuPh	DUI	Control	53.13 ± 2.61^{a}	31.03 ± 1.86^{a}	88.97 ± 3.57^{a}	105.4 ± 4.82^{a}	66.23 ± 3.8^{a}	170.04 ± 5.6^{a}	62.85 ± 1.78^{a}	39.17 ± 1.69	8.58 ± 0.32^{a}	27.04 ± 2.07	62.13 ± 1.81	1.33 ± 0.18^{a}	−1.34 ± 0.21^{a}
		Rotenone	25.15 ± 1.8^{c}	11.65 ± 1^{c}	47.54 ± 3.81^{c}	53.16 ± 3.81^{c}	29.11 ± 2.86^{b}	93.15 ± 6.57^{c}	53.3 ± 2^{ab}	33.35 ± 2.35	6.41 ± 0.36^{ab}	22.13 ± 2.01	59.92 ± 2.33	−1.29 ± 0.15^{b}	−1.6 ± 0.29^{a}
		Antimycin A	11.66 ± 3.06^{d}	5.2 ± 1.34^{d}	17.56 ± 4.6^{d}	20.67 ± 5.69^{d}	10.92 ± 3.14^{c}	29.65 ± 7.82^{e}	36.29 ± 9.49^{b}	26.17 ± 6.88	4.06 ± 1.28^{b}	19.69 ± 5.42	47.55 ± 12.57	−1.39 ± 0.36^{b}	−0.31 ± 0.35^{bc}
		Oligomycin	12.74 ± 2.05^{d}	6.94 ± 0.97^{d}	19.38 ± 3.14^{d}	32.35 ± 5.36^{d}	21.54 ± 4.22^{bc}	50.92 ± 9.37^{d}	59.1 ± 9.07^{ab}	42.49 ± 6.42	4.46 ± 0.75^{b}	25.49 ± 4.07	63.62 ± 8.46	−1.65 ± 0.27^{b}	0.42 ± 0.38^{c}
		Oxamate	41.81 ± 2.32^{b}	25.57 ± 1.45^{b}	72.77 ± 2.7^{b}	88.13 ± 3.93^{b}	57.51 ± 2.94^{a}	147.36 ± 3.74^{b}	67.52 ± 1.73^{a}	40.74 ± 1.73	7.78 ± 0.35^{a}	25.87 ± 1.5	60.19 ± 1.78	0.6 ± 0.12^{a}	−1.05 ± 0.22^{ab}
		F	(4,32) 62.06	(4,40) 72.06	(4,32) 97.94	(4,32) 68.38	(4,32) 53.16	(4,32) 79.50	(4,32) 4.12	(4,32) 2.52	(4,40) 7.78	(4,32) 0.98	(4,32) 0.97	(4,32) 33.5	(4,32) 9.12
		P	**1.27E-14**	**< 2.2E-16**	**< 2.2E-16**	**3.21E-15**	**1.09E-13**	**3.70E-16**	**8.00E-03**	0.06	**9.77E-05**	0.42	0.43	**5.04E-11**	**4.90E-05**
MeMe	SMI	Control	83.12 ± 5.97^{a}	54.59 ± 3.42^{a}	108.18 ± 6.07^{a}	142.24 ± 10.55^{a}	97.4 ± 6.54^{a}	183.29 ± 10.63^{a}	72.13 ± 2.87^{ab}	54.44 ± 2.48	9.55 ± 1.34^{a}	24.61 ± 2.26	75.35 ± 1.66	3.23 ± 0.32^{a}	0.23 ± 0.44
		Rotenone	14.98 ± 2.53^{c}	8.82 ± 1.57^{c}	20.84 ± 3.8^{c}	24.56 ± 4.21^{c}	14.87 ± 2.34^{c}	33.92 ± 6.28^{c}	59.25 ± 9.92^{ab}	46.31 ± 8.55	3.96 ± 0.96^{b}	24.38 ± 4.49	67.87 ± 8.82	−1.8 ± 0.26^{c}	0.9 ± 0.7
		Antimycin A	17.38 ± 1.5^{c}	9 ± 0.77^{c}	23.77 ± 2.72^{c}	28.2 ± 2.8^{c}	15.27 ± 1.35^{c}	38.24 ± 5.03^{c}	59.64 ± 5.63^{ab}	47.66 ± 5.65	3.83 ± 0.91^{b}	23.05 ± 1.47	77.08 ± 2.79	−2.27 ± 0.11^{c}	0.67 ± 0.69
		Oligomycin	19.06 ± 0.9^{c}	8.83 ± 0.4^{c}	24.77 ± 1.41^{c}	31.87 ± 2.5^{c}	16.22 ± 2.06^{c}	41.15 ± 3.3^{c}	53.91 ± 2.4^{b}	43.94 ± 2.13	3.28 ± 0.37^{b}	19.99 ± 1.12	79.97 ± 1.34	−2.38 ± 0.1^{c}	0.52 ± 0.23
		Oxamate	57.2 ± 4.19^{b}	42.1 ± 3.5^{b}	82.28 ± 4.03^{b}	94.25 ± 7.19^{b}	71.12 ± 6.02^{b}	134.24 ± 6.53^{b}	76.96 ± 1.29^{a}	53.2 ± 1.49	7.27 ± 0.42^{a}	25.46 ± 1.64	68.46 ± 1.42	1.45 ± 0.29^{b}	0.38 ± 0.21
		F	(4,32) 76.03	(4,32) 102.26	(4,40) 106.93	(4,32) 75.51	(4,32) 97.26	(4,32) 105.28	(4,32) 3.48	(4,32) 0.93	(4,40) 9.58	(4,32) 0.8	(4,32) 1.6	(4,40) 116.01	(4,32) 0.27
		P	**7.04E-16**	**< 2.2E-16**	**< 2.2E-16**	**7.77e-16**	**< 2.2E-16**	**< 2.2E-16**	**1.70E-02**	0.45	**1.56E-05**	0.53	0.19	**< 2.2E-16**	0.89
NuOb	SMI	Control	105.98 ± 9.69^{a}	81.28 ± 3.79^{a}	123.79 ± 12.47^{a}	231.85 ± 19.55^{a}	192.2 ± 23.9^{a}	266.53 ± 16.58^{a}	82.69 ± 3.77^{a}	69.96 ± 5.68^{a}	7.65 ± 0.69	31.78 ± 1.74^{a}	83.85 ± 3.09	6.23 ± 0.49^{a}	2.07 ± 0.66
		Rotenone	8.69 ± 3.57^{c}	4.25 ± 1.75^{c}	12.83 ± 5.29^{c}	21.91 ± 9.2^{c}	13.23 ± 5.71^{c}	29.57 ± 12.21^{c}	36.52 ± 15.06^{b}	29.23 ± 12.07^{b}	3.09 ± 1.29	13.99 ± 6.28^{b}	44.87 ± 18.38	−1.22 ± 0.51^{bc}	0.28 ± 0.15
		Antimycin A	11.33 ± 1.53^{c}	7.48 ± 0.31^{c}	18.78 ± 4.17^{c}	22.2 ± 5.93^{c}	15 ± 3.85^{c}	39.17 ± 11.67^{c}	73.76 ± 8.93^{a}	50.26 ± 9.9^{ab}	5.29 ± 2.47	38.43 ± 3.05^{a}	65.64 ± 6.17	−1.98 ± 0.23^{c}	0.55 ± 1.38
		Oligomycin	11.19 ± 3.64^{c}	5.8 ± 1.3^{c}	17.87 ± 6.3^{c}	18.87 ± 5.89^{c}	11.24 ± 2.77^{c}	28.96 ± 9.3^{c}	67.73 ± 5.15^{ab}	48.04 ± 4.4^{ab}	3.47 ± 1.87	34.24 ± 4.99^{a}	67.7 ± 5.34	−2.43 ± 0.4^{c}	0.59 ± 0.58
		Oxamate	63.24 ± 21.02^{b}	46.59 ± 15.8^{b}	78.74 ± 23.65^{b}	108.11 ± 35.86^{b}	80.17 ± 28.3^{b}	135.45 ± 39.19^{b}	72.39 ± 5.18^{ab}	57.39 ± 5.6^{ab}	5.87 ± 1.34	25.9 ± 3.52^{ab}	76.5 ± 3.83	1.62 ± 1.6^{b}	1.08 ± 0.62
		F	(4,20) 16.66	(4,20) 21.65	(4,20) 15.09	(4,20) 23.52	(4,20) 21.41	(4,20) 24.44	(4,20) 4.17	(4,20) 3.38	(4,16) 1.31	(4,20) 5.04	(4,20) 2.51	(4,20) 19.69	(4,20) 0.81
		P	**3.71E-06**	**4.91E-07**	**7.75E-06**	**2.53E-07**	**5.38E-07**	**1.85E-07**	**0.012**	**0.028**	0.3	**0.0056**	0.073	**1.03E-06**	0.53
PlMg	SMI	Control	95.2 ± 5.9^{a}	70.29 ± 4.23^{a}	136.98 ± 4.13^{a}	158.06 ± 11.88^{a}	121.15 ± 9.81^{a}	222.89 ± 7.2^{a}	76.78 ± 1.7^{a}	52.29 ± 2.87^{a}	7.96 ± 0.44^{a}	32.59 ± 1.39^{a}	67.45 ± 3.01^{a}	4.44 ± 0.35^{a}	0.03 ± 0.39
		Rotenone	24.25 ± 3.06^{c}	14.03 ± 2.52^{c}	38.11 ± 5.3^{c}	37.3 ± 5.63^{c}	22.47 ± 4.79^{c}	57.54 ± 8.97^{c}	56.5 ± 2.64^{bc}	40.18 ± 2.19^{a}	4.91 ± 0.68^{b}	20.74 ± 1.55^{b}	70.38 ± 2.16^{a}	−1.76 ± 0.3^{d}	−0.35 ± 0.33
		Antimycin A	3.02 ± 1.58^{d}	2.32 ± 1.23^{d}	4.16 ± 2.21^{e}	5.16 ± 2.84^{d}	4.06 ± 2.19^{c}	7.11 ± 4.07^{e}	21.25 ± 11.05^{d}	16.54 ± 8.63^{b}	0.85 ± 0.57^{d}	6.91 ± 3.86^{c}	20.6 ± 10.68^{b}	−0.62 ± 0.32^{c}	0.53 ± 0.33
		Oligomycin	14.91 ± 1.63^{c}	6.5 ± 0.97^{cd}	19.8 ± 2.45^{d}	22.07 ± 2.55^{cd}	10.04 ± 1.61^{c}	29.48 ± 3.93^{d}	43.86 ± 6.3^{c}	34.48 ± 5.08^{ab}	2.54 ± 0.4^{c}	20.77 ± 2.5^{b}	71.46 ± 7.3^{a}	−2.51 ± 0.29^{d}	0.07 ± 0.29
		Oxamate	58.12 ± 4.75^{b}	42.82 ± 4.76^{b}	89.75 ± 6.6^{b}	94.33 ± 9.55^{b}	71.72 ± 9.27^{b}	141.78 ± 12.36^{b}	69.48 ± 4.1^{ab}	45.59 ± 3.43^{a}	7.21 ± 0.65^{a}	26.39 ± 2.19^{ab}	65.75 ± 2.59^{a}	1.43 ± 0.48^{b}	−0.38 ± 0.41
		F	(4,40) 114.19	(4,40) 93.32	(4,40) 172.19	(4,40) 91.35	(4,40) 72.05	(4,40) 172.23	(4,40) 14.13	(4,40) 7.99	(4,40) 39.23	(4,40) 16.78	(4,40) 13.14	(4,40) 64.5	(4,40) 1.16
		P	**< 2.2E-16**	**< 2.2E-16**	**< 2.2E-16**	**< 2.2E-16**	**< 2.2E-16**	**< 2.2E-16**	**2.83E-07**	**7.81E-05**	**2.42E-13**	**3.74E-08**	**6.34E-07**	**< 2.2E-16**	0.33

Table 4.s7. Interaction effect between glycolysis inhibition and chemoattractant addition on sperm motility parameters in five bivalve species, both DUI and SMI. Values are presented as means ± s.e.m. The main effect of the two fixed factors 'treatment' and 'chemoattractants', as well as their interaction, was assessed for each species and each parameter separately through a liner mixed effect model which considered the by-subject variability and the individual variability in the response to egg detection. Simple main effects (indicated by letters in superscript) were determined through a *post hoc* pairwise comparison, with *p*-values adjusted using Holm's correction for multiple testing. Significant differences ($p \leq 0.05$) are shown in bold. 'Control-N', basal sperm motility without chemoattractants; 'Control-ch', basal sperm motility with chemoattractants; 'Oxamate-N', sperm motility in presence of oxamate without chemoattractants; 'Oxamate-ch', sperm motility in presence of oxamate with chemoattractants. ':treat', main effect of factor 'treatment'; ':chem', main effect of factor 'chemoattractants'; ':treat:chem', interaction effect between factor 'treatment' and factor 'chemoattractants'. For abbreviations refer to table 4.s2.

Table s7

Species	Inheritance	Treatment	DAP	DSL	DCL	VAP	VSL	VCL	STR	LIN	ALH	BFC	WOB	PC1	PC2
MyEd	DUI	Control-N	57.96 ± 2.75^{ab}	36.88 ± 1.71	96.86 ± 4.05^{ab}	114.06 ± 4.66	75.41 ± 3.16	184.87 ± 8.19^{ab}	67.57 ± 1.74^{a}	41.82 ± 1.57^{ab}	10.44 ± 0.49	25.59 ± 0.99	61.6 ± 1.59	2.02 ± 0.14^{ab}	−1.38 ± 0.26
		Control-ch	63.07 ± 4.57^{a}	39.98 ± 2.02	111.17 ± 4.18^{a}	110.45 ± 7.16	72.27 ± 3.39	193.4 ± 8.51^{a}	67.15 ± 2.99^{a}	37.61 ± 1.72^{b}	11.14 ± 0.53	25.85 ± 1.58	56.57 ± 2.07	2.3 ± 0.18^{a}	−2.01 ± 0.26
		Oxamate-N	57.16 ± 3.82^{ab}	36.54 ± 1.78	92.86 ± 4.85^{b}	114.33 ± 7.76	75.66 ± 4.86	182.1 ± 8.85^{ab}	68.71 ± 1.25^{ab}	42.89 ± 1.39^{a}	10.4 ± 0.36	26.6 ± 1.55	62.38 ± 1.78	1.99 ± 0.23^{ab}	−1.22 ± 0.2
		Oxamate-ch	48.8 ± 2.3^{b}	34.28 ± 1.62	88.35 ± 4.19^{b}	94.32 ± 3.82	68.11 ± 2.8	164.57 ± 6.52^{b}	74.61 ± 2.18^{b}	43.84 ± 1.99^{a}	10.23 ± 0.38	25.25 ± 1.95	58.09 ± 1.12	1.55 ± 0.14^{b}	−1.17 ± 0.25
		:treat	**$F_{1,20}$=6.7, P=0.017***	$F_{1,20}$=4.11, P=0.056	**$F_{1,20}$=15.66, P=7.7e-04*****	$F_{1,20}$=2.11, P=0.16	$F_{1,20}$=0.38, P=0.54	**$F_{1,20}$=8.96, P=0.007****	**$F_{1,20}$=9.11, P=0.0067****	**$F_{1,30}$=9, P=0.005****	$F_{1,30}$=1.6, P=0.21	$F_{1,30}$=0.04, P=0.84	$F_{1,30}$=0.57, P=0.45	**$F_{1,20}$=8.2, P=0.0095****	**$F_{1,30}$=6.8, P=0.01***
		:chem	$F_{1,10}$=0.16, P=0.7	$F_{1,10}$=0.05, P=0.82	$F_{1,10}$=0.87, P=0.37	$F_{1,10}$=3.44, P=0.09	$F_{1,10}$=2.55, P=0.14	$F_{1,10}$=0.17, P=0.68	$F_{1,10}$=1.6, P=0.23	$F_{1,15}$=1.4, P=0.25	$F_{1,29}$=0.51, P=0.47	$F_{1,13}$=0.17, P=0.67	**$F_{1,23}$=8.9, P=0.006****	$F_{1,10}$=0.14, P=0.71	$F_{1,17}$=2, P=0.17
		:treat:chem	**$F_{1,20}$=5.33, P=0.031***	$F_{1,20}$=3.25, P=0.086	**$F_{1,20}$=7.72, P=0.011***	$F_{1,20}$=2.26, P=0.14	$F_{19.9}$=0.49, P=0.48	**$F_{1,20}$=6.09, P=0.022***	**$F_{1,20}$=4.9, P=0.037***	**$F_{1,30}$=4.5, P=0.04***	$F_{1,30}$=1.4, P=0.24	$F_{1,30}$=0.6, P=0.44	$F_{1,30}$=0.06, P=0.8	**$F_{1,20}$=6.8, P=0.016***	$F_{1,30}$=3.23, P=0.08
RuPh	DUI	Control-N	47.83 ± 3.29	30.06 ± 1.87	79.35 ± 4.24	93.42 ± 6.28^{ab}	63.16 ± 4.04	150.95 ± 8.34^{b}	70.51 ± 1.43	43.56 ± 1.31	7.34 ± 0.4	27.6 ± 1.22	62.31 ± 2.05	0.97 ± 0.23^{ab}	−0.66 ± 0.19
		Control-ch	53.13 ± 2.61	31.03 ± 1.86	88.97 ± 3.57	105.4 ± 4.82^{a}	66.23 ± 3.8	170.04 ± 5.6^{a}	62.85 ± 1.78	39.17 ± 1.69	8.58 ± 0.32	27.04 ± 2.07	62.13 ± 1.81	1.33 ± 0.18^{a}	−1.34 ± 0.21
		Oxamate-N	42.58 ± 3.2	27.53 ± 1.16	68.43 ± 3.79	96.35 ± 6.99^{ab}	67.48 ± 4.5	149.83 ± 5^{b}	72.83 ± 2.71	47.02 ± 1.7	6.62 ± 0.39	27.91 ± 2.32	65.14 ± 3.72	0.8 ± 0.17^{b}	−0.13 ± 0.2
		Oxamate-ch	41.81 ± 2.32	25.57 ± 1.45	72.77 ± 2.7	88.13 ± 3.93^{b}	57.51 ± 2.94	147.36 ± 3.74^{b}	67.52 ± 1.73	40.74 ± 1.73	7.78 ± 0.35	25.87 ± 1.5	60.19 ± 1.78	0.6 ± 0.12^{b}	−1.05 ± 0.22
		:treat	**$F_{1,24}$=13.25, P=0.0013****	**$F_{1,24}$=7.95, P=0.009****	**$F_{1,24}$=19.72, P=1.7e-04*****	$F_{1,16}$=3.68, P=0.07	$F_{1,16}$=0.62, P=0.4	**$F_{1,24}$=7, P=0.014***	$F_{1,24}$=3.4, P=0.07	**$F_{1,16}$=5, P=0.04***	**$F_{1,16}$=7.7, P=0.013***	$F_{1,16}$=0.12, P=0.73	$F_{1,16}$=0.07, P=0.79	**$F_{1,24}$=11.66, P=0.0022****	**$F_{1,16}$=8, P=0.012***
		:chem	$F_{1,12}$=0.72, P=0.4	$F_{1,18}$=0.11, P=0.74	$F_{1,11}$=4, P=0.067	$F_{1,8}$=0.13, P=0.72	$F_{1,8}$=0.98, P=0.35	$F_{1,13.8}$=2.8, P=0.11	**$F_{1,8}$=8.5, P=0.018***	**$F_{1,8}$=8, P=0.02***	**$F_{1,8}$=8.5, P=0.02***	$F_{1,8}$=0.34, P=0.57	$F_{1,8}$=0.83, P=0.38	$F_{1,12}$=0.26, P=0.61	**$F_{1,8}$=9.75, P=0.014***
		:treat:chem	$F_{1,24}$=1.77, P=0.2	$F_{1,24}$=1, P=0.3	$F_{1,24}$=0.75, P=0.39	**$F_{1,16}$=7.31, P=0.015***	**$F_{1,16}$=5.47, P=0.03***	**$F_{1,24}$=5.76, P=0.024***	$F_{1,24}$=0.38, P=0.53	$F_{1,16}$=0.7, P=0.4	$F_{1,16}$=0.02, P=0.87	$F_{1,16}$=0.36, P=0.55	$F_{1,16}$=1.99, P=0.17	**$F_{1,24}$=4.68, P=0.04***	$F_{1,16}$=0.67, P=0.42
MeMe	SMI	Control-N	92.15 ± 6.57	58.32 ± 3.47	115.31 ± 6.13	156.69 ± 11.27	106.29 ± 6.65	194.51 ± 11.01	71.08 ± 2.41	54.88 ± 2.1	7.97 ± 0.49	27.17 ± 1.07^{a}	77.66 ± 1.76	3.59 ± 0.36	0.54 ± 0.27
		Control-ch	83.12 ± 5.97	54.59 ± 3.42	108.18 ± 6.07	142.24 ± 10.55	97.4 ± 6.54	183.29 ± 10.63	72.13 ± 2.87	54.44 ± 2.48	9.55 ± 1.34	24.61 ± 2.26^{ab}	75.35 ± 1.66	3.23 ± 0.32	0.23 ± 0.44
		Oxamate-N	63.84 ± 6.95	41.62 ± 3.43	83.97 ± 8.64	119.88 ± 7.46	85.56 ± 3.56	155.2 ± 8.18	77.77 ± 2.74	56.29 ± 1.97	7.84 ± 0.56	22.97 ± 1.29^{b}	73.86 ± 0.86	2.03 ± 0.33	0.72 ± 0.28
		Oxamate-ch	57.2 ± 4.19	42.1 ± 3.5	82.28 ± 4.03	94.25 ± 7.19	71.12 ± 6.02	134.24 ± 6.53	76.96 ± 1.29	53.2 ± 1.49	7.27 ± 0.42	25.46 ± 1.64^{ab}	68.46 ± 1.42	1.45 ± 0.29	0.38 ± 0.21
		:treat	**$F_{1,24}$=27, P=2.45e-05*****	**$F_{1,24}$=25.13, P=4e-05*****	**$F_{1,24}$=27.24, P=2.4e-05*****	**$F_{1,16}$=31, P=4.25e-05*****	$F_{1,20}$=0.38, P=0.54	**$F_{1,16}$=32.74, P=3.14e-05*****	**$F_{1,16}$=13, P=0.0022****	$F_{1,16}$=0.008, P=0.92	$F_{1,16}$=4.2, P=0.056	$F_{1,24}$=3.3, P=0.08	**$F_{1,16}$=25, P=1.1e-04*****	**$F_{1,24}$=33.27, P=6e-06*****	$F_{1,16}$=0.97, P=0.33
		:chem	$F_{1,9}$=1.53, P=0.24	$F_{1,13}$=0.26, P=0.61	$F_{1,14}$=0.37, P=0.55	$F_{1,8}$=4.72, P=0.061	$F_{1,10}$=2.55, P=0.14	$F_{1,8}$=3, P=0.12	$F_{1,8}$=0.001, P=0.96	$F_{1,8}$=0.8, P=0.39	$F_{1,8}$=0.32, P=0.58	$F_{1,9}$=0.0007, P=0.97	$F_{1,8}$=8.2, P=0.02	$F_{1,15}$=2.41, P=0.14	$F_{1,8}$=1, P=0.34
		:treat:chem	$F_{1,24}$=0.05, P=0.82	$F_{1,24}$=0.52, P=0.47	$F_{1,24}$=0.24, P=0.62	$F_{1,16}$=0.53, P=0.47	$F_{1,20}$=0.49, P=0.48	$F_{1,16}$=0.39, P=0.53	$F_{1,16}$=0.34, P=0.56	$F_{1,16}$=2, P=0.17	$F_{1,16}$=3.4, P=0.08	**$F_{1,24}$=7.5, P=0.011***	$F_{1,16}$=2.1, P=0.16	$F_{1.24}$=0.13, P=0.71	$F_{1,16}$=0.0078, P=0.93
NuOb	SMI	Control-N	102.65 ± 8.88	76.51 ± 6.39	129.03 ± 7.79	193.58 ± 12.73	151.32 ± 12.26^{ab}	242.62 ± 9.6	76.68 ± 3.59	60.87 ± 4.38	7.26 ± 0.46	33.89 ± 1.74	77.28 ± 2.9	5.22 ± 0.53	1.11 ± 0.53
		Control-ch	105.98 ± 9.69	81.28 ± 3.79	123.79 ± 12.47	231.85 ± 19.55	192.2 ± 23.9^{a}	266.53 ± 16.58	82.69 ± 3.77	69.96 ± 5.68	7.65 ± 0.69	31.78 ± 1.74	83.85 ± 3.09	6.23 ± 0.49	2.07 ± 0.66
		Oxamate-N	73.84 ± 10.46	55.39 ± 8.96	93.19 ± 9.61	156.9 ± 14.52	127.66 ± 10.2^{ab}	189.12 ± 12.51	72.34 ± 6.93	57.97 ± 6.61	6.59 ± 0.49	29.21 ± 1.46	75.72 ± 3.95	3.2 ± 0.71	0.95 ± 0.69
		Oxamate-ch	63.24 ± 21.02	46.59 ± 15.8	78.74 ± 23.65	108.11 ± 35.86	80.17 ± 28.3^{b}	135.45 ± 39.19	72.39 ± 5.18	57.39 ± 5.6	5.87 ± 1.34	25.9 ± 3.52	76.5 ± 3.83	1.62 ± 1.6	1.08 ± 0.62
		:treat	**$F_{1,12}$=9.36, P=0.009****	**$F_{1,12}$=9.19, P=0.01***	**$F_{1,12}$=13.15, P=0.003****	**$F_{1,16}$=12.6, P=2e-03****	**$F_{1,16}$=11.32, P=3.9e-03****	**$F_{1,12}$=17.43, P=1.2e-03****	**$F_{1,12}$=6.2, P=0.028***	$F_{1,12}$=4, P=0.06	$F_{1,12}$=3.3, P=0.09	**$F_{1,12}$=6.11, P=0.029***	$F_{1,12}$=1.8, P=0.2	**$F_{1,12}$=13.27, P=3.3e-03****	$F_{1,12}$=1.98, P=0.18
		:chem	$F_{1,5.4}$=0.06, P=0.81	$F_{1,5}$=0.036, P=0.85	$F_{1,4.4}$=0.32, P=0.59	$F_{1,16}$=0.054, P=0.81	$F_{1,16}$=0.02, P=0.87	$F_{1,12}$=0.42, P=0.52	$F_{1,4}$=0.12, P=0.74	$F_{1,4}$=0.2, P=0.66	$F_{1,5}$=0.03, P=0.85	$F_{1,4}$=1.12, P=0.34	$F_{1,4}$=0.86, P=0.4	$F_{1,8.6}$=0.08, P=0.77	$F_{1,4}$=0.27, P=0.62
		:treat:chem	$F_{1,12}$=0.35, P=0.56	$F_{1,12}$=0.54, P=0.47	$F_{1,12}$=0.17, P=0.68	$F_{1,16}$=3.71, P=0.071	**$F_{1,16}$=4.8, P=0.043***	$F_{1,12}$=3.1, P=0.10	$F_{1,12}$=1, P=0.33	$F_{1,12}$=1.5, P=0.22	$F_{1,12}$=0.66, P=0.42	$F_{1,12}$=0.07, P=0.78	$F_{1,12}$=0.76, P=0.39	$F_{1,12}$=2, P=0.18	$F_{1,12}$=1, P=0.32
PlMg	SMI	Control-N	112.4 ± 5.89	82.25 ± 3.92	136.9 ± 4.46^{a}	211.56 ± 14.47	164.82 ± 9.82	252.95 ± 11.04	78.84 ± 1.59	63.03 ± 1.79	7.76 ± 0.33	29.93 ± 1.74	80 ± 2.82^{a}	5.87 ± 0.39	1.32 ± 0.2
		Control-ch	95.2 ± 5.9	70.29 ± 4.23	136.98 ± 4.13^{a}	158.06 ± 11.88	121.15 ± 9.81	222.89 ± 7.2	76.78 ± 1.7	52.29 ± 2.87	7.96 ± 0.44	32.59 ± 1.39	67.45 ± 3.01^{b}	4.44 ± 0.35	0.03 ± 0.39
		Oxamate-N	84.88 ± 6.25	61.34 ± 5.11	117.05 ± 7.66^{b}	138.98 ± 8.14	103.8 ± 7.91	190.55 ± 8.85	72.15 ± 2.37	50.89 ± 2.37	7.67 ± 0.77	29.99 ± 2.29	69.46 ± 1.81^{b}	3.38 ± 0.39	0.02 ± 0.36
		Oxamate-ch	58.12 ± 4.75	42.82 ± 4.76	89.75 ± 6.6^{c}	94.33 ± 9.55	71.72 ± 9.27	141.78 ± 12.36	69.48 ± 4.1	45.59 ± 3.43	7.21 ± 0.65	26.39 ± 2.19	65.75 ± 2.59^{b}	1.43 ± 0.48	−0.38 ± 0.41
		:treat	**$F_{1,20}$=61, P=1.6e-07*****	**$F_{1,20}$=40, P=3.55e-06*****	**$F_{1,30}$=47, P=1.3e-07*****	**$F_{1,20}$=88.22, P=8.97e-09*****	**$F_{1,20}$=90, P=7.14e-09*****	**$F_{1,20}$=86, P=1.1e-08*****	**$F_{1,30}$=7.4, P=0.01***	**$F_{1,30}$=15, P=4.2e-04*****	$F_{1,30}$=0.61, P=0.44	$F_{1,30}$=2.6, P=0.12	**$F_{1,20}$=10, P=0.004****	**$F_{1,20}$=83.88, P=1.35e-08*****	**$F_{1,20}$=7.28, P=0.013***
		:chem	**$F_{1,10}$=9.45, P=0.01***	**$F_{1,10}$=8.11, P=0.01***	$F_{1,10}$=3.72, P=0.08	**$F_{1,10}$=23, P=7.1e-04*****	**$F_{1,10}$=22.1, P=8.3e-04*****	**$F_{1,10}$=19, P=1.4e-03****	$F_{1,13}$=0.73, P=0.4	***F1,27*=11, P=0.002****	$F_{1,10}$=0.04, P=0.84	$F_{1,12}$=0.05, P=0.81	**$F_{1,10}$=7.8, P=0.018***	**$F_{1,10}$=15.66, P=2.6e-03****	**$F_{1,10}$=6.61, P=0.027***
		:treat:chem	$F_{1,20}$=1.34, P=0.26	$F_{1,20}$=0.73, P=0.4	**$F_{1,30}$=7.8, P=0.0088****	$F_{1,20}$=0.37, P=0.54	$F_{1,20}$=0.99, P=0.32	$F_{1,20}$=1.46, P=0.24	$F_{1,30}$=0.01, P=0.9	$F_{1,30}$=1.3, P=0.26	$F_{1,30}$=0.38, P=0.53	$F_{1,30}$=2.6, P=0.11	**$F_{1,20}$=5.4, P=0.03***	$F_{1,20}$=0.73, P=0.4	$F_{1,20}$=1.96, P=0.17