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SYMPOSIUM INTRODUCTION

Beyond the Powerhouse: Integrating Mitonuclear Evolution, Physiology, and Theory in Comparative Biology

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Synopsis Eukaryotes are the outcome of an ancient symbiosis and as such, eukaryotic cells fundamentally possess two genomes. As a consequence, gene products encoded by both nuclear and mitochondrial genomes must interact in an intimate and precise fashion to enable aerobic respiration in eukaryotes. This genomic architecture of eukaryotes is proposed to necessitate perpetual coevolution between the nuclear and mitochondrial genomes to maintain coadaptation, but the presence of two genomes also creates the opportunity for intracellular conflict. In the collection of papers that constitute this symposium volume, scientists working in diverse organismal systems spanning vast biological scales address emerging topics in integrative, comparative biology in light of mitonuclear interactions.

Mitonuclear evolution as integrative biology

The cellular mechanisms that enable aerobic respiration and that sustain complex life arise from the coordinated function of the products of multiple genomes—minimally both a nuclear and a mitochondrial genome (Blier et al. 2001; Rand et al. 2004; Bar-Yaacov et al. 2012). A single functional outcome that is the product of two independently replicating genomes likely necessitates coadaptation between the gene products of the two entities (Lane 2011; Levin et al. 2014; Wolff et al. 2014). Paradoxically, despite the need for the mitochondrial and nuclear genomes to coexist and cooperate, the interests of the two genomes are not identical and selfish conflict between them can alter the course of evolution (Gemmell et al. 2004; Wade and Drown 2016; Havird et al. 2019). The evolutionary processes that shape complex life are hypothesized to be significantly influenced by the outcomes of selection arising from coadaptation, coevolution, and genomic conflict involving mitochondrial and nuclear genes, but mitonuclear processes are only just emerging as a central topic in evolutionary ecology (Sunnucks et al. 2017; Hill 2019a, 2019b).

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The challenge for scientists working to understand macro-features of complex life in the context of the coevolution of mitochondrial and nuclear genes is that such investigations demand understanding and technical knowledge of cell and molecular biology as well as whole-organism processes. Traditionally, biologists focused at one of these levels of organization have paid little attention to discoveries made at the other level (Hagen 1999; Hill 2014). The rift between cell and molecular biology and ecology and evolution reached a peak in the late 20th century when many biology departments at universities fractured into separate cell/molecular departments and ecology/evolution departments, exacerbating a lack of communication between reductionistic and organismal biologists. As genomics and other tools for studying cellular and molecular processes became more broadly accessible to organismal biologists and cell and molecular biologists expanded investigations to non-model species, the walls between cell/molecular and ecology/evolution-focused approaches to biological study began to erode.

It is in the spirit of further eroding division between bottom-up and top-down approaches to understanding the evolution of organisms that we convened our symposium on the evolutionary consequences of mitonuclear coadaptation. Mitonuclear interactions hold the potential to influence our understanding of how major organismal traits evolved across a wide spectrum of biological functions including physiology, life history, genomic architecture, adaptation, and diversification. The symposium contributors use cell/molecular tools to examine the evolution and physiological implications of mitonuclear interactions, but then apply these outcomes to broad questions of organismal ecology and evolution. The papers that comprise this volume of *Integrative and Comparative Biology* reflect the pervasive effects and integrative nature of mitonuclear coadaptation and coevolution (Table 1).

Oxidative stress, mutation, and life history

Eukaryotes rely on mitonuclear communication to set the pace of metabolism both at the level of the whole organism and within cells. As a consequence, whole-organism phenotypes should be related to cellular function (Barja 1998), demonstrating the importance of investigating mitonuclear interactions across multiple biological scales. Jimenez et al. (2019) assess this basic assumption that the "pace of life" should be reflected in mitochondrial function using a comparative study of birds and mammals. Birds have higher basal metabolic rates, higher blood glucose concentration and longer lifespans than similar sized mammals (Hickey 2008), but the literature is equivocal on whether oxidative stress is higher in birds compared with mammals. Jimenez et al. (2019) derived values from the literature for the whole-animal metabolism of 999 species of birds and 1130 species of mammals, and then measured cellular metabolic rates from primary fibroblast cells of a subset of these taxa. They also compared levels of oxidative stress in the focal bird and mammal taxa using separated blood in a group of phylogenetically-corrected birds and mammals. They found that mass-specific metabolic rates were higher in birds compared with mammals. Using primary fibroblast cells, basal metabolism was significantly lower in birds compared with mammals. They measured total antioxidant capacity using plasma and red blood cells and found that circulating lipid damage and catalase activity were significantly lower in birds compared with mammals. This study underscores the fact that whole-animal phenotypes associated with longer lifespan (as those in birds) emerge from aerobic cellular processes (oxidative stress) at a cellular level.

Virtually all studies of mitochondrial function and evolution make specific assumptions about the rate and sources of mutations to the mitochondrial DNA (mtDNA). Organismal biologists have long focused their attention on damage from free radicals (e.g., oxidative damage as investigated by Jimenez et al. 2019), but this is not the sole source of mutations to mtDNA; mutations can also arise via errors during the replication of mtDNA. Mitochondrial turnover—which necessitates mtDNA replication—is independent of the cell cycle, allowing continued mitochondrial replacement in post-mitotic tissues and allowing the rate of replacement to exceed the cellular rate in mitotic tissue (Larsson 2010). Turnover rates can be rapid, for example, the half-life of mitochondria in the human liver is only 2 days (Miwa et al. 2008). Thus, even with repair mechanisms in place, the accumulation of mtDNA mutations is inevitable when mtDNA is copied and copy error can exceed the rate of mtDNA mutation that is caused by oxidative stress (Ameur et al. 2011; Kennedy et al. 2013; Itsara et al. 2014). In this issue, Hood et al. (2019) argue that it is important to consider mitochondrial replication error as an important contributor to variation in the performance and lifespan of individuals. mtDNA replication requires coordination between nuclear-encoded polymerases

Table 1 A summary of the papers that comprise this volume of *Integrative and Comparative Biology*, demonstrating the integrative and comparative nature of mitonuclear studies

Reference (this volume)	Type	Study organism(s)	Scale(s)	Evolutionary topic	Conclusion
Jimenez et al.	Primary research	Birds, mammals	Organismal, cellular, molecular	Aging	Mt phenotypes may underlie differences in lifespan
Hood et al.	Review	Eukaryotes	Organismal, molecular	Mutation rates	Mt replication may play a large role in determining mutation rates
Weaver	Hypothesis	Eukaryotes	Molecular	Complexity, sexual reproduction, mutation rates	AOX may play roles in key evolutionary transitions
Dowling and Adrian	Review	Eukaryotes	Population, organismal, cellular, molecular	Sex-specific effects	Tests of the Mother's curse hypothesis require thoroughness
Montooth et al.	Primary research	Fruit flies	Organismal	Sex-specific effects	The Mother's Curse hypothesis receives mixed support in fruit flies
Havird and McConie	Primary research	Fruit flies, mammals	Molecular	Sex-specific effects	Mother's Curse dynamics do not explain OXPHOS paralog evolution
Sullins et al.	Primary research	Nematodes	Organismal, molecular	Selfishness	Mt heteroplasmy is determined by selection at multiple levels
Ramsey et al.	Primary research	Plants	Population, organismal, molecular	Linkage disequilibrium	Selection for heteroplasmy could mitigate cytonuclear incompatibilities
Ghiselli et al.	Primary research	Molluscs	Tissue, cellular, molecular	Inheritance	Meiotic drive may play a role in the DUI system
Sokolova et al.	Review	Invertebrates	Tissue, cellular, molecular	Environmental adaptation	Mitonuclear interactions may influence hypoxia tolerance
McKenzie et al	Primary research	Fishes	Population, organismal, cellular, molecular	Environmental adaptation, speciation	Geographic variation in mtDNA sequence can be shaped by many factors
Hill	Review	Eukaryotes	Population, molecular	Speciation	Benefits of mt introgression may offset disrupting mitonuclear interactions
Tobler et al.	Review	Eukaryotes	Population, molecular	Environmental adaptation, speciation	Selection on mt function may contribute to reproductive isolation

and mitochondrial-encoded replication cofactors, suggesting that the efficacy of mitonuclear interactions can be key determinants of mitochondrial replication-mediated fitness in many organisms.

A basic assumption by most biologists studying mitonuclear coevolution and coadaptation in complex life is that the respiratory chain, including complexes I, III, and IV, is the only path for aerobic respiration (Hill 2015). As explained in detail by Weaver (2019), however, the alternative oxidase (AOX) is an additional nuclear-encoded oxidoreductase that functions similarly to complex IV of the electron transport system in that it uses electrons from the ubiquinone pool to reduce oxygen to water (McDonald et al. 2009). AOX is a component of the respiratory chain that is widely distributed among eukaryotes but that is often not integrated into physiological and evolutionary research on mitochondria, especially in animals where its distribution is patchy. AOX remains functional under conditions that render complex IV unusable. Weaver (2019) focuses on two primary attributes of AOX: it is resistant to sulfide and its activation prevents excessive free radical production during stress. He hypothesizes that AOX played a role in the evolution of metazoans from simpler eukaryotes by allowing for oxidative

metabolism during the transition from a sulfidic to an oxygenated ocean of the Neoproterozoic era. Based on links between free radical production and sexual reproduction (Hörandl and Speijer 2018) and the role of free radicals in mtDNA mutations, he proposes two additional evolutionary consequences of AOX respiration and its associated decrease in free radical production. AOX could have played a role in the persistence of a facultative asexual reproductive strategy observed in many early branching metazoans, and AOX may also be a mediating factor in the observed low mutation accumulation rates of mtDNA in those taxa. The presence or absence of AOX (and other components of mitochondrial machinery) may, therefore, have implications for key evolutionary transitions in eukaryote life history and evolution.

The Mother's Curse

The necessity of tight mitonuclear coadaptation suggests that there should be broad overlap in the interests of mitochondrial and nuclear genomes in enabling high organismal function. At the same time, however, mitochondria are typically maternally transmitted (but see Ghiselli et al. 2019) and as a consequence, males are typically an evolutionary dead end for mitochondrial genes. Theory predicts that maternal inheritance of mtDNA favors the evolution of male-harming alleles because it renders natural selection blind to mtDNA mutations that harm only males (Cosmides and Tooby 1981; Frank and Hurst 1996; Connallon et al. 2018). Accordingly, over evolutionary time, it is expected that the mitochondrial genome will accumulate a load of male-harming mutations, manifesting in effects on core components of health and life-history of males, a phenomenon called the Mother's Curse (Gemmell et al. 2004). Although over three decades old, there have been few formal tests of this hypothesis, and the field has suffered from the absence of a clear predictive framework.

To redress this, here Dowling and Adrian (2019) provide an overview of the Mother's Curse hypothesis and explain why the Mother's Curse process is most likely to affect traits that exhibit higher levels of sexual dimorphism. They then outline each of three key predictions of the Mother's Curse hypothesis and discuss experimental approaches for testing these predictions, focusing on issues of replication and biological scale. They conclude that while studies to date have provided important insights into the capacity of mitochondrial genomes to accumulate male-harming mutations, these insights come with caveats. First, studies to date have generally been limited to just a handful of model systems. Second, many studies to date have focused on the sex-specific effects of mitochondrial genotypes on just one or two traits and might, therefore, have failed to detect Mother's Curse effects even when these are present within a population or species. And third, tests of the hypothesis have generally been limited by a lack of replication at each of three levels (the number of mitochondrial genotypes sampled, the number of nuclear backgrounds sampled, and replication of the individual genotype); limitations that could also lead to sampling biases and potential errors of inference. Dowling and Adrian (2019) conclude their paper by outlining a roadmap for future tests of the Mother's Curse hypothesis.

In further support of the Mother's Curse hypothesis, Montooth et al. (2019) present new data suggesting that males suffer the negative reproductive consequences of mitonuclear incompatibility, and that these effects are temperature and diet sensitive. However, while the model of mitonuclear incompatibility that they present does have sex-specific fitness effects, the magnitude of these effects is frequently larger in females. They suggest that a productive way forward in studies of the Mother's Curse hypothesis may be to investigate the physiological capacity for sex-specific mtDNA and mitonuclear effects in the context of recent research documenting extensive sexual dimorphism in physiology in the model fruit fly *Drosophila melanogaster* (Millington and Rideout 2018). In this way, developmental physiology may illuminate the scope for sex-specific effects of mtDNA mutations that are a requirement for the Mother's Curse to have ubiquitous effects in populations.

While Montooth et al. (2019) evaluate organismal phenotypes stemming from Mother's Curse, Havird and McConie (2019) consider the role of Mother's Curse in shaping the evolution of nuclear-encoded oxidative phosphorylation (OXPHOS) paralogs. These gene duplicates show remarkable divergence from each other (~50% different in amino acid sequence), yet must interact with a single mitochondrial genome. Because many paralogs show testes-specific gene expression, one hypothesis is that nuclear-encoded OXPHOS duplicates may be a response to Mother's Curse. Testes-specific OXPHOS duplicates may be under selection to offset male harm caused by Mother's Curse mutations in the mitochondrial genome. Examining data from mammals and *Drosophila*, Havird and McConie (2019) conclude that Mother's Curse dynamics may not play a dominant role in shaping the evolution of

testes-specific OXPHOS paralogs. OXPHOS paralogs were found to be evolving under relaxed selection in *Drosophila* and a spatial analysis failed to identify mitonuclear contact sites as being particularly likely to undergo changes in mammals. Alternative processes, independent of mitonuclear interactions, might be more important in shaping the evolution of these highly divergent OXPHOS duplicates. These studies highlight how a focus on conflict in mitonuclear coevolution can increase our understanding of sex-specific processes at the organismal and molecular levels.

Heteroplasmy

The Mother's Curse stems from the uniparental inheritance of mitochondrial genomes, so why is strict maternal inheritance so common in mitochondrial genomes? The alternative biparental inheritance creates a new arena for selfish mitochondria. Having multiple, variable mitochondrial genomes within an organism (i.e., heteroplasmy) can create selection for competition among different mitochondrial lineages within an organism at the expense of individual fitness (Haig 2016; Havird et al. 2019). Selfish mtDNA poses a significant challenge to genome integrity, mitochondrial function, and organismal fitness. Despite implications of selfish mtDNA elements for speciation and other topics in evolutionary biology, the forces controlling their formation, age-related accumulation, and offspring transmission remain largely unknown. Sullins et al. (2019) report the first comprehensive assessment of naturally-occurring selfish mtDNA dynamics during animal development and transmission utilizing *Caenorhabditis briggsae* nematodes—a genetic model that can simultaneously act as a model for pathogenic mitochondrial genome evolution and inheritance. Natural *C. briggsae* isolates harbor varying levels of heteroplasmy (up to ~60%) for a large-scale deleterious deletion affecting the NADH-dehydrogenase 5 gene. The study's comparisons of deletion heteroplasmy levels between parents and offspring were indicative of considerable purifying selection operating to maintain mtDNA genome integrity between generations. However, other results implicated mitonuclear interaction as a likely contributor to observed among-isolate differences in patterns of both mtDNA deletion transmission and age-related accumulation. Taken together, the results imply that natural mtDNA deletion frequencies are likely defined by a balance between recurrent deletion formation as well as selection occurring at gametic and individual levels, all of which may be influenced by mitonuclear interplay.

With heteroplasmy, multiple mitotypes exist within an individual on one nuclear background. When plant mitochondrial genomes undergo recombination between heteroplasmic variants, new allelic combinations, and thus, mitotypes, may be formed. Yet it is presently unknown if heteroplasmy plays a role in patterns of linkage disequilibrium (LD) between nuclear and mitochondrial genes (i.e., mitonuclear LD) and genes within the mitochondrial genome (i.e., mito–mito LD) in natural populations. Ramsey et al. (2019) investigated mitonuclear LD in wild carrot from two geographic regions of the USA, a species known to exhibit occasional biparental inheritance and heteroplasmy. They tested for heteroplasmy and LD by analyzing two mitochondrial genes and simple sequence repeats (SSRs) of 15 nuclear genes. They first assayed the mitochondrial COX1 gene for heteroplasmy. Approximately half of the individuals in each region were heteroplasmic for a silent T/C single nucleotide polymorphism. Ramsey et al. (2019) then calculated mitonuclear LD for heteroplasmic and homoplasmic individuals separately and used a Z-transformation test to determine whether LD differed between them. They show that in one geographical region all five significantly different nuclear SSR–mitotype pairs have higher LD values in heteroplasmic individuals, and in the other region five of six significantly different nuclear SSR-mitotype pairs show the same pattern. They also find that the overall magnitude of LD between COX1 and ATP9 is lower in heteroplasmic individuals in both regions, indicating recombination. Should demographic processes disrupt mitonuclear LD and, therefore, interactions, selection for heteroplasmy could mitigate cytonuclear incompatibilities as heteroplasmy may provide alternative allelic variants to better match with nuclear backgrounds.

Considerations of both cooperation and conflict between co-functioning mitochondrial and nuclear genes become problematic when both sexes can transmit mitochondrial genes to offspring. In this regard, Ghiselli et al. (2019) provide new and fascinating data on the evolutionary consequences of biparental transmission of mitochondria. They studied bivalves with doubly uniparental inheritance (DUI), a condition in which two mitochondrial lineages are present: one following a matrilinear inheritance (F-type) and the other following a patrilinear inheritance (M-type). DUI has been reported so far in ~100 bivalve species. Due to such distinct transmission patterns, the mitochondrial OXPHOS proteins can reach an amino acid sequence divergence of 52% between the F- and M-lineages in the same species. Such divergence, coupled with the natural

heteroplasmy of some tissues, provides a unique opportunity to study mitochondrial inheritance, mitonuclear interactions, and mitochondrial activity in the germline (Milani and Ghiselli 2015). Ghiselli et al. (2019) visualized, for the first time, F- and M-type mitochondrial proteins in the germline and somatic tissues of the DUI species *Ruditapes philippinarum* and found heteroplasmy at the organelle level in undifferentiated germ cells of both sexes and in male soma. On the contrary, as expected, mature gametes turned out to be homoplasmic for the respective sex-specific lineage (F-type in eggs, M-type in sperm). This finding supports a previously advanced hypothesis, according to which the transition from heteroplasmy to homoplasmy would be due to a process of meiotic drive (Milani et al. 2015, 2016). In the light of the new data, Ghiselli et al. (2019) propose a revised model for the DUI system, discussing the interactions of mitochondria with germ plasm and their role in germline development.

Environmental adaptation

The coevolution of the mitochondrial and nuclear genomes not only maintains current metabolic capacities in a population of organisms, but novel mitonuclear combinations can also potentially facilitate adaptation to the environment including environments that would otherwise induce stress. Sokolova et al. (2019) discuss the known and putative mechanisms involved in mitochondrial tolerance to fluctuating oxygen conditions in hypoxia tolerant organisms (predominantly ectotherms). Oxygen fluctuations are an ultimate mitochondrial stressor, and adaptations to hypoxia involve mechanisms that maintain mitochondrial integrity and protein homeostasis, regulate O_2 flux and redox balance during hypoxia, and ensure rapid recovery of the OXPHOS capacity upon reoxygenation. These adaptations require concerted adjustments in mitochondrial- and nuclear-encoded proteins, suggesting that mitonuclear interactions might play a key role in evolution of hypoxia tolerance. However, this aspect of mitonuclear interactions is currently understudied.

In the context of environmental adaptation, McKenzie et al. (2019) studied clines in mitochondrial genotypes in the Atlantic killifish (*Fundulus heteroclitus*). Such clines in mtDNA can be produced as a result of a variety of processes. They are sometimes the result of neutral processes such as secondary contact or sex-biased gene flow. But they can also be the product of extrinsic selection driven by benefits of local adaptation along environmental gradients or by intrinsic factors such as mitonuclear incompatibilities. Distinguishing among these potentially interacting possibilities requires an integrated approach combining population genomic data for both the mitochondrial and nuclear genomes and assessments of mitochondrial function. Atlantic killifish provides a case study where all of these types of data are available. There is a steep cline in mtDNA in this species, through an environmental temperature gradient along the Atlantic coast of North America. There is substantial evidence for the role of neutral demographic forces such as secondary contact in shaping this cline, but there is also evidence of putatively adaptive differentiation in mitochondrial function. Population genomic data reveal signals of weak selection, and potential evidence of mitonuclear incompatibility, but overall this case study highlights the challenges of distinguishing the various factors that can shape geographic variation in mtDNA sequence. This highlights the importance of integrated studies of mitochondrial ecophysiology.

Speciation

Uniparental inheritance and selection pressures act to maintain the tightly coordinated activity of nuclear and mitochondrial genes. For this reason, although these genomes are physically distinct from one another, alleles within them are often found in LD within a population (i.e., mitonuclear LD). This tight linkage is thought to be a consequence of coadaptation between nuclear and mitochondrial genomes within organisms. Divergence in uniquely coadapted sets of *mt* and *N-mt* genes is proposed to be a process of speciation (Gershoni et al. 2009; Burton and Barreto 2012; Hill 2016, 2017). This hypothesis clearly predicts that there should be little flow of mitochondrial genotypes between species (Sloan et al. 2017). The observation of rampant replacement of the mitochondrial genotype of one species with the mitochondrial genotype of another species, particularly when there is little or no change in the nuclear genotypes of either the donor or recipient taxa (Toews and Brelsford 2012), poses a major challenge to the mitonuclear compatibility species concept. Hill (2019b) reviews the evidence for rampant mitochondrial introgression and presents hypotheses for potential benefits of mitochondrial introgression that could theoretically offset the costs of loss of tight mitonuclear coadaptation.

Tobler et al. (2019) further consider a role for mitonuclear genomic interactions in the process of speciation by presenting an overview of the various mechanisms by which mitochondria could

contribute to the evolution of reproductive isolation during ecological speciation. They emphasize cases where divergent selection on mitochondrial function between closely related lineages might be expected to contribute to the formation of various reproductive isolating barriers as a byproduct of adaptation. They highlight key gaps in understanding of the importance and generality of mitochondrially-mediated reproductive isolation at various stages of the speciation process and how such mechanisms interact with other reproductive isolation barriers. They conclude that approaches to the study of mitonuclear speciation that include experimental evolution and a focus on parallel adaptation could help to further our understanding of how reproductive isolation emerges as mitochondria evolve.

Integrative study of mitonuclear interactions

The biggest conclusion from our symposium is that studying mitonuclear interactions demands an integrative framework. The papers in this volume use a wide range of approaches: examining individual residue–residue interactions between mitochondrial and nuclear proteins, using genomic data to map clines in mtDNA and statistical associations between mitochondrial and nuclear genotypes, estimating mutation rates, performing detailed biochemical and physiological characterization of mitochondria, quantifying whole animal life history and physiology, and characterizing patterns of inheritance and heteroplasmy across populations. The implications are equally broad: evolution of genomes, patterns of development, adaptation to different environments, reproductive isolation among populations, and aging processes may all be shaped by mitonuclear interactions. The challenge for mitonuclear biologists will be to unite these diverse approaches and fields in a coherent framework.

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