Title

Assessing the fitness consequences of mitonuclear interactions in natural populations

Journal

Biological Reviews, 94(3)

ISSN

1464-7931

Authors

Hill, Geoffrey E
Havird, Justin C
Sloan, Daniel B
et al.

Publication Date

2019-06-01

DOI

10.1111/brv.12493

Peer reviewed

Assessing the fitness consequences of mitonuclear interactions in natural populations

Geoffrey E. Hill[1*], Justin C. Havird[2], Daniel B. Sloan[3], Ronald S. Burton[4], Chris Greening[5], and Damian K. Dowling[5]

[1]*Department of Biological Sciences, 101 Life Sciences Building, Auburn University, Auburn, AL 36849, USA*

[2]*Department of Integrative Biology, The University of Texas, 2415 Speedway #C0930, Austin TX, 78712, USA*

[3]*Department of Biology, Colorado State University, USA Department of Biology, 1878 Campus Delivery, Colorado State University, Fort Collins, CO 80523, USA*

[4]*Marine Biology Research Division, Scripps Institution of Oceanography, University of California, San Diego, La Jolla, CA 92093, USA.*

[5]*School of Biological Sciences, 18 Innovation Walk, Monash University, Melbourne, VIC 3800, Australia*

Short title: fitness consequences of mitonuclear interactions

[*]Author for correspondence (E-mail: ghill@auburn.edu: Tel.:334 844 9269).

ABSTRACT

Metazoans exist only with a continuous and rich supply of chemical energy from oxidative phosphorylation in mitochondria. The oxidative phosphorylation machinery that mediates energy conservation is encoded by both mitochondrial and nuclear genes, and hence the products of these two genomes must interact closely to achieve coordinated function of core respiratory processes. It follows that selection for efficient respiration will lead to selection for compatible combinations of mitochondrial and nuclear genotypes, and this should facilitate coadaptation between mitochondrial and nuclear genomes (mitonuclear coadaptation). Herein, we outline the modes by which mitochondrial and nuclear genomes may coevolve within natural populations, and we discuss the implications of mitonuclear coadaptation for diverse fields of study in the biological sciences. We identify five themes in the study of mitonuclear interactions that provide a roadmap for both ecological and biomedical studies seeking to measure the contribution of intergenomic coadaptation to the evolution of natural populations. We also explore the wider implications of the fitness consequences of mitonuclear interactions, focusing on central debates within the fields of ecology and biomedicine.

Key words: mitochondria, coadaptation, coevolution, epistatic interactions, gene flow, speciation, mitochondrial medicine, fitness.

CONTENTS

I. INTRODUCTION

Life depends on efficient production of useable energy. The substantial energy needs of most metazoans are met by the oxidative phosphorylation (OXPHOS) system embedded within the inner membrane of mitochondria, which produces approximately 90% of the ATP available to cells (Lane & Martin, 2010). The enzyme complexes that mediate OXPHOS are comprised of

numerous polypeptide subunits. Most of these subunits are encoded by nuclear genes and are transported into mitochondria. However, multiple proton-translocating subunits (13 in bilaterian animals) are encoded by the mitochondrial DNA (mtDNA) (Bar-Yaacov, Blumberg & Mishmar, 2012). Consequently, energy production in eukaryotes must rely on a critical set of interactions between genes that span two distinct genomes (Rand, Haney & Fry, 2004; Wolff *et al.*, 2014; Hill, 2015).

Because the products of mitochondrial genes play a key role in enabling core respiratory processes, it was long assumed that variants that appeared within the mtDNA sequence would be quickly removed by purifying selection (Avise, 2004). This assumption has been supported by analyses of ratios of nonsynonymous (amino acid-changing) to synonymous (putatively silent) mutations across key mitochondrial genes (mt genes) of metazoans (Rand, 2001; Stewart *et al.*, 2008*a*; Nabholz, Ellegren & Wolf, 2013; Popadin *et al.*, 2013; Zhang & Broughton, 2013). Accordingly, a generation of evolutionary biologists, from the 1980s onwards, worked under the purview that the mitochondrial sequence variation segregating within or among populations was selectively neutral (Ballard & Whitlock, 2004; Dowling, Friberg & Lindell, 2008; Ballard & Pichaud, 2014).

By the mid-1990s, however, several studies had emerged that refuted the strict neutrality of variation in mtDNA sequence (Ballard & Kreitman, 1994; Nachman, Boyer & Aquadro, 1994; Nachman *et al.*, 1996; Rand, Dorfsman & Kann, 1994; Pichaud *et al.*, 2012). In subsequent years, a series of experimental studies highlighted numerous cases in which the genetic variation found within the mitochondrial genome was clearly non-neutral (i.e. functional), with pervasive effects on metabolic function (Willett, 2008; Arnqvist *et al.*, 2010; Pichaud *et al.*, 2012; Barreto & Burton, 2013a; Bock, Andrew & Rieseberg, 2014; Wolff *et al.*, 2016) and the expression of

life-history traits (James & Ballard, 2003; Rand, Fry & Sheldahl, 2006; Clancy, 2008; Dowling *et al.*, 2009; Dowling, Meerupati & Arnqvist, 2010; Ma *et al.*, 2016; Roux *et al.*, 2016). Furthermore, these non-neutral mitochondrial effects often exhibited evidence of epistatic interactions with nuclear genes (Dobler *et al.*, 2014; Wolff *et al.*, 2014), consistent with the premise that interactions between mitochondrial and nuclear genomes drive the functionality of OXPHOS.

II. EVIDENCE FOR COEVOLUTION OF MITOCHONDRIAL AND NUCLEAR GENOMES

Research efforts have since aimed to dissect the evolutionary mechanisms that generate functional mitochondrial variation, and much emphasis has been placed on the potential for accumulation of mildly deleterious mutations in mtDNA (Lynch & Blanchard, 1998; Neiman & Taylor, 2009). The notion of a high mitochondrial mutation load runs contrary to the expectation that strong purifying selection would effectively prevent the accumulation of non-neutral variants. However, studies in mutant mouse models have suggested that purifying selection may only be fully effective at removing non-synonymous mtDNA mutations from the female germ line when these mutations confer severely pathogenic effects (Fan *et al.*, 2008; Stewart *et al.*, 2008*b*, *a*). Mitochondrial mutations of moderate effect, including those in transfer RNA (tRNA) and ribosomal RNA (rRNA) genes, have been reported to escape selection and be transmitted across generations (Alston *et al.*, 2017; Barreto *et al.*, 2018). When combined with the observation that mitochondrial genes of many eukaryotes mutate at much higher rates than nuclear genes (Brown, George & Wilson, 1979; Lynch, 1997; Smith & Keeling, 2015; Havird & Sloan, 2016), and that these mutations reside in a genome that has traditionally been thought to

115 experience very low rates of recombination (i.e. an efficient mechanism of preventing mutational
116 accumulation) (Hagström *et al.*, 2014), there would appear to be ample opportunity for mutations
117 to accumulate and contribute to functional mitochondrial variation.

118 If left unchecked, mutational erosion of the mitochondrial genome would quickly lead to
119 degradation of energy production and metabolic homeostasis (Lynch & Blanchard, 1998). It is
120 therefore theorized that mutational erosion should create selection for nuclear genotypes able to
121 offset the negative metabolic effects caused by mtDNA mutations (Rand *et al.*, 2004). Indeed,
122 several studies have now identified signatures of complementary changes in interacting nuclear-
123 encoded genes that have evolved in conjunction with sequence changes in the mitochondrial
124 genome (Osada & Akashi, 2012; Barreto & Burton, 2013*b*; Sloan *et al.*, 2014; Havird *et al.*,
125 2015*b*, 2017; Van Der Sluis *et al.*, 2015; Barreto *et al.*, 2018; Yan, Ye & Werren, 2018). These
126 tandem changes appear consistent with a model of compensatory mitonuclear coevolution. Under
127 this model, the mitochondrial genome would provide a mutational pressure that precipitates
128 coadaptation between mitochondrial and nuclear genomes within populations (Ellison & Burton,
129 2008*b*; Barreto & Burton, 2013*b*; Yee, Sutton, & Dowling, 2013; Havird *et al.*, 2015*b*).

130 The importance of compensatory coevolution between the nuclear and mitochondrial
131 genomes is, however, a topic of current debate (Sloan, Havird & Sharbrough, 2017), and the
132 argument that an asexual and uniparental mode of inheritance makes mtDNA prone to
133 deleterious mutation accumulation has been criticized on both empirical and theoretical grounds
134 (Popadin *et al.*, 2013; Zhang & Broughton, 2013; Cooper *et al.*, 2015; Christie & Beekman,
135 2016). Long-held views regarding the effective haploidy, low effective population size, and
136 inefficient selection of the mitochondrial genome are being challenged (Ballard & Whitlock,
137 2004; Cooper *et al.*, 2015). Indeed, the fact that mtDNA molecules exist in hundreds to

thousands of copies per cell suggest it might be better viewed as a polyploid genome (Greaves & Taylor, 2006). Across eukaryotes, there are variable rates of biparental inheritance of mtDNA and at least occasional recombination between divergent mtDNA molecules (Greiner, Sobanski & Bock, 2015; Ma & O'Farrell, 2015). In bilaterian animals, there is also a genetic bottleneck in mtDNA copy number through the germ line (Stewart & Larsson, 2014), which could provide an effective means by which selection can effectively purge primordial germ cells carrying mtDNA molecules with pathogenic mutations (Burr, Pezet & Chinnery, 2018). Together, these factors (polyploidy, some degree of biparental inheritance, recombination, and a genetic bottleneck during oogenesis) are providing new insights into the dynamics of selection that shape trajectories of mitochondrial genome evolution.

There is also growing interest in the role of *adaptive* changes in mitochondrial genomes, and recent research suggests that a substantial fraction of non-synonymous substitutions in mitochondrial genes may be driven by positive selection (James, Piganeau & Eyre-Walker, 2016). Because of the intimate functional integration between nuclear and mitochondrial genomes, adaptive changes in mtDNA are likely to have epistatic effects and shift selection pressures on the nuclear genome. Indeed, many examples that have been interpreted as supporting a model of compensatory mitonuclear coevolution (Osada & Akashi, 2012; Barreto & Burton, 2013*b*; Sloan *et al.*, 2014; Havird *et al.*, 2015*b*, 2017; Van Der Sluis *et al.*, 2015; Yan *et al.*, 2018) are also consistent with other forms of mitonuclear coevolution that do not depend on the accumulation of deleterious mitochondrial mutations (Sloan *et al.*, 2017). Regardless of the relative contributions of deleterious, neutral, and beneficial changes in triggering the coevolutionary process, natural selection is expected to favour beneficial combinations of alleles

spanning mitochondrial and nuclear genomes and give rise to coadapted mitonuclear genotypes (Rand *et al.*, 2004; Burton, Pereira & Barreto, 2013; Wolff *et al.*, 2014; Hill, 2015).

Herein, we emphasize that further research attention is required to decipher the biological significance of mitonuclear interactions. Despite the ubiquity of co-functioning mitochondrial and nuclear genes, our understanding of the contribution of mitonuclear genetics to metazoan fitness remains incomplete, and most insights are from laboratory-based studies of model organisms. A focus on mitonuclear coadaptation is likely to contribute tangibly to our understanding of basic ecological concepts, such as speciation and the dynamics of sexual conflict (Hill, 2015; Wolff *et al.*, 2016). Furthermore, the implications of such interactions might resonate beyond the evolutionary and ecological sciences, into the realm of biomedicine (Mishmar & Zhidkov, 2010; Wallace, 2010; Dowling, 2014; Gershoni *et al.*, 2014). To inform future research directions, we identify and discuss five themes that have emerged from the study of mitonuclear interactions over the past two decades (Table 1).

II. IMPLICATIONS OF MITONUCLEAR INTERACTIONS SPANNING ECOLOGY AND BIOMEDICINE

It has been proposed that the necessity for mitonuclear coadaptation for cellular respiration may underlie a range of core evolutionary innovations and concepts. These include the evolution of sex and two sexes in eukaryotes (Hadjivasiliou *et al.*, 2013; Havird, Hall & Dowling, 2015*a*), the evolution of a sequestered germ line in bilaterian animals (Radzvilavicius *et al.*, 2016), climate and resource adaptation (Camus *et al.*, 2017; Sunnucks *et al.*, 2017), sexual selection (Hill & Johnson, 2013; Hill, 2018), and speciation (Dowling *et al.*, 2008; Burton & Barreto, 2012; Hill, 2016).

The role of mitonuclear interactions in mediating the process of speciation is currently a major area of scientific research and debate (Hill, 2017; Sloan *et al.*, 2017). It has been proposed that independent coevolution of mt and nuclear genes in isolated populations could lead to uniquely coadapted sets of genes that are not compatible with the coadapted mt and nuclear genes of other populations. If this is the case, gene flow and hybridization events between diverging populations could produce negative phenotypic outcomes due to Dobzhansky–Muller incompatibilities underpinned by mitonuclear interactions (Levin, 2003; Dowling *et al.*, 2008; Gershoni, Templeton & Mishmar, 2009; Burton & Barreto, 2012; Hill, 2017). In theory, therefore, population divergence driven by mitonuclear interactions represents a plausible model underlying the evolution of reproductive isolation between incipient populations, and ultimately speciation. However, the hypothesis that mitonuclear coadapation plays a direct and general role in driving speciation processes remains controversial and requires further investigation (Gershoni *et al.*, 2009; Chou & Leu, 2010; Burton & Barreto, 2012; Bar-Yaacov *et al.*, 2015; Eyre-Walker, 2017; Hill, 2017; Sloan *et al.*, 2017).

The fitness consequences of mitonuclear interactions are also relevant for human medicine. Ongoing research is exploring the significance of diverse mitochondrial haplotypes across human populations (Mishmar *et al.*, 2003; Wallace, 2010). An emerging research focus in biomedicine considers whether human phenotypes are dependent on the nuclear background in which mitochondrial haplotypes are expressed (Levin *et al.*, 2014). Notably, mitonuclear interactions have also been implicated as putative contributors to health outcomes associated with the emerging germline therapy of mitochondrial replacement (Reinhardt, Dowling & Morrow, 2013; Morrow *et al.*, 2015; Dobler *et al.*, 2018) (Fig. 1). Mitochondrial replacement is a modified form of *in vitro* fertilization that could enable prospective mothers that suffer from

mtDNA-induced mitochondrial diseases to produce offspring that are free from the mother's mtDNA mutations (Tachibana *et al.*, 2009, 2013; Craven *et al.*, 2010). The technique pairs a patient's nuclear chromosomes, or fertilized pronuclei, with a healthy complement of donor mitochondrial genes inside the donor's oocyte. Concerns have been raised, however, that the approach may create novel combinations of patient nuclear genotype and donor mtDNA haplotype that have not been previously tested by natural selection and that may lead to unanticipated negative outcomes (Morrow *et al.*, 2015; Dobler *et al.*, 2018). The potential negative effects of producing novel mitonuclear combinations in human oocytes remain widely debated (Reinhardt *et al.*, 2013; Chinnery *et al.*, 2014; Morrow *et al.*, 2015; Sloan, Fields & Havird, 2015; Eyre-Walker, 2017; Rishishwar & Jordan, 2017; Zaidi & Makova, 2018), with a recent meta-analysis presenting evidence that suggests mitonuclear interactions are likely to affect health outcomes in humans, and indeed seem to be associated with stronger effect sizes in humans than other metazoans (Dobler *et al.*, 2018).

III. EMERGING THEMES IN STUDIES OF MITONUCLEAR COADAPTATION

An improved understanding of mitonuclear evolutionary dynamics is required to elucidate the role of mitonuclear interactions in ecological processes such as speciation and biomedical procedures like mitochondrial replacement therapy. Below we detail five themes that we believe should guide future research in this field. These themes have not been synthesized previously into a single framework or readily acknowledged in the current literature. However, we believe each of these themes deserves consideration, particularly when applied to inferences from natural populations and to implications beyond the fields of ecology and evolution.

(1) Theme 1: selection for mitonuclear compatibility should be strong and should exist across all stages of ontogeny

Selection for efficient mitochondrial function, and hence mitonuclear compatibility, is expected to be intense, and it might well begin as early as oogenesis, with massive selection on cells in the germ line (De Fanti *et al.,* 2017; Krakauer & Mira, 1999; Fan *et al.*, 2008; Stewart *et al.*, 2008*a*; Dowling, 2014; Radzvilavicius *et al.*, 2016). When there are multiple mitochondrial genotypes per cell, natural selection becomes inefficient in either eliminating deleterious genotypes or promoting highly functional genotypes (Radzvilavicius *et al.*, 2016). In bilaterian animals, germ line selection on mt genotypes is therefore seemingly facilitated by a well-documented bottleneck in the number of mtDNA molecules per primary oocyte (Wai, Teoli & Shoubridge, 2008; Stewart & Larsson, 2014). This mitochondrial genetic bottleneck enables natural selection to screen oocytes based on their metabolic integrity, underpinned by their mtDNA genotype (which should be fairly homogenous due to the bottleneck) and modulated by an effect of the diploid nuclear genomic background (Cree *et al.*, 2008; Wai *et al.*, 2008). During these stages, there is also a massive cull in the population of oocytes *via* a process called atresia. Together, this provides two levels by which selection might screen for best-functioning mitochondria. Emerging evidence supports the contention that the mitochondria are active within primordial germ cells and developing oocytes (Ge *et al.*, 2012; Kasashima, Nagao & Endo, 2014; Hayashi *et al.*, 2017). Thus, we hypothesize that only those oocytes with full capacity for efficient respiratory function, requiring compatible mitonuclear genotypes, reach maturity (Dumollard, Duchen & Carroll, 2007; Stewart & Larsson, 2014). Germ line selection could well represent a core mechanism favouring the transmission of compatible mitonuclear genotypes across generations (Lane, 2005; Morrow *et al.*, 2015; Radzvilavicius *et al.*, 2016), preventing the inter-

252 generational mutational meltdown of the mitochondrial genome predicted by theory (Lynch *et*
253 *al.*, 1993; Lynch & Blanchard, 1998; Stewart *et al.*, 2008*a*; Cooper *et al.*, 2015), but more data
254 are needed to assess the importance of selection on germ lines. Furthermore, this process can be
255 completed within the developing female foetus during embryogenesis. In humans, this occurs
256 decades before the female is likely to mate and produce her own offspring.
257 In addition, there may be considerable opportunity for selection to act on mitonuclear
258 interactions during the development of cells from spermatogonia to mature sperm cells, but this
259 topic seems not to have been investigated. In humans, a single ejaculate contains approximately
260 10^8 sperm, with only one sperm fertilizing an egg. Again, the difference between a successful
261 and unsuccessful sperm is not random, and strong postcopulatory selection will act on the male
262 ejaculate (Simmons, 2001). Swimming speed and endurance, capacity to cope with chemical
263 barriers, and ability to penetrate the egg faster than competitors dictate success (Snook, 2005;
264 Pizzari, 2009), and sperm derive at least part of the energy that underlies these functions from
265 OXPHOS (Ruiz-Pesini *et al.*, 2007). With few exceptions (Barr, Neiman & Taylor, 2005), the
266 mitochondria that power sperm are not transmitted to offspring. By contrast, the paternal nuclear
267 genome, which includes over 1000 nuclear-encoded mitochondrial (N-mt) genes, is transmitted
268 (Calvo & Mootha, 2010). These N-mt genes create the majority of the mitochondrial proteome,
269 and many interact closely with the mitochondrial-encoded gene products, such that strong
270 selection on mitochondrial function will plausibly lead to strong selection on paternal N-mt
271 genotypes in the sperm that enable cofunction with the common mt genotype of that population.
272 Selection for mitonuclear compatibility should then continue at every developmental stage
273 following fertilization (Chan, 2006; Latorre-Pellicer *et al.*, 2016). For instance, in mammals, not
274 all zygotes will successfully implant in the uterine wall; many developing embryos are

spontaneously aborted; many individuals die during early development; and only a portion of the offspring born into the population will survive to reproductive maturity and succeed in procuring a mate and ultimately in producing viable offspring themselves. The chances of surviving through all of these stages of selection are minimal. The haploid gamete that survives pre-zygotic selection, and then as a diploid genome survives all post-zygotic phases of selection thrown its way, can be considered a one-in-a-million winner in the lottery of life. We propose that at each of these life stages, selection for mitonuclear compatibility could be key. That is, although mitochondrial and nuclear alleles are unlinked and segregate randomly, strong selection through ontogeny could ensure that each adult in the population harbours a fully compatible mitonuclear genotype. The majority of the competition that underlies such selection is, however, difficult to detect unless one makes extremely careful and detailed observations across life stages. Such studies should be a priority for future research.

(2) Theme 2: mitonuclear coadaptation is manifested in mitochondrial physiology

Under a model of mitonuclear coadaptation, mismatching of coevolved mitonuclear genomes should lead to reduced organismal fitness specifically due to compromised mitochondrial function and disturbed bioenergetics (Gershoni *et al.*, 2009). However, given that validation can be operationally very challenging, observed dysfunction in crosses between divergent populations is seldom linked to specific mitonuclear incompatibilities and loss of mitochondrial function. Most experimental designs aimed at disrupting coevolved mitonuclear genotypes, such as crossing individuals from genetically divergent populations, also disrupt coevolved epistatic combinations of nuclear genes, and in such studies nuclear–nuclear rather than mito–nuclear gene interactions might have the largest effect on organismal fitness. While reciprocal crossing

298 designs go some way towards removing the confounding effects of nuclear–nuclear interactions,
299 by shifting the focus onto putative mitonuclear interactions, such designs come with a caveat in
300 species with genetic sex determination because the genotype of offspring of the heterogametic
301 sex will differ across each of the reciprocal crosses. Furthermore, offspring produced by these
302 crosses are prone to effects from other extranuclear sources of variance, such as differences in
303 the microbiome profiles of the mothers or, in some arthropods, cytoplasmic incompatibility
304 caused by *Wolbachia* infection (Werren, Baldo & Clark, 2008; Schaefer, Nadeau & Wray, 2015).
305 One method for isolating mitonuclear effects is to replace the mtDNA from one lineage with
306 the mtDNA from another lineage thereby creating novel mitonuclear gene combinations. Such
307 genomic rearrangement can be achieved either by backcrossing over multiple generations (taking
308 advantage of the maternal inheritance of mtDNA, but biparental inheritance of nuclear genes) or
309 by using genetic tools that suppress recombination to enable chromosome substitution across
310 generations (Dowling *et al.*, 2008). The outcome of such manipulations is the creation of a
311 genetic strain of organism that possesses a novel mitonuclear genotype, in an otherwise intact
312 diploid nuclear background. This combination of approaches allows the researcher to home in on
313 the role of mitonuclear interactions in maintaining organismal function. Such approaches are,
314 however, only possible in study species that are easily propagated in the laboratory environment
315 and that have short generations.
316 One key prediction that can be tested in natural populations is that mitonuclear
317 incompatibilities should have disproportionate effects on mitochondrial function. Moreover,
318 mitochondrial physiology should be affected in a predictable manner according to the specific
319 components that are influenced by incompatibilities (Burton *et al*., 2013). A prime testing ground
320 for assessment of mitonuclear effects distinct from nuclear–nuclear effects is comparison

between OXPHOS complexes composed of both mt- and nuclear-encoded subunits, and complexes with only nuclear-encoded subunits. In many eukaryotes, Complex II (succinate dehydrogenase) is made up entirely of nuclear-encoded proteins, while other complexes responsible for OXPHOS function are chimeric assemblies of nuclear- and mitochondrial-encoded proteins (Rand *et al.*, 2004) (Fig. 2). Complex II function should therefore remain stable (or may even increase as a compensatory measure) regardless of altered mitonuclear interactions, while Complex I (Nicotinamide adenine dinucleotide-dehydrogenase) and Complex IV (cytochrome *c* oxidase) activity are predicted to vary with the mitochondrial genomic background. This prediction was supported in studies with copepods and fruit flies in which mitochondrial and nuclear genotypes from divergent populations were introgressed. In the fruit fly experiment, when hybrids were created that carried nuclear-encoded genes for a tRNA synthetase from *Drosophila melanogaster* and mt-encoded tRNA from *D. simulans*, poor cofunctioning of these non-coadapted mt and N-mt genes caused impairment of translation of mt-encoded OXPHOS subunits with significant effects on Complexes I, III, and IV but no effects on Complex II (Meiklejohn *et al.*, 2013). In the copepod experiment, hybrid crosses between divergent populations of *Tigriopus californicus* reduced activities of OXPHOS Complexes I, III, IV, and V but not Complex II (Ellison & Burton, 2006). These are among the clearest demonstrations of hybrid dysfunction resulting from mito–nuclear interactions because they compellingly indicate that breakdown is only manifested for enzymes under dual control of both nuclear and mitochondrial genomes.

In addition to measuring activities of OXPHOS complexes, a range of other approaches can provide insight into mitochondrial physiology. Notably, production of ATP and generation of reactive oxygen species (ROS) have been shown to vary with mitochondrial genotype (Ellison &

Burton, 2006, 2008*a*; Estes *et al.*, 2011; Barreto & Burton, 2013*a*; Hicks, Denver & Estes, 2013; Barreto, Pereira & Burton, 2015; Latorre-Pellicer *et al.*, 2016). In tractable systems, detailed examination of respiration profiles from isolated mitochondria could also reveal the mechanistic basis for lower fitness in compromised individuals (Chung, Bryant & Schulte, 2017; Mowry *et al.*, 2017; Zhang *et al.*, 2018). While rarely adopted, whole-organism measurements of basal and maximal metabolic rates may also be useful for assessing the fitness consequences of mitonuclear interactions (Sunnucks *et al.*, 2017).

(3) Theme 3: generational delays

Research into the capacity for mitonuclear incompatibilities to evolve in natural populations suggests that, when such incompatibilities emerge, they may not be revealed until F2 and subsequent generations (Burton, Ellison & Harrison, 2006) (Fig. 3). The apparent lack of mitonuclear incompatibilities in the F1 generation from some crosses may arise because offspring receive a full haploid copy of each autosomal chromosome from each parent. Under this model, a full haploid maternal complement of nuclear genes would often be sufficient to maintain mitonuclear-mediated organismal function. As we outlined in Theme 1, a reproductively mature female has survived multifaceted phases of selection, from both pre-fertilization to post-fertilization and across the entire ontogeny. As a result of relentless selection throughout ontogeny, a mother's nuclear genotype should be predicted to function well with her mitochondrial genotype. Therefore, even if the father's nuclear genetic contribution to the offspring genotype exhibits incompatibility with the maternal mitochondrial haplotype, effects of mitonuclear incompatibility may not manifest in the F1 generation because the compatible nuclear alleles provided by the female may mask the effects of less-functional variants provided

by the male (Burton & Barreto 2012; Stelkens, Schmid & Seehausen, 2015). If F1 hybrids are crossed to create F2 hybrids, however, the segregation of diploid nuclear genes will generate recombinant genotypes, with some individuals receiving two paternal copies at a given nuclear locus.

The degree to which incompatibilities are masked in the F1 generation will depend on dominance relationships among alleles (Turelli & Orr, 2000; Raj *et al.*, 2010) because it is expected that both sets of alleles will be expressed in F1s. The degree of masking should also depend on whether the relevant nuclear genes are autosomal or sex-linked (Hill & Johnson, 2013; Hill, 2014). Researchers also face a challenge in separating the effects of any deleterious mitonuclear interactions from the potentially offsetting benefits of heterosis ('hybrid vigour') that are often seen in F1s, and which is attributable to the masking of deleterious recessive mutations within the nuclear genome (Edmands, 2007).

The best description of generational delays in the negative effects of mitonuclear incompatibilities in hybrid crosses comes from studies of the copepod, *T. californicus*, which have no sex chromosomes. When individuals from genetically divergent populations are crossed, F1 offspring typically exhibit a fitness gain relative to the parental populations. In F2 and later-generation recombinants, however, hybrids suffer a fitness cost. Furthermore, full fitness is restored when F2 hybrid females are backcrossed to males from the maternal lineage (Ellison & Burton, 2008*b*). The fitness advantage in the F1 generation in this example can be ascribed to heterosis associated with the creation of offspring exhibiting genome-wide heterozygosity in the diploid nuclear genome. There appear to be no negative effects of interpopulation hybridization at this F1 stage. Yet, F2 offspring clearly have reduced fitness relative to parental populations,

389 and given that fitness outcomes are only restored upon backcrossing with the maternal
390 population, this implicates mitonuclear incompatibilities as drivers of these effects (Fig. 3).
391
392 **(4) Theme 4: mitonuclear incompatibilities can be created by single base substitutions and**
393 **are not limited to protein-encoding genes**
394 The overall genetic divergence of individuals can be a misleading index of mitonuclear
395 compatibility. Many of the changes that distinguish mitochondrial haplotypes are likely to be
396 neutral or nearly so. Only a subset of nuclear gene products are transported to the mitochondrion,
397 and only a small proportion of these nuclear genes whose products function in mitochondria will
398 interact closely with mitochondrial gene products (Burton & Barreto, 2012; Aledo *et al.*, 2014).
399 Thus, if mt and N-mt genes are under strong purifying selection, then there is clearly a potential
400 for substantial divergence in both nuclear and mitochondrial nucleotide sequences with no loss
401 of mitonuclear compatibility. Moreover, given many of the nuclear gene products that function
402 in the mitochondrion also have functions outside of the mitochondrion (Burak *et al.*, 2013;
403 Blumberg *et al.*, 2014; Chatterjee *et al.*, 2016), evolutionary changes to such proteins could be
404 driven by selection that is not related to mitonuclear coadaptation. Conversely, numerous
405 examples now support the contention that mitonuclear incompatibilities leading to loss of fitness
406 could be brought about by a few key changes to either the mitochondrial or nuclear genotype
407 (Aledo *et al.*, 2014; Camus *et al.*, 2015). Indeed, single point mutations in nuclear and
408 mitochondrial genes have been shown to be the basis for mitochondrial dysfunction in
409 heterospecific hybrid crosses (Meiklejohn *et al.*, 2013).
410 To date, most studies on mitonuclear interactions have focused on protein–protein
411 interactions in OXPHOS complexes co-encoded by nuclear and mitochondrial genomes (e.g.

412 (Kwong *et al.*, 2012; Osada & Akashi, 2012; Zhang & Broughton, 2013; Havird *et al.*, 2015*b*).
413 However, the biochemical machinery that enables translation of mitochondrial genes has both
414 nuclear protein components and mitochondrial RNA components, namely tRNAs and rRNAs
415 (Fig. 2B, C). The mitochondrial-encoded RNA components must co-function with nuclear-
416 encoded proteins (Wallace, 2007; Bar-Yaacov *et al.*, 2012; Burton & Barreto, 2012; Sloan *et al.*,
417 2014). The replication and transcription of the mitochondrial genome also involves the
418 interaction of nuclear-encoded proteins with the mtDNA itself (Ellison & Burton, 2008, 2010*a*)
419 (Fig. 2D).

420 Epistasis may occur even when there is no physical interaction of gene products because the
421 coordinated function of mitochondrial and nuclear gene products depends critically on retrograde
422 (mitochondria to nucleus) and anterograde (nucleus to mitochondria) signalling (Moore &
423 Williams, 2005; Woodson & Chory, 2008; Clark, Alani & Aquadro, 2012; Monaghan &
424 Whitmarsh, 2015; Baris *et al.*, 2017). Such signalling requires that both genomes correctly
425 recognize and respond to signals from each other. It should also be noted that new types of
426 mitonuclear interactions are still being discovered; for instance, there is some evidence that mt
427 genomes encode small RNAs (Pozzi *et al.*, 2017) and several small peptides, such as humanin
428 and mitochondrial open reading frame of the 12S rRNA-c (MOTS-c), are encoded by the mt
429 genome (Lee, Yen & Cohen, 2013; Lee *et al.*, 2015). These newly discovered mitochondrial
430 products could play an important role in retrograde signalling. In sum, mitonuclear
431 incompatibilities can be caused by more than just compromised protein–protein interactions.
432

433 **(5) Theme 5: mitonuclear coadaptation is dependent on complex genotype x genotype x**
434 **environment interactions**

There is a tendency to catalogue mitonuclear gene combinations as either high or poorly performing in terms of their phenotypic effects. However, the phenotype that results from any given mitochondrial and nuclear genotype combination will also depend critically on the environment. This view is supported by laboratory studies of invertebrate models, which have confirmed that the performance associated with particular mitonuclear genotypes is routinely contingent on the environmental context (Ellison & Burton, 2006; Dowling, Abiega & Arnqvist, 2007; Dowling *et al*., 2010; Arnqvist *et al*., 2010; Hoekstra, Siddiq & Montooth, 2013; Zhu, Ingelmo & Rand, 2014; Mossman *et al*., 2016; Willett & Burton, 2001, 2003). As an example, in crosses between the fruit flies *D. melanogaster* and *D. simulans*, incompatibilities in the products of mt and nuclear genes slow down larval development and reduce survival at 25°C but have no effect on growth or survival at 16°C (Hoekstra *et al*., 2013).

In addition, studies of spatial variation in mitochondrial haplotype distributions in humans and other metazoans in their natural environments demonstrate that mutational patterns at key protein-coding genes within the mtDNA sequence closely conform to patterns predicted under a scenario of climatic adaptation (Mishmar *et al.*, 2003; Ruiz-Pesini, 2004; Balloux *et al.*, 2009; Cheviron & Brumfield, 2009; Quintela *et al.*, 2014; Silva *et al.*, 2014; Morales *et al.*, 2015; Camus *et al.*, 2017). Indeed, a growing view has emerged that environmental context dependency in mitochondrial disease expression is likely to be common in humans (Mishmar *et al.*, 2003; Wallace, 2005).

For example, emerging evidence supports a role for climatic adaptation in shaping population frequencies of a mtDNA mutation (T3394C) associated with Leber's hereditary optic neuropathy (LHON) in humans, a mitochondrial disease that causes blindness, and which is associated with male biases in penetrance. Ji *et al*. (2012) reported that although T3394C has

arisen multiple times across the human mitochondrial phylogeny, it is highly enriched on haplotypes that are common in high-altitude Asian populations (M9 haplotype in Tibet, and the C4a4 haplotype in the Indian Deccan Plateau). Indeed, the T3394C variant is 22 times more likely to be found at altitudes above 1500 m than among low-altitude Han Chinese populations. Furthermore, functional analyses of transmitochondrial cybrid lines have shown that this mutation causes reductions in mitochondrial complex I activity of between 7 and 28% when expressed on the lowland BC4 and F1 Asian haplotypes, but no such reductions on the M9 haplotype (Ji *et al*., 2012). This result, when coupled with the observations of spatial enrichment of the T3394C variant in high-altitude populations, lends support to the suggestion that the 3394C mutation could well be adaptive at high altitudes, while pathological at low altitudes.

Finally, we note that it is likely that the outcomes of mitonuclear interactions will vary across the sexes, although research into this contention is still in its relative infancy. The two sexes represent very different environments in which mitochondria must function. For example, the female gonads and gamete are metabolically quiescent relative to their male counterparts (Short, 1997; Vaught & Dowling, 2018), with the gametes exhibiting striking differences in both size and mtDNA copy number. Because mitochondria are transmitted through the female lineage, mitochondrial mutations that are male-harming can in theory escape the action of natural selection. Such mutations can therefore increase and linger in populations and be fixed through neutral mechanisms or even spread through positive selection if they confer fitness advantages to females (Frank & Hurst, 1996; Gemmell, Metcalf & Allendorf, 2004; Beekman, Dowling & Aanen, 2014). This ‘mother’s curse’ may be key to explaining why mitochondrial diseases such as LHON exhibit much higher penetrance in males (Yen, Wang & Wei, 2006; Ventura *et al.*, 2007; Milot *et al.*, 2017). Experimental evidence has emerged to indicate that some of the

genetic variation that delineates naturally occurring mtDNA haplotypes in fruit flies (*D. melanogaster*) may exhibit male biases in effects on key life-history traits tied to reproduction and survival (Innocenti, Morrow & Dowling, 2011; Camus, Clancy & Dowling, 2012; Camus *et al.*, 2015; Dowling, Tompkins & Gemmell, 2015; Immonen *et al.*, 2016; Camus & Dowling, 2018). Indeed, some of this mitochondrial genetic variation appears to be overtly sexually antagonistic, augmenting female reproductive outcomes, at cost to males (Camus & Dowling, 2018). The studies of sex biases in mitochondrial genetic variation conducted to date, however, have all compared the sex-specific fitness effects of mtDNA haplotypes when placed against highly controlled nuclear backgrounds lacking segregating genetic variation. While providing proof-of-concept, future studies will need to establish whether male biases in levels of mitochondrial genetic variation underpinning key life-history traits are replicable across diverse nuclear backgrounds. Nonetheless, other case studies supporting the mother's curse hypothesis have come to light in flies (Patel *et al.*, 2016), mice (Nakada *et al.*, 2006), rabbits (Smith, Turbill & Suchentrunk, 2010) and humans (Martikainen *et al.*, 2017) of mtDNA polymorphisms exerting negative effects exclusively on male components of fertility (Vaught & Dowling, 2018).

V. IMPLICATIONS AND OUTLOOK

(1) Implications for understanding speciation

We strongly advocate consideration of mitonuclear interactions in future studies that seek to understand species boundaries in natural populations. As discussed below, rapid advances in fields such as population genomics and molecular modelling increasingly allow inferences to be made about mitonuclear interactions in wild populations of diverse species. However, a deeper understanding of the fitness consequences of these interactions generally demands manipulative

experiments, such as quantitative genetic crosses and mitochondrial respiration measurements. The practical constraints of working with many vertebrate species such as wild bird populations, which produce few offspring and will not breed in captivity, make such experiments highly challenging if not impossible. It can therefore be instructive to consider the study of speciation in animals, notably wild populations of invertebrates, for which such constraints have been overcome.

The aforementioned splash pool copepod, *Tigriopus californicus*, may be the model system in which the role of mitonuclear interactions has been most completely studied (Burton *et al.*, 2013; Yang *et al.*, 2017; Barreto *et al.*, 2018). Many aspects of these studies have been detailed in the above sections of this review. Here we point out two aspects of the biology of this organism that set the stage for development of mitonuclear coadaptation. First, rates of mtDNA substitution are high: Willett (2012) estimated that the rate of substitutions at synonymous sites in *T. californicus* mtDNA is 55-fold higher than the rate in nuclear genes. Second, *T. californicus* populations show strong population structure. Restricted gene flow among populations has resulted in not only high levels of mtDNA divergence across populations, but also the opportunity for uniquely coadapted nuclear genotypes among populations in response to the extensive mtDNA divergence (Barreto *et al.*, 2018).

Numerous studies of mitonuclear interactions have taken advantage of the *Tigriopus* system and these have been discussed within each of the themes developed above. The importance of considering fitness variation across life stages (Theme 1) can at least partially be addressed in this system by determining allelic frequencies of candidate genes in a sample of newly hatched larvae to their frequencies in surviving adults from the same cohort. In an analysis of the alleles of the cytochrome *c* gene (*cytC*; a nuclear gene encoding a protein essential to

527 OXPHOS function), Willett & Burton (2001) observed expected Mendelian genotypic ratios in
528 F2 hybrid larvae, but in adult animals from the same cross they observed that the ratios were
529 skewed in favour of combinations that restored the population-specific coevolved mitonuclear
530 genotypes. In addition to suggesting that *cytC* has coevolved with mitotype, this type of
531 experiment isolates the form of selection as larval-to-adult viability selection. In this particular
532 case, *in vitro* biochemical experiments and site-directed mutagenesis further verified the
533 functional coevolution of *cytC* with mitotype, and demonstrated that only a single amino acid
534 substitution was needed to change dramatically the functional interaction between a nuclear gene
535 and a mitochondrially co-encoded OXPHOS complex (Rawson & Burton, 2002; Harrison &
536 Burton, 2006) (Theme 4).

537 Although there are clear examples of protein–protein interactions underlying
538 mitochondrial dysfunction in *Tigriopus* hybrids, it is also clear that other types of interactions
539 likely contribute to mitonuclear incompatibilities (Theme 4). Ellison & Burton, (2010) found that
540 population mismatches between mtDNA and mitochondrial RNA polymerase resulted in reduced
541 mtDNA gene expression, and Barreto & Burton (2013*b*) found evidence for coevolution in
542 nuclear-encoded ribosomal proteins that interact with rRNA encoded in the mtDNA. Recent
543 work suggests that there is a genome-wide pattern of elevated rates of evolution among nuclear
544 genes known to interact with mtDNA or its gene products compared to nuclear genes lacking
545 those interactions

546 As discussed earlier, the impact of environmental variation on mitonuclear interactions is
547 often overlooked and can be substantial (Theme 5). Willett & Burton (2003) found that the
548 relative fitness of *cytC* genotypes not only depended on mitotype (see above), but also on the
549 thermal environment. The disruptive effect of population mismatches in mtDNA and

mitochondrial RNA polymerase cited above is accentuated under conditions of osmotic stress when the energetic costs of osmoregulation require an upregulation of mitochondrial ATP synthesis (Ellison & Burton, 2008*a*).

These studies with copepods clearly established the negative effects of incompatibilities between mitochondrial and nuclear genes that arise when populations diverge in allopatry, and the potential for such incompatibilities to disrupt gene flow among populations. The implications for speciation are clear. What remains to be established is the relative importance of mitonuclear interactions compared to other potential mechanisms for disruption of gene flow in the process of speciation, and such broader insights will only come with a consideration of mitonuclear interactions in studies of speciation.

(2) Implications for best practices in mitochondrial replacement therapy

It is now well established that the penetrance of numerous disease-conferring mtDNA mutations is affected by a range of modifier alleles that lie within the nuclear genome (Taanman, 2001; Bykhovskaya *et al.*, 2004; Ballana *et al.*, 2007; Davidson *et al.*, 2009; Luo, Hou, & Yang, 2013; Wang *et al.*, 2015; Morrow & Camus, 2017). Thus, signatures of mitonuclear epistasis, moderating disease penetrance and outcomes, are known to be common in human populations.

To date, discussion of the capacity for mitochondrial replacement therapy to lead to compromised mitonuclear function has focused on (1) the anticipation of problems based on understanding patterns of genetic variation and structure within and among human populations, and (2) an assessment of the outcomes of mitochondrial replacement in animal models and humans. A mitonuclear perspective that draws on insights from studies of natural non-human populations should be helpful in developing best practices for performing mitochondrial

replacement techniques in humans. First, following Theme 1, full assessment of the outcome of any combination of mitochondrial and nuclear genes can only come after a complete lifetime because many of the negative consequences of mitonuclear dysfunction might be late-onset diseases like Alzheimer's or Parkinson's diseases (Hudson *et al.*, 2014).

Moreover, researchers should be skeptical of attempts to draw inferences regarding the potential for mitonuclear incompatibilities in humans from assessments of genome-wide covariation between levels of mtDNA and nuclear divergence in the adult population. This is evident from a recent study that attempted to assess the risk of negative outcomes of mitochondrial replacement therapy in humans based on mitonuclear incompatibilities by observing patterns of mitochondrial haplotype and nuclear introgression in humans (Rishishwar & Jordan, 2017). The researchers used the whole mitochondrial and nuclear genome sequences of 2054 "healthy adult" humans available through the 1KGP project (The 1000 Genomes Project Consortium, 2015), which included "five major continental population groups", to assess the possibility that there may be incompatibilities between some mt and N-mt genes between some human populations. The rationale for the analysis was that, if individuals who carry nuclear genes from one population and mitochondrial genes from another population can exist as healthy adults, then admixture of nuclear and mitochondrial genes from divergent populations might not present a substantial risk of mitonuclear incompatibilities in mitochondrial replacement therapy.

There are multiple potential problems associated with such inferences. Firstly, they do not adequately consider the possibility of selection operating against mitonuclear genotypes at earlier life stages (Theme 1). Assessment of healthy adults provides no insights into whether selection had eliminated mitonuclear incompatibilities at earlier life stages in individuals not sampled. Secondly, such inferences are based on genome-wide patterns of mitonuclear association and

596 divergence, and such broad-brushed approaches to assessing mitonuclear genetics overlook the
597 likely scenario that the relevant associations among nuclear genes will be limited to relatively
598 small numbers of key loci that interact with the mt genome (Theme 4). As we have discussed
599 above, mitonuclear incompatibilities might be underpinned by divergence at a small number of
600 sequence sites that are under strong selection for mitochondrial function. Finally, the statement
601 that subjects in the data set were healthy presents an untested assumption because no phenotypic
602 data are presented in the study. Ideally, one would try to link population genomic patterns of
603 mitonuclear genotypes to detailed measures of phenotype (including information on
604 mitochondrial function; Theme 2) (Morales *et al.*, 2015; Baris *et al.*, 2017).
605 While technically challenging, it is important that biomedical researchers seek formally to
606 test the capacity for mitonuclear interactions to affect the outcomes of mitochondrial replacement
607 therapy in non-human primate models, or in human oocytes themselves. In this regard, a recent
608 study by Hyslop *et al*. (2016) provides potential insights into a role of mitonuclear interactions in
609 shaping health outcomes of zygotes following mitochondrial replacement. They utilized a
610 technique known as pronuclear transfer, to move the pronuclei of fertilized zygotes to
611 eunucleated donor embryos, thus creating the opportunity to place the diploid nuclear zygote
612 contributed by one male and female alongside a mitochondrial haplotype contributed by a
613 different female. The authors used this approach to create two types of zygotes. Autologous
614 zygotes were generated by removing and then returning the same pronuclei back into the same
615 zygotes. Autologous zygotes were therefore procedural controls, carrying identical mitonuclear
616 genotypes prior to and following the procedure. Heterologous zygotes were generated by
617 transferring pronuclei from one zygote to another, using donor and parental oocytes that differed
618 in their mtDNA haplogroups. The authors reported lower rates of blastocyst formation in

heterologous zygotes than autologous zygotes, raising the possibility that mitonuclear interactions could be conferring negative effects in the early stages of embryogenesis. That said, the capacity to draw clear inferences from the experiment was somewhat limited by the caveat that creation of heterologous zygotes involved transferring pronuclei from vitrified to fresh oocytes, or *vice versa*, while creation of the autologous zygotes did not.

Humans currently experience substantial gene exchange among most populations around the globe, but this connectivity among human populations is a recent event in the history of *Homo sapiens*. Throughout most of the history of our species, numerous human populations evolved largely in isolation (Akey *et al.*, 2004; Bamshad *et al.*, 2004). A consideration of gene flow among human populations is important because, in a panmictic population, segregation perpetually breaks down genetic associations established by selection (Eyre-Walker, 2017). By contrast, among isolated populations there is a much greater capacity for the evolution of unique genetic associations (Barreto *et al.*, 2018). The isolation of many populations throughout human evolution makes it plausible that mitonuclear incompatibilities might exist in the modern human population.

(3) The future will rely on integration of molecular, biochemical and ecological approaches

Looking to the future, we advocate that integrative approaches are needed to understand the molecular basis and fitness consequences of mitonuclear interactions (Sunnucks *et al.*, 2017). Genotype-to-phenotype links can be developed by combining: (*i*) sequencing of mitochondrial and nuclear genomes to detect sites of selection in populations; (*ii*) molecular modelling and mapping to predict the effects of substitutions on protein structure, function, and interactions;

641 and, where feasible, (*iii*) respirometry and fitness measurements to infer consequences of
642 substitutions at mitochondrial, organismal, and population levels.
643 Molecular modelling is emerging as a particularly valuable tool to make predictions
644 about the effects of mitonuclear interactions on mitochondrial respiration (Grossman *et al.*, 2004;
645 Scott *et al.*, 2011). Driven by recent advances in structural biology, complete three-dimensional
646 structures are now available of the mammalian OXPHOS complexes (Tsukihara *et al.*, 1996;
647 Iwata, 1998; Fiedorczuk *et al.*, 2016; Zhu, Vinothkumar & Hirst, 2016) and the mammalian
648 respirasome supercomplex (Gu *et al.*, 2016; Wu *et al.*, 2016; Guo *et al.*, 2017; Davies, Blum &
649 Kühlbrandt, 2018). It is therefore possible to use these structures to analyse direct molecular
650 interactions between nuclear and mitochondrial gene products. Construction of homology
651 models of sequenced variants of OXPHOS subunits facilitates predictions about how
652 substitutions may affect structure, function, and interactions of OXPHOS complexes. Such
653 approaches have inferred climate-driven positive selection in mitochondrial-encoded Complex I
654 components in a range of animal taxa (Finch *et al.*, 2014; Garvin *et al.*, 2014; Caballero *et al.*,
655 2015). Structural mapping has also recently provided evidence of epistatic interactions between
656 mitochondrial-encoded and nuclear-encoded Complex I variants potentially under climate-driven
657 selection (Garvin *et al.*, 2016; Morales *et al.*, 2018). As we have noted, mitonuclear
658 incompatibilities need not involve direct interactions within multi-subunit complexes (Innocenti
659 *et al.*, 2011; Baris *et al.*, 2017). Nevertheless, the direct molecular interactions between nuclear
660 and mitochondrial gene products remain leading candidates as sites of mitonuclear
661 imcompatibilites. In accordance with Theme 4, homology modelling is also an option to probe
662 protein–DNA and protein–RNA mitonuclear interactions (Bar-Yaacov *et al.*, 2015).

663 It is important to note, however, that molecular understanding of mitonuclear interactions
664 remains in its infancy. The complete atomic resolution of Complex I and the respirasome were
665 only recently resolved (Fiedorczuk *et al.*, 2016; Gu *et al.*, 2016; Zhu *et al.*, 2016; Guo *et al.*,
666 2017) and only low-resolution structures of metazoan ATP synthase have been published (Zhou
667 *et al.*, 2015). As a result, there is limited information from only a handful of model species about
668 the structure, function, and interactions of many OXPHOS subunits and their residues. Thus, it is
669 rarely justified to make detailed mechanistic inferences from molecular modelling, and any
670 predictions should be treated with caution until empirically tested (e.g. respirometry
671 measurements). We anticipate that future advances will increase the predictive power of
672 molecular modelling: higher resolution structures of the respirasome and ATP synthase;
673 improved understanding of how OXPHOS complex structure relates to function at a range of
674 levels and in specific environments; and development of molecular dynamics simulations for
675 these complexes that may reveal the sources of environmental interactions. In turn, such
676 approaches may allow screening for compatible mitonuclear interactions in mitochondrial
677 replacement therapy and help consolidate genotype-to-phenotype links in mitonuclear ecology.
678

679 **VI. CONCLUSIONS**

680 (1) Only in the last couple of decades has the significance of mitochondrial variation, and the
681 interactions of mitochondrial and nuclear genes, been incorporated into a conceptual framework
682 for understanding population structure and speciation.
683 (2) Only a minority of studies consider the potential effects of mitonuclear interactions when
684 assessing adaptation and the genetic basis for variation in individual performance.

(3) Interacting mitochondrial and nuclear genotypes are likely to play a key role in how populations are structured, with implications for the process of speciation and for medical therapies involving recombining mitochondrial and nuclear genotypes.

(4) The key question is how much of an overall effect will arise from mitonuclear interactions *versus* interactions among nuclear genes, and this question can only be answered with a greater research focus on mitonuclear interactions.

(5) Our growing understanding of the coevolution, coadaptation, and co-function of the products of mitochondrial and nuclear genes in natural populations has established a set of themes that should guide further research.

(6) The future lies in the integration of a mechanistic understanding of the biochemical and biophysical consequences of mitochondrial and nuclear genotypes with population biology and ecology.

VII. ACKNOWLEDGEMENTS

Australian Research Council (FT160100022 and DP170100165) and Hermon Slade Foundation (HSF 15/2) to D.K.D., Australia Research Council (DE170100310) to C.G., NIH R01 GM118046 to D.B.S., and NSF DEB1551466 to R.S.B. The authors thank Paul Sunnucks for useful discussions and Adam Eyre-Walker and an anonymous reviewer for making suggestions for improving the manuscript. Author contributions: conceptualization, G.E.H. and D.K.D.; writing, G.E.H., D.K.D., D.B.S., J.C.H., R.S.B., C.G.; figures, J.C.H.

VIII. REFERENCES

AKEY, J.M., EBERLE, M.A., RIEDER, M.J., CARLSON, C.S., SHRIVER, M.D., NICKERSON, D.A. &

708 KRUGLYAK, L. (2004). Population history and natural selection shape patterns of genetic
709 variation in 132 genes. *PLoS Biology* **2**, e286.
710 ALEDO, J.C., VALVERDE, H., RUÍZ-CAMACHO, M., MORILLA, I. & LÓPEZ, F.D. (2014). Protein-
711 protein interfaces from cytochrome c oxidase i evolve faster than nonbinding surfaces, yet
712 negative selection is the driving force. *Genome Biology and Evolution* **6**, 3064–3076.
713 ALSTON, C.L., ROCHA, M.C., LAX, N.Z., TURNBULL, D.M. & TAYLOR, R.W. (2017). The
714 genetics and pathology of mitochondrial disease. *Journal of Pathology* **241**, 236–250.
715 ARNQVIST, G., DOWLING, D.K., EADY, P., GAY, L., TREGENZA, T., TUDA, M. & HOSKEN, D.J.
716 (2010). Genetic architecture of metabolic rate: environment specific epistasis between
717 mitochondrial and nuclear genes in an insect. *Evolution* **64**, 3354–3363.
718 AVISE, J.C. (2004). *Molecular Markers, Natural History, and Evolution*. In *Sinauer, Sunderland,*
719 *MA*. p.
720 BALLANA, E., MERCADER, J.M., FISCHEL-GHODSIAN, N. & ESTIVILL, X. (2007). MRPS18CP2
721 alleles and DEFA3 absence as putative chromosome 8p23.1 modifiers of hearing loss due to
722 mtDNA mutation A1555G in the 12S rRNA gene. *BMC Medical Genetics* **8**, 81.
723 BALLARD, J.W.O. & KREITMAN, M. (1994). Unraveling selection in the mitochondrial genome of
724 *Drosophila*. *Genetics* **138**, 757–772.
725 BALLARD, J.W.O. & PICHAUD, N. (2014). Mitochondrial DNA: more than an evolutionary
726 bystander. *Functional Ecology* **28**, 218–231.
727 BALLARD, J.W.O. & WHITLOCK, M.C. (2004). The incomplete natural history of mitochondria.
728 *Molecular Ecology* **13**, 729–744.
729 BALLOUX, F., HANDLEY, L.-J.L., JOMBART, T., LIU, H. & MANICA, A. (2009). Climate shaped the
730 worldwide distribution of human mitochondrial DNA sequence variation. *Proceedings of*

the Royal Society B: Biological Sciences **276**, 3447–3455.

BAMSHAD, M., WOODING, S., SALISBURY, B.A. & STEPHENS, J.C. (2004). Deconstructing the relationship between genetics and race. *Nature Reviews Genetics* **5,** 598–609.

BAR-YAACOV, D., BLUMBERG, A. & MISHMAR, D. (2012). Mitochondrial-nuclear co-evolution and its effects on OXPHOS activity and regulation. *Biochimica et Biophysica Acta - Gene Regulatory Mechanisms* **1819**, 1107–1111.

BAR-YAACOV, D., HADJIVASILIOU, Z., LEVIN, L., BARSHAD, G., ZARIVACH, R., BOUSKILA, A. & MISHMAR, D. (2015). Mitochondrial involvement in vertebrate speciation? The case of mito-nuclear genetic divergence in chameleons. *Genome Biology and Evolution* **7**, 3322–3336.

BARIS, T.Z., WAGNER, D.N., DAYAN, D.I., DU, X., BLIER, P.U., PICHAUD, N., OLEKSIAK, M.F. & CRAWFORD, D.L. (2017). Evolved genetic and phenotypic differences due to mitochondrial-nuclear interactions. *PLoS Genetics* **13**, 1–23.

BARR, C.M., NEIMAN, M. & TAYLOR, D.R. (2005). Inheritance and recombination of mitochondrial genomes in plants, fungi and animals. *New Phytologist* **168**, 39–50.

BARRETO, F.S. & BURTON, R.S. (2013*a*). Elevated oxidative damage is correlated with reduced fitness in interpopulation hybrids of a marine copepod. *Proceedings of the Royal Society B: Biological Sciences* **280**, 20131521.

BARRETO, F.S. & BURTON, R.S. (2013*b*). Evidence for compensatory evolution of ribosomal proteins in response to rapid divergence of mitochondrial rRNA. *Molecular Biology and Evolution* **30**, 310–314.

BARRETO, F.S., PEREIRA, R.J. & BURTON, R.S. (2015). Hybrid dysfunction and physiological compensation in gene expression. *Molecular Biology and Evolution* **32**, 613–622.

754 BARRETO, F.S., WATSON, E.T., LIMA, T.G., WILLETT, C.S., EDMANDS, S., LI, W. & BURTON,
755 R.S. (2018). Genomic signatures of mitonuclear coevolution across populations of
756 *Tigriopus californicus*. *Nature Ecology and Evolution* **2**, 1250–1257.
757 BEEKMAN, M., DOWLING, D.K. & AANEN, D.K. (2014). The costs of being male: are there sex-
758 specific effects of uniparental mitochondrial inheritance? *Philosophical transactions of the*
759 *Royal Society of London. Series B, Biological sciences* **369**, 20130440.
760 BLUMBERG, A., SAILAJA, B.S., KUNDAJE, A., LEVIN, L., DADON, S., SHMORAK, S., SHAULIAN, E.,
761 MESHORER, E. & MISHMAR, D. (2014). Transcription factors bind negatively selected sites
762 within human mtDNA genes. *Genome Biology and Evolution* **6**, 2634–2646.
763 BOCK, D.G., ANDREW, R.L. & RIESEBERG, L.H. (2014). On the adaptive value of cytoplasmic
764 genomes in plants. *Molecular Ecology* **23**, 4899–4911.
765 BROWN, W.M., GEORGE, M. & WILSON, A.C. (1979). Rapid evolution of animal mitochondrial
766 DNA. *Proceedings of the National Academy of Sciences* **76**, 1967–1971.
767 BURAK, E., YOGEV, O., SHEFFER, S., SCHUELER-FURMAN, O. & PINES, O. (2013). Evolving dual
768 targeting of a prokaryotic protein in yeast. *Molecular Biology and Evolution* **30**, 1563–
769 1573.
770 BURR, S.P., PEZET, M. & CHINNERY, P.F. (2018). Mitochondrial DNA heteroplasmy and
771 purifying selection in the mammalian female germ line. *Development Growth and*
772 *Differentiation* **60**, 21–32.
773 BURTON, R.S. & BARRETO, F.S. (2012). A disproportionate role for mtDNA in Dobzhansky-
774 Muller incompatibilities? *Molecular Ecology* **21**, 4942–4957.
775 BURTON, R.S., ELLISON, C.K. & HARRISON, J.S. (2006). The sorry state of F-2 hybrids:
776 Consequences of rapid mitochondrial DNA evolution in allopatric populations. *American*

Naturalist **168**, S14--S24.

BURTON, R.S., PEREIRA, R.J. & BARRETO, F.S. (2013). Cytonuclear genomic interactions and hybrid breakdown. *Annual Review of Ecology, Evolution, and Systematics* **44**, 281–302.

BYKHOVSKAYA, Y., MENGESHA, E., WANG, D., YANG, H., ESTIVILL, X., SHOHAT, M. & FISCHEL-GHODSIAN, N. (2004). Human mitochondrial transcription factor B1 as a modifier gene for hearing loss associated with the mitochondrial A1555G mutation. *Molecular Genetics and Metabolism* **82**, 27–32.

CABALLERO, S., DUCHÊNE, S., GARAVITO, M.F., SLIKAS, B. & BAKER, C.S. (2015). Initial evidence for adaptive selection on the NADH subunit two of freshwater dolphins by analyses of mitochondrial genomes. *PLoS ONE* **10**, 1–17.

CALVO, S.E. & MOOTHA, V.K. (2010). The mitochondrial proteome and human disease. *Annual Review of Genomics and Human Genetics* **11**, 25–44.

CAMUS, M.F., CLANCY, D.J. & DOWLING, D.K. (2012). Mitochondria, maternal inheritance, and male aging. *Current Biology* **22**, 1717–1721.

CAMUS, M.F. & DOWLING, D.K. (2018). Mitochondrial genetic effects on reproductive success: Signatures of positive intrasexual, but negative intersexual pleiotropy. *Proceedings of the Royal Society B: Biological Sciences* **285**, 20180187.

CAMUS, M.F., WOLF, J.B.W., MORROW, E.H. & DOWLING, D.K. (2015). Single nucleotides in the mtDNA sequence modify mitochondrial molecular function and are associated with sex-specific effects on fertility and aging. *Current Biology* **25**, 2717–2722.

CAMUS, M.F., WOLFF, J.N., SGRÒ, C.M. & DOWLING, D.K. (2017). Experimental evidence that thermal selection has shaped the latitudinal distribution of mitochondrial haplotypes in Australian fruit flies. 1–33.

CHAN, D.C. (2006). Mitochondria: dynamic organelles in disease, aging, and development. *Cell* **125**, 1241–1252.

CHATTERJEE, A., SEYFFERTH, J., LUCCI, J., GILSBACH, R., PREISSL, S., BÖTTINGER, L., MÅRTENSSON, C.U., PANHALE, A., STEHLE, T., KRETZ, O., SAHYOUN, A.H., AVILOV, S., EIMER, S., HEIN, L., PFANNER, N., *ET AL*. (2016). MOF acetyl transferase regulates transcription and respiration in mitochondria. *Cell* **167**, 722–738.e23.

CHEVIRON, Z.A. & BRUMFIELD, R.T. (2009). Migration-selection balance and local adaptation of mitochondrial haplotypes in Rufous-Collared Sparrows (*Zonotrichia capensis*) along an elevational gradient. *Evolution* **63**, 1593–1605.

CHINNERY, P.F., CRAVEN, L., MITALIPOV, S., STEWART, J.B., HERBERT, M. & TURNBULL, D.M. (2014). The challenges of mitochondrial replacement. *PLoS Genetics* **10**, 3–4.

CHOU, J.Y. & LEU, J.Y. (2010). Speciation through cytonuclear incompatibility: insights from yeast and implications for higher eukaryotes. *BioEssays* **32**, 401–411.

CHRISTIE, J.R. & BEEKMAN, M. (2016). Uniparental inheritance promotes adaptive evolution in cytoplasmic genomes. *Molecular Biology and Evolution* **34**, 677–691.

CHUNG, D.J., BRYANT, H.J. & SCHULTE, P.M. (2017). Thermal acclimation and population-specific effects on heart and brain mitochondrial performance in a eurythermal teleost (*Fundulus heteroclitus*). *Journal of Experimental Biology* **220**, 1459–1471.

CLANCY, D.J. (2008). Variation in mitochondrial genotype has substantial lifespan effects which may be modulated by nuclear background. *Aging Cell* **7**, 795–804.

CLARK, N.L., ALANI, E. & AQUADRO, C.F. (2012). Evolutionary rate covariation : a bioinformatic method that reveals co-functionality and co-expression of genes. *Genome Research* **22**, 714–720.

823 COOPER, B.S., BURRUS, C.R., JI, C., HAHN, M.W. & MONTOOTH, K.L. (2015). Similar efficacies
824 of selection shape mitochondrial and nuclear genes in both *Drosophila melanogaster* and
825 *Homo sapiens*. *G3* **5**, 1–45.
826 CRAVEN, L., TUPPEN, H.A., GREGGAINS, G.D., HARBOTTLE, S.J., MURPHY, J.L., CREE, L.M.,
827 MURDOCH, A.P., CHINNERY, P.F., TAYLOR, R.W., LIGHTOWLERS, R.N., HERBERT, M. &
828 TURNBULL, D.M. (2010). Pronuclear transfer in human embryos to prevent transmission of
829 mitochondrial DNA disease. *Nature* **465**, 82–85.
830 CREE, L.M., SAMUELS, D.C., DE SOUSA LOPES, S.C., RAJASIMHA, H.K., WONNAPINIJ, P., MANN,
831 J.R., DAHL, H.H. & CHINNERY, P.F. (2008). A reduction of mitochondrial DNA molecules
832 during embryogenesis explains the rapid segregation of genotypes. *Nature Genetics* **40**,
833 249–254.
834 DAVIDSON, M.M., WALKER, W.F., HERNANDEZ-ROSA, E. & NESTI, C. (2009). Evidence for
835 nuclear modifier gene in mitochondrial cardiomyopathy. *Journal of Molecular and Cellular*
836 *Cardiology* **46**, 936–942.
837 DAVIES, K.M., BLUM, T.B. & KÜHLBRANDT, W. (2018). Conserved in situ arrangement of
838 complex I and III 2 in mitochondrial respiratory chain supercomplexes of mammals, yeast,
839 and plants. *Proceedings of the National Academy of Sciences* **115**, 3024–3029.
840 DE FANTI, S., VICARIO, S., LANG, M., SIMONE, D., MAGLI, C., LUISELLI, D.,
841 GIANAROLI, L. AND ROMEO, G. (2017) Intra-individual purifying selection on
842 mitochondrial DNA variants during human oogenesis. Human Reproduction **32,** 1100–1107
843 DOBLER, R., DOWLING, D.K., MORROW, E.H. & REINHARDT, K. (2018). A systematic review and
844 meta-analysis reveals pervasive effects of the technique of mitochondrial replacement on
845 health. *Human Reproduction Update* **24**, 519–534.

DOBLER, R., ROGELL, B., BUDAR, F. & DOWLING, D.K. (2014). A meta-analysis of the strength and nature of cytoplasmic genetic effects. *Journal of Evolutionary Biology* **27**, 2021–2034.

DOWLING, D.K. (2014). Evolutionary perspectives on the links between mitochondrial genotype and disease phenotype. *Biochimica et Biophysica Acta - General Subjects* **1840**, 1393–1403.

DOWLING, D.K., ABIEGA, K.C. & ARNQVIST, G. (2007). Temperature-specific outcomes of cytoplasmic-nuclear interactions on egg-to-adult development time in seed beetles. *Evolution* **61**, 194–201.

DOWLING, D.K., FRIBERG, U. & LINDELL, J. (2008). Evolutionary implications of non-neutral mitochondrial genetic variation. *Trends in Ecology & Evolution* **23**, 546–554.

DOWLING, D.K., MAKLAKOV, A.A., FRIBERG, U. & HAILER, F. (2009). Applying the genetic theories of ageing to the cytoplasm: Cytoplasmic genetic covariation for fitness and lifespan. *Journal of Evolutionary Biology* **22**, 818–827.

DOWLING, D.K., MEERUPATI, T. & ARNQVIST, G. (2010). Cytonuclear interactions and the economics of mating in seed beetles. *Am Nat* **176**, 131–140.

DOWLING, D.K., TOMPKINS, D.M. & GEMMELL, N.J. (2015). The Trojan female technique for pest control: a candidate mitochondrial mutation confers low male fertility across diverse nuclear backgrounds in *Drosophila melanogaster*. *Evolutionary Applications* **8**, 871–880.

DUMOLLARD, R., DUCHEN, M. & CARROLL, J. (2007). The role of mitochondrial function in the oocyte and embryo. *Current Topics in Developmental Biology* **77**, 21–49.

EDMANDS, S. (2007). Between a rock and a hard place: Evaluating the relative risks of inbreeding and outbreeding for conservation and management. *Molecular Ecology* **16**, 463–475.

ELLISON, C.K. & BURTON, R.S. (2006). Disruption of mitochondrial function in interpopulation

hybrids of *Tigriopus californicus*. *Evolution* **60**, 1382–1391.

ELLISON, C.K. & BURTON, R.S. (2008*a*). Genotype-dependent variation of mitochondrial transcriptional profiles in interpopulation hybrids. *Proceedings of the National Academy of Sciences* **105**, 15831–15836.

ELLISON, C.K. & BURTON, R.S. (2008*b*). Interpopulation hybrid breakdown maps to the mitochondrial genome. *Evolution* **62**, 631–638.

ELLISON, C.K. & BURTON, R.S. (2010). Cytonuclear conflict in interpopulation hybrids: the role of RNA polymerase in mtDNA transcription and replication. *Journal of Evolutionary biology* **23**, 528–538.

ESTES, S., COLEMAN-HULBERT, A.L., HICKS, K. A, DE HAAN, G., MARTHA, S.R., KNAPP, J.B., SMITH, S.W., STEIN, K.C. & DENVER, D.R. (2011). Natural variation in life history and aging phenotypes is associated with mitochondrial DNA deletion frequency in Caenorhabditis briggsae. *BMC Evolutionary Biology* **11**, 11.

EYRE-WALKER, A. (2017). Mitochondrial replacement therapy: are mito-nuclear interactions likely to be a problem? *Genetics* **205**, 1365–1372.

FAN, W., WAYMIRE, K.G., NARULA, N., LI, P., ROCHER, C., COSKUN, P.E., VANNAN, M.A., NARULA, J., MACGREGOR, G.R. & WALLACE, D.C. (2008). A mouse model of mitochondrial disease reveals germline selection against severe mtDNA mutations. *Science* **319**, 958–962.

FIEDORCZUK, K., LETTS, J.A., DEGLIESPOSTI, G., KASZUBA, K., SKEHEL, M. & SAZANOV, L.A. (2016). Atomic structure of the entire mammalian mitochondrial complex I. *Nature* **538**, 406–410.

FINCH, T.M., ZHAO, N., KORKIN, D., FREDERICK, K.H. & EGGERT, L.S. (2014). Evidence of

892 positive selection in mitochondrial complexes I and V of the African elephant. *PLoS ONE*
893 **9**, e92587.
894 FRANK, S.A. & HURST, L.D. (1996). Mitochondria and male disease. *Nature* **383**, 224.
895 GARVIN, M.R., BIELAWSKI, J.P., SAZANOV, L.A. & GHARRETT, A.J. (2014). Review and meta-
896 analysis of natural selection in mitochondrial complex I in metazoans. *Journal of*
897 *Zoological Systematics and Evolutionary Research* **53**, 1–17.
898 GARVIN, M.R., TEMPLIN, W.D., GHARRETT, A.J., DECOVICH, N., KONDZELA, C.M., GUYON, J.R.
899 & MCPHEE, M. V. (2016). Potentially adaptive mitochondrial haplotypes as a tool to identify
900 divergent nuclear loci. *Methods in Ecology and Evolution* **8**, 821–834.
901 GE, H., TOLLNER, T.L., HU, Z., DAI, M., LI, X., GUAN, H., SHAN, D., ZHANG, X., LV, J., HUANG,
902 C. & DONG, Q. (2012). The importance of mitochondrial metabolic activity and
903 mitochondrial DNA replication during oocyte maturation in vitro on oocyte quality and
904 subsequent embryo developmental competence. *Molecular Reproduction and Development*
905 **79**, 392–401.
906 GEMMELL, N.J., METCALF, V.J. & ALLENDORF, F.W. (2004). Mother's curse: the effect of
907 mtDNA on individual fitness and population viability. *Trends in Ecology & Evolution* **19**,
908 238–244.
909 GERSHONI, M., LEVIN, L., OVADIA, O., TOIW, Y., SHANI, N., DADON, S., BARZILAI, N.,
910 BERGMAN, A., ATZMON, G., WAINSTEIN, J., TSUR, A., NIJTMANS, L., GLASER, B. &
911 MISHMAR, D. (2014). Disrupting mitochondrial-nuclear coevolution affects OXPHOS
912 complex I integrity and impacts human health. *Genome Biology and Evolution* **6**, 2665–
913 2680.
914 GERSHONI, M., TEMPLETON, A.R. & MISHMAR, D. (2009). Mitochondrial bioenergetics as a

major motive force of speciation. *Bioessays* **31**, 642–650.

GREAVES, L.C. & TAYLOR, R.W. (2006). Mitochondrial DNA mutations in human disease. *IUBMB Life* **58**, 143–151.

GREINER, S., SOBANSKI, J. & BOCK, R. (2015). Why are most organelle genomes transmitted maternally? *BioEssays* **37**, 80–94.

GROSSMAN, L.I., WILDMAN, D.E., SCHMIDT, T.R. & GOODMAN, M. (2004). Accelerated evolution of the electron transport chain in anthropoid primates. *Trends in Genetics* **20**, 578–585.

GU, J., WU, M., GUO, R., YAN, K., LEI, J., GAO, N. & YANG, M. (2016). The architecture of the mammalian respirasome. *Nature* **537,** 639–643

GUO, R., ZONG, S., WU, M., GU, J. & YANG, M. (2017). Architecture of human mitochondrial respiratory megacomplex I2III2IV2. *Cell* **170**, 1247–1257.e12.

HADJIVASILIOU, Z., LANE, N., SEYMOUR, R.M. & POMIANKOWSKI, A. (2013). Dynamics of mitochondrial inheritance in the evolution of binary mating types and two sexes. *Proceedings of the Royal Society B-Biological Sciences* **280**, 20131920.

HAGSTRÖM, E., FREYER, C., BATTERSBY, B.J., STEWART, J.B. & LARSSON, N.G. (2014). No recombination of mtDNA after heteroplasmy for 50 generations in the mouse maternal germline. *Nucleic Acids Research* **42**, 1111–1116.

HARRISON, J.S. & BURTON, R.S. (2006). Tracing hybrid incompatibilities to single amino acid substitutions. *Molecular Biology and Evolution* **23**, 559–564.

HAVIRD, J.C., HALL, M.D. & DOWLING, D.K. (2015*a*). The evolution of sex: A new hypothesis based on mitochondrial mutational erosion: Mitochondrial mutational erosion in ancestral eukaryotes would favor the evolution of sex, harnessing nuclear recombination to optimize

compensatory nuclear coadaptation. *BioEssays* **37**, 951–958.

HAVIRD, J.C. & SLOAN, D.B. (2016). The roles of mutation, selection, and expression in determining relative rates of evolution in mitochondrial versus nuclear genomes. *Molecular Biology and Evolution* **33**, 3042–3053.

HAVIRD, J.C., TRAPP, P., MILLER, C., BAZOS, I. & SLOAN, D.B. (2017). Causes and consequences of rapidly evolving mtDNA in a plant lineage. *Genome Biology and Evolution* **9**, 323.

HAVIRD, J.C., WHITEHILL, N.S., SNOW, C.D. & SLOAN, D.B. (2015*b*). Conservative and compensatory evolution in oxidative phosphorylation complexes of angiosperms with highly divergent rates of mitochondrial genome evolution. *Evolution* **69**, 3069–3081.

HAYASHI, Y., OTSUKA, K., EBINA, M., IGARASHI, K., TAKEHARA, A., MATSUMOTO, M., KANAI, A., IGARASHI, K., SOGA, T. & MATSUI, Y. (2017). Distinct requirements for energy metabolism in mouse primordial germ cells and their reprogramming to embryonic germ cells. *Proceedings of the National Academy of Sciences* **114**, 8289–8294.

HICKS, K.A., DENVER, D.R. & ESTES, S. (2013). Natural variation in *Caenorhabditis briggsae* mitochondrial form and function suggests a novel model of organelle dynamics. *Mitochondrion* **13**, 44–51.

HILL, G.E. (2014). Sex linkage of nuclear-encoded mitochondrial genes. *Heredity* **112**, 469–470.

HILL, G.E. (2015). Mitonuclear ecology. *Molecular Biology and Evolution* **32**, 1917–1927.

HILL, G.E. (2016). Mitonuclear coevolution as the genesis of speciation and the mitochondrial DNA barcode gap. *Ecology and Evolution* **6**, 5831–5842.

HILL, G.E. (2017). The mitonuclear compatibility species concept. *The Auk* **134**, 393–409.

HILL, G.E. (2018). Mitonuclear mate choice: a missing component of sexual selection theory? *BioEssays* **40**, 1700191.

961 HILL, G.E. & JOHNSON, J.D. (2013). The mitonuclear compatibility hypothesis of sexual
962 selection. *Proceedings of the Royal Society B-Biological Sciences* **280**, 20131314.
963 HOEKSTRA, L.A., SIDDIQ, M.A. & MONTOOTH, K.L. (2013). Pleiotropic effects of a
964 mitochondrial-nuclear incompatibility depend upon the accelerating effect of temperature in
965 Drosophila. *Genetics* **195**, 1129–1139.
966 HUDSON, G., GOMEZ-DURAN, A., WILSON, I.J. & CHINNERY, P.F. (2014). Recent mitochondrial
967 DNA mutations increase the risk of developing common late-onset human diseases. *PLoS*
968 *Genetics* **10**, e1004369.
969 HYSLOP, L.A., BLAKELEY, P., CRAVEN, L., RICHARDSON, J., FOGARTY, N.M.E., FRAGOULI, E.,
970 LAMB, M., WAMAITHA, S.E., PRATHALINGAM, N., ZHANG, Q., O'KEEFE, H., TAKEDA, Y.,
971 ARIZZI, L., ALFARAWATI, S., TUPPEN, H.A., *ET AL*. (2016). Towards clinical application of
972 pronuclear transfer to prevent mitochondrial DNA disease. *Nature* **534**, 383–386.
973 IMMONEN, E., COLLET, M., GOENAGA, J. & ARNQVIST, G. (2016). Direct and indirect genetic
974 effects of sex-specific mitonuclear epistasis on reproductive ageing. *Heredity* **116**, 338–347.
975 INNOCENTI, P., MORROW, E.H. & DOWLING, D.K. (2011). Experimental evidence supports a sex-
976 specific selective sieve in mitochondrial genome evolution. *Science* **332**, 845–848.
977 IWATA, S. (1998). Complete structure of the 11-subunit bovine mitochondrial cytochrome bc1
978 complex. *Science* **281**, 64–71.
979 JAMES, A.C. & BALLARD, J.W.O. (2003). Mitochondrial genotype affects fitness in *Drosophila*
980 *simulans*. *Genetics* **164**, 187–194.
981 JAMES, J.E., PIGANEAU, G. & EYRE-WALKER, A. (2016). The rate of adaptive evolution in animal
982 mitochondria. *Molecular Ecology* **25**, 67–78.
983 JI, F., SHARPLEY, M.S., DERBENEVA, O., ALVES, L.S., QIAN, P., WANG, Y., CHALKIA,

D., LVOVA, M., XU, J., YAO, W. & SIMON, M. (2012). Mitochondrial DNA variant associated with Leber hereditary optic neuropathy and high-altitude Tibetans. *Proceedings of the National Academy of Sciences* **109**, 7391-7396.

KASASHIMA, K., NAGAO, Y. & ENDO, H. (2014). Dynamic regulation of mitochondrial genome maintenance in germ cells. *Reproductive Medicine and Biology* **13**, 11–20.

KRAKAUER, D.C. & MIRA, A. (1999). Mitochondria and germ-cell death. *Nature* **400**, 125–126.

KWONG, S., SRIVATHSAN, A., VAIDYA, G. & MEIER, R. (2012). Is the COI barcoding gene involved in speciation through intergenomic conflict? *Molecular Phylogenetics and Evolution* **62**, 1009–1012.

LANE, N. (2005). *Power, Sex, Suicide: Mitochondria and the Meaning of Life*. Oxford University Press New York.

LANE, N. & MARTIN, W. (2010). The energetics of genome complexity. *Nature* **467**, 929–934.

LATORRE-PELLICER, A., MORENO-LOSHUERTOS, R., LECHUGA-VIECO, A.V., SÁNCHEZ-CABO, F., TORROJA, C., ACÍN-PÉREZ, R., CALVO, E., AIX, E., GONZÁLEZ-GUERRA, A., LOGAN, A., BERNAD-MIANA, M.L., ROMANOS, E., CRUZ, R., COGLIATI, S., SOBRINO, B., *ET AL*. (2016). Mitochondrial and nuclear DNA matching shapes metabolism and healthy ageing. *Nature* **535**, 561–565.

LEE, C., YEN, K. & COHEN, P. (2013). Humanin: a harbinger of mitochondrial-derived peptides? *Trends in Endocrinology and Metabolism* **24**, 222–228.

LEE, C., ZENG, J., DREW, B.G., SALLAM, T., MARTIN-MONTALVO, A., WAN, J., KIM, S.J., MEHTA, H., HEVENER, A.L., DE CABO, R. & COHEN, P. (2015). The mitochondrial-derived peptide MOTS-c promotes metabolic homeostasis and reduces obesity and insulin resistance. *Cell Metabolism* **21**, 443–454.

1007 LEVIN, D.A. (2003). The cytoplasmic factor in plant speciation. *Systematic Botany* **28**, 5–11.
1008 LEVIN, L., BLUMBERG, A., BARSHAD, G. & MISHMAR, D. (2014). Mito-nuclear co-evolution: the
1009 positive and negative sides of functional ancient mutations. *Frontiers in Genetics* **5**, 448.
1010 LUO, L.F., HOU, C.C. & YANG, W.X. (2013). Nuclear factors: roles related to mitochondrial
1011 deafness. *Gene* **520**, 79–89.
1012 LYNCH, M. (1997). Mutation accumulation in nuclear, organelle, and prokaryotic transfer RNA
1013 genes. *Molecular Biology and Evolution* **14**, 914–925.
1014 LYNCH, M. & BLANCHARD, J.L. (1998). Deleterious mutation accumulation in organelle
1015 genomes. *Genetica* **102**, 29–39.
1016 LYNCH, M., BUTCHER, D., BÜRGER, R. & GABRIEL, W. (1993). The mutational meltdown in
1017 asexual populations. *Journal of Heredity* **84**, 339–344.
1018 MA, H., MARTI GUTIERREZ, N., MOREY, R., VAN DYKEN, C., KANG, E., HAYAMA, T., LEE, Y., LI,
1019 Y., TIPPNER-HEDGES, R., WOLF, D.P., LAURENT, L.C. & MITALIPOV, S. (2016).
1020 Incompatibility between nuclear and mitochondrial genomes contributes to an interspecies
1021 reproductive barrier. *Cell Metabolism* **24**, 283–294.
1022 MA, H. & O'FARRELL, P.H. (2015). Selections that isolate recombinant mitochondrial genomes
1023 in animals. *eLife* **4**, e07247.
1024 MARTIKAINEN, M.H., GRADY, J.P., NG, Y.S., ALSTON, C.L., GORMAN, G.S., TAYLOR, R.W.,
1025 MCFARLAND, R. & TURNBULL, D.M. (2017). Decreased male reproductive success in
1026 association with mitochondrial dysfunction. *European Journal of Human Genetics* **25**,
1027 1163–1165.
1028 MEIKLEJOHN, C.D., HOLMBECK, M.A., SIDDIQ, M.A., ABT, D.N., RAND, D.M. & MONTOOTH,
1029 K.L. (2013). An incompatibility between a mitochondrial tRNA and its nuclear-encoded

tRNA synthetase compromises development and fitness in *Drosophila*. *PLoS Genetics* **9**, e1003238.

MILOT, E., MOREAU, C., GAGNON, A., COHEN, A.A., BRAIS, B. & LABUDA, D. (2017). Mother's curse neutralizes natural selection against a human genetic disease over three centuries. *Nature Ecology & Evolution* **1**, 1400–1406.

MISHMAR, D., RUIZ-PESINI, E., GOLIK, P., MACAULAY, V., CLARK, A.G., HOSSEINI, S., BRANDON, M., EASLEY, K., CHEN, E., BROWN, M.D., SUKERNIK, R.I., OLCKERS, A. & WALLACE, D.C. (2003). Natural selection shaped regional mtDNA variation in humans. *Proceedings of the National Academy of Sciences* **100**, 171–176.

MISHMAR, D. & ZHIDKOV, I. (2010). Evolution and disease converge in the mitochondrion. *Biochimica et Biophysica Acta - Bioenergetics* **1797**, 1099–1104.

MONAGHAN, R.M. & WHITMARSH, A.J. (2015). Mitochondrial proteins moonlighting in the nucleus. *Trends in Biochemical Sciences* **40**, 728–735.

MOORE, J.H. & WILLIAMS, S.M. (2005). Traversing the conceptual divide between biological and statistical epistasis: Systems biology and a more modern synthesis. *BioEssays* **27**, 637–646.

MORALES, H.E., PAVLOVA, A., AMOS, N., MAJOR, R., KILIAN, A., GREENING, C. & SUNNUCKS, P. (2018). Concordant divergence of mitogenomes and a mitonuclear gene cluster in bird lineages inhabiting different climates. *Nature Ecology & Evolution* **2**, 1258.

MORALES, H.E., PAVLOVA, A., JOSEPH, L. & SUNNUCKS, P. (2015). Positive and purifying selection in mitochondrial genomes of a bird with mitonuclear discordance. *Molecular Ecology* **24**, 2820–2837.

MORROW, E.H. & CAMUS, M.F. (2017). Mitonuclear epistasis and mitochondrial disease.

Mitochondrion **35**, 119–122.

MORROW, E.H., REINHARDT, K., WOLFF, J.N. & DOWLING, D.K. (2015). Risks inherent to mitochondrial replacement. *EMBO reports* **16**, 541–545.

MOSSMAN, J.A., BIANCANI, L.M., ZHU, C. & RAND, D.M. (2016). Mitonuclear epistasis for development time and its modification by diet in *Drosophila.* **203**, 463–484.

MOWRY, A. V., DONOVIEL, Z.S., KAVAZIS, A.N. & HOOD, W.R. (2017). Mitochondrial function and bioenergetic trade-offs during lactation in the house mouse (*Mus musculus*). *Ecology and Evolution* **7**, 2994–3005.

NABHOLZ, B., ELLEGREN, H. & WOLF, J.B.W. (2013). High levels of gene expression explain the strong evolutionary constraint of mitochondrial protein-coding genes. *Molecular Biology and Evolution* **30**, 272–284.

NACHMAN, M.W., BOYER, S.N. & AQUADRO, C.F. (1994). Nonneutral evolution at the mitochondrial NADH dehydrogenase subunit 3 gene in mice. *Proceedings of the National Academy of Sciences of the United States of America* **91**, 6364–6368.

NACHMAN, M.W., BROWN, W.M., STONEKING, M. & AQUADRO, C.F. (1996). Nonneutral mitochondrial DNA variation in humans and chimpanzees. *Genetics* **142**, 953–963.

NAKADA, K., SATO, A., YOSHIDA, K., MORITA, T., TANAKA, H., INOUE, S., YONEKAWA, H. & HAYASHI, J. (2006). Mitochondria-related male infertility. *Proceedings of the National Academy of Sciences* **103**, 15148–15153.

NEIMAN, M. & TAYLOR, D.R. (2009). The causes of mutation accumulation in mitochondrial genomes. *Proceedings of the Royal Society B: Biological Sciences* **276**, 1201–1209.

OSADA, N. & AKASHI, H. (2012). Mitochondrial–nuclear interactions and accelerated compensatory evolution: evidence from the primate cytochrome c oxidase complex.

Molecular Biology and Evolution **29**, 337–346.

PATEL, M.R., MIRIYALA, G.K., LITTLETON, A.J., YANG, H., TRINH, K., YOUNG, J.M., KENNEDY, S.R., YAMASHITA, Y.M., PALLANCK, L.J. & MALIK, H.S. (2016). A mitochondrial DNA hypomorph of cytochrome oxidase specifically impairs male fertility in *Drosophila melanogaster*. *eLife* **5**, 1–27.

PICHAUD, N., BALLARD, J.W.O., TANGUAY, R.M. & BLIER, P.U. (2012). Naturally occurring mitochondrial DNA haplotypes exhibit metabolic differences: Insight into functional properties of mitochondria. *Evolution* **66**, 3189–3197.

PIZZARI, T. (2009). Sexual selection: sperm in the fast lane. *Current Biology* **19**, R292–R294.

POPADIN, K.Y., NIKOLAEV, S.I., JUNIER, T., BARANOVA, M. & ANTONARAKIS, S.E. (2013). Purifying selection in mammalian mitochondrial protein-coding genes is highly effective and congruent with evolution of nuclear genes. *Molecular Biology and Evolution* **30**, 347–355.

POZZI, A., PLAZZI, F., MILANI, L., GHISELLI, F. & PASSAMONTI, M. (2017). SmithRNAs: could mitochondria "bend" nuclear regulation? *Molecular Biology and Evolution* **34**, 1960–1973.

QUINTELA, M., JOHANSSON, M.P., KRISTJÁNSSON, B.K., BARREIRO, R. & LAURILA, A. (2014). AFLPs and mitochondrial haplotypes reveal local adaptation to extreme thermal environments in a freshwater gastropod. *PLoS ONE* **9**, e101821.

RADZVILAVICIUS, A.L., HADJIVASILIOU, Z., POMIANKOWSKI, A. & LANE, N. (2016). Selection for mitochondrial quality drives evolution of the germline. *PLOS Biology* **14**, e2000410.

RAJ, A., RIFKIN, S.A., ANDERSEN, E. & OUDENAARDEN, A. VAN (2010). Variability in gene expression underlies incomplete penetrance. *Nature* **463**, 913–918.

RAND, D.M. (2001). The units of selection on mitochondrial DNA. *Annual Review of Ecology*

and Systematics **32**, 415–448.

RAND, D.M., DORFSMAN, M. & KANN, L.M. (1994). Neutral and non-neutral evolution of *Drosophila* mitochondrial DNA. *Genetics* **138**, 741–756.

RAND, D.M., FRY, A. & SHELDAHL, L. (2006). Nuclear-mitochondrial epistasis and drosophila aging: Introgression of *Drosophila simulans* mtDNA modifies longevity in *D. melanogaster* nuclear backgrounds. *Genetics* **172**, 329–341.

RAND, D.M., HANEY, R.A. & FRY, A.J. (2004). Cytonuclear coevolution: the genomics of cooperation. *Trends in Ecology & Evolution* **19**, 645–653.

RAWSON, P.D. & BURTON, R.S. (2002). Functional coadaptation between cytochrome c and cytochrome c oxidase within allopatric populations of a marine copepod. *Proceedings of the National Academy of Sciences* **99**, 12955–12958.

REINHARDT, K., DOWLING, D.K. & MORROW, E.H. (2013). Mitochondrial replacement, evolution, and the clinic. *Science* **341**, 1345–1346.

RISHISHWAR, L. & JORDAN, I.K. (2017). Implications of human evolution and admixture for mitochondrial replacement therapy. *BMC Genomics* **18**, 140.

ROUX, F., MARY-HUARD, T., BARILLOT, E., WENES, E., BOTRAN, L., DURAND, S., VILLOUTREIX, R., MARTIN-MAGNIETTE, M.-L., CAMILLERI, C. & BUDAR, F. (2016). Cytonuclear interactions affect adaptive traits of the annual plant *Arabidopsis thaliana* in the field. *Proceedings of the National Academy of Sciences of the United States of America* **113**, 3687–3692.

RUIZ-PESINI, E. (2004). Effects of purifying and adaptive selection on regional variation in human mtDNA. *Science* **303**, 223–226.

RUIZ-PESINI, E., DÍEZ-SÁNCHEZ, C., LÓPEZ-PÉREZ, M.J. & ENRÍQUEZ, J.A. (2007). The role of

the mitochondrion in sperm function: is there a place for oxidative phosphorylation or is this a purely glycolytic process? *Current Topics in Developmental Biology* **77**, 3–19.

SCHAEFER, S., NADEAU, J.H. & WRAY, H.E.G.A. (2015). The genetics of epigenetic inheritance: modes, molecules, and mechanisms. *The Quarterly Review of Biology* **90**, 381–415.

SCOTT, G.R., SCHULTE, P.M., EGGINTON, S., SCOTT, A.L.M., RICHARDS, J.G. & MILSOM, W.K. (2011). Molecular evolution of cytochrome c oxidase underlies high-altitude adaptation in the bar-headed goose. *Molecular Biology and Evolution* **28**, 351–363.

SHARBROUGH, J., HAVIRD, J.C., NOE, G.R., WARREN, J.M. & SLOAN, D.B. (2017). The mitonuclear dimension of Neanderthal and Denisovan ancestry in modern human genomes. *Genome Biology and Evolution* **9**, 1567–1581.

SHORT, R. V (1997). The testis: the witness of the mating system, the site of mutation and the engine of desire. *Acta Paediatrica* **86**, 3–7.

SILVA, G., LIMA, F.P., MARTEL, P. & CASTILHO, R. (2014). Thermal adaptation and clinal mitochondrial DNA variation of European anchovy. *Proceedings of the Royal Society B: Biological Sciences* **281**, 20141093.

SIMMONS, L.W. (2001). *Sperm competition and its evolutionary consequences in the insects*. Princeton University Press, Princeton, N.J.

SLOAN, D.B., FIELDS, P.D. & HAVIRD, J.C. (2015). Mitonuclear linkage disequilibrium in human populations. *Proceedings of the Royal Society B: Biological Sciences* **282**, 20151704.

SLOAN, D.B., HAVIRD, J.C. & SHARBROUGH, J. (2017). The on-again, off-again relationship between mitochondrial genomes and species boundaries. *Molecular Ecology* **26**, 2212–2236.

SLOAN, D.B., TRIANT, D.A., WU, M. & TAYLOR, D.R. (2014). Cytonuclear interactions and

relaxed selection accelerate sequence evolution in organelle ribosomes. *Molecular Biology and Evolution* **31**, 673–682.

SMITH, D.R. & KEELING, P.J. (2015). Mitochondrial and plastid genome architecture: Reoccurring themes, but significant differences at the extremes. *Proceedings of the National Academy of Sciences* **112**, 201422049.

SMITH, S., TURBILL, C. & SUCHENTRUNK, F. (2010). Introducing mother's curse: low male fertility associated with an imported mtDNA haplotype in a captive colony of brown hares. *Molecular Ecology* **19**, 36–43.

SNOOK, R.R. (2005). Sperm in competition: Not playing by the numbers. *Trends in Ecology and Evolution* **20**, 46–53.

STELKENS, R.B., SCHMID, C. & SEEHAUSEN, O. (2015). Hybrid breakdown in cichlid fish. *PLoS ONE* **10**, 1–11.

STEWART, J.B., FREYER, C., ELSON, J.L. & LARSSON, N.-G. (2008*a*). Purifying selection of mtDNA and its implications for understanding evolution and mitochondrial disease. *Nat Rev Genet* **9**, 657–662.

STEWART, J.B., FREYER, C., ELSON, J.L., WREDENBERG, A., CANSU, Z., TRIFUNOVIC, A. & LARSSON, N.G. (2008*b*). Strong purifying selection in transmission of mammalian mitochondrial DNA. *PLoS Biology* **6**, 0063–0071.

STEWART, J.B. & LARSSON, N.-G. (2014). Keeping mtDNA in shape between generations. *PLoS Genet* **10**, e1004670.

SUNNUCKS, P., MORALES, H.E., LAMB, A.M., PAVLOVA, A. & GREENING, C. (2017). Integrative approaches for studying mitochondrial and nuclear genome co-evolution in oxidative phosphorylation. *Frontiers in Genetics* **8**, 25.

TAANMAN, J.W. (2001). A nuclear modifier for a mitochondrial DNA disorder. *Trends in Genetics* **17**, 609–611.

TACHIBANA, M., AMATO, P., SPARMAN, M., WOODWARD, J., SANCHIS, D.M., MA, H., GUTIERREZ, N.M., TIPPNER-HEDGES, R., KANG, E., LEE, H.S., RAMSEY, C., MASTERSON, K., BATTAGLIA, D., LEE, D., WU, D., *ET AL*. (2013). Towards germline gene therapy of inherited mitochondrial diseases. *Nature* **493**, 627–631.

TACHIBANA, M., SPARMAN, M., SRITANAUDOMCHAI, H., MA, H., CLEPPER, L., WOODWARD, J., LI, Y., RAMSEY, C., KOLOTUSHKINA, O. & MITALIPOV, S. (2009). Mitochondrial gene replacement in primate offspring and embryonic stem cells. *Nature* **461**, 367–372.

THE 1000 GENOMES PROJECT CONSORTIUM (2015). A global reference for human genetic variation. *Nature*. http://www.nature.com/nature/journal/v526/n7571/pdf/nature15393.pdf.

TSUKIHARA, T., AOYAMA, H., YAMASHITA, E. & TOMIZAKI, T. (1996). The whole structure of the 13-subunit oxidized cytochrome c oxidase at 2.8 angstrom. *Science* **272**, 1136.

TURELLI, M. & ORR, H.A. (2000). Dominance, epistasis and the genetics of postzygotic isolation. *Genetics* **154**, 1663–1679.

VAN DER SLUIS, E.O., BAUERSCHMITT, H., BECKER, T., MIELKE, T., FRAUENFELD, J., BERNINGHAUSEN, O., NEUPERT, W., HERRMANN, J.M. & BECKMANN, R. (2015). Parallel structural evolution of mitochondrial ribosomes and OXPHOS complexes. *Genome Biology and Evolution* **7**, 1235–1251.

VAUGHT, R.C. & DOWLING, D.K. (2018). Maternal inheritance of mitochondria: implications for male fertility? *Reproduction* **155**, R159–R168.

VENTURA, D.F., GUALTIERI, M., OLIVEIRA, A.G.F., COSTA, M.F., QUIROS, P., SADUN, F., DE NEGRI, A.M., SALOMÃO, S.R., BEREZOVSKY, A., SHERMAN, J., SADUN, A.A. & CARELLI, V.

(2007). Male prevalence of acquired color vision defects in asymptomatic carriers of Leber's hereditary optic neuropathy. *Investigative Ophthalmology and Visual Science* **48**, 2362–2370.

WAI, T., TEOLI, D. & SHOUBRIDGE, E.A. (2008). The mitochondrial DNA genetic bottleneck results from replication of a subpopulation of genomes. *Nature Genetics* **40**, 1484–1488.

WALLACE, D.C. (2005). The mitochondrial genome in human adaptive radiation and disease: On the road to therapeutics and performance enhancement. *Gene* **354**, 169–180.

WALLACE, D.C. (2007). Why do we still have a maternally inherited mitochondrial DNA? Insights from evolutionary medicine. *Annual Review of Biochemistry* **76,** 781–821.

WALLACE, D.C. (2010). Bioenergetics, the origins of complexity, and the ascent of man. *Proceedings of the National Academy of Sciences* **107**, 8947–8953.

WANG, F., HUANG, G., TIAN, H., ZHONG, Y., SHI, H., LI, Z., ZHANG, X., WANG, H. & SUN, F. (2015). Point mutations in KAL1 and the mitochondrial gene MT-tRNA cys synergize to produce Kallmann syndrome phenotype. *Scientific Reports* **5**, 13050.

WERREN, J.H., BALDO, L. & CLARK, M.E. (2008). *Wolbachia*: master manipulators of invertebrate biology. *Nature Reviews Microbiology* **6**, 741–751.

WILLETT, C.S. (2008). Significant variation for fitness impacts of ETS loci in hybrids between populations of *Tigriopus californicus*. *Journal of Heredity* **99**, 56–65.

WILLETT, C.S. (2012). Quantifying the elevation of mitochondrial DNA evolutionary substitution rates over nuclear rates in the intertidal copepod *Tigriopus californicus*. *Journal of Molecular Evolution* **74**, 310–318.

WILLETT, C.S. & BURTON, R.S. (2001). Viability of cytochrome c genotypes depends on cytoplasmic backgrounds in *Tigriopus californicus*. *Evolution* **55**, 1592–1599.

1214 WILLETT, C.S. & BURTON, R.S. (2003). Environmental influences on epistatic interactions:
1215 viabilities of cytochrome c genotypes in interpopulation crosses. *Evolution* **57**, 2286–2292.
1216 WOLFF, J.N., LADOUKAKIS, E.D., ENRÍQUEZ, J.A. & DOWLING, D.K. (2014). Mitonuclear
1217 interactions: evolutionary consequences over multiple biological scales. *Philosophical*
1218 *Transactions of the Royal Society B: Biological Sciences* **369**, 20130443.
1219 WOLFF, J.N., PICHAUD, N., CAMUS, M.F., COTE, G., BLIER, P.U. & DOWLING, D.K. (2016).
1220 Evolutionary implications of mitochondrial genetic variation: mitochondrial genetic effects
1221 on OXPHOS respiration and mitochondrial quantity change with age and sex in fruit flies.
1222 *Journal of Evolutionary Biology* **29**, 736–747.
1223 WOODSON, J.D. & CHORY, J. (2008). Coordination of gene expression between organellar and
1224 nuclear genomes. *Nature Reviews Genetics* **9**, 383–395.
1225 WU, M., GU, J., GUO, R., HUANG, Y. & YANG, M. (2016). Structure of mammalian respiratory
1226 supercomplex I1III2IV1. *Cell* **167**, 1598–1609.e10.
1227 YAN, Z., YE, G. & WERREN, J. (2018). Evolutionary rate coevolution between mitochondria and
1228 mitochondria-associated nuclear-encoded proteins in insects. *bioRxiv*, 288456.
1229 YANG, M., HE, Z., SHI, S., WU, C., HILL, C., DIEGO, S., JOLLA, L., WALLIS, G.P., ARNTZEN, J.W.,
1230 VENDITTI, C., MEADE, A., PAGEL, M., VAN DOREN, B.M., CAMPAGNA, L., HELM, B., ET AL.
1231 (2017). Speciation genomics and a role for the Z chromosome in the early stages of
1232 divergence between Mexican ducks and mallards. *Evolution* **11**, 1–21.
1233 YEE, W.K.W., SUTTON, K.L. & DOWLING, D.K. (2013) In vivo male fertility is affected by
1234 naturally occurring mitochondrial haplotypes. *Current Biology* **23**, R55--R56. Elsevier.
1235 YEN, M.-Y., WANG, A.-G. & WEI, Y.-H. (2006). Leber's hereditary optic neuropathy: a
1236 multifactorial disease. *Progress in Retinal and Eye Research* **25**, 381–396.

ZAIDI, A.A. & MAKOVA, K.D. (2018). Mito-nuclear effects uncovered in admixed populations. *bioRxiv*, 349126.

ZHANG, F. & BROUGHTON, R.E. (2013). Mitochondrial-nuclear interactions: compensatory evolution or variable functional constraint among vertebrate oxidative phosphorylation genes? *Genome Biology and Evolution* **5**, 1781–1791.

ZHANG, Y., BRASHER, A.L., PARK, N.R., TAYLOR, H.A., KAVAZIS, A.N. & HOOD, W.R. (2018). High activity before breeding improves reproductive performance by enhancing mitochondrial function and biogenesis. *The Journal of Experimental Biology*, jeb.177469.

ZHOU, A., ROHOU, A., SCHEP, D.G., BASON, J. V., MONTGOMERY, M.G., WALKER, J.E., GRIGORIEFFNIKO, N. & RUBINSTEIN, J.L. (2015). Structure and conformational states of the bovine mitochondrial ATP synthase by cryo-EM. *eLife* **4**.

ZHU, C.T., INGELMO, P. & RAND, D.M. (2014). GxGxE for lifespan in *Drosophila*: mitochondrial, nuclear, and dietary interactions that modify longevity. *PLoS Genetics* **10**, e1004354.

ZHU, J., VINOTHKUMAR, K.R. & HIRST, J. (2016). Structure of mammalian respiratory complex I. *Nature* **536**, 354–358.

Table 1. Five themes in the study of mitonuclear interactions

1. **Relentless selection for mitonuclear compatibility across ontogeny**. Mitonuclear interactions have fitness consequences at multiple stages of development, which may result in compounding effects in filtering out maladapted mitonuclear genotypes.

   Prediction: the frequencies of mitochondrial (mt) and nuclear-encoded mitochondrial (N-mt) genes are predicted to change across life stages via selection for mitonuclear compatibility and functionality.

2. **Mitonuclear coadaptation is manifested in mitochondrial physiology**. The localized role of mt gene products within the mitochondria leads to the expectation that deleterious effects of maladapted mitonuclear genotypes will be mediated by changes in mitochondrial function.

   Prediction: incompatibilities in coadapted sets of mt and N-mt genes will have effects targeted to the physiological and biochemical properties of mitochondria.

3. **Generational delays**. Mitonuclear incompatibilities may be shielded by dominance in the F1 generation and may be affected by sex linkage.

   Prediction: the negative effects of novel combinations of mitochondrial and nuclear genes may not be evident until F2 and later generations.

4. **Mitonuclear incompatibilities need not involve protein–protein interactions or myriad substitutions.** Although most attention has focused on the protein–protein interactions that occur within oxidative phosphorylation (OXPHOS) complexes, there are many other arenas for mitonuclear interactions, including mitochondrial translation, transcription, and DNA replication. Single changes in mt or nuclear genes can also cause severe incompatibilities. Moreover, because many or most of the sequence changes that contribute to divergence in nuclear and mt genes may be neutral, the actual variants responsible for mitonuclear incompatibilities likely represent a small subset of total sequence change.

   Prediction: mitonuclear incompatibilities can be caused by a small number of variants that need not change amino acid sequence and that may not be proportional to overall sequence divergence.

5. **Mitonuclear coadaptation is dependent on complex genotype × genotype × environment interactions**. Mitochondrial function and physiology is highly context dependent, so the signatures of mitonuclear coadaptation are likely to be as well.

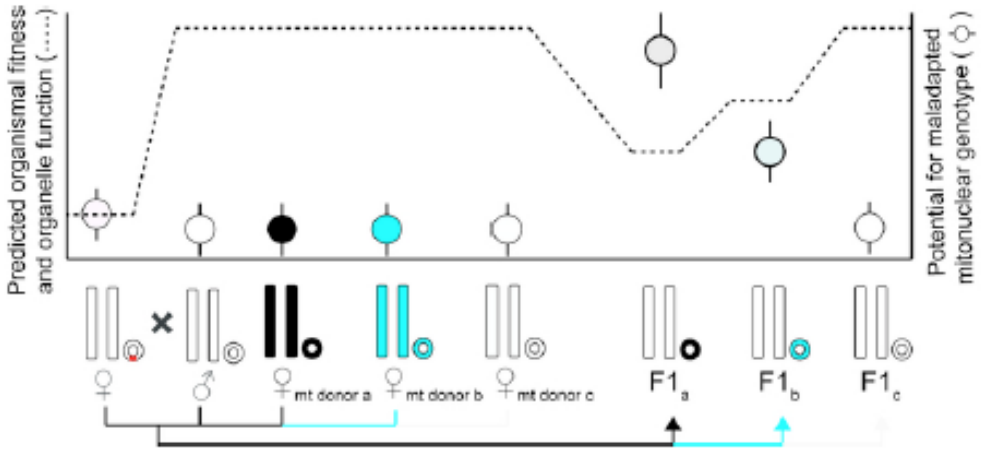
   Prediction: the outcomes of genetic interactions between mitochondrial and nuclear genomes will be dependent on the genetic [*via* epistasis involving other mtDNA and nuclear single nucleotide polymorphisms (SNPs)], physiological (e.g. the sex in which the mtDNA is expressed) and abiotic environment.

Figure Legends:

Fig. 1. Predicted organismal fitness, organelle function, and potential for maladapted mitonuclear genotypes during mitochondrial replacement therapy. Three possible mitochondrial donors are shown, yielding variable degrees of conceivable mitonuclear incompatibilities. Importantly, deleterious mtDNA mutations (shown in red) have *known* fitness consequences, while those resulting from mitonuclear incompatibilities are *predicted* and likely complex.

Fig. 2. Examples of mitonuclear interactions.: (A) multisubunit protein complexes of the electron transport chain, (B) mitochondrial ribosomal RNA (rRNA) and nuclear ribosomal proteins of the mitochondrial ribosome, (C) mitochondrial tRNA-Thr and nuclear threonyl-tRNA synthetase, and (D) mitochondrial DNA and nuclear DNA polymerase gamma. Non-interacting mitochondrial-encoded components are shown in green, nuclear-encoded components are in yellow, and interacting residues that physically contact residues encoded by both genomes are in red. All models are from mammals, except C which is from yeast. Interacting residues were identified following Sharbrough *et al.* (2017). PDB accessions used in structural depictions are 5LNK, 1ZOY, 1BGY, 1V54, 5ARA, 3J9M, 4YYE, and 5C51.

Fig. 3. Predicted organismal fitness and organelle function across generations. In the F1 generation, mitonuclear incompatibilities may generally be masked by retention of a maternal allele. Most mitonuclear incompatibilities are predicted to occur in F2 or later generations. After Burton *et al*. (2013).



1328

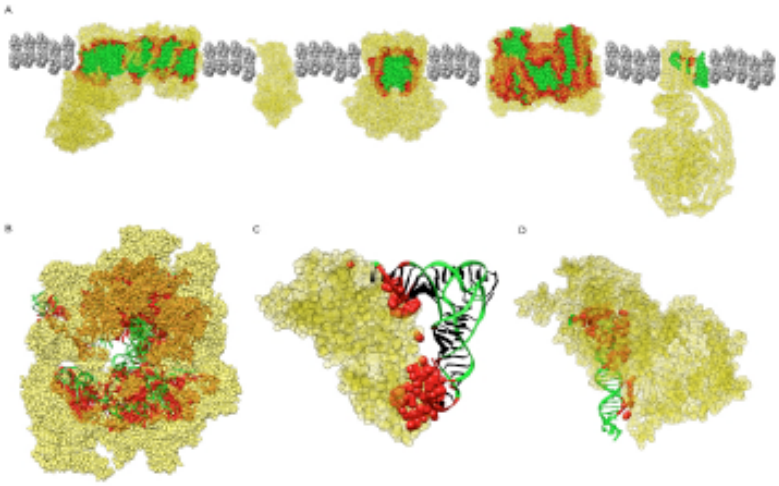
1329

1330 Figure 1

1331

1332

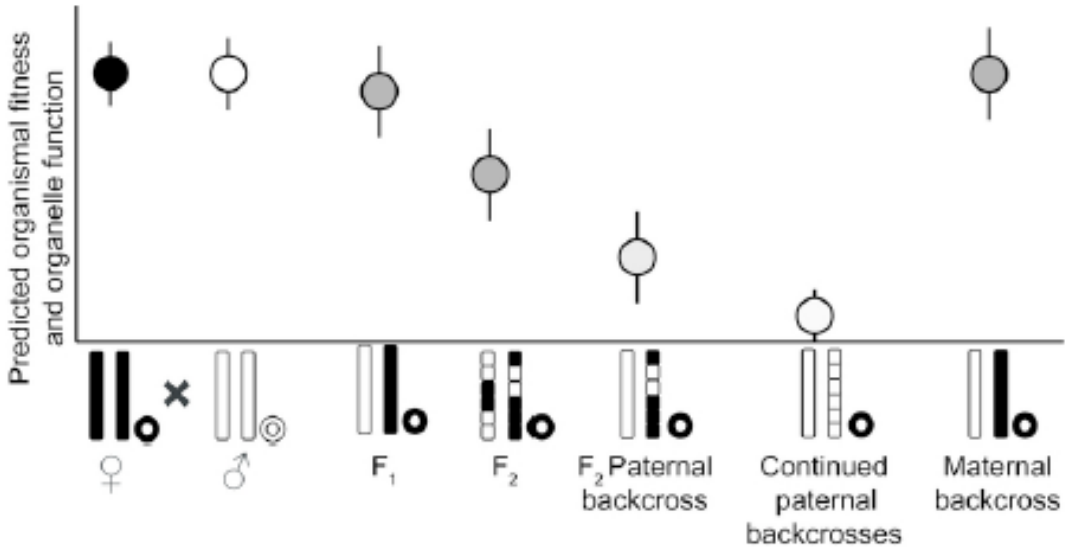
1333



1334

1335 Figure 2

1336



1337

1338

1339 Figure 3.