Molecular Ecology (2017) **26**, 2212–2236 doi: 10.1111/mec.13959

INVITED REVIEWS AND SYNTHESES

The on-again, off-again relationship between mitochondrial genomes and species boundaries

DANIEL B. SLOAN, JUSTIN C. HAVIRD and JOEL SHARBROUGH
Department of Biology, Colorado State University, Fort Collins, CO 80523, USA

Abstract

The study of reproductive isolation and species barriers frequently focuses on mitochondrial genomes and has produced two alternative and almost diametrically opposed narratives. On one hand, mtDNA may be at the forefront of speciation events, with coevolved mitonuclear interactions responsible for some of the earliest genetic incompatibilities arising among isolated populations. On the other hand, there are numerous cases of introgression of mtDNA across species boundaries even when nuclear gene flow is restricted. We argue that these seemingly contradictory patterns can result from a single underlying cause. Specifically, the accumulation of deleterious mutations in mtDNA creates a problem with two alternative evolutionary solutions. In some cases, compensatory or epistatic changes in the nuclear genome may ameliorate the effects of mitochondrial mutations, thereby establishing coadapted mitonuclear genotypes within populations and forming the basis of reproductive incompatibilities between populations. Alternatively, populations with high mitochondrial mutation loads may be rescued by replacement with a more fit, foreign mitochondrial haplotype. Coupled with many nonadaptive mechanisms of introgression that can preferentially affect cytoplasmic genomes, this form of adaptive introgression may contribute to the widespread discordance between mitochondrial and nuclear genealogies. Here, we review recent advances related to mitochondrial introgression and mitonuclear incompatibilities, including the potential for cointrogression of mtDNA and interacting nuclear genes. We also address an emerging controversy over the classic assumption that selection on mitochondrial genomes is inefficient and discuss the mechanisms that lead lineages down alternative evolutionary paths in response to mitochondrial mutation accumulation.

Keywords: co-evolution, cytonuclear interactions, introgressive hybridization, mitochondrial introgression, mutation accumulation, reproductive isolation

Received 16 October 2016; revision received 16 November 2016; accepted 18 November 2016

Background

Speciation and species boundaries are often intertwined with cytoplasmic genetics. Classic breeding experiments dating back to the work of J. G. Kölreuter in the 18th century (reviewed in Mayr 1986) have demonstrated that the reproductive barriers that prevent hybridization between isolated populations are often asymmetric, meaning that the extent of incompatibilities in hybrid offspring depends on which species acted as the maternal vs. paternal parent (Turelli & Moyle 2007). Effects of mitochondrial DNA (mtDNA) and other cytoplasmically inherited genomes (i.e. plastids and bacterial endosymbionts) represent one likely cause of these asymmetries in reciprocal crosses. Because Darwin (1859) was one of the first to recognize the widespread nature of these asymmetries, the phenomenon has been termed 'Darwin's corollary' (Turelli & Moyle 2007). More recent investigations have revealed that the small number of genes housed in mitochondrial genomes can play a disproportionately large role in reproductive isolation (Gershoni *et al*. 2009; Burton & Barreto 2012; Burton *et al*. 2013; Hill 2016).

Correspondence: Daniel B. Sloan, Fax: 970 491 0649; E-mail: dan.sloan@colostate.edu

At the same time, the rise of molecular systematics and the widespread use of mitochondrial markers in 'barcoding' efforts have demonstrated that discordance between mitochondrial and nuclear genealogies is commonplace and often results from mtDNA introgression between otherwise well-defined species (Chan & Levin 2005; Toews & Brelsford 2012). Such observations lead to the seemingly paradoxical conclusion that mitochondrial genomes play a central role in creating species boundaries but then blatantly disregard them. Although the occurrence of mitonuclear incompatibilities and mitochondrial introgression are both supported by extensive bodies of literature, these fields remain somewhat disconnected, perhaps explaining why this paradox is only rarely acknowledged (Boratyński *et al.* 2011; Burton & Barreto 2012; Toews & Brelsford 2012). Our goal in this review was to synthesize these two fields, with a particular focus on deleterious mutation accumulation in mtDNA as a potential link between these seemingly unrelated and even contradictory biological patterns.

This review is divided into five sections. First, we describe and elaborate on the model of compensatory mitonuclear co-evolution (Rand *et al.* 2004; Osada & Akashi 2012; van der Sluis *et al.* 2015), which has been hypothesized to result in a disproportionate role for mtDNA in the evolution of reproductive isolation and the origin of species (Gershoni *et al.* 2009; Burton & Barreto 2012). In its boldest form, this line of argument has led to the recent proposal of a 'mitonuclear compatibility species concept' (Hill 2016). Second, we review the adaptive and nonadaptive mechanisms that can promote preferential introgression of mitochondrial genomes. We focus on the possibility that adaptive introgression of foreign mtDNA may occur in species or populations that have suffered from accumulation of deleterious mitochondrial mutations (Llopart *et al.* 2014; Hulsey *et al.* 2016). Third, we review recent evidence for cointrogression of mtDNA and nuclear genes that are targeted to the mitochondria (Beck *et al.* 2015; Boratyński *et al.* 2016), which is a potential mechanism to achieve 'the best of both worlds' by introducing adaptive mitochondrial haplotypes without disrupting coevolved mitonuclear interactions. However, we also identify some of the population genetic hurdles that are likely to impede cointrogression in many circumstances. Fourth, we revisit classic evolutionary assumptions about the reduced efficacy of selection in asexual mitochondrial genomes (Muller 1964; Lynch & Blanchard 1998; Neiman & Taylor 2009), because these assumptions play a central role in the models discussed in this study but have been called into question by recent studies (Popadin *et al.* 2013; Cooper *et al.* 2015). Finally, we discuss mechanisms that may result in contrasting evolutionary responses to mitochondrial mutation accumulation, laying out testable hypotheses and directions for future research.

Mitonuclear incompatibilities and species boundaries

Mitochondrial genes in Bateson–Dobzhansky–Muller incompatibilities

One of the cornerstones of the field of speciation genetics is a model based on epistatic interactions between two or more loci (Coyne & Orr 2004). The central concept is that changes in individual loci can accumulate independently in isolated populations or sequentially in the same population in such a way that certain combinations of alleles were never 'tested' by natural selection (Fig. 1) (Orr 1995). If such untested combinations produce harmful interactions, then hybridization will result in sterility, inviability or other reductions in fitness. These interactions are known as Dobzhansky–Muller or Bateson–Dobzhansky–Muller incompatibilities (BDMIs) after the model's early proponents (Orr 1996). The appeal of this model is that it provides a mechanism by which hybrid sterility/inviability can evolve without individual populations traversing 'fitness valleys'.

In principle, BDMIs can involve any pair of genes as long as it is possible for hybridization to generate novel combinations of alleles at those loci. Given that mtDNA accounts for only a tiny fraction of eukaryotic genes, the null expectation is that most BDMIs should occur among nuclear loci and that mitonuclear incompatibilities should be unusual. For example, in an animal with ~20 000 protein-coding genes of which 13 are found in the mitochondrial genome, only ~0.1% of all possible pairwise interactions would be mitonuclear. Counter to this expectation, mitonuclear interactions have been identified as an important source of reproductive barriers in numerous and diverse eukaryotic lineages (Table 1). The many examples of reproductive isolation in plants associated with plastid-nuclear incompatibilities further underscore a role for cytoplasmic–nuclear interactions in speciation (Levin 2003; Greiner *et al.* 2011). Evidence of cytonuclear incompatibilities in hybrids has come from a combination of crossing experiments, cellular manipulations to generate cytoplasmic hybrids (cybrids), site-directed mutagenesis of genes involved in mitonuclear interactions, mitochondrial/plastid biochemistry, protein structural modelling and phylogenetic/population genetic analyses. These observations have led many to conclude that cytoplasmic genomes play a disproportionate role in reproductive isolation (although, to our knowledge, no meta-analyses have yet been performed to provide statistical support

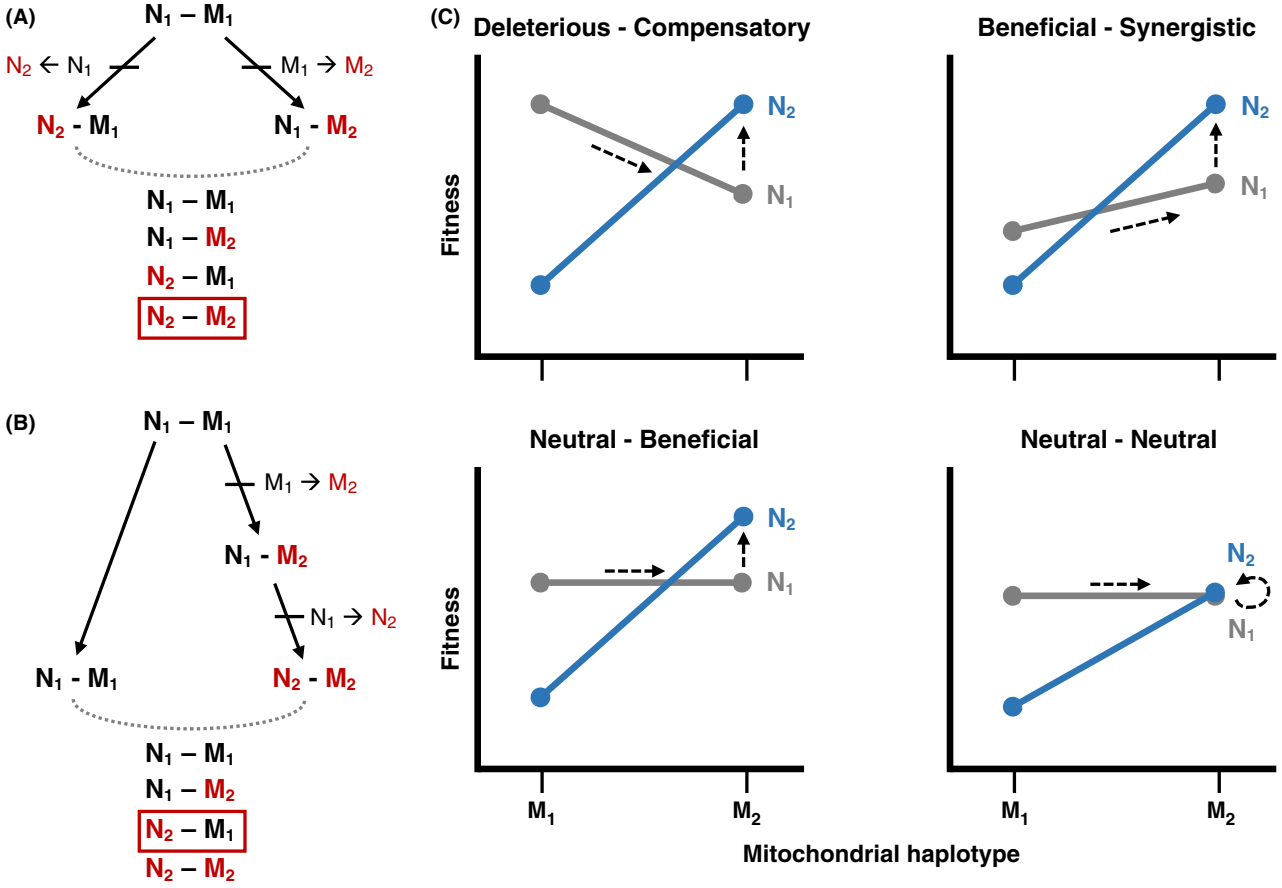


Fig. 1 Bateson–Dobzhansky–Muller incompatibilities and mitonuclear epistasis. (A) The BDMI model applied to a mitonuclear interaction. N_1 and N_2 are the ancestral and derived nuclear alleles, respectively. M_1 and M_2 are the ancestral and derived mitochondrial alleles, respectively. For simplicity, nuclear genotypes are written as haploid. The curved, dotted line indicates hybridization and admixture, which produces all possible combination of mitonuclear genotypes. The genotype that had not previously been 'tested' by selection is boxed in red. (B) Although BDMIs are conventionally described as resulting from substitutions that occur in parallel in two different populations (see panel A), the same logic can apply to two sequential substitutions within the same population (Orr 1995). This distinction is important because much of the theory related to mitonuclear interactions involves co-evolutionary pressures that are expected to produce mitochondrial and nuclear changes in the same lineage. Once again, the curved, dotted line indicates hybridization/admixture, and the previously 'untested' mitonuclear genotype is boxed in red. (C) Examples of mitonuclear epistasis in which an initial mitochondrial change (from M_1 to M_2) can make a nuclear change (from N_1 to N_2) that previously would have been deleterious now be beneficial or at least neutral. The ancestral and derived nuclear alleles are shown in grey and blue, respectively. Black arrows indicate the direction of evolutionary change. The examples differ based on the fitness effects of the initial mitochondrial change (deleterious, neutral or beneficial) and of the subsequent nuclear changes. The examples do not represent a comprehensive set of all possible arrangements, but any of these paths (and others) would arrive at coadapted mitonuclear genotypes that can be disrupted by hybridization.

for disproportionality). This body of work has been the subject of extensive reviews (Levin 2003; Gershoni *et al.* 2009; Greiner *et al.* 2011; Burton & Barreto 2012; Burton *et al.* 2013; Reinhardt *et al.* 2013; Hill 2015), so we direct readers to those for a more detailed summary and literature references.

The compensatory mitonuclear co-evolution model

Why is it then that mitochondrial genomes are thought to play a disproportionate role in the evolution of reproductive isolation? Prevailing theory points to multiple key features of mtDNA inheritance that can result in rapid co-evolution with the nucleus and the build-up of coadapted mitonuclear genotypes. In particular, mitochondria are typically transmitted in a uniparental fashion, making them effectively haploid and asexual. Both of these characteristics are expected to reduce effective population size (N_e), which will increase the rate at which polymorphisms sort out into genetically structured populations (Harrison 1989). Asexual inheritance and low N_e are also thought to reduce the

efficiency with which selection can remove deleterious mutations from the population (Muller 1964; Lynch & Blanchard 1998; Neiman & Taylor 2009) (see 'Deleterious Mutation Accumulation and the Efficacy of Selection in Cytoplasmic Genomes' below for a detailed discussion of this topic). In many eukaryotic lineages, mitochondrial genomes also experience much higher mutation rates than the nucleus (Brown *et al.* 1979; Denver *et al.* 2000; Lynch *et al.* 2008). The combination of a large mutational input and the reduced ability of selection to filter those mutations may act as a one-two punch that can lead to a high fixation rate for weakly deleterious alleles.

While high mutation rates and uniparental inheritance are common features of mitochondrial genomes, it is important to note that the genetics of mitochondria vary tremendously across eukaryotic lineages. Mitochondrial mutation rates span orders of magnitude (Nabholz *et al.* 2009; Richardson *et al.* 2013), and there are many exceptions in which mitochondrial genomes are inherited biparentally and undergo sexual recombination (Barr *et al.* 2005). Therefore, any attempt to describe a 'universal' model of mitonuclear co-evolution may fall short. It is noteworthy that some of the strongest evidence for the effects of mitochondrial genomes in speciation comes from organisms with exceptionally high mitochondrial mutation rates such as the copepod *Tigriopus* and the parasitoid wasp *Nasonia* (Oliveira *et al.* 2008; Willett 2012).

Another key feature of mitochondrial genetics is the extensive interaction between mitochondrial gene products and nuclear-encoded proteins. Mitochondrial genomes encode anywhere from 1 to 100 genes (Burger *et al.* 2013; Petersen *et al.* 2014), but the function of mitochondria can depend on >1000 different proteins (Calvo & Mootha 2010), the vast majority of which are encoded by mitochondrially targeted genes in the nucleus (N-mt genes). Essential cell functions such as cellular respiration and mitochondrial translation are mediated by intimate interactions between mitochondrial and N-mt gene products that form multisubunit complexes such as ribosomes and OXPHOS enzymes (Fig. 2; Table 2). The division of labour between the mitochondrial and nuclear genomes largely results from the history of endosymbiotic gene transfer into the nucleus during eukaryotic evolution (Timmis *et al.* 2004).

Observations of high rates of mitochondrial mutation accumulation have led to the hypothesis that selection favours changes in N-mt proteins that compensate for deleterious mitochondrial substitutions and maintain the stability and function of multisubunit complexes

Table 1 Examples of systems with evidence of a cytonuclear basis of reproductive isolation or cytonuclear effects on fitness. In some cases, the evidence remains indirect and/or speculative

Taxon	References
Animals	
Arthropods (*Acanthoscelides*, *Callosobruchus*, *Encarsia*, *Drosophila*, *Nasonia*, *Tigriopus*)	Sackton *et al.* (2003), Dowling *et al.* (2007b), Ellison *et al.* (2008), Niehuis *et al.* (2008), Meiklejohn *et al.* (2013), Đorđević *et al.* (2015), Gebiola *et al.* (2016), Immonen *et al.* (2016)
Nematodes (*Caenorhabditis*)	Zhu *et al.* (2015), Chang *et al.* (2016)
Vertebrates (*Anguilla*, *Ambystoma*, Centrarchidae, *Chamaeleo*, *Eopsaltria*, *Mus*, *Passer*)	Nagao *et al.* (1998), Bolnick *et al.* (2008), Gagnaire *et al.* (2012), Lee-Yaw *et al.* (2014), Trier *et al.* (2014), Bar-Yaacov *et al.* (2015), Morales (2016)
Fungi	
Yeast (*Saccharomyces*)	Zeyl *et al.* (2005), Lee *et al.* (2008), Chou *et al.* (2010), Paliwal *et al.* (2014), Spirek *et al.* (2015)
Plants	
Angiosperms (numerous)	Reviewed in Levin (2003), Greiner *et al.* (2011)

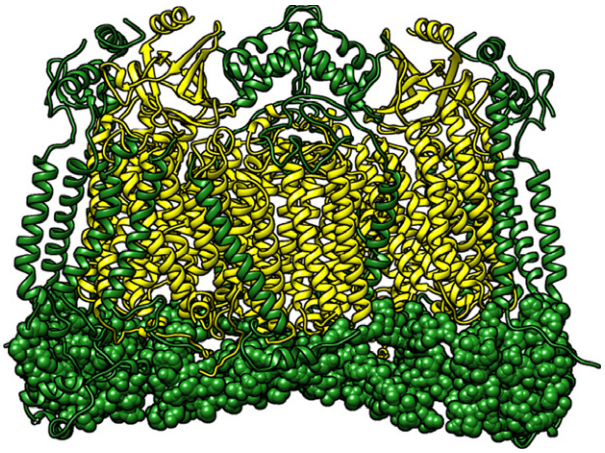


Fig. 2 An example of intimate interactions between mitochondrial and N-mt proteins: 3D structure of the OXPHOS complex IV (cytochrome c oxidase) isolated from bovine heart tissue (PDB accession 1OCC). Three mitochondrially encoded subunits (COX1, COX2 and COX3) are shown in yellow, while 10 nuclear-encoded subunits (COX4, COX5A, COX5B, COX6A2, COX6B1, COX6C, COX7A1, COX7B, COX7C, COX8B) are shown in green. COX5 subunits, which have been implicated in cointrogression with an mtDNA haplotype that has moved between *Drosophila* species (Beck *et al.* 2015), are drawn with green spheres. The remaining subunits are drawn with ribbons.

Table 2 Examples of interactions between nuclear and cytoplasmic gene products at the molecular level and related studies of the potential for co-evolutionary dynamics resulting from these interactions

Interaction	References
Mitochondria	
OXPHOS complexes	Sackton *et al.* (2003), Willett & Burton (2004), Ellison & Burton (2006), Ellison *et al.* (2008), Liu *et al.* (2009), Osada & Akashi (2012), Parmakelis *et al.* (2013), Gershoni *et al.* (2014), Havird *et al.* (2015), J. C. Havird, P. Trapp, C. Miller, I. Bazos & D. B. Sloan (in review)
Ribosomes	Liu *et al.* (2009), Barreto & Burton (2013), Sloan *et al.* (2014b), J. C. Havird, P. Trapp, C. Miller, I. Bazos & D. B. Sloan (in review)
tRNA maturation	Meiklejohn *et al.* (2013), Pett & Lavrov (2015), Adrion *et al.* (2016)
mRNA binding/ processing/ regulation	Fujii *et al.* (2011)
Transcription	Ellison & Burton (2010)
DNA replication/repair	Ellison & Burton (2010), J. C. Havird, P. Trapp, C. Miller, I. Bazos & D. B. Sloan (in review)
Plastids	
Photosynthetic enzymes	Kanevski *et al.* (1999), Gong *et al.* (2012), Sehrish *et al.* (2015)
CLP Caseinolytic Protease	Rockenbach *et al.* (2016)
Acetyl-CoA Carboxylase	Rockenbach *et al.* (2016)
Ribosomes	Sloan *et al.* (2014b), Weng *et al.* (2016)
tRNA maturation	Duchêne *et al.* (2005)
mRNA binding/ processing/regulation	Hayes & Mulligan (2011)
RNA polymerase (PEP)/ transcription	Zhang *et al.* (2015)
DNA replication/repair	Zhang *et al.* (2016)
Other Bacterial Endosymbionts	
Photosynthetic enzymes (*Paulinella*)	Nowack *et al.* (2016)
Amino acid, peptidoglycan and vitamin biosynthesis pathways (insect endosymbionts)	Hansen & Moran (2011), Husnik *et al.* (2013), Sloan *et al.* (2014a)

(Rand *et al.* 2004; Osada & Akashi 2012; van der Sluis *et al.* 2015). This relationship is expected to be asymmetric because the larger N_e and typically sexual mode of inheritance in the nuclear genome should result in more efficient selection acting both in favour of beneficial changes and against deleterious changes. Therefore, adaptive changes in the nucleus would tend to compensate for deleterious changes in the mitochondrial genomes rather than vice versa. This compensatory co-evolution model is supported by findings that N-mt proteins tend to evolve more rapidly than other nuclear-encoded proteins (Barreto & Burton 2013; Sloan *et al.* 2014b; Havird *et al.* 2015). Even so, there are important alternative interpretations that must be taken into account. In particular, it has been suggested that N-mt genes are less functionally constrained than other nuclear genes and, therefore, likely to tolerate more amino acid substitutions even in the absence of positive selection (Barreto & Burton 2013; Nabholz *et al.* 2013; Zhang & Broughton 2013; Sloan *et al.* 2014b; Pett & Lavrov 2015; Adrion *et al.* 2016). For example, it is likely that selection for translational efficiency is less intense on the enzymatic machinery (i.e. ribosomal proteins and aminoacyl tRNA synthetases) that functions in the mitochondria than on the corresponding cytosolic machinery that is responsible for translating the enormous number of transcripts originating from the nucleus (Barreto & Burton 2013; Sloan *et al.* 2014b; Pett & Lavrov 2015; Adrion *et al.* 2016).

One of the more direct tests of the compensatory co-evolution model was performed by Osada & Akashi (2012), who showed that mitochondrial and nuclear substitutions in primates preferentially occur at pairs of interacting amino acids. Their work also revealed that the nuclear substitutions tend to occur after the mitochondrial substitutions. Harrison & Burton (2006) have also used site-directed mutagenesis to show that some examples of interpopulation hybrid breakdown in the copepod *Tigriopus* are the result of amino acid substitutions in N-mt genes.

Comparing related species with highly heterogeneous mitochondrial mutation rates can also provide a framework for powerful and well-controlled tests for compensatory co-evolution because increased mitochondrial mutation rates are expected to elicit stronger selection on N-mt genes. A number of lineages of flowering plants (e.g. the genera *Plantago*, *Pelargonium* and *Silene*) have been identified as having unusually high and variable mitochondrial mutation rates (Mower *et al.* 2007). We have found that *Silene* species with recent accelerations in mitochondrial mutation rate have experienced correlated increases in the rate of amino acid substitution in interacting N-mt genes but not in the rest of the nuclear genome (Sloan *et al.* 2014b; Havird *et al.* 2015; J. C. Havird, P. Trapp, C. Miller, I. Bazos & D. B. Sloan, in review). More generally, rates of amino acid substitution in N-mt genes tend to be higher in lineages with

faster mitochondrial mutation rates across eukaryotes (Havird & Sloan 2016). Together, these alternative lines of research support the hypothesis that mitochondrial mutation accumulation creates selection for subsequent (and potentially compensatory) changes in the nucleus.

Beyond the compensatory mitonuclear co-evolution model

The predicted outcome of compensatory mitonuclear co-evolution is coadapted or 'matched' mitonuclear genotypes. This coadaptation model is consistent with observations from diverse eukaryotic lineages that hybridization often exposes mitonuclear BDMIs (Table 1). Even so, it is important to recognize that not all modes of mitonuclear co-evolution must be driven by deleterious changes in mtDNA and compensatory changes in nuclear genes. Considering alternative models is especially relevant to groups such as angiosperms, in which cytonuclear incompatibilities are commonly observed (Levin 2003; Greiner *et al.* 2011). Angiosperms maintain a large number of cytoplasmic genes that engage in interactions with the nucleus (e.g. a typical total of 24-41 protein-coding genes in the mitochondrial genome and ~80 in the plastid genome), but these lineages should be less susceptible to deleterious mutation accumulation because of relatively low mitochondrial and plastid mutation rates (Wolfe *et al.* 1987; Drouin *et al.* 2008).

Mitochondrial substitutions that are neutral or adaptive may also facilitate subsequent changes in N-mt genes that would have been deleterious on the ancestral mitochondrial background. In fact, there is a wide range of scenarios that could promote the evolution of mitonuclear BDMIs based on sign epistasis (i.e. when the direction of a nuclear mutation's effect on fitness depends on the mitochondrial background) (Fig. 1). Researchers have taken advantage of genetic model systems such as *Drosophila* and *Saccharomyces* to generate a full matrix of different pairwise combinations of mitochondrial and nuclear genotypes (Zeyl *et al.* 2005; Dowling *et al.* 2007a,b; Paliwal *et al.* 2014; Zhu *et al.* 2014; Mossman *et al.* 2016). These efforts have shown extensive mitonuclear epistasis for many fitness-related measures that can exhibit diverse interaction patterns (Fig. 1). Many of these experiments have been conducted in two or more environments and have revealed that patterns of mitonuclear epistasis are often context-dependent (Dowling *et al.* 2007a; Arnqvist *et al.* 2010; Hoekstra *et al.* 2013; Paliwal *et al.* 2014; Zhu *et al.* 2014; Mossman *et al.* 2016). The presence of such 'G × G × E interactions' indicates that the effects of disrupting coadapted mitonuclear genotypes may vary across environments.

An additional layer of complexity results from the fact that mitochondrial genomes are naturally prone to conflicting levels of selection. Changes in mtDNA that increase the probability of mitochondrial transmission may be favoured by selection even when they come at the expense of organismal fitness. For example, mitochondrial genome copies with large deletions may hamper cellular respiration but still spread because these deletion mutants have an intracellular replication advantage (Taylor *et al.* 2002; Clark *et al.* 2012; Phillips *et al.* 2015). In species with maternally inherited mitochondria, mtDNA mutations that have deleterious effects only in males are able to spread because mtDNA transmission is not dependent on male fitness. Such alleles may even be *favoured* by selection if they have beneficial effects in females (i.e. sexually antagonistic selection). Proposed by Frank & Hurst (1996), this phenomenon is often referred to as 'mother's curse' (Gemmell *et al.* 2004). Cytoplasmic male sterility in flowering plants is a classic example of this conflict (Touzet & Budar 2004; Fishman & Willis 2006; Fujii *et al.* 2011; Beekman *et al.* 2014). The mother's curse hypothesis is also supported by experimental findings that mitochondrial genetic variation in *Drosophila* has larger phenotypic effects on males than on females (Innocenti *et al.* 2011; Camus *et al.* 2012). The presence of selfish mitochondrial elements creates selection on the nucleus to counteract or mitigate their effects. Therefore, such elements may be effectively silenced within a population but exposed by interpopulation crosses (e.g. Fishman & Willis 2006; Yee *et al.* 2013). In summary, there are diverse evolutionary pressures, extending well beyond the conventional model of compensatory mitonuclear co-evolution, that can lead to 'matched' mitonuclear genotypes that are sensitive to disruption by hybridization.

The mitonuclear compatibility species concept

The use of mitochondrial loci in 'barcoding' studies implies that mitochondrial markers have value in defining species and species boundaries. However, under the compensatory co-evolution model, mtDNA is more than diagnostic of species identity; it can play a causal role in establishment of new species. Accordingly, Hill (2016) has proposed a novel species concept based on mitonuclear compatibility: 'divergence in mitochondrial genotype is the basis for speciation, and therefore, mitochondrial genotype defines a species… A species is a population that is reproductively isolated from other populations by incompatibilities in uniquely coadapted mt and N-mt genes'. Hill & Johnson (2013) have also argued that some of the classic behavioural and morphological traits associated with sexual selection act to

reinforce the reproductive barriers established by mitonuclear incompatibilities. They propose that species-specific ornamentation patterns in birds and other animals convey important information so that females can choose a mate that will transmit N-mt genes that are compatible with her own mitochondrial genotype. They also hypothesize that male ornamentation serves as an indicator of male quality that is closely tied to mitochondrial function. These provocative hypotheses raise a number of interesting evolutionary questions; however, it is a substantial extrapolation to go from the conclusion that mitochondrial genes play a disproportionate role in generating reproductive isolation to the argument that species are defined by their mitochondrial genotypes. As we discuss below, any placement of mtDNA at the centre of a species concept must be reconciled with the rampant introgression of mtDNA across species boundaries that has been observed in many eukaryotic lineages.

Mitochondrial introgression

Widespread discordance between nuclear and mitochondrial genealogies

The pervasive use of molecular markers in population genetic and phylogenetic studies has revealed that nuclear and cytoplasmic genealogies often conflict (Rieseberg & Soltis 1991; Currat *et al.* 2008). This 'mitonuclear discordance' is not a rare phenomenon. Toews & Brelsford (2012) attempted to generate an unbiased estimate of the frequency of conflicting mitochondrial and nuclear genealogies in animals by sampling a set of 61 studies that utilized both nuclear and mitochondrial markers. Their analysis revealed evidence of discordance in 11 of those studies (18%). Similarly, Funk & Omland (2003) found evidence of species-level paraphyly or polyphyly in 23% of surveyed animal mtDNA studies, although some of these cases are likely artefacts of incorrect taxonomy (McKay & Zink 2010).

One possible explanation for discordance among gene trees is incomplete lineage sorting (Degnan & Rosenberg 2009), in which ancestral polymorphisms are initially maintained in multiple lineages after species splits and then undergo differential patterns of fixation. The net result is a difference between gene tree topologies and the overall species tree. However, incomplete lineage sorting is unlikely to be the predominant cause of mitonuclear discordance in cases in which there is a general agreement among large numbers of nuclear loci but discordance with mitochondrial genealogies. Because of the reduced N_e in mitochondrial genomes, polymorphisms in mtDNA are expected to sort out relatively rapidly by genetic drift and, therefore, be less likely to conflict with a consensus (nuclear) species tree (Good *et al.* 2015).

A more likely cause of mitonuclear discordance in these circumstances appears to be introgression, which can often act in an asymmetric fashion between populations and can differentially affect nuclear vs. mtDNA (Chan & Levin 2005; Toews & Brelsford 2012). In fact, there are many cases in which mitochondrial introgression appears to occur with little or no detectable movement of nuclear genes (e.g. Bernatchez *et al.* 1995; Zieliński *et al.* 2013; Pons *et al.* 2014; Good *et al.* 2015). There is even evidence that mitochondrial introgression has shaped the evolutionary history of our own closest relatives. The genealogies of humans and related hominins exhibit mitonuclear discordance (Reich *et al.* 2010), and recent findings suggest that Neanderthals may have experienced an introgression event that replaced their ancestral mitochondrial background after they diverged from Denisovans (Meyer *et al.* 2014, 2016). When juxtaposed with evidence that mtDNA often plays a role in establishing species boundaries, observations of widespread mitonuclear discordance pose perplexing questions about the forces that promote mitochondrial introgression across those boundaries.

Nonadaptive causes of mitochondrial introgression

There are many reasons to expect introgression to preferentially affect mtDNA, even in the absence of selection pressures that favour a specific mitochondrial haplotype (Box 1). Likewise, there are potential nonadaptive explanations for the asymmetrical or unidirectional patterns of introgression that are frequently observed. These mechanisms generally follow from the fact that mtDNA is preferentially or exclusively transmitted by females in many species. Therefore, biological differences in the behaviour or genetics of the two sexes can differentially affect the movement of mitochondrial vs. nuclear genes across species and population boundaries. Examples of the nonadaptive mechanisms that promote asymmetric and preferential introgression of mtDNA are detailed in Box 1.

Adaptive mitochondrial introgression

There is increasing evidence for the functional importance of naturally occurring mitochondrial polymorphisms (Ballard & Whitlock 2004; Dowling *et al.* 2008; Galtier *et al.* 2009; Bock *et al.* 2014; Dobler *et al.* 2014). Mitochondrial genetic effects have been linked to numerous and diverse fitness-related traits, such as energy production (Kenney *et al.* 2013), ageing (Bender *et al.* 2006), neuronal function (Kraytsberg *et al.* 2006)

Box 1. Nonadaptive Causes of Asymmetric and Preferential Introgression of mtDNA

Although adaptive hypotheses are often proposed to explain observed patterns of mtDNA introgression, preferential movement of mitochondrial genes across species boundaries likely occurs even in the absence of selection. Nonadaptive mechanisms can also produce highly asymmetrical introgression from one species to another without gene flow in the opposite direction. The mechanisms described below are all contingent on uniparental inheritance of mtDNA such that behavioural and genetic differences between the sexes can translate into differences in the relative mobility of mitochondrial vs. nuclear genes.

- Sex-Biased Dispersal: Differences in the propensity of males and females to migrate can affect rates of mitochondrial vs. nuclear gene flow. Male dispersal results in movement of nuclear genes but not mtDNA. Conversely, female immigrants make a disproportionate contribution to mitochondrial allele frequencies in a population. Analogous effects are also associated with differences in dispersal rates of pollen vs. seeds in plant populations (McCauley 1995). Therefore, sex-biased dispersal can have important (but not necessarily intuitive) effects on the relative rates of nuclear and mitochondrial introgression. For example, Currat *et al.* (2008) modelled admixture at the interface of an expanding range front and found that introgression into the invading species preferentially occurs at loci with *low* rates of intraspecific gene flow. The interpretation of this result is that introduced alleles are less likely to get swamped out by continued migration from the core of the range when intraspecific gene flow is limited. Under this model, introgression would be biased towards mtDNA in species with male-biased dispersal (e.g. many mammals) and towards nuclear DNA in species with female-biased dispersal (e.g. many birds), which was supported by a subsequent meta-analysis (Petit & Excoffier 2009).
- Sex Bias in Hybrid Offspring (Haldane's Rule): Introgression of genes across species boundaries requires that hybrid offspring are viable and capable of mating with the parental species. J.B.S. Haldane's (1922) classic observation that hybrid sterility preferentially occurs in the heterogametic sex (i.e. males in XY sex determination systems and females in ZW sex determination systems) has important implications for rates of mitochondrial introgression. For example, in ZW species (e.g. birds), the fact that hybrid females are likely to be sterile may preclude mitochondrial introgression even if nuclear introgression can still occur via male hybrids. Conversely, Haldane's rule may contribute to disproportionate introgression of mtDNA in XY systems (e.g. mammals). Interestingly, the combination of female-biased dispersal and ZW sex determination may act as a dual hindrance that makes mitochondrial introgression unusually rare in birds (Petit & Excoffier 2009; but see Toews & Brelsford 2012 for conflicting evidence about prevalence in birds). Therefore, because mtDNA introgression may be relatively rare in birds, it might not be a coincidence that the mitonuclear compatibility species concept emerged, in part, based on the observed effectiveness of mitochondrial barcoding for species delimitation in the field of ornithology (Hill 2016). The effects of sex bias can also be extended to systems that result in the exclusive production of female offspring (e.g. cytoplasmic male sterility in plants (Schnable & Wise 1998) and reproductive manipulation by *Wolbachia* and other intracellular bacteria in insects (Werren *et al.* 2008). Such cases can result in rapid introgression of mtDNA into a population, whereas introgression of nuclear genes is inhibited by the necessity for repeated backcrossing with males from that population (Hurst & Jiggins 2005).
- Asymmetric Reproductive Incompatibilities from Reciprocal Crosses (Darwin's Corollary): While Haldane's rule pertains to asymmetries between male and female offspring from hybrid crosses, an extension of this rule (known as Darwin's corollary) holds that reciprocal crosses between two species often result in unequal degrees of hybrid sterility or inviability (Turelli & Moyle 2007). Such asymmetries will naturally lead to biased or unidirectional transfer of mtDNA from one species to another. For example, if hybridization between species A and species B fails because of either pre- or postzygotic barriers when species A acts as the maternal parent, then mtDNA introgression can exclusively occur from B to A and not vice versa (assuming maternal inheritance).
- Sexual Selection: As noted above, asymmetric reproductive incompatibilities can drive preferential introgression of mtDNA. This same logic applies when sexual selection, male competition and female choosiness result in higher rate of hybridization for rare female migrants than rare male migrants (Rieseberg *et al.* 1996; Chan & Levin 2005).
- Haplodiploidy: Recent modelling work has indicated that haplodiploid species may be especially prone to biased introgression of mtDNA (Patten *et al.* 2015). In these systems, females are diploid but males develop from unfertilized eggs and are haploid. Therefore, hybrid males can only be produced by hybrid females. The fact that hybrid males do not even exist until after hybrid females are already reproducing creates a potential bias towards female-mediated movement of genes across species boundaries. As above, such female biases may drive preferential introgression of mtDNA.

and thermoregulation (Fangue *et al.* 2009). In hindsight, early arguments that mtDNA can serve as a neutral marker may have been based more on expediency than biology and presently serve as little more than a straw man. Some of the clearest evidence for the functional importance of mitochondrial polymorphisms comes from studies that measure the phenotypic effects of placing different mitochondrial haplotypes onto the same nuclear background(s). Studies that include multiple nuclear backgrounds (e.g. Paliwal *et al.* 2014; Zhu *et al.* 2014) are especially valuable in helping to partition the additive and epistatic components of mitochondrial genetic variation (reviewed in Dobler *et al.* 2014).

Because mitochondrial genetic variation can affect fitness, it can be subject to selective sweeps (James *et al.* 2016). Such selective effects might also have the potential to extend across species boundaries in the form of mitochondrial introgression events. A meta-analysis of animal studies found that adaptive mitochondrial introgression is one of the predominant interpretations of mitonuclear discordance, as adaptive causes were hypothesized by the original authors of more than one-third of the studies included in the meta-analysis (Toews & Brelsford 2012). Towards that end, mitochondrial haplotypes have been identified as the potential basis for thermal tolerance and local adaptation to different climatic conditions (Bernatchez *et al.* 1995; Mishmar *et al.* 2003; Melo-Ferreira *et al.* 2005; Dowling *et al.* 2008; Morales *et al.* 2015). In addition, selection need not act on mtDNA itself to promote introgression, as mitochondrial variants can hitchhike with the adaptive/selfish spread of other cytoplasmic elements such as plastids or *Wolbachia* (Jiggins 2003). While population genetic modelling is often used to reject nonadaptive scenarios and conclude that there must have been a driving effect of selection to produce observed levels of introgression, direct functional tests for fitness effects associated with introgressed mitochondrial haplotypes are unfortunately still rare (e.g. Aubert & Solignac 1990; Blier *et al.* 2006; Boratyński *et al.* 2011; Deremiens *et al.* 2015). Therefore, the vast majority of cases of purported adaptive mitochondrial introgression are based on indirect evidence or solely on speculation.

Deleterious mutation accumulation and adaptive mitochondrial replacement

Apparent cases of adaptive mitochondrial introgression are often hypothesized to be the result of selection for locally adapted or novel/beneficial mitochondrial haplotypes. However, some researchers have proposed an alternative form of adaptive introgression in which a foreign mitochondrial haplotype sweeps through a population that has accumulated a large 'load' of deleterious mitochondrial mutations (Llopart *et al.* 2014; Hulsey *et al.* 2016). This phenomenon is expected to predominate in small or isolated populations that are most prone to deleterious mutation accumulation (Lynch *et al.* 1995). Accordingly, Llopart *et al.* (2014) argued that the introgression of *Drosophila yakuba* mtDNA into *D. santomea* populations is the result of the history of mutation accumulation in *D. santomea*, owing to its smaller N_e. Likewise, Hulsey *et al.* (2016) recently reported evidence of rapid mitochondrial introgression in the cichlid fish *Herichthys minckleyi*, which is narrowly endemic (i.e. low N_e) and was shown to have a history of relaxed/inefficient selection on its mitochondrial genome. This line of argument is also relevant to other observed cases of asymmetric mitochondrial introgression into species that are endangered or have restricted geographical distributions, such as the Adriatic sturgeon *Acipenser naccarii* (Ludwig *et al.* 2003) or the crotaphytid lizard *Crotaphytus reticulatus* (McGuire *et al.* 2007).

The concept of replacing mutationally loaded genomes has also been extended to other endosymbiotic systems. In particular, there is growing evidence that many ancient obligate endosymbionts in insects undergo a cycle of deleterious mutation accumulation followed by replacement or supplementation with an entirely novel bacterial endosymbiont (Bennett & Moran 2015; Husnik & McCutcheon 2016). This replacement process may also be carried out at the level of individual genes. There are many examples of mitochondrial genes (e.g. ribosomal proteins and tRNAs) that have been replaced by distantly related homologs in the nucleus (Adams *et al.* 2002; Pett & Lavrov 2015), which is remarkable in the light of the previous discussion about disrupting coadapted interactions because (bacterial-like) mitochondrial genes and their (archaeal-like) nuclear counterparts are separated by billions of years of evolution.

The displacement of mutationally loaded mitochondrial haplotypes by introgression fits into a broader view of hybridization as a mechanism to replace damaged or null alleles that have become fixed in a population/species (Ellstrand & Schierenbeck 2000; Rieseberg 2009). This replacement model provides the motivation for the applied practice of 'genetic rescue' in conservation efforts, in which foreign individuals are introduced to boost genetic variation in small and endangered populations (Ingvarsson 2001; Whiteley *et al.* 2015). The contrasting views of mitochondrial introgression vs. mitonuclear incompatibilities have been highlighted in debates over whether to preferentially introduce females during the implementation of genetic rescue programmes. In particular, it has been argued that introducing females may be beneficial because they can

contribute mitochondrial haplotypes that carry fewer deleterious mutations and, therefore, have the potential to adaptively introgress into the recipient population (Gemmell & Allendorf 2001). Alternatively, the introduction of foreign mtDNA may result in mitonuclear incompatibilities that reduce reproductive fitness in the population and hinder genetic rescue efforts (Havird *et al.* 2016).

Mitonuclear cointrogression: the best of both worlds?

Evidence for correlated introgression of mtDNA and interacting N-mt genes

Mitochondrial introgression – regardless of whether it is driven by adaptive or nonadaptive forces – comes at the risk of breaking up coadapted mitonuclear genotypes. Mitonuclear cointrogression is one potential mechanism to mitigate the deleterious consequences of disrupting epistatic interactions (Burton & Barreto 2012). This process would involve the preferential introgression of N-mt genes along with introgressing mtDNA. The possibility of cointrogression is intriguing because it suggests populations might be able to benefit from adaptive mitochondrial introgression without suffering the costs of mitonuclear incompatibilities.

To date, evidence of mitonuclear cointrogression remains limited, but recent studies have pointed to possible examples of this phenomenon. For example, Beck *et al.* (2015) found that mitochondrial introgression from *Drosophila yakuba* into *D. santomea* was accompanied by preferential introgression of N-mt genes that encode subunit V of the mitochondrial cytochrome c oxidase complex (Fig. 2). Similarly, in the Eastern Yellow Robin of Australia (*Eopsaltria australis*), an apparent history of adaptive mitochondrial introgression correlates with the introgression of a 15.4 Mb region of chromosome 1A that exhibits suppressed recombination and is highly enriched for N-mt genes (Morales 2016; Morales *et al.* 2016). In addition, effects on body mass and basal metabolic rate associated with crosses in bank voles (*Myodes glareolus*) carrying either native or introgressed mitochondrial haplotypes have been interpreted as possible evidence for cointrogressing N-mt genes (Boratyński *et al.* 2016). Results from experimental populations of the copepod *Tigriopus californicus* are also consistent with the potential for mitonuclear cointrogression in natural populations. Pritchard & Edmands (2013) found that hybrid swarms generated by experimental crosses between divergent populations of *T. californicus* evolved towards a higher degree of matching between mitochondrial and nuclear genotypes over time.

Inhibition of N-mt gene introgression between populations with divergent mtDNAs

Our focus in this review has been on cases in which mitochondrial introgression occurs despite limited nuclear gene flow. It is important to note, however, that the opposite pattern also occurs. In many systems, sharp population or species boundaries can be delineated based on mtDNA variation even in the presence of substantial nuclear introgression across those boundaries (Currat *et al.* 2008; Petit & Excoffier 2009). In these cases, there is the potential for selection on mitonuclear interactions to shape patterns of nuclear gene flow in a way that is analogous to cointrogression. Specifically, when there is strong differentiation between populations based on mitochondrial genotypes, N-mt genes may be less likely to introgress than other nuclear genes because they are more prone to cause deleterious epistatic effects on a foreign mitochondrial background (Burton *et al.* 2013).

For example, at the geographical boundaries between the hybrid Italian sparrow *Passer italiae* and its parent species, mtDNA and N-mt genes were both found to exhibit steeper clines than other loci (Trier *et al.* 2014). A similar pattern was observed in a hybrid zone between two subspecies of the Atlantic killifish *Fundulus heteroclitus*, in which mtDNA and the two N-mt genes included in the study exhibited the steepest and most concordant clines (McKenzie *et al.* 2016). Bar-Yaacov *et al.* (2015) also relied on this principle to identify candidate N-mt genes that may be involved in key mitonuclear interactions in a subspecies of Israeli chameleons (*Chamaeleo chamaeleon recticrista*) that exhibits a sharp mitochondrial cline associated with ancient marine geographical barrier despite a lack of differentiation based on nuclear markers or morphology.

In summary, if mitonuclear incompatibilities affect rates of gene flow, then patterns of mtDNA population structure may more closely resemble those for N-mt genes than for other nuclear loci. Correlations between mtDNA and N-mt genes could result either from cointrogression or from disproportionate inhibition of N-mt gene introgression between populations with divergent mitochondrial genotypes.

Barriers to mitonuclear cointrogression and maintenance of mitonuclear linkage disequilibrium

Although the possibility of cointrogressing mitochondrial and N-mt genes has intuitive appeal, there are reasons to be sceptical that mitonuclear cointrogression is widespread in natural populations. In particular, it is difficult for selection to maintain associations between

mitochondrial and nuclear alleles (i.e. mitonuclear linkage disequilibrium) because mitochondrial and nuclear loci are not physically linked. In fact, the null expectation for a random-mating population is that 50% of mitonuclear linkage disequilibrium will be lost every generation during introgression (Arnold 1993). Therefore, the ability of an N-mt allele to introgress will depend on its fitness effects on both native and foreign mitochondrial backgrounds. After an initial migration/admixture event, introgressing alleles are likely to be at low frequency. Therefore, within a small number of generations, an N-mt allele will predominantly be found on the native mitochondrial background in the resident population rather than on the introgressing mitochondrial background with which it was introduced. Even if there is selection to maintain mitonuclear interactions, any benefits associated with that N-mt allele being 'matched' with the introgressing mitochondrial haplotype may be overwhelmed by incompatibilities with resident haplotypes until the introgressing mtDNA rises to high frequency in the population (Fig. 3). Such effects may limit the extent to which N-mt genes preferentially cointrogress with mitochondrial genes or even make N-mt genes *less* likely than random/neutral nuclear markers to cointrogress with mtDNA (Fig. 3).

These hurdles by no means preclude the possibility of mitonuclear cointrogression, however, as there are many factors that might increase the likelihood of cointrogression, including inbreeding, asymmetric mitonuclear incompatibilities, and recurring bouts of admixture after an introduced mitochondrial haplotype has already reached high frequency in the resident population (Arnold *et al.* 1988; Asmussen *et al.* 1989; Dean & Arnold 1997; Cruzan & Arnold 1999; Wade & Drown 2016). It is also important to note that the hurdles that limit the potential for cointrogression may not apply equally to cases where there is preferential selection *against* introgression of N-mt genes (see examples of Italian sparrows, Atlantic killifish and Israeli chameleons described above). When there is large divergence in mtDNA between populations, an introduced N-mt allele will have to interact almost exclusively on the 'mismatched' mitochondrial background in the resident population. Therefore, more extensive sampling

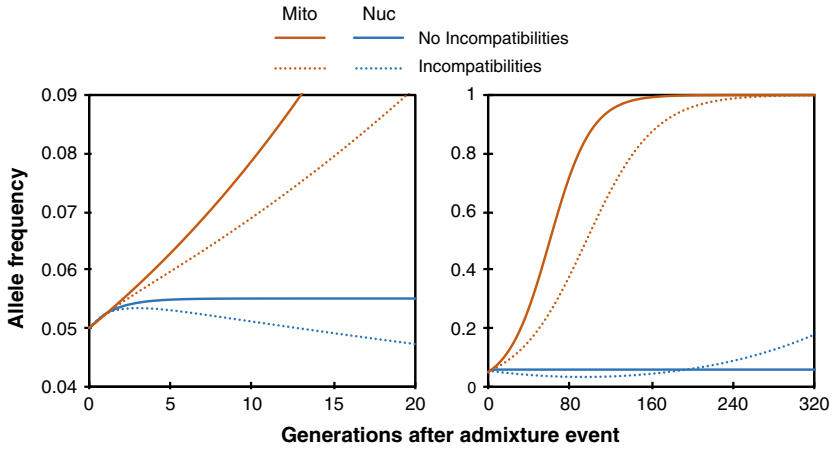


Fig. 3 Barriers to cointrogression for unlinked loci. A deterministic (i.e. infinite population size) and random-mating model for the introgression of mitochondrial (orange) and nuclear (blue) alleles after a one-time admixture event that introduces foreign alleles at a frequency of 5%. The two plots show the same results on two different timescales (note the differences in scales for both axes). The introduced mitochondrial haplotype is favoured by selection and undergoes adaptive introgression. The selection coefficient for the introduced mtDNA (s_m) is 0.05. The solid lines depict a scenario in which the nuclear locus is neutral. Under this scenario, the introduced nuclear allele increases in frequency over the first few generations by hitchhiking with the introgressing mtDNA. But its frequency plateaus as mitonuclear LD is rapidly eliminated by random mating. The dotted lines depict a scenario in which there is a symmetrical mitonuclear incompatibility between the native and introduced genotypes. The epistatic selection coefficient (s_i) acting against 'mismatched' mitonuclear genotypes is −0.02. These incompatibilities are assumed to exhibit incomplete dominance, so heterozygotes experience half of this cost (−0.01). Under this scenario, the rate of spread of the introduced mtDNA is slower because of its negative interactions with native nuclear alleles, but it nevertheless introgresses rapidly because of the strong selection on it (s_m is still 0.05). After the short initial hitchhiking phase, the introduced nuclear allele that is 'matched' to the introgressing mtDNA actually starts to decline in frequency because of its harmful interactions with the native mtDNA, which remains far more abundant in early generations. The introduced nuclear allele does not begin to increase in frequency until the foreign mtDNA spreads to >50% in the population, creating a very long lag. Under a more realistic model with a finite population size, the introduced nuclear allele would be at risk of stochastic loss from the population because of its early decline in frequency and the long lag before there is any selection in favour of it. The code used to generate these models is provided as supplementary material.

might reveal that it is relatively common for the introgression of N-mt genes to be preferentially inhibited between populations with sharply divergent mitochondrial haplotypes.

Deleterious mutation accumulation and the efficacy of selection in cytoplasmic genomes

Theoretical expectations for deleterious mutation accumulation in asexual cytoplasmic genomes

Our interpretation of mitonuclear incompatibilities and mitochondrial introgression has leaned heavily on the assumption that mitochondrial genomes are prone to the accumulation of deleterious mutations. Because purifying selection is the dominant selective force acting on anciently conserved OXPHOS enzyme complexes (Castellana *et al.* 2011), a reduced efficacy of selection would be associated with a higher rate of amino acid substitutions. However, recent studies examining statistical patterns of sequence evolution and population genetic variation have raised doubts about whether mitochondrial genomes suffer from inefficient selection (Mamirova *et al.* 2007; Popadin *et al.* 2013; Zhang & Broughton 2013; Cooper *et al.* 2015). Therefore, it is timely to revisit the theoretical and empirical evidence that has been generated in the analysis of mutation accumulation in mitochondria and other cytoplasmic genomes.

Expectations for mitochondrial mutation accumulation are rooted in a large body of theory regarding the genetic consequences of biparental inheritance and sexual recombination primarily because cytoplasmic genomes often lack these genetic phenomena. Some of the most prominent hypotheses to explain the evolution of sex relate to its role in improving the efficacy of selection for beneficial mutations and against deleterious mutations. By generating novel combinations of alleles at different loci, sex has the potential to separate out beneficial mutations that happen to have occurred on backgrounds with high loads of deleterious mutations (Peck 1994). In the absence of sex, the entire genome remains completely linked such that loci cannot be selected independently from their genomic background (i.e. the Hill-Robertson Effect), thereby reducing N_e throughout the genome (Hill & Robertson 1966). As a result, weakly deleterious mutations are more likely to spread by genetic drift or by hitchhiking with beneficial alleles undergoing selective sweeps (Birky & Walsh 1988). Muller's Ratchet (Muller 1964; Felsenstein 1974) is an additional mechanism of mutation accumulation and involves the stochastic loss (either by drift or by mutation) of the most fit genotype from an asexual population (i.e. the one carrying the fewest deleterious mutations). In the absence of sexual reproduction, this high-fitness genotypic class cannot be restored without fortuitous beneficial mutations. Therefore, asexual genomes (such as those found in many mitochondrial lineages) are expected to suffer mutational deterioration through the sequential and irreversible 'clicking' of this ratchet.

Although these genetic consequences are thought to be a general feature of asexual genomes, susceptibility to deleterious mutation accumulation can also be affected by other factors. For example, as discussed above, inefficient selection against deleterious mutations can be worsened by a large mutational input in genomes with high mutation rates, which is often (but not always) the case for mtDNA (Brown *et al.* 1979; Wolfe *et al.* 1987; Lynch *et al.* 2008) (Fig. 4). In addition, mutation accumulation depends on the intensity of selection. For example, a reduced intensity of selection may explain accumulation of nonsynonymous substitutions in mtDNA of organisms with reduced metabolic demands such as salamanders (Chong & Mueller 2013) and flightless birds (Shen *et al.* 2009). In the extreme, mitochondrial genomes have been eliminated entirely in some anaerobic eukaryotic lineages that do not depend on the electron transport chain and OXPHOS for producing ATP (reviewed in Gray 2012). Deleterious mutation accumulation in asexual genomes should also depend heavily on N_e. Large populations can potentially benefit from more efficient selection and a greater probability of convergent or back mutations even in the absence of sexual recombination (Lynch & Blanchard 1998). In this respect, the fact that uniparental inheritance and transmission bottlenecks render mitochondrial genomes effectively haploid (even though they are present in many copies per cell) may increase their susceptibility to mutation accumulation (Neiman & Taylor 2009).

Empirical evidence for deleterious mutation accumulation in mitochondrial and other cytoplasmic genomes

One of the most striking and common patterns in the field of molecular evolution is that endosymbiotic bacteria and organelles undergo genome reduction and accelerated rates of sequence evolution (Gray *et al.* 1989; McCutcheon & Moran 2012) (Fig. 4). These changes likely reflect three major causes: 1) relaxed selection on genes that become redundant in an intracellular environment (Andersson *et al.* 1998), 2) the reduced efficacy of selection that results from the lower N_e and asexual inheritance that are typical of an endosymbiotic lifestyle (Lynch 1996; Moran 1996), and 3) accelerated mutation rates (Brown *et al.* 1979;

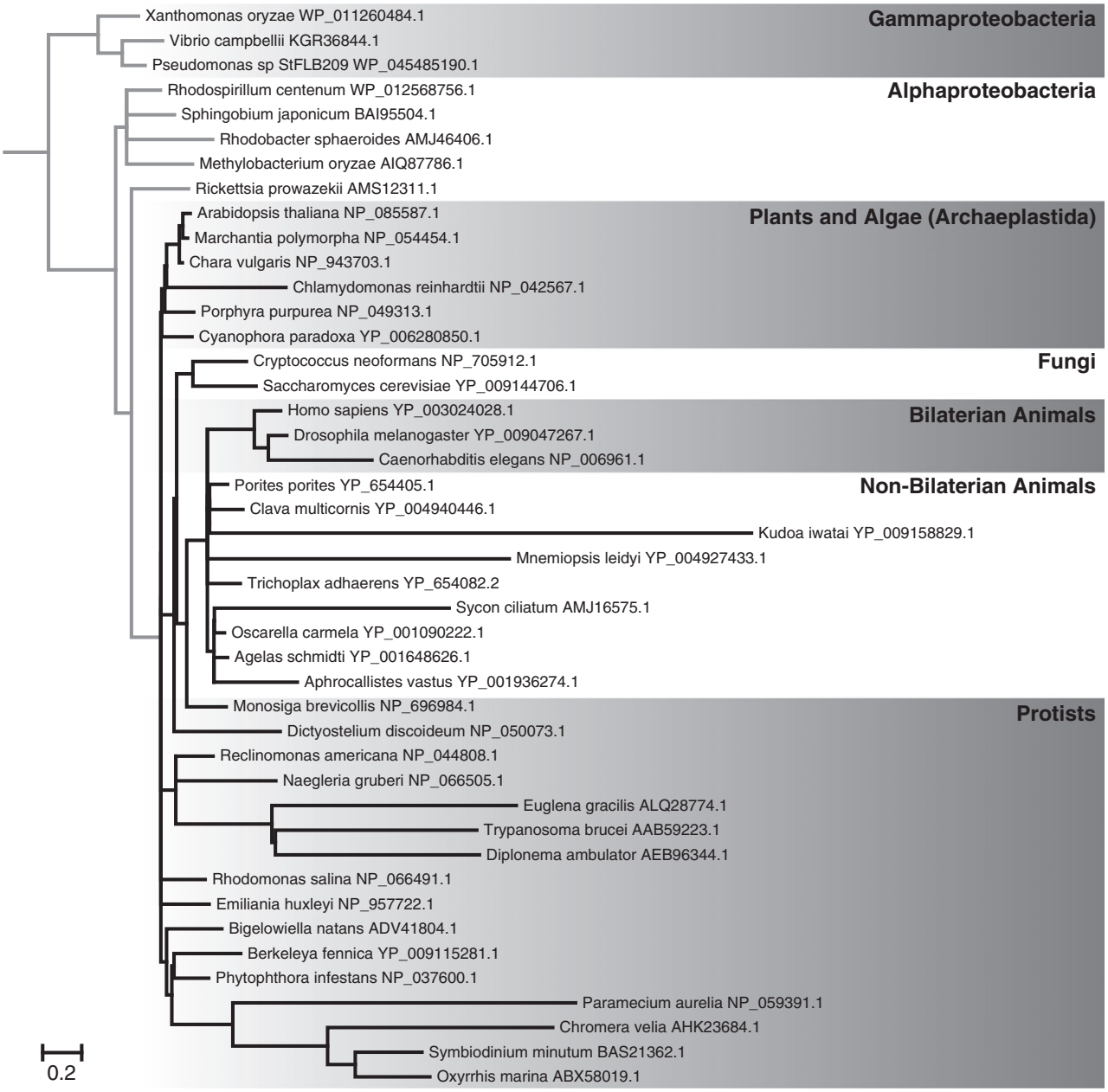


Fig. 4 Accelerated and variable rates of protein sequence evolution in mitochondria. Branch lengths are scaled based on the proportion of amino acid substitutions in translated *cox1* genes sequences from select bacterial and mitochondrial genomes (grey and black branches, respectively). Sequences were aligned with MAFFT (E-INS-i), and divergent N- and C-terminal portions of the alignment were manually trimmed. Branch length estimates were then generated in PAML v4.9a, using an LG+G model and a topology that was constrained based on published sources (Burki 2014; Lavrov & Pett 2016).

Moran *et al.* 2009). Statistical evidence based on rates of nonsynonymous vs. synonymous sequence divergence (d_N/d_S) and population genetic variation has provided some support for the interpretation that cytoplasmic genomes experience inefficient selection. For example, Lynch & Blanchard (1998) concluded that mitochondrial genes are subject to elevated levels of d_N/d_S relative to nuclear genes indicative of a more porous 'selective sieve' (but see below for discussion of recent findings that call this conclusion into question particularly in animals). More broadly, diverse bacterial lineages have exhibited an increase in d_N/d_S upon transitioning from free-living to obligately endosymbiotic lifestyles (Kuo *et al.* 2009). The effects of asexual inheritance in enabling deleterious mutation accumulation are also supported by examples

of eukaryotic species or specific regions of eukaryotic genomes (e.g. Y chromosomes) that have transitioned to asexual modes of inheritance (Kaiser & Charlesworth 2010; Neiman *et al.* 2010; Tucker *et al.* 2013; Hollister *et al.* 2015; Sharbrough *et al.* 2016).

While investigations of sequence divergence and population genetics have advanced our understanding of the efficacy of selection in cytoplasmic genomes, it is easy to lose track of functional biology when scrutinizing statistical patterns of nucleotide substitutions. There are many aspects of molecular function in mitochondrial and other cytoplasmic genomes that point towards a history of deleterious mutation accumulation. For example, Lynch (1996, 1997) showed that mitochondrial tRNAs typically have lower thermodynamic stability than tRNAs found in the nucleus or in free-living bacteria. In fact, mitochondrial tRNAs are seemingly deformed in many lineages, often exhibiting 'armless' structures that lack key functional elements found in tRNAs from almost all other genomes (Watanabe *et al.* 2014). Mitochondrial OXPHOS and ribosomal genes have also undergone reductive evolution, which may have necessitated the recruitment of novel nuclear-encoded proteins to compensate for these changes (van der Sluis *et al.* 2015).

These patterns of functional degradation also appear to extend to other endosymbionts. In many of the ancient and obligate intracellular bacteria found in insects, genes are so divergent and degenerated that they require massive expression of chaperones to achieve proper protein folding and functionality (Moran 1996). For example, early analysis of protein expression in the obligate *Buchnera* endosymbiont from aphids identified a protein expressed at extremely high levels (Ishikawa 1984). This protein was named Symbionin because it was presumed to play a central and perhaps novel function in facilitating the symbiosis between host and bacteria. Further investigation, however, revealed the Symbionin was simply GroEL (Kakeda & Ishikawa 1991), a chaperone that aids in protein folding and is widely conserved in both prokaryotes and eukaryotes. The apparent functional decline of obligate bacterial endosymbionts is consistent with the repeated recruitment of novel symbiotic partners either in complementary roles or as outright replacements for ancestral endosymbionts (Bennett & Moran 2015; Husnik & McCutcheon 2016).

Mitochondrial and other cytoplasmic genomes also exhibit some of the most extreme and bizarre examples of gene/genome architecture ever identified (Campbell *et al.* 2015; Smith & Keeling 2015). Most changes to the 'universal' genetic code are found in mitochondria or other obligate bacterial endosymbionts, and nucleotide compositions are extraordinarily biased in many of these genomes, affecting protein sequence evolution (Perna & Kocher 1995; Knight *et al.* 2001; McCutcheon & Moran 2012). In many eukaryotic lineages, generating intact mitochondrial protein-coding sequences requires extensive RNA editing or *trans*-splicing of numerous independently transcribed loci (e.g. Burger *et al.* 2016; Lavrov *et al.* 2016). While complex genome architectures and gene expression systems might have beneficial regulatory roles, many of these complexities are likely non-adaptive and even deleterious but have become 'locked in' by a process described as 'constructive neutral evolution' (Covello & Gray 1993; Stoltzfus 1999; Lynch *et al.* 2006; Gray *et al.* 2010).

It is important to emphasize that degeneration of cytoplasmic genomes is unlikely to be caused by reduced efficacy of selection alone. The transition to an intracellular environment is expected to reduce functional constraint on many cellular processes. One line of evidence that reduced *intensity* (rather than efficacy) of selection plays a substantial role is that the nuclear-encoded proteins required for mitochondrial translation (e.g. ribosomal proteins and aminoacyl tRNA synthetases) evolve much more rapidly than the cytosolic proteins required for translation of nuclear transcripts (Barreto & Burton 2013; Sloan *et al.* 2014b; Pett & Lavrov 2015; Adrion *et al.* 2016). Because both sets of genes are located in the nucleus, this pattern cannot be explained by the differences in N_e or sexual recombination that distinguish mitochondrial and nuclear genomes. Instead, the N-mt genes appear to be under less functional constraint and, therefore, more tolerant of amino acid substitutions (although positive selection resulting from co-evolution with mitochondrial genes is also a likely contributing factor; Barreto & Burton 2013; Sloan *et al.* 2014b).

Doubts about reduced efficacy of selection in mitochondrial genomes

The notion that mitochondrial genomes are subject to deleterious mutation accumulation because of their typically asexual and uniparental mode of inheritance has become so engrained in the field of molecular evolution that it is often taken for granted. Therefore, recent studies questioning this conclusion have the potential to profoundly alter our understanding of mitochondrial evolution (Mamirova *et al.* 2007; Popadin *et al.* 2013; Zhang & Broughton 2013; Cooper *et al.* 2015). These studies are largely based on the observation that d_N/d_S values in animals are often substantially lower for mitochondrial genes than for nuclear genes. Assuming that purifying selection is the predominant force acting on the genes, this pattern is exactly the opposite of what would be expected if selection on mtDNA is less

efficient than on nuclear genes. It is worth noting that this pattern was also observed in a much earlier comparative study of d_N/d_S values in nuclear vs. mitochondrial genomes. Lynch & Blanchard (1998) found that, unlike in plants and fungi, d_N/d_S values from mitochondrial genes were lower than those from nuclear genes in vertebrates. But the authors largely dismissed this finding as an artefact and concluded that the permissive accumulation of nonsynonymous substitutions was a general feature of mitochondrial genomes.

A recent expansion of this analysis, however, confirmed that the relationship between mitochondrial and nuclear d_N/d_S values can vary wildly across eukaryotic lineages and is generally tilted towards lower mitochondrial d_N/d_S values in animals (Havird & Sloan 2016). Most of the variance in the relationship between mitochondrial and nuclear d_N/d_S values could be explained by differences in the relative mutation rate between genomes. Although dividing by d_S is thought to be an appropriate normalization to control for differences in mutation rates, these findings suggest that a great deal of caution is needed when making direct comparisons between d_N/d_S values from genomes with large differences in the rate and spectrum of mutations. Comparisons between nuclear and mitochondrial d_N/d_S values are further complicated by the fact that they cannot involve a direct comparison between the same genes (in contrast to comparisons between orthologous genes in endosymbiotic bacteria and their free-living relatives; Kuo *et al.* 2009).

A recent population genetic analysis of mitochondrial and nuclear variation in humans and *Drosophila* has also raised doubts about the classic assumption of reduced efficacy of selection in mitochondrial genomes (Cooper *et al.* 2015). This and related analyses of selection on mtDNA (Rand & Kann 1996; Nachman 1998; James *et al.* 2016) are largely based on a McDonald–Kreitman framework, in which the ratio of nonsynonymous to synonymous changes is compared for fixed differences between species vs. segregating polymorphisms within species (McDonald & Kreitman 1991). The results of McDonald–Kreitman tests can be summarized by the neutrality index (NI) (Rand & Kann 1996). NI values less than one indicate an excess of nonsynonymous substitutions between species and a substantial effect of positive selection, whereas NI values greater than one indicate an excess of nonsynonymous polymorphisms within species and a predominance of purifying selection. Cooper *et al.* (2015) found that differences in NI values between mitochondrial and nuclear genes were negligible, suggesting that the efficacy of selection may be similar in both genomes. However, despite the fact that NI values are a standard tool for detecting the relative contributions of positive vs. purifying selection, it may be more challenging to use these values to draw clear conclusions about the *efficacy* of selection. Inefficient selection can increase the spread of weakly deleterious mutations at both intra- and interspecific levels, so it is not immediately clear whether a reduction in the efficacy of selection would result in increases or decreases in the NI. Indeed, the theoretical relationships between N_e and NI values are complex (Weinreich & Rand 2000; Welch *et al.* 2008; Betancourt *et al.* 2012). Despite the similarity in NI values between mitochondrial and nuclear genomes, Cooper *et al.* (2015) found clear evidence of a lower N_e in the mitochondrial genome. Further complicating matters is the fact that meta-analyses of data from animal mitochondrial and nuclear genomes have alternatively concluded that NI values are higher in the mitochondrial genome (Weinreich & Rand 2000), lower in the mitochondrial genome (Bazin *et al.* 2006), or essentially the same in the mitochondrial and nuclear genomes (Cooper *et al.* 2015).

Although there are reasons to be cautious about interpreting recent studies that question whether mitochondrial genomes are prone to mutation accumulation, these studies raise important points that warrant investigation. The observation that d_N/d_S values for animal mtDNA are very low (often <0.1) is compelling and, at a minimum, indicates that the vast majority of deleterious mutations are being successfully filtered out by selection. It is also important to note that much of the strongest evidence for the degradation of cytoplasmic genomes is based on comparisons relative to free-living bacteria. In contrast, current uncertainties pertain more to the amount of ongoing mutation accumulation in mitochondrial genomes relative to the nucleus. These are different comparisons and may naturally yield different results.

It is also possible that the historical process of mutation accumulation and mitochondrial genome degradation has been arrested in some eukaryotic lineages. Two features of animal mitochondrial genomes may be relevant in this respect. First, bilaterian animals have evolved extremely small mitochondrial genomes, which typically contain only 13 protein-coding genes (Boore 1999). With reduced gene content, the costs of asexuality may decline as the effects of selection on linked sites are lessened. Second, transmission of animal mitochondria is often associated with a 'germline bottleneck'. Although any bottleneck in mtDNA copy number is expected to lower N_e and further contribute to genetic drift, there is evidence that this bottleneck can act in a nonrandom fashion and selectively filter deleterious mutations that are segregating within heteroplasmic cells (Rand 2008; Stewart *et al.* 2008; Vevea *et al.* 2014). In this case, selection at the intracellular level may be

reinforcing organismal-level selection that benefits the transmission of nuclear genes. Therefore, it is possible that germline bottlenecks serve to increase rather than decrease the efficacy of selection on mtDNA (Christie & Beekman 2017). Finally, it is important to note that, even in cases where mitochondrial genomes do not suffer from a reduced efficiency of selection, they still may experience more fixation of amino acid substitutions because of a higher rate of mutational input (Fig. 4), resulting in opportunities for epistatic interactions and mitonuclear coadaptation (Fig. 1).

Open questions and future directions

Are mitochondrial genomes truly exceptional or disproportionate with respect to reproductive barriers and introgression?

We have highlighted long-standing narratives from the literature that mtDNA is not 'just another locus' when it comes to key evolutionary processes. For example, it has been argued that, relative to nuclear genes, mtDNA disproportionately contributes to BDMIs (Burton & Barreto 2012) and preferentially introgresses across species boundaries (Chan & Levin 2005). However, many such conclusions have been extrapolated from case studies and have not been tested in an unbiased statistical fashion. Fortunately, the genomic data are now available to enable rigorous statistical tests and meta-analyses to answer the following questions. Are mitochondrial genomes (and N-mt genes) statistically overrepresented among the set of loci involved in reproductive incompatibilities? Is genealogical incongruence between mitochondrial and nuclear loci more common than between two arbitrary nuclear loci? Obtaining quantitative (and not just qualitative) answers to these questions is important. After all, it is possible that mitonuclear incompatibilities and mitochondrial introgression are both statistically disproportionate phenomena but still rare in an absolute sense. In that case, the clashing narratives regarding incompatibilities and introgression might not be paradoxical at all; they may each simply apply to their own small subset of lineages.

Are mitochondrial genomes prone to deleterious mutation accumulation?

Recent studies suggesting that mitochondrial genomes do not experience deleterious mutation accumulation represent a potentially significant departure from classic models of mutation accumulation in asexual genomes (Mamirova *et al.* 2007; Popadin *et al.* 2013; Zhang & Broughton 2013; Cooper *et al.* 2015). Because this work has exclusively focused on the mitochondrial genomes of bilaterian animals, we suggest that a top priority should be to extend these studies to other eukaryotic lineages. Evidence already exists that the relationship between sequence evolution in mitochondrial vs. nuclear genomes may vary dramatically across eukaryotic lineages (Lynch & Blanchard 1998; Havird & Sloan 2016). The growing availability of genomic and transcriptomic data sets from diverse eukaryotes provides an opportunity to examine how signatures of selection (e.g. d_N/d_S and NI) for mitochondrial and nuclear genes vary based on properties such as relative mutation rates, modes of inheritance and relative levels of gene expression. But such studies should be accompanied by efforts to establish methods for confidently estimating the efficacy of selection, particularly when making comparisons between genomes with highly divergent mutation rates. These efforts may depend on obtaining accurate measures of mutation load and the distribution of fitness effects of new mutations for both mitochondrial and nuclear genes.

Is replacement of mutationally loaded mitochondrial haplotypes a common cause of adaptive introgression?

Apparent cases of adaptive mitochondrial introgression are often interpreted as being the result of selection for local adaptation to an environmental condition such as climate. But an alternative form of adaptive introgression could arise based on selection to replace mitochondrial haplotypes in populations that have experienced a build-up of deleterious mitochondrial mutations (Llopart *et al.* 2014; Hulsey *et al.* 2016). This hypothesis is associated with a number of testable predictions (Box 2).

Do mitonuclear interactions preferentially maintain associations between mtDNA and N-mt genes?

The field of population genetics has reached an important turning point in that analyses of natural populations are no longer restricted to a handful of genetic markers. It is now possible and common to achieve truly genomewide sampling in populations undergoing hybridization and introgression (Harrison & Larson 2014, 2016; Abbott *et al.* 2016; Payseur & Rieseberg 2016). The resulting data sets present the opportunity for rigorous tests of the hypothesis that mitonuclear incompatibilities shape patterns of introgression across the nuclear genome (Trier *et al.* 2014; Bar-Yaacov *et al.* 2015; Beck *et al.* 2015; McKenzie *et al.* 2016; Morales 2016). Specific predictions include the following:

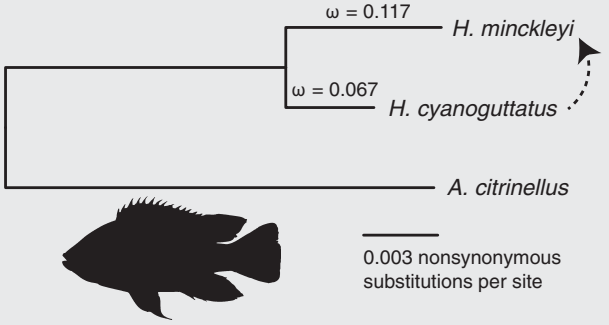
1 In cases of mitochondrial introgression, N-mt genes will preferentially cointrogress relative to background rates of introgression for nuclear markers.

Box 2. Testing for deleterious mutation accumulation and adaptive mitochondrial replacement

If selection to replace mitochondrial haplotypes with high mutation loads is a pervasive force, ongoing cases of adaptive introgression should exhibit clear asymmetries between donor and recipient populations/species. In particular, recipient populations should exhibit:

- Smaller (effective) population sizes as assessed by census data and/or genetic diversity measures
- A greater build-up of fixed nonsynonymous variants in mitochondrially encoded proteins (see figure), which can be assessed using relative rate tests and phylogenetic analysis of branch lengths as implemented in widely used tools such as PAML (Yang 2007) and MEGA (Kumar *et al.* 2016)
- Specific signatures of relaxed or inefficient selection in the mitochondrial genome based on predicted effects on both beneficial and deleterious mutations (decreased and increased probabilities of fixation, respectively) (Wertheim *et al.* 2015)
- Inefficient purging of deleterious mutations in the mitochondrial genome as indicated by elevated proportions of nonsynonymous variants among the mitochondrial polymorphisms segregating within species

Box 2 Figure. An example of introgression that is consistent with the possibility of mitochondrial mutation load selecting for adaptive replacement. The recipient species (*Herichthys minckleyi*) exhibits significantly higher nonsynonymous divergence (from the outgroup *Amphilophus citrinellus*) than the donor species (*H. cyanoguttatus*) based on Tajima's relative rate test ($P = 0.009$). *Herichthys minckleyi* also has a lower N_e and shows evidence of relaxed selection on the mitochondrial genome (Hulsey *et al.* 2016). Nonsynonymous branch lengths and ω values (i.e. d_N/d_S) were estimated in PAML using a concatenation of all mitochondrial protein-coding genes. The arrow indicates the direction of mitochondrial introgression. The cichlid image is from PhyloPic (M. Tan).



2 In cases of nuclear introgression and the maintenance of sharp clines for mitochondrial haplotypes, gene flow at N-mt loci will be more highly restricted relative to background rates of introgression for nuclear markers.
3 Because scenario 1 requires cointrogression of unlinked loci, the statistical associations between mtDNA and N-mt genes will tend be more robust in scenario 2 than in scenario 1.

These predictions would especially apply to the subset of N-mt genes that are involved in the most direct interactions with mitochondrially encoded gene products and mtDNA itself (Table 2). Identification of key interactions can be informed by 3D structural models of mitonuclear complexes such as ribosomes and OXPHOS enzymes (Osada & Akashi 2012; Havird *et al.* 2015), and these efforts are aided by a growing catalog of solved protein structures, especially those from diverse eukaryotic lineages. The above predictions can be tested by examining both outlier loci and average values for measures such as rates of introgression, cline shape in hybrid zones and mitonuclear linkage disequilibrium (Sloan *et al.* 2015; McKenzie *et al.* 2016; Morales 2016). These population genetic approaches could also be extended to a phylogenomic level to test the prediction that mitochondrial trees should be more concordant with the topologies of N-mt gene trees than with those of random nuclear genes (e.g. as measured by Robinson–Foulds distance).

Are population genetic patterns supported by direct measures of fitness and mitochondrial function?

Although there is an extensive body of literature identifying potential cases of adaptive mitochondrial introgression, direct tests of the fitness and functional effects of

Table 3 Predicted conditions that would favour alternative evolutionary responses to mitochondrial mutation accumulation

	Conditions promoting...	
	Compensatory co-evolution and mitonuclear incompatibilities	Adaptive mitochondrial introgression/replacement
Mitochondrial mutation rates	Higher rates	Lower rates
Effective Population Size	Large or symmetric population sizes	Small or asymmetric population sizes
Population Subdivision	More subdivision	Less subdivision
Divergence Time	More ancient divergence	More recent divergence
Cotransmission of nuclear and mitochondrial loci (e.g. inbreeding)	More cotransmission	Less cotransmission
Constraints on mitochondrial function	Intense constraints	Relaxed constraints

different mitochondrial haplotypes are far less common in studies of introgression. The generation of cybrids through direct cellular manipulations or crossing designs enables such functional analyses, which should be a priority in concluding that mitochondrial introgression is the result of selection given the many nonadaptive mechanisms that can mimic the effects of adaptive introgression (Box 1). These analyses are also crucial in providing functional insights into the nature of mitonuclear incompatibilities. In particular, there is a need for fully factorial experiments that examine all pairwise combinations among a set of nuclear and mitochondrial backgrounds. Such experiments can disentangle additive and epistatic effects and have largely been limited to a small number of model systems such as yeast and *Drosophila* (e.g. Paliwal *et al.* 2014; Zhu *et al.* 2014).

What makes some lineages prone to mitonuclear incompatibilities while others experience rampant mitochondrial introgression?

As we have highlighted in this review, the abundant evidence for both mitonuclear incompatibilities and mitochondrial introgression poses somewhat of a paradox. If, as we contend, these seemingly contrasting patterns can actually represent two alternative solutions to the same problem of mitochondrial mutation accumulation, we are faced with the question of why certain lineages may be more likely to evolve compensatory changes in the nucleus while others undergo mitochondrial replacement via introgression. It is important to emphasize that these scenarios are not necessarily mutually exclusive and that both could occur within a single lineage over time in response to different mitochondrial mutations, although replacement should become a less viable option with increasing divergence as mitonuclear complexes become more coadapted. We suggest that there are a number of testable hypotheses regarding the factors that predispose species to these alternative evolutionary responses (Table 3).

Addressing each of these questions will represent an important step forward in disentangling the complex relationship between mitochondrial genomes and species boundaries. Fortunately, the recent and dramatic increase in the availability of population genomic data sets makes many of the above approaches feasible on scales that were previously unimaginable.

Data accessibility

No new data sets were generated in writing this review. The code used to make Fig. 3 is provided as supplementary material.

Acknowledgements

This work was motivated in large part by the presentations and discussions at the symposium on 'Co-evolving Genomes: Cooperation and Conflict in Cytonuclear Interactions' at the Evolution 2016 meetings in Austin, TX. We thank the Society for the Study of Evolution for sponsoring that symposium and all the presenters for their stimulating and insightful contributions. We are grateful for encouragement from Louis Bernatchez to submit this review. We also thank two anonymous reviewers, Lisa Angeloni, Damian Dowling, Matt Hahn, Geoff Hill, Hernán Morales, Nancy Moran, Rachel Mueller, Maurine Neiman, and many members from some of their laboratory groups as well as our own laboratory group for helpful comments on earlier versions of this manuscript. Our research on mitonuclear interactions and co-evolution is supported by Colorado State University, the National Science Foundation (MCB-1412260) and a postdoctoral fellowship from the National Institutes of Health (F32GM116361).

References

Abbott RJ, Barton NH, Good JM (2016) Genomics of hybridization and its evolutionary consequences. *Molecular Ecology*, **25**, 2325–2332.

Adams KL, Daley DO, Whelan J, Palmer JD (2002) Genes for two mitochondrial ribosomal proteins in flowering plants are derived from their chloroplast or cytosolic counterparts. *Plant Cell*, **14**, 931–943.

Adrion JR, White PS, Montooth KL (2016) The roles of compensatory evolution and constraint in aminoacyl tRNA synthetase evolution. *Molecular Biology and Evolution*, **33**, 152.

Andersson SG, Zomorodipour A, Andersson JO *et al.* (1998) The genome sequence of *Rickettsia prowazekii* and the origin of mitochondria. *Nature*, **396**, 133–140.

Arnold J (1993) Cytonuclear disequilibria in hybrid zones. *Annual Review of Ecology and Systematics*, **24**, 521–554.

Arnold J, Asmussen MA, Avise JC (1988) An epistatic mating system model can produce permanent cytonuclear disequilibria in a hybrid zone. *Proceedings of the National Academy of Sciences of the United States of America*, **85**, 1893–1896.

Arnqvist G, Dowling DK, Eady P *et al.* (2010) Genetic architecture of metabolic rate: environment specific epistasis between mitochondrial and nuclear genes in an insect. *Evolution*, **64**, 3354–3363.

Asmussen MA, Arnold J, Avise JC (1989) The effects of assortative mating and migration on cytonuclear associations in hybrid zones. *Genetics*, **122**, 923–934.

Aubert J, Solignac M (1990) Experimental evidence for mitochondrial DNA introgression between *Drosophila* species. *Evolution*, **44**, 1272–1282.

Ballard JWO, Whitlock MC (2004) The incomplete natural history of mitochondria. *Molecular Ecology*, **13**, 729–744.

Barr CM, Neiman M, Taylor DR (2005) Inheritance and recombination of mitochondrial genomes in plants, fungi and animals. *New Phytologist*, **168**, 39–50.

Barreto FS, Burton RS (2013) Evidence for compensatory evolution of ribosomal proteins in response to rapid divergence of mitochondrial rRNA. *Molecular Biology and Evolution*, **30**, 310–314.

Bar-Yaacov D, Hadjivasiliou Z, Levin L *et al.* (2015) Mitochondrial involvement in vertebrate speciation? The case of mitonuclear genetic divergence in chameleons. *Genome Biology and Evolution*, **7**, 3322–3336.

Bazin E, Glémin S, Galtier N (2006) Population size does not influence mitochondrial genetic diversity in animals. *Science*, **312**, 570–572.

Beck EA, Thompson AC, Sharbrough J, Brud E, Llopart A (2015) Gene flow between *Drosophila yakuba* and *Drosophila santomea* in subunit V of cytochrome c oxidase: A potential case of cytonuclear co-introgression. *Evolution*, **69**, 1973–1986.

Beekman M, Dowling DK, Aanen DK (2014) The costs of being male: are there sex-specific effects of uniparental mitochondrial inheritance? *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, **369**, 20130440.

Bender A, Krishnan KJ, Morris CM *et al.* (2006) High levels of mitochondrial DNA deletions in substantia nigra neurons in aging and Parkinson disease. *Nature Genetics*, **38**, 515–517.

Bennett GM, Moran NA (2015) Heritable symbiosis: the advantages and perils of an evolutionary rabbit hole. *Proceedings of the National Academy of Sciences of the United States of America*, **112**, 10169–10176.

Bernatchez L, Glémet H, Wilson CC, Danzmann RG (1995) Introgression and fixation of Arctic char (*Salvelinus alpinus*) mitochondrial genome in an allopatric population of brook trout (*Salvelinus fontinalis*). *Canadian Journal of Fisheries and Aquatic Sciences*, **52**, 179–185.

Betancourt AJ, Blanco-Martin B, Charlesworth B (2012) The relation between the neutrality index for mitochondrial genes and the distribution of mutational effects on fitness. *Evolution*, **66**, 2427–2438.

Birky CW, Walsh JB (1988) Effects of linkage on rates of molecular evolution. *Proceedings of the National Academy of Sciences of the United States of America*, **85**, 6414–6418.

Blier PU, Breton S, Desrosiers V, Lemieux H (2006) Functional conservatism in mitochondrial evolution: insight from hybridization of arctic and brook charrs. *Journal of Experimental Zoology Part B*, **306**, 425–432.

Bock DG, Andrew RL, Rieseberg LH (2014) On the adaptive value of cytoplasmic genomes in plants. *Molecular Ecology*, **23**, 4899–4911.

Bolnick DI, Turelli M, Lopez-Fernandez H, Wainwright PC, Near TJ (2008) Accelerated mitochondrial evolution and "Darwin's corollary": asymmetric viability of reciprocal F1 hybrids in Centrarchid fishes. *Genetics*, **178**, 1037–1048.

Boore JL (1999) Animal mitochondrial genomes. *Nucleic Acids Research*, **27**, 1767–1780.

Boratyński Z, Alves PC, Berto S *et al.* (2011) Introgression of mitochondrial DNA among *Myodes* voles: consequences for energetics? *BMC Evolutionary Biology*, **11**, 355.

Boratyński Z, Ketola T, Koskela E, Mappes T (2016) The sex specific genetic variation of energetics in bank voles, consequences of introgression? *Evolutionary Biology*, **43**, 37–47.

Brown WM, George M, Wilson AC (1979) Rapid evolution of animal mitochondrial DNA. *Proceedings of the National Academy of Sciences of the United States of America*, **76**, 1967–1971.

Burger G, Gray MW, Forget L, Lang BF (2013) Strikingly bacteria-like and gene-rich mitochondrial genomes throughout jakobid protists. *Genome Biology and Evolution*, **5**, 418–438.

Burger G, Moreira S, Valach M (2016) Genes in hiding. *Trends in Genetics*, **32**, 553–565.

Burki F (2014) The eukaryotic tree of life from a global phylogenomic perspective. *Cold Spring Harbor Perspectives in Biology*, **6**, a016147.

Burton RS, Barreto FS (2012) A disproportionate role for mtDNA in Dobzhansky-Muller incompatibilities? *Molecular Ecology*, **21**, 4942–4957.

Burton RS, Pereira RJ, Barreto FS (2013) Cytonuclear genomic interactions and hybrid breakdown. *Annual Review of Ecology, Evolution, and Systematics*, **44**, 281–302.

Calvo SE, Mootha VK (2010) The mitochondrial proteome and human disease. *Annual Review of Genomics and Human Genetics*, **11**, 25–44.

Campbell MA, Van Leuven JT, Meister RC *et al.* (2015) Genome expansion via lineage splitting and genome reduction in the cicada endosymbiont Hodgkinia. *Proceedings of the National Academy of Sciences of the United States of America*, **112**, 10192–10199.

Camus MF, Clancy DJ, Dowling DK (2012) Mitochondria, maternal inheritance, and male aging. *Current Biology*, **22**, 1717–1721.

Castellana S, Vicario S, Saccone C (2011) Evolutionary patterns of the mitochondrial genome in metazoa: Exploring the role of mutation and selection in mitochondrial protein–coding genes. *Genome Biology and Evolution*, **3**, 1067–1079.

Chan KMA, Levin SA (2005) Leaky prezygotic isolation and porous genomes: rapid introgression of maternally inherited DNA. *Evolution*, **59**, 720–729.

Chang C-C, Rodriguez J, Ross J (2016) Mitochondrial–nuclear epistasis impacts fitness and mitochondrial physiology of

interpopulation *Caenorhabditis briggsae* hybrids. *G3: Genes| Genomes| Genetics*, **6**, 209–219.

Chong RA, Mueller RL (2013) Low metabolic rates in salamanders are correlated with weak selective constraints on mitochondrial genes. *Evolution*, **67**, 894–899.

Chou J-Y, Hung Y-S, Lin K-H, Lee H-Y, Leu J-Y (2010) Multiple molecular mechanisms cause reproductive isolation between three yeast species. *PLoS Biology*, **8**, e1000432.

Christie JR, Beekman M (2017) Uniparental inheritance promotes adaptive evolution in cytoplasmic genomes. *Molecular Biology and Evolution*, doi: 10.1093/molbev/msw266.

Clark KA, Howe DK, Gafner K *et al.* (2012) Selfish little circles: transmission bias and evolution of large deletion-bearing mitochondrial DNA in *Caenorhabditis briggsae* nematodes. *PLoS ONE*, **7**, e41433.

Cooper BS, Burrus CR, Ji C, Hahn MW, Montooth KL (2015) Similar efficacies of selection shape mitochondrial and nuclear genes in both *Drosophila melanogaster* and *Homo sapiens*. *G3: Genes| Genomes| Genetics*, **5**, 2165–2176.

Covello PS, Gray MW (1993) On the evolution of RNA editing. *Trends in Genetics*, **9**, 265–268.

Coyne JA, Orr HA (2004) *Speciation*. Sinauer Associates, Inc, Sunderland, Massachusetts.

Cruzan MB, Arnold ML (1999) Consequences of cytonuclear epistasis and assortative mating for the genetic structure of hybrid populations. *Heredity*, **82**, 36–45.

Currat M, Ruedi M, Petit RJ, Excoffier L (2008) The hidden side of invasions: massive introgression by local genes. *Evolution*, **62**, 1908.

Darwin C (1859) *On the Origin of Species*. John Murray, London.

Dean R, Arnold J (1997) The effects of unidirectional incompatibility on cytonuclear disequilibria in a hybrid zone. *Genetica*, **101**, 215–224.

Degnan JH, Rosenberg NA (2009) Gene tree discordance, phylogenetic inference and the multispecies coalescent. *Trends in Ecology & Evolution*, **24**, 332–340.

Denver DR, Morris K, Lynch M, Vassilieva LL, Thomas WK (2000) High direct estimate of the mutation rate in the mitochondrial genome of *Caenorhabditis elegans*. *Science*, **289**, 2342–2344.

Deremiens L, Schwartz L, Angers A, Glémet H, Angers B (2015) Interactions between nuclear genes and a foreign mitochondrial genome in the redbelly dace *Chrosomus eos*. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, **189**, 80–86.

Dobler R, Rogell B, Budar F, Dowling DK (2014) A meta-analysis of the strength and nature of cytoplasmic genetic effects. *Journal of Evolutionary Biology*, **27**, 2021–2034.

Đorđević M, Savković U, Lazarević J, Tucić N, Stojković B (2015) Intergenomic interactions in hybrids between short-lived and long-lived lines of a seed beetle: Analyses of life history traits. *Evolutionary Biology*, **42**, 461–472.

Dowling DK, Abiega KC, Arnqvist G (2007a) Temperature-specific outcomes of cytoplasmic-nuclear interactions on egg-to-adult development time in seed beetles. *Evolution*, **61**, 194–201.

Dowling DK, Friberg U, Hailer F, Arnqvist G (2007b) Intergenomic epistasis for fitness: within-population interactions between cytoplasmic and nuclear genes in *Drosophila melanogaster*. *Genetics*, **175**, 235–244.

Dowling DK, Friberg U, Lindell J (2008) Evolutionary implications of non-neutral mitochondrial genetic variation. *Trends in Ecology & Evolution*, **23**, 546–554.

Drouin G, Daoud H, Xia J (2008) Relative rates of synonymous substitutions in the mitochondrial, chloroplast and nuclear genomes of seed plants. *Molecular Phylogenetics and Evolution*, **49**, 827–831.

Duchêne A-M, Giritch A, Hoffmann B *et al.* (2005) Dual targeting is the rule for organellar aminoacyl-tRNA synthetases in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences of the United States of America*, **102**, 16484–16489.

Ellison CK, Burton RS (2006) Disruption of mitochondrial function in interpopulation hybrids of *Tigriopus californicus*. *Evolution*, **60**, 1382–1391.

Ellison CK, Burton RS (2010) Cytonuclear conflict in interpopulation hybrids: the role of RNA polymerase in mtDNA transcription and replication. *Journal of Evolutionary Biology*, **23**, 528–538.

Ellison CK, Niehuis O, Gadau J (2008) Hybrid breakdown and mitochondrial dysfunction in hybrids of *Nasonia* parasitoid wasps. *Journal of Evolutionary Biology*, **21**, 1844–1851.

Ellstrand NC, Schierenbeck KA (2000) Hybridization as a stimulus for the evolution of invasiveness in plants? *Proceedings of the National Academy of Sciences of the United States of America*, **97**, 7043–7050.

Fangue NA, Richards JG, Schulte PM (2009) Do mitochondrial properties explain intraspecific variation in thermal tolerance? *Journal of Experimental Biology*, **212**, 514–522.

Felsenstein J (1974) The evolutionary advantage of recombination. *Genetics*, **78**, 737–756.

Fishman L, Willis JH (2006) A cytonuclear incompatibility causes anther sterility in Mimulus hybrids. *Evolution*, **60**, 1372–1381.

Frank SA, Hurst LD (1996) Mitochondria and male disease. *Nature*, **383**, 224.

Fujii S, Bond CS, Small ID (2011) Selection patterns on restorer-like genes reveal a conflict between nuclear and mitochondrial genomes throughout angiosperm evolution. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 1723–1728.

Funk DJ, Omland KE (2003) Species-level paraphyly and polyphyly: frequency, causes, and consequences, with insights from animal mitochondrial DNA. *Annual Review of Ecology, Evolution, and Systematics*, **34**, 397–423.

Gagnaire PA, Normandeau E, Bernatchez L (2012) Comparative genomics reveals adaptive protein evolution and a possible cytonuclear incompatibility between European and American Eels. *Molecular Biology and Evolution*, **29**, 2909–2919.

Galtier N, Nabholz B, Glemin S, Hurst GD (2009) Mitochondrial DNA as a marker of molecular diversity: a reappraisal. *Molecular Ecology*, **18**, 4541–4550.

Gebiola M, Kelly SE, Hammerstein P, Giorgini M, Hunter MS (2016) "Darwin's corollary" and cytoplasmic incompatibility induced by *Cardinium* may contribute to speciation in Encarsia wasps (Hymenoptera: Aphelinidae). *Evolution*, **70**, 2447–2458.

Gemmell NJ, Allendorf FW (2001) Mitochondrial mutations may decrease population viability. *Trends in Ecology & Evolution*, **16**, 115–117.

Gemmell NJ, Metcalf VJ, Allendorf FW (2004) Mother's curse: the effect of mtDNA on individual fitness and population viability. *Trends in Ecology & Evolution*, **19**, 238–244.

Gershoni M, Templeton AR, Mishmar D (2009) Mitochondrial bioenergetics as a major motive force of speciation. *BioEssays*, **31**, 642–650.

Gershoni M, Levin L, Ovadia O *et al.* (2014) Disrupting mitochondrial-nuclear coevolution affects OXPHOS complex I integrity and impacts human health. *Genome Biology and Evolution*, **6**, 2665–2680.

Gong L, Salmon A, Yoo M-J *et al.* (2012) The cytonuclear dimension of allopolyploid evolution: an example from cotton using Rubisco. *Molecular Biology and Evolution*, **29**, 3023–3036.

Good JM, Vanderpool D, Keeble S, Bi K (2015) Negligible nuclear introgression despite complete mitochondrial capture between two species of chipmunks. *Evolution*, **69**, 1961–1972.

Gray MW (2012) Mitochondrial evolution. *Cold Spring Harbor Perspectives in Biology*, **4**, a011403.

Gray MW, Cedergren R, Abel Y, Sankoff D (1989) On the evolutionary origin of the plant mitochondrion and its genome. *Proceedings of the National Academy of Sciences of the United States of America*, **86**, 2267–2271.

Gray MW, Lukeš J, Archibald JM, Keeling PJ, Doolittle WF (2010) Irremediable Complexity? *Science*, **330**, 920–921.

Greiner S, Rauwolf U, Meurer J, Herrmann RG (2011) The role of plastids in plant speciation. *Molecular Ecology*, **20**, 671–691.

Haldane JBS (1922) Sex ratio and unisexual sterility in hybrid animals. *Journal of Genetics*, **12**, 101–109.

Hansen AK, Moran NA (2011) Aphid genome expression reveals host-symbiont cooperation in the production of amino acids. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 2849–2854.

Harrison RG (1989) Animal mitochondrial DNA as a genetic marker in population and evolutionary biology. *Trends in Ecology & Evolution*, **4**, 6–11.

Harrison JS, Burton RS (2006) Tracing hybrid incompatibilities to single amino acid substitutions. *Molecular Biology and Evolution.*, **23**, 559–564.

Harrison RG, Larson EL (2014) Hybridization, introgression, and the nature of species boundaries. *Journal of Heredity*, **105**, 795–809.

Harrison RG, Larson EL (2016) Heterogeneous genome divergence, differential introgression, and the origin and structure of hybrid zones. *Molecular Ecology*, **25**, 2454–2466.

Havird JC, Sloan DB (2016) The roles of mutation, selection, and expression in determining relative rates of evolution in mitochondrial vs. nuclear genomes. *Molecular Biology and Evolution.*, **33**, 3042–3053.

Havird JC, Whitehill NS, Snow CD, Sloan DB (2015) Conservative and compensatory evolution in oxidative phosphorylation complexes of angiosperms with highly divergent rates of mitochondrial genome evolution. *Evolution*, **69**, 3069–3081.

Havird JC, Fitzpatrick S, Funk WC *et al.* (2016) Sex, mitochondria, and genetic rescue. *Trends in Ecology and Evolution*, **31**, 96–99.

Hayes ML, Mulligan RM (2011) Pentatricopeptide repeat proteins constrain genome evolution in chloroplasts. *Molecular Biology and Evolution*, **28**, 2029–2039.

Hill GE (2015) Mitonuclear ecology. *Molecular Biology and Evolution*, **32**, 1927–1937.

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

Hill GE, Johnson JD (2013) The mitonuclear compatibility hypothesis of sexual selection. *Proceedings of the Royal Society Biological Sciences B*, **280**, 20131314.

Hill WG, Robertson A (1966) The effect of linkage on limits to artificial selection. *Genetical Research*, **8**, 269–294.

Hoekstra LA, Siddiq MA, Montooth KL (2013) Pleiotropic effects of a mitochondrial–nuclear incompatibility depend upon the accelerating effect of temperature in *Drosophila*. *Genetics*, **195**, 1129–1139.

Hollister JD, Greiner S, Wang W *et al.* (2015) Recurrent loss of sex is associated with accumulation of deleterious mutations in *Oenothera*. *Molecular Biology and Evolution*, **32**, 896–905.

Hulsey CD, Bell KL, García-de-León FJ, Nice CC, Meyer A (2016) Do relaxed selection and habitat temperature facilitate biased mitogenomic introgression in a narrowly endemic fish? *Ecology and Evolution*, **6**, 3684–3698.

Hurst GDD, Jiggins FM (2005) Problems with mitochondrial DNA as a marker in population, phylogeographic and phylogenetic studies: the effects of inherited symbionts. *Proceedings of the Royal Society of London B: Biological Sciences*, **272**, 1525–1534.

Husnik F, McCutcheon JP (2016) Repeated replacement of an intrabacterial symbiont in the tripartite nested mealybug symbiosis. *Proceedings of the National Academy of Sciences of the United States of America*, **113**, E5416–E5424.

Husnik F, Nikoh N, Koga R *et al.* (2013) Horizontal gene transfer from diverse bacteria to an insect genome enables a tripartite nested mealybug symbiosis. *Cell*, **153**, 1567–1578.

Immonen E, Collet M, Goenaga J, Arnqvist G (2016) Direct and indirect genetic effects of sex-specific mitonuclear epistasis on reproductive ageing. *Heredity*, **116**, 338–347.

Ingvarsson PK (2001) Restoration of genetic variation lost–the genetic rescue hypothesis. *Trends in Ecology & Evolution*, **16**, 62–63.

Innocenti P, Morrow EH, Dowling DK (2011) Experimental evidence supports a sex-specific selective sieve in mitochondrial genome evolution. *Science*, **332**, 845–848.

Ishikawa H (1984) Characterization of the protein species synthesized in vivo and in vitro by an aphid endosymbiont. *Insect Biochemistry*, **14**, 417–425.

James JE, Piganeau G, Eyre-Walker A (2016) The rate of adaptive evolution in animal mitochondria. *Molecular Ecology*, **25**, 67–78.

Jiggins FM (2003) Male-killing Wolbachia and mitochondrial DNA: selective sweeps, hybrid introgression and parasite population dynamics. *Genetics*, **164**, 5–12.

Kaiser VB, Charlesworth B (2010) Muller's ratchet and the degeneration of the *Drosophila miranda* neo-Y chromosome. *Genetics*, **185**, 339–348.

Kakeda K, Ishikawa H (1991) Molecular chaperon produced by an intracellular symbiont. *Journal of Biochemistry*, **110**, 583–587.

Kanevski I, Maliga P, Rhoades DF, Gutteridge S (1999) Plastome engineering of ribulose-1, 5-bisphosphate carboxylase/oxygenase in tobacco to form a sunflower large subunit and tobacco small subunit hybrid. *Plant Physiology*, **119**, 133–142.

Kenney MC, Chwa M, Atilano SR *et al.* (2013) Mitochondrial DNA variants mediate energy production and expression levels for CFH, C3 and EFEMP1 genes: implications for age-related macular degeneration. *PLoS ONE*, **8**, e54339.

Knight RD, Freeland SJ, Landweber LF (2001) Rewiring the keyboard: evolvability of the genetic code. *Nature Reviews Genetics*, **2**, 49–58.

Kraytsberg Y, Kudryavtseva E, McKee AC *et al.* (2006) Mitochondrial DNA deletions are abundant and cause functional

impairment in aged human substantia nigra neurons. *Nature Genetics*, **38**, 518–520.

Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, **33**, 1870–1874.

Kuo CH, Moran NA, Ochman H (2009) The consequences of genetic drift for bacterial genome complexity. *Genome Research*, **19**, 1450–1454.

Lavrov DV, Pett W (2016) Animal mitochondrial DNA as we don't know it: mt-genome organization and evolution in non-bilaterian lineages. *Genome Biology and Evolution*, **8**, 2896–2913.

Lavrov DV, Adamski M, Chevaldonné P, Adamska M (2016) Extensive mitochondrial mRNA editing and unusual mitochondrial genome organization in calcaronean sponges (Calcarea, Porifera). *Current Biology*, **26**, 86–92.

Lee HY, Chou JY, Cheong L *et al.* (2008) Incompatibility of nuclear and mitochondrial genomes causes hybrid sterility between two yeast species. *Cell*, **135**, 1065–1073.

Lee-Yaw JA, Jacobs CGC, Irwin DE (2014) Individual performance in relation to cytonuclear discordance in a northern contact zone between long-toed salamander (Ambystoma macrodactylum) lineages. *Molecular Ecology*, **23**, 4590–4602.

Levin DA (2003) The cytoplasmic factor in plant speciation. *Systematic Botany*, **28**, 5–11.

Liu SL, Zhuang Y, Zhang P, Adams KL (2009) Comparative analysis of structural diversity and sequence evolution in plant mitochondrial genes transferred to the nucleus. *Molecular Biology and Evolution*, **26**, 875–891.

Llopart A, Herrig D, Brud E, Stecklein Z (2014) Sequential adaptive introgression of the mitochondrial genome in *Drosophila yakuba* and *Drosophila santomea*. *Molecular Ecology*, **23**, 1124–1136.

Ludwig A, Congiu L, Pitra C *et al.* (2003) Nonconcordant evolutionary history of maternal and paternal lineages in Adriatic sturgeon. *Molecular Ecology*, **12**, 3253–3264.

Lynch M (1996) Mutation accumulation in transfer RNAs: Molecular evidence for Muller's ratchet in mitochondrial genomes. *Molecular Biology and Evolution*, **13**, 209–220.

Lynch M (1997) Mutation accumulation in nuclear, organelle, and prokaryotic transfer RNA genes. *Molecular Biology and Evolution*, **14**, 914–925.

Lynch M, Blanchard JL (1998) Deleterious mutation accumulation in organelle genomes. *Genetica*, **103**, 29–39.

Lynch M, Conery J, Burger R (1995) Mutation accumulation and the extinction of small populations. *American Naturalist*, **146**, 489–518.

Lynch M, Koskella B, Schaack S (2006) Mutation pressure and the evolution of organelle genomic architecture. *Science*, **311**, 1727–1730.

Lynch M, Sung W, Morris K *et al.* (2008) A genome-wide view of the spectrum of spontaneous mutations in yeast. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 9272–9277.

Mamirova L, Popadin K, Gelfand MS (2007) Purifying selection in mitochondria, free-living and obligate intracellular proteobacteria. *BMC Evolutionary Biology*, **7**, 17.

Mayr E (1986) Joseph Gottlieb Kölreuter's contributions to biology. *Osiris*, **2**, 135–176.

McCauley DE (1995) The use of chloroplast DNA polymorphism in studies of gene flow in plants. *Trends in Ecology & Evolution*, **10**, 198–202.

McCutcheon JP, Moran NA (2012) Extreme genome reduction in symbiotic bacteria. *Nature Reviews Microbiology*, **10**, 13–26.

McDonald JH, Kreitman M (1991) Adaptive protein evolution at the Adh locus in Drosophila. *Nature*, **351**, 652–654.

McGuire JA, Linkem CW, Koo MS *et al.* (2007) Mitochondrial introgression and incomplete lineage sorting through space and time: phylogenetics of crotaphytid lizards. *Evolution*, **61**, 2879–2897.

McKay BD, Zink RM (2010) The causes of mitochondrial DNA gene tree paraphyly in birds. *Molecular Phylogenetics and Evolution*, **54**, 647–650.

McKenzie JL, Dhillon RS, Schulte PM (2016) Steep, coincident, and concordant clines in mitochondrial and nuclear-encoded genes in a hybrid zone between subspecies of Atlantic killifish, Fundulus heteroclitus. *Ecology and Evolution*, **6**, 5771–5787.

Meiklejohn CD, Holmbeck MA, Siddiq MA *et al.* (2013) An Incompatibility between a mitochondrial tRNA and its nuclear-encoded tRNA synthetase compromises development and fitness in *Drosophila*. *PLoS Genetics*, **9**, e1003238.

Melo-Ferreira J, Boursot P, Suchentrunk F, Ferrand N, Alves PC (2005) Invasion from the cold past: extensive introgression of mountain hare (*Lepus timidus*) mitochondrial DNA into three other hare species in northern Iberia. *Molecular Ecology*, **14**, 2459–2464.

Meyer M, Fu Q, Aximu-Petri A *et al.* (2014) A mitochondrial genome sequence of a hominin from Sima de los Huesos. *Nature*, **505**, 403–406.

Meyer M, Arsuaga JL, de Filippo C *et al.* (2016) Nuclear DNA sequences from the Middle Pleistocene Sima de los Huesos hominins. *Nature*, **531**, 504–507.

Mishmar D, Ruiz-Pesini E, Golik P *et al.* (2003) Natural selection shaped regional mtDNA variation in humans. *Proceedings of the National Academy of Sciences of the United States of America*, **100**, 171–176.

Morales HE (2016) *Genomic and Phenotypic Adaptation in a Widespread Passerine*. PhD Dissertation, Monash University, Clayton.

Morales HE, 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.

Morales HE, Sunnucks P, Joseph L, Pavlova A (2016) Perpendicular axes of incipient speciation generated by mitochondrial introgression. *BioRxiv* doi: 10.1101/072942.

Moran NA (1996) Accelerated evolution and Muller's rachet in endosymbiotic bacteria. *Proceedings of the National Academy of Sciences of the United States of America*, **93**, 2873–2878.

Moran NA, McLaughlin HJ, Sorek R (2009) The dynamics and time scale of ongoing genomic erosion in symbiotic bacteria. *Science*, **323**, 379–382.

Mossman JA, Biancani LM, Zhu CT, Rand DM (2016) Mitonuclear epistasis for development time and its modification by diet in *Drosophila*. *Genetics*, **203**, 463–484.

Mower JP, Touzet P, Gummow JS, Delph LF, Palmer JD (2007) Extensive variation in synonymous substitution rates in mitochondrial genes of seed plants. *BMC Evolutionary Biology*, **7**, 135.

Muller HJ (1964) The relation of recombination to mutational advance. *Mutation Research*, **106**, 2–9.

Nabholz B, Glémin S, Galtier N (2009) The erratic mitochondrial clock: variations of mutation rate, not population size, affect mtDNA diversity across birds and mammals. *BMC Evolutionary Biology*, **9**, 54.

Nabholz B, Ellegren H, Wolf JB (2013) High levels of gene expression explain the strong evolutionary constraint of mitochondrial protein-coding genes. *Molecular Biology and Evolution*, **30**, 272–284.

Nachman MW (1998) Deleterious mutations in animal mitochondrial DNA. *Genetica*, **102**, 61–69.

Nagao Y, Totsuka Y, Atomi Y *et al.* (1998) Decreased physical performance of congenic mice with mismatch between the nuclear and the mitochondrial genome. *Genes & Genetic Systems*, **73**, 21–27.

Neiman M, Taylor DR (2009) The causes of mutation accumulation in mitochondrial genomes. *Proceedings of the Royal Society B: Biological Sciences*, **276**, 1201–1209.

Neiman M, Hehman G, Miller JT, Logsdon JM, Taylor DR (2010) Accelerated mutation accumulation in asexual lineages of a freshwater snail. *Molecular Biology and Evolution*, **27**, 954–963.

Niehuis O, Judson AK, Gadau J (2008) Cytonuclear genic incompatibilities cause increased mortality in male F2 hybrids of Nasonia giraulti and N. vitripennis. *Genetics*, **178**, 413–426.

Nowack ECM, Price DC, Bhattacharya D *et al.* (2016) Gene transfers from diverse bacteria compensate for reductive genome evolution in the chromatophore of *Paulinella chromatophora*. *Proceedings of the National Academy of Sciences of the United States of America*, **113**, 12214–12219.

Oliveira DC, Raychoudhury R, Lavrov DV, Werren JH (2008) Rapidly evolving mitochondrial genome and directional selection in mitochondrial genes in the parasitic wasp Nasonia (Hymenoptera: Pteromalidae). *Molecular Biology and Evolution*, **25**, 2167–2180.

Orr HA (1995) The population genetics of speciation: the evolution of hybrid incompatibilities. *Genetics*, **139**, 1805–1813.

Orr HA (1996) Dobzhansky, Bateson, and the genetics of speciation. *Genetics*, **144**, 1331–1335.

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.

Paliwal S, Fiumera AC, Fiumera HL (2014) Mitochondrial-nuclear epistasis contributes to phenotypic variation and coadaptation in natural isolates of *Saccharomyces cerevisiae*. *Genetics*, **198**, 1251–1265.

Parmakelis A, Kotsakiozi P, Rand D (2013) Animal mitochondria, positive selection and cyto-nuclear coevolution: insights from pulmonates. *PLoS ONE*, **8**, e61970.

Patten MM, Carioscia SA, Linnen CR (2015) Biased introgression of mitochondrial and nuclear genes: a comparison of diploid and haplodiploid systems. *Molecular Ecology*, **24**, 5200–5210.

Payseur BA, Rieseberg LH (2016) A genomic perspective on hybridization and speciation. *Molecular Ecology*, **25**, 2337–2360.

Peck JR (1994) A ruby in the rubbish: beneficial mutations, deleterious mutations and the evolution of sex. *Genetics*, **137**, 597–606.

Perna NT, Kocher TD (1995) Patterns of nucleotide composition at fourfold degenerate sites of animal mitochondrial genomes. *Journal of Molecular Evolution*, **41**, 353–358.

Petersen J, Ludewig AK, Michael V *et al.* (2014) *Chromera velia*, endosymbioses and the rhodoplex hypothesis–plastid evolution in cryptophytes, alveolates, stramenopiles, and haptophytes (CASH lineages). *Genome Biology and Evolution*, **6**, 666–684.

Petit RJ, Excoffier L (2009) Gene flow and species delimitation. *Trends in Ecology & Evolution*, **24**, 386–393.

Pett W, Lavrov DV (2015) Cytonuclear interactions in the evolution of animal mitochondrial tRNA metabolism. *Genome Biology and Evolution*, **7**, 2089–2101.

Phillips WS, Coleman-Hulbert AL, Weiss ES *et al.* (2015) Selfish mitochondrial DNA proliferates and diversifies in small, but not large, experimental populations of *Caenorhabditis briggsae*. *Genome Biology and Evolution*, **7**, 2023–2037.

Pons JM, Sonsthagen S, Dove C, Crochet PA (2014) Extensive mitochondrial introgression in North American Great Black-backed Gulls (*Larus marinus*) from the American Herring Gull (*Larus smithsonianus*) with little nuclear DNA impact. *Heredity*, **112**, 226–239.

Popadin KY, Nikolaev SI, Junier T, Baranova M, Antonarakis SE (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.

Pritchard VL, Edmands S (2013) The genomic trajectory of hybrid swarms: outcomes of repeated crosses between populations of *Tigriopus californicus*. *Evolution*, **67**, 774–791.

Rand DM (2008) Mitigating mutational meltdown in mammalian mitochondria. *PLoS Biology*, **6**, e35.

Rand DM, Kann LM (1996) Excess amino acid polymorphism in mitochondrial DNA: contrasts among genes from Drosophila, mice, and humans. *Molecular Biology and Evolution*, **13**, 735–748.

Rand DM, Haney RA, Fry AJ (2004) Cytonuclear coevolution: the genomics of cooperation. *Trends in Ecology & Evolution*, **19**, 645–653.

Reich D, Green RE, Kircher M *et al.* (2010) Genetic history of an archaic hominin group from Denisova Cave in Siberia. *Nature*, **468**, 1053–1060.

Reinhardt K, Dowling DK, Morrow EH (2013) Mitochondrial replacement, evolution, and the clinic. *Science*, **341**, 1345–1346.

Richardson AO, Rice DW, Young GJ, Alverson AJ, Palmer JD (2013) The "fossilized" mitochondrial genome of Liriodendron tulipifera: ancestral gene content and order, ancestral editing sites, and extraordinarily low mutation rate. *BMC Biology*, **11**, 29.

Rieseberg LH (2009) Evolution: replacing genes and traits through hybridization. *Current Biology*, **19**, R119–R122.

Rieseberg LH, Soltis DE (1991) Phylogenetic consequences of cytoplasmic gene flow in plants. *Evolutionary Trends in Plants*, **5**, 65–84.

Rieseberg LH, Whitton J, Linder CR (1996) Molecular marker incongruence in plant hybrid zones and phylogenetic trees. *Acta Botanica Neerlandica*, **45**, 243–262.

Rockenbach KD, Havird JC, Monroe JG *et al.* (2016) Positive selection in rapidly evolving plastid-nuclear enzyme complexes. *Genetics*, **204**, 1507–1522.

Sackton TB, Haney RA, Rand DM (2003) Cytonuclear coadaptation in *Drosophila*: disruption of cytochrome c oxidase activity in backcross genotypes. *Evolution*, **57**, 2315–2325.

Schnable PS, Wise RP (1998) The molecular basis of cytoplasmic male sterility and fertility restoration. *Trends in Plant Science*, **3**, 175–180.

Sehrish T, Symonds VV, Soltis DE, Soltis PS, Tate JA (2015) Cytonuclear coordination is not immediate upon allopolyploid formation in *Tragopogon miscellus* (Asteraceae) allopolyploids. *PLoS ONE*, **10**, e0144339.

Sharbrough J, Luse M, Boore JL, Logsdon JM, Neiman M (2016) Radical changes persist longer in the absence of sex. *BioRxiv*, doi: 10.1101/049924.

Shen Y-Y, Shi P, Sun Y-B, Zhang Y-P (2009) Relaxation of selective constraints on avian mitochondrial DNA following the degeneration of flight ability. *Genome Research*, **19**, 1760–1765.

Sloan DB, Nakabachi A, Richards S *et al.* (2014a) Parallel histories of horizontal gene transfer facilitated extreme reduction of endosymbiont genomes in sap-feeding insects. *Molecular Biology and Evolution*, **31**, 857–871.

Sloan DB, Triant DA, Wu M, Taylor DR (2014b) Cytonuclear interactions and relaxed selection accelerate sequence evolution in organelle ribosomes. *Molecular Biology and Evolution*, **31**, 673–682.

Sloan DB, Fields PD, Havird JC (2015) Mitonuclear linkage disequilibrium in human populations. *Proceedings of the Royal Society B: Biological Sciences*, **282**, 20151704.

van der Sluis EO, Bauerschmitt H, Becker T *et al.* (2015) Parallel structural evolution of mitochondrial ribosomes and OXPHOS complexes. *Genome Biology and Evolution*, **7**, 1235–1251.

Smith DR, Keeling PJ (2015) Mitochondrial and plastid genome architecture: Reoccurring themes, but significant differences at the extremes. *Proceedings of the National Academy of Sciences of the United States of America*, **112**, 10177–10184.

Spirek M, Polakova S, Jatzova K, Sulo P (2015) Post-zygotic sterility and cytonuclear compatibility limits in *S. cerevisiae* xenomitochondrial cybrids. *Frontiers in Genetics*, **5**, 454.

Stewart JB, Freyer C, Elson JL *et al.* (2008) Strong purifying selection in transmission of mammalian mitochondrial DNA. *PLoS Biology*, **6**, e10.

Stoltzfus A (1999) On the possibility of constructive neutral evolution. *Journal of Molecular Evolution*, **49**, 169–181.

Taylor DR, Zeyl C, Cooke E (2002) Conflicting levels of selection in the accumulation of mitochondrial defects in Saccharomyces cerevisiae. *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 3690–3694.

Timmis JN, Ayliffe MA, Huang CY, Martin W (2004) Endosymbiotic gene transfer: Organelle genomes forge eukaryotic chromosomes. *Nature Review Genetics*, **5**, 123–135.

Toews DP, Brelsford A (2012) The biogeography of mitochondrial and nuclear discordance in animals. *Molecular Ecology*, **21**, 3907–3930.

Touzet P, Budar F (2004) Unveiling the molecular arms race between two conflicting genomes in cytoplasmic male sterility? *Trends in Plant Science*, **9**, 568–570.

Trier CN, Hermansen JS, Saetre GP, Bailey RI (2014) Evidence for mito-nuclear and sex-linked reproductive barriers between the hybrid Italian sparrow and its parent species. *PLoS Genetics*, **10**, e1004075.

Tucker AE, Ackerman MS, Eads BD, Xu S, Lynch M (2013) Population-genomic insights into the evolutionary origin and fate of obligately asexual *Daphnia pulex*. *Proceedings of the National Academy of Sciences of the United States of America*, **110**, 15740–15745.

Turelli M, Moyle LC (2007) Asymmetric postmating isolation: Darwin's corollary to Haldane's rule. *Genetics*, **176**, 1059–1088.

Vevea JD, Swayne TC, Boldogh IR, Pon LA (2014) Inheritance of the fittest mitochondria in yeast. *Trends in Cell Biology*, **24**, 53–60.

Wade MJ, Drown DM (2016) Nuclear–mitochondrial epistasis: a gene's eye view of genomic conflict. *Ecology and Evolution*, **6**, 6460–6472.

Watanabe Y, Suematsu T, Ohtsuki T (2014) Losing the stem-loop structure from metazoan mitochondrial tRNAs and co-evolution of interacting factors. *Frontiers in Genetics*, **5**, 109.

Weinreich DM, Rand DM (2000) Contrasting patterns of nonneutral evolution in proteins encoded in nuclear and mitochondrial genomes. *Genetics*, **156**, 385–399.

Welch JJ, Eyre-Walker A, Waxman D (2008) Divergence and polymorphism under the nearly neutral theory of molecular evolution. *Journal of Molecular Evolution*, **67**, 418–426.

Weng ML, Ruhlman TA, Jansen RK (2016) Plastid-nuclear interaction and accelerated coevolution in plastid ribosomal genes in Geraniaceae. *Genome Biology and Evolution*, **8**, 1824–1838.

Werren JH, Baldo L, Clark ME (2008) Wolbachia: master manipulators of invertebrate biology. *Nature Reviews Microbiology*, **6**, 741–751.

Wertheim JO, Murrell B, Smith MD, Pond SLK, Scheffler K (2015) RELAX: detecting relaxed selection in a phylogenetic framework. *Molecular Biology and Evolution*, **32**, 820–832.

Whiteley AR, Fitzpatrick SW, Funk WC, Tallmon DA (2015) Genetic rescue to the rescue. *Trends in Ecology & Evolution*, **30**, 42–49.

Willett CS (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 CS, Burton RS (2004) Evolution of interacting proteins in the mitochondrial electron transport system in a marine copepod. *Molecular Biology and Evolution*, **21**, 443–453.

Wolfe KH, Li WH, Sharp PM (1987) Rates of nucleotide substitution vary greatly among plant mitochondrial, chloroplast, and nuclear DNAs. *Proceedings of the National Academy of Sciences of the United States of America*, **84**, 9054–9058.

Yang Z (2007) PAML 4: Phylogenetic Analysis by Maximum Likelihood. *Molecular Biology and Evolution*, **24**, 1586–1591.

Yee WKW, Sutton KL, Dowling DK (2013) In vivo male fertility is affected by naturally occurring mitochondrial haplotypes. *Current Biology*, **23**, R55–R56.

Zeyl C, Andreson B, Weninck E (2005) Nuclear-mitochondrial epistasis for fitness in *Saccharomyces cerevisiae*. *Evolution*, **59**, 910–914.

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

Zhang J, Ruhlman TA, Sabir J, Blazier JC, Jansen RK (2015) Coordinated rates of evolution between interacting plastid and nuclear genes in Geraniaceae. *Plant Cell*, **27**, 563–573.

Zhang J, Ruhlman TA, Sabir JS *et al.* (2016) Coevolution between nuclear-encoded DNA replication, recombination, and repair genes and plastid genome complexity. *Genome Biology and Evolution*, **8**, 622–634.

Zhu CT, Ingelmo P, Rand DM (2014) G×G×E for lifespan in *Drosophila*: mitochondrial, nuclear, and dietary interactions that modify longevity. *PLoS Genetics*, **10**, e1004354.

Zhu Z, Lu Q, Zeng F, Wang J, Huang S (2015) Compatibility between mitochondrial and nuclear genomes correlates with the quantitative trait of lifespan in *Caenorhabditis elegans*. *Scientific Reports*, **5**, 17303.

Zieliński P, Nadachowska-Brzyska K, Wielstra B *et al.* (2013) No evidence for nuclear introgression despite complete mtDNA replacement in the Carpathian newt (*Lissotriton montandoni*). *Molecular Ecology*, **22**, 1884–1903.

D.B.S. drafted the majority of the paper and performed analyses in Figs 3 and 4. J.C.H. drafted portions of the paper, generated Fig. 2, and performed analysis in Box 2. J.S. drafted portions of the paper and assisted in the generation of Fig. 2.

Supporting information

Additional supporting information may be found in the online version of this article.

Appendix S1 Perl script used to generate model data presented in Fig. 3.