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Review

Mitochondrial genetic variation as a potential mediator of intraspecific behavioural diversity

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Mitochondrial genes play an essential role in energy metabolism. Variation in the mitochondrial DNA (mtDNA) sequence often exists within species, and this variation can have consequences for energy production and organismal life history. Yet, despite potential links between energy metabolism and the expression of animal behaviour, mtDNA variation has been largely neglected to date in studies investigating intraspecific behavioural diversity. We outline how mtDNA variation and interactions between mitochondrial and nuclear genotypes may contribute to the expression of individual-to-individual behavioural differences within populations, and why such effects may lead to sex differences in behaviour. We contend that integration of the mitochondrial genome into behavioural ecology research may be key to fully understanding the evolutionary genetics of animal behaviour.

Highlights

Mitochondria contain their own genomes. These genomes play a central role in energy production. Individuals within populations can differ in their mitochondrial DNA sequence, and these genetic differences are known to influence energy metabolism and organismal life history.

Little research has investigated how mitochondrial genetic variation within species contributes to the expression of behavioural diversity. This is a significant oversight, considering that energy metabolism can influence the expression of behavioural traits.

We outline evidence that suggests that a comprehensive understanding of how and why individuals consistently differ from one another in their behaviour will require integration of mitochondrial genetics into animal behaviour research. We suggest that such a synthesis will promote avenues for future research that will be key to understanding intraspecific behavioural diversity.

Why mitochondrial genetic variation matters for understanding behavioural diversity

Mitochondria are vital to organismal energy production. These cellular organelles contain their own DNA (mtDNA), which encodes enzyme complexes that are crucial to energy production via **aerobic metabolism** (see Glossary) [1,2]. Furthermore, the mitochondrial genomes of animals typically have a much higher mutation rate than that of their nuclear counterparts [3,4]. Given that aerobic metabolism is the principal process by which eukaryotes produce energy, it was traditionally assumed that strong **purifying selection** would prohibit any functional (i.e., phenotype-modifying) mutations from accumulating within the mtDNA sequence [5–7]. Any mtDNA sequence variation (hereafter mtDNA variation) that did exist was, therefore, assumed to be selectively neutral and have little effect on **mitochondrial function**.

However, while the efficacy of purifying selection on the mtDNA sequence is well supported [8,9], studies have now shown that mtDNA variation routinely exists within populations; these variants occur across distinct mtDNA **haplotypes** that can be maintained at intermediate frequencies and confer clear fitness differences [10]. Despite these insights, very little research has investigated whether mtDNA haplotype variation may contribute to the expression of individual behavioural differences. We contend that this is a significant oversight, considering that behavioural expression is dependent on energy production, and that individual variation in organismal physiology is thought to contribute to the emergence of individual-to-individual behavioural differences [11–14]. While the contribution of sequence variation in the nuclear genome to the regulation of behavioural variation is beyond doubt, and the fields of behavioural ecology and quantitative genetics have successfully partnered for decades, the role of mtDNA variation has been largely neglected in animal behaviour research. In our review, we outline the need to better integrate mitochondrial genetics into behavioural ecology, thereby bridging a disciplinary divide between these scientific fields that we believe will advance our understanding of intraspecific behavioural diversity.

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Mitochondrial genomes underpin energy production, metabolism, and life history

Mitochondrial genetics and energy production: how does mtDNA influence mitochondrial function?

Mitochondria are small organelles found within the cells of virtually all eukaryote life. These organelles are primarily responsible for cellular energy production and contain their own diminutive genome (e.g., >3 billion nuclear DNA base pairs versus ~16 500 mtDNA base pairs in humans). In **bilaterian animals**, the mitochondrial genome is strictly maternally inherited and typically encodes for 37 gene products (13 protein-coding, 22 transfer RNAs, and two ribosomal RNAs) that play important roles in mitochondrial function and protein translation [4,15,16] (Figure 1). In addition, other peptides and small RNAs have recently been described that are transcribed by the mtDNA and that potentially affect mitochondrial function [17–21]. All of the 13 protein-coding genes found within mtDNA contribute to the production of essential enzyme complexes involved in oxidative phosphorylation (OXPHOS) – a key component of aerobic metabolism which involves the use of oxygen and chemical energy released by dietary nutrients to fuel the conversion of adenosine triphosphate (ATP) [4,22,23]. Given that ATP is the principal source of energy used for cellular processes, gene products of the mitochondrial genome thus play a crucial role in regulating organismal energy production.

Mitochondrial genetic variation within species can mediate energy metabolism and life-history traits

It is now clear that sequence variation in the mtDNA routinely exists within species and that this variation can alter mitochondrial function [24,25]. For example, Wolff *et al*. investigated mitochondrial function in 12 naturally occurring mtDNA haplotypes that were derived from geographically distinct *Drosophila melanogaster* populations and placed into a standardised (i.e., isogenic) nuclear background [26]. The authors reported that mtDNA haplotype influenced both mitochondrial bioenergetics and the quantity of mitochondria [26] – a finding that has been supported in similar studies with both vertebrate [27–30] and invertebrate [1,2,15,16,31–34] species. Likewise, variation among naturally occurring mtDNA haplotypes within species has been shown to influence whole-organism metabolic rate [35,36], as well as the production of reactive oxygen species (ROS) [1,27,31,32,37], which are a class of highly reactive molecules thought to affect organismal life history [38,39]. Furthermore, mtDNA variation can exist between individuals within the same population and this variation may also have functional consequences [10,40]. For example, whole-organism metabolic rate differed among three mtDNA haplotypes that occur in sympatry within

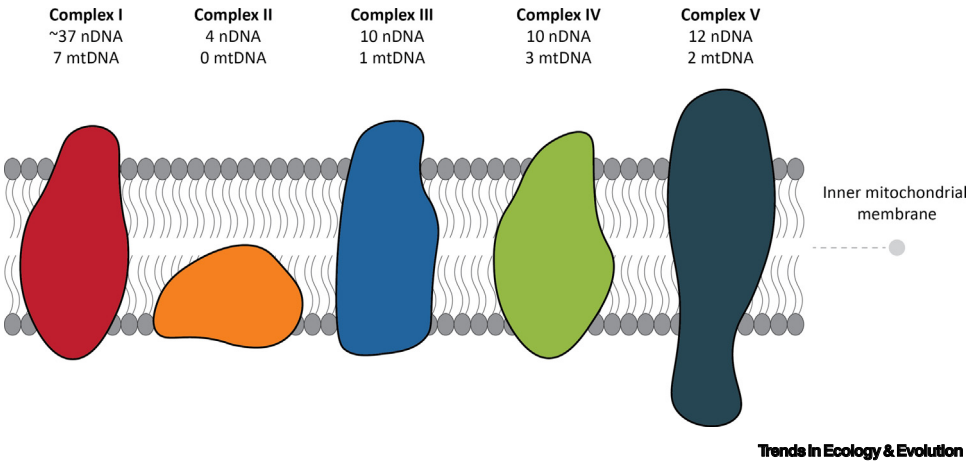

Figure 1. Mitochondrial and nuclear-encoded proteins contribute to energy production. Simplified illustration outlining the contribution of subunits encoded by the mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) to the protein complexes (I–V) involved in the electron transport system and subsequent organismal energy production.

Glossary

Aerobic metabolism: a series of metabolic reactions that occur within the mitochondria involving the use of oxygen and potential energy stored in nutrients – sugars, fatty acids, or amino acids – to fuel the production of cellular energy in the form of ATP.
Bilaterian animals: animals that have bilaterally symmetrical body plans (i.e., have a left and right side) during embryonic development. Includes all vertebrates, arthropods, molluscs, and many other animal groups.
Genetic admixture: when genotypes from once genetically isolated ancestral populations interbreed.
Haplotype: a specific set of alleles that are usually inherited together. Because mitochondrial genes are inherited maternally, and generally lack recombination, they exist and are inherited together as distinct mtDNA haplotypes.
Isofemale line: the descendants of a single, wild-collected gravid female that are allowed to interbreed. Isofemale lines are commonly used to investigate the genetic basis of trait expression within species – particularly in *Drosophila* flies.
Mitochondrial function: the suite of biological processes performed by the mitochondria. These processes involve, but are not limited to, the production of cellular energy through oxidative phosphorylation, cellular signalling, heat generation, and apoptosis.
Mitochondrial introgression: the outcome of interbreeding whereby mtDNA from one genetic lineage is incorporated into and coexpressed with nuclear DNA from a separate genetic lineage.
Mito-nuclear interaction: epistatic interactions between genes located within the nuclear and mitochondrial genomes. These interactions are necessary for fundamental biological processes that influence energy production and organismal physiology.
Mother's curse: a hypothesis that predicts the accumulation of male-harming mutations in the mtDNA. This is due to the maternal inheritance of mitochondria resulting in selection only being able to act on mtDNA variants in females.
Negative frequency-dependent selection: where the relative fitness of a DNA variant is inversely associated with its frequency within the population, resulting in rare genotypes having a

Drosophila subobscura fruit fly populations [41]. Further, two major mtDNA haplotypes found within a population of Atlantic killifish (*Fundulus heteroclitus*) displayed differences in nuclear allele frequency, with the authors reporting an association between mito-nuclear genotype and individual-to-individual differences in mitochondrial function [28]. Together, these results suggest that individual-to-individual variation in the mtDNA sequence within populations may have substantial consequences for mitochondrial function and energy production.

Moreover, the effects of mtDNA variation on energy metabolism have been linked to how species allocate resources to various life history traits. For example, the influence of mtDNA haplotype variation on mitochondrial quantity and gene expression is associated with reproduction and longevity in *D. melanogaster* [16]. Comparable findings have also been observed in other invertebrate species where mtDNA haplotype has been shown to mediate fecundity, egg size, and lifespan [2,31,34,42,43]. Furthermore, studies have reported associations between mtDNA haplotype and litter size, muscle area, and growth rate in domestic pigs (*Sus scrofa domesticus*) [44,45], as well as between mtDNA haplotype and fertility in captive populations of male European brown hares (*Lepus europaeus*) [46]. Together, this research suggests that within-population variation in mtDNA sequence may have substantial consequences for individual life history.

Interactions between nuclear and mitochondrial genes in energy metabolism

Aerobic metabolism is also dependent on genes found within the nuclear genome. Indeed, approximately 80 of the nuclear-encoded proteins that are transported into the mitochondria contribute subunits of the respiratory enzymes that comprise OXPHOS [3,23]. Organismal energy production is, thus, dependent on the coordinated interaction between mitochondrial and nuclear genes found within eukaryotic cells [3,23,47]. Indeed, much research has now reported that the effects of mtDNA variation on mitochondrial function are dependent on the particular nuclear genotype with which the mtDNA variation coexists [4,23,47]. Incompatibilities between the mitochondrial and nuclear genotypes can negatively affect the function of mitochondrial enzyme complexes [48,49] and in some cases, even single point mutations between interacting loci in the mitochondrial and nuclear genomes may be sufficient to compromise energy production [49]. The functional consequences of these **mito-nuclear interactions** are thought to result in the coevolution of mitochondrial and nuclear genomes, with selection acting to maintain compatible genotype combinations within populations [23,47]. Thus, the studies conducted to date suggest that nuclear genetic variants may interact with mtDNA haplotypes to produce individual differences in mitochondrial function within populations.

How is functional mtDNA variation maintained within populations?

Evolutionary theorists have puzzled over the conditions by which functional variation in the mitochondrial genome may be maintained within populations, and numerous studies have modelled the conditions under which a joint polymorphism in the mtDNA and nuclear genome can be upheld via mito-nuclear fitness interactions [50–53]. While these theoretical studies have found that the conditions promoting a stable polymorphism are generally narrow (restricted to cases of strong differential selection across life stage or sexes, or placement of the interacting nuclear loci on the X chromosome [50–53]), some empirical studies screening for mito-nuclear fitness interactions within populations have reported that mtDNA haplotypes segregating naturally (and at intermediate frequencies) in these populations may interact epistatically with different nuclear backgrounds to shape fitness related traits (reviewed in [10]). This empirical research thus suggests that epistatic interactions between the mitochondrial and nuclear genomes may contribute to the maintenance of functional variation in the mtDNA sequence (and at the interacting nuclear loci) within populations [10].

fitness advantage over more common genotypes.
Pace-of-life syndromes: coevolved suites of life history, physiological, and behavioural traits within populations (e.g., more active individuals with higher metabolisms and faster growth rates) due to trade-offs in resource allocation to either current or future reproduction.
Purifying selection: the removal of deleterious DNA mutations at loci under selection, resulting in reduced genetic diversity and the elimination of harmful variants from a population.

Moreover, recent research has suggested that mtDNA polymorphisms may be maintained within populations under **negative frequency-dependent selection**. Studies leveraging experimental evolution in *D. subobscura* fruit flies and *Callosobruchus maculatus* beetles observed that the fitness of competing mtDNA haplotypes within laboratory populations was inversely associated with the relative frequencies of each haplotype [54–56]. Further, the thermal environment has been linked to the fitness of mtDNA haplotypes in both *D. melanogaster* and *C. maculatus*, suggesting that spatio-temporal variation in environmental conditions may also act to maintain mtDNA variation within populations [36,42,57–59].

Collectively, the research presented earlier highlights that mtDNA variation can be maintained within populations, and that this variation can have functional consequences for organismal energy production. When segregating at intermediate frequencies, even relatively small amounts of within-population variation in the mtDNA sequence may interact with both nuclear genetic variants and environmental conditions to influence the expression of individual-to-individual differences in physiology and life history traits.

Mitochondrial genetic variation and the expression of behavioural diversity

Linking variation in behaviour, physiology, and life history

Research spanning diverse taxa has found that individuals often differ from one another in their behaviour, with some individuals, for example, being more active or risk-prone than other conspecifics within the population [60,61]. These consistent individual differences in behaviour (often termed behavioural types or personality traits) have important evolutionary consequences, as they are usually heritable [62] and influence survival [63] and reproductive success [64,65]. Further, individual behavioural differences are thought to affect key ecological process such as the distribution, persistence, and productivity of populations, dispersal dynamics, and even disease transmission [66]. Thus, understanding the factors that contribute to the expression of consistent individual-to-individual behavioural differences has important implications for species ecology and evolution.

The expression of individual-to-individual behavioural differences within populations is thought to be, in part, driven by life-history trade-offs in resource allocation to either current or future reproduction [11,61]. These trade-offs may generate variation between individuals in their energy requirements, and thus subsequent resource acquisition and behaviour, ultimately resulting in the coevolution of suites of life-history, physiological, and behavioural traits (i.e., **pace-of-life syndromes**) [11,13,61]. While empirical support for pace-of-life syndromes has been mixed [67], recent meta-analyses have found that the behaviour expressed by an individual often covaries (albeit only moderately) with its metabolic rate [12,68], as well as body mass and body size [68]. Studies across a variety of species have also demonstrated correlations between individuals in their behaviour and life-history [69–71]. While the presence and magnitude of these associations may differ between species and depend on the specific behavioural and physiological/life-history traits measured [12,72], it is clear that individual variation in energy production and allocation partly contribute to the expression of individual-to-individual behavioural differences.

Importantly, mtDNA variation and mito-nuclear interactions may influence these life-history trade-offs and pace-of-life syndromes, with potential consequences for individual-to-individual behavioural differences. For example, accumulating evidence suggests that mtDNA variation may have negative pleiotropic effects on the phenotype, with some mtDNA haplotypes increasing performance of one trait at the expense of another [16,35]. Indeed, mtDNA haplotypes that confer increased metabolic rate in male *D. melanogaster* are associated with reduced longevity [35]. These effects may also differ across males and females, with recent examples suggesting that

mtDNA haplotypes that increase the performance of a trait in one sex may decrease trait performance in the opposite sex (i.e., intersexual pleiotropy) [35,73]. Thus, mtDNA haplotypes likely play a role in mediating life-history trade-offs and subsequent pace-of-life syndromes, suggesting that mtDNA variation within populations may influence the expression of individual-to-individual behavioural differences (Figure 2).

Behaviour and mtDNA: understanding how mtDNA haplotypes generate intraspecific behavioural diversity

Given that mtDNA variation and mito-nuclear interactions can mediate organismal physiology and life-history, and that variation in these traits is thought to influence behaviour, it is not surprising that mtDNA haplotypes have been shown to influence intraspecific behavioural diversity [74–77]. Using strains of seed beetles (*C. maculatus*), in which mtDNA haplotypes of three allopatric populations had been placed against target nuclear genotypes from the same populations to create nine distinct combinations of mitochondrial and nuclear genotypes, Løvlie *et al.* found that mito-nuclear interactions shaped activity levels among experimental populations [77]. Individual seed beetle activity was also associated with both body mass and lifespan among experimental populations, and the relationship between these traits across individual beetles was influenced by their combined mito-nuclear genotype [77]. Similarly, the relationship between behaviour and basal metabolic rate was linked to mtDNA haplotype in several bank vole (*Myodes glareolus*) populations, and this effect varied between the sexes [78]. While research in laboratory mice identified little evidence for an effect of mtDNA haplotype on wheel-running behaviour [79], previous work in *D. melanogaster* found a substantial influence of mtDNA variation on locomotor activity [75,76], with one study reporting that mtDNA haplotype explained ~20% of the variance in activity levels among experimental populations [76].

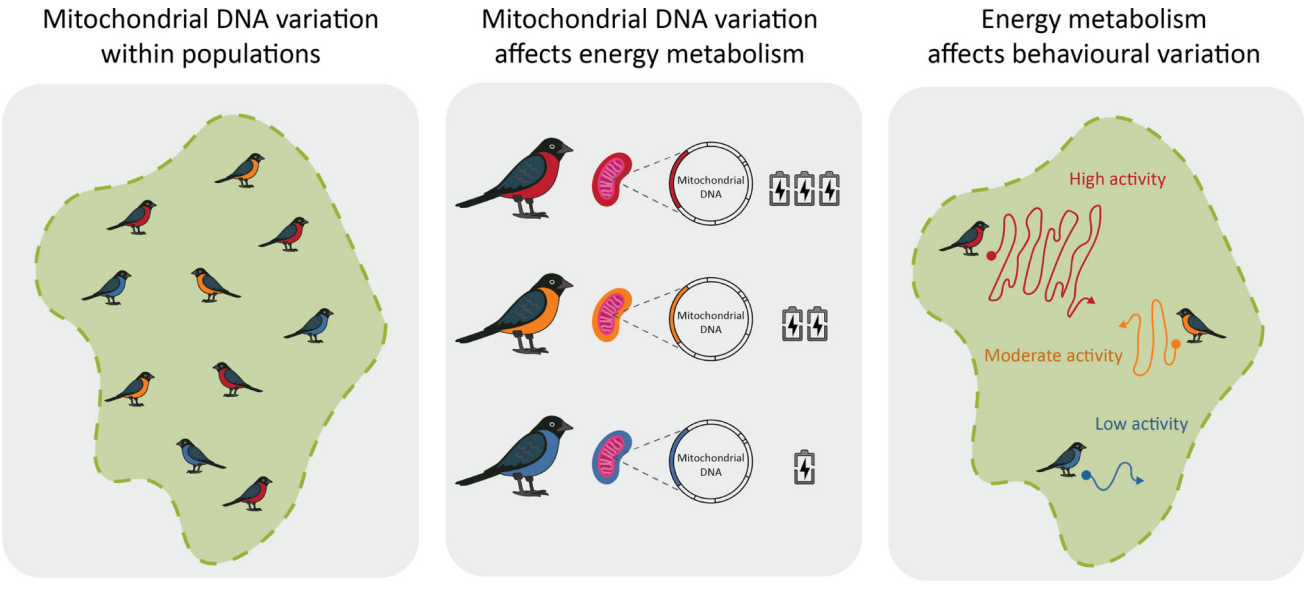

Figure 2. Mitochondrial genetic variation can influence intraspecific behavioural diversity. Simplified illustration of how mitochondrial DNA (mtDNA) haplotype variation within a hypothetical population of birds may influence energy metabolism, and subsequent individual-to-individual differences in activity rates. Birds with different plumage colours (i.e., red, orange, and blue) are used here as a visual illustration of mtDNA haplotype variation within the population (i.e., but this is not intended to suggest that mtDNA haplotypes influences plumage colour polymorphism). We also note that, while not included here, for simplicity, the effects of mtDNA variation on energy metabolism are often mediated by epistatic interactions with nuclear genetic variation. These epistatic interactions, along with negative-frequency dependent selection and environmental variation, may maintain mtDNA haplotype variation within populations.

Moreover, mtDNA haplotype variation has recently been linked to individual-to-individual behavioural differences within populations; Ueno and Takahashi described two broad haplotype groups coexisting within four populations of *Drosophila immigrans* flies [80]. They collated a set of **isofemale lines**, and measured each for locomotor activity, reporting an association between haplotype groups and locomotor activity across the lines [80]. While the approach used in the study was unable to control for possible confounding covariation in nuclear genes across the lines, the results suggest a possible link between within-population mtDNA variation and individual-to-individual differences in behaviour.

Furthermore, feedback loops between an individual's intrinsic state (e.g., body condition, metabolic rate, residual reproductive value etc.) and their behaviour are also thought to mediate individual-to-individual behavioural differences within populations [81]. For example, high condition individuals with greater energy reserves may be able to afford to take greater risks when foraging, thereby further increasing their body condition (and vice versa for low condition individuals [81]). Mitochondrial genomes may influence the expression of individual-to-individual behavioural differences via these state-behaviour feedbacks when mtDNA haplotypes and mito-nuclear interactions influence mitochondrial function and subsequent physiological and life-history traits. However, these suggestions require future testing (Box 1).

Taken together, this research highlights a role for mtDNA haplotypes in generating behavioural differences among populations. Moreover, mtDNA variation and mito-nuclear interactions may also generate within-population variation in physiology and life-history traits (e.g., [41]), with preliminary evidence suggesting that such effects may contribute to the expression of individual-to-individual differences in behaviour (e.g., [76,80]). However, further work which appropriately partitions behavioural variation among-individuals within populations, and tests for covariance with mtDNA haplotype, will be needed to clarify these links (see 'Future directions' section).

Mother's curse: maternal inheritance of mitochondria and sex differences in the expression of behaviour

Maternal inheritance of mtDNA and the sex-specific expression of behaviour

Mitochondria (and therefore mitochondrial genomes) are strictly maternally inherited in the majority of animal taxa [82,83]. This has evolutionary implications for the accumulation of functional mtDNA variation within species [82–85]. In theory, maternal transmission of mitochondria results in a 'sex-specific selective sieve', whereby evolutionary responses to selection on mtDNA will proceed only through females (with exception of species in which males affect the fitness of their sisters through kin selection or inbreeding [86]). Selection is, therefore, effectively blind to mtDNA variants that are deleterious in males, when these same variants are neutral or nearly neutral in females. This selective sieve should lead to the accumulation of male-biased mutation loads in the mitochondrial genome (i.e., '**mother's curse**' [83,85,87,88]).

Experimental support for the existence of male-biased mutation loads in the mitochondrial genome has emerged through a number of studies [35,82,87–89]. Research in *D. melanogaster* has uncovered male biases in the effect of mtDNA haplotype variation on trait expression, consistent with the premise that these haplotypes harbour mutation loads conferring male-biased effects [35,87–89]. For example, effects of mtDNA haplotype on wing size, longevity, aging, and metabolic rate were larger in male *D. melanogaster*, relative to their female counterparts [35,87,89]. The accumulation of male-biased mutation loads is likely to contribute to sexual dimorphism in behaviour, with mtDNA haplotypes linked to sex differences in the expression of behaviour in males and females [76,78,80]. Further, increased functional mtDNA variation in males

Box 1. State-behaviour feedbacks and mtDNA variation

Dynamic feedback loops between an individual's intrinsic state (e.g., body condition, metabolic rate, residual reproductive value etc.) and their behaviour are thought to influence behavioural differences between individuals [81]. We contend that mitochondrial haplotypes may play a part in facilitating these state-behaviour feedbacks due to the potential role of mtDNA in influencing mitochondrial structure and function, as well as mtDNA copy number within cells [16,99]. Mitochondria are known to plastically alter their structure, position, and number within cells to facilitate ATP production in energetically demanding tissues [100]. For example, the proportion of inter-membrane junctions between adjacent mitochondria increases in skeletal muscle following exercise [101], and changes in inner mitochondrial membrane structure can influence energy production [102]. Previous research in European beewolf wasps (*Philanthus triangulum,* Figure I) has demonstrated that females whose flight muscles had a higher inner mitochondrial membrane density were more efficient at provisioning their offspring [103], suggesting that variation in mitochondrial structure and position may influence animal performance [100]. Such associations may result in feedback loops, whereby changes to mitochondrial structure and density in individuals with high foraging activity for example, may facilitate increased energy production, leading to subsequent high activity rates and greater resource acquisition. The production of ROS during OXPHOS may also influence feedback loops, due to their role in cellular signalling and oxidative stress [38,39]. Thus, to the extent that mitochondrial function and density, as well as ROS production, are mediated by mtDNA haplotype variation, mtDNA has the potential to influence individual-to-individual behavioural differences though state-behaviour feedback loops. Future work using distinct mtDNA haplotypes will be needed to better understand whether mtDNA variation can influence state-behaviour associations.

Figure I. European beewolf (*Philanthus triangulum*). Photo credit: Shutterstock/Keith Hider.

may also result in the expression of greater individual-to-individual behavioural differences, when compared with females. However, this suggestion warrants further empirical research.

It is also worth highlighting that mtDNA haplotypes that are explicitly sexually antagonistic (e.g., benefiting females while harming males) can be maintained within populations and may even become fixed under positive selection [90]. Indeed, recent evidence has found that mtDNA haplotypes that increase metabolic rate [35] or reproductive traits [73] in female *D. melanogaster* are associated with decreased trait expression in male conspecifics. These findings further suggest

that mtDNA variation may result in sex differences in physiological and life history traits, with potential consequences for sexual dimorphism in behaviour within populations.

Implications of mother's curse for mitochondrial introgression

The accumulation of male-harming mutations in mtDNA has been hypothesized to place the nuclear genome under strong selective pressure for compensatory adaptations that rescue depressed male function [23,47]. The consequent co-evolution of nuclear and mitochondrial genomes is predicted to then lead to sex-specific fitness consequences when these co-adapted states are disrupted, whereby males will be the sex that experiences the greatest fitness depression upon disruption [82]. Indeed, laboratory research using breeding lines that place different mtDNA haplotypes into separate nuclear backgrounds has shown that breaking apart coevolved mito-nuclear genomes often has distinct effects in males and females, although not always in the direction predicted by the 'Mother's curse' [76,89,91]. The sex-specific effects of mito-nuclear disruption may be especially pertinent during periods of **genetic admixture** and hybridization between previously isolated populations (e.g., biological invasions and secondary contact). Here, mtDNA haplotypes may be expressed without their coevolved nuclear compensatory functions, potentially resulting in disproportionately large and negative effects on male performance when male-harming mtDNA mutations are exposed during **mitochondrial introgression** [92]. Indeed, analysis of foraging performance in long-toed salamanders (*Ambystoma macrodactylum*) from a secondary contact zone between two distinct genetic lineages revealed that lineage-mismatched combinations of mitochondrial and nuclear genotypes (mito-nuclear mismatch) negatively affected feeding behaviour in males, but not females [93]. Further, mito-nuclear mismatch also reduced fecundity, development rate, and running speed in both male and female leaf beetles (*Chrysomela aeneicollis*) from a naturally occurring admixed population, suggesting that mitochondrial introgression can also negatively affect the behaviour and life-history of both males and females alike [94]. How mitochondrial introgression in natural systems influences the expression of individual-to-individual behavioural differences, and how this differs between males and females remains largely unknown and demands further attention. To this end, biological invasions – where previously isolated populations have been introduced into a new range – could serve as useful model systems (Box 2).

Box 2. Invasive species as a model for understanding the behavioural consequences of mitochondrial introgression

Much research has demonstrated the potential negative effects of mito-nuclear incompatibilities on organismal performance [23,47] and outlined why such effects are predicted to be more pronounced in males [82,83,89]. However, many of the existing studies have used artificially created laboratory lines that combine distinct mito-nuclear genotypes [34,77,89,91,104], with research on natural systems undergoing mitochondrial introgression in the wild less common (but see [92]). How mitochondrial introgression in natural systems affects behavioural diversity is, thus, unclear. Invasive species may be a promising model in addressing these research questions (e.g., [105]). As an example, delicate skinks (*Lampropholis delicata*) have recently been introduced (~ 1980s) into Lord Howe Island (Australia) [106]. The invasive populations are descended from four different native-range clades in Australia and contain seven distinct mtDNA haplotypes (Figure I [106,107]). These seven haplotypes differ in the sequence of their *ND2* and *ND4* mitochondrial protein-coding genes (maximum sequence divergence between haplotypes is 8.3% [106,107]), which both play a key role in OXPHOS. Despite the island's small size (~14.55 km^2), the haplotypes are spatially structured, with some invasive populations containing only a single mtDNA haplotype (i.e., no genetic admixture), while other populations contain up to four distinct haplotypes (i.e., genetic admixture) [106]. Interestingly, male skinks from admixed populations display marginally increased within-population variation in sprint speed compared with both non-admixed invasive populations and native-range conspecifics [108]. Similarly, while male skinks from the non-admixed populations increased their average sprint speed following introduction, this was not seen in conspecifics from admixed populations [108]. Population differences in nuclear genetic variance likely contribute to these effects. However, whether the mtDNA haplotypes differ in their bioenergetics, and whether mito-nuclear interactions play a part in the observed differences in male behavioural diversity in invasive populations of this species, or any other, is yet to be resolved.

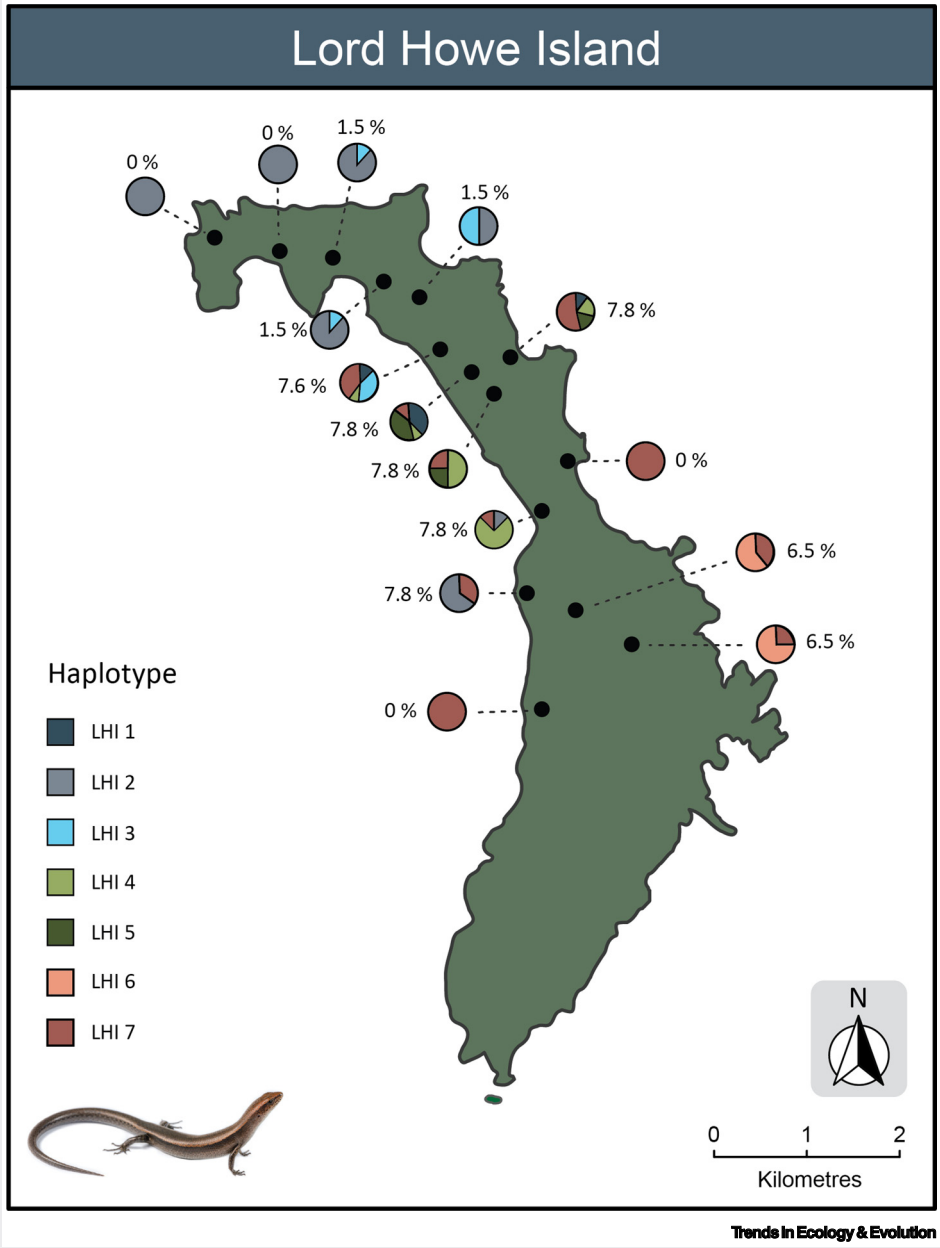

Figure I. Schematic diagram of the distribution and relative abundance of mitochondrial haplotypes among invasive delicate skink (*Lampropholis delicata*) populations on Lord Howe Island, adapted with permission from [106,109]. Percentages show the maximum sequence divergence among mitochondrial haplotypes present in each invasive population (8.3% sequence divergence across all Lord Howe Island populations). Relative mitochondrial haplotype frequencies indicated in pie charts are presented for illustrative purposes (see [106,107] for description of haplotype frequencies). Photo insert credit: Jules Farquhar.

Future directions

It is clear that mtDNA variation and mito-nuclear interactions within species can affect organismal performance [23,40,47]. The majority of previous studies have focused on quantifying how mito-

nuclear interactions mediate physiological and life-history trait expression [2,26,32,34,35,41,87]. While headway has been made into understanding how such effects influence intraspecific behavioural diversity [75–78,80], integration of mitochondrial evolutionary genetics into animal behaviour research is still lacking. Moreover, the work that has been done has largely focussed on the role of mtDNA variation in generating behavioural differences among-populations. More research investigating links between individual differences in ecologically relevant behavioural traits and levels of mtDNA variation that have been observed to segregate within populations is needed (Box 3). Further, the relative contribution of additive mtDNA effects and mitonuclear epistasis in contributing to individual differences in behavioural expression is currently not clear. This knowledge gap arises from the limitations of previous studies in this field that have typically placed mtDNA haplotypes collected from divergent lineages/populations alongside two or three highly controlled (inbred) nuclear backgrounds, observing some level of functional epistasis. Whether functional epistasis in this context rules out the possibility for additive mitochondrial effects when measured at a population-level (across all of the nuclear genetic alleles that segregate within a population) remains largely unaddressed. In addition, understanding the potential contribution of mtDNA haplotype variation to state-behaviour feedback loops in generating individual-to-individual behavioural differences will also be a promising topic for further research.

Moreover, much research has found that the influence of mtDNA variation and mito-nuclear interactions is often context-dependent. Indeed, the effect of mtDNA variation on performance can be altered by life-stage [26,95], diet [31,96], temperature [95,97], and social context [75], suggesting that mitochondrial genetic variation may be routinely sensitive to mtDNA × environment (G × E) or even complex mtDNA × nuclear DNA × environment (i.e., G × G × E) interactions. Understanding the

Box 3. Translating theory into practice: next steps

Testing many of the ideas laid out in this paper will require new research efforts. The increased availability of genomic sequence data in combination with greater cross-disciplinary training and collaboration has endowed behavioural ecologists with improved molecular and bioinformatic literacy, emphasizing the feasibility of integrating genomic technologies into behavioural research to understand the role of mitochondrial genetics in animal behaviour. Indeed, datasets are likely to already exist for well-studied, pedigreed populations where whole DNA sequences of individuals within the pedigree are available and behavioural traits also scored for the same individuals. For example, European populations of great tits (*Parus major major*; Figure I) have been extensively studied both in the wild and in the laboratory over the past several decades to investigate the underlying genetics of personality traits [110–112]. Individual differences in exploratory behaviour are thought to be heritable in these populations and maintained via spatio-temporal heterogenous selection [113,114]. Recently, high-density SNP microarrays with around 500 000 SNPs have become available in this species [111]. Using these techniques, researchers have identified an estimated 3253 [95% confidence interval (CI) = 315–8499] SNPs as potentially explaining some variation in exploratory behaviour [111]. Interesting, ~46 different mtDNA haplotypes have also been identified within European great tit populations [115], but whether these haplotypes contribute to behavioural expression is not clear.

Leveraging already-existing sequence data from these populations to screen for mtDNA variation would provide a valuable first step in understanding associations between mtDNA haplotypes and behavioural expression. Further, where populations have good pedigree information and maternal lineages are well-known, the maternal inheritance of mtDNA means that not all individuals within the population necessarily need to be sequenced. Here, the combination of pedigree information and mtDNA haplotype data from known maternal lineages may facilitate quantitative genetic approaches that can accurately partition the contribution of mtDNA haplotypes to the expression of individual-to-individual behavioural differences. Investigating interactions between maternal mtDNA haplotype and paternal (i.e., sire) identity in these quantitative genetic models could provide key information on how mito-nuclear interactions and incompatibilities between mitochondrial and nuclear genotypes may influence behavioural expression, and whether such effects are sex-specific. Laboratory studies may also further help to uncover whether mtDNA haplotypes present in these populations affect mitochondrial bioenergetics, whole-organism physiology, and/or ROS production. Such research will provide important information about the relative importance of additive mtDNA variation and epistatic interactions between the mitochondrial and nuclear genomes in contributing to the expression of individual-to-individual behavioural differences within-populations. Last, studies in non-model species that allow the establishment of quantitative genetic breeding designs and the use of similar approaches to those used in *Drosophila* flies and *Callosobruchus* beetles (e.g., the creation of novel genetic strains that mix different mtDNA and nuclear genotypes) would be useful additions for understanding the ubiquity of mtDNA effects and mito-nuclear interactions in mediating within-population behavioural variation.

Figure I. European great tit (*Parus major major*). Photo credit: Shutterstock/Ondrej Prosicky.

conditions in which mtDNA variation and epistatic interactions between mitochondrial and nuclear genomes influence the expression of behavioural diversity will be a key area for future research.

Finally, different mtDNA haplotypes can exist within the same individual (termed heteroplasmy) and these haplotypes can be maintained within individuals through tissue-specific selection [98]. A growing body of evidence has shown that heteroplasmy can have substantial consequences for organismal health and performance [10]. How heteroplasmy influences behaviour and whether variation in heteroplasmy across individuals affects the expression of individual-to-individual behavioural differences is not clear and will require further work.

Concluding remarks

The mitochondrial genome plays a central role in energy production and metabolism, which in turn influences organismal life history and behaviour. Despite this, the mitochondrial genome has largely been neglected in behavioural ecology research, with only a relatively small number of studies investigating how mtDNA haplotype variation influences intraspecific behavioural diversity. While the contribution of the nuclear genome to animal behaviour is now well established, a greater focus on the role of mtDNA haplotype variation and mito-nuclear interactions in the expression of behavioural variation may be key to fully understanding the evolutionary genetics of animal behaviour (see Outstanding questions).

Outstanding questions

How do additive and epistatic mitochondrial genotypic contributions influence individual-to-individual behavioural differences within populations, and does this differ between males and females?

Does mtDNA haplotype variation and mito-nuclear interactions within populations maintain individual-to-individual behavioural differences via state-behaviour feedback loops?

Under what environmental conditions do mtDNA haplotypes and mito-nuclear interactions influence the expression of intraspecific behavioural diversity?

Do mtDNA haplotypes and mito-nuclear interactions influence an individual's capacity to respond to environmental change (i.e., within-individual behavioural plasticity)?

How does mitochondrial introgression in natural systems influence the expression of behavioural traits, and are the effects different in males and females?

Mitochondria also play important roles in growth and development, apoptosis, and intra/inter-cellular signalling (among other functions). What range of mitochondrial functions does mtDNA variation influence, and how does this affect behavioural expression?

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Declaration of interests

We have no competing interests to declare.

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