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Maternal transmission, sex ratio distortion, and mitochondria

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Abstract – In virtually all multicellular eukaryotes, mitochondria are transmitted exclusively through one parent, usually the mother. In this short review, we discuss some of the major consequences of uniparental transmission of mitochondria, including deleterious effects in males, and selection for increased transmission through females. Many of these consequences, particularly sex ratio distortion, have well-studied parallels in other maternally transmitted genetic elements, such as bacterial endosymbionts of arthropods. We also discuss the consequences of linkage between mitochondria and other maternally transmitted genetic elements, including the role of cytonuclear incompatibilities in maintaining polymorphism. Finally, as a case study, we discuss a recently discovered maternally transmitted sex ratio distortion in an insect that is associated with extraordinarily divergent mitochondria.

Keywords – cytoplasmic male sterility, doubly uniparental inheritance, reproductive parasitism, symbiosis, *Wolbachia*

/Body

By virtue of their symbiotic origin, mitochondria are special (1). They have retained their own genome (with a few interesting exceptions), despite the fact that the vast majority of mitochondrial proteins are encoded in the much larger nuclear genome. A functioning organelle thus requires the tight regulation and coordination of two genomes with very different properties, histories, and locations. In addition, mitochondrial genomes reproduce asexually and are cytoplasmically inherited, typically through one sex – usually the female. This mode of transmission differs from most nuclear genomes, and has important consequences on an organism's fitness. There have been many excellent reviews on the different evolutionary trajectories of mitochondrial and nuclear genomes, including how these can result in genetic conflicts and incompatibility (e.g. 2-10). In this short review, we focus on the relationship between mitochondria and sex ratio distortion. We discuss how maternal transmission can drive the evolution of mitochondria (and other symbionts) that increase the frequency of females. We also consider how linkage between mitochondria and other maternally transmitted genetic elements, such as sex ratio distorters, can result in cytonuclear incompatibilities that may ultimately affect the persistence of the distorter. Finally, as a case study, we discuss a recently discovered maternally transmitted sex ratio distortion in a booklouse that is associated with extraordinarily divergent mitochondria.

In almost all multicellular eukaryotes, as well as many unicellular ones (i.e. microbial eukaryotes), transmission of mitochondria is strictly uniparental (4, 11). This is not just a consequence of eggs being much larger than sperm, as mitochondria are transmitted exclusively via males in some lineages, such as many conifer species (12). Organisms have independently evolved diverse, sophisticated strategies to target and destroy mitochondria from the opposite sex, even in species with sperm containing very few mitochondria (11, 13, 14). This is best explained as a mechanism of control by the host, to reduce conflict and to prevent the spread of selfish mitochondria (5, 15). Uniparental inheritance prevents mixing of different cytoplasmic lineages, and is thus expected to reduce competitive interactions between mitochondrial variants. Even with strict uniparental transmission, many generations of asexual reproduction within a host may

allow mitochondrial genomes that have a replication advantage but that are ultimately deleterious to increase in frequency. In general, the frequency and fitness consequences of such selfish mitochondria have been little studied, although these have been documented in diverse organisms, including nematodes and yeast (16, 17). In humans, there are many documented cases of mitochondrial genomes with deleterious mutations reaching high frequencies within an individual, with negative health consequences (18).

Uniparental transmission means that one sex is an evolutionary dead end, and this plays a major role in shaping the evolutionary trajectory of mitochondria (19, 20). For most multicellular organisms, transmission of mitochondria is maternal, and we focus the rest of our discussion on this mode of inheritance. First, the combination of asexual reproduction and small, serially bottlenecked populations has resulted in the persistent accumulation of slightly deleterious mutations in mitochondria (21). This pattern has been observed across a wide range of organisms, and in addition to mitochondria, we also see it in maternally transmitted microbial endosymbionts (22). This phenomenon is exacerbated in the non-transmitting sex: a major consequence of maternal transmission of mitochondria is that mutations that are deleterious in males can reach high frequencies if they are neutral (or advantageous, or even slightly deleterious) in females (20, 23; Figure 1a). This helps explain why male infertility in humans is commonly due to mitochondrial mutations (24, 25). A recent beautiful study in *Drosophila melanogaster* fruit flies demonstrated the pervasive effects of this hidden mitochondrial variation on male fitness (26). The authors established fly lines with different mitochondrial genomes but the same nuclear genetic background. Despite the fact that these flies differed only with respect to their tiny mitochondrial genomes, there were large fitness consequences, but only in males. One mitonuclear combination resulted in male sterility, and in all combinations gene expression in males (but not females) was radically altered, with over a thousand nuclear genes affected, especially in male-specific tissues.

Another striking consequence of maternal transmission is sex ratio distortion (Figure 1b). Maternally transmitted lineages that increase the frequency of females will be favoured by selection; this may result in conflicts between cytoplasmic and nuclear genes over

optimal offspring sex ratios (27, 28). Female-biased sex distortion is best known in maternally transmitted microbial symbionts of arthropods (18, 29, 30), in which at least five different lineages of intracellular bacteria, such as *Wolbachia*, *Rickettsia* and *Spiroplasma* (31-33), and one lineage of intracellular microbial eukaryote, *Microsporidia* (34), have independently evolved the ability to manipulate reproduction in a wide range of hosts. These symbionts distort sex ratios in three sophisticated ways: by causing infected females to reproduce asexually (parthenogenesis-induction), by transforming infected males into females (feminization), or by killing the sons of infected females early in development (male-killing). These strategies have different predicted equilibrium frequencies and evolutionary outcomes. For example, symbionts that induce parthenogenesis are more likely to become fixed in a population, since males are no longer required for reproduction. Although there has been much recent work on reproductive manipulators, we are probably only at the tip of the iceberg in describing the diversity of manipulators, since only terrestrial arthropods have been surveyed in any detail. Interestingly, many strategies that have been predicted to occur (19, 35), such as symbionts that distort sex ratios by preventing fertilization of Y chromosome-bearing sperm, have not yet been discovered.

What about sex ratio distortion by organelles? As far as we are aware, there are no known cases of distortion by plastids (and there has been relatively little work on consequences of uniparental transmission and sex-specific effects of plastids). On the other hand, mitochondria that distort sex ratios are well known – this is extremely common in plants, in a phenomenon called cytoplasmic male sterility (5, 36). Cytoplasmic male sterility has evolved independently hundreds of times in hermaphroditic plant species, and in many different ways, from causing sterile or inviable pollen, to preventing the proper development of male reproductive organs. This results in an individual that is female. Cytoplasmic male sterility has been studied in great detail, in part because of its agricultural importance as an effective tool to prevent selfing. Two additional features of cytoplasmic male sterility stand out. First, its genetic basis is striking, as it typically involves the evolution of novel mitochondrial genes (5, 36. 37), as opposed to accumulation of sex-specific deleterious mutations in genes that are already present,

although the novel genes have often incorporated truncations and fusions of other mitochondrial genes. Second, cytoplasmic male sterility has repeatedly been followed by the evolution of nuclear genes that suppress male sterility. In many cases, both sterility and suppressor genes become fixed in a population, such that sterility is only uncovered through genetic crosses between different populations. There is also evidence for evolutionary arms races between sterilizing and suppressing genes (38), highlighting the importance of conflict in shaping the evolution of sex ratio distortion. Although the genetic basis of a number of cytoplasmic male sterility and nuclear suppressor systems is now known, the mechanisms involved are still poorly understood.

Why are there no known cases of mitochondrial sex ratio distortion in animals, or for that matter, in any organisms other than plants? Another way of asking this question is whether there is something special about plants and their mitochondria. Plant mitochondrial genomes are incredibly dynamic (39-41) and have a great propensity for horizontal transfer and acquisition of novel genes, as seen in the many different ways cytoplasmic male sterility has evolved. Indeed, the first documented case of lateral gene transfer in eukaryotes that did not involve mobile genetic elements was in plant mitochondria (42). This was recently shown to be taken to an extreme degree in *Amborella trichopoda*, whose enormous ~4 Mb mitochondrial genome has acquired the equivalent of six foreign mitochondrial genomes from algae, mosses, and other flowering plants (43). Another recent study showed that some mitochondrial tRNAs in liverworts were likely acquired from *Chlamydia* (44). Yet horizontal transfer in mitochondrial genomes is not unique to plants, and has been reported in diverse lineages, including fungi, sponges, and corals, often associated with mobile introns (45-50). We would not be surprised if many more cases of mitochondrial horizontal transfer will be reported, including transfers from intracellular microbial endosymbionts, which include many known sex ratio distorters, occur in high numbers within cells and in close proximity to mitochondria, and are common sources of transfer to nuclear genomes (51). One lineage of endosymbionts in ticks, *Candidatus* Midichloria mitochondrii, is even known to reside within mitochondria (52). In sum, we do not see any clear reason why we should not find mitochondrial distortion in lineages other than plants. Since cytoplasmic male sterility in

plants is always found in association with hermaphroditism, perhaps a useful place to start to look would be in lineages that contain hermaphrodites.

One especially promising lineage to study mitochondrial involvement in sex distortion is that of some bivalves. These are the only animals that are known to deviate from uniparental transmission of mitochondria (53, 54). Some bivalves have two distinct types of mitochondria. One type is transmitted from mothers to all their offspring (sons and daughters), while the other is transmitted exclusively from fathers to sons. This unusual mode of mitochondrial transmission is called doubly uniparental inheritance and it has been speculated that it evolved from paternal mitochondria escaping targeted destruction by the host (55). One fascinating consequence of doubly uniparental inheritance is that the two types of mitochondria are very different – they have different dimensions, tissue distributions, and are highly divergent at the sequence level. Strikingly, both mitochondrial types have acquired new genes (55-57), confirming that animal mitochondrial genomes are capable of evolving novelty. Little is known about the function of these novel genes, although recent studies have shown that they are transcribed and translated into proteins (58). Although they have no clear homologs, it has been suggested that at least one of the novel genes may have a viral origin (55, 57, 58). Understanding the function of these novel genes will not only gain insight into the mechanism of doubly uniparental inheritance, but it may also shed light on how sex itself is determined in bivalves, as this is not yet known. Interestingly, it has been speculated that mitochondria themselves might determine sex, as only males contain male-specific mitochondria and their unique genes (56). Finally, unusual sex ratio distortion has been documented in *Mytilus* mussels and *Ruditapes* clams (57, 59, 60), with individuals from the same population producing female-biased, male-biased, or 50:50 sex ratios. In *Mytilus*, female bias appears to be under maternal nuclear control. Sex ratio distortion in bivalves is an intriguing system to look for antagonistic interactions between distorting mitochondria and nuclear suppressors, similar to cytoplasmic male sterility in plants.

Another consequence of maternal transmission is that all genetic entities that are exclusively maternally transmitted, such as organelles, endosymbionts, and female-

184 limited (W) sex chromosomes, are in perfect linkage (8; Figure 1c). As a result, their
185 evolutionary fates are bound together. This has been studied in great detail in symbiont-
186 mitochondria associations (61, 62), particularly with respect to the population genetic
187 consequences of co-transmission; in contrast, there have been few studies on W
188 chromosome-mitochondria associations (63), probably because until recently there have
189 been few available W chromosome markers. Mitochondrial markers are often used to
190 track and to age maternally transmitted microbial symbiont infections, including sex ratio
191 distorters. Many studies have shown that symbionts decrease mitochondrial genetic
192 variation as they spread through the population, replacing uninfected individuals with
193 infected ones, along with their associated mitochondrial genome (61, 64-66). At the same
194 time, the effective population size of mitochondria in the remaining uninfected
195 individuals will be greatly reduced, further affecting variation. This phenomenon is
196 especially common in symbionts that cause cytoplasmic incompatibility, in which
197 uninfected females produce few or no offspring when they mate with infected males. As
198 a result, infected females are at a reproductive advantage over their uninfected
199 counterparts and rapidly replace them (67), purging mitochondrial variation.
200
201 On the other hand, it has also been shown that mitochondrial polymorphisms can persist
202 in a population due to linkage with inherited symbionts (62, 68). For example, the
203 ladybird beetle *Adalia bipunctata* is polymorphic both for mitochondrial haplotypes and
204 at least two strains of male-killing *Rickettsia* (62). The deeply divergent mitochondria
205 suggest that these male-killer infections are old, but it is not known how or why both the
206 male-killers (and their mitochondrial partners) have persisted. In some cases, inherited
207 symbionts and their associated mitochondrial partner have been introduced into a new
208 host via hybridization. For example, the fly *Drosophila quinaria* harbours two extremely
209 divergent mitochondria (69). One is perfectly linked with infection with a strain of the
210 symbiont *Wolbachia* (whose effect on its host is not known, but it does not appear to
211 cause cytoplasmic incompatibility or sex ratio distortion), and it is suggested that this
212 mitochondrial haplotype actually came from a now extinct species that was the original
213 host for this *Wolbachia*.
214

What are the functional consequences of mitochondrial polymorphism and linkage? A number of studies have begun to examine functional differences between mitochondrial variants, with consequences on host fitness. For example, a recent study in the neotropical pseudoscorpion *Cordylochernes scorpioides* found that trade-offs explained the persistence of two divergent mitochondrial genomes; while males carrying one of these genomes had higher sperm competitive ability, females with this mitochondrial genome had reduced sexual receptivity (70). In warblers, hybridization has resulted in the introgression of a mitochondrial variant that is associated with differences in flight efficiency and migratory potential (71). Little work has been done on functional consequences of linkage between mitochondria and symbionts, and how a symbiont's persistence and spread depend on its mitochondrial partner (and vice versa) is generally not known. Perhaps the most detailed work has been in *Drosophila simulans*, which segregates numerous mitochondrial haplotypes with different respiration efficiencies, and that are linked to different strains of *Wolbachia* (72, 73). Some of the mitochondria associated with symbionts appear to be so deeply divergent that one might wonder how and whether they are co-adapted with the nuclear genome. Studies in a wide range of organisms, including copepods (74) and wasps (75), have shown that rapid coevolution between mitochondria and nuclear genomes can result in hybrid mitonuclear incompatibilities, and we might expect symbionts and other sex ratio distortions to be constrained by similar incompatibilities.

CASE STUDY: EXTRAORDINARY SEX RATIO DISTORTION AND MITOCHONDRIAL POLYMORPHISM IN AN INSECT

We recently found an unusual case of extreme sex ratio distortion in a booklouse. Booklice are the closest free-living relatives of parasitic lice; both are members of the insect order Psocodea (76). The distortion occurs in a recently discovered sexual booklouse that is closely related to *Liposcelis bostrychophila* (Psocodea: Liposcelidae), a worldwide pest of stored grains and domestic kitchens that reproduces via apomictic parthenogenesis and is universally infected with a *Rickettsia* endosymbiont (77-80). The sexual form is not a pest; we collect it under dead yucca leaves and leaf litter in the Chiricahua Mountains of southeastern Arizona. Morphologically, the sexual form is

246 virtually indistinguishable from *L. bostrychophila* (80). Because they are genetically
247 distinct and reproductively isolated by virtue of their mode of reproduction, we refer to
248 the sexual form as *L.* nr. *bostrychophila*.
249
250 When we confirmed that the sexual form is obligately sexual (i.e. virgin females will
251 never produce offspring), we were surprised to find that our lab cultures of *L.* nr.
252 *bostrychophila* were polymorphic for two types of females. One type of female never
253 produces sons, while the other produces a mixed sex ratio (Figure S1). The inheritance of
254 this extreme sex ratio distortion is strictly maternal (i.e. females whose mothers produced
255 only daughters will do the same, whereas females whose mothers produced sons and
256 daughters will produce a mixed sex ratio). Although, in our experience, this
257 polymorphism can be stably maintained in mixed lab cultures, we now culture the two
258 types of females separately, adding males from the 'normal' line to mate with 'distorter'
259 females every generation. We have ruled out the possibility that 'distorter' females are
260 gynogenetic sperm parasites (i.e. parthenogenetic lineages that require male sperm from a
261 close relative to trigger development) (81), by recovering paternal alleles in the daughters
262 of 'distorter' females (Figure S2).
263
264 Because the distortion is maternally inherited, we searched for maternally transmitted
265 microbial symbionts that might be causing it, as these are of course well known in
266 insects. Despite extensive searches, using targeted and untargeted molecular screens, as
267 well as microscopy, we have found no evidence of a microbial symbiont. (This is in
268 contrast to the asexual *L. bostrychophila*, which harbors *Rickettsia*.) We also find no
269 evidence for male-killing in the 'distorter' line, as there is no difference in the number of
270 eggs that hatch and ultimately develop into adults compared to 'normal' females
271 (generalized linear model: df=18, P=0.113). Instead, we were surprised to find that
272 mitochondrial genes in the 'distorter' and 'normal' lines are highly divergent. We
273 focused our efforts on sequencing the mitochondrial genomes of these two different lines,
274 in order to explore the possibility that the distortion might be caused by mitochondria.
275

Sequencing the mitochondrial genomes of 'distorter' and 'normal' individuals proved to be quite a surprise (and also quite a challenge) (Figure 2). Not only were they incredibly divergent (ranging from ~53-77% similarity at protein-coding genes) but they also had radically different gene order and genome structure. Both 'distorter' and 'normal' individuals had multipartite mitochondrial genomes, consisting of at least 5 and 7 minicircles, respectively. Minicircle mitochondrial genomes have been documented in a number of Psocodea (82), ranging from two in *Liposcelis bostrychophila* and two other *Liposcelis* species (83, 84) (although another species, *L. decolor*, has a single 'canonical' chromosome [85]), all the way to the extreme case in the human louse, *Pediculus humanus*, whose mitochondrial genome consists of 18 minicircles (86). While many other mitochondria (and plastids) have evolved minicircle genome architecture (87, 88), in arthropods this organization is apparently restricted to Psocodea. Mitochondrial genes in Psocodea also appear to evolve extremely rapidly, in both parasitic and free-living species (76, 89). Much work remains to be done to understand how sex is determined in *L.* nr. *bostrychophila* and to identify the genetic basis of the extreme sex distortion, such as whether it is caused by mitochondria or by another female-limited portion of the genome, perhaps a B chromosome or a distorting X chromosome. Little is known about sex determination in *Liposcelis* and other Psocodea; this lineage typically exhibits XO male sex determination (90).

The degree of divergence between the 'normal' and 'distorter' mitochondrial genomes is striking, and we hypothesize that it has functional consequences. Interestingly, we have found a consistent morphological difference between 'distorter' and 'normal' mitochondria. We first uncovered this difference while performing electron microscopy to search for microbes that might be causing the sex-ratio distortion. We did not find any microbes, but instead found that mitochondria in the paired rectal glands (91) of 'distorter' females look unusual (Figure 3). In 'normal' females, these glands are packed with mitochondria that are tightly associated with membrane, forming mitochondrial-scalariform junction complexes. These complexes are only known in arthropods, and are commonly found in the rectum, where they play an important role in ion transport and osmoregulation (92, 93). In the rectal glands of 'distorter' females of the same age, on the

other hand, there are few or no scalariform junctions, and mitochondria have few or no cristae and irregular shapes; this morphology is reminiscent of aged or damaged mitochondria (94, 95). Mitochondria in other tissues in 'distorter' females do not appear different from 'normal' females. We speculate that the unusual mitochondria in 'distorter' rectal glands may be a result of cytonuclear incompatibilities that are exposed in these tissues because they are so metabolically active (and packed with mitochondria). Further support for cytonuclear incompatibilities in 'distorter' females comes from the observation that they have a reduced lifespan relative to 'normal' females (coxph: df=1, $P<0.001$; Figure 4); this is also intriguing given the mitochondrion's well-known role in longevity (72). Thus even if mitochondria are not the cause of the sex ratio distortion, incompatibilities between 'distorter' mitochondria and the nuclear genome may play a major role in shaping how the distortion persists in the wild (and in our mixed lab cultures), as we might otherwise expect 'distorter' females to overtake their 'normal' counterparts since they only produce females, which would then lead to extinction.

CONCLUSION

We predict that the coming years will see the discovery of many novel cases of sex ratio distortion, such as the extreme case in booklice described here, as well as the discovery of non-plant mitochondrial distorters. This will be spurred in part by the growing realization of the importance of microbial symbionts in shaping the ecology and evolution of multicellular organisms. It will also be facilitated by the ease of DNA sequencing, which will make it much easier to develop markers for sex chromosomes and selfish genetic elements. Of course, the easiest place to start looking for interesting systems is in cases of deeply divergent mitochondrial polymorphisms, and this will be facilitated by the (fortuitous) choice of the mitochondrial cytochrome c oxidase gene as the marker of choice in animal DNA barcoding studies that are currently cataloguing the planet's biodiversity (96). Finally, we speculate that the persistence of many sex ratio distortion systems, as well as other interesting and unusual reproductive systems with maternal inheritance (97, 98), may be affected by mitonuclear incompatibilities.

338

339 Methods

340

341 *Insect Rearing* – Distorter and normal females were kept in separate cultures in glass jars
342 (125ml). We used a 1:10 (by weight) mixture of Rice Krispies (Kellogg's) and Cracked
343 Wheat (Bob's Red Mill) to rear insects. Colonies were maintained at 75% relative
344 humidity and 27°C. We added males to distorter female colonies weekly to ensure
345 females had an opportunity to mate.

346

347 *Mitochondrial Sequencing and Annotation* – We sequenced the mitochondrial genome of
348 distorter and normal females with a combination of Illumina and Sanger sequencing. For
349 Illumina sequencing, we extracted DNA from ethanol-preserved distorter and normal
350 females (~35 pooled individuals/line) using a Qiagen DNeasy Blood and Tissue kit.
351 Libraries for each line were constructed and sequenced by Beckman Coulter Genomics,
352 providing ~4×10^7 100 bp PE reads per line. Draft assemblies for each line were
353 generated with Ray v. 2.20 (k = 31; 99). We searched assemblies for mitochondrial genes
354 using tblastx, with sequenced *L. bostrychophila* mitochondrial genomes as queries (83).
355 Pieces of retrieved genes (400-800 bp) were then used as seeds in mitoBim (100;
356 proofreading mode) to corroborate and extend minicircles, prior to validation by PCR and
357 Sanger sequencing (see Table S1 for primer sequence and PCR conditions). We
358 sequenced most of the PCR products directly but in some cases products were cloned
359 using StrataClone PCR cloning kits (Agilent Technologies). All Sanger sequencing was
360 carried out with total DNA extractions from 16 females in 60ul of PrepMan Ultra (Life
361 Technologies). We annotated the mitochondrial protein coding regions by extracting
362 open reading frames longer than 120bp from the minicircle assemblies using getorf
363 (EMBOSS) and using blastp searches against the non-redundant protein (nr) database
364 (NCBI). We manually identified rRNA coding regions using Geneious version 6.1 by
365 performing nucleotide alignments using the default parameters with the rRNA coding
366 regions from *L. bostrychophila*. Mitochondrial genome sequences have been deposited in
367 GenBank under the following accessions: KP641133, KP657691-657699, and
368 KP671844-671845.

We also completed a series of PCR reactions in individual booklice to explore mitochondrial variation within each female type. For eight individual females of each female type (i.e. 'distorter' or 'normal'), we amplified five different regions of the mitochondrial genome that were expected to range in size from 1200-3000 bp. Single female DNA extractions were carried out in 20ul of PrepMan Ultra. For all five regions, all eight females produced a single band of the expected size, suggesting that there is little within-type variation.

Microscopy – Adult insects were processed using standard TEM methodology (101); double-fixation and embedding into Epon. For light microscopy, 0.5 um sections were stained in Richardson's Stain (Azure II and Methylene Blue in Borax solution). 85 nm thick TEM sections were stained in uranyl acetate and lead citrate and viewed in a Hitachi H7000 TEM at 75 kV. Images were captured using an AMT 2k x 2k CCD camera.

Longevity and Male-killing – We set up jars containing 150 late instar females of each type ('distorter' or 'normal') along with 75 males. At the end of this period the females were reproductively mature and mated. We then transferred the females into approximately 5g of cracked wheat to lay eggs. After 24 hours the females were transferred to another jar containing 5g of cracked wheat. After we removed the females from egg laying jars, 10 eggs from the jar were transferred into a petri dish (35mm diameter) containing 0.7g of Rice Krispies and cracked wheat. We prepared two petri dishes containing 10 eggs every day for each female type. We did this for 5 days resulting in 10 replicate containers for each female type.

Three weeks after the eggs were laid, we began checking for adults. We recorded when individuals completed development and transferred females into a new petri dish containing 0.7g of food. Females raised in the same petri dish were kept together as adults. We recorded when males completed development but then discarded them. We checked females approximately 3 times a week and recorded female longevity (from the date eggs were laid until death) as well as the number and sex of individuals that developed from each container.

400 We analyzed data using Rstudio version 3.1.0 (102). We performed a survival
401 analysis for the data assessing longevity of females with the package survival (103) using
402 a Cox proportional hazards (coxph) model. We assessed whether the different female
403 types differed in longevity, clustering individuals by container. We also assessed whether
404 there was any evidence of male killing by examining whether there was a difference in
405 the number of individuals (males and females) that developed from a container depending
406 on the type of individuals in the container.
407
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413 who has taught us so much about *Liposcelis*.
414
415 Figure Legends
416
417 Figure 1. Three possible consequences of maternal transmission of mitochondria (and
418 other maternally transmitted organelles and symbionts). A) Mutations that are deleterious
419 in males can become common if they do not decrease female fitness. B) Maternally
420 transmitted lineages that increase the frequency of females will be favoured by selection.
421 C) Mitochondria and maternally transmitted symbionts are linked, such that symbionts
422 that spread in a population will bring their associated mitochondrial haplotype along with
423 them. Different colors represent different haplotypes. Mt = mitochondria, S = symbiont.
424
425 Figure 2. 'Distorter' (A) and 'normal' (B) *Liposcelis* nr. *bostrychophila* have radically
426 different mitochondrial genome order and organization. Protein-coding and ribosomal
427 genes are labeled; genes on the forward/complementary strand are on the outside/inside
428 of the circles. Similarity between genes ranges from 53-80%: ATP6 (75.4%), ATP8
429 (65.4%), CO1 (76.6%), CO2 (73.9%), CO3 (70.6%), COB (76.8%), ND1 (76.1%), ND2
430 (73.8%), ND3 (76.8%), ND4 (72.4%), ND4L (75.9%), ND5 (70.9%), ND6 (53.1%), 12S

(80.1%), 16S (80.1%). While all circles have been closed using PCR, a few have not been completely sequenced, and these are indicated by the small gaps. 'Normal' minicircle sizes (minicircles are named for their largest gene): 16S (3265 bp), ND4 (3426 bp), CO1 (3147 bp), ATP6 (2714 bp), ND6 (1354 bp), ND1 (1275 bp), ND5 (>3717 bp). 'Distorter' minicircle sizes: 16S (2746 bp), ND4 (5312 bp), CO1 (5626 bp), 12S (2131 bp), ND5 (~4600 bp).

Figure 3. Distinctive mitochondrial morphology in rectal glands of 'distorter' females. A) TEM of 4-week old 'normal' female rectal gland, showing mitochondria with intact cristae, many intact scalariform junctions (i.e. parallel plasma membranes), and even ground substance (i.e. cytosol) between the two. B) Close-up of previous image. C) TEM of 4-week old 'distorter' female rectal gland, showing abnormal mitochondria with fragmented, electron dense material within and few cristae, few intact scalariform junctions, and condensed ground substance between the two. D) Close-up of previous image. E) & F) Light micrograph of sagittal section of a 'normal' female with arrows to indicate the location of the glands. Scale bar in A-D = 500 nm, and in E,F = 50 um.

Figure 4. 'Distorter' *Liposcelis* nr. *bostrychophila* females have a significantly shorter lifespan than 'normal' females (coxph: df=1, $P<0.001$; n=51 and 32 for 'distorter' and 'normal' females, respectively). Crosses indicate censored data (two individuals were lost during the experiment, and one individual survived past the end of the experiment).

Figure S1. Offspring sex ratio of 'normal' and 'distorter' *Liposcelis* nr. *bostrychophila*, (n=10 containers of each type).

Figure S2. Gene flow between 'distorter' females and males. Chromatograms of two linked SNPs at a putative phosphodiesterase gene showing that 'distorter' females inherit paternal alleles. PCR conditions: 95°C×3min, (94°C×1min, 54°C×1min, 72°C×1.5min)×35, 72°C×10min. Primers (phos1F–TCCCTTCCGTCAATAAATGC and phos1R–AATGTTCGAAATGCCGAGTC) amplify a 627 bp product.

Table S1. Primer sequences and PCR conditions for mitochondrial Sanger sequencing.

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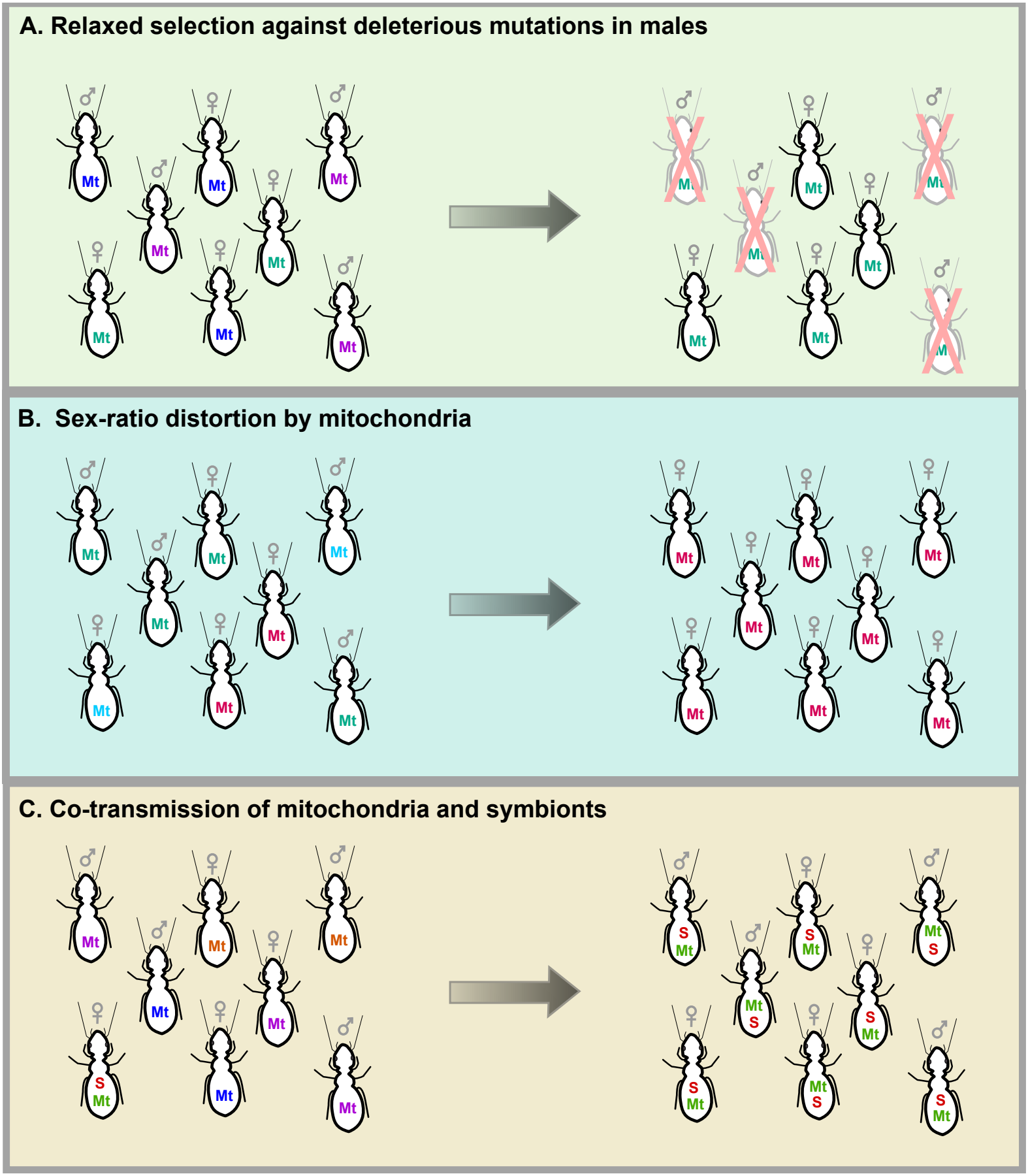
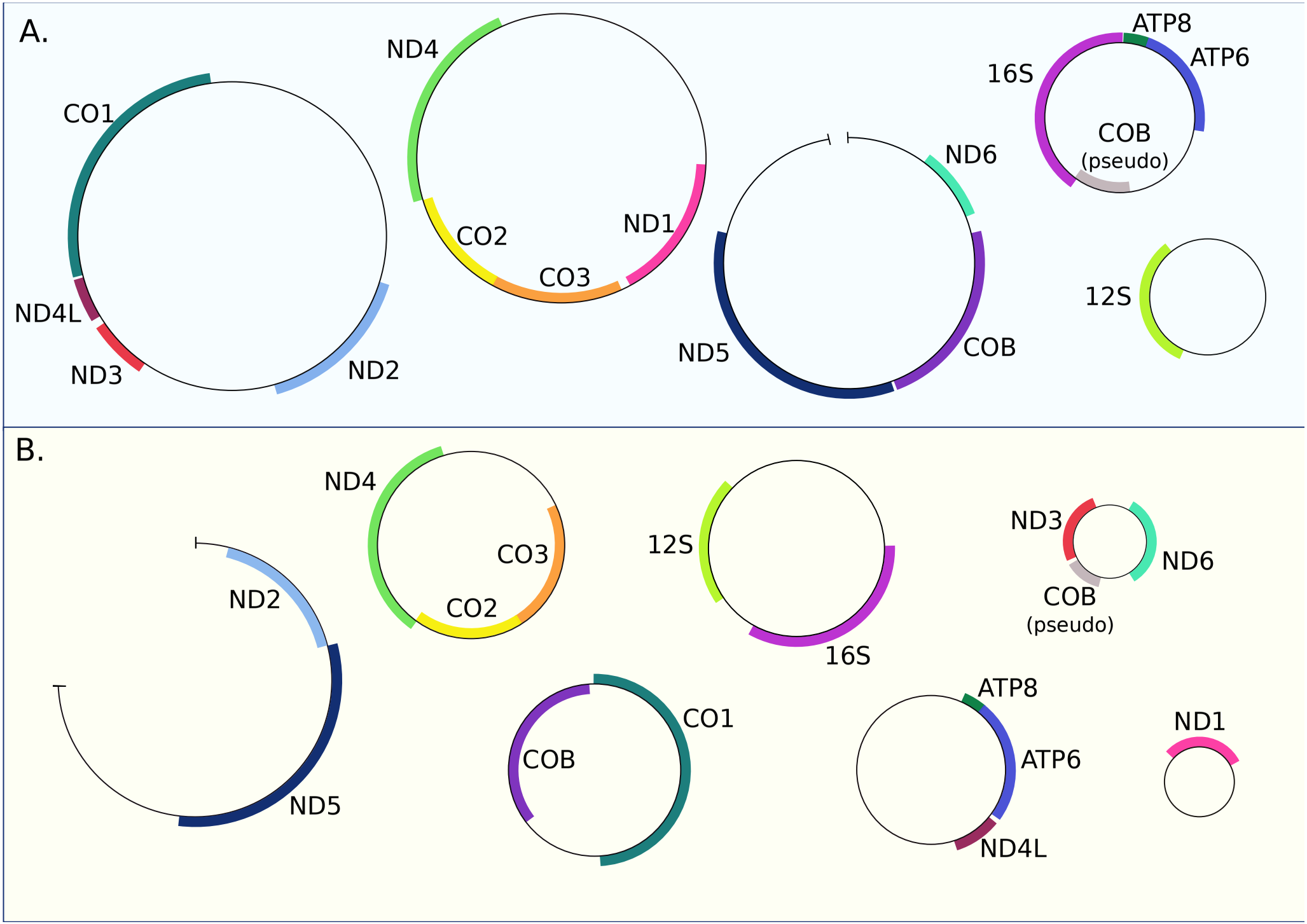

A. Relaxed selection against deleterious mutations in males
B. Sex-ratio distortion by mitochondria
C. Co-transmission of mitochondria and symbionts
Mt
S
Mt


A.
CO1
ND4
ATP8
ATP6
16S
COB
(pseudo)
ND6
CO2
ND1
CO3
ND4L
ND3
ND2
ND5
COB
12S
B.
ND4
CO3
CO2
ND2
12S
16S
ND3
ND6
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(pseudo)
CO1
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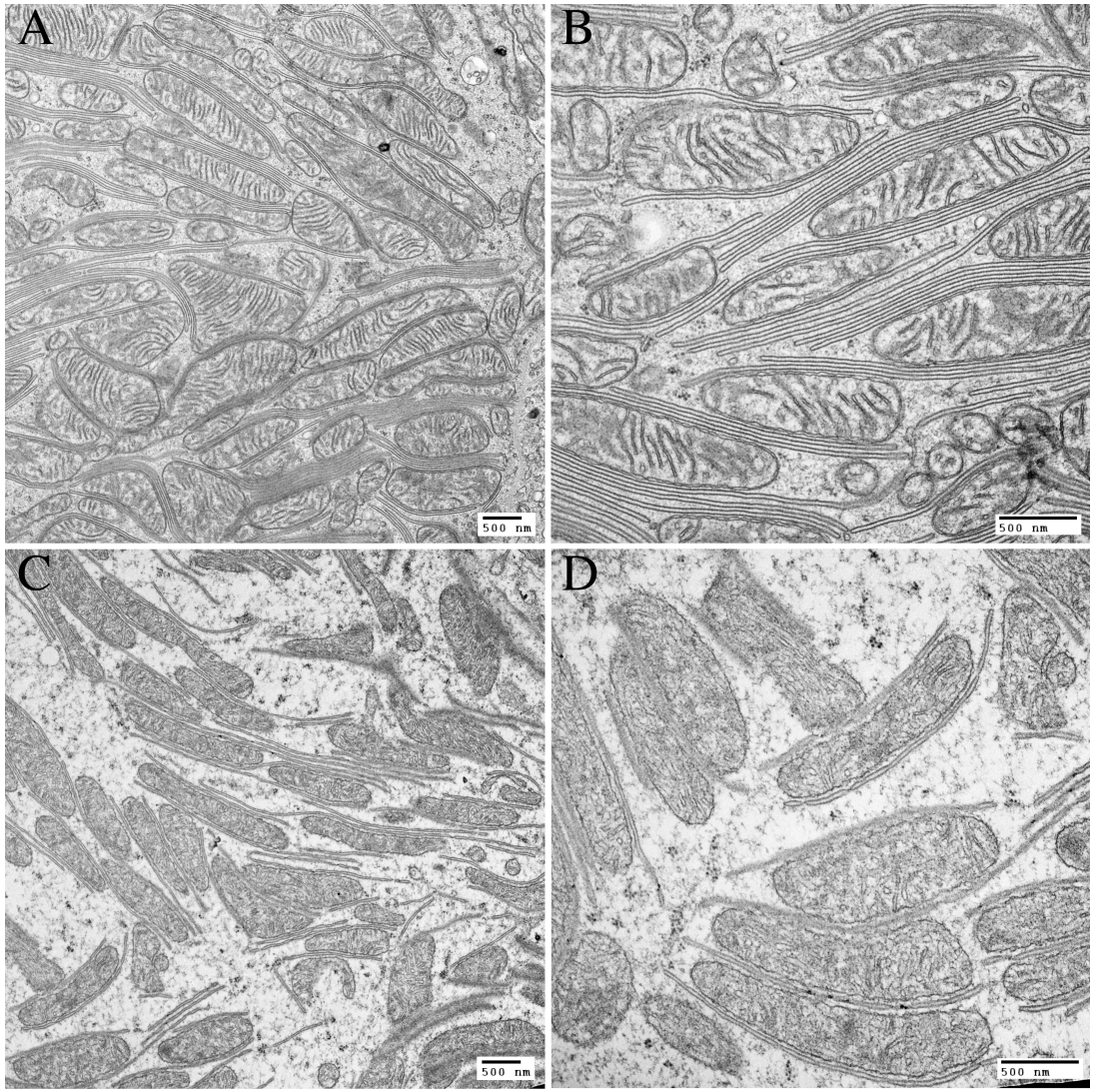
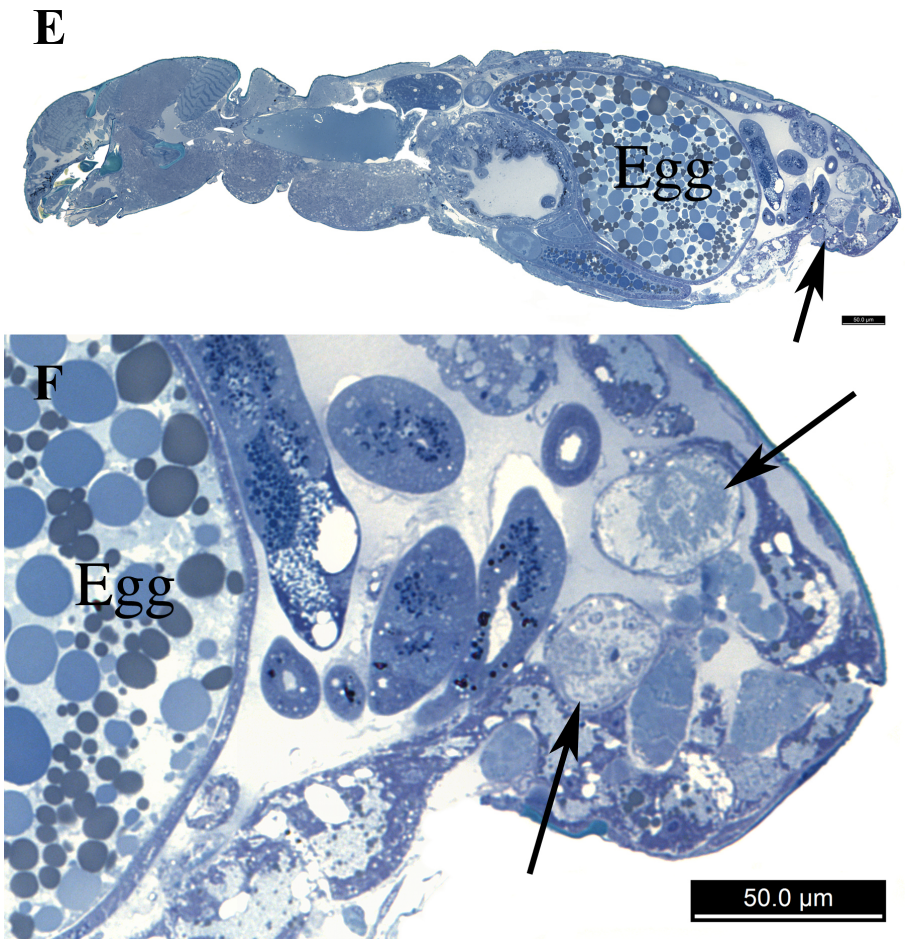
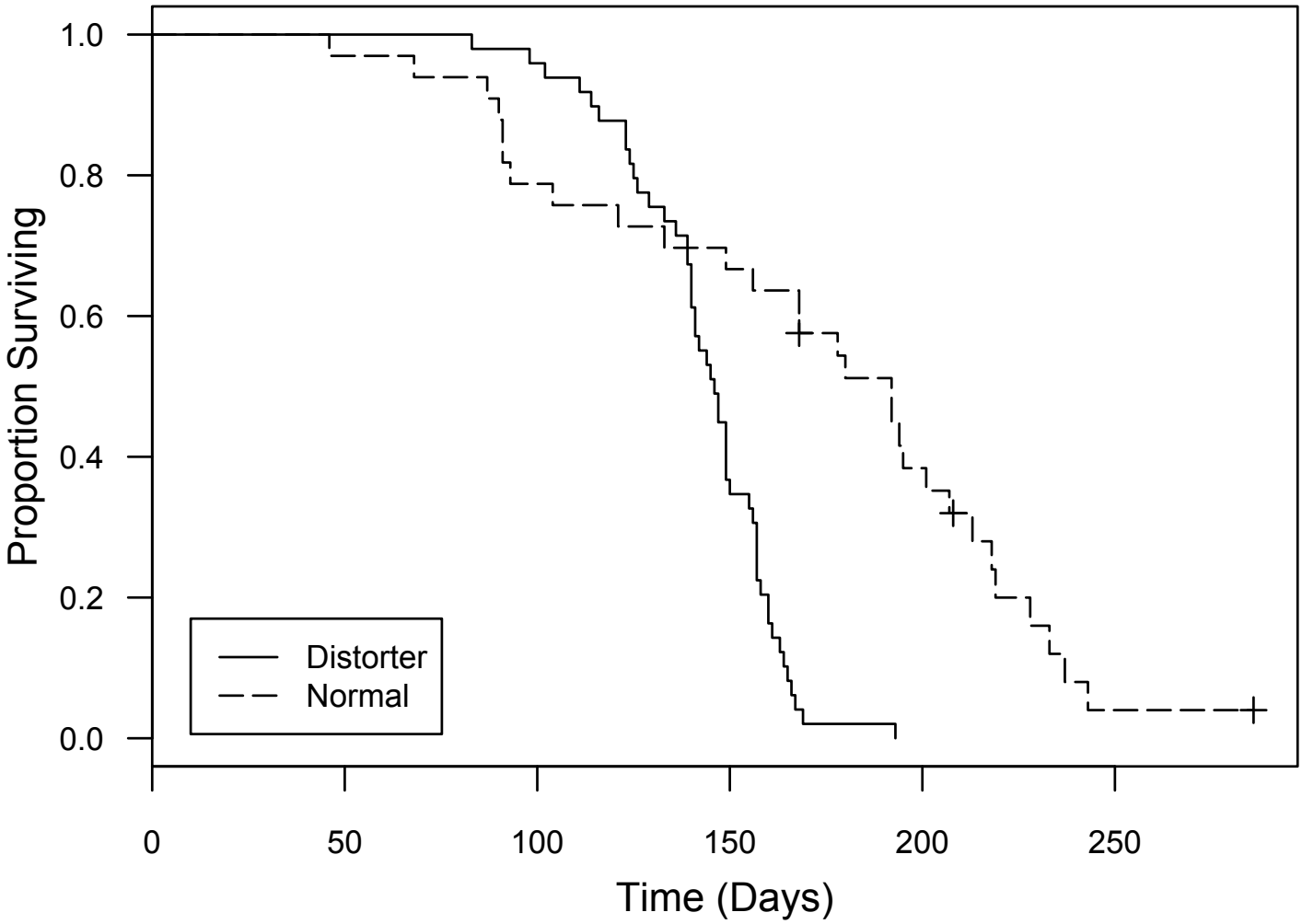
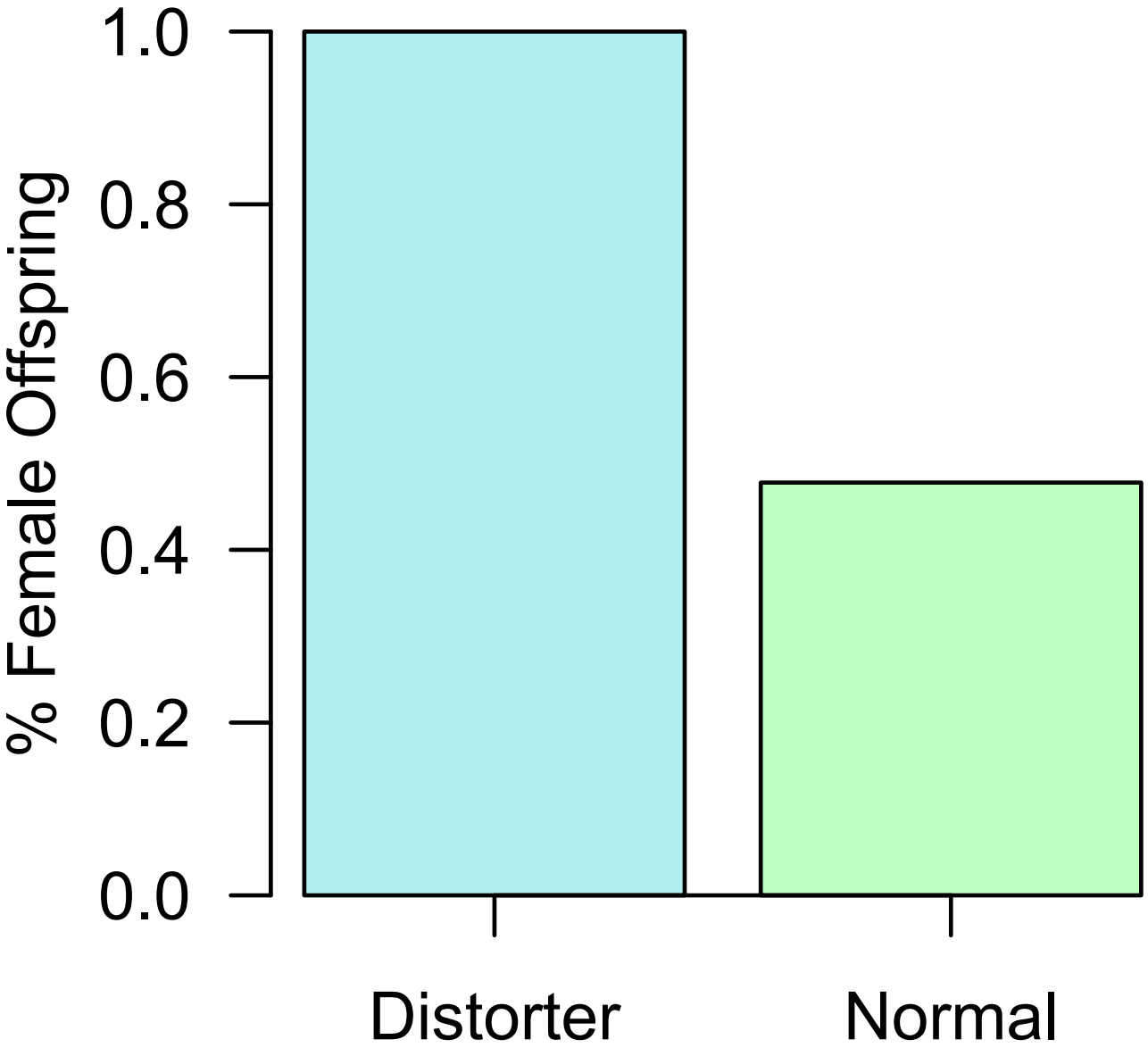
ND5
ND4L

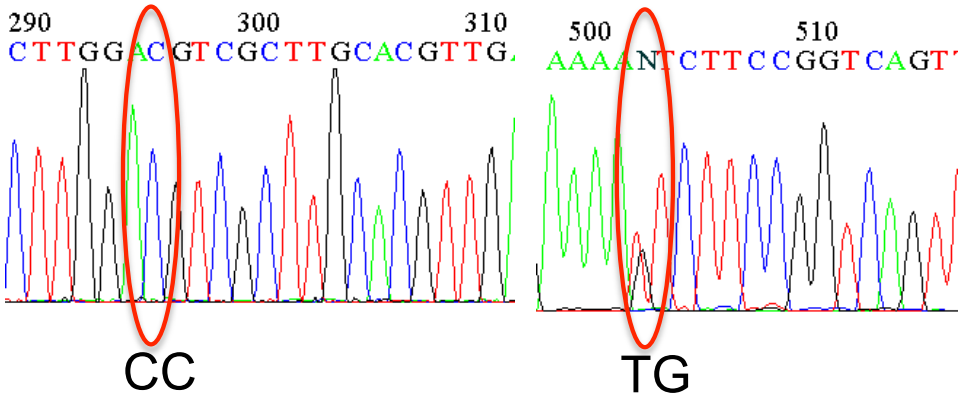

A
500 nm
B
500 nm
C
500 nm
D
500 nm


E
Egg
F
Egg
50.0 µm

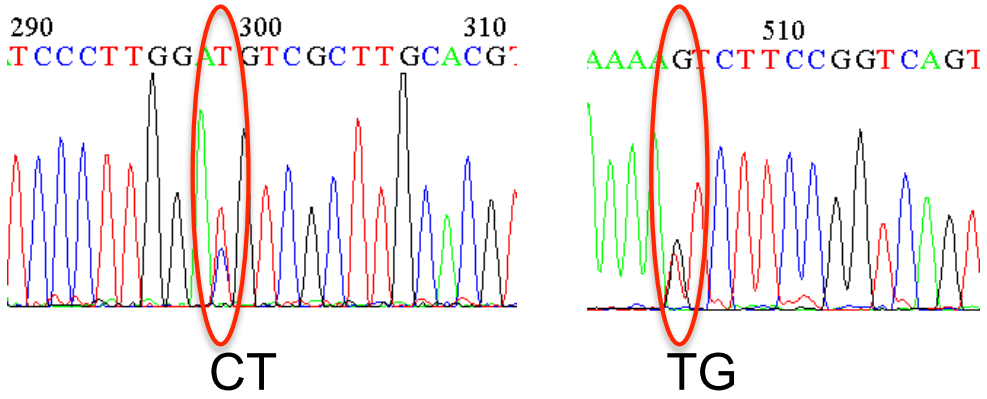

1.0
0.8
0.6
0.4
0.2
0.0
Proportion Surviving
Distorter
Normal
0
50
100
150
200
250
Time (Days)


% Female Offspring
1.0
0.8
0.6
0.4
0.2
0.0
Distorter
Normal

Mother
(from ‘distorter’ line)



Father



Daughter

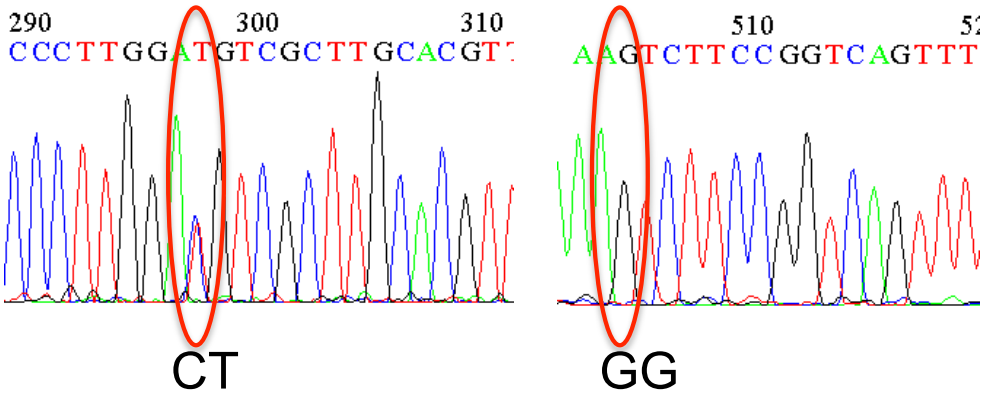


Table S1

Female Type	Minicircle[1]		Forward Primer	Sequence	Reverse Primer	Sequence	Additional information
Normal	CO1		MCO1F1	GAATTTCCTTTCACCTTCATGG	MCO1R1	ACTAGCAGGTTTCCCACGTC	95°C×3min, (94°C×1min, 59°C×1min, 72°C×1.5min)×35, 72°C×10min
			MCO1F2	AATGGTTCACCCCGTACCTG	MCO1R2	CCCATGAAGGTGAAAGGAAA	95°C×3min, (94°C×1min, 59°C×1min, 72°C×1.5min)×35, 72°C×10min
			MCO1F3	GGGGAAATGAGGGATCAAAT	MCO1R3	GTTTTGTCCCGCATAAGGAA	95°C×3min, (94°C×1min, 58°C×1min, 72°C×4min)×35, 72°C×10min
	ND4		MND4F1	TGCAGTCCATGAAAGCCTGT	MND4R1	GATGCTAATCCTGGGCGACT	95°C×3min, (94°C×1min, 52°C×1min, 72°C×1.5min)×35, 72°C×10min
			MND4F2	AGTCGCCCAGGATTAGCATC	MND4R2	ACAGGCTTTCATGGACTGCA	93°C×3min, (93°C×20sec, 60°C×1min, 68°C×5min)×35, 68°C×5 min
			MND4F3	TGGTCTGCAAGATTCCGTTAAAA	MND4R3	CAAGCCCATGACCGTGAAAT	93°C×3min, (93°C×20sec, 58°C×1min, 68°C×3min)×35, 68°C×5 min
	ND1		MND1F1	GCTTATCCTCGTTTGCGATT	MND1R1	ACGAAAATTCCATGCCCCCA	95°C×3min, (94°C×1min, 60°C×1min, 72°C×1.5min)×35, 72°C×10min
			MND1F2	CTCCCTTTGATTTGGCAGAA	MND1R2	ACAGGCTCAAGGAGGAATGA	95°C×3min, (94°C×1min, 58°C×1min, 72°C×1.5min)×35, 72°C×10min
	ATP6		MATP6F1	TTACCCGGATATGGATTGGA	MATP6R1	GAACACAAAGGGCAACAACC	95°C×3min, (94°C×1min, 58°C×1min, 72°C×4min)×35, 72°C×10min
			MATP6F2	CAAGGCCGCAATTATGAAAT	MATP6R2	GGGGAATGATCGTGAAAGAA	93°C×3min, (93°C×20sec, 56°C×1min, 68°C×5 min)×35, 68°C×5 min
	ND6		MND6F1	TCCATGACTTTAGAGTTTGAATGAGG	MND6R1	GAAAATGATTTGCGGGAAGA	95°C×3min, (94°C×1min, 59°C×1min, 72°C×1.5min)×35, 72°C×10min
			MND6F2	TTCCCGCAAATCATTTTCTC	MND6R2	AAAAGTGATTATGAGGCCACCAA	95°C×3min, (94°C×1min, 59°C×1min, 72°C×1.5min)×35, 72°C×10min
	16S		M16SF1	TGGCGGCTTTTATTCACATT	M16SR1	TGGGGTTACCCTGAACTCAT	95°C×3min, (94°C×1min, 58°C×1min, 72°C×4min)×35, 72°C×10min
			M16SF2	GCCGCAGTAAATTGTGCCAA	M16SR2	CAAACCGCCCGTCACTTCTA	95°C×3min, (94°C×1min, 54°C×1min, 72°C×2min)×35, 72°C×10min
	ND5		MND5F1	CAATGAAGGTGGTATCCCCATA	MND5R1	GTCACCTTTTCTGGCGACTC	93°C×3min, (93°C×20sec, 56°C×1min, 68°C×5 min)×35, 68°C×5 min
Distorter	CO1		FCO1F1	TAATGCCCAAGTCCGGATGG	FCO1R1	TGCTCACACAATGAACCCCA	93°C×3min, (93°C×20sec, 58°C×30sec, 68°C×6min)×35, 68°C×5min
			FCO1F2	CAACCCCCAAAAACCCATTC	FCO1R2	AATCAACGGGAACACAAGGT	95°C×3min, (94°C×1min, 58°C×1min, 72°C×2min)×35, 72°C×10min
	ND4		FND4F1	TAACCGCACTAGAACCCCCA	FCO1R1	TGAACTGGGGCCTCAACATG	93°C×3min, (93°C×20sec, 58°C×30sec, 68°C×6min)×35, 68°C×5min
			FND4F2	TCACATGGGTTTTTATCCCCTTT	FCO1R2	GGAATTTGAGTATGTCCCTTCC	95°C×3min, (94°C×1min, 58°C×1min, 72°C×2min)×35, 72°C×10min
	16S		FATP6F1	AGAGTGATTGGAAGGGCAAC	FATP6R1	CATCGAGGTCGCAATCATAA	95°C×3min, (94°C×1min, 58°C×1min, 72°C×1.5min)×35, 72°C×10min
			FATP6F2	ACAGCCGCAGTAAATTGTGC	FATP6R2	TCTAGGCATGTCCTACCCTGA	95°C×3min, (94°C×1min, 58°C×1min, 72°C×1.5min)×35, 72°C×10min
	ND5		FND5F1	CGTGCCTTGTGGAATGGTT	FND5R1	TCGAATATCTTGCCACCCGG	95°C×3min, (94°C×1min, 54°C×1min, 72°C×1.5min)×35, 72°C×10min
		*	FND5F2	CCGGGTGGCAAGATATTCGA	FND5R2	CAACCATTCCACAAGGCACG	93°C×3min, (93°C×20sec, 58°C×30sec, 68°C×6min)×35, 68°C×5min

	FND5F3	TGAACGATCTAAAACTCGAAGAA	FND5R2	CAACCATTCCACAAGGCACG	95°C×3min, (94°C×1min, 59°C×1min, 72°C×1.5min)×35, 72°C×10min
12S	F12SF1	TGCCCTGTTCAAGAAAATTG	F12SR1	TTACTCGGCGAAAGCTTCAT	93°C×3min, (93°C×20sec, 58°C×1min, 68°C×3min)×35, 68°C×5 min
	F12SF2	ATGAAGCTTTCGCCGAGTAA	F12SR2	TCGGGTGATAACCTACAACCA	95°C×3min, (95°C×20sec, (63°C-53°C)×30sec, 72°C×1.3min)×10, (95°C×20sec, 55°C×30sec, 72°C×1.3min)×24, 72°C×5min

1- Minicircles are named for the largest gene in that circle

* Not fully sequenced