Selfish drive can trump function when animal mitochondrial genomes compete

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Abstract

Mitochondrial genomes compete for transmission from mother to progeny. We explored this competition by introducing a second genome into *Drosophila melanogaster* to follow transmission. Competitions between closely related genomes favored those functional in electron transport, resulting in a host-beneficial purifying selection[1]. Contrastingly, matchups between distant genomes often favored those with negligible, negative or lethal consequences, indicating selfish selection. Exhibiting powerful selfish selection, a genome carrying a detrimental mutation displaced a complementing genome leading to population death after several generations. In a different pairing, opposing selfish and purifying selection counterbalanced to give stable transmission of two genomes. Sequencing of recombinant mitochondrial genomes revealed that the non-coding region, containing origins of replication, governs selfish transmission. Uniparental inheritance prevents encounters between distantly related genomes. Nonetheless, within each maternal lineage, constant competition among sibling genomes selects for super-replicators. We suggest that this relentless competition drives positive selection promoting change in the sequences influencing transmission.

Natural selection culls populations of compromising mutations and favors traits that enhance organismal fitness. Nuclear genes have a relatively uniform exposure to natural selection as a result of regimented replication and segregation. In contrast, unconstrained competition among multiple mitochondrial genomes creates alternative ways to select for fit genomes, as well as opportunities for selfish genomes to increase in abundance.

In heteroplasmic lines of *Drosophila*, mitochondrial genomes harboring mutations detrimental to oxidative phosphorylation (OXPHOS) function suffered a transmission disadvantage, even when complemented by co-resident wild-type genomes. The resulting

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purifying selection is based on competition among genomes within the organism rather than survival of fit organisms[1,2]. However, a selfish advantage might also bias transmission and provide an evolutionary drive (i.e. selfish drive)[3–5]. For example, in *S. cerevisiae*, preferential replication promotes inheritance of hypersuppressive petite mitochondrial DNA (mtDNA)[6–8]. In multicellular organisms, defective mitochondrial genomes carried in animals can also benefit from selfish drive to enhance their propagation[9–14].

To study head-to-head competition between mitochondrial genomes, we used cytoplasmic transplantation to bypass uniparental inheritance, which normally prevents encounters between unrelated genomes. In the resulting heteroplasmic lines, purifying selection dominated competition between closely related genomes[1], but here we show that when distantly related genomes compete, a selfish selection drives success of genomes having negligible, or negative consequences to the fly. Stable heteroplasmy can occur when selfish selection benefits one genome and purifying selection benefits the other. Our findings indicate that competition among mitochondrial genomes influences their evolutionary trajectory.

We impose selection for OXPHOS function by raising the temperature of a line carrying a marked genome with a temperature sensitive lethal mutation in the cytochrome c oxidase subunit I gene (*mt:ND2^{del1}* + *mt:CoI^{T300I}*) (Figure 1a). When paired with a related wild-type genome in heteroplasmic strains, selection reduced the transmission of temperature sensitive genome due to an intra-organismal competition among mitochondria genomes[1,2]. In contrast, when paired with the *ATP6[1]* genome[15,16], a diverged *D. melanogaster* mitochondrial genome with numerous sequence differences (Figure 1), the temperature sensitive genome completely displaced the *ATP6[1]* genome[17]. Unlike purifying selection, this displacement occurred at either 25 °C or 29 °C, hence was not substantially influenced by the ability of the genome to contribute to OXPHOS (Figure 1a). Even at the restrictive temperature, the population expanded as long as the *ATP6[1]* genome provided complementing *mt:CoI* function. However, after several generations, just as the *ATP6[1]* genome disappeared, the entire stock died. We conclude that the temperature sensitive genome has a transmission advantage despite carrying a detrimental allele that leads to lethality.

Based on our recent demonstration of recombination among mitochondrial genomes[17], we genetically mapped the sequence distinctions making the temperature sensitive genome the stronger competitor. Exchange events transferring these sequences should produce an enhanced *ATP6[1]* genome that would persist to give surviving flies. When five heteroplasmic lines were followed at 29 °C, one line gave surviving progeny carrying a recombinant genome (Figure 1a). This recombinant has the majority of the *ATP6[1]* coding sequence including the functional *mt:CoI* allele, but carried the entire non-coding segment and a small segment flanking coding sequence from the temperature sensitive genome (Figure 1b)[17]. Later, we isolated another recombinant with the entire coding sequence derived from the *ATP6[1]* genome but with the non-coding sequence from the temperature sensitive genome (Supplementary Figure 1a). At first, the surviving heteroplasmic flies had a low relative abundance of the recombinant genome and a preponderance of the temperature sensitive genome. Over subsequent generations, the relative abundance of recombinant

genomes increased, showing their ability to successfully compete with the co-existing temperature sensitive genome (Figure 1c, Supplementary Figure 1b & c). These changes in the relative ratios of the mitochondrial genomes (as well as those described below) occurred in the absence of change in the total copy number of mitochondrial genomes. These data show that acquisition of the non-coding region from the temperature sensitive genome is sufficient to endow the recombinant with an improved ability to compete against the temperature sensitive genome. We conclude that, at least in this pairing of genomes, the difference in selfish drive maps to the non-coding region of the mitochondrial genome.

The non-coding region of the mitochondrial genome, also known as the control region or the regulatory region, contains the origins of replication, and is the most variable sequence for many metazoan species[18–21]. The *D. melanogaster* version is AT-rich (> 90%) and large (~4.6 kb), and is mainly (> 90%) composed of five tandem type I repeats and four tandem type II[22] (Supplementary Figure 2a). Within *D. melanogaster*, this region exhibits frequent nucleotide and length polymorphisms[23–25] (e.g. Supplementary Figure 2a & b), and the divergence is more extensive in other *Drosophila* species[26,27] (e.g. Supplementary Figure 2c). For instance, *D. yakuba* mtDNA (NC_001322) has a shorter (~1 kb) and diverged non-coding region. We thus introduced mitochondrial genomes from other *Drosophila* species into *D. melanogaster* and examined their ability to compete.

We first introduced cytoplasm of *D. yakuba* (diverged ~10 MY) embryos into *D. melanogaster* embryos carrying the temperature sensitive genome (Figure 2a). However, no lines retaining the *D. yakuba* mitochondrial genome were recovered. On the other hand, at the restrictive temperature, where selection favored retention of the *D. yakuba* genome, two of 50 injected females gave viable progeny heteroplasmic for the *D. yakuba* genome and a persisting temperature sensitive genome. The *D. yakuba* genome was carried at a low but stable level (~4%) for many generations (> 100) under constant temperature selection (Figure 2b).

Expression of mitochondrially-targeted PstI selectively eliminated *D. melanogaster* mtDNA (site at mt7496) giving lines containing only the *D. yakuba* genome (Figure 3a). These lines (named *D. mel* (mito-*yakuba*)) were viable and, in our measures, at least as robust as wild-type flies (Figure 3b & c). This was surprising because examples of nuclear-mitochondrial incompatibility[28–31] had suggested that mismatches between the nuclear genes and the foreign mitochondrial genes would compromise OXPHOS and hence the *D. mel* (mito-*yakuba*) flies. Instead, the result shows that the only incompatibility is between the two mitochondrial genomes, apparently the result of unequal competition. Similarly, incompatibility between mitochondrial genomes had been noted to influence transfer of ape mtDNA into human tissue culture cells[12].

In the above heteroplasmic line, the *D. yakuba* genome benefits from purifying section at 29 °C because it provides a functional *mt:CoI* gene. Disappearance of *D. yakuba* mtDNA within two generations at the permissive temperature shows the importance of this advantage (data not shown). In contrast, the *D. melanogaster* temperature sensitive genome, whose OXPHOS function is compromised by mutation, is sustained by selfish drive. These two selections oppose and balance each other to maintain stable transmission of the two

genomes. Since purifying selection operates during oogenesis to limit transmission of the temperature-sensitive genome[1], this stage-limited selection against the temperature sensitive genome ought to give the *D. yakuba* genome a transient relative advantage. Indeed, at the restrictive temperature, newly deposited eggs have an especially high abundance of the *D. yakuba* genome. At the permissive temperature, eggs showed no increase, arguing that this increase depends on selection against the temperature sensitive electron transport defect (Figure 2c). The relative abundance of *D. yakuba* genome then declined during development, only to increase again during oogenesis (Figure 2c). Thus, selfish selection favoring the *D. melanogaster* genome influences competition at many stages of the life cycle, while the purifying selection favoring the *D. yakuba* genome is largely restricted to oogenesis[1,2,32]. This temporal distinction might underlie previously observed oscillations in the relative abundance of other heteroplasmic genomes[33].

To further demonstrate that *D. melanogaster* mitochondrial genomes benefits from a stronger selfish drive, we introduced other *D. melanogaster* mitochondrial genotypes, many of which do not support organismal vigor as effectively as the *D. yakuba* genome (Figure 3b & c)[16,32,34], into the *D. mel* (mito-*yakuba*) line. These tested *D. melanogaster* genomes outcompeted the *D. yakuba* genome at 25 °C (Figure 3d), an outcome detrimental to the fitness of the flies.

Notably, the competitive strengths of *D. yakuba, ATP6[1]* and the temperature sensitive genomes do not fall on a simple hierarchy: *D. yakuba* mtDNA is displaced by *ATP6[1]*, suggesting that it is the weaker competitor, yet, the *D. yakuba* genome is sustained in conjunction with the temperature sensitive genome at the high temperature while the *ATP6[1]* genome is eliminated. Apparently, competitive strength is not determined by potency of a single factor.

When we re-introduced the temperature sensitive genome into the *D. mel* (mito-*yakuba*) line, heteroplasmy was again stabilized at the restrictive temperature, but with a higher ratio of the *D. yakuba* genome (Figure 3d). Since the sequence of the *D. yakuba* mitochondrial genome was unchanged, the ratio change is likely due to accumulation of nuclear modifiers, which have previously been suggested to influence competition among mitochondrial genomes[35–37]. The modification of this balance between selfish selection and purifying selection acting on competing genomes ought to be sensitive to minor perturbations of either selection. It will be interesting to explore how nuclear genes modify the competition between mitochondrial genomes.

The native genome is not always the winner in interspecies heteroplasmic lines. For instance, when we introduced *D. mauritiana* (diverged ~2mya) mtDNA (*maI*), it replaced the *D. melanogaster* genomes within a few generations (Supplementary Figure 3). A different *D. mauritiana*/*D. melanogaster* heteroplasmic line behaved similarly[38]. Previously, De Stordeur ranked mitochondrial haplotypes from *D. simulans* and *D. mauritiana* for their potency to displace each other[39]. While these findings suggest that mismatches in competitive strength are common among diverged genomes, the role of selfish drive in determining the outcome in these competitions has yet to be determined.

While the mechanism of selfish drive is unknown, localization of the trait to the non-coding region constrains possibilities. The non-coding sequence is unlikely to influence drive by complex actions, such as evasion of mitophagy or localizing mitochondria to the germline. In contrast, the localization of the two origins for asymmetric replication of the mtDNA (Supplementary Figure 2a) suggests a link between replication and competitive strength. While specific sequence features characterize the start sites of replication[40], associated sequences show numerous SNPs and variations in the number of repeat sequences (Supplementary Figure 2a & c). A previous study of large laboratory fly populations detected instability in repeat number and tracked transient heteroplasmy in individual flies to show that longer variants of the non-coding regions tended to increase in abundance during transmission[24]. We suggest that this finding is related to our observations of selfish drive. Though little is known about the control of *Drosophila* mtDNA replication, the control region is likely to influence copy control, primer synthesis and initiation efficiency. Even a subtle gain in replication, amplified over many rounds of genome doubling, would result in a large competitive advantage. We thus hypothesize that selfish drive can be equated with replicative drive.

Each new mutation that enhances mtDNA transmission can displace genomes without this asset. The displacement raises the bar for the next mutation to produce a super-competitor that will again take over in an unending 'arms race'[41]. Thus, as described in the 'red queen' hypothesis, we propose that a constant competition among co-existing mitochondrial genomes creates an intraorganismal positive selection for super-replicators during evolution. Uniparental inheritance confines each super-replicator to a lineage, fragmenting a species into multiple lineages within which local competition independently selects for winning mitochondrial genomes[42]. Positive selection accelerates sequence divergence, and the non-coding sequences of mtDNA diverge especially fast[42–44]. Divergence in the size of this region by alteration of repeat number is widespread[45–50]. This complicates alignment of these sequences between species making quantification of change problematic. Using sequences of twelve *D. melanogaster* wild isolates[25], we show that divergence of the non-coding sequence exceeds neutral changes (Supplementary Figure 2b), a signature of positive selection. Thus, selfish selection, a positive selection, drives rapid change of noncoding sequences, while purifying selection, a negative or conserving selection, limits change of the coding sequences.

To conclude, our findings show that competition between distantly related mitochondrial genomes can be dominated by the selfish drive of a genome rather than its contribution to the fitness of an organism. As a caution, the incompatibilities observed suggest that the success of planned efforts to treat mitochondrial diseases using mitochondrial donors may well depend on the competitive strength of the donor's mitochondrial genome.

Online Methods

Fly stocks

The *D. melanogaster* mutant alleles *mt:ND2^{del1}* and *mt:CoI^{T300I}* were previously described[1]. These alleles were present either alone, or on a double mutant genome *mt:ND2^{del1}* + *mt:CoI^{T300I}*. Flies homoplasmic for the *ATP6[1]* mitochondrial genome was kindly provided

by Dr. Michael Palladino (University of Pittsburgh, U.S.). *D. mauritiana* and *D. yakuba* flies were obtained from *Drosophila* species stock center, San Diego. Flies with different mitochondrial genomes were backcrossed to Canton S for 10–30 generations to homogenize the nuclear background. Other strains used included UAS-mito-PstI and nos-Gal4. The stocks were cultured at 18–25 °C on standard fly medium.

Establishment of heteroplasmic lines

Poleplasm transplantation was used to generate heteroplasmic flies as previously described[1]. For the *mt:ND2^del1* + *mt:CoI^T300I* / *ATP6[1]* line ('+' indicates alleles on the same genome and '/' indicates the co-residence of the two indicated genomes), *ATP6[1]* flies were used as the recipient during poleplasm transplantation in order to obtain lineages with high initial abundance of the *ATP6[1]* genome. Numerous female progeny (G0) from injected embryos were individually crossed to *mt:ND2^del1* + *mt:CoI^T300I* males for 2 days at 25 °C. After progeny collection, mothers were sacrificed for total DNA extraction and the proportion of *ATP6[1]* genome was estimated by qPCR as described below. The progeny (G1) of the mothers were either maintained at 25 °C, or shifted to 29 °C and maintained at 29 °C for multiple generations.

For the *D. yakuba*/*mt:ND2^del1* + *mt:CoI^T300I* line, cytoplasm from *D. yakuba* embryos was transplanted into the *mt:ND2^del1* + *mt:CoI^T300I* embryos and eclosed adults were kept at 29 °C to select for flies with the *D. yakuba* genome. By doing this, two independent lines were established and both stably transmitted *D. yakuba* mtDNA (~4%) from generation to generation at 29 °C. Subsequently, a mitochondrially-targeted restriction enzyme, mito-PstI, was expressed in the germline of the two heteroplasmic lines to eliminate the *mt:ND2^del1* + *mt:CoI^T300I* genome, as only the *D. melanogaster* mtDNA contain a PstI site. Through this, several lines with only wild-type *D. yakuba* mtDNA were established. The *D. mel (mito-yakuba)* line was then used a recipient for subsequent cytoplasm transplantations.

For lines heteroplasmic for *D. mauritiana* and *D. melanogaster* mtDNA, cytoplasm from *D. mauritiana* embryos was transplanted into *D. melanogaster* embryos homoplasmic for wild-type mtDNA, *mt:ND2^del1* + *mt:CoI^T300I*, or *mt:ND2^del1*. Several G0 mothers were crossed to wild-type mtDNA, *mt:ND2^del1* + *mt:CoI^T300I*, or *mt:ND2^del1* males respectively for 2 days at 25 °C to produce G1 females in order to establish independent lineages.

Phenotypic analysis of flies with different mitochondrial genotypes

Flies homoplasmic for *mt:ND2^del1* + *mt:CoI^T300I*, *ATP6[1]*, *mt:ND2^del1* and *D. yakuba* mitochondrial genome were backcrossed to Canton S males for at least 10 generations. To assay the lifespan, newly eclosed flies were separated by sex and over 80 flies of each sex were used to follow each population in each condition. To avoid crowding, these were housed at a density of 10 flies per vial or less throughout the analysis (Supplementary Table 1). The flies were transferred to fresh vials and survivorship was recorded every two days at both 25 °C and 29 °C. The climbing assay was performed as previously described[1]. Briefly, 20 flies (backcrossed for 30 generations) of various ages were transferred to a plastic cylinder (22 cm long, 1.5 cm diameter) with a mark 10 cm line from bottom. After 1 h for acclimation, the flies were knocked down to the bottom by gently tapping the tubes. The

time required for 50% of the flies to climb to the marked 10 cm line was recorded. Three trials were conducted for each group, and three groups were used for each genotype. For all the above phenotypic studies, individual flies were picked randomly and the climbing assay was performed blind to avoid cognitive bias in scoring. For statistical analysis, Log-rank test and unpaired Student's t-test was performed for the survivorship and climbing assay data, respectively, in order to compare flies homoplasmic with different mitochondrial genotypes.

DNA isolation

Total DNA was extracted from adults as described preiously[1]. Frequencies of mitochondrial genotypes were measured in individual founding females (G0) and their further generations via qPCR. When populations were analyzed, we extracted DNA from groups of 40 individuals.

Sequencing the *D. yakuba* mtDNA

Three long-range PCR reactions using Expand Long Template PCR system (Roche) were performed using the total DNA from *D. yakuba* and *D. mel* (*mito-yakuba*) as template: mt186 – 7519, mt7229 – 14797 and mt12822 – 400 with the following program: 1 cycle of 93 °C for 3 min, 30 cycles of 93 °C 15 s, 50 °C 30 s, 60 °C 8 min, and 1 cycle of 60 °C for 10 min. Primers were designed to give full coverage of the *D. yakuba* mitochondrial genome (Supplementary Table 2) for sequencing by QuintaraBio (Albany, CA).

qPCR Parameters

qPCR assays were performed as described previously[1]. Briefly, the total mtDNA copy number in heteroplasmic flies was measured by qPCR of a 52 bp region (mt361 – 412) present in all mtDNA genotypes (primer mt361F and mt412R, Supplementary Table 2). Primers cognate to the *mt:ND2* loci of specific genomes were designed to measure copy number of genomes with *ATP6[1]*, or *D. yakuba*, or *D. mauritiana* genomes without amplifying a product from the *D. melanogaster mt:ND2^{del1}* allele: these were used for qPCR of a 51 bp from the *mt:ND2* region of these genomes (See Supplementary Table 2 for primers). Standard curves were constructed using a series of 10-fold dilutions of purified PCR fragment containing both the common region and *ATP6[1]*, or *D. yakuba*, or *D. mauritiana mt:ND2* region. The efficiency of the 2 primer sets was normalized each time by comparing total mtDNA copy number estimated for the same wild-type DNA sample. qPCR was performed with the following reaction conditions: 95 °C for 10 min, 40 cycles of 95 °C 30 s and 48 °C 30 s. For each 20 μl qPCR reaction, 1% of a fly's total DNA was used as template. The Ct values used ranged from 13 to 33 and each reaction was repeated 3 times or more. To distinguish the *ATP6[1]* genome from the *D. yakuba* mtDNA, two different sets of primers were designed for the qPCR assay (Supplementary Table 2): mt6237F and mt6314R as the common primers; and mt6652F and mt6811R as primers specific for recognizing *D. yakuba* mtDNA.

Monitoring abundance of the *D. yakuba* mitochondrial genome during development

Four females heteroplasmic for *D. yakuba* and *mt:ND2^{del1}* + *mt:CoI^{T300I}* genomes were individually crossed (in separated vials) to *mt:ND2^{del1}* + *mt:CoI^{T300I}* males for 2 days at

29 °C. The mothers of each vial were transferred to new vials to collect eggs for 16 h at 29 °C. Subsequently, the mothers and half of the collected eggs were sacrificed to measure the relative abundance of *D. yakuba* genome via qPCR described above. The rest of the eggs were allowed to developed into late 3rd instar larvae at 29 °C before they were sacrificed to measure the relative abundance of *D. yakuba* genome. To examine the abundance of *D. yakuba* mtDNA in newly laid eggs when oogenesis occurs at 22 °C, late 3rd instar larvae were shifted from 29 °C to 22 °C, and the eggs were collected at 22 °C and sacrificed to measure the abundance of *D. yakuba* genome.

Quantitative analyses of *D. mauritiana* mitochondrial genome based on restriction cleavage

For lines heteroplasmic for *D. mauritiana* wild-type mtDNA and *D. melanogaster* wild-type mtDNA, the sequence difference between the two genomes did not allow performance of qPCR. *Thus, we* quantified the relative amount of genomes distinguished by restrictions enzyme cleavage site by comparing the ratios of diagnostic restriction fragments. Total DNA was collected from 40 adult flies of various heteroplasmic lines from generation 1 to generation 4 (for generation 0, total DNA was collected from the mother after it was mated with males at 25 °C for two days to lay eggs). A mtDNA region (mt11517 – 12529) was amplified by PCR (30 cycles of 95 °C 30 s, 50 °C 30 s and 60 °C 60 s). The PCR products were then digested completely with XhoI under the conditions recommended by the supplier (NEB). The digested DNA was separated by gel electrophoresis, and the ratio of cut and uncut DNA was estimated by measuring the intensity of bands using ImageJ.

Southern analysis

Southern blotting was used to detect the recombinant genome and monitor the length variation in the non-coding region of the mitochondrial genomes. It was performed as described in Ma and O'Farrell[17]. In brief, digested DNA was separated on a 0.8% agarose gel by electrophoresis and transferred to Hybond N+ membrane by the capillary method. The blot was hybridized with PCR-generated probes (mt1577 – 2365 or mt21 – 400, see Supplementary Table 2) that were labeled with DIG-11-dUTP.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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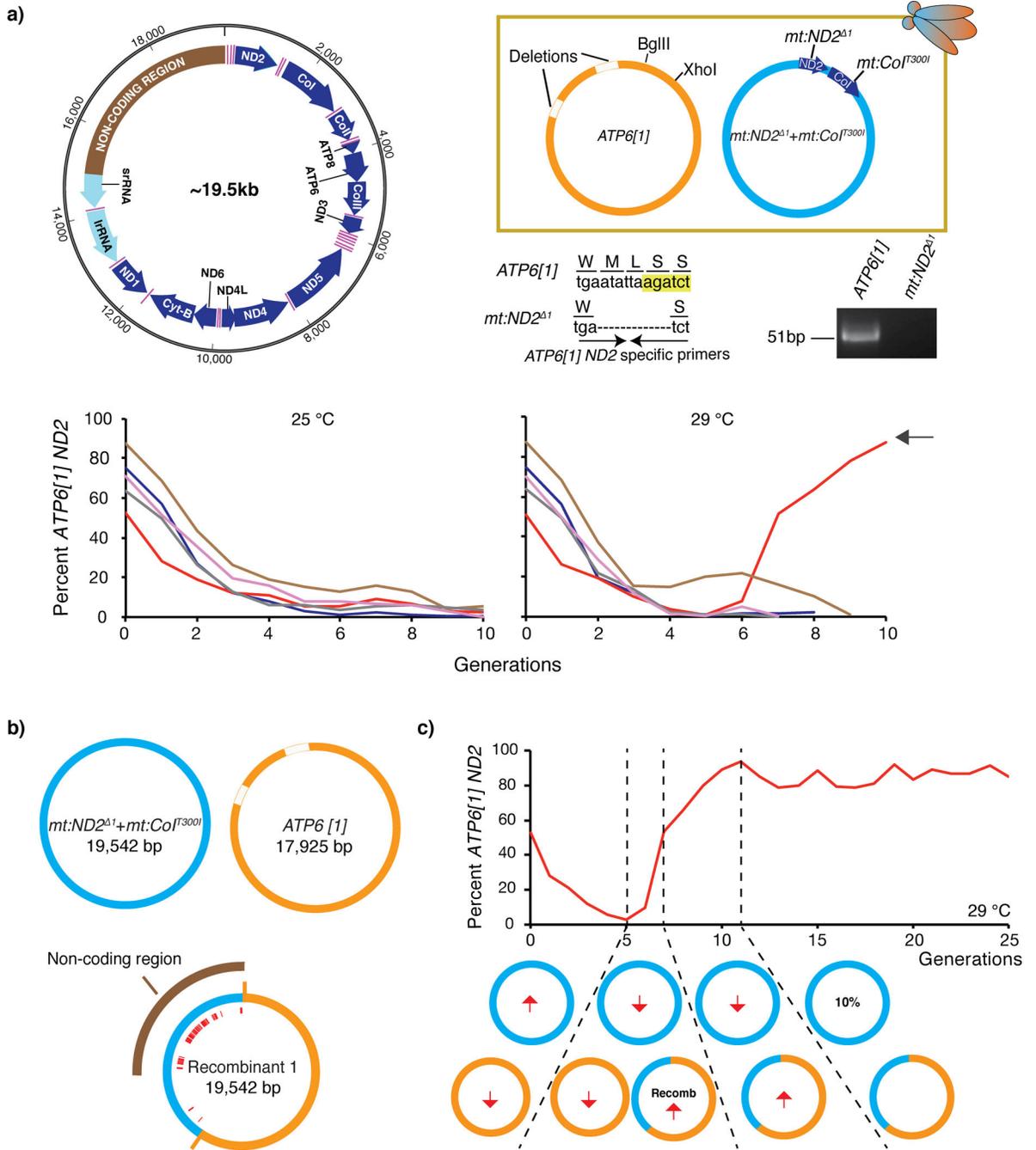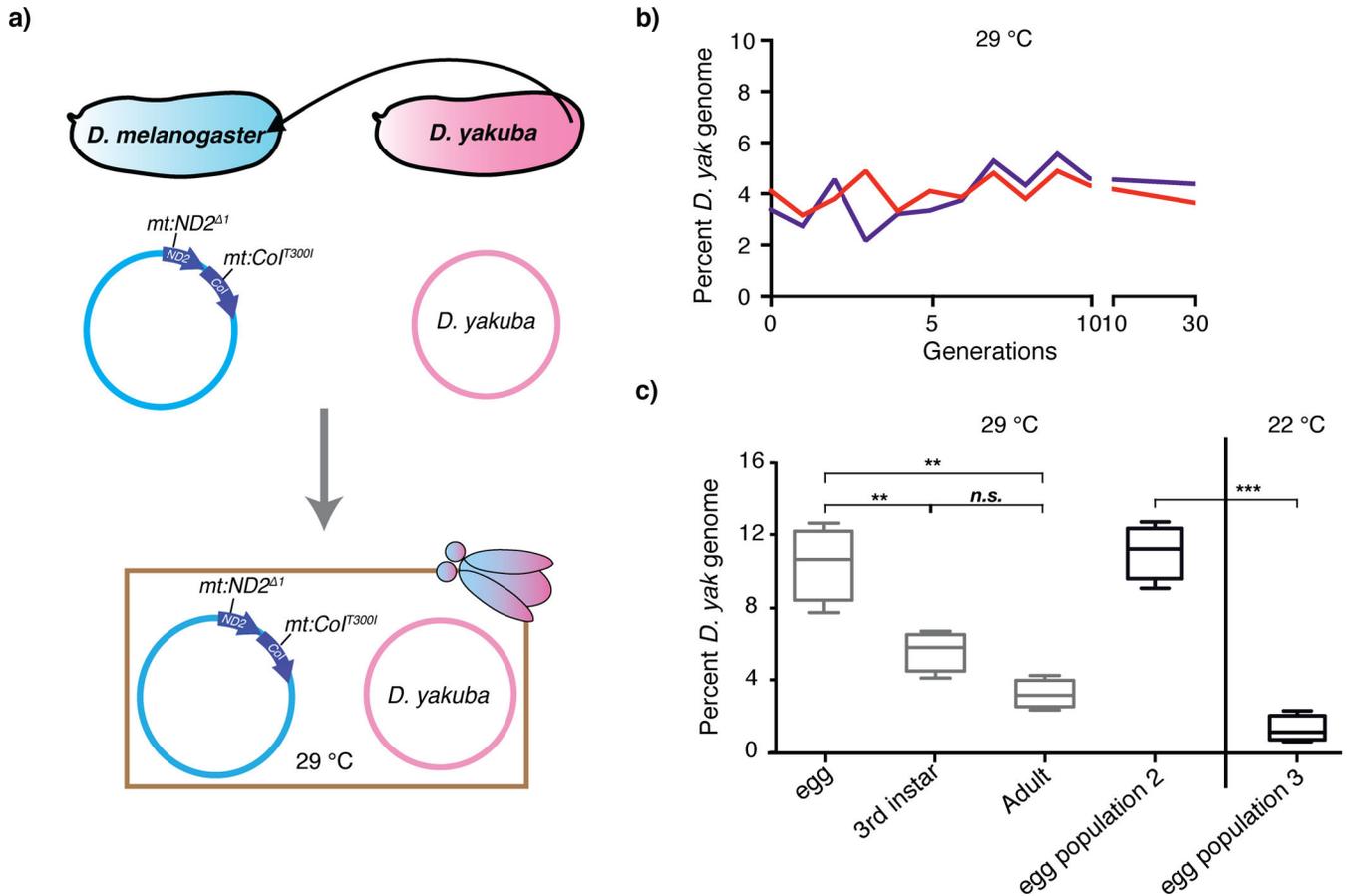
Figure 1.

Selection based on selfish drive in a heteroplasmic line containing the *ATP6[1]* genome and the temperature sensitive double-mutant: *mt:ND2^{del1}* + *mt:CoI^{T300I}*. **(a)** Decline of the *ATP6[1]* genome when co-existing with *mt:ND2^{del1}* + *mt:CoI^{T300I}*. A schematic (upper left) of *D. melanogaster* mitochondrial genome with protein coding genes (blue), rDNA loci (light cyan), tRNAs (pink) and the non-coding region (brown). Key features distinguishing the *ATP6[1]* and temperature sensitive genomes are indicated (upper right panel). A PCR primer set that selectively amplifies the intact *ND2* locus of the *ATP6[1]* genome is

indicated (BglII site, yellow highlight). The relative abundance of the *ATP6[1]* genome as assessed by qPCR for five lines maintained at 25 °C and 29 °C for multiple generations. After the *ATP6[1]* abundance fell to a low level (illustrated), the flies at 29 °C started to die (not shown), but in one line a few survivors expanded and showed an increasing abundance in a genome with the *ATP6[1] ND2* region (red line, black arrow). **(b)** The map of the recombinant genome sequenced by PacBio SMRT technology. Red lines indicate the distribution of SNPs characteristic of *mt:ND2^{del1}* + *mt:CoI^{T300I}* genome that are present in the recombinant. The *ATP6[1]* genome also lacks ~1.6 kb of the non-coding region[17]. **(c)** The transmission of the recombinant genome was favored when paired with the temperature sensitive genome. The directional arrows indicate how the abundance of a particular genotype was increasing or decreasing at any given generation.

Figure 2.

Stable transmission of the *D. yakuba* mtDNA in the *D. melanogaster* nuclear background. **(a)** A heteroplasmic line was established by transferring cytoplasm of *D. yakuba* embryos into embryos carrying the *mt:ND2del1* + *mt:CoIT300I* genome. **(b)** The proportion of *D. yakuba* mtDNA was maintained at ~4% for over 30 generations in two independent heteroplasmic lines at 29 °C. **(c)** The abundance of the *D. yakuba* mtDNA oscillated during development: high in newly deposited eggs, declined during development and rose in oogenesis to reach a high level in eggs again at 29 °C. The first three entries come from analysis of different stages of the lifecycle across one generation. As expected for a stably propagated stock, an analysis of eggs collected at a different time (egg population 2) gave the same relative abundance for the *S. yakuba* genome. However, when mothers were shifted to 22 °C at the end of 3rd instar larval stage so that oogenesis occurred at the permissive temperature, the eggs laid had a reduced abundance of the *D. yakuba* genome (egg population 3). Results are means ± SD (n = 4 for each data point). Unpaired Student's t-test was performed to compare the difference in the abundance of *D. yakuba* mtDNA between newly deposited eggs, 3rd instar larvae and adult flies (** = $p < 0.01$, *** = $p < 0.001$).
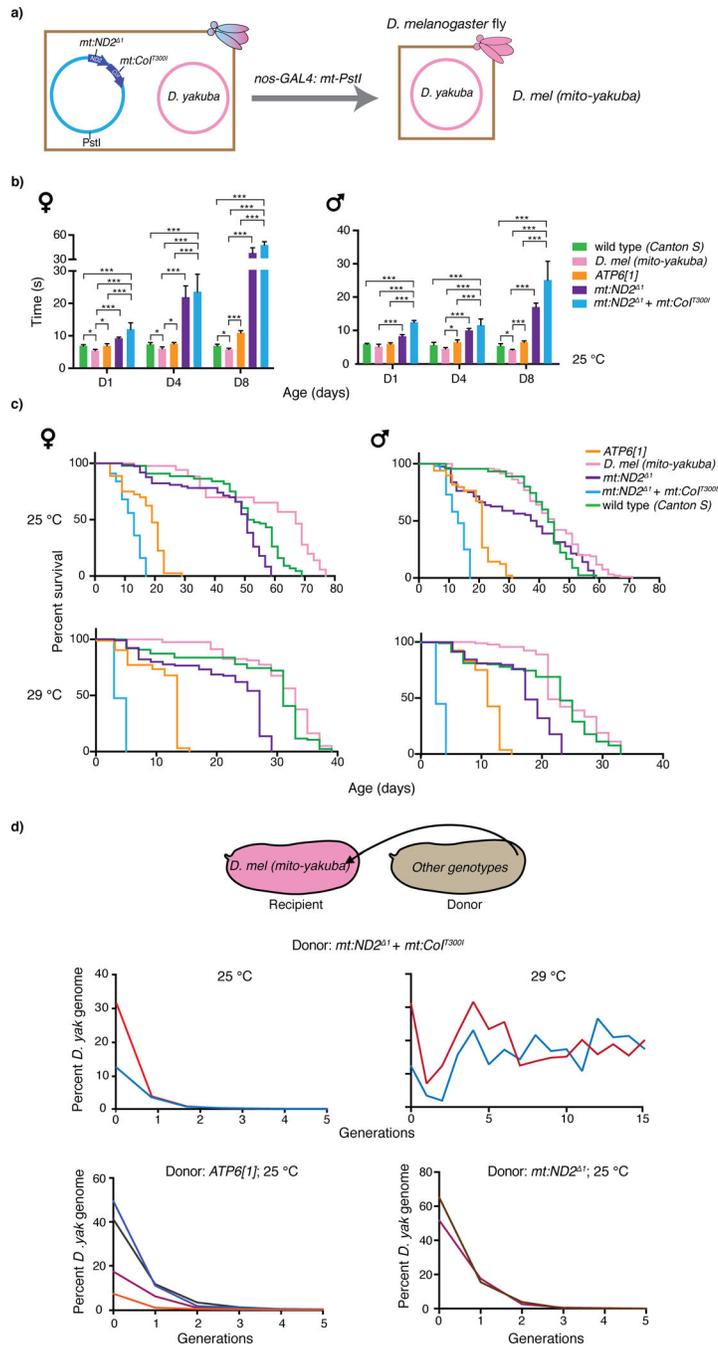
Figure 3.
Cross species analysis of functional conservation and competitive strength of mitochondrial genomes. **(a)** The *D. melanogaster* genome was eliminated from a heteroplasmic line by expressing PstI that is targeted to mitochondria. **(b)** *D. mel* (mito-*yakuba*) flies climb faster than *D. melanogaster* flies carrying various native mitochondrial genomes. Time (means ± SD, n = 3) required for 50% of flies (25 °C) of the indicated age (D = days) and sex to climb to a prescribed height after being gently knocked down was recorded. Significance of differences is based on unpaired Student's t-test (* = $p < 0.05$, ** = $p < 0.01$, *** = $p <$

0.001). **(c)** Lifespan of congenic flies with different mitochondrial genotypes at 25 °C and 29 °C. Survivorship was recorded every two days (n > 80, see Supplementary Table 1a). The *D. yakuba* mitochondrial genome supports robust survival that exceeds that supported by several native genomes (see Supplementary Table 1b & c for statistical analysis). **(d)** The *D. yakuba* mitochondrial genome was quickly outcompeted by various *D. melanogaster* genomes at 25 °C. Native mitochondrial genomes were introduced into the *D. mel* (mito-*yakuba*) line, and the relative abundance of the *D. yakuba* genome was followed over generations by qPCR (see Online Methods). The differently colored lines represent independently produced heteroplasmic lines. The *D. yakuba* mtDNA was only maintained when partnered with the temperature sensitive genome at 29 °C.