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Title: Selfish drive trumps function when animal mitochondrial genomes compete

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Abstract

A poorly understood competition among mitochondrial genomes determines which genomes populate cells, and which are transmitted. We introduced mitochondrial genomes from distant strains or species into *D. melanogaster* embryos. In several pairings, a functionally compromised genome, apparently benefitting from an overpowering selfish drive, outcompeted an uncompromised genome for transmission. In some of these cases, selfish drive led to the complete elimination of the functional genome over several generations with lethal consequences, while in others the advantage conferred by selfish drive was counterbalanced by a functional advantage in the other genome, resulting in stable transmission of two mitochondrial genomes. By isolating recombinant mitochondrial genomes, we mapped selfish-drive to the non-coding region that includes the origins of replication. We suggest that mitochondrial genomes evolve under the influence of both a purifying selection[1-5] that conserves function in the coding regions, and a selfish selection for enhanced replication that promotes divergence of noncoding sequences. Uniparental inheritance isolates mitochondrial genomes in distinct lineages, and divergence of selfish drive proceeds independently within each lineage. The differences in selfish drive can have profound consequences when genomes of different lineages are combined as we have done here, and as planned in the treatment of human mitochondrial diseases[6-8].

Natural selection culls populations of compromising mutations and favors traits that enhance organismal fitness. If all else is equal, this selection guides evolution. However, transmission can be an unfair game. Selfish genetic elements, which enhance their own transmission relative to the rest of an individual's genome, can arise and spread even if neutral or damaging[9-22]. Transmission of nuclear genes is managed by a segregation system that limits opportunities for selfish behavior, biasing evolution toward natural selection. In contrast, mitochondrial genomes have a 'relaxed' mode of replication and random segregation. These differences increase opportunities for the spread and persistence of selfish mitochondrial variants. For example, in *S. cerevisiae*, where the mitochondrial genomes are inherited from both parents, preferential inheritance of hypersuppressive petite mtDNA has been reported apparently due to preferential replication[23-25]. In multicellular organisms, variant mitochondrial genotypes arising by mutation will be favored by within-organism selection if they have increased replication, or if they provide cells a proliferative advantage[26]. If such variant genomes arise in the germline, gains made in the individual will lead to preferential transmission that will provide an evolutionary drive. This evolutionary drive is somewhat contained by uniparental inheritance, which prevents spread of such successful genomes beyond the lineage in which they arise[27]. Nonetheless, within each lineage mitochondrial genomes that outcompete neighbors should overtake and succeed. However, despite expectation that selfish drive would be an influential factor in mtDNA evolution, reported examples of selfish mtDNA for transmission are extremely rare in animals[28,29]. Additionally, little is known about how the interplay of selections based on function and selfish drive influences the persistence and abundance of defective mitochondrial genomes in an organism and in a population[30-32].

Here, by making heteroplasmic flies carrying diverged *Drosophila* mitochondrial genomes, we show direct evidence that selection based on 'selfish drive' can promote destructive gains in the prevalence of a functionally defective genome. We then mapped the selfish drive to the non-coding region of the mitochondrial genome by isolating relevant recombinant genomes. Moreover, we show that the selfish-drive can interact with purifying selection to maintain a defective genome in a population in partnership with a functional genome (balanced heteroplasmy). These results suggest that selfish drive is an important factor defining the trajectory of mitochondrial genome evolution.

We first observed selection based on selfish drive when analyzing the segregation behavior of marked genome with a temperature sensitive allele of the cytochrome oxidase I gene, $mt{:}ND2^{del1}$ + $mt{:}CoI^{T300I}$ that we refer to as the temperature sensitive genome (Figure 1). Previously, we and another lab showed that when the temperature sensitive genome was combined with a closely related wild type genome, its abundance declined over generations at restrictive temperature and this purifying selection was due to competition among mitochondrial genomes during oogenesis[2,4]. Unexpectedly, when the temperature sensitive genome was partnered with the *ATP6[1]* genome, a diverged *D. melanogaster* genome distinguished by numerous sequence polymorphisms and a

shorter AT-rich region, the temperature sensitive genome completely displaced the *ATP6[1]* genome after several generations at either 25°C or 29°C (Figure 1A)[33]. This occurred despite the fact that flies homoplasmic for *ATP6[1]* are relatively healthy, and apparently more robust and fertile than flies with the temperature sensitive genome at either temperature (Figure 1B and [34]). At 29°C, the loss of the *ATP6[1]* genome led to a crisis: as long as the *ATP6[1]* genome was modestly abundant, the population expanded because the *ATP6[1]* genome provides *wild type mt:CoI* function, but in subsequent generations all the flies died as the functional *ATP6[1]* genome disappeared. We conclude that the temperature sensitive genome achieves a selective advantage without providing an advantage to the organism.

Since the *ATP6[1]* genome is distinguished by numerous sequence polymorphisms and a shorter AT-rich region, its failure to thrive in heteroplasmic combination could be attributed to any of these differences. As described in a separate communication, we have shown that recombination among mitochondrial genomes occurs[33]. The death of the heteroplasmic stocks provided a selection for recombinants carrying the drive region of the temperature sensitive genome and the functional *mt:CoI* allele of the *ATP6[1]* genome. When five heteroplasmic lines were followed at 29°C, one line gave surviving progeny that contained a recombinant genome (Figure 1B). The recombinant genome contained the majority of the *ATP6[1]* coding sequence including the functional *mt:CoI* allele, and the entirety of the non-coding segment plus a small segment of flanking coding sequence from the temperature sensitive genome (Figure 1C)[33]. This combination of sequences endowed the recombinant with an ability to compete well for maintenance, as well as providing function: traits that allowed it to persist and ultimately increase in abundance to become the dominant genome (Figure 1D). Later, we isolated another recombinant with the entire coding sequence derived from the *ATP6[1]* genome but with a restriction fragment length characteristic of the regulatory region from the temperature sensitive genome (Figure S1A). Southern analysis and qPCR showed that this recombinant also became the dominant genome in later generations (Figure S1B & C). Together these data show that non-coding sequences from the temperature sensitive genome are sufficient to endow the recombinant with strong drive. 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, which contains the origins of replication, is the most variable region in the mitochondrial genomes of many species[35-38]. The *D. melanogaster* version is super AT-rich (>90%) and especially large with five tandem type I repeats and four tandem type II repeats that make up > 90% of its ~4.6 kb extent[39] (Figure S2A). Mitochondrial genomes in this species not only exhibit frequent nucleotide changes in this region, but also exhibit length polymorphisms (e.g. Figure S2A and [40,41]). Other *Drosophila* species also show extensive divergence in the regulatory region (Figure S2B and [42,43]). We introduced mitochondrial genomes from other *Drosophila* species into *D. melanogaster* and examined their ability to compete.

We first made a heteroplasmic line containing mtDNA from both *D. melanogaster* and *D. yakuba*, species that diverged ~10 mya. We introduced cytoplasm of *D. yakuba* embryos into *D. melanogaster* embryos carrying the temperature sensitive genome (Figure 2A), but the efficiency of retaining the *D. yakuba* mitochondrial genome after transfer was very low and no stable lines were recovered. Thus, we applied selection against the temperature sensitive genome to select for flies retaining the *D. yakuba* genome. Among the 50 injected females that were kept at 29°C, two produced viable and fertile progeny. These progeny gave two lines that maintained the *D. yakuba* genome in heteroplasmic combination with the persisting temperature sensitive genome. The *D. yakuba* genome was carried at a low but stable level (<5%) for many generations as long as temperature selection against the temperature sensitive genome was maintained (Figure 2B).

Maintenance of both genomes appears to be due to a balance of two selections: The *D. yakuba* genome gains a selective advantage at 29°C because it provides a functional *mt:CoI* gene, but apparently another factor gave the *D. melanogaster* genome an advantage that allowed it to persist despite its functional deficit at the high temperature. In accord with this balance of selections, *D. yakuba* mtDNA disappeared from these lines within two generations at permissive temperature. From previous work, we know that selection against the temperature sensitive genome occurred mainly during oogenesis at restrictive temperature[2]. If, in contrast to this time-limited selection for function, the selective advantage of the *D. melanogaster* mitochondrial genome acted through the life cycle, we might see oscillations in the relative abundances of the two genomes. In particular, after enjoying the selection for function during oogenesis, we expected the *D. yakuba* genome to be at a high level of abundance in newly deposited eggs. Indeed, the proportion of *D. yakuba* genome in heteroplasmic lines oscillated within one generation: it was highest in newly deposited eggs and then decreased during development, only to increase again during oogenesis (Figure 2C), suggesting that the competition between the two genomes played out, at least in part, during somatic growth. We propose that the advantage incurred by selfish drive can influence competition at many stages of the life cycle, while the functional selection is more restricted to times during oogenesis. This temporal distinction might be responsible for previous observations of oscillations in the relative abundance of heteroplasmic genomes[44].

Since the *D. yakuba* genome had no PstI recognition site, whereas the temperature sensitive genome had a PstI site at mt7496, we expressed a mitochondrially-targeted PstI in the germline of the heteroplasmic lines to completely eliminate *D. melanogaster* mtDNA (Figure 3A). Interestingly, flies homoplasmic for *D. yakuba* genome (called *D. mel* (*mito-yakuba*)) were not only viable but also as healthy as *wild type* flies at various temperatures (Figure 3B). This was surprising because various examples of nuclear-mitochondrial incompatibility have been described[45-48], and thus we initially thought that the selective disadvantage of the *D. yakuba* genome was the result of functional deficits resulting from a mismatch between the nuclear genes and mitochondrial genes

contributing to electron transport. Instead, the finding that the *D. yakuba* mitochondrial genome worked well once we had eliminated the *D. melanogaster* genome showed that the only substantial defect of the *D. yakuba* genome was in its ability to compete with the endogenous genome.

Another cross species combination of mitochondrial genomes gave a very different type of result. When we introduced a *D. mauritiana* (diverged ~2mya) mitochondrial genome (*maI*) into *D. melanogaster* flies, *D. mauritiana* genome completely replaced the *D. melanogaster* genomes within a few generations at 25°C (Figure S3). A similar observation has been made when a different *D. melanogaster*/*D.mauritiana* heteroplasmic line was generated[49]. Thus, while one would expect the *D. melanogaster* mitochondrial genome to be optimized for function in its resident background, it was the weaker competitor, again suggesting that competitive success might not be based entirely on function.

To further examine the basis of competition, we re-introduced *D. melanogaster* mitochondrial genomes into the *D. mel* (*mito-yakuba*) line. All of several *D. melanogaster* genomes tested outcompeted the *D. yakuba* genome, even the relatively poor *D. melanogaster* competitor genome, *ATP6[1]* (Figure 3C). This is not selection based on function because *D. melanogaster* genomes that took over (*e.g.* the temperature sensitive genome, *mt:ND2*^{del1}, and the *ATP6[1]* genomes) gave lines that were far less healthy than *D. mel* (*mito-yakuba*) flies (Figure S4 and [34,50,51]). The *D. yakuba* genome appeared to have an intrinsic replicative/transmission disadvantage, perhaps associated with its diverged and shorter (~1kb) non-coding region. It should be noted that competitive strength of *D. yakuba*, *ATP6[1]* and the temperature sensitive genome do not fall on a simple hierarchy: the *D. yakuba* genomes is displaced by *ATP6[1]*, suggesting that it is weaker, yet, when these genomes are paired with the temperature sensitive genome, only the *D. yakuba* genome can be maintained at high temperature, suggesting that it is the more successful competitor. This suggests competitive strength is determined by more than the potency of a single factor or interaction.

We observed some adaptation in the *D. mel* (*mito-yakuba*) line after several generations. Re-introducing the temperature sensitive genome at the restrictive temperature gave balanced heteroplasmy with a higher ratio of *D. yakuba* genome compared to the two original lines (Figure 3C). Since the sequence of the *D. yakuba* mitochondrial genome remained unchanged (data not shown), the adaptation is likely due to nuclear modifiers. This finding shows that nuclear genes modulate the ability of mitochondrial genomes to succeed in competition. It will be interesting to explore the nature of this interaction between nuclear and mitochondrial genomes.

While the mechanism of selfish drive is unknown, its localization to the non-coding region constrains the possibilities. The non-coding sequences are not likely to influence drive by complex actions such as evasion of mitophagy or localizing mitochondria to the

germline. Furthermore, since selfish drive is independent of function, control of the transcription of the coding sequences, which would affect function, is not likely to be responsible. In contrast, the non-coding region has a direct involvement in replication. The noncoding region includes the two origins for asymmetric replication of the mitochondrial DNA (Figure S2A). These origin sequences are substantially conserved, and are associated with repeat sequences. Polymorphisms in repeat number and nucleotide changes distinguish the genomes examined in this study (Figure S2A). Little is known about the control of mitochondrial replication in *Drosophila*; nonetheless, we might anticipate that sequences throughout the control region would contribute to replication functions such as copy control, primer synthesis and initiation efficiency. Even subtle changes could result in a large competitive advantage because differences in replication would be amplified over many rounds of genome doubling. We thus hypothesize that selfish drive can be equated with replicative drive.

If selfish selection prevails in the non-coding regions of mitochondrial genomes, while purifying selection is more pronounced in the coding regions, the evolutionary trajectory of these two regions might differ. Indeed, variations in the length of mitochondrial genomes within large laboratory fly populations has been attributed to differences in the non-coding region where longer variants, presumably with an increased number of repeat sequences, exhibited preferential transmission[41]. In addition, recurrent mutations at specific sites in the non-coding region related to replication were found to occur independently in multiple somatic tissues and individuals in humans[26]. Comparisons among related species also show that non-coding regions of mitochondrial genomes evolve much more rapidly than the coding region[52-54]. These distinctive behaviors of non-coding sequences could be partially explained by a positive selection for variants with increased selfish drive. In contrast, purifying selection would preserve the sequences of coding sequences. Thus, the divergence pattern of the mitochondrial genome is consistent with the idea that selfish selection and purifying selection have largely distinct targets, non-coding and coding sequences, respectively.

Figure Legends

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 base pairs indicated on the outer circle. Protein coding genes are indicated in red, rDNA loci in green and the non-coding regulatory region in brown. The key features distinguishing the *ATP6[1]* and temperature sensitive genome are indicated (upper right panel) and a PCR primer set that selectively amplifies the intact *ND2* locus of the *ATP6[1]* genome is indicated. The relative abundance of the *ATP6[1]* genome as assessed by qPCR with specific and general primers is shown (lower panels) for five lines maintained at 25°C and 29°C for multiple generations. After the *ATP6[1]* abundance fell to low level, the flies at 29°C started to die, except for one lineage (red line), which not only survived at the restrictive temperature, but also showed an increasing abundance in a genome with the *ATP6[1] mt:ND2* region. B) Phenotypic analysis of flies homoplasmic for either the *ATP6[1]* or *mt:ND2*^{del1}+*mt:CoI*^{T300I} genome. Note that for a wild type *Canton S* stock, mean survival was about 50 days (Figure 3). Survivorship was recorded every two days at both temperatures. For the climbing test, the time required for 50% of the flies in a population of the indicated age (growing at 25°C) and sex to climb to a prescribed height in a graduated cylinder after being gently knocked down to the bottom was recorded. By day 8, *mt:ND2*^{del1}+*mt:CoI*^{T300I} flies were more or less immobilized for a long time after they were knocked to bottom of the cylinder, so the data were not included in the graph. Results are means ± SD (n = 3 for each data point). C) 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 (two type I repeats and two type II repeats)[33]. D) 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* mitochondrial genome in the *D. melanogaster* nuclear background. A) A heteroplasmic line was established by transferring cytoplasm of *D. yakuba* embryos into embryos carrying the *mt:ND2*^{del1} + *mt:CoI*^{T300I} 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 *D. yakuba* mtDNA was highest in newly deposited eggs and then decreased during development in four independent lines at 29°C. Results are means ± SD (n = 3 for each data point).

Figure 3: Conservation in function of the mitochondrial genome cross-species, but divergence in the ability to compete. A) The *D. melanogaster* genome was eliminated

from a heteroplasmic line by expressing PstI that is targeted to mitochondria. B) The lifespan and the climbing ability of the *D. mel* (*mito-yakuba*) line are similar to *D. melanogaster* flies with the *wild type* mtDNA. C) *D. yakuba* mitochondrial genome was quickly outcompeted by various *D. melanogaster* genomes at 25°C. After establishment of the *D. mel* (*mito-yakuba*) line, cytoplasm transplantation was performed using *mt:ND2*^{de1l}*+mt:CoI*^{T300I}, *ATP6[1]* and *mt:ND2*^{de1l} embryos as donors and the relative abundance of the *D. yakuba* genome was followed over generations by qPCR (see methods). The differently colored lines represent independently produced heteroplasmic lines: these vary in starting abundance of the *D. yakuba* mtDNA, which reflects the degree of success of the cytoplasmic transfer. The *D. yakuba* mtDNA was only maintained when partnered with the temperature sensitive genome at 29°C.

Online Methods

Fly stocks

The *D. melanogaster* mutant alleles *mt:ND2*^{del1} and *mt:CoI*^{T300I} were previously described [2,55]. 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 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 out-crossed to *Canton S* for 10 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 and the method was described in [2]. 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 *mt:ND2*^{del1} + *mt:CoI*^{T300I}/*mt:D. yak* 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* mitochondrial genome (~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* mitochondrial genome 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 the *mt:ND2*^{del1} + *mt:CoI*^{T300I}/*mt:D. mau* line, cytoplasm from *D. mauritiana* embryos was transplanted into the *mt:ND2*^{del1} + *mt:CoI*^{T300I} or *mt:ND2*^{del1} embryos. Several G0 mothers were crossed to *mt:ND2*^{del1} + *mt:CoI*^{T300I} males 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 10 generations. To assay

the lifespan, newly eclosed flies were separated by sex and around 10 flies were placed in one vial. The flies were transferred to fresh vials and survivorship was recorded every two days at both 25°C and 29°C. At least 100 flies were used to plot the longevity curve. The climbing assay was performed as described in Ma et al[2]. Basically, 20 flies 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 phenotypical studies, individual flies were picked randomly and no blinding was done.

DNA isolation:

Total DNA was extracted from adults as described in Ma et al[2]. 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 3min, 30 cycles of 93°C 15s, 50°C 30s, 60°C 8 min, and 1 cycle of 60°C for 10 min. Primers were designed all around the *D. yakuba* mitochondrial genome (Table S1) for sequencing by QuintaraBio (Albany, CA).

qPCR Parameters:

qPCR assays were performed as described in Ma et al[2]. Basically, the total mtDNA copy number of heteroplasmic flies was measured by qPCR of a 52 bp region (mt361-mt412) present in all mtDNA genotypes (primer mt361F and mt412R, Table S1). To measure copy number of genomes with *ATP6[1],* or *D. yakuba*, or *D. mauritiana* allele of *mt:ND2* in the presence of $mt{:}ND2^{del1}$, qPCR of the 51 bp *ATP6[1],* or *D. yakuba*, or *D. mauritiana mt:ND2* region was performed (See Table S1 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. To distinguish the *ATP6[1]* genome from the *D. yakuba* mtDNA, two different sets of primers were designed for the qPCR assay (Table S1): mt6237F and mt6314R as the common primers; and mt6652F and mt6811R as primers specific for recognizing *D. yakuba* mtDNA.

Monitoring abundance of *D.yakuba* 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. Subsequently, the mothers and half of the collected eggs were sacrificed to measure the abundance of *D. yakuba* genome via qPCR described above. The rest of the eggs were allowed to developed into late 3rd instar larvae before they were sacrificed to measure the abundance of *D. yakuba* genome.

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[33]. Basically, 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 Table S2) that were labeled with DIG-11-dUTP.

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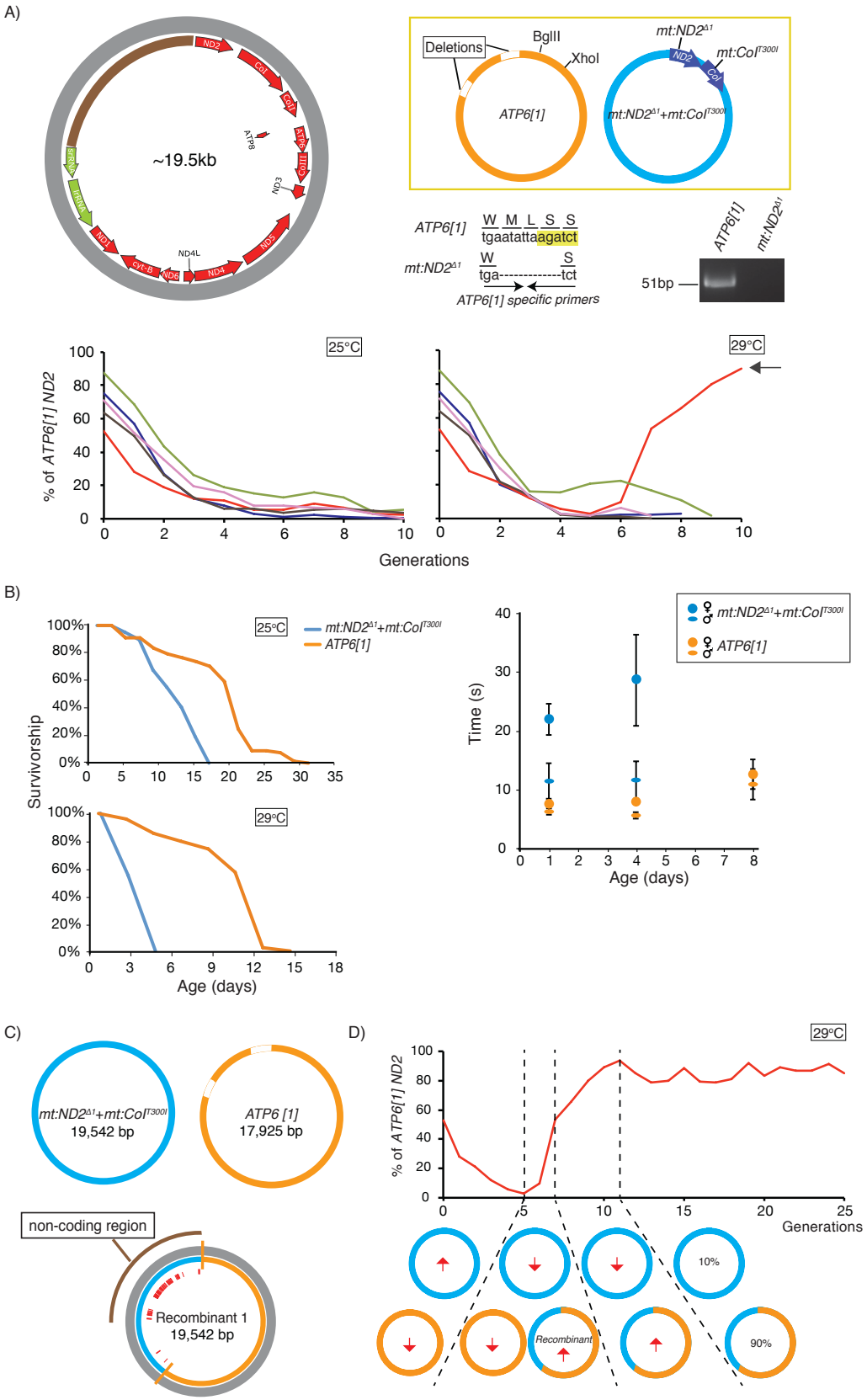
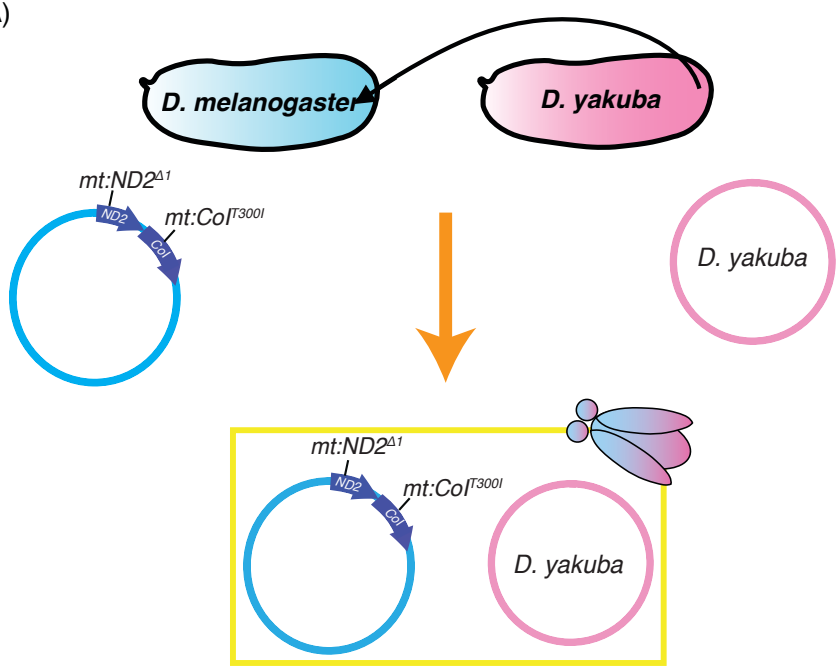
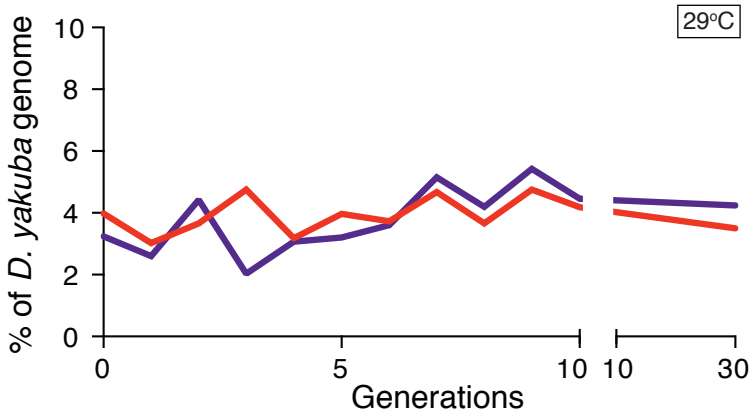

A)
~19.5kb
ND2
COI
COII
ATP8
ATP6
COIII
ND3
ND5
ND4
ND4L
ND6
CYT-B
ND1
16SrRNA
12SrRNA
Deletions
BglII
XhoI
ATP6[1]
mt:ND2Δ1
mt:ColT300I
mt:ND2Δ1+mt:ColT300I
ATP6[1] W M L S S tgaatattaagatct
mt:ND2Δ1 W S tga----------tct
ATP6[1] specific primers
51bp
25°C
29°C
% of ATP6[1] ND2
Generations
B)
25°C
29°C
mt:ND2Δ1+mt:ColT300I
ATP6[1]
Survivorship
Age (days)
Time (s)
C)
mt:ND2Δ1+mt:ColT300I
19,542 bp
ATP6 [1]
17,925 bp
non-coding region
Recombinant 1
19,542 bp
D)
29°C
% of ATP6[1] ND2
Generations
10%
Recombinant
90%

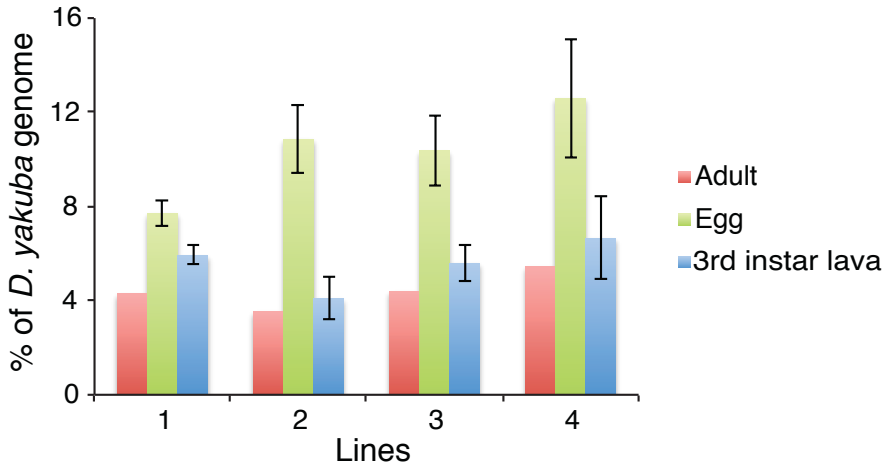
A)

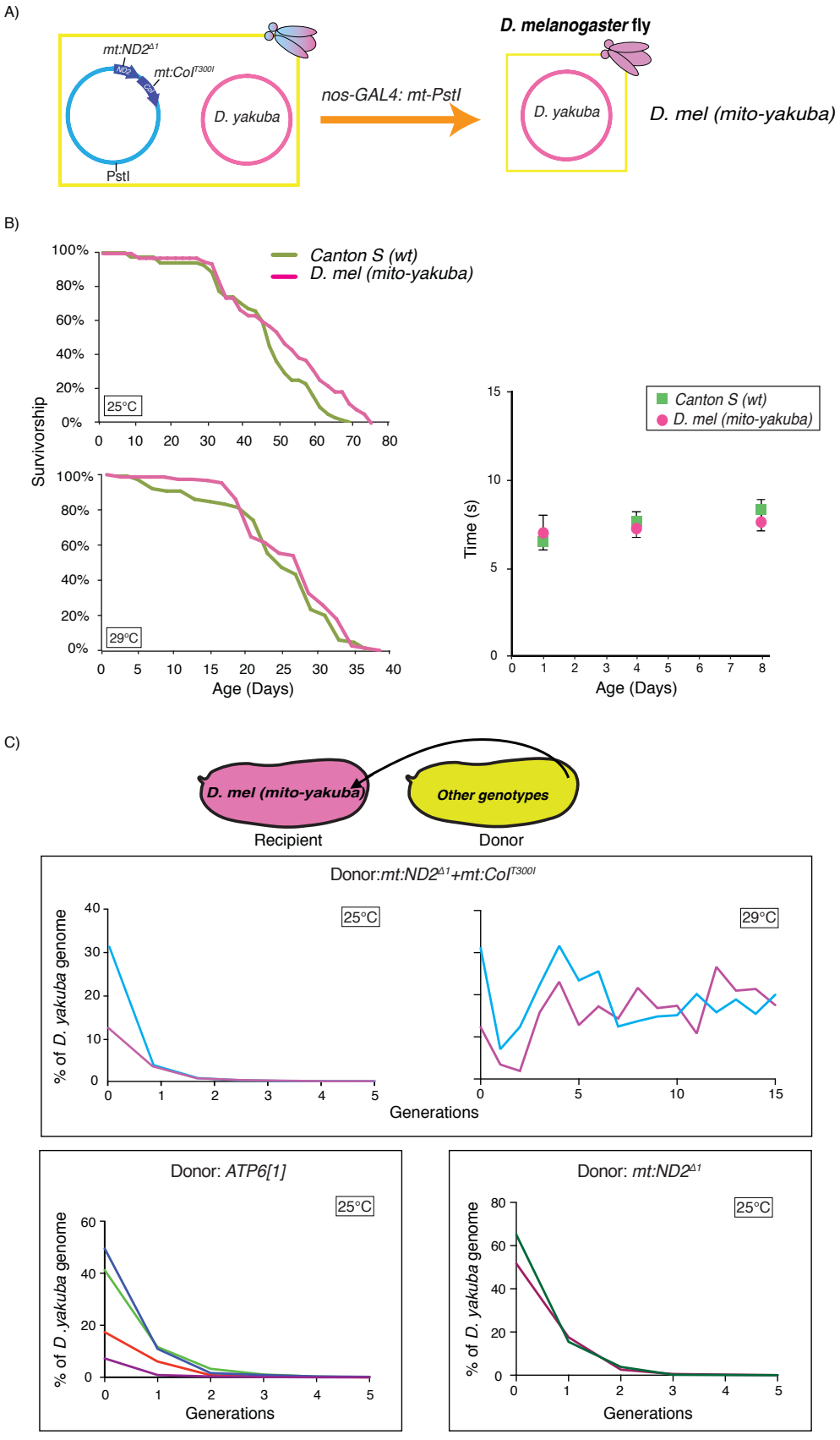

D. melanogaster
D. yakuba
mt:ND2Δ1
mt:ColT300I
ND2
Col
D. yakuba
mt:ND2Δ1
mt:ColT300I
ND2
Col
D. yakuba

B)


29°C
% of D. yakuba genome
0
2
4
6
8
10
0
5
10
10
30
Generations

C)


% of D. yakuba genome
0
4
8
12
16
1
2
3
4
Lines
Adult
Egg
3rd instar lava


A)
mt:ND2Δ1
mt:ColT300I
ND2
COI
PstI
D. yakuba
nos-GAL4: mt-PstI
D. melanogaster fly
D. yakuba
D. mel (mito-yakuba)
B)
Canton S (wt)
D. mel (mito-yakuba)
Survivorship
25°C
29°C
Age (Days)
Time (s)
Age (Days)
C)
D. mel (mito-yakuba)
Other genotypes
Recipient
Donor
Donor:mt:ND2Δ1+mt:ColT300I
% of D. yakuba genome
25°C
29°C
Generations
Donor: ATP6[1]
Donor: mt:ND2Δ1