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Περιβαλλοντική Βιολογία ENVIRONMENTAL BIOLOGY

Consequences of mito-nuclear interactions in life history traits of zebrafish

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Introduction

The most important function of mitochondria is oxidative phosphorylation (OXPHOS). They also participate in other functions such as cell death, signalling, synthesis of amino acid, nucleotides and lipids, ion homeostasis, cell motility and proliferation. Hence, mutations in mtDNA have been associated with severe human disorders. These diseases are quite common and can be severe, eventually leading to death. At the moment, they cannot be cured. They only can be prevented with mitochondrial replacement therapy (MRT).

Even though MRT is a promising technique, questions have been raised about the potentially harmful genetic effects of mismatch mito-nuclear interactions (combination of the recipient's nuclear genome with the donor's mitochondrial genome) and heteroplasmy (remains of a small amount of mutated mtDNA in the oocyte along with the wild type mtDNA). These questions need more research with an experimental model system that will be closer to mammals, but will be much easier to handle. Zebrafish (*Danio rerio*) is a particularly suitable candidate for this role because of its easy maintenance and continuous, and almost infinite, supply of eggs.

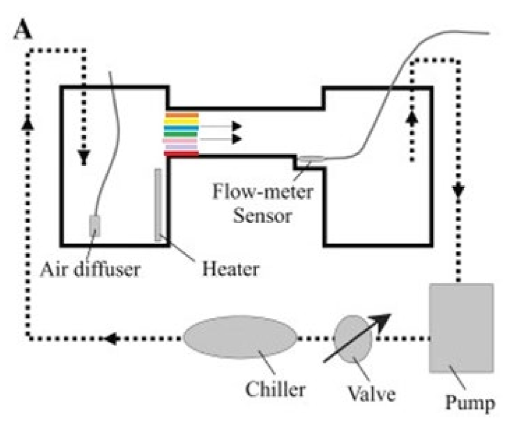
Experimental design

6 strains of *Danio rerio*:
AB, SJD, TL, WIK, Wag, Casper

3 species of genus Danio:
Danio nigrofasciatus
Danio albolineatus
Danio aesculapii

genetic loci	primers	sequence (5'-3')	size of DNA part
16sRNA	16SAR	CGCCTGTTTATCAAAAACAT	614
	16SBR	CCGGTYTGAACTCAGATCAYGT	
COX1	LCO1490	GGTCAACAAATCATAAAGATATTGG	708
	HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	

- 15 pairs: 5AB, 5SJD, 5(♀SJD x ♂AB)
- ~100 eggs/strain
- ~ 20 days rearing (length ~ 10-12 mm)
- placed in the swimming tunnel for 15 min at 2 TLs^{-1} water velocity increased every 15 min at a rate of 3 TLs^{-1}
- critical swimming speed measurements



- 20 pairs: 4AB, 4SJD, 4(♀SJD x ♂AB)
- 57-60 eggs/pair
- 8-24hpf: removing unfertilized and dead eggs
- 48hpf: video for heart rate measurements, photos of hatched larvae every 30 mins for total length at hatching measurements
- mortality and hatching rates measurements

Table 1. Estimations of evolutionary divergence in mitochondria between sequences of 4 strains and 2 species. The number of base differences per site from between sequences are shown.

	strain/species	1	2	3	4	5
1	*D. rerio* AB					
2	*D. rerio* TL	0,23%				
3	*D. rerio* WIK	0,71%	0,67%			
4	*D. rerio* SJD	0,75%	0,71%	0,04%		
5	*D. nigrofasciatus*	6,43%	6,47%	6,77%	6,82%	
6	*D. albolineatus*	25,09%	25,04%	25,08%	25,14%	25,28%

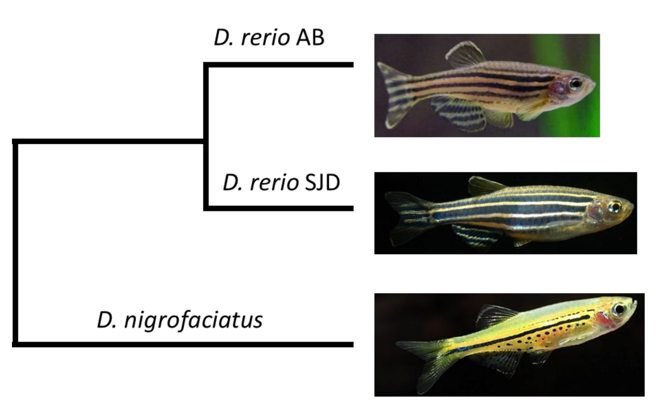


Figure 1. Morphological view of strains and species which were selected for further research with designated genetic distances.

Purpose of research

This thesis contains the first steps in using zebrafish as an experimental model for studying mito-nuclear interactions and heteroplasmy. These steps include:

- finding suitable strains and species to create chimeric strains with nuclear and mitochondrial DNAs from different strains or species;
- characterizing the "default" life history traits of zebrafish paternal strains and their hybrids (swimming capacity, heart rate, total length at hatching, growth rate, mortality and hatching rates).

Results

The genetic distances were calculated pairwise for all strains and species combinations between 6 strains and 3 species. Genetic distances showed that the genetically most distant strains are AB and SJD (0,75%) and the genetically closest species are *D. rerio*'s AB and *D. nigrofasciatus* (6,43%) (Table 1, Figure 1). Hence, research continued with these 3 species/strains and their hybrids.

There was no correlation between relative critical swimming speed (RU_{crit}) and total length (TL) of the fish, hence RU_{crit} did not change with the change in TL in any strain (Figure 2a). RU_{crit} of SJD is significantly lower than of AB strain (p=0.02): 10.5 ± 0.9 TL s^{-1} for AB and 10.0 ± 0.9 TL s^{-1} for SJD (Figure 2b).

Embryos of AB strain had significantly higher heart rate (HR) than the embryos of the other strains (p=0): 218.3 ± 6.9 beats per minute (bmp) versus 198.6 ± 9.3 bmp for SJD and 197.7 ± 8.8 bmp for their hybrids (Figure 2c).

Also, there was a significant difference in TL at hatching between all the strains (p≈0): 3.6 ± 0.1 mm for AB, 3.4 ± 0.1 mm for SJD x AB and 3.2 ± 0.1 mm for SJD (Figure 2d).

Regarding mortality and hatching rates, statistical analysis did not show any significant difference in neither between strains, nor between replicates (p>0.05) (Figure 3a,b).

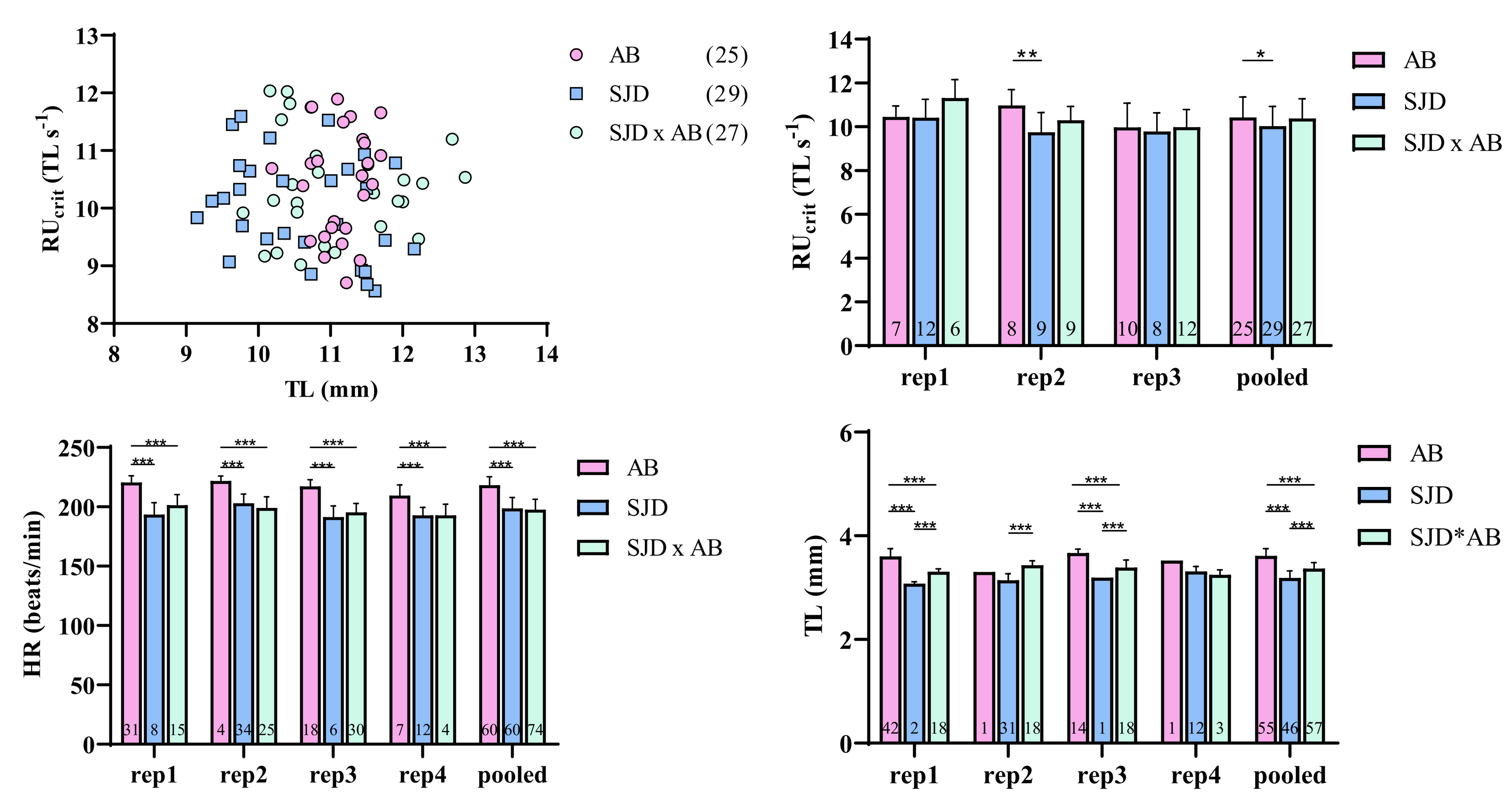


Figure 2. a) Correlation of RU_{crit} and TL. b) RU_{crit} per strain and replicate. c) HR of 48 hpf embryos per strain and replicate. d) TL at hatching per strain and replicate. Error bars for all graphs equal to 1 SD.

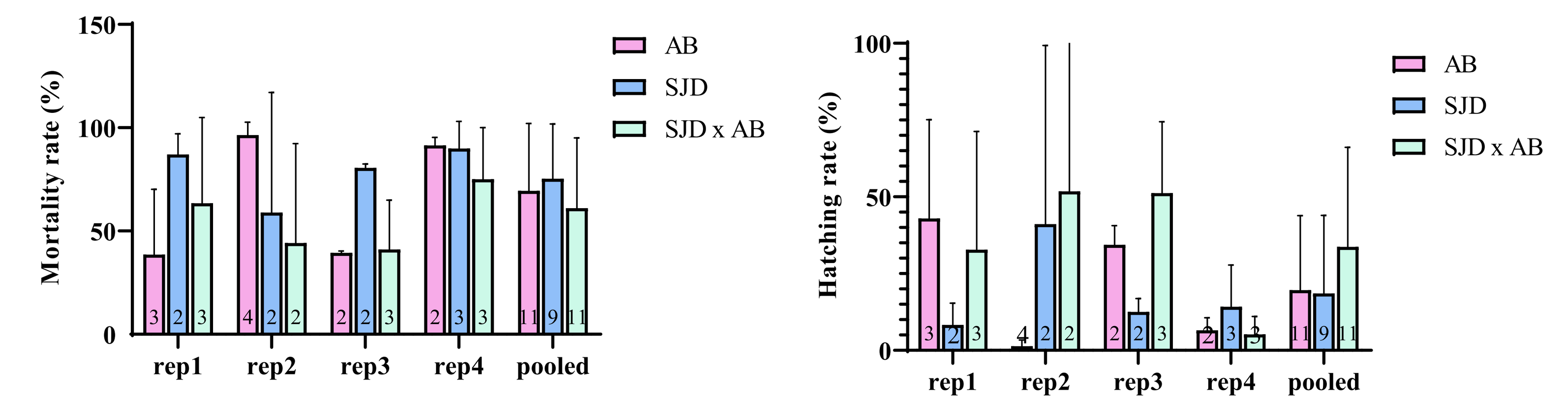


Figure 3. a) Mortality rate per strain and replicate. b) Hatching rate per strain and replicate. Error bars for all graphs equal to 1 SD.

Discussion

There is a possibility that mitochondrial polymorphism might be the reason SJD had lower swimming performance than AB. However, we can only make a hypothesis because swimming capability is also strongly related with many phenotypic factors.

There are not so many studies about the comparison of HR of embryos of different zebrafish strains/species at 48 hpf. The vast majority of studies usually use AB strain just as a control group for studying effects of various environmental factors. In our study we characterized the "default" heart rate for future studies, and future objective might be to learn whether the higher heart rate of AB strain is due to differences in mtDNA between strains.

We successfully excluded during our experiments all factors that affect TL at hatching, we can possibly suggest that the difference in TL between strains might be due to "strain" factor as well. Also, it would be useful to verify these results for embryos hatched at 72 hpf as well as to check whether there any difference in TL at hatching between early-hatched (48-72 hpf) and later-hatched (72-96 hpf) embryos with respect to strain.

As results of this study, it occurred that mortality and hatching rate did not differ with respect of the strain. However, other studies presented that hatching rate in zebrafish should be about 80-100%, mortality rate of zebrafish should be no more that 10-15%. Our results showed different values that might be explained by the fact that we only used random 50-60 eggs from each pair, and other studies used mass spawning.

Conclusions

This thesis examined and selected the *D. rerio's* strains (AB and SJD) and species (*D. nigrofasciatus*) which are suitable for the study of mitochondrial interactions and heteroplasmy in chimeric zebrafish. For that study, strain/species-specific primers were created and experiments for life history trait measurements of zebrafish were done. For now, the "default" life history traits of strains AB, SJD and their hybrid are standardized. The technique of *in vitro* fertilization was successfully implemented in the study and we intend to start main research as soon as possible.

Table 2. Conclusive table. Absence of the same letter shows that there is no significant difference in this life history trait between strains .

strain	heart rate	total length at hatching	critical swimming speed	mortality & hatching rates
AB	a	a	a	a
SJD	b	b	b	a
♀SJD x ♂AB	b	c	ab	a

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ΤΜΗΜΑ ΒΙΟΛΟΓΙΑΣ
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