

# Unraveling the Adaptive Significance of Mitochondrial Genome Variability of *Drosophila obscura* †

Pavle Erić <sup>1,\*</sup>, Aleksandra Patenković <sup>1</sup>, Katarina Erić <sup>1</sup>, Marija Tanasković <sup>1</sup>, Slobodan Davidović <sup>1</sup>, Mina Rakić <sup>1,2</sup>, Marija Savić Veselinović <sup>2</sup>, Marina Stamenković-Radak <sup>2</sup> and Mihailo Jelić <sup>2</sup>

<sup>1</sup> Department of Genetics of Populations and Ecogenotoxicology, Institute for Biological Research “Siniša Stanković” – National Institute of the Republic of Serbia, University of Belgrade, Bulevar Despota Stefana 142, 11060 Belgrade, Serbia.

<sup>2</sup> Faculty of Biology, University of Belgrade, Studentski trg 16, 11000 Belgrade, Serbia.

\* Correspondence: pavle.eric@ibiss.bg.ac.rs; Tel.: +381112078334

† Presented at the 1st International Electronic Conference on Entomology (IECE 2021), 1–15 July 2021; Available online: <https://iece.sciforum.net/>.

**Abstract:** *Drosophila obscura* is a very common fruit fly inhabiting European forests. This species has a large number of mitochondrial haplotypes of *Cyt b* gene. We used experimental lines of *D. obscura* to test the adaptive significance of intra-population variability of the mitochondrial genome (mtDNA) and selective forces that maintain it. We chose three isofemale lines with distinct mitochondrial haplotypes of *Cyt b* gene from each of the two populations sampled in Serbia. Using backcrossing, we created nine experimental lines for each population with all combinations of mtDNA haplotypes and nuclear genetic backgrounds (nuDNA). Individuals of both sexes were tested separately for desiccation resistance at two temperatures. Cox proportional hazards model, with four factors: mtDNA, nuDNA, sex and temperature was used to analyze the survival data. In some comparisons we noticed significant effect of mtDNA on desiccation resistance, while all of them showed significant effect of interaction between mitochondrial and nuclear genome. Temperature in interaction with mtDNA or mito-nuclear genotype more frequently showed significant effect on desiccation resistance compared to sex in interaction with mtDNA or mito-nuclear genotype. Our results show adaptive significance of intra-population variation of mtDNA and importance of interactions between mitochondrial and nuclear genome on fitness. Temperature specific mito-nuclear interaction rather than sex-specific selection on mito-nuclear genotypes maintains mtDNA variability in this model species.

**Keywords:** *D. obscura*; *Cyt b* gene; desiccation resistance; mtDNA; variability

**Citation:** Erić, P.; Patenković, A.; Erić, K.; Tanasković, M.; Davidović, S.; Rakić, M.; Savić Veselinović, M.; Stamenković-Radak, M.; Jelić, M. Unraveling the adaptive significance of mitochondrial genome variability of *Drosophila obscura*, in Proceedings of the 1st International Electronic Conference on Entomology, 1–15 July 2021, MDPI: Basel, Switzerland, doi:10.3390/IECE-10522

Published: 1 July 2021

**Publisher’s Note:** MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



**Copyright:** © 2021 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

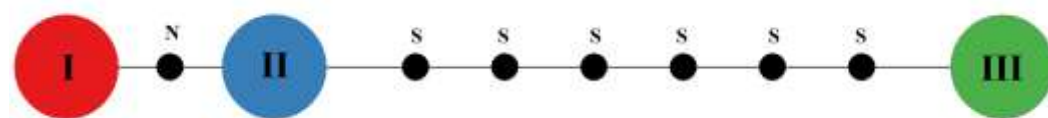
Variation in the mtDNA has long been considered selectively neutral [1–2]. This view was based solely on the properties of the mitochondrial genome (mtDNA), it is haploid, there is no dominance and its inheritance is mostly uniparental. Although it generally codes a small number of genes, their products are enrolled in very important processes which enable eukaryotic cells their high energy efficiency. Hence, variation in mtDNA that affects an organism’s fitness would swiftly be eliminated or fixed by natural selection.

However, the modern view of selective processes that act on mtDNA takes into consideration functional interrelations between mitochondrial and nuclear genomes (nuDNA) [3,4]. Interactions between the two genomes are complex and encompass several important biological processes such as cellular respiration, mtDNA replication, transcription, and translation. All of these processes require sequences coded from both genomes [5–8].

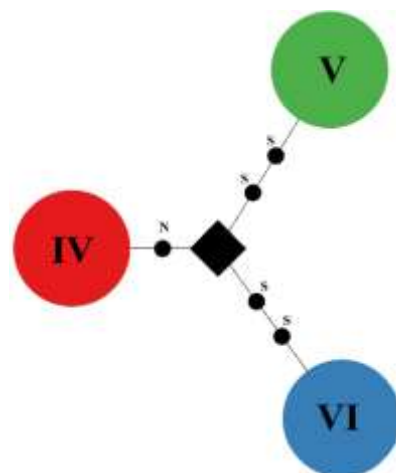
The adaptive inter-population variability in mtDNA is easily explained with the different selective regimes in different populations. However, the existence of stable intra-population variability is quite problematic to explain. While theoretical models predict the action of either negative frequency-dependent selection [8–9] or sex-specific selection on mito-nuclear genotypes [10], empirical research in this field lacks. Some data show that spatial or temporal variation in the environment, through the action of selection on mito-nuclear genotypes, could maintain intra-population variation in mtDNA [3,11]. We used a set of experimental lines of *D. obscura* to test differences in desiccation resistance, which is an important fitness component. We tested the influence of nuDNA, sex, and temperature on desiccation resistance for bearers of different mtDNA haplotypes.

## 2. Materials and Methods

We chose three isofemale lines with distinct mitochondrial haplotypes of the *Cyt b* gene from each of the two populations sampled in Serbia (Figures 1 and 2) Those lines were maintained in the laboratory on a standard corn-meal medium for multiple generations. Using 14 generations of backcrossing, we created nine experimental lines for each population with all combinations of mtDNA haplotypes and nuclear genetic backgrounds (nuDNA). Backcrossing procedure included mating of 10 virgin females of specific haplotype with twice as many virgin males with the desired nuclear genetic background. For each specific genotype, more than 30 individuals of both sexes were tested separately for desiccation resistance at two temperatures (16 °C and 19 °C). Flies were inspected in small plastic tubes hourly after the experiment was set. Cox proportional hazards model, with four factors: mtDNA, nuDNA, sex, and temperature was used to analyze the survival data. Starting from the full model with all the interactions of the four factors, we subtracted term by term, in order to identify the minimal adequate model, having the lowest Akaike information criterion (AIC) score, in both populations and all genotype interactions.



**Figure 1.** Haplotypes I, II and III from the first population. Small black circles are mutations, N-nonsynonymous; S-synonymous.



**Figure 2.** Haplotypes IV, V and VI from the second population. Small black circles are mutations, N-nonsynonymous; S-synonymous.

### 3. Results

Due to the proportional hazards assumption being violated, in the first population, we had to stratify the data by both temperature and nuDNA factors, while in the second population all the variables satisfied the proportional hazards assumption, and stratification was not necessary. In both populations, females generally survived longer than their counterpart males. Flies of both sexes survived longer on the lower temperature in both populations, as was expected.

In the first population, we noticed a significant effect of mtDNA genotype on desiccation resistance in two out of three pairwise comparisons, not being significant in comparison II vs III which differ by six mutations although all synonymous. The mtDNA x nuDNA interaction significantly influenced the survival time in all three comparisons. While sex was a significant factor for survival under desiccation stress in all three comparisons, mtDNA x sex was significant in two comparisons, and for the combination of mtDNA x nuDNA x sex significance was not observed. Both Genotype by temperature interactions (mtDNA x temp and mtDNA x nuDNA x temp) were statistically significant in two out of three comparisons in the first population. Interaction between mtDNA, sex, and temperature was highly significant in all comparisons. Results of the Cox proportional hazards model factors and their significance for the three pairwise comparisons in the first population are given in Table 1.

**Table 1.** The effect of mitochondrial haplotype (mt), nuclear genetic background (nu), sex, temperature and their interaction on desiccation resistance for the first population of *D. obscura*.

Comparisons	I vs. II				I vs. III				II vs. III			
	loglik	Shisq	df	p	loglik	Shisq	df	p	loglik	Shisq	df	p
mt	-2531.4	9.2404	1	**	-2536.2	19.5678	1	***	-2566.1	0.3299	1	ns
nu	strata	strata	-	-	strata	strata	-	-	strata	strata	-	-
temp	strata	strata	-	-	strata	strata	-	-	strata	strata	-	-
sex	-2521.6	19.5695	1	***	-2530.5	11.5509	1	***	-2563.9	4.3986	1	*
mt*nu	-2506.3	30.534	1	***	-2521.5	17.9675	1	***	-2562	3.8502	1	*
mt*sex	-2505.4	1.8009	1	ns	-2516.2	10.6697	1	**	-2556.4	11.1001	1	***
mt*t	-2503.6	3.7593	1	.	-2513.9	4.5404	1	*	-2551.5	9.806	1	**
mt*nu*sex	-2503.4	0.2691	2	ns	-2513.6	0.5384	2	ns	-2551.1	0.8387	2	ns
mt*nu*t	-2484.3	38.1587	1	***	-2513	1.2665	1	ns	-2548.7	4.8792	1	*
mt*sex*t	-2476.7	15.2555	2	***	-2505	15.9898	2	***	-2534	29.3755	2	***

Loglik - log likelihood; Shisq - shisquared value; df - degrees of freedom; ns - non-significant; . -  $p < 0.06$ ; \* -  $p < 0.05$ ; \*\* -  $p < 0.01$ ; \*\*\* -  $p < 0.001$ ; strata - variable is stratified.

In the second population, there was no need for stratification in our models. Both nuDNA and temperature were highly significant in all three comparisons. Mitochondrial haplotype significantly influenced survival in only one pairwise comparison, while the genotype interaction (mtDNA x nuDNA) significantly influenced resistance to desiccation stress in all comparisons. Interestingly, in the second population both sex and mtDNA x sex had a significant effect on desiccation resistance only in one pairwise comparison, while the combination of mtDNA x nuDNA x sex, was conversely significant in two of the three pairs. The second population showed a significant influence of genotype by temperature interaction (mtDNA x Temp as well as mtDNA x nuDNA x Temp) in all three comparisons. Sex x temp x mtDNA interaction term was highly significant in two comparisons. Full results of the Cox models' factors and their significance for the three pairwise comparisons in the second population are given in Table 2.

**Table 2.** The effect of mitochondrial haplotype (mt), nuclear genetic background (nu), sex, temperature and their interaction on desiccation resistance for the second population of *D. obscura*.

Comparisons	IV vs. V				IV vs. VI				V vs. VI			
	loglik	Shisq	df	p	loglik	Shisq	df	p	loglik	Shisq	df	p
mt	-2873.1	2.0812	1	ns	-3278.7	4.0711	1	*	-2798.6	0.09	1	ns
nu	-2853.4	39.269	1	***	-3219	119.3312	1	***	-2780.6	36.1704	1	***
temp	-2770.2	166.5078	1	***	-3149.5	138.9328	1	***	-2764.8	31.5955	1	***
sex	-2769	2.4158	1	ns	-3149.4	0.3154	1	ns	-2761.2	7.0618	1	**
mt*nu	-2764.4	9.0753	1	**	-3144.6	9.5929	1	**	-2756.8	8.7382	1	**
mt*sex	-2764.4	0.0271	1	ns	-3142.3	4.5495	1	*	-2756.8	0.0614	1	ns
mt*t	-2757.4	14.0732	1	***	-3139.6	5.4025	1	*	-2754	5.6945	1	*
mt*nu*sex	-2754.3	6.0943	2	*	-3132.8	13.5174	2	**	-2752	3.9551	2	ns
mt*nu*t	-2749.2	10.3658	2	**	-3126.8	11.9788	2	**	-2739.8	24.443	2	***
mt*sex*t	-2748.8	0.7144	2	ns	-3117	19.7199	2	***	-2731.5	16.576	2	***

loglik - log likelihood; Shisq - shi squared value; df - degrees of freedom; ns - non-significant; \* -  $p < 0.05$ ; \*\* -  $p < 0.01$ ; \*\*\* -  $p < 0.001$ ; strata - variable is stratified.

#### 4. Discussion

Our results have several important findings. They show that mtDNA variants (on their own) have a significant effect on fitness, which was measured by desiccation resistance. The desiccation resistance is an important fitness component determining which fruit flies cope with harsh environmental conditions of water deprivation in dry months in natural habitats. This way our results support a body of growing evidence of non-neutrality of mtDNA variability [4,12,13], and more importantly they give evidence for the adaptive significance of intra-population variation in mtDNA [14–16]. All pairwise comparisons showed a significant effect of interaction between mitochondrial and nuclear genome. This result is not surprising considering that the *Cyt b* gene is part of respiratory complex III which also includes subunits coded by nuDNA. Therefore, subunits coded from different genomes have to be co-adapted for the proper energy production in mitochondria. If one haplotype is combined with a non-matching nuclear genetic background, a decrease in fitness is expected.

With females of *D. obscura* being generally bigger than males, it is expected that sex is a significant factor in all comparisons, with larger females surviving longer in dry conditions. In some of the isofemale lines from second population, sex ratio skewed towards females was observed, consequently fewer males were available for the experiment. This deviation from equal sex ratio could be the result of a mutation on the X chromosome, that is lethal to all the male carriers, and in females it decreases their fitness, so in the experiment we had only healthy males and some females with the mutation.

The sex to genotype interaction on fitness is observed in several pairwise comparisons. The significant interaction between mtDNA and sex is in line with the Mother’s Course Hypothesis [17,18]. Due to maternal inheritance of mtDNA, mutations that are harmful only in males, having no effect or being beneficial to females, cannot be eliminated by natural selection. Interaction between sex and mito-nuclear genotype is observed only in two cases, indicating a difference in direction or strength of selection on mito-nuclear genotypes depending on the sex of the individual. This type of selection is marked as one of the balancing mechanisms that maintain stable intra-population variation in mtDNA [10], but there is limited evidence that empirically supports this theoretical ground [19,20].

On the other hand, the temperature in interaction with mtDNA or mito-nuclear genotype in almost all pairwise comparisons showed a significant effect on desiccation resistance. Different haplotypes on their own or in interaction with nuclear genetic background have different fitness values depending on the environment. Previous studies

have shown this sort of genotype by environment interactions [20–23]. The abundance of this type of interaction in our data suggests that genotype-by-environment interactions are important for maintaining stable intra-population mtDNA variability in nature.

**Supplementary Materials:** Power point presentation: Pavle Eric presentation.pdf

**Author Contributions:** Conceptualization, M.J. and P.E.; methodology, M.J. and P.E.; software, P.E.; validation, P.E., A.P., K.E., M.T., S.D., M.R., M.S.V., M.S.R. and M.J.; formal analysis, P.E., A.P., K.E., M.T., S.D., M.R., M.S.V., M.S.R. and M.J.; investigation, P.E., A.P., K.E., M.T., S.D., M.R., M.S.V., M.S.R. and M.J.; resources, P.E., A.P., K.E., M.T., S.D., M.R., M.S.V., M.S.R. and M.J.; data curation, P.E., M.J.; writing—original draft preparation, M.J. and P.E.; writing—review and editing, P.E., A.P., K.E., M.T., S.D., M.R., M.S.V., M.S.R. and M.J.; visualization, P.E. and M.J.; supervision, M.S.R.; project administration, M.S.R. and M.T.; funding acquisition, M.S.R. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia, grant number 451-03-9/2021-14/200178 for MR, MSV, MSR, and MJ and 451-03-9/2021-14/200007 to PE AP, KE, MT, SD, and MR.

**Informed Consent Statement:** Not applicable

**Data Availability Statement:** Data are available on request from the corresponding authors

**Acknowledgments:**

**Conflicts of Interest:** “The authors declare no conflict of interest.” “The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results”.

## References

- Ballard, J.W.; Kreitman, M. Is mitochondrial DNA a strictly neutral marker? *Trends Ecol Evol* **1995**, *10*, 485–488.
- Avise, J.C.; Arnold, J.; Ball, R.M.; Bermingham, E.; Lamb, T.; Neigel, J.E.; Reeb, C.A.; Saunders, N.C. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. *Ann Rev Ecol System* **1987**, *18*, 489–522.
- Wolff, J.N.; Ladoukakis, E.D.; Enriquez, J.A.; Dowling, D.K. Mitonuclear interactions: evolutionary consequences over multiple biological scales. *Philos T Roy Soc B* **2014**, *369*, 20130443.
- Dowling, D.K.; Friberg, U.; Lindell, J. Evolutionary implications of non-neutral mitochondrial genetic variation. *Trends Ecol Evol* **2008**, *23*, 546–554.
- Rand, D.M.; Haney, R.A.; Fry, A.J. Cytonuclear coevolution: the genomics of cooperation. *Trends Ecol Evol* **1995**, *19*, 12645–12653.
- Meiklejohn, C.D.; Holmbeck, M.A.; Siddiq, M.A.; Abt, D.N.; Rand, D.M.; Montooth, K.L. An incompatibility between a mitochondrial tRNA and its nuclear-encoded tRNA synthetase compromises development and fitness in *Drosophila*. *PLoS Genet* **1995**, *9*, e1003238.
- Ellison, C.K.; Burton, R.S. Cytonuclear conflict in interpopulation hybrids: the role of RNA polymerase in mtDNA transcription and replication. *J Evol Biol* **2010**, *23*, 528–538.
- Novičić, Z.K.; Sayadi, A.; Jelić, M.; Arnqvist, G. Negative frequency dependent selection contributes to the maintenance of a global polymorphism in mitochondrial DNA. *BMC Evol Biol* **2020**, *20*, 20.
- Gregorius, H.R.; Ross, M.D. Selection with gene-cytoplasm interactions. I. Maintenance of cytoplasm polymorphisms. *Genetics* **1984**, *107*, 165–178.
- Babcock, C.S.; Asmussen, M.A. Effects of differential selection in the sexes on cytonuclear polymorphism and disequilibria. *Genetics* **1996**, *142*, 839–853.
- Dowling, D.K.; Abiega, K.C.; Arnqvist, G. Temperature-specific outcomes of cytoplasmic-nuclear interactions on egg-to-adult development time in seed beetles. *Evolution* **2007**, *61*, 194–201.
- Ballard, J.W.O.; Pichaud, N. Mitochondrial DNA: more than an evolutionary bystander. *Funct Ecol* **2014**, *28*, 218–231.
- Dowling, D.K.; Evolutionary perspectives on the links between mitochondrial genotype and disease phenotype. *BBA Gen Subj* **2014**, *1840*, 1393–1403.
- Katwa, S.D.; Ballard, J.W.O. Sympatric *Drosophila simulans* flies with distinct mtDNA show age related differences in mitochondrial metabolism. *Insect Biochem Mol Biol* **2007**, *37*: 923–932.
- Pichaud, N.; Ballard, J.W.O.; Tanguay, R.M.; Blier, P.U. Naturally occurring mitochondrial DNA haplotypes exhibit metabolic differences: insight into functional properties of mitochondria. *Evolution* **2012**, *66*: 3189–3197.
- Maklakov, A.A.; Friberg, U.; Dowling, D.; Arnqvist, G.; Within-population variation in cytoplasmic genes affects female lifespan and ageing in *Drosophila melanogaster*. *Evolution* **2006**, *60*: 2081–2086.
- Gemmell, N.J.; Metcalf, V.J.; Allendorf, F.W. Mother's curse: the effect of mtDNA on individual fitness and population viability. *Trends Ecol Evol* **2004**, *19*, 238–244.

18. Dowling, D.K.; Adrian, R.E. Challenges and prospects for testing the mother's curse hypothesis. *Integr Comp Biol* **2019**, *59*, 875–889.
19. Jelić, M.; Arnqvist, G.; Novičić, Z.K.; Kenig, B.; Tanasković, M.; Anđelković, M. Stamenković-Radak, M. Sex-specific effects of sympatric mitonuclear variation on fitness in *Drosophila subobscura*. *BMC Evol Biol* **2015**, *15*, 1–9.
20. Dowling, D.K.; Friberg, U.; Hailer, F.; Arnqvist, G. Intergenomic epistasis for fitness: within-population interactions between cytoplasmic and nuclear genes in *Drosophila melanogaster*. *Genetics* **2007**, *175*, 235–44.
21. Dowling, D.K.; Abiega, K.C.; Arnqvist, G. Temperature-specific outcomes of cytoplasmic-nuclear interactions on egg-to-adult development time in seed beetles. *Evolution* **2007**, *61*, 194–201.
22. Arnqvist, G.; Dowling, D.K.; Eady, P.; Gay, L.; Tregenza, T.; Tuda, M.; Hosken, D.J. Genetic architecture of metabolic rate: environment specific epistasis between mitochondrial and nuclear genes in an insect. *Evolution* **2010**, *64*, 3354–3363.
23. Rand, D.M.; Mossman, J.A.; Zhu, L.; Biancani, L.M.; Ge, J.Y. Mitonuclear epistasis, genotype-by-environment interactions, and personalized genomics of complex traits in *Drosophila*. *IUBMB life* **2018**, *70*, 1275–1288.