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MOLECULAR PROFILE OF THE *D. melanogaster* MUTANT GENOTYPE w¹¹¹⁸ IN THE PRESENCE OF VARIABLE AMOUNT OF DEUTERIUM (D)

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Abstract

The *Drosophila melanogaster* w¹¹¹⁸ mutant line was used to identify the effect of deuterium (D) on DNA synthesis. D concentrations ranged from 30ppm to 96.89% (low and very high amount respectively). Five generations of flies were bred on culture media prepared with 6 concentrations of D. For each generation the DNA was analyzed, and its variability was established. The results showed a small involvement of D in the successive synthesis of nuclear DNA.

Key words: *D. melanogaster* mutant genotype w¹¹¹⁸; molecular profile; Deuterium presence

Introduction

In nature the stable isotope of hydrogen namely deuterium (D) is present in very small amounts (135-150ppm). In artificially produced water the amount of D could be smaller as in depleted water (DDW, below 130ppm) or higher as heavy water (HW, over 200ppm). The D importance aroused when different positive or negative biological processes were discovered.

The most important results are cancer treatment (1, 2, 3) UV radiation protection (4) and plant animal and insect improvement (5). The background of D influence is still not clarified. Based on the known data, we have followed the influence of different D amount to organism evolution, cell division and the nuclear DNA.

The main goal was to establish the modifying influence of D upon nuclear DNA to drosophila's offspring which have grown in media with suboptimal, optimal (as in nature) or very high amount of D.

Materials and Methods

Biological Material. The mutant strain w¹¹¹⁸ of *D. melanogaster* was used. The fruit flies were grown in the laboratory conditions at 25°C and 60-75% humidity on standard medium prepared in different solutions with variable amount of D. In the growth media the D concentration was: low (30ppm), 140ppm as in our lab and high 500ppm, 2.07%, 13.26% and 96.89% (1% =10.000ppm). The D depleted water was produced at the National Research-Development Institute for Cryogenic and Isotopic Technologies ICSI Râmnicu Vâlcea; heavy water with a concentration of 96.89% was produced by Drobeta Turnu Severin Plant.

Working Method. The DNA extraction was done in three replications based on the modified Doyle and Doyle method (6). Amplification of DNA samples was done with RAPD (Random Amplified Polymorphic DNA) and ISSR (Inter Simple Sequence Repeats) primers. The DNA extracted from each variant/generation was amplified successively with 4 RAPD and 8 ISSR primers (Table 1). The DNA fractionated into the gels was read with VisionWorks®LS (UVP England). The dimensions for all detected fragments were established in comparison with the fragments of the molecular weight marker.

Table 1. The ISSR and RAPD primers used in experiment

No.	ISSR	Sequence of primers (5'-3')	No.	RAPD	Sequence of primers (5'-3')
1	UBC807	(AG) ₈ T	1	P1	ACACAGAGGG
2	UBC808	(AG) ₈ C	2	P16	TCGGCGGTTC
3	UBC820	(GT) ₈ C	3	P17	CTGCATCGTG
4	UBC853	(AG) ₈ YT	4	OPW 19	CCATTCCCCA
5	A2	(AC) ₅ TG			
6	A7	(AG) ₁₀ T			
7	A 13	(GT) ₆ CC			
8	A 17	(GTG) ₃ GC			

Results and discussions

A number of 211 different alleles were identified (151 generated by ISSR markers and 50 by RAPD markers), with an average of 17.6 alleles/primer.

The genetic similarity was high for the individuals of different generations, even if they grew up on culture media with different concentrations of deuterium their (Fig.1).

The dendrogram highlights two main groups, labeled A and B. The group A includes the G1 and G2 generations. Group B is divided into two subgroups: the first subgroup (B1) includes the G3 and the second one (B2) include G4 and G5.

Whether the distribution of variants and generations was random in the dendrogram of each primer, the one that cumulates the ISSR and RAPD primes DNA profile showed an orderly distribution by generations and in each of them by 6 concentration of D.

The ISSR and RAPD dendrogram pointed out high genetic similarity of each generation of w^{1118} individuals. The DNA of each generation that grew up on media with small normal and high concentration of deuterium formed a cluster.

In each cluster branch the natural D amount (140ppm) was the start point. The artificial concentrations of 30ppm and 500ppm D occupied in all clusters the second and the third positions.

In G1-G3 the number of branches in the cluster was constant 6. Due to the lethality of individuals raised on medium with high D concentration 13.2622% the G4 have had five branches.

Noteworthy is the positional instability of the DNA formed in the presence of the concentration of 2.07% D

The molecular profile of the descendants in G3 was arranged in correlation to the D concentration: from 30ppm to 96.89%.

It seems that after 3 generations of development on the same D content the molecular pattern of the DNA is willing according to the D concentration.

Fig. UPGMA clustering of w^{1118} lineage using ISSR & RAPD primers

G3 is the critical point at which individuals adapt their molecular profile to "unusual" living conditions.

The descendants of different generations, even if they grew up on medium with different concentrations of deuterium, had a high genetic similarity.

The correlation coefficients between ISSR and RAPD primers concerning to the genetic similarity of w^{1118} descendants was high and significant passing $p < 0.001$.

The variance overall value pointed out a genetic low variability inside of the generation and high between generations. The F test concerning the bands of the primers were without significance for G1 and G2 and were significant for all variables starting with G3 ($p < 0.01$).

Discussions:

This paper was focused on the “changes” of DNA in the mutant genotype *D. melanogaster* w¹¹¹⁸ under the influence of the variable amount of D.

In the literature there are no works with which to compare our own results.

The UPGMA dendrogram clusters pointed the root position of the tree between groups A and B. The clades position showed the evolution of the individuals from each generation. Genetically, group A is the oldest. It is natural for such a turning point to appear in which individuals get adapted their entire cellular and informational activity to a new environment, the presence of D, which leads to a modified evolutionary line. Such an adaptive behavior was described in the disruptive selection for different new environment (9, 10).

Compared to the DNA formed by flies under normal evolutionary conditions (140ppm), the used ISSR and RAPD primers showed that there are no significant changes in the molecular profile up to G3.

The descendants of 13.2622 %D were affected by sterility and didn't generate new offspring's. The G4 parents from 500ppm, 2.07% 96.89% D survived without capability to do successors. We suppose that very high concentrations of D did not produce significant changes in the molecular profile of DNA but induced incompatible cellular modification with life.

The major difference, which led to the fracture of the molecular profile in two distinct cluster groups, occurs in G3, which was also found in the phenotypic analysis of descendants (11).

Based on the reduced survival of females, we considered that the earliest and most important disturbances occur in ovogenesis. Significant but undirected changes between the genders indicate reduced ability of D to alter the evolution of the X and Y chromosomes. In general, low concentrations of D have improved phenotypic parameters, viability, motility and fertility, which is a definite support for the use of DDW in human and animal life and therapy. Almost all biological processes were repressed when D concentrations were high. Even if in the ontogeny and organogenesis of the adults of the w¹¹¹⁸ line abnormalities were observed in their molecular profile, no fundamental changes were noticed.

In the 5 successive generations there were not observed mutants as it was suggested by Bartel and Varshavsky (1988; 12).

Conclusions

The organized experiment did not meet our assumption.

The tree's root is positioned between the 2 groups, from the genetically point of view group A is the oldest.

Molecular determination indicated almost the same molecular model in each generation.

No detectable change in the DNA profile of w¹¹¹⁸ genotype was found due to D accumulation

Even if in the ontogeny and organogenesis of the adults of the w¹¹¹⁸ line abnormalities were observed in their molecular profile, no fundamental changes were noticed.

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