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THE INVOLVEMENT OF DEUTERIUM PRESENCE IN THE *Drosophila melanogaster* EVOLUTION I. EFFECTS OF DEUTERIUM CONCENTRATIONS UPON THE *White* (w^{1118}) GENOTYPE

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***Abstract**: To determine the action of different deuterium concentrations on the phenotype of drosophila individuals, the w^{1118} genotype was used over 5 generations. D concentrations ranged from 30ppm up to 24.22% [1%= 1,000ppm], creating 6 gradients. The observation has been done at: female prolificacy, larvae motility, pupation height, number of female and male adults and finally sex ratio was establish. The obtained data were statistically processed. Generally the low percent of D (30ppm) improved the average lifespan of descendants and had a favorable effect on all their developmental traits. Compared to the control (140ppm) the reaction of the individuals was divided into 3 groups: - significantly better than it when the amount of D was small (30ppm); - significantly lower if the concentration of D was high (24.22%) and - higher than the control, but without statistical assurance, at all other concentrations. If in larvae and adults the amount of D was unexpectedly high in the DNA the amount of D remained at the natural state as in control.*

Keywords: Deuterium, *Drosophila melanogaster* w^{1118} genotype, phenotype reaction.

1. Introduction

Environmental factors as well as hydrogen isotopes can significantly affect the organism biological parameters [18]. The water is an interchangeable mixture of hydrogen and oxygen isopologues. The hydrogen-related isotopologues are: *light water* or *normal water* (H_2O), *semi-heavy water* (HDO), *heavy water* (D_2O), and *super-heavy water* or *tritiated water* (T_2O) [25]. The oxygen isotopologues of water are light and heavy ^{16}O and ^{17}O&^{18}O respectively [10]. Their peculiarities are used as marker for authenticity of food and other economic purposes [9]. The ratio of D/H in water shows substantial geographical variation, which could affect the plants and animal's evolution [4]. The spatial isotopic distribution in tap

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waters reflects the regional variation of isotopes in the local environment [3], with considerable variation in Timisoara during seasons. The D as well as other stable isotopes are influenced by latitude, humidity, and the seasonal temperature [15, 27].

The importance of isotopologues is given by numerous studies in different fields: in the nature and the urban environment [13], from precipitation [8] solid part of water [31] in vapors [1] in organisms [17] in methods of work [33] animals and human health [26].

The isotopic signature of water changes through phase transitions in the atmospheric water cycle and temperature [7]. In our 2011 determinations we found that the amount of D in the laboratory drinking water was almost constant in the spring months (12.04/142.3ppm; 04.05/142.7ppm; 09.05/142.4ppm) and lower in July (29.07/140.7ppm). Distilled water had a content of 139.4ppm D. Under natural conditions D stabilizes rapidly from low or high concentrations to the value specific to the geographical area.

There are only a few works about the D involved in the drosophila lifespan, fertility and the D content in the descendants [12 30]. However, it is unclear whether D content has an impact in the drosophila's phenotype and whether it might alter it.

Studying the ontogeny fertility and variability of drosophila is helpful to understand the effect of the D amount changes in our life quality.

In our experiment the Control was the normal/light water ($H_2{}^{16}O$).

Stable isotopologues of water have slightly different physical and chemical properties which require a different latent energy for the operation of the aqueous phase. Both have preferential characteristics: evaporation and condensation at light and heavy water respectively (Table 2).

Main goal: To point out the modifying influence of D upon drosophila phenotype w^{1118}.

2. Materials and Methods

The biological material*:* *D. melanogaster* is an appreciated model for knowing human behavior and especially in neurodegenerative studies [14]. *Drosophila melanogaster* L. is a model organism for genetic studies, being used for testing drugs, food, toxins, pollution, and human degenerative illnesses. Approximately 60% of human genes are identical to those of drosophila: 1) the main signaling pathways and molecular mechanisms are conserved in both organisms; 2) many aspects of development and behavior are similar to both kingdoms. For her easily altered particularities the mutant w^{1118} genotype of *D. melanogaster* was used. The short lifespan (8.5 days at 25°C), a good viability the ease of maintenance, made this a perfect for our study. The population was maintained on life cycles on standard environment at 25°C, humidity 65-70% (provided by drip with mixture of D) and constant lighting since1990. Generations of "deuterated" offspring were obtained by passing individuals from G0 (normal environment) on the prepared medium in variants with variable amounts of D. The fundament of this experiment consisted in the presumption that D accumulation in the offspring's is linked with the amount of D from their growth media. Each generation, G1 - G5, was generated by parental forms (♀3: ♂3) from offspring raised successively in the specific concentration of D. Parents were eliminated 72 hours after the start of each experiment.

Deuterium administration. The virgin individuals "head of generation" were raised on the culture medium with D in variable quantity. It was the same standard food, but D replaced H_2O (145ppm) to create the following deuteration treatments: low amount: deuterium-depleted water (30ppm) and semy-heavy water (500ppm, 2.07%, 13.26%, and 22.5%, 96.89%). The different amount of D in the growth medium was obtained by volumetric mixture between 30ppm and 96.98% (Table 1).

Table 1. Experimental variants D content in ppm and %.

No.	Variants	D content		Water category
		ppm	%	
1	V1	140	0.0140	Light water[19]
2	V2	30	0.0030	Deuterium depleted water
3	V3	500	0.0500	Semi heavy water
4	V4	20.700	2.0700	Semi heavy water
5	V5	132,622	13.2622	Semi heavy water
6	V6	242,200	24.2200	Semi heavy water

The ingredients of the culture medium were processed into mixtures with D in the concentrations mentioned above. We consider that in the case of our experiment the contribution of D to the subjects is made by ingestion but also by respiration. Successive generations, marked with "G1", "G2", up to "G5", were quantified in 3 repetitions.

The differences among the parameters of different waters are useful in understanding food ingestion, maggots' motility and last but not least the flies better fodder preparation.

During the hatching period within 24 hours adult flies of both sexes were collected and counted.

Table 2. Physical properties of isotopologues of water used for our purposes

Proprieties	Normal water (140ppm)	DDW	Semi heavy water	Heavy water
Boiling point (ºC)	100.0[20]	100.0[22]	100.7[21]	101.4[20]
pH	7.0[20]	7.0	7.27[21]	7.44[20]
Density (g/ml)	0.998[20]	0.995[22]	1.05[21]	1.11[20]

The following parameters were evaluated.
1) D amount in different ontogenesis phases and in DNA;
2) The locomotors ability of larvae and adults to adapt to the different deuterium content in the;
3) Prolificacy and sensitivity of females/males to different concentrations of deuterium;
4) Molecular investigation at the adults (the second part of this investigation).
A total of 9,112 flies were observed in studies of the relationship between w^{1118} genotype and D amount.
The present paper refers to the phenotypic responses of the offspring of the w^{1118} line at the D variation in their environment.

3. Results and Discussions

The chapter material and working method showed how the series of descendants were obtained (G1-G5).
Depending on the applied environmental factors, the Drosophila’s lifespan will be influenced in different ways. In Drosophila, morphological and behavioral traits are influenced by environment favorable or harmful factors. This presumably is caused by an increase/decrease in the rate of physiological and metabolic processes [28].

Variation of D in different lifespan phases and in DNA
In order to know how D accumulates in adults and to see if the cell has the capacity to filter and stop the various "noxious substances/D", the content of D in the adults of the 5th generation and the DNA extracted from them was determined (Table 3).

The 5th larvae generation had a less content compared to the growth media (142.86 ± 9.4<145ppm). Unexpected was the high amount of D into the larvae formed in 30ppm medium (125.52 ± 8.3>30ppm).

Table 3. D contents of larvae, imago and DNA at w^{1118} genotype the 5th generation grown successively on different concentrations of D*
(1% = 10.000ppm)

Variants: types of water	The D content			
	of growth medium	in larvae (ppm)	in adults (ppm and %)	DNA samples (ppm)
Light	145ppm	142.86 ± 9.4	151.2±3.0ppm	141.5±3.0
Deuterium depleted (DDW)	30ppm	125.52 ± 8.3	4.678±0.094%	141.8 ±3.0
Semi-heavy	500ppm	No results	244.5±5.0ppm	141.7±3.0
Semi-heavy	24.22%	No results	8.615±0.172 %	142.3±3.0

The reported uncertainty is calculated using a magnification factor k = 2, which corresponds to a confidence level of approximately 95%.

Comparing the D content of their growth medium with larvae it can be considered that the body's cells have the ability to filter non-specific "products" and to maintain the organism at the normal evolutionary parameters; it means larval have the ability to adapt their cells to D amount from the environment for their benefit.

In adults, the D content was extremely high compared to their growth environment and that of the larvae. Also it is incomprehensible why there is a bioaccumulation of D in adults who came from a growth medium with a low D content (30ppm). We assume that the accumulation of D in adults indicates its storage in the exoskeleton structure by replacing H or by accumulation in its transformed cells, tissue in which the water is almost non-existent. It is also possible that the structure of the exoskeleton chooses for stronger bonds than those of H. By similarity we mention Magozzi's studies made on vertebrates which pointed out in the absence of isotopic variation in the environment the ^{2}H and ^{18}O values of keratin vary dependent on the artificial habitat created [19]. A similar process of different elements accumulation in chitine was described by Boden et al. [2].

In nuclear DNA extracted from adults, the D content was lower compared to the larval and of adults' cells, with values close to a "natural" amount (141.5±3ppm). There are no differences between the D content in DNA molecules and in the culture medium. Regardless of whether the entire life cycle took place in environments with D in a similar amount as in nature (145ppm) or in the presence of an excessively low or excessively high content (30ppm respectively 24.22%), in the DNA was not accumulated D. In comparison to control in the nuclear DNA of deuterated-adults the deuterium amount increased slightly varying 0.3ppm/30ppm; 0.2ppm /500ppm and 0.8ppm/24.22% D. It was 142.3ppm into DNA of adults grew in the highest concentration of D (24.22%).

***Survival index** (SI)* it is a sum of reactions from different stages of development of individuals, to the “death” that can be used to estimate the body's response to different environmental conditions. From the many traits that condition the survival rate, we chose those that were considered more eloquent.

The ontogenetic evolution In the developmental stage of drosophila's are two distinct stages of morphogenesis: larval stages and metamorphosis into adults. These two stages of development are related to the embryonic stage, which is a component of other ontogenetic pathways [21]. During the drosophila development, the environment acts as a timer in the body growth (genetically determined), and in the duration of its progress (plasticity of the phenotype).
Among larval stage at the D variants and control no significant differences were established. The pupae and adult stages were shorter than the control. According to the control the life span was more accelerated till 2.07% when it was short. From 13.26% showed a return process, being almost to the normal. It is intriguing how the first hatched adults were female and the last hatched were male. Such a finding contradicts the observations made by us in other conditions.

The lifespan of *Drosophila suzukii* (Matsumura) was the most similar to our experiment. In D. *suzukii the* ontogenetic cycle included first instars larvae (3-7 days) pupation (4-15 days) and adult (20-30 days)[11]. Comparing the results obtained in our experiment (Table 4) with the lifespan of *D. suzukii* we found that in the larvae stage appeared the most significant changes. The pupae stage was likely to *S. suzukii*. The life span was longer only at the control.

Table 4. The period of time for each ontogenetic stage (days)

D content	The larvae stage				Pupae stage	Hacking the:				Life span duration
	1st	2nd	3rd	total		the first adult		the last adult		
						days	Gender*	after	Gender*	
145ppm	2	1	8	11	15	10	Female	4 (14)	Male	40
30ppm	1	1	8	10	10	7	Female	3 (10)	Male	30
500ppm	2	1	8	11	9	5	Female	1 (6)	Male	26
2.07%	1	2	4	7	10	5	Female	1 (6)	Male	23
13.26%	1	2	5	8	9	7	Female	1 (8)	Male	25
24.22%	2	2	8	12	12	9	Female	1 (10)	Female	34

* The first and the last hatched descendant

The locomotors activity During the 2-3 days after leaving the culture medium, the motility of the larvae was gradual. In all variants the migration was progressive; sometimes even the larvae climbed above the point of insertion of the pupae, after which they descended. Such behavior has not been mentioned and we cannot explain it. Compared to the control, the motility of the larvae was more active and the insertion height of the pupae was higher at the 30ppm variant (Figure 1; x_{G1-G5}=4.66±0.14 cm).

The highest extra amount of D (24.22%) the motility of the larvae and the pupae fixation was reduced with 20% (x_{G1-G5}=2.88±0.50 cm). The height of pupation was relatively the same in the variants in which D was low or in excess.

Under the investigated conditions, larvae locomotors activity varied much in the first generations (G1-G2). The larvae accommodation to the unusual medium started from G3. From G4 a stability process took place, the pupation highest was similar between D variants.

Taken in account the general average/generation it is revealed a pronounced differences between the insertion height of the pupae of G1-G3 (x_{G1}=3.74±0.36, x_{G2}=3.74±.027, x_{G3}=3.71±0.43 cm). From G4 the highest of pupation is up 4 and 5 cm (x_{G4}=4.08±0.40 and x_{G5}=5.97±0.19 cm).
The particular behavior of offspring revealed a favorable influence of excess of D.

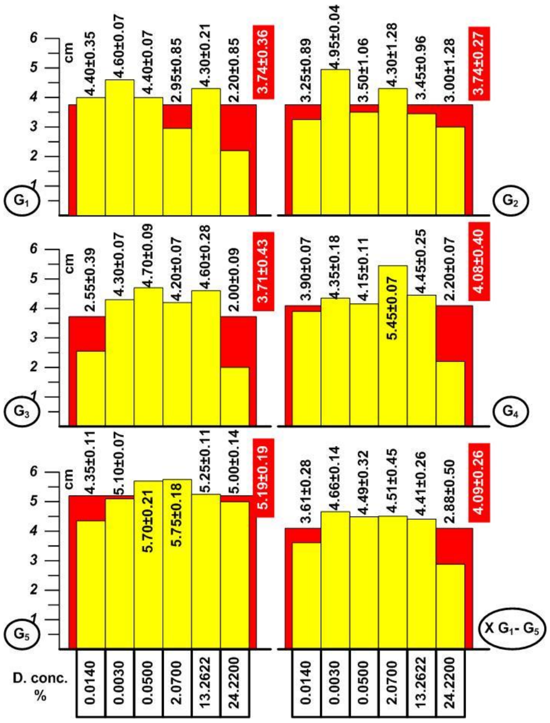

Figure 1. Larval motility and height of pupation for different concentrations of deuterium

Comparing the reaction values of line w^{1118} with other 3 lines and 3 local (wild) populations it was found that the pupation height was the highest at a concentration of 30ppm (4.5 cm and 4.27 cm respectively) and the lowest at the concentration of 24.22% (2.66 cm and 3.35 cm, respectively). Overall, the reaction of wild genotypes was slightly superior to drosophila lines (3.94 cm < 3.67 cm) but without signification (d=0.27 cm).

Population size was closely correlated with the concentration of D in the culture medium. The negative effect of D was revealed at the concentration of 2.29% at which the lethality index was 50%.

The evolution of the size of the populations from the five successive and different generations (Figure 2) highlighted: high variability within each variant; random increase up to G3; from G3 a tendency of “accommodation” of the offspring to the increased concentrations of D were observed; In G4, the individuals show a significant tolerance to the presence of D in the culture medium.

The selected traits appears to be suitable for the study of such interactions, since we assume that they are extremely sensitive to a myriad of environmental factors, ranging from D to temperature or radiation [34, 5].

Gender and D amount*:* gender specific survivorship and mortality rates pointed out a better survivorship and lower mortality for female compared to males at ages up to 24 h after emergence. As observed in previous studies of *Drosophila* and at other organisms [24, 23], age-specific mortality rates is increased for females [22].

Unlike other *Drosophila* population and genotypes we worked with all variants of the w^{1118} line, the first offspring were female and the last were male; with one exception, the concentration of 24.22% (Table 4).

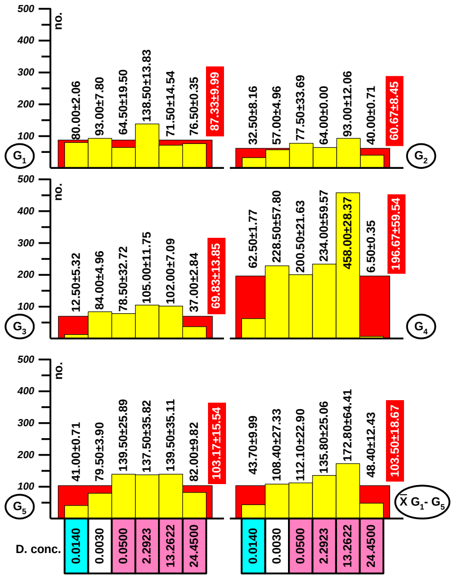

Figure 2. The population size at drosophila w^{1118} genotypes of different generations formed into environment with variable concentrations of D

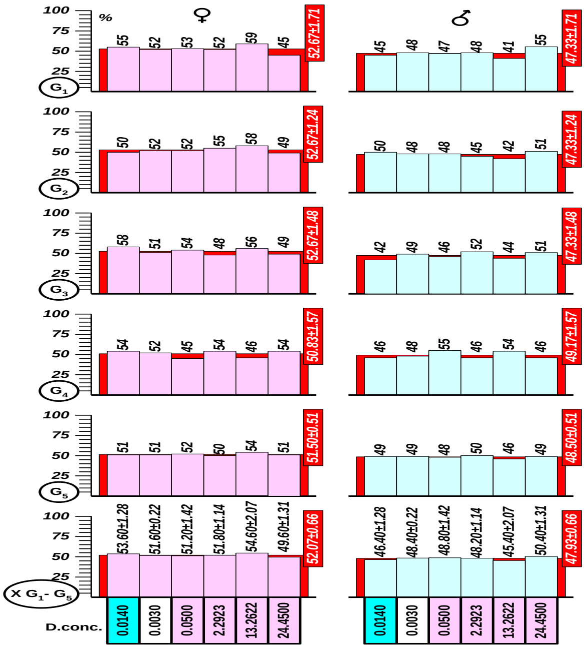

Figure 3. Proportion of females and males in populations of different generations formed into environment with variable concentrations of D

There are no data to show that the w^{1118} genotype has another way of ranking the genders.
The average proportion of females and males in the population of all generations was in favor of XX sex (52.07 + 0.66> 47.93 + 0.66%; Figure 3).
Compared to Control, in all variants the number of females was lower and, only at the concentration of 13.2622% their number was higher.
As shown in Table 4 and Figure 3, the mean life span of females was higher compared to the males by 4%, as is no typical in mixed-sex populations [16, 29].

To date, there are no studies evaluating the effect of D on drosophila
Repeating the study to other genotypes (local populations or standard lines of drosophila) it was determined similar effects of D on their life cycle and behavior.

Since mortality was slightly affected by D means that different generation descendants may display a reaction norm for lifespan.

D had an effect of accelerating the metabolism of different ontogenetic stages, which ultimately led to the shortening of life span without affecting the phenotypic architecture of individuals. Of the thousands of adults observed, no cases of Petit or other phenotypic defects were found as observed in other experiments.

The D amount in the nuclear DNA of individuals grown at concentration 24.22% increased relatively insignificant compared to the D content of the medium and of adults. Against to the control the content of D "stored" in the nuclear DNA of adults raised insignificant ($d_{30ppm-C}$=+0.2ppm and $d_{24.22\%-C}$=+0.8ppm).

The actual causes of deuterium-environment-organism interactions remain unknown for most drosophila genotypes, but may reflect significant changes in their physiological determinants and we presume that the genetic background was not affected.

The distinction is important, because the theory of evolution is entirely dependent upon the assumption of genetic variants that have individual-specific effects on survival [20, 32, 6].

The drosophila's behavior could be used to quantify the D involvement at other organisms, comparing their patterns and of specie-specificities in the presence of D excess.

Conclusions

(1) Low percentages of D added to the regular diet of *D. melanogaster* improved mean life span without an effect on fecundity.

(2) However, D had an adverse effect on development time and showed toxicity as the dose was increased.
(3) Compared to the control (0.0140%) the motility of the individuals was separated into 3 groups:
- significantly superior to the control when the amount of D was small (30ppm);
- significantly lower if the concentration was high (24.22%) and
- higher than the control, but without statistical assurance, at all other concentrations.
(4) Regardless of generation, the large amount of D significantly reduced the motility of larvae (24.22%).
(5) The proportion of the two sexes in "naturalized" populations to the excess D in their living environment was not changed.
(6) The most significant result of this study is that the body/cell has the ability to maintain its DNA almost intact.
(7) Thus, in *Drosophila melanogaster*, even if D increases greatly in their living environment it has itself a limited influence to cause significant increase of damages.

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