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STUDIES ON *DROSOPHILA MELANOGASTER* L. ROMANIAN ECOTYPES HIGHLIGHT THE DIFFERENCES AMONG *DROSOPHILA* ECOTYPES ASSESSING THE MORPHOLOGICAL TRAITS AFTER TRAPPING

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Abstract. In comparison with three standard Drosophila strains 19 Romanian ecotypes were investigated. The ecotypes were collected from areas differentiated by anthropic utilization and environmental conditions. Even though populations of D. melanogaster are not really isolated it has been identified phenotypic and genotypic evident polymorphism. There are compared the quantitative and behavior traits.

Keywords: Drosophila ecotypes; behaviour; fertility, body size

Introduction

Concerns of knowledge and characterization of the ecotypes in Romania are few and sporadically were made (1; 2). In almost all works mutants or isogenic lines were used. In 1943 Radu in collaboration with Catsch and Kanellis (1943 and 1967) communicated the results of radiation on *Drosophila* lines (3). Tudose (1992) and Gavrilă (1996) used mutant genotypes of fruit fly as "model" for their studies. A tentative to collect and to characterize the Romanian *Drosophila* ecotypes was initiated on USAMVB Department of Genetics since 1984. Later in 2008 due to a research program (PNII - 52158 - TRICHOAS) the previous stock made Romanian ecotypes (5) and classical straits (5) were enriched with other 11 ecotypes.

For a cosmopolite organism as *Drosophila* the environment specificity is a frame in which it has to survive to adapt and to evolve or to extinct. The fruit fly physiological particularities are influenced by the environment changes and indicate its adaptability vs. vulnerability (4; 5; 6). The ability to colonize multiple sites is an indication of the biological success of many species as well as on *Drosophila*. For *Drosophila* usually the number of habitats within a geographical area is large. If there is a competition, however, it may be reduced by natural selection by means of adaptation in the available sites (7). In those new niches the fruit fly is obligated to adapt its phenotype and behavior to those specific conditions. This is the cause of the improved

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changes and if they are fixed are leading to the evolved populations of fruit flies (8).

In the fruit fly species the clinal variability was a subject of intense study (9, 10; 11, 12, 13). The *Drosophila* species exhibits high viability across a wide range of growth conditions. The limit of temperatures varies from 10 > 14°C to 28 > 33°C. Adult fertility is maintained across a somewhat narrower range (12 > 14°C to 28 > 30°C; 14). For aridity, individual maximum limits depend on the nature of the flies being 24h. The forms selected for desiccation (reduced humidity to 10%) survive only few hours (15). In some *Drosophila* species the limits might be modified by plastic responses (16, 17).

Previous studies on *Drosophila* species have shown that inherent morphology is highly species specific, and differs among ecotypes (e.g., tropical, continental, hilly, forest etc).

We expected to be a variation among and within Romanian ecotypes of *Drosophila* generated by the environmental peculiarities expressed in the heterogeneity of the morphological traits and individuals' behavior.

The population of fruit flies developed in the "anthropic" sites is made up of individual phenotypes perfectly adapted to the particular environment. In our case ecological site is defined as the larvae specific feeding conditions, the adults foraging, assortment of fruits, the space localization, weather conditions and radiation.

In this paper, we address the following questions: [1] how is the individual's behavioral response to different constraints conditions; [2] there are additional "events"?; [3] how is the population structure and the fertility of *Drosophila* ecotypes in the first generations after trapping?; [4] are body size particularities connected to sexual dimorphism and site of origin?.

The Experimental and Environmental Details

Generally, populations vary and constantly adapts to their environment: food, the seasonality, land forces, and other "constraints" factors. In order to characterize and highlight the existing differences among ecotypes it is necessary to know the specificity of local environment. This is the reason to give each developmental site description of *Drosophila* populations. In **Tab. 1** are indicated the locations and the number of ecotypes along with some environmental particularities.

The *Drosophila* clones were collected in different niche located in:

- **Alba**, **Arad** and **Timiş** Counties, situated in the Northern-Southern West part where the climate is more gentle, with Mediterranean influence, rainfall varying from 800mm, 590mm and 620mm respectively;

- **Dolj** and **Constanta** Counties situated in South and South East part of Romania with an arid climate and powerful tendencies of desertification; the average of precipitations are 630 and 450 mm respectively; and

- **Gorj** County surrounded by Southern Carpathians mountains has a moderate-continental climate and a weak Mediterranean influence. The annual average temperatures vary between 6-10°C and rainfall between 600-800 mm.

In the collection localities the environment is more or less different from the general pattern of the county. Their peculiarities are shown further.

From ***Roşia Montana*** (Alba County, 46°18'N and 23°08'/ 850-1000 MASL) 2 ecotypes were collected (**RM**-North and **RM**-Church). Roşia Montană is situated in the Apuseni Mountains in the Red Valley with gentile climate even if the 6.0°C temperature average is under favorable limits. The predominant soils are *Eutric Cambisols* and *Lepti eutric Cambisols* weakly acidic and acidic respectively. The area is surrounded by forests. The amount of rain is more than 900mm (**Tab.1**). Rosia Montana is famous for their gold mines, uranium, and other rare and heavy metals. The radioactivity of soil is higher than 1100 Bq/kg. Amount of 430 Bq/kg/fresh materials were detected in plants (**ANPM** 2009; 18). In leaves the concentration of heavy metals was higher than normal level (Ni, Pb, Cu, Zn; 19). The cultivated plants are vines, plums, apples, pears trees and in forest are plenty of berries. The environment and food for larva and adults fruit fly development was abundant but we presume being apart due to the heavy metals presence. Huge populations of fly were moving in gardens and orchards. To collect *Drosophila* was not a problem. We hope to identify tolerant genotypes for heavy metals and other stress conditions. In lab conditions only RM-Ch survived. The wild population was a mix made from normal and big individuals (RM-**n** and RM-**B**).

Socodor (Pedological Research Centre), ***Nadab*** and ***Barzava*** communes are located in **Arad** County. The Socodor and Nadab are situated on Solonetz basic and ultra basic pH >8.5 soils associated with flat lands in a climate with hot – dry summers. The rainfall varied from 430 to 590mm respectively. It is a farming and horticulture ecosystem. The main crops are corn, cabbage, tomatoes, plums, apples, and vines. In August in vineyards a lot of fruit flies are foraging. From Socodor and Nadab we collected 4 and 2 "ecotypes" respectively and finally due to small differences among them preserved only one mix sample for each location. We presume to identify tolerance to salt and heat genotypes.

The Barzava is a uranium mining locality where the radionuclides presence is higher than the admissible average concentration for our Country (Tab.1; Bq/Kg dm; **ANPM.ro**). It is surrounded by hills covered with forests. The *haplic luvisol* is very acid (pH – 5.13 – 6.2). The climate is continental - temperate with an average of temperatures 6 - 10°C and in summer shall be 18

-20°C. Precipitation ranges 600 to 800 mm. Plums, mirabelle, apples and different vegetables are the main cultivated plants. In the woods and glades are growing crab apples and cherries and dog roses. We collected an ecotype in the proximity of greened uranium mines (Barzava 11) and of the forest (Barzava Forest). In both places the amount of visiting fruit flies was small. It seems to be an unfavorable ecosystem for fruit fly.

Timisoara and **Sag of Timiş** are located in southeastern Pannonian Plain. For the temperate continental climate of Timisoara the main characteristic consists in diversity and irregularities of atmospheric processes. Warm air masses blowing from the Adriatic Sea and Atlantic Ocean are bringing a lot of rain. In the spring and summer the temperatures are terrible. The polar – continental air coming from East, from the beginning of September, is temperate by Mediterranean cyclones. The average annual temperature and rains are 10.6°C and 629mm respectively. Even that the desertification process is visible. In "Timisoara" point of collection the presence of radionuclides is varying from low (Ra-226) to high (Ac-228 [Th-232] and K-40; **Tab.1**). Around Timisoara a strong agricultural, horticultural vineyards and fruit growing area are. For *Drosophila* the stress of climatic conditions is moderated by excess of food. From June the fruit fly is omnipresent. Since 2008 and 2009 Timisoara and Sag ecotypes are preserved.

The **Giubega** (43°98' N – 44°05'E), **Motatei** (23°61' N – 23°77'E) and **Bucovat/Palilula** (44°17' N – 23°43E) are situated in the South part of Dolj County. This is the hottest and driest area from Ro. Generally is a plain land (30 – 35m MASL) only Palilula is in gentile hilly – wooded ecosystem (350m MASL). For Giubega and Motatei generally the climate is continental with weak Mediterranean influence but both localities are situated in proximity of sandy soils of the "Dolj desert". In the summer the temperatures are extremely high. +10.5°C is the average of annual temperature associated with 610mm/year precipitations not uniformly distributed. The radionuclides concentration is higher than minimum admissible for Romania. Even if the Bucovat/Palilula is considerate having a high amount of radiation the radionuclides presence in the point of collection was low (**Tab.1**). In the 3 ecotypes collected in Dolj areas we hope to identify tolerant genotypes for desiccation and resistant to high temperature and radiation (20).

Tab. 1 General description of the environment in which *Drosophila* ecotypes have evolved

no.	Ecotype	County	Ecosystem	Altitude mMASL	Latitude N	Longitude E	Soil	Rain (mm) Humid (%)	T°C	Radionuclides 1)Natural	2)Artificial
1	Oregon [R]	TM	Lab conditions CONTROL	90	45°45'	21°13'	Standard culture medium	70 – 80 %	22 – 25	Addm. limits m X	Max
2	White [w^{1118}]									Ra-226 14.04 34.99	72.91
3	Ebony [e]									Ac-228 4.65 30.40 K-40 104.60 444.08	51.27 618.79
4	Timisoara		Agriculture Horticulture.	90			Cambic-chernozem	629 mm	10.6	30.5<X; 42.5>X; 650>Max	14.2±17.0#
5	Sag*			85	45°39'	21°10'		584 mm	10.9		
6	Socodor	AR	Agriculture Horticulture	94	46°31'	21°26'	Solonetz pH>8.5	430 mm	10.4	29.9<X; 36.7>X; 609>X	9.36±0.54#
7	Nadab			89	46°29'	21°31'		590 mm			
8	Barzava** 11		Horticulture Agriculture	200	46°07'	21°59'	Haplic luvisol pH = 5.7	720 mm	8.2	962.1>Max; 47.2>X; 421>X	37.9±32.1#
9	Barzava** Forest		Forest	210	46°07'	21°59'				43.6>X; 45.9>X; 540>X	59.1±27.9#
10	Roşia Montana **n****	AB	Horticulture	930	46°18'	23°07'	Cambisols	900 mm	6	Soil radioactivity> 1100Bq/kg	
11	Roşia Montana **B****										
12	Bucovat***	DJ	Forest Horticulture.	130	44°16'	23°42'	Preluvosoil	638 mm	10	15.5<X; 30.6>X; 291<X	28.6±1.45#
13	Giubega		Agriculture Horticulture	81	44°07'	23°25'	Reddish eutricambosol	570 mm	10.5	23.5<X; 28.6<X; 371<X	29.0±3.45#
14	Motatei			69	44°05'	23°12'					
15	Târgu Jiu City	GJ	Urban/Horti.	205	45°03'	23°17'	Preluvosoil; reddish subtype	620 mm	9.7		
16	Peşteana		Horticulture (submontane)	215	44°51'	23°18		600 mm	10		
17	Rovinari****			201	44°54'	23°10'				26.5<X; 25.3<X; 375<X	2.53<#
18	Urdari			146	44°47'	23°17'				35.5>X; 43.9<X; 586>X	55.8±2.53#
19	Plopsoru Grey		Cellar for fruit fermentation	140	44°47'	23°21'	Preluvosoil moderate acid	550 mm	10	23.5<X; 31.8>X; 504>X	127.7±5.67 >#
20	Plopsoru Black **n**										
21	Plopsoru Black **B**										
22	Cernavoda	CT	Fruit market	50	44°37	28°08'	Kastanoziom mildly alkaline	450 mm	11	30.1<X; 52.2>Max; 430<X	

ecosystem anthropic modified**: * thermal plant; ** mining activity; *** high natural radioactivity; * mining activity and power plant
MASL: Meters above sea level;
1) **Natural Radionuclide** Ra-226; Ac-228; K-40 Bq/Kg.soil/0-40cm; 2) **Artificial Radionuclides** Cs-137 Chernobyl;
m, X, Max represents the minimum, average and maximum radionuclides limits admitted in Romania;
Maximum average / Country
n =normal; **B** = big individuals

More than a quarter of ecotypes were collected from **Gorj County**. It is an interesting area for its anthropic modified environment and a particular climate. Widely used is coal mining and power plant business. A special attention was given to human life and health monitoring (18).The soil analysis pointed out the amount of natural radionuclides Ra-226, Ac-228 and K-40 being between minimum and medium admissible in Romania. In comparison to Urdari and Plopsoru in the Rovinari area the natural and artificial radioactivity is low. The highest artificial radioactivity generated by Cs-137 was in Plopsoru. Due to large fields of orchards the environment is proper for fruit fly. Outside or in the basement the moist and warm environment was provided by plums, mirabelle, apples and grapes in fermentation. We presume an special activation of gene complex in survived individuals providing them a better suitability to the environment conditions noticeable in phenotype. Plopsoru Black sustains our presumption.

Cernavoda (Constanta County) is one of the warmest cities in Romania. There is moderate subtropical climate with maritime and some continental influences. In summer the days and nights are very warm and dry. In autumn the days can be warmer than June. Winter can be very windy and cool. Winter arrives much later than in other Counties weather is mild with 8°C to 12°C. The large area of vineyards, orchard and vegetable gardens represents a “paradise” for *Drosophila*. From Cernavoda area 2 “ecotypes” were collected from fruit market and cellar of grapes fermentation. Only Cernavoda (fruit market) was used in present work.

Some events in the collecting work have generated a lot of questions yet unresolved. These are the Plopsoru Black ecotype and the "big" individuals collected from Rosia Montana and Plopsoru.

Materials and Methods

19 wild *Drosophila* ecotypes were compared with Oregon (R), White 1118 (w^{1118}) and Ebony (e) stock strains. Flies were raised in glass vials on standard medium at 25/22^{o}C. Throughout this work the ecotypes are always designated with the place name of collection. For each question under investigation the method of working is presented.

Statistic work: Using two-factor analysis of variance has been possible to establish the hierarchy among ecotypes dependent of their average size. The sexual dimorphism was determinate and the size difference between male and female individuals was evaluated. Averages were compared using Least Difference test (LD) (21). The significance of differences was expressed based on alphabetic symbols, being considered as significant (at p - 0.05; A>B>C>D>E).

Results and Discussions

The primary screening followed wild fruit fly collected from natural conditions in September on gardens, basements, forest from different localities and from minimum 5 points (replications). The wild adults were caught in 800cmc glass vials containing bananas in fermentation and "*Drosophila* standard medium".

To prevent the constraints effects as well as the reduce space, inbreeding effect and the new diet 10:10 outdoor adults were the "starter" of a new generation. The "*in situ*" parents have lay eggs during five days, after which they have been eliminated. The hatched individuals formed the first "*ex situ*" generation (G1) further preserved in laboratory conditions. Each generation has been initiated from a couple of 10♀:10♂; maintained at a size of at least 100 individuals.

The final result consists in a collection of ecotypes that have a high probability of phenotypic and behavioral similarity. In Plopsoru village in the same place two forms were captured.

In the first part of work the G1 and G2 generations were evaluated (**Tab.2**).

To understand the phenotype and behavior difference of flies, grown in outdoor and in limited space (jars) the adult's fertility body and wings sizes were was evaluated in G1 and G2. The viability, the motility, and the inbreeding effect were also assessed.

After the fly capture, in the lab conditions the population size stability was followed. On 50 of adults the body size was evaluated. Daily the emerged adults male and female were counted. Finally the evolution of population growth/day and total amount of adults were established (**Tab.2**).

Tab. 2. The fertility of *Drosophila* individuals originating from *in situ* (natural conditions) **and *ex situ*** (laboratory conditions) **estimated by the size of progeny formed in the first generation of (G1) and the second (G2)** [10♀: 10♂]

no.	Ecotypes / The size and population structure	The first generation after trapping (G1)			The grown generation in lab. conditions (G2)			The % decrease of fertility in G2		
		Total	♀	♂	Total	♀	♂	Total	♀	♂
1	Oregon [R]	-	-	-	116 ± 39.8	61	55	-	-	-
2	White [w1118]	-	-	-	104 ± 22.3	48	56	-	-	-
3	Ebony [e]	-	-	-	173 ± 15.9	80	93	-	-	-
4	Timisoara [North]	498 ± 7.5	238	260	416 ± 3.6	210	206	83.53	88.24	79.23
5	Sag* [Timis]	232 ± 92	106	116	186 ± 12.0	100	86	80.17	94.34	74.14
6	Socodor	423 ± 28.0	203	220	324 ± 16.1	161	163	76.59	78.31	74.09
7	Nadab	448 ± 14.5	198	250	403 ± 8.7	206	200	89.95	97.53	80.00
8	Barzava** 11	206 ± 16.8	100	106	84 ± 21.1	39	45	40.78	39.00	42.45
9	Barzava forest**	116 ± 12.8	49	67	76 ± 56	33	43	65.52	67.45	64.18
10	Rosia Montana** Normal [n]	519 ± 4.0	248	271	134 ± 5.3	73	61	*25.82*	*29.44*	*22.51*
11	Rosia Montana** Big [B]	84 ± 19.0	41	43	18 ± 15.2	10	8	*21.43*	*24.39*	*18.60*
12	Bucovat***	378 ±59.0	183	195	178 ± 16.0	87	101	47.09	47.54	51.78
13	Giubega	364 ± 3.6	173	198	218 ± 4.8	110	108	59.89	63.58	54.55
14	Motatei	336 ± 2.5	158	178	241 ± 3.1	119	122	71.73	75.32	68.54
15	Targu Jiu [City]	346 ± 2.5	166	180	204 ± 3.7	104	100	58.96	62.65	55.56
16	Pesteana	445 ± 19.0	221	224	268 ± 9.4	129	139	60.22	58.37	62.05
17	Turceni****	233 ± 13.0	115	118	200 ± 14.5	102	98	85.84	88.69	83.05
18	Urdari	315 ± 5.2	152	163	244 ± 4.8	120	124	77.46	78.94	76.07
19	Plopsoru Gray	253 ± 16.0	112	141	213 ± 17.8	101	112	84.19	90.18	79.43
20	Plopsoru Black Normal [n]	26 ± 18.3	8	18	59 ± 17.9	21	38	**226.92**	**262.50**	**211.11**
21	Plopsoru Black Big [B]	15 ± 24.1	6	9	21 ± 58.0	9	12	**140.00**	**150.00**	**133.3**
22	Cernavoda	324 ± 3.1	161	163	116 ± 5.2	55	61	35.80	34.16	37.42
	$\overline{x} \pm s_{\overline{x}}$	339.8 ± 27.1	161.4 ± 13.2	178.1 ± 14.2	219.1 ± 23.9	109.3 ± 12.3	110.6 ± 11.8	120.7 ±52.1	52.1 ± 25.5	67.6 ± 70.0
% of male and female in population		100	47.54	52.46	100	49.90	50.10			

1. The *Drosophila* behavior during collection and accommodation to laboratory conditions

Only a small part of individuals “preferred” to use standard or banana food. The flies visited the vials but did not “enjoy” the medium used for capture. A mixture of banana and grapes was the proper food for adults to laying eggs. The traps with standard medium were useful only in unfavorable conditions and for a long time for capture. Even if there is a large genetic variation for food-related traits (22) the analyzed wild ecotypes exhibited a selective response. It seems that at Romanian wild ecotypes the gustatory perception was selected for specific food. **Ueno** et al 2001 (23) demonstrated that the gustatory phenotype modification took place when a single-nucleotide in Gr5a gene was changed. Only Ala218Thr DNP occurs naturally in the *Gr5a* gene polymorphism. The gustatory behavior is still under investigation (24).

After capturing the adults were extremely anxious. Duration to adapt to the limited space varied a few days and for some genotypes never. After a Brownian movement they rested on the plug (the upper part of jar) and then went down to the culture medium. In this period of "orientation" many individuals did not survived. The fly lethality could be caused by the limited space, anxiety, and reproductive constraints (inbreeding).

2. Apart events notification

An unusual situation appeared at Rosia Montana and Plopsoru ecotypes. The natural populations were a mixture of normal and big individuals. In the Plopsoru population were observed grey and among they a few black individuals

The big individuals were separated from the normal form and mated apart.

They survived only for a few generations. During this time the population size continually decreased and finally disappeared.

We wanted to establish the phenotypic difference between normal and big individuals; between female and male; and ecotypes, Rosia Montana and Plopsoru Black originated in two different Counties The statistical work pointed out significant differences between big and normal individuals and female and male averages [$LSD_{1\%}$ <d=0.68 and <d=0.726 mm respectively]. It was an insignificant difference between Rosia Montana and Plopsoru Black ecotypes [$LSD_{5\%}$>d=0.048]. We presume that the big individuals appeared in very large populations and are the result of larval **particular/over** food.

The *ex situ* conditions favored the Plopsoru Black individuals (**Tab.2**). With each generation the population was larger. Our observations pointed out their particularities. They are black with an evident sexual dimorphism. The eggs bear 6-9 appendages. The life cycle is long (23-25 days at 25°C; 25). It is a philo-darkness form very sensitive to inbreeding. The Plopsoru Black formed a particular strain. Up to now its taxonomic position was not clarified. Accidentally in the large populations big individuals emerged. It was the reason to suppose that in large population it is possible to appear and survive big individuals. In case of Plopsoru ecotypes we supposed the action of disruptive selection. The first result was the population divergence in grey and black. The polymorphism created two subpopulations with completely different traits which finally were sexual isolated

3. The fertility evolution of *Drosophila* ecotypes

The shift from a "free" environment evolution to laboratory constraints growing conditions is a major stress for all individuals. The fertility is the most sensitive reply of organism pointing out its homeostasis. It was comparatively assessed the fertility of individuals coming from the natural environment with those formed in laboratory conditions. The determination was made through the number of new emerged progeny (**Tab.2**). The G1 populations emerged from *in situ* parents with a high fertility. In G1 the offspring growth varied from 116±12.8 to 519±4.0 on Barzava Forest and Rosia Montana respectively. Almost all ecotypes were similar as fertility (x±sx =339.8±27.1). In the second generation (G2) the fertility dropped down from 76±5.6 to 416±3.6 at Barzava Forest and Timisoara respectively. Generally the fertility of "*ex situ*" parents was diminished (x ± sx = 219.1±23.9). In the both generations the average of male individuals was higher than that of female (178.1 ±14.2 > 161.4 ±13.2 and 110.6 ±11.8 > 109.3 ±12.3). In G2 the sex ratio was close to normal (1:1). For all of the followed ecotypes the body size decreased with larval density.

Our results are in concordance with other studies. **Billeter** et al., (2012; 26) mentioned that the changes in "social context" had dramatic effects on reproductive success. The observations emphasized the Rosia Montana, Bazava and Bucovat ecotypes homeostasis. The ecotypes evolved in radioactive hilly environment and their transfer in lab conditions strongly influenced the G2 fertility. **Messina** (1991; 27) emphasized "even in the absence of plasticity, in a spatially distributed population in a uniform environment there is always some positive selection on dispersal rate due to local kin competition" It is known that changes in temperature almost always affect vital parameters of *Drosophila* populations such as viability, fertility, development time and other factors that influence the rate of population growth and survival (28)

4. The Body Size in Ecotypes and Sexual Dimorphism

Body size is a major fitness-related that contributes to adaptation successful. The body size of *Drosophila* natural populations in relation with different environmental factors was a subject of studies (29).

In our case the large trait variability among ecotypes imposes to establish the differences among ecotypes and its significance. 25 males and 25 female was the subject of measurement. Only Plopsoru Black made an exception. The results of statistical analysis are shown in **Tab. 3**.

Tab.3. Variance Analysis for the involvement of male and female body size and the ecotype upon the average size of Romanian Drosophila "landraces"

Source of variation	Sum of Squares	Degree of freedom	Mean square	F	Probability
Repetitions	0.696	2	0.348	3.74	
body growth of male and female [**A**]	12.243	1	12.243	131.71**	0.008
Residual [**A**]	0.186	2	0.093	1.25	
Landraces [**B**]	18.092	21	0.862	11.56**	<.001
Individuals x Landraces	1.197	21	0.057	0.77	0.752
Residual [**B**]	6.258	84	0.075		
Total	38.672	131			

A - sex of individuals: a^1- female and a^2- male
B -genotypes [19 landraces and 3 strains]

The analysis of variance [**Tab 3**] reveals the significant differences [**] between individuals [males/females] and among ecotypes.

The hierarchical analysis of variance outlined in **Tab. 4** illustrates the differences of general body size of adults and the trait similitude between females and males.

Tab. 4 The involvement of individual female and male size on the general habitus of *Drosophila* population originated in various environmental sites

no	no.of Eco.	Landraces	♀&♂individuals size [mm]				The general size [mm]		
			Female	signif	Male	signif	$\bar{x} \pm s_{\bar{x}}$	signif	$S_{\%}$
1	20	Plopsoru Black [B]	x 4.60	a	y 3.83	a	4.22±0.23	A	13.45
2	10	Rosia Montana [B]	4.30	ab	3.77	a	4.03±0.14	AB	8.68
3	18	Urdari	x 4.07b	b	y 3.50	ab	3.78±0.22	B	14.52
4	9	Sag	x 3.70	bc	y 3.10	bc	3.40±0.15	C	11.00
5	8	Timisoara North	x 3.70	bc	y 3.07	bc	3.38±0.16	C	11.43
6	3	**Ebony [e]**	x 3.70	bc	y 3.03	cd	3.37±0.16	C	11.38
7	19	Plopsoru Grey	x 3.70	bc	y 2.97	cd	3.33±0.17	CD	12.82
8	4	Socodor	*x 3.63*	c	y 2.97	cd	3.30±0.16	CDE	11.97
9	11	Rosia Montana [n]	x 3.50	cd	x 3.10	bc	3.30±0.09	CDE	6.91
10	6	Barzava 11	x 3.50	cd	*y 2.93*	cde	*3.22±0.13*	CDEF	9.91
11	21	Plopsoru Black [n]	x 3.50	cd	y 2.77	cdef	3.13±0.21	CDEFG	16.96
12	7	Barzava Forest	x 3.30	cdef	x 2.93	cde	3.12±0.10	CDEFG	10.70
13	17	Turceni	x 3.37	cde	x 2.70	cdef	3.03±0.16	DEFG	12.97
14	13	Giubega	x 3.27	cdef	y 2.77	cdef	3.02±0.13	EFG	10.36
15	1	**Oregon [R]**	x 3.47	cd	y 2.53	ef	3.00±0.24	EFG	20.00
16	5	Nadab	x 3.30	cdef	y 2.60	def	2.95±0.20	FGH	16.71

17	15	Targu Jiu [City]	x 3.13	def	x 2.77	cdef	2.95±0.16	FGH	13.00
18	16	Pesteana	x 3.27	cdef	x 2.63	def	2.95±0.19	FGH	15.72
19	12	Bucovat	x 3.33	cdef	y 2.53	ef	2.93±0.20	FGH	16.79
20	22	Cernavoda	x 2.97	ef	x 2.87	cde	2.92±0.18	FGH	15.09
21	14	Motatei	x 3.37	cde	y 2.40	f	2.88±0.24	GH	20.51
22	2	**White 1118 [w[1118]]**	x 2.93	f	y 2.43	f	2.68±0.14	H	12.78
23		$\bar{x} \pm s_{\bar{x}}$	**3.53±0.06**		**2.92±0.05**		**3.22±0.05**		
24		**CV%**	**12.97**		**15.22**		**16.86**		

-Individuals - $LSD_{5\%}$= 0.23 mm $LSD_{1\%}$= 0.53 mm $LSD_{0,1\%}$= 1.68 mm (X,Y,Z)
-Landraces - $LSD_{5\%}$= 0.31 mm $LSD_{1\%}$= 0.41 mm $LSD_{0,1\%}$= 0.54 mm (A,B,C)
-Individuals x Landraces - $LSD_{5\%}$= 0.46mm $LSD_{1\%}$= 0.59 mm $LSD_{0,1\%}$= 0.77 mm (x, y, z)
-Landraces x Individuals- $LSD_{5\%}$= 0.44 mm $LSD_{1\%}$= 0.59 mm $LSD_{0,1\%}$= 0.76 mm (a, b, c)

On all ecotypes the body size of females was larger compared with the males (3.53±0.06>2.92±0.05). The body size varied from 2.93 to 4.07mm on female and 2.46 to 3.50mm on males. The results of variance analysis indicated that only 36.4% and 45.6% female and male ecotypes pointed out a larger body size than the overall body size average/sex (3.53±0.06mm and 2.92±0.05mm). The analyzed ecotypes showed moderate variability in terms of body size in females and males of *Drosophila* [10 <CV%> 20]. It was found that for more than a quarter of the examined ecotypes the overall effects for both sexes were small and without significance (x / x). Their similarity or insignificant sexual dimorphism appears to be caused by hilly forests and orchards environment, a limited possibilities of movement, associated with low temperatures. However, the body size among individuals in certain specific physical environments was large and significant (**Tab. 4**). It was concluded that knowledge of quantitative trait, in our case the body size, is directly associated with fitness for fertility. An exception was Rosia Montana Big.

Other similar studies on body weight pointed out the effect of disruptive selection only in assortative mating (without complete sexual isolation between the subpopulations). These experiments demonstrated that developmental temperatures, both within and between generations, influence territorial success of flies (30).

Conclusions

The Romanian studied ecotypes emphasized a large variability in quantitative traits as well as fertility and body size.

Even if the fruit fly populations are not real isolated it has been identified interpopulational and intrapopulational phenotypic polymorphism.

The presence of two forms of *Drosophila* in the same niche [Plopsoru] pointed out their commune origin separated due to disruptive selection.

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