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Variability of *Drosophila melanogaster* radius incompletus trait expressivity under the food fortification by folate-methionine cycle metabolites

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The aim of study was to determine how the excess of certain (key) metabolites of folate-methionine cycle (folic acid, methionine, betaine) in the diet affects *Drosophila melanogaster* radius incompletus trait expressivity. Folate metabolism is a supplier of one-carbon chemical groups to the number of vital cell processes (purine nucleotides biosynthesis, methionine regeneration, DNA, RNA and proteins methylation) and, therefore, it is directly related to the gene activity regulation. This prompted the supplements choice for study. For the experiment we used *D. melanogaster* stock with *radius incompletus* (*ri*) gene mutation. The expressivity of radius incompletus trait appeared to be a convenient model with well-studied genetic control to evaluate the effect of various factors. It has been established that an excess of folic acid, betaine or methionine in culture medium affects *D. melanogaster* radius incompletus trait expressivity. The direction and strength of effect depend on the sex of individual developed in the medium with one of mentioned supplements. Females appeared to be more sensitive. Each of the dietary supplements used was characterized by a specific effect on studied trait expressivity: under betaine influence we observed a significant decrease in the trait expression in females but not in males; under folic acid action sex-specific differences stayed stable; development in the medium with methionine excess resulted in a decrease of the studied index mean values with an increase of the individuals' variability. Evaluation in terms of fluctuating asymmetry proved more stable development of males in changing conditions, as compared to females.

Key words: *metabolites of folate-methionine cycle, expressivity, radius incompletus trait, Drosophila melanogaster.*

Мінливість експресивності ознаки radius incompletus *Drosophila melanogaster* при додаванні в їжу метаболітів фолатного-метіонінового циклу

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Дана робота мала на меті установити, яким чином надлишок деяких (ключових) метаболітів фолатно-метіонінового циклу (фолієва кислота, метіонін, бетаїн) в раціоні *Drosophila melanogaster* позначається на експресивності ознаки radius incompletus. Обмін фолатів є постачальником одновуглецевих хімічних груп для низки життєво важливих процесів клітини (біосинтез пуринових нуклеотидів, регенерація метіоніну, метилування ДНК, РНК, білків), а отже безпосередньо стосується регуляції активності генів. Саме це обумовило вибір домішок для проведення дослідження. Експеримент проводили із використанням лінії *D. melanogaster* з генною мутацією *radius incompletus* (*ri*). Експресивність ознаки ri виявилась зручною моделлю з добре вивченим механізмом генетичного контролю для оцінки впливу різних факторів. Встановлено, що надлишок фолієвої кислоти, метіоніну або бетаїну в поживному середовищі впливає на експресивність ознаки ri *D. melanogaster*. Напрямок та сила впливу залежать від статі особини, яка пройшла повний цикл розвитку на середовищі з домішкою, більш чутливими є самки. Кожна з уживаних домішок мала специфічний вплив на експресивність досліджуваної ознаки: під впливом бетаїну істотно знижувався прояв у самиць, але не у самців; за дії фолієвої кислоти у досліджуваній концентрації зберігаються стать-специфічні розбіжності у значеннях показника; на середовищі з метіоніном спостерігалось зниження середніх значень показника та збільшення варіативності особин за ним. Оцінка за показником флуктуючої асиметрії підтвердила більш стабільний розвиток самців в умовах, що змінюються, порівняно із самками.

Ключові слова: *метаболіти фолатно-метіонінового циклу, експресивність, ознака radius incompletus, Drosophila melanogaster.*

Изменчивость экспрессивности признака radius incompletus *Drosophila melanogaster* при добавлении в пищу метаболитов фолатно-метионинового цикла

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Цель данной работы – установить, каким образом избыток некоторых (ключевых) метаболитов фолатного-метионинового цикла (фолиевая кислота, метионин, бетаин) в рационе *Drosophila melanogaster* сказывается на экспрессивности признака radius incompletus. Обмен фолатов является поставщиком одноуглеродных химических групп для ряда жизненно важных процессов клетки (биосинтез пуриновых нуклеотидов, регенерация метионина, метилирование ДНК, РНК и белков), а, следовательно, непосредственно касается регуляции активности генов. Именно это обусловило выбор добавок для проведения исследования. Эксперимент проводили с использованием линии *D. melanogaster* с генной мутацией *radius incompletus* (*ri*). Экспрессивность признака ri *D. melanogaster* оказалась удобной моделью с хорошо изученным механизмом генетического контроля для оценки влияния различных факторов. Установлено, что избыток фолиевой кислоты, метионина или бетаина (в заданных концентрациях) в питательной среде влияет на экспрессивность признака ri *D. melanogaster.* Направление и сила действия зависят от пола особи, которая прошла полный цикл развития на среде с добавкой. Более чувствительными являются самки. Каждая из использованных добавок характеризовалась специфическим действием на экспрессивность исследуемого признака: под воздействием бетаина существенно снизилось его проявление у самок, но не у самцов; при действии фолиевой кислоты в заданной концентрации сохраняются пол-специфические различия; на среде с метионином наблюдалось снижение средних значений изучаемого показателя при увеличении вариативности особей. Оценка по показателю флуктуирующей асимметрии подтвердила более стабильное развитие самцов в изменяющихся условиях, по сравнению с самками.

Ключевые слова: *метаболиты фолатно-метионинового цикла, экспрессивность, признак radius incompletus, Drosophila melanogaster.*

Introduction

At adaptation to changing environment the multicellular organism metabolism regulation is achieved by complex interactions between tissues and individual cells. One of the mechanisms of such interactions is the gene activity regulation by presence or absence of certain substances, as the expression of each gene is controlled by a complex interplay of hormonal, neuronal factors and factors of supply. The gene expression control by nutrients availability is well described in prokaryotes (Fafournoux et al., 2000; England et al., 2010) and in lower eukaryotes (De Caterina, Madonna, 2004). Similar studies are conducted in mammals. It has been shown that carbohydrates, fatty acids, sterols, as well as minerals and vitamins are involved in regulating the expression of individual genes (Towle, 1995; Foufelle et al., 1998; Pe'gorier, 1998; Vaulont et al., 2000; Duplus et al., 2000; Grimaldi, 2001). However, the mechanisms involved in the control of higher eukaryotes gene expression by components of the diet (especially by amino acids) are not fully understood (Fafournoux et al., 2000; Bruhat, Fafournoux, 2001; Kilberg, Barbosa-Tessmann, 2002; Mordier et al., 2002). The special importance of this type of regulation is that, on one hand, multicellular organisms cannot synthesize all the amino acids and, on the other one, they have no an amino acids depot (like for lipids or glucose) (Averous et al., 2003).

Folate-methionine cycle is a complex cascade process controlled by enzymes that utilize folic acid derivatives as coenzymes. It is an important part of the primary metabolism of cells because it is a supplier of one carbon chemical groups for a number of vital cellular processes, like purine nucleotides biosynthesis, methionine regeneration and DNA, RNA and proteins methylation, etc. The remains of one atom of carbon entering the folate-methionine metabolism, are formed in the catabolism of certain amino acids (serine, glycine, histidine), in choline catabolism, in the detoxification of formaldehyde and in utilization of formic acid and its salts (byproducts of cellular metabolism) (Michal, 1999). That's why we chose three important metabolites of this cycle (methionine, folic acid and betaine) to test their ability to influence trait expressivity in *Drosophila melanogaster*, probably through the regulation of correspondent gene expression.

Folic acid (pteroylglutamic acid, $C_{19}H_{19}N_7O_6$) is one of B group vitamins. It is built of 6-methylpteridine residue, para-aminobenzoic and L-glutamic acids (M=441.4 g/mol; T_m=250°C). In foods (animal products, fresh herbs, yeast) it is found in conjugate form (folate), and only after the conversion (by the enzyme gamma-glutamyl hydrolase) in the body it acquires the capacity for absorption in the proximal small intestine, followed by folate monoglutamate restoration to tetrahydrofolic acid (THF) having vitamin activity (The vitamins…, 2008). Folate metabolism is known to affect DNA stability in two ways. The first one is de novo nucleotides synthesis. Low levels of 5,10-methylenetetrahydrofolate lead to tymidylate synthesis inhibition. As a result, the ratio dUMP/dTMP increases raising the likelihood of false incorporation of dUMP while DNA synthesis. Removing dUMP by DNA glycosylaze can lead to single- and double-stranded breaks. Moreover unbalanced nucleotide pool interferes with the repair, resulting in DNA damage (Woods, 1964). The second way is to produce SAM (S-adenosylmethionine). The lack of SAM in the cell results in insufficient DNA methylation and, in turn, in violation of chromosome segregation and abnormal gene expression (Firso, Choi, 2005). Hypermethylation of tumor suppressor genes promoter regions (as well as hypormethylation of promoter sites of proto-oncogenes, repeated DNA sequences, dispersed retrotransposons, endogenous retroviral elements, etc.) can cause selective growth and transformation of cells underlying carcinogenesis (Ma et al., 1999; Ehrlich, 2002; Fetisova et al., 2007). Folate deficiency and dysfunction of enzymes that metabolize homocysteine lead to accumulation of the last one in cells, as well as to an increased overall level of this compound in plasma (Davis, Uthus, 2004). Homocysteine has a strong toxic, atherogenic and trombofilic effects (Fetisova et al., 2007). Hyperhomocysteinemia and homocystinuria are also associated with increased risk of neural tube defects, ectopic lens, complications during pregnancy, osteoporosis (Kluijtmans et al., 2003; Dutta et al., 2005).

Methionine is one of the most important dietary compounds that are not synthesized in the body. It is one of the essential amino acids, so it must be constantly ingested with food. It is also usual first amino acid of the protein and the basis of many vital substances. Methionine in the body goes into the amino acid cysteine synthesis (as a sulfur source), which is a glutathione precursor. The latter is involved in toxins removal and protects the liver. Methionine is needed in the body under B12 vitamin deficiency. Violation of cysteine formation from methionine is one of the causes of neurological disorders in vitamin B12 deficiency. Methionine also serves as the chemical groups and elements source for the biosynthesis of epinephrine and choline. The lack of active methionine violates the synthesis of choline and choline-containing phospholipids (lecithin and sphingomyelin) which are nerve tissue components (Firso, Choi, 2005). The special role of this amino acid in metabolism is due to the fact that it contains moving methyl group ($-CH_3$), which can be transmitted to other compounds. In connection with such broad metabolic demand, methionine is often used in many human diseases and conditions treatment (Steinberg, 1984; Lieber, 2002; Krzystanek et al., 2011).

Betaine (trimethylglycine) is a natural compound (discovered by C.Scheibler in sugar beet (*Beta vulgaris*) juice in 1866) that is found in animals and plants. For the time betaine was thought to be completely inactive. But later it was found that as a component of food for farm animals and birds it plays a significant role of labile methyl groups source for homocysteine methylation in the liver (Craig, 2004). The body of birds and animals cannot synthesize methyl groups, and pulls them with nutrition only. Moreover, in birds, for example, there is no choline oxidase for betaine production (Zeisel et al., 2003; Craig, 2004), so its role as a full feeding factor equals in importance to the presence of vitamins and other biologically useful compounds in food. In addition, compared with other compounds, the efficiency of betaine is higher because it is an active methylating form of choline (Obeid, 2013; Wang et al., 2014). That is it may be an alternative way for normal methylation reactions and it functions as an alternative methyl groups donor in the recovery of methionine from homocysteine. Thus, betaine can replace defects in methylation reactions caused by malfunction of folate cycle or by the lack of vitamin B12. Betaine is the only known source of methyl groups for this key pathway. In the case of folate cycle violations (failure, methotrexate induced depression) participation of betaine in the homocysteine transformation to methionine becomes decisive (Obeid, 2013; Wang et al., 2014). Betaine can also replace SAM as a donor of methyl groups for direct methylation of phosphatidylethanolamines that is an alternative way of phosphatidylcholine formation. It is believed that due to the involvement into these two reactions betaine affects intermediate metabolism.

As far as *D. melanogaster* was used as a model organism we have checked for some evidence that substances added to the larvae culture medium (amino acids – modified glycine and methionine – and folic acid) affect flies longevity and fertility. As to the folic acid effects in *D. melanogaster* the information is contradictory. There are publications that favor the ability of fruit flies to synthesize this vitamin (Venters, 1971). However, there is also an idea that folate synthesis in insects depends on their germ endosymbiotic microorganisms (Douglas et al., 2001). The other group of scientists (Grandison et al., 2009) defined the particular impact of methionine in flies longevity and fertility. The addition of 10 essential amino acids to the dietary feed in various combinations led to the following effects: if the amount of methionine is the "standard" the fertility will be high, if the amount of methionine is the "dietary" the fertility will be low, in spite of the amount of other amino acids. Longevity, on the contrary, was reduced in flies reared in the medium with "standard" amount of methionine and other essential amino acids. All other combinations: (1) high methionine, low others; (2) low methionine, high others; (3) both low – resulted in a long life. What exact essential amino acids methionine interacts with the authors could not figure out. Apparently, the interaction between the components of the food is obviously quite complex (Grandison et al., 2009).

The key folate-methionine cycle metabolites (amino acid methionine, a derivative of the amino acid glycine – betaine, and folic acid) were selected for the study because the folate exchange is a supplier of one carbon atom chemical groups for a number of vital cellular processes such as purine nucleotides biosynthesis, methionine regeneration and DNA, RNA and proteins methylation, and the balance of amino acids and vitamins affects flies longevity and fertility.

Quantitative traits genetics is one of the traditional lines of classic genetic research. Due to high genetic complexity of quantitative traits it is, for now, behind other genetic branches. Except the lack of information about the mechanisms of genetic control of quantitative traits, the ways of their formation in individual development, population dynamics responses to direct and indirect pressure of artificial selection and the role in the species evolution are also insufficiently explored. Here we will analyze radius incompletus trait expressivity as a quantitative trait resulted from gene mutation that violates venation of *D. melanogaster* wing plate.

The second radial wing vein of *D. melanogaster* is a good model for quantitative traits studying. It is the last of longitudinal wing veins being formed at prepupa stage. The *radius incompletus* (*ri*) mutation is one of a series of *knirps* gene alleles, located in 3–47.0. It suppresses the formation of central part of radial (L2) wing vein. Instead, the normal allele of this gene (kni^{+}) provides a complete vein formation. The length of the distal and proximal sites varies depending on genetic and environmental factors (Ratner, Vasil'eva, 2000; Zakharenko et al., 2010). This mutation is actively investigated because it is an example of a complex pattern of a quantitative trait inheritance. It is assumed that this system contains a gene of main effect limiting trait morphogenesis (classical Mendelian gene), polygenes affecting main effect gene expression, and mobile genetic elements (MGE). It is consistent with modern views on a typical genetic system of quantitative trait determination:

1) Oligogene (or main effect gene), that is required for the formation of trait. If oligogene limits characteristics formation in morphogenesis, its manifestation becomes dependent on polygenes influences.

2) Polygenic modifiers, each of which is not necessary for the trait formation, but together they can alter the expressivity significantly. It is important that polygenic modification is applicable only to certain oligogenes. In other words, only in this case the phenomenon of incomplete penetrance and variable expressivity appears. Among the properties of polygenes we can mention their dispersed distribution through the genome, a small individual effect on the trait, the lack of their own distinct phenotypic manifestations, allelic diversity. Various polygenes make different contributions into the trait formation, and these contributions can be multidirectional. The system of gene *knirps* expression is thought to be an example of classic polygenic system (Ratner, Vasil'eva, 2000).

3) MGE. There are several hypotheses of MGE influence on traits manifestation: the hypothesis of MGE genetic drift at selection; the hypothesis of modifier MGE influence on the mode of complex polygenes display as far as MGE include functional sites potentially interfering nearby (and other) genes; the hypothesis of the “pattern-champion” of polygenes that contributes significantly to the quantitative trait phenotype whereas MGEs act as markers that are subjected to selection (Ratner et al., 2003). Polygenic structure and MGE start to affect the expression only when some olygogene becomes the one who limits the expression (Vasilyeva et al., 2008; Ratner et al., 2003). The system of different MGE patterns can be analyzed as a universal genomic system of "soft" control for polygenic quantitative traits modifications, including expressivity.

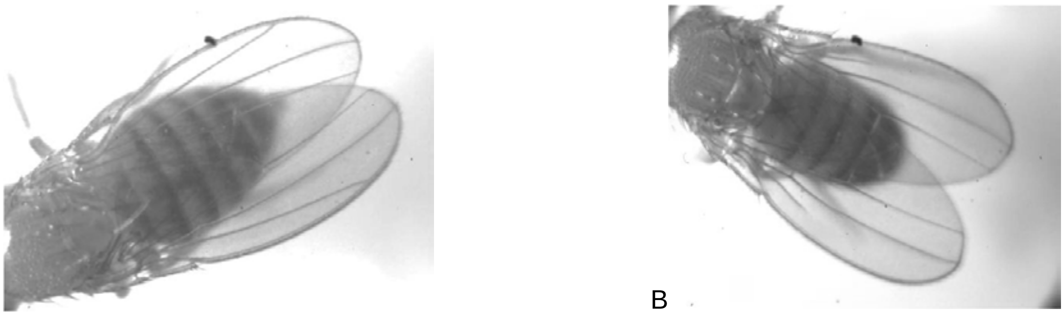
Thus, based on the above, the aim of this work is to establish how the excess of some folate-methionine cycle metabolites in *D. melanogaster* diet affects the expressivity of radius incompletus trait.

Materials and methods

As the material for the study we used *D. melanogaster radius incompletus* – $knirps^{radius\ incompletus}$ – stock from the Collection of drosophila stocks of Genetics and Cytology Department of V.N.Karazin Kharkiv National University that is among objects of Ukraine National Heritage. This mutation is characterized by 100% penetrance and variable expressivity. The stock used is characterized by stable phenotypic manifestation: the presence of proximal part of radial wing vein only (Fig. 1).

Individuals from the control group were reared on the standard corn-meal medium under the constant temperature (t=23±1°C) and humidity conditions. To simulate the excess of folate-methionine cycle components in feeding medium the following supplements were used: pharmacological drug "Folic Acid" (Acidum Folicum) produced by "Technologist" (c. Uman), tablets of 0.005 g (composition: active ingredient – folic acid; supporting ingredients – potato starch, sugar, calcium stearate), was added to the culture medium in its final concentration for feeding larvae 1 mg/ml; pharmacological drug "Methionine" produced by "Kyiv Vitamin Factory", coated tablets (composition: active substance – methionine 250 mg; supporting ingredients – potato starch 44 mg, methylcellulose 3 mg, stearic acid 3 mg, wheat 18.556 mg, sucrose

62.486 mg, magnesium carbonate 18.264 mg, talc 0.496 mg, refined sunflower oil 0.097 mg, beeswax 0.097 mg, azorubine 0.004 mg), washed from the coat before addition to the culture medium in its final concentration for feeding larvae 1 mg/ml; biologically active supplement "Betaine" (Finland), yellowish powder (composition: active ingredient – trimethylglycine; supporting ingredients – inositol), was added to the culture medium in its final concentration for feeding larvae 5%. The active drug concentrations were established experimentally (Volkova et al., 2010, 2013).



A B

Fig. 1. The phenotypes of *Drosophila melanogaster*: A. wild type; B. radius incompletus (×20)

ri trait expressivity was evaluated in imagoes survived in the medium with supplement, or control by the ratio of the L2 proximal fragment length to the projection of its full length. The result was expressed as a fraction. The measurements were carried out in photographs of imagoes made using a stereoscopic microscope (Delta Optical NTX-3C), digital camera (Sigeta UCMOS 3100 3.1MP) and Image analysis® software. Image processing was performed using the ImageJ® software. For each group of comparison from 20 to 73 measurements were done. Totally there were 16 groups of comparison dependently on the sex of individual, left or right wing, nature of supplement. The total number of examined veins was 740. For the parameter measured an arithmetic average and its error (through the standard deviation) were calculated. To evaluate the impact of various factors on the studied characteristic variation ANOVA was used. The power of factors influences (η^2) was evaluated by Plokhinskiy method (Atramentova, Utevska, 2007). To analyze statistical relationships between characteristics measured Spearman correlation was used. Statistica 6.0® was used for calculations.

The level of developmental stability of Drosophila individuals under the effect of factors was studied by the indicator "fluctuating asymmetry." This indicator was calculated by one of the commonly used formulas for each experimental group, for males and females separately (Zakharov, 1982):

$$\delta_d^2 = \frac{\sum (d_{1-r} - M_d)^2}{n-1},$$

where: $M_d = \frac{\sum d_{1-r}}{n}$ – mean difference between sides;

$d_{1-r} = \frac{2(d_l - d_r)}{d_l + d_r}$ – difference of trait values between left (l) and right (r) sides.

Results and discussion

The impact of folate-methionine cycle metabolites excess on Drosophila radius incompletus trait expressivity

The analysis of experimental data established sex-specific reaction of *D. melanogaster* individuals on various supplements to the culture medium (Fig. 2). For example, among the females of control group we observed an asymmetry in the manifestation of trait studied, the average ri expressivity in the left wing was 0.33±0.02, and in the right one 0.30±0.01. At betaine and methionine supplements these differences passed away. In the case of betaine supplement the expressivity varied as 0.32±0.004 and 0.31±0.004 for the left and right wings respectively. In the case of methionine it was 0.32±0.01 for each of the wings. In the case of the development in the medium with the folic acid excess the expressivity of trait studied in females enhanced (0.35±0.01 and 0.36±0,01 for the left and right wings respectively) compared with the control group, but the asymmetry disappears.

Males in the control group, by contrast, are characterized by more homogeneous values of the index (in the left wing 0.31±0.01; in the right wing 0.32±0.01). Rearing in the medium with betaine excess did not lead to significant changes (0.31±0.003 in both wings). As a folic acid effect we observed elevation of the asymmetry in trait manifestation. In the experimental group the average expressivity on the left wing was 0.33±0.01, and in the right one 0.31±0.01. At the same time, the methionine excess also led to increased asymmetry, but with lower means (0.31±0.01 and 0.30±0.01 for the left and right wings respectively).

ANOVA confirmed the effect of medium supplement (F=10.80, p<0.05), of sex of individuals (F=24.75, p<0,05) and of these two factors interaction (F=3.59, p<0.05) on the ri trait expressivity (Table 1).

Moreover, among the factors in this experiment the presence of some supplement in the medium had the most powerful effect on ri expressivity (η^2=0.04; p<0.05). The second effect size we observed for the sex of individual (η^2=0.03; p<0.05). The weakest effect the interaction of these two factors had (η^2=0.01; p<0.05). Despite the fact that the strength of the impact of these factors is rather small, it is in each case is statistically significant. So folate-methionine cycle metabolites studied modify the expressivity of radius incompletus trait in *D. melanogaster* and, possibly, the expression of the corresponding gene. The low values of effect powers may indicate the influence through some intermediaries (modifier genes and their products).

The analysis of each supplement effects separately showed the following. The adding of betaine (5%) to the nutrient medium reduces the manifestation of ri trait in females significantly but not in males (Fig. 3, A). ANOVA revealed the statistically significant effect of supplement presence (F=13.87, p<0.05), of sex (F=14.84, p<0.05) and of these two factors interaction (F=6.49, p<0.05) on the trait expressivity.

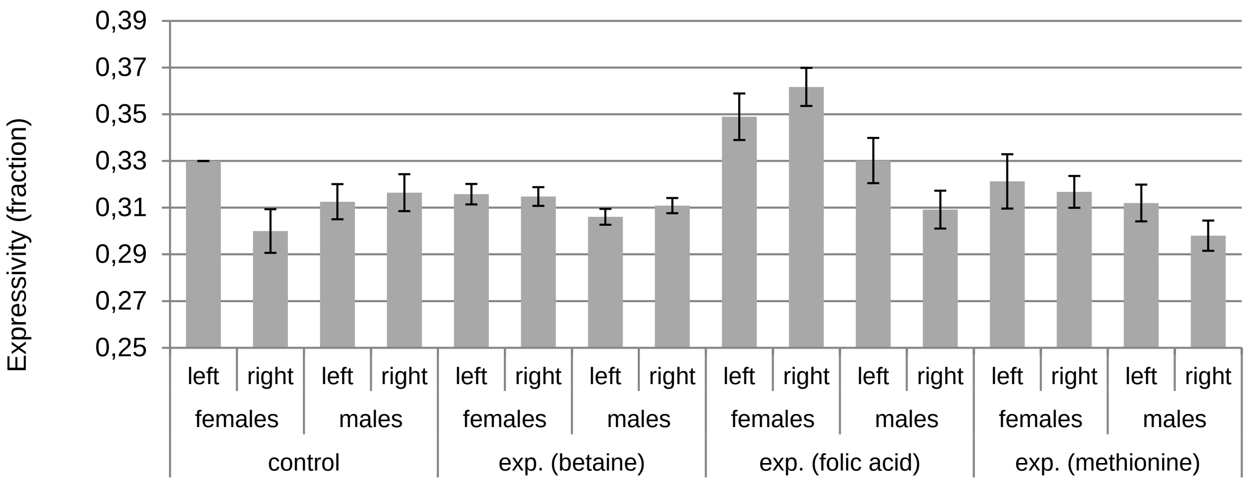


Fig. 2. Variation of radius incompletus trait expressivity depending on folate-methionine cycle metabolites excess in the medium

Table 1.

The effect of various factors on radius incompletus trait expressivity (ANOVA results)

	SS	D. F.	MS	F	p
Supplement	**0,097**	**3**	**0,033**	**10,80**	**<0,05**
Sex	**0,074**	**1**	**0,074**	**24,75**	**<0,05**
Wing	0,003	1	0,003	1,08	>0,05
Supplement VS Sex	**0,032**	**3**	**0,011**	**3,59**	**<0,05**
Supplement VS Wing	0,003	3	0,001	0,36	>0,05
Sex VS Wing	0,001	1	0,001	0,18	>0,05
Supplement VS Sex VS Wing	0,020	3	0,007	2,22	>0,05
Uncontrollable factors	2,178	725	0,003		

Note: SS – uncorrected variance (sum of squares); D. F. – degrees of freedom; MS – corrected variance (mean square); F – Fisher criterion; p – level of significance.

The folic acid supplement (1 mg/ml) led to increased means of parameter studied both in males and females. But these changes were accompanied by high level of variability and thus the most pronounced were sex differences with higher values for females (Fig. 3, B). ANOVA showed a statistically significant effect of the sex on expressivity (F=22.342, p<0.05), but showed neither supplement presence nor the influence of these two factors interaction. This fact requires further study. By the way, the hypervitaminosis by folic acid, in insects particular, is rarely discussed in the literature. As to *D. melanogaster* specifically, despite its widespread use as a genetic model organism, many aspects of its folate metabolism are still not clear. First, the controversial ability of insects to synthesize folates that was mentioned above (Venters, 1971). In addition, there are studies showing an increase in vitality, fertility, growth and intensity of fruit flies development at higher folate levels. In the case of folate absence in food, they cannot finish their metamorphosis (Singh, Brown, 1957; Golberg et al., 1945). The same authors but later showed that if the food of an adult female contains no folate the most of eggs she produces would be nonviable (Sang, King, 1961). Other authors found that in this species the need in folates derived from food increases when larvae are reared in the medium with low concentration of nucleic acids, high concentration of proteins and glycine, or reduced serine content (Geer, 1963). And, of course, a variety of negative effects of the folate inhibitors proves the important role of folic acid in securing many biological functions in Drosophila (Affleck et al., 2006).

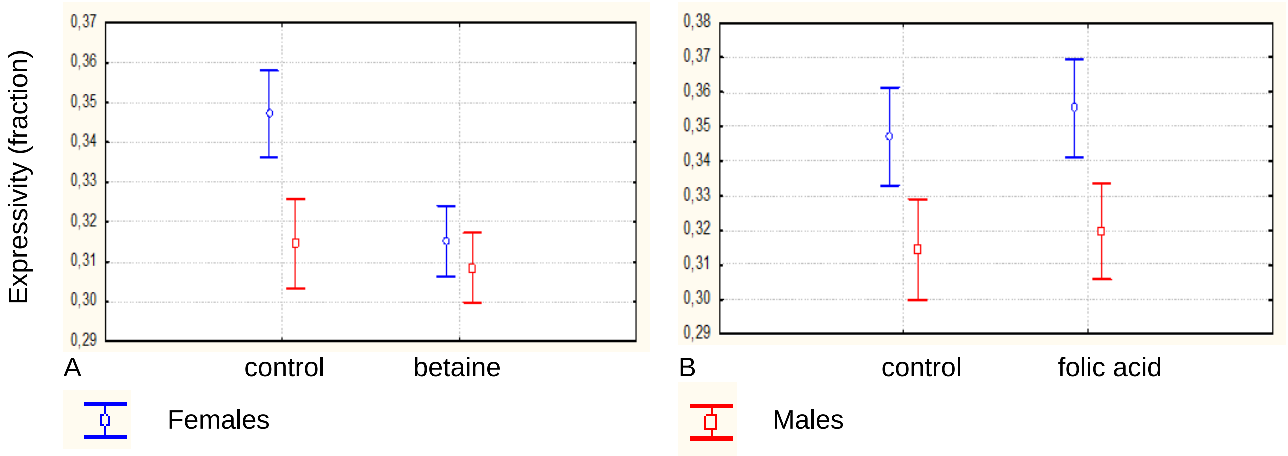


Fig. 3. The effect of betaine (A) and folic acid (B) on *D. melanogaster* radius incompletus trait expressivity

Methionine supplement at 1 mg/ml concentration reduced the means of parameter studied both in males and females. But, as in the case of folic acid effect, we observed considerable variability in the trait manifestation accompanied even by disappearance of sex differences (Fig. 4). ANOVA showed statistically significant impact of methionine presence (F=4.417, p<0.05) and gender (F=6.454, p<0.05) factors on the ri expressivity, but did not reveal the effect of the these two factors interaction.

Based on the results, we can say that of three studied folate-methionine cycle metabolites betaine has the most effective influence on the radius incompletus trait expressivity.
Presumable reason is that betaine (trimethylglycine) has three labile methyl groups and is a derivative of the simplest amino acid involved in the synthesis of many other amino acids.

The lack of statistically significant impact of the factor "wing" (left or right) and of the interaction of this factor with the other in any combination (Table 1) establishes the fact that the fluctuating asymmetry exactly takes place in manifestation of *D. melanogaster* radius incompletus trait. Therefore, this feature can be used for biological indication. Especially because *D. melanogaster* is a cosmopolitan species, living synanthropically and thus individuals are exposed to the same factors that people in the same areas.

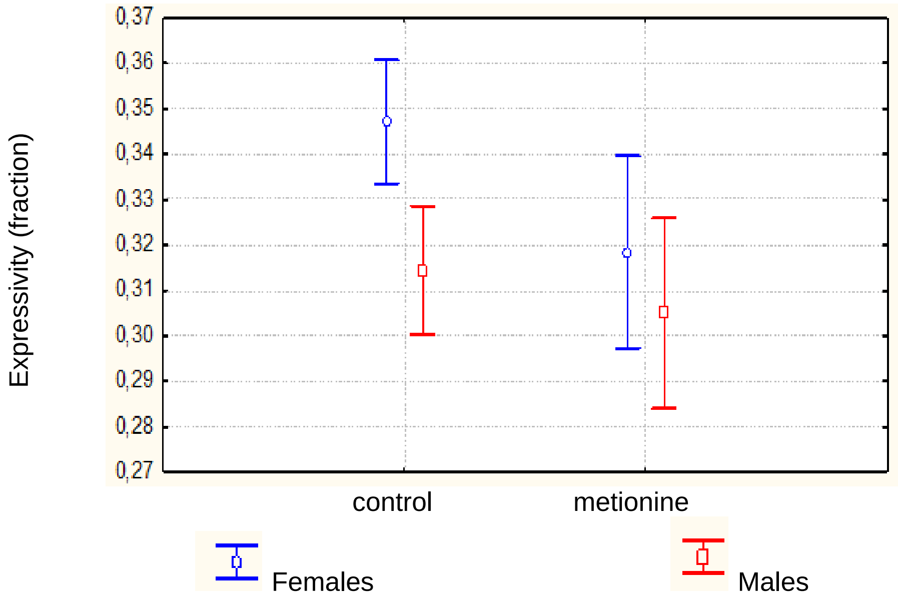


Fig. 4. The methionine effect on *D. melanogaster* radius incompletus trait expressivity

The effect of folate-methionine cycle metabolites excess on developmental stability of drosophila individuals

According to the value of radius incompletus trait expressivity we calculated (Table 2) the index of fluctuating asymmetry for females and males separately for each experimental group (Zakharov, 1982). This index characterizes the stability of organism development (Graham et al., 2010).The developmental stability as the ability of the body to develop without abnormalities is the indicator of natural population state. The most accessible way to evaluate the stability level is the determination of fluctuating asymmetry of bilateral morphological characters (Sokolova, Sharlayeva, 2006). But by this indicator one can consider only the stability in respect to certain characteristic. After the analysis of a large number of different structures it becomes possible to reveal general patterns, common for the group of features that vary, that to some extent may allow to determine the stability of development of organisms as a whole (Zakharov, 1982).

Table 2.

Fluctuating asymmetry coefficient for the radius incompletus trait expressivity under the use of various supplements

Experimental group	Sex	Fluctuating asymmetry coefficient
Control	Females	0.066
	Males	0.034
Betaine	Females	0.014
	Males	0.013
Folic acid	Females	0.484
	Males	0.072
Methionine	Females	0.035
	Males	0.026

In populations existing in certain environmental conditions through rather long period a certain level of developmental stability is maintained (Shadrina, Volpert, 2006). Abnormalities are usually observed in populations existing at the limit of species ecological capabilities, as well as in the area of hybridization of different forms, adapted to different environmental conditions. Nonspecificity of the reaction such as instability in response to different changes of environmental conditions allows using this characteristic for bioindication of anthropogenic effects on the environment (Zakharov, 1982).

Our results suggest that the development of drosophila males (in particular the formation of their wing venation) is more stable comparatively to females under the conditions of excess of certain folate-methionine cycle metabolites in the nutrient medium.

We also calculated the correlation (Spearman correlation coefficient) between the average value of expressivity index of radius incompletus trait and fluctuating asymmetry coefficient (r_S=0.88; p=0.003). That is there is a statistically significant strong direct relationship between two indexes measured under the experimental conditions. Thus, based on the results obtained in this experiment, certain folate-methionine cycle metabolites excess in larval nutrient medium has sex-specific effect on the degree of manifestation of radius incompletus mutation in *D. melanogaster*.

Conclusions

D. melanogaster radius incompletus trait expressivity is a convenient model with well-studied mechanism of genetic control and can be used to evaluate the effects of various factors. Changing the diet by addition of folate-methionine cycle components to the nutrient medium affects the radius incompletus trait expressivity in *D. melanogaster* (F=10.80, p<0.05). The direction and force of the effect depends on the sex (F=24.75, p<0.05) of the individual who passed the full cycle of development in the environment with the supplement. Females are more sensitive. Each of the supplements used is characterized by a specific effect on the trait expressivity: betaine (5%) significantly reduces the manifestation in females but not in males; under the folic acid (1 mg/ml) sex-specific differences are kept; methionine (1 mg/ml) reduces the average values of parameters studied but increases the variability of individuals. The analysis in terms of fluctuating asymmetry confirmed more stable development of males in changing conditions (with the addition of folate-methionine cycle metabolites) comparatively to females.

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