ISSN 0206-5657. Вісник Львівського університету. Серія біологічна. 2014. Випуск 66. С. 85–90
Visnyk of the Lviv University. Series Biology. 2014. Issue 66. P. 85–90

УДК 57.017.64 : 57.021

GENOTOXIC EFFECT OF THE INFLUENCE OF FOLIC ACID ON HIBRID AND MUTANT STRAINS OF *DROSOPHILA MELANOGASTER*

A. Marvin, O. Antosyuk, S. Shihova

Ural Federal University named after the first President of Russia B.N. Yeltsin
19, Mira St., Ekaterinburg 620002, Russia
e-mail: antosuk-olga@mail.ru

Drosophila melanogaster is a model object often used in different investigations including genetic and toxicological investigations. The influence of folic acid on many possess such as: vitality, organogenesis, development of organisms is always presents. Posited that the influence of folic acid is different depend from parent form of *Drosophila melanogaster*.

Keywords: Folic acid, morphometric analysis, hybrid, dihydrofolatereductase.

Drosophila is convenient model object, both in genetic, and in embryological researches. The big range of mutations of drosophila allows more detail studying biological effect of individual genes. One of the most studied model systems, allowing quality and quantitative analyzing, are *vestigial* (*vg*) and *scalloped* (*sd*) mutations. Mutation of vg has violations in development already at an egg stage since DNA and protein synthesis is broken. In 1935 R. Goldshmidt showed that at interlinear hybrids (wt×*vg*) have damages like "cutting" on a wing during a selection the frequency of similar damages increases [4]. Also, in 1947 R. Khesin studied maternal effect and found reliable dependence of duration of development of egg and duration of a stage of a post-embryonal development in the drosophila, which based on from what strain there were females [7]. In the researches was shown that the frequency of occurrence of damage like "cutting" on a wing was very different: 0,2–74,4% [5]. Variability of this indicator is caused by biochemical distinctions of amount of dihydrofolatereductaseat strains of wild type of drosophila.

It is known that to 75% of genes of the drosophila are ontological genes of human, and this genes may serve as cause of various diseases, such as Alzheimer's disease or schizophrenia [6, 8].

Thus, the objective of this research was reduced to the following: on the example of interlinear hybrids and mutant strains of the drosophila to define influence of folic acid on ontogenesis.

Materials and methods

In our work the intersrtain`s hybrids received by crossing of 11 strains of wild type (*MMM*, *Aramil*, *Bios-3*, *Giovanni*, *New World*, *Host*, *Sudak*, *Belgorod*, *Chelyabinsk*, *Oregon R*, *Canton-S*) to the mutant strain *vestigial*, and also mutant strains were used: *Beadex*, *cut*, *scalloped*, *vestigial*, *Unicum* ("Unicum" is received in laboratory of Ecological genetics of animals UrFU by long selection to wing`s damages like the "cutting"("–" selection to F30 and "+" selection after F30 since the moment of emergence of a "white" mutation from the "scalloped" strain). The early stars larvae were located on Alderston's medium (yeast of 25 g, glucose of 25 g, an agar – 2 g) containing folic acid (20 mg/kg medium), and after that the frequency of occurrence of offspring's mortality at different stages and were calculate and average individual fertility of individuals were analyzed. Females and males used in pairs which were located in vessels with the agar environment in number of 25, and every day in during 10–12 days the eggs were calculated, then the frequency of occurrence of embryonic mortality of offspring at early and late stages of development in Petri's dishes, and also the frequency of occurrence of post-embryonic mortality was analyzed. The results were analyzed by program Statistica 6.0 (used criterion of Student

(P≤0,05)). The morphometric analysis of a wing was carried out on wings of adult individuals with "Universal Desktop ruler" program were used and measured 18 linear and 6 two-dimensional (the areas squares) wing`s parameters in millimeters. Then the received values were analyzed by the discriminant analysis.

Results and discussion

The first part of work developed to the model which is most suitable for studying of influence of folic acid on parameters of viability, such as: the frequency of occurrence of damages of a wing like "cutting" and a wing`s morphometry. The reciprocal crossing of 11 strains of wild type to the *vestigial* were carried out. Results of this experiment are given in table 1. This table of average values from 4–5 repeats included. The analysis of hybrids of the first generation shows variability of frequency of occurrence of damages of a wing like "cutting" (Fig. 1) like it was received before J. Goux with colleagues in 1973 [4].

Table 1

Parameters of viability of mutant srtains of *Drosophila melanogaster*
(Concentration of folic acid 20 mg/kg of medium)

Strain/ Parameter	Mean individual fecundity (folic acid)	Mean individual fecundity (control)	Frequency of early embryonic mortality (folic acid)	Frequency of early embryonic mortality (control)	Frequency of late embryonic mortality (folic acid)	Frequency of late embryonic mortality (control)
Vestigial	15.27±2.26	4.16±1.00	18.66±5.83	35.16±4.29	5.83±0.29	3.42±0.91
Cut	18.68±2.77	12.34±1.42	9.07±1.99	9.15±4.08	0.99±0.25	4.95±1.94
Scalloped	19.54±2.07	7.16±1.61	15.78±2.41	38.17±6.30	5.50±1.22	8.00±1.42
Beadex	12.97±2.03	7.73±1.23	9.62±2.56	43.12±4.51	2.44±0.61	6.24±1.02
«Unicum»	21.21±1.66	19.34±4.02	10.54±2.16	11.83±0.23	4.00±0.79	5.48±0.35

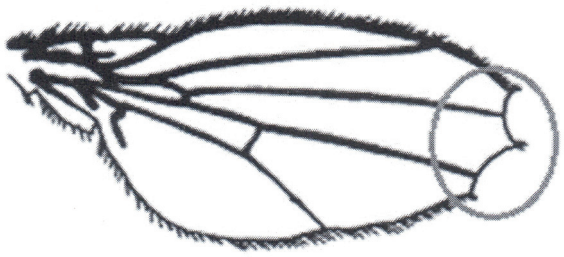

Fig. 1. Damages like "cutting" on the wings of hybrid strains (wt×*vg*).

In our research this values were 1,2–36,0% whereas in J. Goux works were 0,2–74,4%. Frequency of occurrence of damages like "cutting" on a wings demonstrated that every strain of wild type is very different. It means that the frequency of occurrence of the damages of a wing aroused from influence of vg and to some extent which strain of wild type used. Different strains of wild type demonstrated different value of dihydrofolatereductase enzyme. The largest frequency of occurrence of damages on wings in each of 11 crossings with the strain of wild type is observed in that case when as a maternal organism was the *vg* (maternal effect). In F4 by way "+" selections the frequency of occurrence of damages like "cutting" of the wings considerably increases, and the maternal effect is minimized, and combinative variability became the base on chromosome level for selection. Apparently from table 1 in a course the reciprocal crossings were found, namely: when we used as a maternal form of the *vg* mutant strain we found damage of a wing at males more quantity than at females practically in all crossings (Tabl. 1).

Probably, in during of long selection of the X-chromosome of *vg* strain the genes modifiers accumulate in homozygote. The gene of *vg* of hybrids is already presented in a heterozygote

form, and genes modifiers can quite change an expression and a penetration of vg. The largest frequency of damages is found in experiments with use of strains: *Giovanni*, *New World*, *Host*, *Chelyabinsk* and *Sydak*. The further researches held on to influence of folic acid, are given on the *Giovanny* and *Host* strains. The analysis of mean individual fertility of the *Giovanny* and *Host* strains showed more higher then mean individual fecundity of vestigial mutant strain: in the *Giovanni* strain 22,52±1,64, in the *Host* 48,63±1,76, whereas in the vg 13,17±1,33. Life expectancy of the *vg* strain of 2 weeks, whereas at strains of wild type of 1 month to the 3rd. The mean individual fertility of hybrids of the first generation is also reduced rather control group: *vg×Giovanni* 14,07±1,48, *vg×Host* 20,92±1,33, also the frequency of occurrence of embryonic mortality at early and late stages of development considerably increases in comparison with control group. The embryonic mortality of *vg×Giovanni* strain increases to 26,82 (control 0,96) at early stages of development and to 4,65 (control 0,46) at late stages. The embryonic mortality of *vg×Host* strain at early stages of development obtained 23,52 (control 5,94), and at late stages-5,82 (control 1,04). The parameters of viability of hybrids, which have been grown up on the medium with folic acid show ambiguous influence of folic acid, so to the *vg×Host* strain folic acid doesn't render any noticeable positive effect, even fertility slightly decreases and the frequency of occurrence of embryonic mortality increases. And to the *vg×Giovanni* strain folic acid renders the expressed positive effect reducing embryonic mortality: at an early stage of development to 0,91 (control 1,76), and on late-0,44 (control 0,49). Thus, distinction of strains of wild type and what they are a basis for hybrids of the first generation, providing difference in work dihydrofolatereductase, and respectively a frequency of occurrence of damages of wing like "cutting" is confirmed.

The morphometric analysis of a wing showed that hybrid strains have obvious differences in parameters of wings unlike the strains of wt participating in the reciprocal crossings (Fig. 2). Wings' difference of parameters at the hybrid strains: *vg×Host* and *Host×vg*, and also *vg×Giovanni* and *Giovanni×vg* attests to influence of maternal effect on processes of a morphogenesis of a wing (Fig. 3). The folic acid influences on wings' parameters at both hybrid strains (Fig. 4).

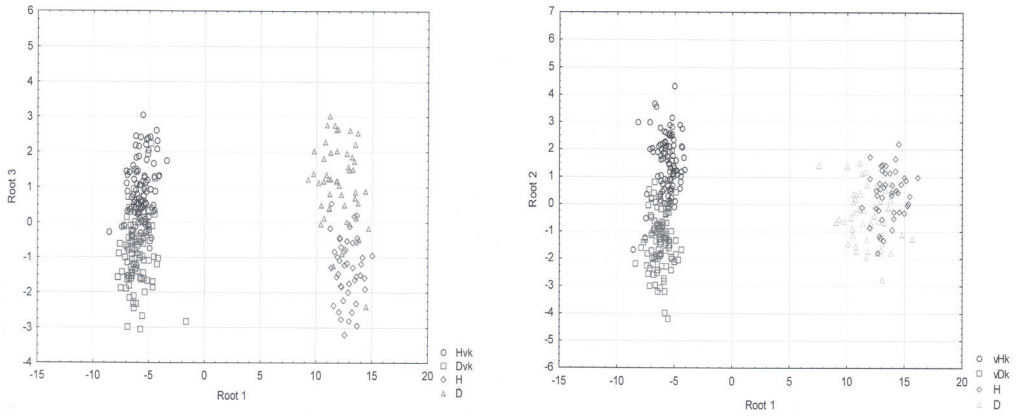

Fig. 2. Chart of dispersion of values (*Hvk-Host×vg*; H- Host wt; *Dvk- Giovanni×vg*; D-Giovanni wt)-left, Chart of dispersion of values (*vHk-vg×Host*; *H-Host* wt; vDk- *vg×Giovanni*; D-Giovanni wt)-right.

The ambiguous effect of influence of folic acid at mutant strains is also expressed. In the vestigial and cut strains the positive effect of folic acid for certainly is shown (Statistica 6.0, Student's criterion) increases mean individual fertility and reduces the frequency of occurrence of embryonic mortality (Tab. 1). In the presence of folic acid fertility increases only, and the frequency of embryonic mortality changes within control values in the scalloped and *Beadex* strains.

Besides, in the *Unicum* strain such indicator as the frequency of occurrence of embryonic mortality is has observed negative effect of presence of folic acid. It was shown that in the *Beadex* strain after influence by folic acid many indicators of a wing including both its length and all areas of the wings also change covered. In the scalloped strain only 4 wings` linear parameters change: AD, AM, KF and FC, insignificant (fig. 5) by the size. Thus, ambiguity of influence of folic acid can be observed at the level of generative and somatic cells, and at the level of also mutant strains, and strains of wild type react to presence of folic acid differently, showing linear distinctions which can consist in various quantity dihydrofolatereductase.

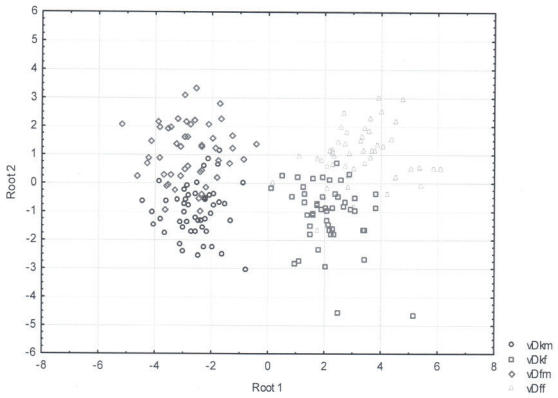

Fig. 3. Chart of dispersion of values (Hvk-*Host*×*vg*; Dvk-*Giovanni*×*vg*; vHk- *vg*×Host; vDk- *vg*×*Giovanni*).

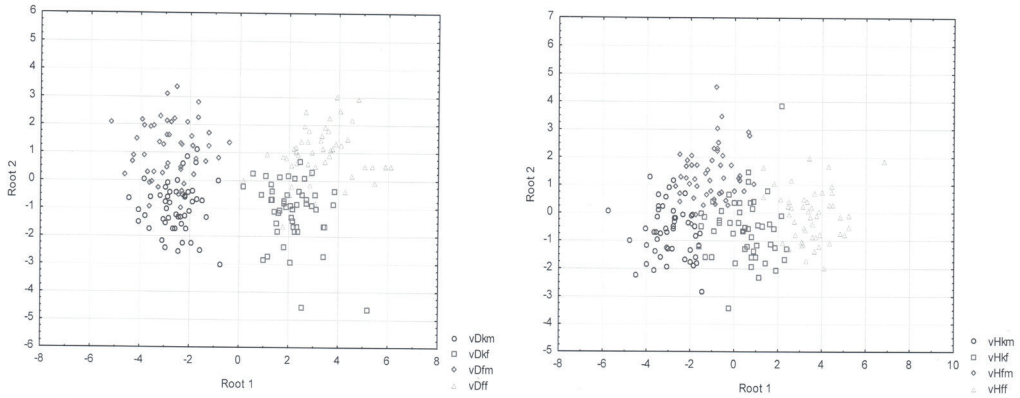

Fig.4. Chart of dispersion of values (vDk-*vg*×*Giovanni*; vDf-*vg*×*Giovanni* on folic acid; m-male, f-female)-left, Chart of dispersion(vHk-vgHost; vHf-*vg*×Host on folic acid; m-male, f-female)-right.

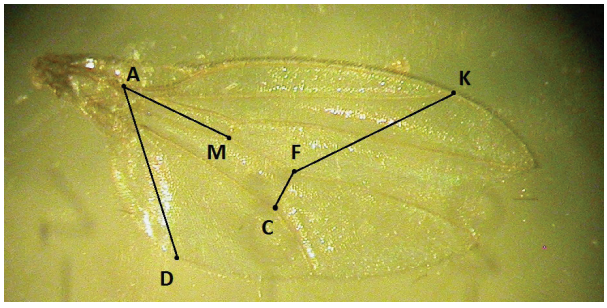

Fig. 5. The wing of strain ‘scalloped’ after the influence of folic acid (changed AD, AM, KF and FC).

Thus, hybrid individuals of *vg*×wt or wt×*vg* have excellent indicators of viability and wings` parameters, partly, due to existence of maternal effect. Also probably, due to so-called incomplete domination of *vg* at heterozygotes changes of wings' parameters of rather control strains of wild type, and also changes of a number of indicators of viability is observed. These differences are based on a difference of strains of wild type among themselves, and also various activity of enzyme dihydrofolatereductase. Besides, influence of folic acid impact on ontogenesis stages that the share of each strain of wild type necessary for a positive effect can be also the result of linear distinctions in normal conditions, and also because of various need of a certain amount of folic acid is noted that also is observed and also on mutant strains.

The authors would like to thank Ekaterina Burnatova and Yuliya Ginzburg for their help in literature and mathematic parts.

REFERENCES

1. *Antosyuk O. N., Krysova L. V., Davidenko K. A.* et al. The influence of Methotrexate on instability of genome of *Drosophila melanogaster* 2010 // Drosophila in the experimental genetics and biology: The 2nd International conf. Theses. 2010. P. 17–20.
2. *Butzbach K., Epe B.* Photogenotoxicity of folic acid // Free Radic. Biol. Med. 2013. Vol. 13. P. 571–576.
3. *Deng H., Hughes S., Bell J., Simmonds A.* Alternative Requirements for vestigial, scalloped, and Dmef2 during muscle Differentiation in *Drosophila melanogaster* // Mol. Biol. Cell. 2009. Vol. 20. N 1. P. 256–269.
4. *Goldschmidt R.* Gen und Aussencharkter III // Biol. Zentralbl. 1935. P. 55–535.
5. *Goux J.* Variationda la penetrance et de la dominance an locus vestigial chez la drosophile // Comptes Rendus de l`academie des Scienes de Paris. 1973. N 277. P. 2413–2415.
6. *Hirth F. Drosophila melanogaster* in the study of human neurodegeneration // CNS and Neurological Disorders-Drug Targets. 2010. Vol. 9. P. 504–523.
7. *Khesin R. B.* Maternal effect of *Drosophila melanogaster*. Prevalence of maternal effect // DAN USSR (Rus). 1947. Vol. 58 .P. 667–670.
8. *Lenz S., Karsten P., Schulz J., Voigt A.* Drosophila as a screening tool to study human neurodegenerative diseases // J. Neurochem. 2013. Vol. 127. P. 453–460.

Стаття: надійшла до редакції 24.01.14

доопрацьована 24.03.14

прийнята до друку 12.05.14

ГЕНОТОКСИЧНИЙ ЕФЕКТ ВПЛИВУ ФОЛІЄВОЇ КИСЛОТИ НА ГІБРИДНІ Й МУТАНТНІ ЛІНІЇ ДРОЗОФІЛИ

А. Марвін, О. Антосюк, С. Шихова

Уральський Федеральний університет
імені першого Президента Росії Б. М. Єльцина
вул. Миру, 19, Єкатеринбург 620002, Росія
e-mail: antosuk-olga@mail.ru

Drosophila melanogaster – модельний об'єкт, який використовують у різноманітних дослідженнях, зокрема, генетичних і токсикологічних. Вплив фолієвої кислоти

завжди наявний і діє на такі процеси, як, наприклад, життєздатність, органогенез, розвиток цілісного організму. Встановлено, що вплив фолієвої кислоти різний і залежить від батьківської форми дрозофіли.

Ключові слова: фолієва кислота, морфометричний аналіз, гібрид, дигідрофолатредуктаза.

ГЕНОТОКСИЧЕСКИЙ ЭФФЕКТ ВОЗДЕЙСТВИЯ ФОЛИЕВОЙ КИСЛОТЫ НА ГИБРИДНЫЕ И МУТАНТНЫЕ ЛИНИИ ДРОЗОФИЛЫ

А. Марвин, О. Антосюк, С. Шихова

Уральский Федеральный университет
имени первого Президента России Б.Н. Ельцина
ул. Мира, 19, Екатеринбург 620002, Россия
e-mail: antosuk-olga@mail.ru

Drosophila melanogaster – модельный объект, используемый в различных исследованиях, включая генетические и токсикологические. Влияние фолиевой кислоты всегда присутствует, и действует на такие процессы, как, например, жизнеспособность, органогенез, развитие целостного организма. Установлено, что воздействие фолиевой кислоты различно и зависит от родительской формы дрозофилы.

Ключевые слова: фолиевая кислота, морфометрический анализ, гибрид, дигидрофолатредуктаза.