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knirps and *cubitus interruptus* loci interaction in wing veins pattern formation in *Drosophila melanogaster* (morphology analysis)

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We studied the expressivity of radius incomletus and cubitus interruptus traits of *Drosophila melanogaster* imagoes, depending on the individual's genotype, sex, and analyzed wing. It has been established that the presence of kni^{ri} mutation leads to the change of cubitus interruptus trait expressivity (partial suppression of mutant phenotype at the background of general venation pattern changings). On the contrary the presence of ci^{1} mutation in the genotype does not affect the radius incompletus trait expressivity. Males demonstrate more pronounced expressivity of both traits than females. No bilateral asymmetry in the expressivity of corresponding mutant features has been detected. Standing on the results obtained we suppose that *knirps* and *cubitus interruptus* loci do interact in wing veins pattern formation, and *knirps* is the regulatory factor for *cubitus interruptus* expression.

Key words: *Drosophila melanogaster, expressivity, radius incompletus, cubitus interruptus, nonallelic genes interactions.*

Взаимодействие локусов *knirps* и *cubitus interruptus* при формировании паттерна жилкования крыла *Drosophila melanogaster* (анализ морфологии)

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Мы изучили экспрессивность признаков radius incomletus и cubitus interruptus у имаго *Drosophila melanogaster*, в зависимости от генотипа особи, пола и анализируемого крыла. Было установлено, что присутствие мутации kni^{ri} приводит к изменению экспрессивности признака cubitus interruptus (частичная супрессия мутантного фенотипа на фоне изменения общего паттерна жилкования). Наоборот, наличие мутации ci^{1} в генотипе не влияет на экспрессивность признака radius incomletus. Самцы характеризуются большей экспрессивностью обоих признаков, чем самки. Не выявлено билатеральной асимметрии в экспрессивности соответствующих мутантных фенотипов. Исходя из полученных результатов, мы полагаем, что локусы *knirps* и *cubitus interruptus* взаимодействуют при формировании особенностей жилкования крыла, и *knirps* является фактором регуляции экспрессии *cubitus interruptus*.

Ключевые слова: *Drosophila melanogaster, экспрессивность, radius incompletus, cubitus interruptus, взаимодействие неаллельных генов.*

Взаємодія локусів *knirps* та *cubitus interruptus* при формуванні патерну жилкування крила *Drosophila melanogaster* (аналіз морфології)

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Ми вивчили експресивність ознак radius incomletus і cubitus interruptus в імаго *Drosophila melanogaster*, залежно від генотипу особини, статі та аналізованого крила. Було встановлено, що присутність мутації kni^{ri} призводить до зміни експресивності ознаки cubitus interruptus (часткова супресія мутантного фенотипу на тлі змін загального патерну жилкування). Навпаки, наявність мутації ci^{1} в генотипі не впливає на експресивність ознаки radius incomletus. Самці характеризуються більшою експресивністю обох ознак, ніж самки. Не виявлено білатеральної асиметрії в експресивності відповідних мутантних фенотипів. Виходячи з отриманих результатів, ми вважаємо, що локуси *knirps* і *cubitus interruptus* взаємодіють при формуванні особливостей жилкування крила, і *knirps* є чинником регуляції експресії *cubitus interruptus*.

Ключові слова: *Drosophila melanogaster, експресивність, radius incompletus, cubitus interruptus, взаємодія неалельних генів.*

Introduction

One of the most actively studied questions in modern genetics is the investigation of systems of quantitative traits genetic control. In addition to poor understanding of quantitative traits genetic control

mechanisms, ways of their developmental formation and population dynamics responses to direct and indirect artificial selection pressure, their role in the evolution of species also is not described completely.

Various genes interactions within the genotype affect significantly the overall genotype norm of reaction. As a result physiological manifestations of the same gene in the same environmental conditions may differ in individuals with different genotypes. The gene can manifest itself as a dominant one in some individuals but have no manifestation in others. The degree of phenotypic expression of the gene can vary significantly (even to its 100% penetrance). The manifestation of traits with variable expressivity depends not only on external and internal (genetic) environment, they are also usually characterized by developmental variability resulted from the errors or alternative metabolic circuits in genotype realization (Vorobyova, Gogadze, 2001).

The genetic factors are very important for the formation of traits with variable expressivity. In the continuation of genotype effects studies on the expressivity of some of them (radius incompletus, vestigial, Bar, eyeless, and others) in Drosophila melanogaster (Zolotykh, Nekrasova, 2004; Kirpichenko et al., 2002; Philiponenko et al., 2008) this work has been carried out.

It should be said that both *knirps* and *cubitus interruptus* loci are well studied and their interactions with other genes are described rather completely (Gene Dmel\kni…, Gene Dmel\ci…), but the information about their interaction with one another is rather reduced (Diaz-Benjumea, Garcia-Bellido, 1990). Thus, the aim of this work is to evaluate the features of interaction between *knirps* and *cubitus interruptus* mutant loci breaking wing venation in *Drosophila melanogaster*. To accomplish this goal the following tasks were formulated: (1) to analyze radius incomletus and cubitus interruptus traits expressivity, depending on the genotype, the sex of individuals, and analyzed wing (left or right), (2) to analyze the mutual influence of mutations on correspondent phenotypes expressivities, (3) to establish if there any correlation between changes in traits studied.

Materials and methods

We used three *Drosophila melanogaster* stocks: *knirps* (*kni^{ri}*), *cubitus interruptus^{1}* (*ci^{1}*) and the double mutant *kni^{ri}; ci^{1}* from the Collection of drosophila stocks of Genetics and Cytology Department of V.N.Karazin Kharkiv National University that is among objects that constitute Ukraine National Heritage (fig. 1). Since the stocks carrying the mutations may have modifiers which influence their phenotype, we have outcrossed them to wild type (Canton-S) before study.

kni^{ri} *ci^{1}* *kni^{ri}; ci^{1}*

Fig. 1. Phenotypic expression of mutations tested

Individuals of all stocks were reared in the standard corn-meal medium under the constant temperature (t=23±1°C) and humidity conditions. During the first day after imagoes emerge they were separated according to the sex and were holding separately on temporary food till the wing morphology analysis (not longer than 3 days).

Traits expressivity was evaluated individually both in right and left wings as the ratio of the length of the missing portion of the correspondent vein to the projection of its full length. We analyzed 40 individuals of each sex. Measurements were carried out in digital photographs of imagoes wings, made by means of 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. The result was performed as a fraction. Statistica 6.0® was used for statistics analysis (ANOVA, Pearson correlation).

Results and their discussion

The index of radius incompletus trait expressivity (fig. 2) in *kni^{ri}* stock for females varies as 0,38±0,01 both on the left and the right wings. Males of the same stock are characterized by higher values of the index supported by low level (but statistically insignificant; t=1,4; P>0,05) of bilateral asymmetry: 0,46±0,01 – in the left wing and 0,44±0,01 – in the right one. The asymmetry of manifestation of this trait was tested because previously it has been shown by us in radius incompletus males (Philiponenko et al., 2008). In this study, it was found that the proportion of males with the most profound manifestation of radius incompletus trait is significantly higher than the proportion of females of the same class.

The presence of *cubitus interruptus* mutation in the genotype of radius incompletus individuals only slightly reduces the radius incompletus trait expressivity (fig. 2). At the background of general decline in expressivity there is a slight change in the asymmetric expression of the mutation in females (0,36±0,02 – left wing and 0,35±0,02 – right wing) probably resulted from the variability level increase in general that can be indirectly indicated by an increase of the standard error of the mean (as it relates to the indication of the variance). The differences between the expressivity of the trait in males on different sides of the body, on the contrary, is not changed: 0,45±0,02 – left wing and 0,43±0,01 – the right wing.

ANOVA confirmed the dependence of the radius incompletus trait expressivity on the sex of the individual (F=49,23; P<0,001) that may indicate the modifying effect of sex chromosomes genes in the genetic control system of the radial wing veins formation.

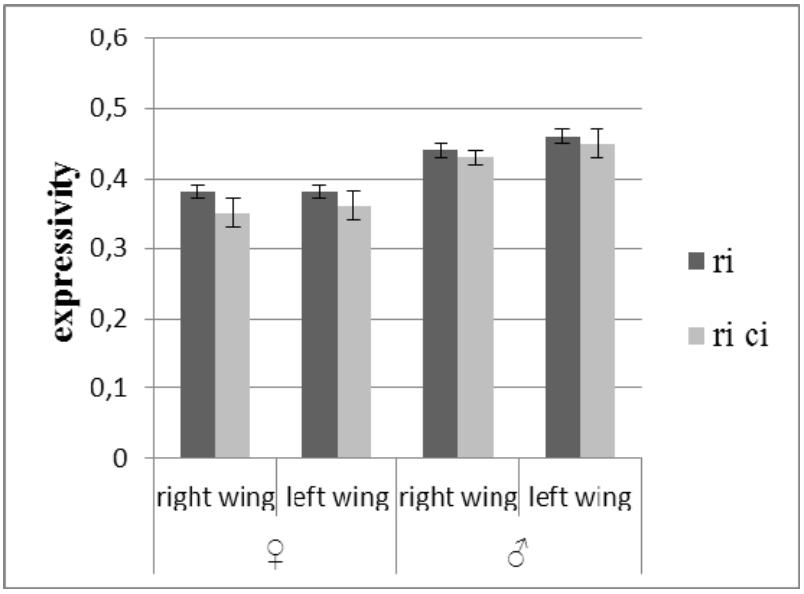


Fig. 2. radius incompletus trait expressivity depending on the genotype, sex and wing analyzed (n=40)

The system of *knirps* gene expression is the example of classical polygenic systems. It contains the main effect gene (*knirps*) and a large number of modifier genes (polygenes), each of which makes rather small contribution to the expressivity of the trait. The size variation of two (proximal and distal) vein fragments depends on the action of modifier genes (Vasilyeva, Ratner, 2000). More than 50% of Drosophila genome is represented by the genes with the function of quantitative traits modifiers (Vasilyeva, 2004). Individuals, which we refer to a particular class, probably, have a different combination of modifier genes alleles.

The variability of cubitus interruptus trait expressivity depending on the same factors (genotype, sex and side of the body) is characterized by several different parameters (fig. 3). The presence of *kni^{ri}* mutation

significantly alters the degree of manifestation of *ci*^{1}: trait expressivity decreases in both sexes on both wings.

In females cubitus interruptus expressivity in the left wing is changed from 0,43±0,03 to 0,27±0,03 (t=3,8; P<0,01), and in the right one – from 0,41±0 04 to 0,27±0,03 (t=2,8; P<0,01). Existed originally slight asymmetry is absent in the double mutants. In males, there are less pronounced changes: in the left wing the expressivity changes from 0,48±0,02 to 0,41±0,04, and in the right one – from 0,50±0,02 to 0,39±0,03. But it should be noted that in males the asymmetry of changings is more pronounced.

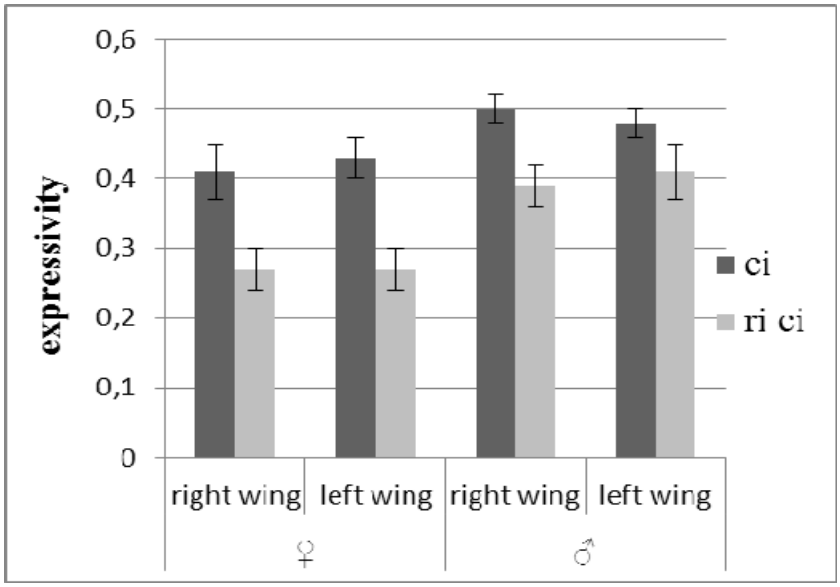
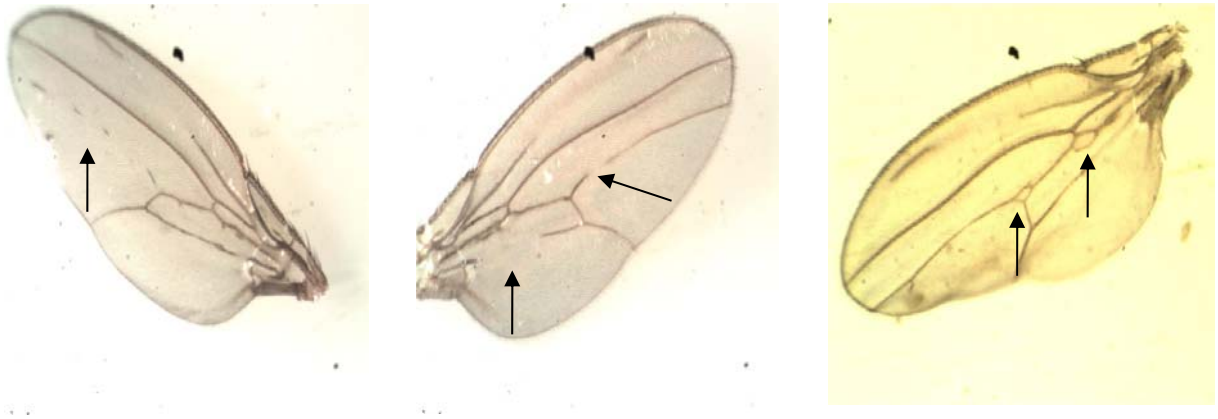


Fig. 3. cubitus interruptus trait expressivity depending on the genotype, sex and wing analyzed (n=40)

ANOVA has proved the dependence of cubitus interruptus trait expressivity on the genotype (i.e., the presence of additional mutation affecting the veins pattern formation) – F=33,18; P<0,001 – and on the sex of individuals – F=22,93; P<0,001. There is also the correlation between the these two traits expressivity changings: for females on the right wing r=-0,32; P<0,05, and for males on the same wing r=0,32; P<0,05).



L4 vein fragmentation L5 interruption Deltas formation

Fig. 4. Wing veins pattern changings in double mutants *kni*^{ri}*;ci*^{1}

It should be also said that besides changes directly in the cubital vein (the gap reduction) the presence of *kni*^{ri} mutation in the genotype of *ci*^{1} mutants causes an increase in the frequency of individuals with

complex venation anomalies (absence or fragmentation of other veins, abnormal cells, etc.) (fig. 4), which partly confirms the hypothesis about the importance of kni^{ri} expression product for the regulation of ci^{1} functioning.

It is known that in Drosophila *ci* locus is necessary for normal development. This gene is expressed in all cells of anterior compartments, in embryos and in imaginal discs (Schwartz et al., 1995). And it is the regulatory one for the two genes controlling segmentation – *Hedgehog* and *decapentaplegic*. Thus, the results of this study fulfill this picture with the evidence of cubitus interruptus and radius incompletus genetic systems interaction.

Conclusions

It was found that all three *D. melanogaster* stocks examined (kni^{ri}, ci^{1} and kni^{ri}; ci^{1}) are characterized by persistent intersexual differences in correspondent mutations expressivities. And the traits expressivities in males are higher than in females. None of the genotypes studied revealed any bilateral asymmetry in the expressivity of corresponding mutant traits. It is shown that the presence of ci^{1} mutation in the genotype does not affect radius incompletus trait expressivity. But it was found that the presence of kni^{ri} mutation changes cubitus interruptus trait expressivity: the magnitude of the gap between the fragments veins decreases, but the frequency of individuals with complex venation anomalies (absence or fragmentation of other veins, abnormal cells) arises also.

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