



## OPPONENT'S REVIEW ON BACHELOR/DIPLOMA \* THESIS

**Name of the student:** Anna Rázková

**Thesis title:** The effect of CG18446 on the specification of circulating immune cells in *Drosophila melanogaster*

**Supervisor:** RNDr. Alena Krejci, Ph.D.

**Referee:** Adam Bajgar, Ph.D.

**Referee's affiliation:** The University of South Bohemia, Department of molecular biology and genetics, Laboratory of integrative physiology

	Point scale <sup>1</sup>	Points
<b>(1) FORMAL REQUIREMENTS</b>		
<b>The extent of the thesis</b> (for bachelor theses min. 18 pages, for masters theses min. 25 pages), <b>balanced length of the thesis parts</b> (recommended length of the theoretical part is max. 1/3 of the total length), <b>the logical structure of the thesis</b>	0-3	3
<b>Quality of the theoretical part (review)</b> (number and relevancy of the references, recency of the references)	0-3	2
<b>Accuracy in citing of the references</b> (presence of uncited sources, uniform style of the references, use of correct journal titles and abbreviations)	0-3	2
<b>Graphic layout of the text and of the figures/tables</b>	0-3	3
<b>Quality of the annotation</b>	0-3	3
<b>Language and stylistics, complying with the valid terminology</b>	0-3	2
<b>Accuracy and completeness of figures/tables legends</b> (clarity without reading the rest of the text, explanation of the symbols and labeling, an indication of the units)	0-3	2
<b>Formal requirements – points in total</b>		<b>17</b>
<b>(2) PRACTICAL REQUIREMENTS</b>		
<b>Clarity and fulfillment of the aims</b>	0-3	3
<b>Ability to understand the results, their interpretation, and clarity of the results, discussion, and conclusions</b>	0-3	2
<b>Discussion quality – interpretation of the results and their discussion with the literature</b> (absence of discussion with the literature is not acceptable)	0-3	2
<b>Logic in the course of the experimental work</b>	0-3	3
<b>Completeness of the description of the used techniques</b>	0-3	2

\* Choose one

<sup>1</sup> Mark as: 0-unsatisfactory, 1-satisfactory, 2-average, 3-excellent.

The experimental difficulty of the thesis, independence in experimental work	0-3	2
Quality of experimental data presentation	0-3	2
The use of up-to-date techniques	0-3	3
The contribution of the thesis to the knowledge in the field and the possibility to publish the results (after eventual supplementary experiments)	0-3	2
Practical requirements – points in total		21
<b>POINTS IN TOTAL (MAX/AWARDED)</b>	<b>48</b>	<b>38</b>

**Comments of the reviewer on the student and the thesis:**

The aim of the project is to analyze the effect of a mutation in gene CG18446 on the hematopoiesis, differentiation, and mobilization of *Drosophila* immune cells in response to genetically simulated immune system challenge. The experiments are well-designed and the approaches are reasonable. Making these experiments in enough biological replications and with standardized genetic background could bring very valuable data perfectly fitting ongoing project in the mentor's laboratory. The project is another example of showing *Drosophila* as a simple and elegant model organism for the solution of complicated biological questions *in vivo*.

**The thesis itself** consists of typical chapters of expected range and often of good quality. Nevertheless, there are some particular points that can be easily corrected by one more reading if the author would pay more attention to both stylistics of the text as well as the interpretation of data and their presentation.

I would like to praise section called **Summary** where the author made a good introduction into her motivations, aiming, techniques, and outcomes of the thesis in a few sentences – which really helps to understand and orient in the rest of the text.

**Introduction** of the thesis is well-organized and clearly leads the reader to the motivation of the research team to set up following experiments, while elucidates what is already known about the problematics. Some facts are presented a little bit chaotically with misleading interpretations, which is probably result of not very deep knowledge of the problematics (which is completely understandable in student of bachelor degree). Some chapters (like "Lifecycle") seem to be pointless in the context of the thesis itself, while some important topics are missing such as "Role of JAK/STAT directly in lymph gland", "Description of HopTum phenotypes in context of already published research", "Principle of melanotic reaction" (Gal4>UAS, Immune response to wasp infestation). Contrary to my criticism, I would evaluate the introduction as very good.

**Aims of the thesis** are well-defined and clear, even though I would omit the explanation of the selected approaches here.

Part **Material and Methods** is brief (in positive meaning) with good enough description of experiments allowing their easy recapitulation. Nevertheless, two concrete parts of the methods make the results very confusing and disturbing:

1. The first is a **description of genotypes** analyzed in particular experiments. Genotypes of flies used in the thesis are not satisfactorily described the same as concrete genotypes used in particular experiments. Author described which fly lines were crossed for progeny used in particular experiments rather than final genotype, which makes the interpretation and understanding of the data very hard.
2. The second point is very atypical use of asterisks for marking statistical significance making the reading of result very confusing.

The **results** are well-written and perfectly organized. Although, interpretation of some

experiments is complicated by not complete clarity of genotypes used in the experiments. Since the aim of the thesis is to describe the effect of a mutation in CG18446 gene on phenotype induced by another mutation (Hop Tum), I would suggest to carry out experiments describing the effect of CG18446 mutation on hematopoiesis in comparison to wild-type flies too. To see whether there is the effect only in response to activation or in response just to triggered differentiation. Part of the work has been made on a confocal microscope where the ratio of cells was calculated from confocal pictures – unfortunately, the pictures are not present in the thesis at all – even though they could represent the most attractive part of the thesis. Not complete clarity of the data (at least for me) raised several questions I'm going to ask at the end of my review.

In the **discussion**, the data and outcomes of the thesis are very well described and interpreted but their comparison with current knowledge is poor or almost missing.

In summary, the thesis is focused on the important question about the regulation of hematopoiesis and data can help us to understand the general regulatory mechanisms of hematopoietic niche maintenance and differentiation. The author learned several interesting techniques from Drosophila genetics to microscopy analysis on both inverted fluorescent and confocal microscope same as work with literature and preparation of publication outcome. In this view, the work Anna made, is more than satisfactory on the level of bachelor student degree.

**Suggestions and questions, to which the student has to answer during the defense.**

**Mistakes, which the students should avoid in the future:**

1. In **results** author used MEDIANS for a description of data gained by counting of cells – why? Particularly when using T-tests for statistics, working with means. What median actually represents and when is it better contrary to mean and standard deviation?
2. How can be explained the huge difference in data sets between plots 7-12-16?
3. Hop-tum genetic construct is present on the X chromosome (present in females in two copies while in males in just one copy) – is there any difference in case of females and males in the phenotypes. If not - by which mechanism is it caused?
4. The disadvantage of using mutants is the presence of the mutation during the whole development – are there some developmental phenotypes connected with both – a gain of function mutation Hop-Tum and loss of function mutation CG18446 – in comparison to wild-type flies?
5. Gene CG18446 is expressed in all immune tissues (such as lymph gland; circulating hemocytes and fat body). By which tool/approach can author dissect what tissue is crucial for the partial rescue of Hop-tum induced phenotypes presented in the thesis.

**Conclusion:**

In conclusion, I

**r e c o m m e n d**

**the thesis for the defense and I suggest the grade Very good which can be improved by presentation and discussion during the defense itself.<sup>2</sup>**

In České Budějovice date 7/1/2018

Adam Bajgar, Ph.D.