Přírodovědecká fakulta
Faculty of Science

Jihočeská univerzita v Českých Budějovicích
University of South Bohemia in České Budějovice

OPPONENT'S REVIEW ON BACHELOR/DIPLOMA* THESIS

Name of the student: **Verena Fettinger**

Thesis title: **Influence of Juvenile Hormone and its Receptor on the Immune system during Metamorphosis of Drosophila melanogaster**
Supervisor: **prof. Marek Jindra, Ph.D.**

Referee: **RNDr. Pavlína Věchtová, Ph.D.**
Referee`s affiliation: **Faculty of Science, University of South Bohemia in České Budějovice & Institute of Parasitology, Biology Centre CAS**

	Point scale[1]	Points
(1) FORMAL REQUIREMENTS		
Extent of the thesis (for bachelor theses min. 18 pages, for masters theses min. 25 pages), **balanced length of the thesis parts** (recommended length of the theoretical part is max. 1/3 of the total length), **logical structure of the thesis**	0-3	1.5
Quality of the theoretical part (review) (number and relevancy of the references, recency of the references)	0-3	1
Accuracy in citing of the references (presence of uncited sources, uniform style of the references, use of correct journal titles and abbreviations)	0-3	0.5
Graphic layout of the text and of the figures/tables	0-3	1
Quality of the annotation	0-3	3
Language and stylistics, complying with the valid terminology	0-3	1.5
Accuracy and completeness of figures/tables legends (clarity without reading the rest of the text, explanation of the symbols and labeling, indication of the units)	0-3	0
Formal requirements - points in total		**8.5**
(2) PRACTICAL REQUIREMENTS		
Clarity and fulfillment of the aims	0-3	2
Ability to understand the results, their interpretation, and clarity of the results, discussion, and conclusions	0-3	1
Discussion quality - interpretation of the results and their discussion with the literature (absence of discussion with the literature is not acceptable)	0-3	1.5
Logic in the course of the experimental work	0-3	3

* Choose one

[1] Mark as: 0-unsatisfactory, 1-satisfactory, 2-average, 3-excellent.

Completeness of the description of the used techniques	0-3	2
Experimental difficulty of the thesis, independence in experimental work	0-3	2
Quality of experimental data presentation	0-3	1
The use of up-to-date techniques	0-3	2
Contribution of the thesis to the knowledge in the field and possibility to publish the results (after eventual supplementary experiments)	0-3	2
Practical requirements - points in total		**16.5**
POINTS IN TOTAL (MAX/AWARDED)	**48**	**(25)[2]**

Comments of the reviewer on the student and the thesis:

1) There are too few references used to build this thesis. In particular, only 7 peer-reviewed publications were used to build the entire thesis, including introduction and discussion! Most information used in the introduction are sourced from a Flybase database, which is not appropriate for any thesis. For example, all the information about antimicrobial peptides were obtained in Flybase database although there are many peer-reviewed publications that describe these peptides equally well, such as for example Hui-Yu, 2014. Insect Antimicrobial Peptides and Their applications (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4083081/).

2) An extensive Introduction and Result part is presented within the Method section. This is the case of sections "Experimental assembly" and "*Drosophila melanogaster* genotypes" which should both be a part of Introduction chapter and subchapters "Testing the kit", "Testing the Trizol Method", "Comparing the "Triozol Method" to the RNeasy kit", and "Testing cDNA synthesis" should be a part of the Results chapter.

3) A molecular-weight size marker description is missing in all figures presenting the results of agarose gel electrophoresis.

4) Names of chemicals used for building this thesis are given arbitrarily. For example "iQ SYBR kit" instead of "iQ SYBR Green Supermix", or are even missing, such as in case of cDNA synthesis method description.

5) In the method chapter on page 32, there is a reference missing for the information that DmEcad is a housekeeping gene for *D. melanogaster*.

6) A description of x-axis is missing in all graphs. Based on the information provided in Methods, I assume it should be delta/delta Ct?

7) I believe that it is a general consensus that the reference for both tables and figures should be provided in the preceding text, which I did not find anywhere in the text and which is another formal defect of this work.

8) Both the table and figure headings should be self-explanatory.

[2] Enter the number of points awarded.

Unfortunately, this is not the case of this work. I was not able to understand the content of each graph and table without a long search through entire work in order to find the respective description if any.

9) The results are presented within large graphs and tables extending 17 pages! This way of data presentation is very unfortunate as it disables data comparison and contextual interpretation. In the future, I highly recommend a more concise presentation, such as combining several graphs into one large graph with parallel bars describing expression of each antimicrobial peptide. The same rearrangement would be very helpful with corresponding tables.

10) An extensive result part is present in a Discussion chapter. In fact, the results that are presented in the discussion chapter in a subchapter "My findings" are just a summary of results that are extensively described in a Result chapter. These results are not put into context with existing literature and thus it does not make a sense to place them to Discussion.

11) I don't understand the purpose of a chapter "Figures" on page 60, particularly when the list of tables in the thesis follows and no reference to a single figure is presented here.

12) Instead, I would appreciate a list of abbreviations as it took me about 25% of entire time dedicated for reading and understanding the thesis to search for the meaning of each abbreviation in the text of the thesis.

13) References are not written in the correct form. Especially the last three references either contain extra information or are missing some details or both. Moreover, the very last reference refers to some webpage. Proper thesis should use mainly peer-reviewed publication to support the presented data and not some random webpage. Web page reference can only be used in very specific examples such as citing a database, or software, etc. I am convinced that An Introduction to *Drosophila melanogaster* can be found in plenty of peer-reviewed publications.

14) Referencing the Flybase is also not correctly formulated, moreover, there is a word "accessed" written in German.

15) There are many more formal flaws, inaccurate formulations, typos and grammar mistakes in this work, however, it is out of the time range to discuss them here and I believe that they could be easily found upon closer revision of this thesis by any reader.

Suggestions and questions, to which the student has to answer during the defense. Mistakes, which the students should avoid in the future:

1) In the Result chapter in tables 17-23 and figures 44-50, a comparison of a "wild type-like" genotype with mutant *w gce*^{2,5k} and *w Met*^{27} is made in order to assess potential up- or down-regulation of antimicrobial peptides expression in these mutants. Why was the *yw* genotype of *D. melanogaster* considered a "wild type"? From the introductory part, I

understood that this genotype carries mutation "yellow" leading to a non-wild type yellow cuticle colour and "white" leading to a non-wild type white eyes colour. Why was not an actual wild type fly used for the comparison?

2) I could not find a description of Met receptor function in *w Met*^{27} and Gce receptor function in *D. melanogaster w gce*^{2,5k} genotype, which is quite inconsistent because other mutations are described properly. Could you, please, provide a description of the function of these receptors and a phenotypes that result from the mutations in these genes?

3) I could not find any information about a standard deviation that is calculated from experimental replication in the bar graphs. In bar graphs, it is usually presented as "whiskers" and expresses a statistical reliability of the presented data. In fact, I could not find any information about biological replicates being used for the generation of the expression data at all and it would be advisable to mention that in both method and result part. Could you, please, clarify whether a biological replication of the expression was used and if not, what was the reason for it?

4) In the Results chapter in the subchapter "Developmental profiles of AMP mRNA expression in control and JH receptor mutants" the relative expression of antimicrobial genes from samples isolated using "RNeasy micro kit" was calculated using different housekeeping gene expression data. In particular, Att-A was normalized to DmEcad, while the remaining genes' expression data were normalized using RP49. The expression data of antimicrobial genes collected from samples isolated using the "Trizol method" were all normalized to RP49 gene expression only. What was the reason for this?

5) In the Result chapter, there is very little information written in the subchapter "Expression of transgenic AMP reporter construct in vivo". Although the author states that the experiment did not work as expected I am convinced that this process is still worth mentioning in this thesis as it would show the extent of experimental work that the author had performed to produce any data for the thesis irrespective of their success. I believe that a detailed explanation of this experimental procedure would also be useful in order to understand all the obstacles that the author encountered to produce either positive or negative results.

6) In the Discussion chapter on page 57 the author compares her results with unpublished data provided by prof. Marek Jindra presented in Figure 58. However, there is no information available about the process used for the generation of these data and thus a reader cannot evaluate the validity of such comparison. Additionally, the heading of Figure 58 does not state what is actually depicted on the figure, such as for example an agarose gel with products of semiquantitative PCR, and thus this comparison is of no value for the discussion in this presented form. The same problem is present in a discussion with unpublished data presented in figure 59. Could the author describe in more details what can we see in figures 58 and 59?

Conclusion:

This thesis in this presented form does not comply with formal requirements for bachelor thesis. The structure of the thesis is confusing and the flow of ideas is not continuous, many ideas and results are scattered throughout all chapters and are often repeated several times. The number of peer-reviewed references is unacceptable.

Conversely, the topic of the thesis is very interesting, the amount of work, as well as the quality and scientific value of the results, is good. I also appreciate the author's ability to discuss the results and to suggest possible explanations for the observed data and contrasts in existing literature.

It is thus very unfortunate that this work is presented in a nearly unreadable way and with so many formal and factual mistakes.

In conclusion, I

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

the thesis for the defense and I suggest the grade .[3]

In **České Budějovice** date **23. 07. 2020**

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signature

[3] You can suggest a grade, which can be modified during the defense based on the presentation. However, if the reviewer is not present at the defense, the grade will not be counted. Grades: excellent (1). Very good (2), Good (3), Unsatisfactory/failed (4).