

Institute of Biochemistry and
Biophysics PAS, Pawińskiego 5A, 02-106
Warsaw, Poland

Review of the doctoral dissertation by
Joanna Karolina Palka, M.Sc.
entitled

“Effects of the reproductive system on the evolution of fitness-related traits in *Caenorhabditis elegans* under different environmental conditions”

The doctoral dissertation of Joanna Karolina Palka M.Sc. was conducted in Genomics and Experimental Evolution Group at the Institute of Environmental Sciences of Jagiellonian University in Krakow, under the supervision of Zofia Prokop, Ph.D., DSc Habil..

Assessment of the merit of dissertation

The origin of sex and sexual dimorphism is a classical and fundamental question of biology. The biological function of sexual dimorphism is still unclear, although it is relatively well understood why recombination is beneficial. Since the times of Charles Darwin, who discovered "the fast male evolution", i.e., the rapid evolution of secondary male traits, evolutionary biologists have hypothesized that males are involved in rapid adaptations of populations. The rapid male evolution has been later described in different animals and unicellular parasites, e.g., *Plasmodium*. However, there are green algae, the male traits of which do not evolve rapidly. To sum up, the scientific question is fundamental.

The doctoral student applied modern technics to study this question. In the presented study, the worm *C. elegans* is used as a model organism. This species is androdiecious, i.e., worms are mainly hermaphrodites reproducing by self-fertilizing. However, there are males which can fertilize hermaphrodites. Likely, a mechanism based on self-fertilization evolved recently, as the majority of species in the genus *Caenorhabditis* are dioecious- with females and males reproducing by obligatory outcrossing. The doctoral student constructed mutants with hermaphrodites replaced by functional females. It was achieved by introgression of the *fog-2(q71)* allele blocking sperm production by hermaphrodites.

Using this excellent model, the author tested the following hypotheses (see page 22):

“The first hypothesis assumed that changing the reproductive system would impose selective pressure on traits associated with outcrossing, leading to the re-evolution of traits degenerated by the selfing syndrome.

The second hypothesis was related to adaptation to the new environment depending on the reproductive system. The rate of adaptation in the new environment was expected to be higher in obligatory outcrossing populations, compared to populations reproducing almost exclusively through self-fertilization.”

These hypotheses were tested using experimental evolution. Wild worms and mutants were cultured at standard (20°C) and modified temperatures (24°C).

It turns out that after over 100 generations of evolution in the altered reproductive system, the majority of populations did not show signs of recovery from selfing syndrome in terms of fertilization rate and fitness. However, in five of them, there were indications of recovery.

I found these observations very sound and unexpected, as it is likely that selfing phenotype evolved due to gene deletions-androdieious worm species have smaller genomes. This is a classical mechanism named "Less is more". **Therefore, it is unlikely that worms regain lost genes during the presented experiment. I am curious which genes the androdieious worm species lost. One can answer this question using public databases.**

The evolutionary experiment did not confirm the second hypothesis. It revealed that genetic background has an impact on the evolutionary response. The doctoral student hypothesized that the small genetic diversity of experimental populations has an impact on the outcome of this study. Likely, more diverse obligatory outcrossing populations adapt more rapidly to new ecological niches than self-fertilizing ones.

Additionally, the graduate student developed a novel computer method for fitness analysis-the Convolutional Neural Network based on image. Such networks are widely used for image analysis. It turns out that this is an excellent tool for this kind of studies.

Formal evaluation of PhD thesis

The thesis has a typical form. At the beginning, there is an introductory part containing an abstract, a general introduction, and the aims of the thesis.

The general introduction describes the scientific question and the applied model organism. I have one critical remark here. In my opinion, the doctoral student should introduce the "fast male evolution" phenomenon.

Results are shown in three chapters. Actually, they are manuscripts of the following scientific papers:

1. Palka, J. K., Dyba, A., Brzozowska, J., Antoł, W., Sychta, K., & Prokop, Z. M. (2023). Evolution of fertilization ability in obligatorily outcrossing populations of *Caenorhabditis elegans*. *PeerJ*, *11*, e15825.
2. Genetic background modulates the effects of reproductive system and temperature in experimentally evolving *Caenorhabditis elegans* populations (probably not published yet)
3. Palka, J. K., Fiok, K., Antoł, W., & Prokop, Z. M. (2020). Competitive fitness analysis using convolutional neural network. *Journal of Nematology*, *52*(1), 1-15

I found very confusing the presented concept of experimental blocks in chapter 1. I am afraid that it is not clear for someone, like me, who has no experience with worms. I should be grateful for some clarification concerning these blocks during the public defense.

The general conclusions are the final part of the thesis.

To sum up, the thesis is well written. It describes the question, its significance, experimental results, and conclusions.

Conclusion

Summarizing the above, I conclude that the stated scientific objectives significantly lead to the development of present knowledge and contribute to the original solution of a scientific problem. It shows that the re-evolution of traits degenerated by the selfing syndrome sometimes occurs. For me, this is unexpected, and I found this result very sound. As I wrote, I should be grateful for providing more information about genes lost during the evolution of self-fertilization during public defense.

I am particularly impressed by the quality of the presented bioinformatic study at an exceptional level among experimental molecular biologists.

The presented thesis satisfies all the formal and customary requirements for a Ph.D. in molecular biology/evolution.

Due to the high scientific value of the results that provide new information expanding the current state of knowledge in the field of sex evolution and the fact that some results have already been published, I also recommend considering it as an outstanding dissertation Ph.D. - thesis with honors. I am particularly impressed by her bioinformatic study. It is exceptional among experimental doctorate students.

Szymon Kaczanowski

Associate professor
Institute of Biochemistry and Biophysics



Signed by /
Podpisano przez:

Szymon
Kaczanowski
Instytut Biochemii i
Biofizyki PAN

Date / Data:
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Instytut Biochemii i Biofizyki PAN
Pawińskiego 5A, 02-106
Warszawa, Polska

**Wniosek końcowy recenzji doktoratu magister Joanny Palki w języku polskim
pt: “Effects of the reproductive system on the evolution of fitness-related traits in
Caenorhabditis elegans under different environmental conditions”**

Podsumowując uważam, że zrealizowanie badań przedstawionych w rozprawie doktorskiej magister Joanny Palki przyczyniło się do istotnego poszerzenia aktualnego stanu wiedzy dotyczącego ważnego problemu naukowego. Uważam też, że zdumiewające jest to, że w czasie eksperymentu ewolucyjnego czasami dochodzi do odtworzenia cech adaptacyjnych zdegenerowanych w „syndromie samozapłodnienia”. Jak napisałem już wcześniej, w moim przekonaniu obserwacja ta jest istotna. Dlatego, chcę zadać doktorantce pytanie: jakie geny zostały utracone podczas ewolucji samozapłodnienia u nicienia *C. elegans*.

Szczególnie wysoko oceniam jakość przedstawionych w rozprawie analiz bioinformatycznych na poziomie nie spotykanym wśród doktorantów prowadzących badania eksperymentalne.

Oceniana rozprawa doktorska spełnia wszystkie wymagania zwyczajowe oraz stawiane przez ustawodawcę. Wnoszę o dopuszczenie doktorantki magister Joanny Palki do dalszych etapów obrony doktoratu. Ponieważ uzyskane wyniki badań mają wysoką wartość naukową i są częściowo już opublikowane, proponuje także rozważenie nagrodzenia wykonanej rozprawy według zwyczajów panujących w Uniwersytecie Jagiellońskim.

Szymon Kaczanowski

Profesor Instytutu
Biochemii i Biofizyki PAN



Signed by /
Podpisano przez:

Szymon
Kaczanowski
Instytut Biochemii i
Biofizyki PAN

Date / Data:
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