Swedish University of Agricultural Sciences
Uppsala BioCenter
Department of Plant Biology
Linnean Center for Plant Biology

To: Student Affairs Division, Charles University
Dr. Tomáš Fér

Dr. Adrien Sicard
Swedish University of Agricultural Sciences
Uppsala BioCenter; Room D450
Almas Allé 5; PO-Box 7080
SE-75007 Uppsala, Sweden

Tel. +46 18 67 3324
Email: Adrien.sicard@slu.se

Evaluation Report of the PhD thesis submitted by Ömer İltas entitled " Consequences of the mating system shift on the evolution of sexual traits in flowering plants"

This manuscript describes and discusses the research conducted by Ömer İltas during his doctoral studies under the supervision of Prof. Dr. Clément Lafon Placette at the Department of Botany at Charles University. During his PhD, Ömer studied the impact of mating system changes on the evolution of sexual traits in plants. Sexual selection has been shown to operate in a wide variety of organisms, including plants. Yet, the extent to which it influences plants' phenotypic and genomic evolution has largely been discussed at the theoretical level, primarily due to the lack of comprehensive model organisms showing variation in the intensity of sexual selection. Ömer exploited evolutionary shifts in reproductive plants and recent developments in genomic techniques to challenge long-standing hypotheses on the influence of sexual selection on organismal evolution. He argued convincingly that gametophytic selection is considerably reduced in self-fertilizing compared to outcrossing organisms due to their high homozygosity levels, thus offering a comparison point to investigate the role of sexual selection on plant evolution. Using independent mating system transitions in the *Brassicaceae* with various divergence times between lineages with contrasting mating systems, Ömer assessed the impact of shifts in the intensity of sexual selection on sexual performance traits, co-evolutionary dynamics between male and female attributes, and sex-specific resource allocation strategies in seeds.

The introduction opens by presenting transitions in mating systems in plants and their known or expected consequences on gametophytic evolution, emphasizing the major influences of sexual selection and how these should be affected by transitions to selfing. The final part of the introduction discusses the influence of mating system transitions on parental conflict in hybrids and how they may contribute to the establishment of reproductive barriers. The introduction reads very well, providing a comprehensive overview of the field and focusing on the most relevant information for the work presented in the thesis. It also highlights current knowledge gaps, providing strong motivation for the work conducted.

After the introduction, the thesis clearly outlines the objectives and provides well-justified approaches before discussing the main results and conclusions. The methods section does not delve

into detailed descriptions but rather offers justifications for the choice of methodologies and research directions. This approach is much appreciated and significantly improves the narrative of the thesis. The final section on the results and discussion provides an overview of the results, acknowledges limitations in their interpretations, and offers perspectives. This could perhaps have been more developed, as it mostly discusses each manuscript independently. A broader reflection on the connections between the different chapters of the thesis and their implications for the general understanding of sexual selection in plants would have been appreciated. Five manuscripts corresponding to different chapters were presented in the annex of the thesis.

Overall, the thesis reads very well and is written in very good English. Great care has been taken with the text and the organization of the thesis. The methods have been described with precision and clarity. Ömer has conducted an extensive body of work, successfully implementing challenging methods and yielding significant original findings:

Article 1 focuses on understanding whether sexual selection can rapidly drive the differentiation of pollen performance. To this end, pollen germination competence was assessed in *A. lyrata*, comparing young selfing populations with obligate outcrossing populations. While the populations investigated introduce confounding factors associated with population history, they represent a thoughtful choice to investigate the short-term effects of variation in the intensity of sexual selection. Phenotypic analyses are complemented by an elegant transcriptomic analysis that allowed the identification of genes differentially expressed between selfers and outcrossers in elongating pollen but also pollinated pistils. The experiments and analyses were rigorously conducted, the results well interpreted, and the limitations of the study clearly outlined. It demonstrated that differences in pollen performance exist between recently diverged lineages with contrasting mating strategies, suggesting that variation in the intensity of sexual selection can promote rapid divergence in pollen performance. These results are original and provide valuable conceptual advances to the field, naturally leading to a high-quality publication in *Plant Cell Physiology*. I would have personally appreciated further discussion on how the molecular data could explain the phenotypic differences observed and what evolutionary scenarios might trigger such rapid divergence in pollen performance traits.

The second manuscript investigates the evidence for runaway selection in plants, focusing on style length and pollen competition as a case study. It compiles a thorough survey of the literature describing evidence of correlated changes in style length and pollen growth rate associated with variation in the intensity of sexual selection. Additionally, it employs mathematical modeling to demonstrate that coevolution between stigma and pollen is possible and tests selective parameters that may influence this coevolution. The manuscript further discusses plausible underlying mechanisms and population genomics approaches to identify them. This manuscript is well-written and provides a valuable synthesis of current knowledge. The modeling approach is a significant advance in the field, incorporating more realistic assumptions about the selective forces at play. Although further simulations could have been conducted to assess the robustness of the conclusions under different assumptions, the study provides clear indications of the conditions under which runaway selection can occur, offering valuable testable hypotheses. The manuscript could have benefited from deeper discussion about the extent to which the in silico results align with empirical observations. The narrative would also have been strengthened by discussing alternative selective pressures that could influence the traits investigated, particularly female functions.

The third manuscript combines microscopic analyses with proteomics to study differences in pollen structure and function following the transition to self-fertilization in plants. To this end, Ömer focused on three sister species in the Brassicaceae. This study demonstrated that pollen from selfers tends to have smaller pollen coat areas compared to outcrossing species and suggested that differences in pollen coat structure coincide with the evolution of the selfing syndrome. It also demonstrated functional convergent evolution among mating system pairs, at least for the composition of pollen coat proteins. These interpretations are robust; the experiments have been rigorously conducted and analyzed. This study is the first to suggest a link between mating system evolution and pollen structure. Also only few studies had, so far, quantified the pollen proteome. The manuscript reads very well and appears mostly complete. I have no doubt it will be published in a reputable journal. A more direct assessment of the effects of phylogeny or mating system signals, along with population genetic analyses, could nicely complement the study and test further hypotheses on the underlying evolutionary triggers.

In manuscript 4, Ömer investigated how rapidly variation in the intensity of parental conflict and sexual selection among lineages can trigger postzygotic barriers associated with seed developmental defects. Here as well, Ömer exploited recent transitions to selfing in *Arabidopsis lyrata* to address this question. Reciprocal crosses were performed to assess the effect of parental genomes on hybrid fitness. He demonstrated that endosperm-based hybrid incompatibility, reminiscent of imbalances between parental contributions, had already evolved between these lineages. He also suggested that other factors, likely linked to ecological adaptation or evolutionary divergence, contributed. This study is highly valuable because it demonstrates that the balance between parental genome contributions can evolve very quickly and that the timing of endosperm cellularization is largely influenced by the parental genome, ultimately impacting seed size. It further suggests that factors beyond sex-specific interests also contribute to the evolution of parental contributions. Further discussion on how ecological and population genetic properties could trigger this evolution would have been beneficial in the discussion. This manuscript is of high quality and has led to a publication in the renowned scientific journal *Evolution*.

The fifth study analyzes polymorphic patterns across selfer and outcrosser populations in *A. lyrata* to test theoretical expectations about the evolution of imprinted genes. The study is well-designed and employs advanced population genomics and phylogenetic methods. The results are not fully consistent with an accelerated evolutionary rate for imprinted genes but rather suggest more intense purifying selection. The results also indicate that mating system changes have limited or at least non-rapid effects on the evolution of imprinted genes, which is unexpected. Non-imprinted endosperm genes show signs of diversifying selection in autogamous species, suggesting that ecological pressures may also drive endosperm evolution. These findings suggest a different perspective on the current theoretical understanding of parental conflict in plants. This manuscript is well-advanced but could still benefit from further polishing. There are a couple of formatting issues, and supplemental tables that were not provided. The narrative could be improved, particularly by commenting on the choice of approaches and providing further motivation for the chosen control gene sets. The descriptions of the results are accurate but sometimes difficult to follow.

These are all important achievements within the field and demonstrate great commitment and excellent methodological skills. Furthermore, Ömer İltas presented accurate interpretations as well as a detailed critical analysis of the results obtained. This indicates very good analytical capabilities and great scientific maturity. Ömer's work has led to two publications as first author so far, with other first-authorship manuscripts nearly ready for submission. While minor

improvements could be made in integrating discussions and expanding theoretical reflections, the thesis is of high scientific quality and meets the standards for a doctoral degree. Therefore, I most strongly recommend Ömer İltas's thesis for defense.

Sincerely,

Adrien Sicard