

**Review of the PhD thesis submitted by Hosam Osama Mohamed Nageeb Elansary:
Organellar DNA diversity in some ornamental plants related to reproduction system
and life strategy**

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I. Generally comments

Ph. D. Thesis of Mr. Elansary is presented in synthetic form. Diversity in organellar DNA was analyzed on various geographical scales from intrapopulation level to whole distribution range using three models in this thesis. The mode of inheritance of organellar DNA was also analyzed in some of these models in natural populations or in hybridization experiments. Gynodioecy, breeding system typical for studied model *Silene vulgaris*, is recently intensively studied by various research groups, namely from the ecological, evolutionary and genetic viewpoint. One these excellent teams is research group of Helena Štorchová who was supervisor of Mr. Elansary. Thus subject of thesis represents one of the topics of current research.

The results of thesis are based on:

- (i) 331 wild plants = progeny of ten open pollinated maternal plants collected in Kováry population (Czech Republic);
- (ii) 1007 plants of controlled crosses of *Silene vulgaris* = progenies of 29 females from Kováry populations pollinated by hermaphrodites from geographically distant populations;
- (iii) 48 individuals of *Silene latifolia* representing eight European populations from Sweden, Denmark, the Netherlands, and Germany.
- (iv) ca 150 plants of *Aldrovanda vesiculosa* from 18 populations representing the most of world-wide distribution range of this species

Wide spectrum of methods was used in the thesis, the key results are based namely on molecular methods of PCR-RFLP, DNA sequencing, and Southern-RFLP.

II. Comments, notes and questiones:

1. "*Number of mitochondria per cell*"

What is usual number of mitochondria in plant (Angiosperm) oosphere comparing to those in pollen generation cell. Is there any reason for paternal mode of mitochondrial genome inheritance based on bias in this ratio at least in some species?

2. "*Number of mitochondrial DNA units per mitochondria*"

What is the dynamics in the mitochondrial DNA copy numbers per mitochondrion during plant life? If the fluctuation in the copy number is present during the plant ontogeny, is there chance for the bottle-neck-effect in the shift in frequency of haplotypes in heteroplasmic mitochondria?

3. "*Interspecific and intraspecific hybridization in relation to organellar inheritance*"

Is there any difference in the mode of chloroplast or mitochondrial inheritance between interspecific and intraspecific crosses in plants or animals? How frequent is recent natural interspecific hybridization in *Silene*? Is there any evidence for the role of ancient interspecific hybridization during the evolution of *Silene*?

4. "*Sampling design for paternal inheritance in natural population*"

The detection of paternal inheritance of organellar DNA in natural population (open progeny) of *Silene vulgaris* could be better designed (in my opinion) by analysis of sample pairs, one mother + one its child, than by analysis of "families" as was used in thesis. The analysis of families contains redundant number of pseudo-replications. Chance for detection of rare type of inheritance is much lower than in pairing design of sampling if the same amount of analyses carried out (i.e. 165 mother-child pairs vers. 10 families including 331 samples). On the other hand, your less effective sampling design gave the positive result which is quite enough for confirmation of parental transmtion of organellar DNA in you model population.

5. "*Gender of maternal plants of Silene vulgaris*"

If you studied the percentage of "strange" haplotypes in progeny of ten mother plants from natural population. Did you know gender of mother plants? How frequent is selfing in hermaphrodites of this genus? Maybe, if maternal gender was unknown (I could overlook that information in text of thesis), the relation between KovC haplotype and female frequency could be determined or biased by gender of maternal plants? Such potential bias is well visible in the controlled crosses with female mothers (table 11).

6. "Gender shift"

In the chapter M&M (p. 48), there is mentioned the shifting of gender in gynodioecious *Silene vulgaris* (from female status to cosexual). In table 10 on p. 87, there are such plants documented in all "families" in relatively high frequencies from 9 to 44 %. If there is nuclear-cytoplasmic regulation of sex expression, what mechanism is responsible for such shift (beside of explanation presented in discussion on p. 117)? Is such shift known also in other gynodioecious species? What is usual frequency of gynomonecious individuals in *Silene vulgaris* populations?

7. "Distant or proximal crosses"

In the chapter 5.2. (p 90) is mentioned that females from Kováry were pollinated by hermaphrodites from the same or from geographically distant localities in controlled crossing experiments. However, table 11 describing crossing design includes just distant crossing? Could you explain whether proximal crosses were used? If yes, did you find some effects of distant nuclear restorers on sex ratio in hybrid progeny?

8. "Effect of reproduction mode to polymorphism of organellar DNA"

In p. 11 of Abstract is mentioned that you "demonstrated that different reproduction mode did affect the level of polymorphism in organellar DNA." In fact you found three different levels of polymorphism in three species differing in reproduction mode. However, these species also differ in many other features of their biology, ecology, morphology, etc. Do you think that these differences are also responsible for DNA polymorphism?

9. "Silene vulgaris as ornamental and edible plant"

Studied models are presented as ornamental plants in thesis title. Despite the fact that every plant (as well as every woman) could be considered to be nice (in own way), I am convinced that none from studied species *Silene vulgaris*, *S. latifolia* and *Aldrovanda vesiculosa* could be

considered to be ornamental plant. Similarly, on p. 24 you mentioned edible uses of *Silene vulgaris*. I could not imagine, edible use of that species. Could you better specify it?

10. "*Submitted papers*"


In the abstract of your thesis, two papers are mentioned as submitted, but they are not included in your thesis as chapters or supplements. Could you demonstrate them during the defense at least in the manuscript form?

11. "*Text similarity*"

Regarding to my specialization (botany) which is far from genetics, some introductory parts of thesis were difficult to understand for me. In such cases I try to find some additional information for better understanding as it usual using Google. It was surprising for me that during that checking I have found high similarity or the identity between some paragraphs in introductory part of thesis and paragraphs in published papers and handbooks: e.g. the paragraph in the end of page 19 and the beginning of page 20 comparing to the third introductory paragraph in the paper of Abdelnoor et al. 2003 (PNAS, 100/10: 5968-5973) or two paragraphs in the page no 23 comparing to the paragraphs in the page 34 of the chapter genetics in the book Progress in Botany 67 (Esser et al. 2004). Could you explain it?

III. Conclusion

Thesis of Hosam Osama Mohamed Nageeb Elansary constitutes on my opinion a very valuable addition to genetics of gynodioecy and study of organellar DNA dynamics. The candidate has demonstrated very good skills in molecular methods, sampling strategy, and results presentation. I recommend to accept this thesis for the doctor degree after its successful defense.


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Brno, August 23, 2009