

## 7. Conclusions

The purpose of this Ph.D. work was oriented towards two points.

1. The analysis of organellar diversity among three plant species (*Silene vulgaris*, *Silene latifolia* and *Aldrovanda vesiculosa*). The three plant species had different morphological and physiological characters. The focus here was on two physiological points.
  - 1.1. The reproduction system which is directly correlated with the mitochondrial DNA (in the case of CMS). This point was presented in the comparison between *Silene vulgaris* and *Silene latifolia*.
  - 1.2. The different life strategy which is directly correlated with the organellar DNA diversity. This point was presented by the comparison between the two *Silene* species from one side and *Aldrovanda vesiculosa* from the other side. The later usually propagate vegetatively and live in different media (water), it present different life strategy under the umbrella of angiosperms.
2. The analysis of the inheritance of mitochondrial and chloroplast genome in *Silene vulgaris*. The study of this inheritance was facilitated by the high polymorphism available in the organellar DNA. This polymorphism could be detected by the different molecular markers used in this study. These molecular markers included either, gene coding regions markers previously used by D.E.McCauley and his team (McCauley *et al.*, 2005; McCauley *et al.*, 2007; Pearl *et al.*, 2009) or non-coding regions markers like Southern-RFLP. These variable markers gave us larger insight into the inheritance of organellar DNA.

Indeed the evidence on rare paternal inheritance of organellar genomes was confirmed in *S. vulgaris* as found in previous studies. The high mtDNA variation was visible among individuals and within individual. The within individual variation achieved by Southern-RFLP is novel and had not been found before using this method. The large sampling size and the types of mitochondrial markers (*atp1* and *cox1*) in Southern-RFLP were not applied before. Only *cox1* was analyzed by Southern-RFLP in previous studies in *S. vulgaris* (Olson and McCauley, 2002; Štorchová and Olson, 2004). The limited paternal inheritance of mitochondrial genomes in *S. vulgaris* alone does not explain the high diversity achieved here, which required consideration of other processes like mitochondrial sorting and substoichiometric shifting to understand this phenomenon.

*The core conclusions of this Ph.D. study could be summarized in two points:*

### 1. Organellar DNA diversity vary among species of angiosperms

- a. The comparison between organellar diversities of *S. vulgaris* and *S. latifolia* confirmed that the later has higher organellar diversity in both of coding and non-coding regions. *S. vulgaris* is gynodioecious plant whereas *S. latifolia* is a dioecious plant species. This study gave evidence that the breeding system is predictive of organellar DNA polymorphism. The higher polymorphism found in *S. vulgaris* than *S. latifolia* was explained by the balancing selection which acts on CMS factors in gynodioecious *S. vulgaris*. *S. vulgaris* harbors many old haplotypes compared to dioecious species *S. latifolia*. These ancient haplotypes resulted from long term balancing selection. In addition, mtDNA polymorphism and divergence in *Silene vulgaris* could be further increased also by mutation rate heterogeneity which is a characteristic of mtDNA.
- b. Organellar DNA uniformity was found among accessions of aquatic carnivorous plant *A. vesiculosa* from four continents. The tiny variation in cpDNA discovered in *A. vesiculosa* in this study is novel, that it represents the first documented sequence variation among *A. vesiculosa* accessions. *A. vesiculosa* belongs to Caryophyllales, and is therefore distantly related to *Silene*. It was proposed that long-distance dispersal, frequent vegetative

propagation and maybe also recent bottleneck and slow mutation rate contribute to genetic uniformity observed in this specie.

## **2. Rare paternal inheritance and other processes like mitochondrial sorting and substoichiometric shifting may explain the high variation in mtDNA in *S. vulgaris***

- a.** The rare paternal inheritance of mtDNA was confirmed in this study in *S. vulgaris* natural population. There was no paternal inheritance detected in the controlled crosses between geographically distant populations which could be interpreted by the block of paternal transmission that could happen at any stages of Gametogenesis, Fertilization and Postfertilization. It may also reflect the large geographic and presumably genetic distance between the nuclear genomes of both parents.
- b.** The among and within individual variation could be explained by two point of views:
  - i. Mitochondrial sorting which exist in heteroplasmic individuals like *S. vulgaris*. The process includes random transfer of different numbers of copies of each allele from one mitochondria and cell to the next. The final result of it is a change in the frequency of mitochondrial alleles within different plant tissues.
  - ii. Substoichiometric shifting which include changes in the relative frequencies of different sublimons in the mtDNA. These sublimons could have rapid and dramatic changes in relative copy number of portions of the mitochondrial genome over the time of one generation and usually involves only a single subgenomic DNA molecule. This process could change the plant phenotype.Paternal inheritance cannot be excluded because it could happen in parallel with these processes. Further investigation could enhance our understanding of these phenomena.

Next step in the current project of Helena Štorchová as suggested by her is to understand the forces which shape genetic variation in mitochondrial genomes of *S. vulgaris* by determination of sequences corresponding to the bands, observed in complex RFLP patterns of *atp1* flanking regions in several families. This task is facilitated by the knowledge of the complete sequence of mitochondrial genome in *S. vulgaris*, which will be soon available. H.Štorchová will search the sequence of complete genome for repeats and estimate, whether recombination in these repeats could be responsible for creation of new fragments flanking the *atp1* gene. Then, probes derived from these repeats will be prepared and the plants showing within-individual variation will be tested again.