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Mitochondrial genomes of the plant genus *Silene* in the context of the evolution of plant mitochondria

Mitochondriální genomy rodu *Silene* v kontextu evoluce rostlinných mitochondrií

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The aims of the doctoral thesis

1. Population genetic studies of organellar markers in the gynodioecious plant species *Silene vulgaris.*

2. The assembly and annotation of complete mitochondrial genomes of *Silene latifolia* and *Silene vulgaris* as the key for the understanding the extremely high rearrangement rates in mitochondrial genomes of *Silene vulgaris.*

3. The impact of mitochondrial genome fluidity on the transcription of mitochondrial genes of the *Silene* species.

4. The identification of mitochondrial features associated with cytoplasmic male sterility in *Silene vulgaris* using comparative transcriptomic studies.

5. *Silene vulgaris* as a suitable model for the investigation of cytoplasmic male sterility in wild species.

INTRODUCTION

Mitochondria and the origin of eukaryotic cell

The origin of eukaryotes was undoubtedly one of the most important events in the evolution of life on Earth. It is explained by endosymbiotic theory articulated already by Mereschkowsky (1905) in the beginning of 20th century and later reintroduced and developed by Margulis (1970). This theory proposes that mitochondria and plastids are the descendants of once free-living proteobacteria and cyanobacteria, respectively, which entered an ancestral host cell and became its permanent endosymbionts. Multiple evidence supports this idea (reviewed by Roger and Muñoz-Gómez (2017)). Both mitochondria and plastids harbor genomic DNA, ribosomes of prokaryotic type, and electron-transport chain (ETC) proteins similar to bacteria.

Whereas the origin of mitochondria was understood, how the nucleus might have arisen is still the matter of debate (Martin and Muller 1998, Lindmark a Muller 1973, Karnkowska et al. 2016, Hampl et al. 2019). Despite of this uncertainty, general agreement exists, that mitochondria are tightly associated with the origin of eukaryotic cell.

Mitochondrial function

Mitochondria serve as miniature power plants supplying the cell with ATP, a general energetic currency. Inner mitochondrial membrane hosts the components of ETC and the enzymes responsible for ATP synthesis. The tricarboxylic acid (TCA) cycle is localized in mitochondrial matrix and serves as the source of reduced NADH for the electron transport chain. It also produces carbon skeletons for the biosynthesis of various amino acids and other compounds. Mitochondria represent an important compartment where essential catabolic and anabolic reactions occur. The biosynthesis of iron-sulphur (Fe-S) clusters (Webert et al. 2014), the beta oxidation converting fatty acids to acetyl-CoA, which enters the TCA cycle (Schulz 1991), the synthesis of serine from glycine, or the degradation of branched amino acids or proline in plant mitochondria (Szal a Podgórska 2012) are well-known examples.

Mitochondria possess DNA which is transcribed into mRNA showing some prokaryotic features (e.g. lacking polyA tails). They also contain ribosomes and conduct proteosynthesis.

However, the vast majority of proteins necessary for mitochondrial metabolism are encoded in the nucleus and imported to mitochondria. Only a small portion of mitochondrial proteome is encoded by mitochondrial genome. The set of mitochondrial genes includes some components of ETC or ribosomal proteins. It varies across eukaryotic evolutionary lineages but surprising similarities can be found even between plants and humans (tobacco and human mitochondrial genomes share the *nad1*, *nad2*, *nad3*, *nad4*, *nad4L*, *nad5*, *nad6*, *cob*, *cox1*, *cox2*, *cox3*, *atp6*, *atp8* genes). The largest number of mitochondrial genes (66 protein coding genes), was found in jakobids, the separate protist branch, where gene number reduction in mitochondria slowed its pace (Burger et al. 2013).

The number of encoded genes has changed during the evolution of mitochondrial genomes. In addition, the number and type of introns, the extent and origin of intergenic regions, genomic rearrangements and synteny vary across the tree of life (**Table 1**). Organelles of some species can modify (edit) nascent RNAs to alter their sequences. Cytidine-to-uracil (C-to-U) editing is common in higher plant mitochondria and chloroplasts, whereas the opposite uracil-to-cytitine editing occurs more often in some ferns and hornworts (Rüdinger et al. 2009). No editing has been discovered in mitochondria of green algae (Charophytes and Chlorophytes), which suggests that editing arose as a consequence of terrestrial life style in plants (Turmel et al. 2013).

Evolutionary trends in plant mitochondrial genomes

Mitochondrial genomes of higher plants and animals are profoundly different. Animal mitochondrial genomes are small, compact, gene-dense, contaning intron-less genes (except for cnidaria). For example, human mitochondrial genome is 16 kb long and contains 37 genes, 13 of them coding for proteins (Taanman 1999). In contrast, mitochondrial genomes of higher plants are large (about 200kb – 12 Mb, Sloan et al. 2012), containing long intergenic regions, genes with introns, and multiple promoters (Kühn et al. 2005). They undergo frequent recombination, resulting in extensive genomic rearrangements, including the transfer of DNA from nucleus or chloroplasts. Despite of size and structure variation, mitochondrial DNA sequences evolve very slowly, except for several lineages with accelerated mutation rates (Mower et al. 2007). Some

Table 1. Plant mitochondrial genomes. The number of edits refers to C > U RNA editing, if it is not stated otherwise. The number of trans-splicedI introns type II is given in parentheses following the total number of introns type II.

	GB Accession	Size	Genes	Intron I	Intron II	Edits	Reference
Chlorophyta							
Nephroselmis olivacea	NC_008239	45,223	66	4	0	0	Turmel et al. 1999
Ostreococcus tauri	NC_008290	44,237	63	0	0	0	Robbens et al. 2007
Charophyceae and relatives							
Chara vulgaris	NC_005255	67,737	69	14	13	0	Turmel et al. 2003
Chaetosphaeridium globosum	NC_004118	56,574	68	9	2	0	Turmel et al. 2002
Closterium baillyanum	KF060940	152,089	71	20	11	0	Turmel et al. 2013
Roya obtusa	KF060943	69,465	69	0	2	0	Turmel et al. 2013
Bryophyta							
Marchantia polymorpha	NC_001660	186,609	70	7	25	0	Oda et al. 1992
Pleurozia purpurea	NC_013444	168,526	68	7	21	a few	Wang et al. 2009
Physcomitrella patens	NC_007945	105,340	66	3	24	11	Rüdinger et al. 2009
Phaeoceros laevis	NC_013765	209,482	43	0	32	> 600	Xue et al. 2010
Anthoceros agrestis	MK087647	227,925	46	4	40	496 C > U, 403 U > C	Gerke et al. 2020
Lycophyta							
Isoetes engelmannii	HQ616410-434	> 57 571	40	3	27	1782 C > U, 222 U > C	Grewe et al. 2011
Selaginella moellendorffii	JF338143–147	>183 000	20	1	36(3)	2152	Hecht et al. 2011
Huperzia squarrosa	JQ002659	413,530	66	0	32	300-600	Liu et al. 2012
Ferns							
Psilotum nudum	KX171638-9	628,553	68	1	26	965 (19 U > C)	
Ophioglossum californicum	NC_030900.1	372,339	63	0	20	1014 (58 U > C)	Guo et al. 2017
Gymnospermae			**no U > C editing in seed plants**				
Cycas taitungensis	AP009381	414,903	71	0	26(5)	1,214	Chaw et al. 2008
Gingko biloba	KM672373	346,544	67	0	25(5)	1,405	Guo et al. 2016
Welwitschia mirabilis	KT313400	978,846	40	0	15(11)	99	Guo et al 2016; Fan et al. 2019
Taxus cuspidata	MN593023	468,924	46	0	15(11)	974	Kan et al. 2020
Pinus taeda	MF991879	1,191,054	59	0	26(13)	> 1000	Direct submission to Genbank
Angiospermae							
Amborella trichopoda	KF754799-784	3,866,039	63	0	25(6)	835	Rice et al. 2013
Liriodendron tulipifera	KC879625-661	553,721	64	0	25(5)	781	Richardson et al.2013
Magnolia biondii	MN206019	967,100	64	0	25(5)	NA	Dong et al. 2020
Nicotiana tabacum	NC_006581	430,597	57	0	23(6)	463	Sugiyama et al. 2005
Arabidopsis thaliana	BK010421	367,808	49	0	23(5)	441	Sloan et al. 2018
Oryza sativa	NC_007886	490,521	54	0	23(6)	481	Tian et al. 2006
Spirodela polyrhiza	NC_017840.1	228,493	57	0	21(6)	~ 600	Wang et al. 2012
Viscum scurruloideum	KT022222-3	> 65 873	25	0	3(1)	150-300	Skippington et al. 2015
Caryophyllales							
Beta vulgaris	NC_002511	368,801	50	0	20(6)	357	Kubo et al. 2000
Silene latifolia	HM562727	253,413	36	0	19(6)	287	Sloan et al.2010
Silene vulgaris	JQ771300-305	361,139	34	0	19(6)	298	Sloan et al. 2012b
Silene noctiflora	JF750482 +	6,728,000	32	0	18(6)	189	
Silene conica	JF750534 +	11,318,000	30	0	19(6)	182	Sloan et al. 2012a

trends can be repeatedly tracked in major clades of seed plants. For example, elevated substitution rate, loss of genes, and loss of editing sites occurred in parallel in gymnosperms (*Welwitschia*), and in angiosperms (*Silene*, *Geranium*).

The evolutionary trend toward increasing size of mitochondrial genome associated with the inflation of non-coding intergenic regions, but not with the increase in gene number, may be recognized in plants. However, this view is oversimplified. The evolution of mitochondrial genome in plants is highly dynamic with periods of stasis and a sudden appearance of novel features.

Mitochondrial genomes of algae and bryophytes

Mitochondrial genomes of Chlorohytes are small, compact, with conserved gene order, and a variable number of introns. Gene content varies among taxonomic lineages, but the gene set is relatively stable, with infrequent gene losses (**Table** 1). The taxonomic group Zygnematophycae is a sister group of land plants. These algae and their relatives e.g. Charophycae exhibit a conserved structure of mitochondrial genome, but show notable variation in genome size (e.g. *Closterium* mitochondrial genome is comparable in size to bryophyte mitochondrial genome). The sequenced charophycean mitochondrial genomes vary in the number and position of introns.

Two types of introns exist in mitochondria. Group I introns are spliced out in a linear form, whereas group II introns are removed in a form of lariat, similarly to nuclear introns. They have a conserved secondary structure, which suggests their possible function as ribozymes (Lang et al. 2007). Group I and group II introns are mobile elements, the former use DNA-mediated homing mechanism, whereas the latter propagate through RNA intermediates. Both intron groups require special enzymes for the proliferation – endonucleases or maturases. The evolutionary instability of the mitochondrial intron set is very high in Charophyceae – there are only 2 introns in *Roya*, but 27 introns in *Chara*. The species also differ in the ratio of group I and group II introns and in intron size (Turmel et al. 2013)

The important evolutionary innovation, which has not been observed in algae, appeared with emergence of land plants: RNA editing. Its extent is modest in liverworts, but large in hornworts (**Table 1**). Other features of bryophyte mitochondrial genomes are conserved among bryophytes and quite similar to Charophyceae – e.g. gene content and order of some genes. In addition to high editing extent, hornworts show some trends similar to higher plants. Their mitochondrial genomes lost the genes, e.g. encoding ribosomal proteins, which were often lost in various angiosperm lineages. Hornworts have less conserved gene order than other bryophytes. These characteristics of mitochondrial genomes are consistent with the position of hornworts as a sister groups of tracheophytes (Libertin et al. 2018).

Mitochondrial genomes of lycophytes and ferns

Lycophytes represent the sister group to all other vascular plants. Their mitochondrial genomes possess many features typical for tracheophytes – rearranged gene order, high level of RNA editing, insertions of plastid and nuclear DNA, presence of trans-spliced introns. Trans-splicing puts together the parts of the same intron located in various positions on the organellar genome. It makes possible to produce a functional mRNA from two or even three genic fragments scattered on the genome. The emergence of trans-splicing correlates with the acceleration of mitochondrial genomic rearrangements in vascular plants.

The most archaic mitochondrial genome among lycophytes (and all tracheophytes) is that of *Huperzia squarrosa* (Liu et al. 2012). It retains a conserved cluster of genes for ribosomal protein, shows lower level of editing, and no trans-spliced introns. Two other lycophytes with completely sequenced mitochondrial genomes (**Table 1**) exhibit extremely high frequency of editing, reaching more than 2100 sites in *Selaginella*. In contrast with the evolutionary trend in angiosperms, RNA editing in basal clades of lycophytes and ferns is lower than in later branching clades. RNA editing by C-to-U conversion prevails in lycophytes and ferns, but reverse editing U-to-C, which is absent in angiosperms, occurs quite often in ferns (Knie et al. 2016).

Two fern species with completely sequenced mitochondrial genomes – *Ophioglossum californicum* and *Psilotum nudum* – harbor protein coding gene content similar to the set of 41 genes inferred to be present in the ancestral mitochondrial genome of seed plants (Guo et al.

2017). A specific gene loss occurred in *O. californicum*, where *ccm* genes responsible for cytochrom c maturation were absent in both mitochondria and nucleus. Both fern species contain numbers of repeats in their mitochondrial genomes, but exhibit a very low rate of intramolecular recombinations and genome rearrangements. **This documents that the mere existence of repeats is not sufficient to ensure homologous recombination in plant mitochondrial genomes**, but additional factors e.g. specific enzymes are required.

Mitochondrial genomes of gymnosperms

Our knowledge about gymnosperm mitochondrial genomes lagged behind angiosperms. Complete sequences were obtained from five species only (**Table 1**), mitochondrial genome of *Picea glauca* was published as a draft (Jackman et al. 2016). *Gingko biloba*, *Welwitschia mirabilis* (Guo et al. 2016), *Cycas taitungensis* (Chaw et al. 2008), and *Pinus taeda* (direct submission MF991879) possess mitochondrial genomes of moderate size with 41 protein coding genes and 25 or 26 introns, 6, 5, or 13 of them, respectively, trans-spliced. This gene content is close to the inferred ancestral mitochondrial genome of seed plants. The gymnosperm species contain numerous editing sites converting C to U, but not in the opposite way.

In sharp contrast with the previous species, the mitochondrial genome of *Welwitschia mirabilis* (Guo et al. 2016) is larger (nearly 1 Mb), but it harbors much less genes. Although the knowledge of gymnosperm mitochondrial genomes is very limited compared with angiosperms, analogous evolutionary trends may be recognized in the two phylla – gene losses, reduction in editing sites, elevated evolutionary rates in some lineages, size expansion.

Mitochondrial genomes of angiosperms

The mitogenomes of flowering plants are fluidic, extremely variable in gene content and order, substitution rate, size, content of repeats, number of editing sites, and extent of foreign DNA incorporation. This variation is the highest in eudicots and monocots, whereas basal angiosperms harbor more conserved, „fossilized“ mitochondrial genomes, as documented by *Nymphaea colorata* (Dong et al. 2018), *Liriodendron tulipifera* (Richardson et al. 2013), or *Magnolia biondii* (Dong et al. 2020) **(Table 1)**. The two mitochondrial genomes of early flowering plants are of moderate size and possess 64 – 65 genes similarly to gymnosperms. They

contain more than ten conserved gene clusters, some of them dated back to the origin of mitochondria (*rrn18- rrn5*).

Amborella trichopoda is considered to be a sister taxon to all other angiosperms (Soltis et al. 2011). Besides some conserved features, as gene content, shared with other mitogenomes of early flowering plants, it shows a unique propensity to extensive horizontal gene transfer (HGT). Large pieces and even entire mitogenomes of green algae, mosses and other flowering plants were incorporated to the *Amborella* mitogenome, which was enlarged five times compared with *N. colorata* or *L. tulipifera* (Rice et al. 2013). Although most foreign genes are not expressed and most likely pseudogenized, they are maintained in *Amborella* mitochondrial genome for a very long time.

HGT is a common phenomenon in flowering plant mitochondria, being particularly frequent among parasitic plants, which often acquired mitochondrial genes from their host. Extensive acquisition of host gene was described in the holoparasitic plant *Lophophytum mirabile* (Sanchez-Puerta et al. 2017), which replaced 80 % of its mitochondrial genes by the host-derived homologs.

Some parasitic plants harbor mitochondrial genomes reduced in size owing to the loss of multiple genes. The mitogenome of hemiparasitic mistletoe *Viscum scurruloideum* is the smallest among angiosperm mitochondrial genomes investigated by now (Skippington et al. 2015) (**Table 1**); it lost the complex I genes. This loss is most likely associated with the harnessing glycolysis, not respiration, as a main source of ATP.

Huge mitogenomes of the family Cucurbitaceae (up to 3 Mb) represent the opposite site of the broad size range of angiosperm mitochondrial genomes (Alverson et al. 2010). The size expansion was achieved by the increase in intron length, by expansion of long and short repeats, or by the transfer of plastid and nuclear DNA, whereas the gene content was not substantially changed.

Although the core gene content of plant mitochondria did not change very much, multiple parallel losses of some mitochondrial genes, e.g. encoding succinate dehydrogenases (*sdh*) or ribosomal proteins were observed. The genes were either transferred to the nucleus

and transcribed from nuclear promoters, or were replaced by nuclear genes (Adams a Palmer 2003).

Extreme variation in mitochondrial substitution rate (proxy of mutation rate) across flowering plants represents the most remarkable phenomenon associated with angiosperm mitogenome. Synonymous substitution rates vary by four orders of magnitude, being highly accelerated e.g. in genera *Plantago*, *Geranium, Silene*, *Carex* or *Ajuga* (Mower et al. 2007; Zhu et al. 2014). After more than a decade of research on the species with accelerated mitochondrial mutation rate, the explanation of this phenomenon remains mysterious.

We not only do not know the reason of sudden changes in mutation rates in plant mitochondrial genomes, but we also do not fully understand the consequences of high mutation rates. The mutation burden hypothesis (Lynch et al. 2006) posits, that fast evolving animal mitogenomes are small because of the cost associated with the maintenance of introns/intergenic regions under high mutation rate. In contrast, plant mitogenomes may "afford" larger size owing to slow mutation rate and lower selection pressure. However, this hypothesis is at odds with mitogenomes in some lineages (*Silene* – Sloan et al. 2012, *Cucumis* – Alverson et al. 2010), which exhibit both size expansion and acceleration of mutation rates.

Mitochondrial genomes of the genus *Silene*

Exponential increase in the number of completely sequenced mitochondrial genomes makes possible to describe the evolution of mitochondrial genome across plant lineages, but the driving forces behind this evolution remain still largely unknown. Why did flowering plants end up with mitochondrial genomes so drastically distinct from animals?

The analysis of mitochondrial genomes in plant lineages with high variation in mitogenome structure and mutation rate among closely related species may contribute to the understanding causes and consequences of this variation. The genus *Silene* comprises many species distributed around the globe with diverse reproduction systems – hermaphroditism, gynodioecy, dioecy. They harbor mitogenomes extremely variable in size, structure, extent of rearrangements, RNA editing, and mutation rate, which evolved in a relatively short time of less than 15 milions years (Sloan et al. 2009). Owing to its genetic and life style diversity, the genus

Silene may stand in all angiosperms. **The processes and trends discovered in *Silene* mitochondria will help to understand the evolution of mitochondrial genomes in flowering plants.**

Silene latifolia has a small mitogenome of about 253 kb with slow substitution rate, which can be visualized like a single circular molecule, so called master circle. It contains six large repeats, mediating equilibrium intramolecular recombination (Sloan et al. 2010). It is similar to other plant mitogenome, except for less tRNA and ribosomal protein genes. *S. latifolia* is a dioecious species, important for the understanding sex chromosome evolution.

In contrast, *Silene conica* and *Silene noctiflora* possess the largest mitogenomes reported in plants (11.3 and 6.6Mb, respectively) and exhibit extremely fast evolutionary rates of not only mitochondrial genomes (Sloan et al. 2012a), but also of plastid genomes (Sloan et al. 2014). Mitogenomes of both species exhibit a complex multipartite structure consisting of many chromosomes, either autonomous or mutually recombining.

Silene vulgaris belongs to slowly evolving *Silene* species (but still with a bit higher mutation rate than most other angiosperms) with mitochondrial genome of only about 430 kb in size. However, it shows unprecedented intraspecific variation of mitochondrial DNA. Five completely sequenced mitogenomes of this species differ not only in structure, intergenic sequences and gene order, but also in the number of genes (Sloan et al. 2012b, Štorchová et al. 2018). High polymorphism of *S. vulgaris* mitogenome may be related to its reproduction system gynodioecy, in which hermaphrodites and females co-exist. The gender is encoded by the interaction of mitochondrial and nuclear genes (Hanson a Bentolila 2004). Balancing selection acting on mitochondrial genes is assumed to be responsible for a high polymorphism of mitochondrial genomes.

The analysis of additional *Silene* species with gynodioecious reproduction system, which evolved in parallel, would test this hypothesis, but the polymorphism was studied in single genes only (Touzet and Delph 2009). The assembly of complete mitogenomes of *Silene* species is complicated by the presence of many recombining repeats, which cause frequent genomic

rearrangements. The application of third generation sequencing generatig long reads will solve this problem.

The physical structure of mitochondrial DNA in angiosperms

Mitochondrial and plastid DNA associated with a large array of the proteins involved in replication, recombination, transcription, splicing, editing and ribosome assembly form a complex structure called nucleoid. Plant mitochondrial nucleoids contain e.g. PolI-like DNA polymerases, DNA primase-helicase TWINKLE, RecA-like recombinases RECA2 and RECA3, several types of dsDNA or ssDNA-binding proteins, or the recombination surveillance factor MutS-like homolog (MSH1) (Davila et al. 2011, Gualberto et al. 2014).

The copy number of mitochondrial genomec per mitochondria and their composition vary across plant organs and tissues. Some mitochondria possess uncomplete mitochondrial genomes missing several genes (Woloszynska et al. 2006), others, e. g. in senescing leaves, do not contain any DNA et all (Oldenburg a Bendich 2015).

Despite of a common way of visualisation of plant mitochondrial genomes as single master circles, their realistic physical structure is much more complex. Electron microscopic studies of plant mitochondrial DNA showed the mixture of linear, branched and small circular subgenomic molecules, both single- and double-stranded (Oldenburg a Bendich 1996, Oldenburg and Bendich 2015). How are such complex structure faithfully replicated is not currently known. Possible mechanisms involve recombination-dependent replication and replisome assembly, which requires primers synthesis (Brieba 2019, Morley et al. 2019). Small circular chromosomes may be replicated by means of rolling circle, as documented by the oligomers found in the mitochondrial genome of *Silene vulgaris* (Sloan et al. 2012a, Štorchová et al. 2018).

Although major knowledge about the structure of angiosperm mitochondrial genome was gained by investigating the model plant *Arabidopsis thaliana* owing to a large collection of mutations in mitochondria-targeted proteins available (Shedge et al. 2007, Davila et al. 2011, García-Medel et al. 2019), the studies of *Silene* species significantly deepened our

understanding the evolutionary forces shaping plant mitochondrial genomes (Sloan et al. 2010, Sloan et al. 2012a, Sloan et al. 2012b, Štorchová et al. 2018).

Repair of mitochondrial DNA in angiosperms

Electron movement through ETC in the course of oxidative respiration results in the production of reactive oxygen species (ROS), which may damage DNA. A highly oxidative environment of mitochondrial matrix would cause a high DNA mutation rate if the mutations were not suppressed or corrected by DNA repair. Two repair mechanisms operate in plant mitochondria – base excision repair (BER) and the repair of double-strand breaks (DSB).

Whereas nucleotide mismatches often occur due to replication errors, DSBs predominantly result from the oxidative damage of DNA. Its repair cannot utilize sequence of the complementary strand and incorporate the correct nucleotide as in case of BER. Instead, the resection at break points generates single-strand DNA, which finds out the regions of longer homology in the mitochondrial genome, where it replaces the original strand, forming so called D loop (García-Medel et al. 2019). Then new replication starts at both 3'ends of the break points. After filling the gaps and ligation, the complex is resolved with or without crosseover (reviewed by Chevigny et al. 2020).

Alternatively, the broken ends are joint together through non-homologous end-joining (NHEJ) or microhomology-mediated end-joining (MMEJ), the processes requiring no or only a very short region of homology. These two repair mechanisms produce deletions, they may also join non-corresponding break points, which leads to genomic rearrangements. It depends on the proteins stabilizing single-strands of resected DNA, whether the repair relying on longer homologous regions, or NHEJ or MMEJ takes place. The ssDNA-binding proteins SSBs support repair associated with homologous recombination, whereas the WHY proteins from the WHIRLY family or organellar single-stranded binding (OSB) proteins direct the repair through NHEJ or MMEJ (Garcia-Medel et al. 2019). The MSH1 protein, which prevents the recombination across the regions with imperfect homology, plays an important role in recombination-mediated repair. The frequency of mitochondrial genome rearrangements increases under MSH1 misfunction or absence (Shedge et al. 2007).

The perfect correction of DSBs requires long regions of homology, which are not always available. The alternative pathways NHEJ and MMEJ generate deletions and/or rearrangements with possibledeleterious effects. As the repair correcting DSBs by homologous recombination is the prominent repair mechanism in plant mitochondria, frequent occurrence of repeats and genomic expansion of angiosperm mitochondrial genomes may be the consequences of DNA repair. The efficient repair of DSBs also secures low mutation rate observed in plant mitochondrial genomes (Christensen 2018). The very high mutation rate associated with huge mitochondrial genomes in some lineages, for example in *Silene conica* and *Silene noctiflora* (Sloan et al. 2012a) may be explained by a failure of a DNA repair mechanism in mitochondria, which results in both elevated mutation rate and genome expansion. More research is needed to clarify this question, because very little information is available about mitochondrial DNA repair in *Silene*.

Cytoplasmic male sterility (CMS)

NHEJ or MMEJ may sometimes paste together the pieces of functional mitochondrial genes, which gives rise to chimeric genes encoding chimeric proteins. They may interfer with native proteins, most often the members of ETC in the inner mitochondrial membrane (Hanson and Bentolila, 2004), which is harmful for mitochondrial function. As the cell energy demand is the highest during pollen maturation, the expression of chimeric genes affects the production of viable pollen, which results in anther abortion and male sterility. The expression of chimeric genes may be influenced by nuclear factors (Rf), capable to restore male fertility (Fujii a Small 2011, Barkan et al. 2012). Plant individuals, not producing pollen develop the female (F) gender, and those with restored male fertility become hermaphrodites (H). In this case, male sterility is encoded by two factors – cytoplasmic (mitochondrial chimeric gene) and nuclear (*Rf* genes). It is called cytoplasmic male sterility (CMS) and it represents a special case of mitochondrial-nuclear interaction). The two genders - females and hermaphrodites – constitute the basis of the plant breeding system gynodioecy (Olson a McCauley 2002), which is the second most widespread angiosperm reproduction system after hermaphroditism.

CMS has a great economic importance in agriculture, being utilized for the production of hybrid seed. It is described in rice (Chakraborty et al. 2015), sugar beet (Darracq et al. 2011), or sunflower (Balk a Leaver 2001). Much less is known about CMS in wild plants. *Silene vulgaris* has emerged as a model system for the investigation of gynodioecy and CMS in wild populations (Bernasconi et al. 2009). It was used in numerous population genetic studies, although its CMS genes have remained unidentified. The first candidate CMS gene in *Silene vulgaris* (*Bobt*) was described in our lab (Štorchová et al. 2012, Štorchová et al. 2018). *Silene vulgaris* represents a suitable model to investigate the relationships between mitochondrial genome evolution and CMS in plants. One of its advantages is the existence of multiple CMS types associated with various mitochondrial haplotypes (Olson et al. 2019). We investigated two CMS types of *S. vulgaris* in greater detail – the haplotypes KRA and KOV. The male sterility in the KRA type is most likely caused by the chimeric gene *Bobt* (Štorchová et al. 2018), whereas the cause of male sterility in the haplotype KOV, associated with long non-coding RNA, is not known (Stone et al. 2017). We have also compared cytoplasmic transcriptomes of female and hermaphroditic flowers and found numerous differentially expressed genes, many of them related to oxidative stress (Štorchová et al. under preparation). In sharp contrast, plastid-encoded transcriptomes were nearly identical between the genders, despite of the prominent differences in the expression of the nuclear-encoded proteins targeted to plastids (Krüger et al. 2019). The study of CMS of *S. vulgaris* therefore represents a promising approach in the research of the signaling from the nucleus to the organelles and *vice versa* (anterograde and retrograde signaling, respectively), the fundamental phenomenon of the gene expression regulation in eukaryotes**. The variability of reproduction systems, saltatory evolution of mitochondrial genomes and the richness of interactions between organelles and nucleus qualify *Silene* species.**

METHODS

We developed some novel methods and improved the existing protocols in the course of our long-time efforts to clarify mitochondrial structure and function in the plant genus *Silene*. First, we focused on wet lab methods, later also on bioinformatic procedures.

DNA extraction

The reliable method of DNA extraction is the prerequisite for the successful study of genetic markers or the structure of organellar genomes, particularly when non-model species are investigated. There are several commercial kits suitable for plant DNA extraction, but the protocols developed in the lab are cheaper and easier to modify or to scale according to specific experimental needs. *Silene vulgaris* leaves have thick cuticle, low proportion of living cells and a broad array of secondary compounds. Our original method of DNA extraction used sorbitol treatment, which removed secondary compounds before the application of chaotropic solvent CTAB (Štorchová at al. 2000). This method provided a high yield of non-degraded DNA with all tissues of *S. vulgaris*, which was beneficial particularly in Southern hybridization requiring high amount of clean intact genomic DNA. Our sorbitol DNA extraction method has become widely used in many labs around the world, particularly in teams working with wild plant populations and lacking rich financial resources to purchase expensive DNA extraction kits.

Reference genes to evaluate gene expression in *Silene vulgaris*

The selection of suitable reference genes with stable invariable expression across the experimental conditions for the normalization of transcript levels in gene expression studies is indispensable for accurate analyses by quantitative real-time PCR (RT-qPCR). We identified two housekeeping genes *SvACT* (coding for actin) and *SvGAPDH* (coding for glyceraldehyde-3-phosphate dehydrogenase) as stable references applicable on both random-primed and oligo(dT)-primed cDNA in *Silene vulgaris* (Kolouškova et al. 2017). Random-primed cDNA facilitates the estimation of the levels of both nuclear-encoded and organelle-encoded transcripts (lacking polyA tails) in the same RNA specimen, a key benefit in the studies of cyto-nuclear interactions.

Bioinformatic pipelines for the correct mapping of plant mitochondrial transcriptomes

We tested several bioinformatic methods for the best performance of read mapping to the reference mitochondrial genome with aim to consider the complex way of splicing (including trans-splicing) of plant mitochondrial RNA. We selected GSNAP (Genomic Short-read Nucleotide Alignment Program) as the most convenient and accurate tool for this purpose. We also defined

„transcription islands“ (intergenic transcribed features), using sliding window calculation of read coverages relative to the coverage of coding regions. Lastly, we developed the bioinformatic protocol allowing to distinguish whether editing preceded splicing or *vice versa*, based on the counting of edited nucleotides in the reads spanning the splice junction and derived from the spliced *versus* unspliced transcripts (Stone at al. 2017). We applied our bioinformatic approaches in the analyses of *S. vulgaris* transcriptomes, but they may be used in any plant species.

RESULTS

High variation of gender ratio, mitochondrial and plastid markers in natural populations of *Silene vulgaris* in Europe, its native range

Silene vulgaris has become a favored model for the study of gynodioecy (the reproduction system, where females and hermaphrodites co-occur in the same population) and cytoplasmic male sterility (CMS) since the last decade of the past century. The investigation of plastid and mitochondrial markers (McCauley 1998, Olson and McCauley, 2002) revealed very high diversity of organellar DNA. The high within-species polymorphism of organellar markers was found to be associated with gynodioecious breeding system (Touzet and Delph 2009). It might have been caused either by balancing selection maintaining diverse CMS genes and corresponding haplotypes in the population for a long time (Städler and Delph 2002), or by sequential selective sweeps introducing the haplotypes associated with novel CMS genes (Ingvarsson and Taylor 2002).

We performed the population genetic study of eight populations of *S. vulgaris* from Central Europe (Czech Republic, Germany, and Austria) and discovered the extremely high diversity of mitochondrial DNA, expressed as a high proportion of private alleles, present in a single population (Štorchová and Olson 2004). This diversity exceeded the previously described diversity of US populations (Olson and McCauley, 2002), which may be explained by the reduction of genetic richness in the introduced range.

We studied RFLP polymorphism of mitochondrial DNA, which reflected both sequence and structural differences among the mitochondrial haplotypes. In addition, we analyzed the plastid genome variation using the sequence of the *trnH* - *psbA* intergenic region. This region encodes RNA hairpin in the *psbA* 3' UTR, which causes frequent homoplastic inversions (Štorchová and Olson 2007). The mitochondrial and plastid markers were linked, but not completely. This observation agreed with the mode of inheritance of the two organelles. Mitochondria and plastids are maternally inherited, but a rare paternal inheritance (or paternal leakage) was described in *S. vulgaris* for both mitochondria and plastids (Pearl et al. 2009). When mitochondrial genomes from two parents co-exist in the same cell, the fusion of organelles followed by DNA recombination may occur. Thus, the plant mitochondrial markers occasionally exhibit incomplete linkage.

We also reported a very low proportion of females in the populations of *S. vulgaris* from the above-timberline sites in the Allgauer Alps, which were nearly 100% hermaphroditic (Štorchová and Olson 2004). The origin of these high-mountain populations is unknown. The detailed phylogeographic study of *S. vulgaris* by Sebasky et al. (2016) described the geographic pattern from the South East to the North West linked to the Neolithic expansion of agriculture. However, this study did not include the populations from high European mountains. We have recently sequenced complete plastid genomes from high mountains *S. vulgaris* and estimated their basal position in the phylogenetic tree of *S. vulgaris* haplotypes (Krüger et al. 2019). This suggested that high mountains might have been refuges for the ancestral populations of *S. vulgaris*, which migrated uphill and downhill in glacial and interglacial periods, respectively. The genetic distance between lowland and high mountain *S. vulgaris* populations is also supported by a lower viability of the progeny from their mutual crosses, demonstrated by a low germination rate (Olson et al. 2019).

If our hypothesis is correct, it opens an attractive possibility, that high mountain populations of *S. vulgaris* harbor ancestral mitochondrial genomes, from which the highly rearranged mitochondrial genomes of lowland populations of *S. vulgaris* evolved. We plan to test this hypothesis by sequencing the complete mitochondrial genomes of *S. vulgaris* from various locations in the Alps and other high European mountains.

The prominent structural differences in mitochondrial DNA documented by Southern-RFLP in European populations of *S. vulgaris* suggested extensive rearrangements and inspired subsequent sequencing and assembly of complete mitochondrial genomes of *S. vulgaris* (Sloan et al. 2012).

The assembly of complete mitochondrial genomes in *Silene* species revealed extreme variation in the sequences, configurations and gene numbers

Unlike gene-dense plastid genomes with conserved size and structure, the highly rerranged and extremely variable plant mitochondrial genomes represent a challenge for assembly efforts. The mitochondrial genome of *Silene latifolia* was among the first angiosperm mitochondrial genomes completely sequenced (Sloan et al. 2010). It was rather small (253 kb), but frequently recombining across six large repeats. Our lab contributed with Southern hybridization experiments proving ongoing equilibrium recombination across each of the repeats. The mitochondrial genome of *S. latifolia* lost many genes encoding ribosomal proteins or tRNAs, which were transferred to the nucleus. Its substitution rate was comparable with other plant mitochondrial genomes. The mitochondrial genome of *S. latifolia* exemplifies the evolutionary trend of the gradual loss of the genes involved in proteosynthesis and their transfer to the nucleus.

The mitochondrial genome of *S. vulgaris*, the sister species of *S. latifolia*, differed remarkably and surprisingly from that of *S. latifolia*. First of all, no single mitochondrial genome represented this species. Instead, the haplotypes with distinct Southern- RFLP patterns differed profoundly in mitochondrial genome structure, gene order, extent of plastid DNA insertions, and even in gene number, namely in the presence or absence of the *rpl5* gene. We sequenced the complete mitochondrial genomes of four *S. vulgaris* haplotypes (Sloan et al. 2012) and added the fifth haplotype later (Štorchová et al. 2018). However, even five completely sequenced mitochondrial genomes did not capture the entire mitochondrial metagenome of *S. vulgaris*. About 10 % of the mitochondrial sequences provided no hit by blast search, which indicated the existence of so far undiscovered mitochondrial DNA in this species. The substitution rate of

protein-coding genes in *S. vulgaris* mitochondria is faster compared with *S. latifolia*, but much slower than in the super-fast evolving species *S. conica* and *S. noctiflora* (Sloan et al. 2012b)

The mitochondrial genomes of *S. vulgaris* are multipartite, composed of several chromosomes, which are either autonomous, not recombining with other chromosomes, or non-autonomous, occasionally recombining with the others. One of the chromosomes was always much larger than the rest of them. The mitochondrial genomes may be visualised as circular molecules, but this view is oversimplified, as documented by Southern hybridizations (Sloan et al. 2012). Some parts of mitochondrial DNA exist as single-stranded or complex branched structures. All five completely sequenced mitochondrial genomes of *S. vulgaris* contained a small chromosome (2.5 kb -7 kb) with zero gene content. It carried the region of sequence homology among all the haplotypes, which function, if any, is currently unknown. The small chromosomes exist in several multimeric structures as open circles, or supercoiled or linear molecules. They resemble bacterial plasmids, but no genes or replication origins were recognized in their sequences (Štorchová et al. 2018). The presence of the small chromosome-specific sequnces in all the sequenced haplotypes and also in additional, so far unassembled mitochondrial genomes of *S. vulgaris* suggests their functional importance, which shall be revealed by further research.

The huge mitochondrial genomes of the fast evolving species *S. conica* and *S. noctiflora* have a complex multipartite structure. The number of their chromosomes is much higher than in *S. vulgaris*, reaching up to 60 -70, and varies among the individual populations (Sloan et al. 2012b).

The diversity of mitochondrial genomes among *Silene* species is enormous, ranging from small, simple, slowly evolving genome of *S. latifolia* to giant, multipartite, fast evolving genomes of *S. conica* and *S. noctiflora*. The mitochondrial genome of *S. vulgaris* stands between the two extremes, but it is distinguished by its extraordinary intraspecific variation. The complete mitochondrial genomes were assembled only in the minority of *Silene* species. The causes and the consequences of the structural and sequence variation among the mitochondrial genomes of a single plant genus, as well as their mode of replication and the impact on mitochondrial

function, are unknown. We have just started to discover the secrets of plant mitochondrial DNA. *Silene* species represent the most appropriate models to be investigated.

The transcription profiles of mitochondrial genes of *Silene vulgaris* are influenced by the mitochondrial genomic environment as well as by the nuclear background

The rearrangements of mitochondrial genomes primarily affect intergenic regions. The recombination across coding sequences is likely selected against to preserve gene function, although it may occur either (Štorchová et al. 2018). However, the rearrangements of intergenic DNA influence gene expression, when they target gene flanks at 5' or 3' UTR (untranslated transcribed regions). We may expect the variation in mitochondrial transcript starts and termini.

To test this hypothesis, we analyzed the progeny of *S. vulgaris* individuals collected in the field and observed the variation in the number and size of bands generated by Northern hybridization with the probes derived from two essential mitochondrial genes - *atp1* and *cox1*, coding for ATP synthase subunit 1 and cytochrome oxidase 1, respectively. The transcript pattern estimated by Northern hybridization strongly correlated with Southern RFLP, which implied that genomic configuration was responsible for the variation in RNA banding pattern. The plants with identical restriction sites in gene flanking regions showed also identical transcript pattern (Elansary et al. 2010).

The transcription of plant mitochondrial genes is predominantly controlled at posttranscriptional level by RNA processing, but transcription starts in the promoter region also play a role (Stoll et al. 2013). We adopted RNA circularization and primer extension, and identified three 5' ends of the *atp1* transcripts in the KOV haplotype, which were most likely generated by the combination of RNA processing and transcription initiation (Muller and Storchova, 2013). Interestingly, the proportion of individual transcripts with specific 3` end depended on the nuclear background. The transcript with long 5` end appeared only in a part of the progeny from the crosses between the KOV female and the pollen donor with different haplotype. Its inheritance did not correspond to the Mendelian single locus inheritance, but it was rather dictated by epistatic interactions.

This example demonstrates how useful can be *S. vulgaris* as the model for the study of the regulation of mitochondrial gene expression in plants. Unfortunately, the complete nuclear genome of *S. vulgaris* is not yet available, which makes difficult to identify the nuclear genes influencing the transcription in mitochondria. If the genome assembly is missing, transcriptome reference can be used for the comparison between the contrasting conditions. However, the analyzis of transcriptome regarding the mitochondria-targeted transcripts is constrained by frequent incorrect assembly of PPR (Pentatricopeptide Repeat) transcripts, when short reads are used. The huge plant family of PPR genes includes hundreds of members participating in organellar processes such as RNA editing, RNA splicing, RNA transcription initiation or processing (Gutmann et al. 2020). Very recently, the sequencing long reads derived from cDNA by PacBio method (IsoSeq) was introduced, but it is so far very expensive method, which was not available in previous years. In the following research we have therefore concentrated on the global transcriptomic studies based on short Illumina reads.

Mitochondrial and plastid transcriptomes of *Silene vulgaris* and *Silene noctiflora*

After performing the transcription studies of single genes, we adopted RNASeq to describe mitochondrial and also plastid transcriptomes of *Silene* globally. We analyzed the mitochondrial transcriptomes of young flower buds from three females and three hermaphrodites (full siblings) carrying the same haplotype. We found the high overall similarity in read coverages and RNA editing rates among the individual transcriptomes (Stone et al. 2017, Štorchová et al. 2018). Our results were in agreement with recent findings of Niazi et al. (2019), who described the coordination mechanism, which controls and „buffers“ mitochondrial transcriptomes under variable intramitochondrial (redox state) and cytoplasmic (ROS, ion concentrations, nuclear-encoded proteins targeted to mitochondria etc.) conditions. Variation in transcript initiation or processing, which we observed e.g.in the *atp1* gene transcription (Müller and Štorchová, 2013) did not affect the general expression of the respective genes.

We detected surprisingly high read coverage of introns, sometimes higher than of adjacent exons. It means, that the spliced introns did not undergo fast degradation, but persisted in mitochondria for a longer period. This notion is in line with the possible function of mitochondrial introns as ribozymes, which is also supported by their complex and stable

secondary structure (Bonen a Vogel 2001). We have also determined the order of splicing and RNA editing at the global level, using our RNASeq data and a newly developed pipeline. We found that RNA editing preceded splicing in most cases. There were only few exceptions, when splicing was required to generate the target site for the editing recognition factor (Stone et al. 2017).

The comparison of female and hermaphrodite mitochondrial transcriptomes of the haplotype KOV revealed only one feature differentially expressed between the two genders, which might be responsible for the induction of male sterility in this haplotype of *S. vulgaris*. It was non-coding RNA about 400 nt long, expressed about 15 times higher in females (Stone et al. 2017). It was the first report of mitochondrial non-coding RNA associated with CMS in plants. However, it is not clear, whether this RNA represents the cause or rather the consequence of CMS. More research is necessary to clarify this point.

We utilized our experience in plant mitochondrial transcriptome construction for the analysis of transcription in *Silene noctiflora*, the plant with a giant mitochondrial genome (6.7 Mb), split into many chromosomes. We detected transcription in several autonomous chromosomes carrying no coding sequence, which may imply their functionality (Wu et al. 2015).

As both plastid and mitochondrial transcripts lack polyA tails, we could have used our RNASeq data generated without polyA enrichment also for the construction of plastid transcriptomes of the respective haplotype. We revealed no differences in read coverage or editing between the genders in six plants. Alike mitochondria, plastids maintained their transcriptional processes at stable level, despite multiple differences in expression of organelle-targeted proteins recorded between the genders (Štorchová et al., in preparation).

The advantage of *Silene vulgaris* as the model for the investigation of Cytoplasmic male sterility

Mitochondrial CMS genes are often chimeric, composed of pieces of standard mitochondrial genes and/or regions of unknown origin. They are well studied in crops, whereas their recognition in wild

plants is delayed. We found the chimeric gene *Bobt* consisting of the fragments of *atp1* and *cox2* in the haplotype KRA of *S. vulgaris*. It was upregulated in females, which makes it a promising CMS candidate gene, the first one in *S. vulgaris* (Štorchová et al. 2012). The *Bobt* gene was co-transcribed with the essential gene *cob* coding for cytochrome b.

To clarify the context of the CMS gene in the KRA haplotype, we assembled the complete mitochondrial genome of this haplotype. It consisted of seven chromosomes. Two of them were autonomous and occurred as multimeric circles in higher copy numbers than the large recombining chromosomes. The *cob* gene occurred in two genomic configurations. It was either transcribed from its own promoter or it was dependent on the co-transcription with the CMS gene *Bobt*. The flip-flop mechanism ensured by the homologous recombination across the *cob* coding region exchanged the two configurations. The frequency of each configuration did not correlate with gender (Štorchová et al. 2018), but it might have been influenced by heat stress. Inhibition of the CMS gene transcription in hermaphrodites downregulated also *cob*, which would negatively impact mitochondrial function. The "free *cob*" configuration shall be preferred under stressful conditions to ensure sufficient *cob* expression even in hermaphrodites with inhibited *Bobt* transcription. This interesting possibility is currently under investigation. The *cob* - *Bobt* interchange is one of infrequent examples of possible functional importance of homologous recombination in plant mitochondria, which is otherwise considered to be a side product of DNA repair (Chevigny et al. 2020).

Several unrelated CMS factors exist in crop species or in metapopulations of wild plants (Darracq et al. 2011). Each CMS gene can be restored by its matching *Rf* gene(s), not by other *Rf*. How are the CMS determinants and *Rf* factors distributed in natural populations is not known. We crossed *S. vulgaris* plants collected in Europe and USA and determined the ratio of females in individual crosses (Olson et al. 2019). We found remarkable consistency in gender ratio among the progenies from various fathers, no matter how far its population of origin was located. This observation suggests the global distribution and lasting persistence of at least some *Rf* genes. Only single population collected in the Alps above-timberline site produced distinct gender ratios, being incapable to restore females from other (lowland) populations. The

high mountain populations of *S. vulgaris* are genetically distant from lowland plants (Krüger et al. 2019). They are the focus of our current interest because they carry ancestral mitochondrial genomes, which may clarify the origin and evolution of extremely variable and rearranged mitochondrial genomes of *S. vulgaris.*

CMS has been intensively studied in crops, which passed genetic bottleneck in the process of domestication. They contain only a part of genetic diversity encountered in wild species. To fully understand CMS, we shall turn to natural populations in the wild. Owing to the variability of mitochondrial genomes, the abundance of genetic markers, easy cultivation and crossing, and a broad distribution area, *S. vulgaris* becomes the best model for the investigation of CMS in wild species.

SUMMARY

Unlike the small, compact, intron-less and conserved mitochondrial genomes of animals, higher plants possess large mitochondrial genomes, varying profoundly not only in size, but also in gene content and order, in nucleotide substitution rates and in the RNA editing, which post-transcriptionally modifies the sequences of RNAs and often reverses the mutations in coding sequences. RNA editing emerged in land plants, it is very abundant in hornworts, then it was probably secondarily lost in liverworts. Gene order has been at least patchily preserved in algae, mossess, lycophytes and ferns, and also in some gymnosperms and basal angiosperms. Eudicots and monocots increased mitochondrial genomic rearrangement rate and size variation. However, a single feature remains conserved in flowering plants compared to algae and mosses - the number and position of introns.

Some angiosperm lineages exhibit gene losses and reduction in RNA editing, as well as the acceleration in substitution rates, sometimes reaching incredibly high values (Sloan et al. 2012a). Similar evolutionary trends (gene reduction, size increase) could be observed in gymnosperms, but the evidence is scarce because very few complete mitochondrial genomes have been assembled in gymnosperms to date.

Given the colorful variety of angiosperm mitochondrial genomes, it is difficult to expect that a single plant genus would make possible to study all the aspects of mitochondrial genome evolution in flowering plants. However, such genus exists – it is *Silene* (campion) !

Some *Silene* species such as *S. latifolia* harbor the mitochondrial genome of standard size and conventional rearrangement rate, but with reduced gene number and editing sites (Sloan et al. 2010). Its sister species *S. vulgaris* is remarkable by extreme rearangements and within-speceis varition of mitochondrial genomes (Sloan et al. 2012b). It is a gynodioecious plant, with females and hermaphrodites occurring in the same populations. *S. vulgaris* became a very suitable model to study CMS in wild populations (Stone et al. 2017, Olson et al. 2019) after the first candidate CMS factors had been revealed (Štorchová et al. 2012, Štorchová et al. 2018). Other *Silene* species, *S. conica* and *S. noctiflora*, carry the largest mitochondrial genomes with extremely high evolutionary rates (Sloan et al. 2012a). Their genomes are split to many chromosomes, some of them without annotated genes, but being still transcribed (Wu et al. 2015).

Owing to its propensity to encode CMS, the structure of mitochondrial genome is tightly interwoven with plant reproduction system. *S. latifolia* is a dioecious plant with XY sex chromosomes. Its gynodioecios sister *S. vulgaris* makes possible to investigate, whether dioecy and gyndioecy evolved in parallel, or whether dioecy arose from gynodioecy, and to follow the impact of a changed reproduction system on mitochondrial genome.

The genus *Silene* provides the possibility to analyze not only the transition from gynodioecy to dioecy, but also the parallel evolution of dioecy terminating with male heterogamety XY in *S. latifolia*, or female heterogamety ZW in *Silene otites*. The mitochondrial genomes of the section *Otites* are totally unknown, their assembly promises interesting findings. **In conclusion, the research of mitochondrial genomes and their relationship with reproduction system in *Silene* is still in its infancy and very worthy to proceed!**

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LIST OF PAPERS included In DSc Thesis : The set of **16** papers

Helena Štorchová is first and/or corresponding author (*) in **12** of them, senior author in **1** paper, contributing author in **3** papers

1. **Methods**

Štorchová H*, Hrdličková R, Chrtek JJr, Tetera M, Fitze D, Fehrer J (**2000**) An improvement of DNA isolation from plants collected in the field and conserved in saturated NaCl/CTAB solution. Taxon 49:79–84. (IF_{2000} = 0.863; Citations: 123; without autocitations: 105)

Kolouškova P, Stone JD, **Štorchová H*** (**2017**) Evaluation of reference genes for reverse transcription quantitative real-time PCR (RTqPCR) studies in Silene vulgaris considering the method of cDNA preparation. PloS One 12:e0183470. (IF_{2017} = 2.74; Citations: 0)

2. **High variation of gender ratio, mitochondrial and plastid markers in natural populations of *Silene vulgaris* in Europe, its native range**

Štorchová H*, Olson MS (**2004)** Comparison between mitochondrial and chloroplast DNA variation in the native range of *Silene vulgaris.* Mol Ecol 13:2909-2919. (IF_{2004} = 4.375; Citations:28; without autocitations:19)

Štorchová H*, Olson MS **(2007)** The architecture of the chloroplast *trnH-psbA* non-coding region in angiosperms*.* Plant Syst Evol 268:235-256. (IF_{2007} = 1.492; Citations:46; without autocitations: 45)

3. **The assembly of complete mitochondrial genomes in the *Silene* species revealed extreme variation in the sequences, configurations and gene numbers**

Sloan DB, Alverson AJ, **Štorchová H**, Palmer JD, Taylor DR (**2010**) Extensive loss of translational genes in the structurally dynamic mitochondrial genome of the angiosperm

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Sloan DB**,** Müller K**,** McCauley DE, Taylor DR, **Štorchová H** (senior author) (**2012**) Intraspecific variation in mitochondrial genome sequence, structure, and gene content in *Silene vulgaris,* an angiosperm with pervasive cytoplasmic male sterility. New Phytol 196:1228–1239. (IF_{2012} = 6.736; Citations:61; without autocitations: 54)

4. The transcription profiles of mitochondrial genes of *Silene vulgaris* are influenced by the mitochondrial genomic environment as well as by the nuclear background

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5. Mitochondrial and plastid transcriptomes of *Silene vulgaris* and *Silene noctiflora*

Wu ZQ, Stone JD, **Štorchová H,** Sloan DB (**2015**) High transcript abundance, RNA editing, and small RNAs originating from intergenic regions in the massive mitochondrial genome of the angiosperm *Silene noctiflora*. BMC Genomics 16: 938. (IF_{2015} = 3.867; Citations: 21; without autocitations: 19)

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Krüger M, Abeyawardana OAJ, Juříček M, Krüger C, **Štorchová H*** (2019) Variation in plastid genomes in the gynodioecious species *Silene vulgaris*. BMC Plant Biol 19:568. (IF_{2019} = 3.497; Citations: 2; without autocitations: 2)

6. The advantage of *Silene vulgaris* as the model for the investigation of Cytoplasmic male sterility

Štorchová H, Müller K, Lau S, Olson MS (**2012**) Mosaic origins of a complex chimeric mitochondrial gene in *Silene vulgaris*. PLoS One 7:e30401. (IF_{2012} = 2.074; Citations: 9; without autocitations: 2)

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7. Reviews

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