Elo, Annakaisa

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Key words: flowering, *APETALA1, FRUITFULL*, *LEAFY, BpMADS3*, *BpMADS4*, *BpFULL1*, chloroplast mutator, *AtMSH1*, plant mitochondria, substoichiometric shifting, gene clusters, protein targeting, *AtPolγ1*, *AtPolγ2*, signal sequences

Accelerating the flowering of trees would be extremely useful because the long generation time makes the breeding and genetic research of trees difficult. Birch has proven to be an excellent model species for studying and manipulating flowering in trees. One of the main aims of the current study has been to characterize birch homologs for *APETALA1* (*AP1*), *FRUITFULL* (*FULL*), and *LEAFY* (*LFY*). These are genes known to control floral initiation and early phases of flower development in *Arabidopsis*.

Three cDNAs representing the *AP/SQUA* group of MADS-box genes, designated *BpMADS3*, *BpMADS4* and *BpMADS5* (*Betula pendula* MADS), were isolated and characterized. *BpMADS3* is phylogenetically most closely related to *AP* but, in contrast to *AP1,* has expression in late developmental stages. Ectopic expression of *BpMADS3* resulted in early flowering in *Arabidopsis* and tobacco. The tobacco plants expressing *BpMADS3* flowered early and vigorously and stayed very small, apparently producing inflorescences from every axillary bud. The expression of *BpMADS4* starts at a very early stage of both male and female inflorescence development. In later development, it becomes expressed in the bracts and in the flower initials. Expression was also detected in all the vegetative tissues tested, including the roots. The ectopic expression of *BpMADS4* accelerates flowering dramatically in *Arabidopsis*, tobacco and in birch. The birch line that flowered the earliest, started about 11 days after rooting, the saplings being only 3 cm high. The birches transformed with the *BpMADS4* antisense construct showed remarkable delay in flowering and the number of flowering individuals was reduced. Two of the transformed lines did not show any signs of flower development during the course of the two years of the study.

BpMADS5 (later renamed *BpFULL1*) has a high sequence similarity to *BpMADS4* and is expressed from the very early stages of inflorescence development. It continues to be expressed in fruit development suggesting a similar function to the *Arabidopsis* gene *FRUITFULL*. The ectopic expression of *BpFULL1* resulted in early flowering in *Arabidopsis* and tobacco but its effect in birch was weak and inconsistent. The clearest phenotypic changes in birch lines overexpressing *BpFULL1* were abnormal branching of the inflorescences, elongation of the inflorescence axil and the development of extra male organs in the flowers.

In additon to the MADS box genes described above, a birch homolog for the *LEAFY* and *FLORICAULA* genes, *BpFLO,* was isolated. *BpFLO* is expressed in inflorescences and in all the vegetative tissues tested except the roots. Ectopic expression *of BpFLO* resulted in early flowering in *Arabidopsis* but not in tobacco. In birch, the effects of the overexpression of *BpFLO* on the time of initiation of flowering seemed to depend on the genetic background used in the transformation. In one of the transformed clones used, the average time of initiation of flowering was accelerated compared to controls but in the second clone, the average initiation time was delayed.

These results prove that *BpMADS3*, *BpMADS4* and *BpFULL*, despite their high sequence homology, have differentiated but likely redundant roles in birch flower initiation and development. Their roles in birch flower development are generally similar to those of their homologs in other species. The most important finding of this study is that it is possible to either accelerate or suppress flowering in birch by overexpressing or suppressing a single birch gene, *BpMADS4*. The *BpMADS4* is also a very promising candidate for accelerating flowering in other plant species.

The second part of this study concentrates on processes unique to a plant cell. The mitochondrion plays crucial roles in many processes in the life and development of a plant. Flower development and

mitochondrial processes are likely to be co-regulated since the maternal transmission of the mitochondrial population into the following generation takes place during the development of female gametophytes. The genomic control of the mitochondrial processes during development is still mostly uncharacterized.

Mutation of the *Arabidopsis* nuclear *CHM* gene results in a green-white leaf variegation that, in subsequent generations, displays maternal inheritance. It was originally suggested that it causes plastid mutations and was named the *chloroplast mutator*. However, it was shown later that the appearance of the variegation phenotype is accompanied by a specific rearrangement of mitochondrial DNA which includes amplification of a specific molecule encoding a chimeric sequence. The aim of this study was to characterize the mitochondrial genome instability and nuclear-mitochondrial interaction, and to investigate how the nuclear gene locus *CHM* controls mitochondrial genome structure in *Arabidopsis*. Additionally, it was the aim to characterize the gene locus behind the *chm* mutant phenotype in *Arabidopsis*.

Using DNA markers designed on the basis of information from classical mapping experiments, the *CHM* locus was mapped on *Arabidopsis* chromosome III. The candidate *CHM* gene was shown to encode for a protein related to the MutS that is involved in mismatch repair and DNA recombination. The isolated cDNA encoded for a mitochondrial targeting presequence, and a confocal microscopy analysis revealed the mitochondrial localization of the protein. This study confirms that *CHM* (*AtMSH1*) maintains mitochondrial genome stability in *Arabidopsis*. Despite the homology with mismatch repair proteins, the main function of *AtMSH1* is likely to be in controlling recombinational activity in the mitochondrial genome.

Mapping of the *CHM* locus on *Arabidopsis* chromosome III revealed that the surrounding chromosomal region is unusually rich in genes supposed to be involved in mitochondrial DNA and RNA maintenance. The tight linkage of the genes behind mtDNA metabolism suggests that they are very likely to be co-regulated (e.g. through imprinting). Before this study, nearly all of the putative plant genes for mitochondrial DNA replication and repair were unidentified, and this survey revealed several candidates putatively involved in these functions. Evidently, an extensive interchange of organellar targeting presequences has occurred between unrelated proteins suggesting that intergenic recombination has been the major mechanism in the acquisition of organelle-targeting capacity for the genes in this region. The duplication of gene loci, and even larger chromosome regions, seems to be a common event resulting in functional gene duplicates. In several instances, gene duplication events have resulted in mitochondrial, plastid, and dual targeting forms of a nearly identical protein. In summary, it can be conluded that protein targeting in plant organelles is flexible and under constant evolution. In several examples, two cell organelles share the genes coding for proteins involved in their genome maintenance functions. This study reveals that the genomes of the nucleus, the mitochondria and the plastids have evolved together in a dynamic and tightly controlled process in plants.

Annakaisa Elo, Faculty of Biosciences, University of Joensuu, P.O.Box 111, FIN-80101 Joensuu, Finland

ABBREVIATIONS

AP1	*APETALA1*
AtMSH1	*Arabidopsis thaliana* mitochondrial MutS homolog
bp	base pair
BpFULL1	*Betula pendula* FRUITFULL-like 1
BpMADS	*Betula pendula* MADS
BpFLO	*Betula pendula* FLO
CHM	*CHLOROPLAST MUTATOR*
CMS	cytoplasmic male sterility
eu	eudicot
FLO	*FLORICAULA*
FUL	*FRUITFULL*
GFP	green fluorescent protein
LFY	*LEAFY*
mt	mitochondria
nt	nucleotide
UTR	untranslated region

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is mainly based on the following publications, referred in the text by Roman numerals I-IV. In addition some previously unpublished data is included.

I Elo A, Lemmetyinen J, Turunen M-L, Tikka L and Sopanen T. 2001. Three MADS box genes similar to APETALA1 and FRUITFULL from silver birch (Betula pendula). Physiologia Plantarum 112: 95-103

II Elo A, Lemmetyinen J, Novak A, Keinonen K, Porali I, Hassinen M and Sopanen T. 2007. BpMADS4 has a central role in the inflorescence initiation in silver Birch (Betula pendula, Roth). Submitted.

III Abdelnoor RV, Yule R, Elo A, Christensen AC, Meyer-Gauen G and Mackenzie SA. 2003. Substoichiometric shifting in the plant mitochondrial genome is influenced by a gene homologous to MutS. Proceedings of the National Academy of Science of the United States of America 100:5968-5973

IV Elo A, Lyznik A, Gonzales DO, Kachman SD and Mackenzie SA. 2003. Nuclear genes that encode mitochondrial proteins for DNA and RNA metabolism are clustered in the Arabidopsis genome. Plant Cell 15:1619-1631

1. INTRODUCTION

Reproduction is a crucial event in the life cycle and evolution of a plant and in the survival of plant species. Consequently, the timing and placing of flowering, as well as the phases of flower development are tightly controlled.

From a human point of view, flowering, followed by fruit and/or seed formation, is the most important trait of many plant species. Advances in understanding the genetic basis of flowering induction and flower development have facilitated attempts to manipulate flowering by genetic modification. Reducing the time to the initiation of flowering would be especially desirable for tree breeders since the long generation time makes tree breeding extremely slow and tedious. Similarly, methods of preventing flowering and inducing sterility have also been most welcomed in attempts to prevent the spread of transgenes from genetically manipulated plants. Birch has proven to be an excellent model species for studying and manipulating flowering in trees. Previous studies show that the flowering of birch can be efficiently inhibited (Lemmetyinen, 2003; Lännenpää, 2005). This study shows that the flowering of birch can also be accelerated.

The transition to the reproductive phase, floral organ development, and production of pollen and especially the production of seed and fruit are highly demanding on energetic and metabolic resources. To accommodate the increased energetic and metabolic burden, the activities of chloroplasts and mitochondria must be coordinated and upregulated. Flower development seems to be controlled by the nuclear genome. However, dysfunction of the mitochondrial genome has also been documented to severely distort reproductive development in several cases.

Although the basis of the regulation of flower development has been extensively studied, very little is known about the maintenance, organization and transmission of the mitochondrial genome in higher plants. Flower development and these mitochondrial processes are likely to be co-regulated since the maternal transmission of the mitochondrial population into a following generation takes place during the development of female gametophytes.

The mitochondrion can be thought of as a central regulator of several cellular functions such as metabolism, programmed cell death, and intracellular signaling. In higher plants, mitochondrial functions and behavior have been influenced by the plant cell's unique context of having two separate cell organelles for energy metabolism. Co-evolution of mitochondria and chloroplasts has expanded and refined protein targeting capacity, and dual targeting of organellar proteins seems to be common. The mitochondrial genomes of plants have acquired structural and maintenance features distinct from their animal counterparts.

1.1. Gene regulation during inflorescence initiation

1.1.1. Preparing to flower

Plant development is a continuous process during which a plant goes through distinct phases. The major developmental transitions preceding flowering are:

1) juvenile to adult
2) adult to reproductive maturity

The juvenile and adult phases are defined according to whether or not the shoot is competent to flower. After germination, during the juvenile phase, plants undergo shoot maturation and are incompetent to reacting to the environmental or internal signals which trigger flowering in a mature plant (Lawson and Poethig, 1995; Mártinez-Zapater and Martín-Trillo, 2002). Several plant species have distinct physiological or morphological features in their juvenile and adult phases such as a leaf shape which is typical of the juvenile phase (Lawson and Poethig, 1995; Telfer et al., 1997). In appropriate environmental conditions, a plant undergoes the floral transition and becomes reproductive.

Very little is known about the transition from the juvenile to the adult or about the vegetative phase change. On the basis of information gathered from different plant species, it seems that plants do not have a clear, distinct switch from the juvenile to the adult phase. Instead, reproductive competence is gained through a gradual change controlled by a combination of genetic and environmental signals (e.g. Martín-Trillo and Martínez-Zapater, 2002). In annuals, this phase change

occurs within weeks or days after germination whereas trees can stay juvenile for decades. A developmental reprogramming of shoot apical meristems takes place before sexual reproduction occurs in plants. This transition from the vegetative to the reproductive phase is influenced by environmental signals such as day length and temperature. In *Arabidopsis*, the genes known to regulate the phase change from juvenile to adult include, for example, *HASTY* (Telfer and Poethig, 1998) and *CONSTANS* and *FCA* are examples of genes regulating the change from the vegetative to the reproductive phase , (e.g. Blazquez and Weigel 2000; Mouradov et al., 2002).

1.1.2. Transition to flowering

Flowering is a fundamental developmental process and its proper timing and placing is crucial for successful reproduction. The timing is controlled in response to physiological and environmental signals, and flower initiation results from the balance between reproductive competence and flowering inducing factors (Martín-Trillo and Martínez-Zapater, 2002). The transition to flowering occurs in the apical shoot meristems. They are reprogrammed after induction to either produce an inflorescence or floral meristems, which give rise to floral organs.

The main rule in the genetic regulation of plant reproduction is that a plant will flower if the flowering is not repressed. The characterization flowering time mutants in *Arabidopsis* has shed light on the mechanisms that activate flowering (e.g. Blazquez, 2000; Sung et al., 2003). Several floral repressors that block the transition from vegetative to inflorescence development have been characterized in early flowering, loss-of-function mutants. In *Arabidopsis*, it can be deduced that *EMF1* and *2* are required for vegetative development since loss-of-function mutants produce flowers without developing rosette leaves. Thus the normally functioning *EMF1* and *2* genes repress the formation of the inflorescences and are needed for vegetative development (Sung et al., 2003). Other known floral repressor genes include *CONSTANS* which transduces the environmental signals to the floral promoter genes, and *TERMINAL FLOWER* (*TFL*), which negatively regulates the floral promoting genes *LEAFY* (*LFY*) and *APETALA1* (*AP1*) (reviewed e.g., by Sung et al., 2003). The *Antirrhinum* ortholog for *AP1* is *SQUAMOSA* (*SQUA*) and the *LFY* ortholog is *FLORICAULA* (*FLO*) (Coen et al., 1990; Huijser et al., 1992). Typically, plants carrying mutated versions of these genes develop shoots instead of flowers. Accordingly, in *Arabidopsis*, the ectopic expression of either the *AP1* or the *LFY* leads to early flower development (Mandel and Yanofsky, 1995). In poplar, constitutive expression of the *LFY* gene from *Arabidopsis* resulted in early formation of flowers (Weigel and Nilsson, 1995). More complete reviews of the genes influencing flowering time are presented by, for example, Blázquez (2001), Lemmetyinen and Sopanen (2004) and Lemmetyinen (2003).

In *Arabidopsis*, *FRUITFULL* (*FUL,* formerly known as *AGL8*), together with *LFY*, is among the earliest known genes that determine the role of the inflorescence meristem (Mandel and Yanofsky 1995a; Hempel et al., 1997; Ferrándiz et al., 2000a). FUL appears to maintain the identity of the inflorescence meristem and promote floral meristem specification. Interestingly, *FUL* is also required for the proper development of the cauline leaf and later in carpel and fruit development (Gu et al., 1998; Ferrándiz et al., 2000b). *DEFH28* seems to have a corresponding function to *FUL* in the meristem and fruit development of *Antirrhinum* (Müller et al., 2001), whilst the function of the more homologous gene *AmFUL* is yet to be described (Litt and Irish, 2003). Of the genes described above, *AP1*, *CAL* and *FUL* are members of the closely related *AP1/FUL* gene family (I; Litt and Irish, 2003; this study).

In the next phase of development, the inflorescence meristem gives rise to floral meristems. The known genes in *Arabidopsis* that control the developmental switch between inflorescence and floral meristems include *AP1, CAULIFLOWER (CAL), LFY* and *FUL*. As well as specifying floral meristem identity, *AP1* has been implicated in sepal and petal identity (Weigel and Meyerowitz, 1994). *CAL* seems to have a redundant role with *AP1* but it seems to be a result of duplication and is therefore unique in the family *Brassicaceae* (I, Purugganan, 1998).

1.1.3. Floral organ identity

Flower development is a pattern formation process which is thought to be equivalent to animal development. Floral organs start to develop on the flanks of the floral meristems. The formation starts from the outer whorl (sepals), followed by the initials of petals, stamens and finally carpels. During the past decade, intensive genetic and molecular analyses have revealed much of the genetic activity behind initiation and development of flowers (reviewed e.g. in Ma et al., 1994; Theissen et al., 2000; Jack, 2001a; Lemmetyinen and Sopanen, 2004). Much of this research was conducted using *Antirrhinum majus* and *Arabidopsis thaliana* as model species. Especially in *Arabidopsis,* reverse genetics has proven to be a powerful tool. Work with known floral mutations has lead to a simplified ABC model of flower development (Weigel and Mayerowitz, 1994). The ABC model describes three classes of homeotic genes, which act on three overlapping domains of the meristem in order to determine the floral organ identity of four concentric whorls. Class A genes, *AP1* and *AP2*, control the identity of sepals and these genes together with the activity of class B genes, *AP3* and *PISTILLATA,* specify petals. The stamen is identified by the class B genes functioning together with the class C gene, *AGAMOUS* (*AG*). The function of *AG* alone specifies the identity of the carpel and the determinate development of a flower. In addition to these so called classical ABC genes, several other genes such as *AGL11* and *SEPALLATA1-3* have been found to be involved in floral organ development (e.g. Jack, 2001b; Lemmetyinen and Sopanen, 2004; Theissen 2001).

Identification of the genes that determine the inflorescence and floral meristem identities, and the characterization of the ABC function has revealed that, with a few exceptions, these genes are members of the family of MADS box transcription factors. Several of these gene products act as either hetero- or homodimers or even larger complexes (reviewed e.g. by Theissen et al., 2000). Several of the homologs of these genes that regulate either the identity of the meristem or floral organ development have been identified in various tree species including conifers. For a more detailed review, see Lemmetyinen and Sopanen (2004).

1.1.4. Birch flower development

In contrast to herbaceous annual species such as *Arabidopsis*, the juvenile phase before sexual reproduction can last several decades in trees (e.g. Peña et al., 2001). During this juvenile phase trees do not flower even in favorable environmental conditions. Trees typically show genetic variation in the length of the juvenile phase, and birch is a good example. Birch lines obtained through selection flower within months instead of the usual 5-10 years (Stern, 1961; Lemmetyinen et al., 1998). Birch is also a typical perennial, which means that reproductive development is restricted to only some parts of the plant, and so in favorable environmental conditions it can flower every year.

The structure of the birch flower differs significantly from that of the model plants. Birch flowers are typical for wind pollinated species and thus highly reduced in structure. Flowers develop on inflorescences ("catkins"), so flowering involves two transitions: first the vegetative meristem turns into an inflorescence meristem and then the inflorescence meristem eventually gives rise to floral meristems.

Birch has unisexual flowers that develop on separate male and female inflorescences which are on the same tree. The flowers develop in groups of three in the axil of three fused scales (Fig. 1). The male flower consists only of a simple perianth (tepals), which is thought to be a fusion of sepals and petals, and of two stamens. A study by Lemmetyinen (2003) suggests that tepals in male flowers are equivalents of sepals rather than petals. The female flower has only an ovary with two stigmata and has no signs of tepals (Fig. 1). Flowers of both sexes are supported by scales which are thought to be equivalents of cauline leaves. The key questions have been how the ABC function genes are regulated during birch flower development, and what regulates the development of the sex of the birch flowers.

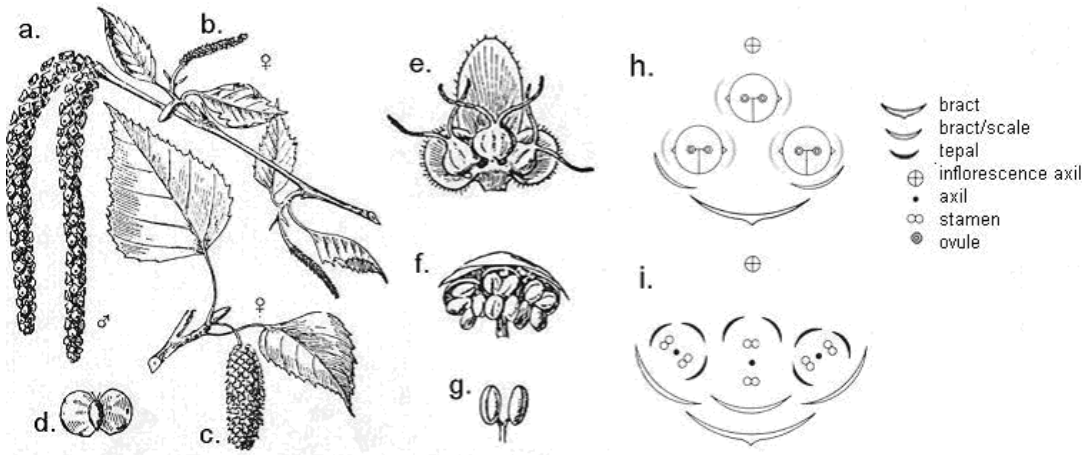

Figure 1. Structures of the birch female and male inflorescences and flowers: a. mature male inflorescence, b. young, erected female inflorescence, c. seed bearing female inflorescence, d. seed, e. female flower, f. male flower, g. stamen with two filaments, h. scheme of a female flower, i. scheme of a male flower. According to Strassburger et al., 1971, and Macdonald and Mothersill, 1987).

Birch flower development lasts about one year, proper environmental signals (over wintering) being crucial to the development. The male inflorescences start to develop in May, soon after the previous flowering, and emerge in June. The development of the female inflorescences starts in late July and they emerge in the following spring shortly before anthesis.

The focus in this study is on the early phases of birch inflorescence development and on the induction of inflorescene and floral meristems. The major questions are: how is the reproductive competence gained, and what are the major genes regulating inflorescence initiation? Genes that are expressed later in birch inflorescence and flower development have been studied in more detail by Lemmetyinen (2003) and Lännenpää (2005), for example.

1.1.5. Manipulation of the flowering in trees

The long generation time, the inability to regulate the flowering time, and even large size have been the major restrictions to breeding and molecular genetic research of trees. Suppression of the juvenile phase and accelerated flowering can, in some cases, be achieved by manipulating environmental conditions. For example, birch seedlings can be induced to flower in months instead of years by growing them in intense, continuous light and an elevated level of CO_2 (Lemmetyinen et al., 1998). Hormonal treatments could also be used to induce flowering (Meilan, 1997). However, these treatments are only effective for some species and other, more effective means are needed. The most promising results have been achieved by regulating the functioning of the genes that control flowering. The acceleration of the initiation of flowering in trees was first achieved by overexpressing *Arabidopsis LFY* in hybrid aspen. It flowered in only a few months in tissue culture and, in soil, it flowered after only six to seven months, instead of the several years that it usually takes (Weigel and Nilsson, 1995). However, the inflorescences of these plants were abnormal. The flowers were sterile and the roots were defected. Precocious flowering of poplar was achieved in later experiments by overexpressing endogenous poplar *PTLF* but the results were variable and inconsistent (Rottmann et al., 2000). A stronger acceleration of flowering was obtained by overexpressing a poplar homolog for *FLOWERING LOCUS T* (*FT*), *PtFT1*, in *Poplar*. In this experiment, transformed male poplars initiated flowerlike structures in 4 weeks instead of the normal 8 to 20 years (Böhlenius et al., 2006). In citrus, overexpression of either *AP1* or *LFY* resulted in production of fertile, normal flowers within one year, instead of the normal six to ten years

(Peña et al., 2001). This early flowering phenotype was inherited by the progeny and, interestingly, the transgenic plants maintained their annual flowering cycle.

One of the main aims of manipulating flowering in trees is to produce sterility by preventing the formation of the inflorescences, flowers or the sexual organs of the flowers (Lemmetyinen and Sopanen, 2004). The goals and the advantages of the possible sterility vary (e.g. Strauss et al., 1995). When cultivating genetically modified plants in the field, the spreading of transgenes is generally unaccepted and should be prevented. The most rational means for achieving this is to inhibit the flowering of the transgenic plants using biotechnical approaches. The prevention of flowering and the benefits of achieving it are studied and reviewed in detail by, for example, Lemmetyinen et al. (2001), Lemmetyinen et al. (2004), Lemmetyinen (2003), Lemmetyinen and Sopanen (2004) and Lännenpää (2005).

However, as concluded by Martín-Trillo and Martínez-Zapater (2002), the approaches to manipulating flowering are mostly trial and error experiments because a deeper understanding of the reproductive mechanisms is pending.

1.2. Plant mitochondria

1.2.1. The plant mitochondrial genome

The mitochondrial genome in higher plants displays unique properties and behavior but relatively little is understood about mitochondrial DNA maintenance and transmission. Despite their large size, plant mitochondrial genomes encode only a fraction of the information required for their biogenesis and function. In the *Arabidopsis* mitochondrial genome, 57 genes have been identified which encode the components of complexes I to V, cytochrome c biogenesis, rRNAs, ribosomal proteins, tRNAs, and a few open reading frames (Unseld et al., 1997). A distinct feature of plant mitochondrial genome organization is the prominent role of recombination. Recombinationally active, repeated sequences subdivide the genome into a number of different, highly redundant, subgenomic molecules so that the mtDNA is essentially heteroplasmic (Mackenzie and McIntosh, 1999; Fauron et al., 1995; Kmiec et al., 2006; Fig. 2). Ectopic (non-homologous) recombination also occurs, and there is evidence of DNA exchange occurring at sites sharing as little as 7 nucleotides of homology. These events can result in intragenic repeats, giving rise to novel open reading frames and expressive gene chimeras (Andre et al., 1992). This genomic plasticity distinguishes the plant mitochondrion from the mammalian and fungal ones.

DNA replication may be conducted by a rolling circle mechanism, and experimental difficulties identifying replication origins have led to the suggestion of recombination-mediated replication initiation (Backert and Börner, 2000). Neither the origins of DNA replication nor the components of the mitochondrial DNA replication apparatus have been well defined in plants or fungi. It is clear, however, that recombination is an important component of mitochondrial genome maintenance in these systems.

In most plants and animals, the transmission pattern is strictly maternal. Whilst the selective advantage of uniparental cytoplasmic inheritance is a subject of debate, it is generally agreed that the sharp reduction of mitochondrial numbers in the ovule/ovum during gametogenesis represents a significant population bottleneck. This bottleneck is a developmental window in which dramatic shifts in mitochondrial gene frequencies occur (Bergstrom and Pritchard, 1998). The plant mitochondrial genome is rendered unusually variable in structure by a phenomenon called substoichiometric shifting (Fig. 2). It was first reported in maize (Small et al., 1987) as the stable presence of subgenomic mitochondrial DNA molecules within the genome at near-undetectable levels. The process appears to be highly dynamic; mitochondrial genomic shifting involves rapid and dramatic changes in the relative copy number of portions of the mitochondrial genome within one generation (Janska et al., 1998).These substoichiometric forms have been estimated to occur at levels as low as one copy per 100-200 cells (Arrieta-Montiel et al., 2001). Generally, the rapid shifting process involves only a single subgenomic DNA molecule, which often contains recombination-derived chimeric sequences, and the process is apparently reversible (Janska et al., 1998; Kanazawa et al., 1994). Genomic shifting can alter the plant

phenotype because the process activates or silences mitochondrial sequences located on the shifted molecule. Observed phenotypic changes have included plant tissue culture properties (Kanazawa et al., 1994), leaf variegation and distortion (Sakamoto et al., 1996) and spontaneous reversion to fertility in cytoplasmic male sterile crop plants (Janska et al., 1998; Small et al., 1989). It has been postulated that substoichiometric shifting may have evolved to permit the species to create and retain mitochondrial genetic variation in a silenced but retrievable form (Small et al., 1989).

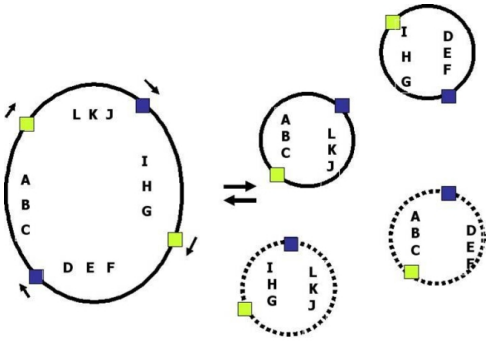

Figure 2. Scheme of mitochondrial DNA heteroplasmy and shifting. The mitochondrial genome is subdivided into a collection of molecules through intragenic recombination. Some of these subgenomic mtDNA molecules are present at near-undetectable levels (presented here with a dashed line) but their relative copy numbers can change rapidly and drastically in only one generation in a phenomenon called substoichiometric shifting.

1.2.2. Nuclear-mitochondrial interaction in plants

In plants, the relative copy number of recombination-derived subgenomic DNA molecules within mitochondria is controlled by nuclear genes and a genomic shifting process can result in suppression of their differential copy number to near-undetectable levels. Mitochondrial substoichiometric shifting has been shown to be under nuclear gene control in at least two cases, involving the Fr gene in Phaseolus vulgaris (Janska and Mackenzie, 1993) and the *CHM* gene in Arabidopsis (Martínez-Zapater et al., 1992; Redei, 1973). Amplification of a mitochondrial subgenomic DNA molecule that carries a chimeric gene sequence is associated with pollen sterility in the bean plant (Johns et al., 1992; Abad et al., 1995). This phenomenon is reversible (Janska et al., 1998). Subsequent studies showed that substoichiometric shifting of the plant mitochondrial genome is truly controlled by the nucleus. The nuclear gene controlling the subgenomic copy number was designated *Fr* (Janska et al., 1998). The genomic rearrangement effected by Fr involves copy number suppression of a subgenomic DNA molecule to low levels only detectable by polymerase chain reaction.

In *Arabidopsis*, a nuclear gene has been found to influence mitochondrial substoichiometric shifting in an apparently similar manner. Mutation of the nuclear *CHM* gene results in a green-white leaf variegation that, in subsequent generations, displays maternal inheritance (Martínez-Zapater et al., 1992; Fig 3). The gene was originally named the *chloroplast mutator* because it was first thought to cause plastid mutations it (Redei, 1973). However, it was shown later that the appearance of the variegation phenotype is accompanied by a specific rearrangement of mitochondrial DNA (Martínez-Zapater et al., 1992) that includes amplification of a specific DNA molecule encoding a chimeric sequence (Sakamoto et al., 1996). Genetic analysis suggests that the wild type form of *CHM* actively suppresses the copy number of the subgenomic molecule carrying the chimeric sequence. Loss of proper function of the *CHM* gene, characterized by two available EMS-derived mutant alleles *chm1-1*, *chm1-2* (Redei 1973) and a tissue culture-derived mutant allele, *chm1-3* (Martínez-Zapater et al., 1992; Fig 3), results in rapid and specific copy number amplification of the subgenomic molecule. This produces the observed leaf variegation. A recent study found a second *Arabidopsis* nuclear gene, *OSB1* (*Organellar Single-stranded DNA Binding Protein 1*), to control the stoichiometry of alternative mtDNA forms (Zaegel et al., 2006). An *AtOSB1* T-DNA insertion caused a variegated leaf phenotype and mtDNA alterations in a very similar manner to *CHM,* suggesting a closely related function between these genes (Zaegel et al., 2006). In both the bean and the

Arabidopsis systems, the identified nuclear genes appear to be involved in active copy number suppression of mutant mitochondrial DNA molecules.

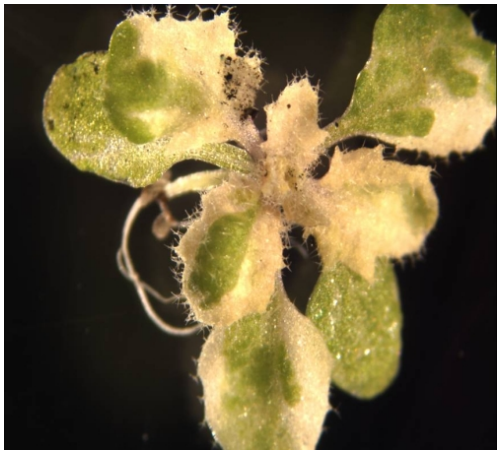

Figure 3. The variegated phenotype in *Arabidopsis* caused by the *chm1-3* mutant allele. The distorted leaf shape is associated with the phenotype (Picture by D.O. Gonzales).

1.2.3. Evolution of mitochondrial protein targeting

It has been proposed that mitchondria have an endosymbiotic origin from an ancestor common to the rickettsial subdivision of the *α-Proteobacteria* (Andersson et al., 1998; Gray et al., 1999; Emelyanov, 2001). Evidence suggests that during eukaryotic evolution, the proto-mitochondrial form relinquished much of its genetic complement to the nucleus by a continuing process of inter-organellar gene transfer. These events apparently occur via processed RNA intermediates that must acquire, following integration to the nuclear genome, a promoter and targeting presequence for nuclear functioning (reviewed by Brennicke et al., 1993; Martin and Herrmann, 1998; Adams et al., 1999; Palmer et al., 2000). The additional components are likely to be acquired by intergenic recombination (Brennicke et al., 1993; Kadowaki et al. 1996). Individual plant species would thus represent evolutionary intermediates in an ongoing process of gene transfer from the mitochondrion to the nucleus (Brennicke et al., 1993; Gray, 1995; Mackenzie and McIntosh, 1999).

The identification of nuclear genes that encode mitochondrial proteins has allowed more detailed investigation of their origins and likely evolutionary paths. Apparently, evolutionary processes have led to a complex network of interorganellar protein traffic that originates from nuclear genes encoding mitochondrial and plastid proteins. Although a relatively small number of genes have been studied in detail, several examples exist of proteins capable of dual targeting to mitochondrial and chloroplast compartments (Small et al. 1998; Peeters and Small, 2001; Christensen et al., 2005), and of inter-organellar gene substitution. In the latter case, nuclear genes of plastid origin are now found to encode for mitochondrial (Adams et al., 2002) or cytosolic (Krepinsky et al., 2001) proteins. Similarly, nuclear genes of mitochondrial origin can be found which encode cytosolic (Mireau et al., 1996) or plastid proteins (Gallois et al., 2001). Further, genes originating in plastids exist in the mitochondrial genome (Joyce and Gray, 1989). There is no defining amino acid sequence or pattern to distinguish mitochondrial and plastid targeting but the general properties have been reviewed by Peeters and Small (2001), for example. Plant mitochondrial targeting is conditioned by a fairly long (~40 to 60 amino acids) N-terminal peptide. It is usually rich in Arg and Ser, with a number of aliphatic residues such as Leu and Ala to form an amphiphilic α-helix. The chloroplast targeting peptide is generally long (averaging 58 amino acids), rich in Ser and Ala, and low in acidic amino acids.

1.2.4. The mitochondrial influence in plant reproductive development

Since developmental regulators could modulate basic metabolic processes, do plants also exploit metabolism for control of development (Skinner et al., 2001)? It is rational to think that the genes regulating flower development and mitochondrial genome maintenance are tightly co-regulated. This is because proper allocation of energetic and metabolic resources is crucial for the transition to the reproductive phase, for floral organ development and for production of male (pollen) and female gametes, and especially for the subsequent seed and fruit development. The activities of chloroplasts and mitochondria must be

coordinated and up regulated in order to accommodate the increased energetic and metabolic burden (Skinner et al., 2001). Indeed, both the amount of mitochondria and the levels of several nuclear encoded mitochondrial RNAs have been shown to increase in developing flowers (Huang et al., 1994, Mackenzie and McIntosh, 1999). Accordingly, reproductive development has been shown to be particularly sensitive to changes in mitochondria. Mutations affecting mitochondrial function have been reported to result in disruption or degeneration of ovules (Skinner et al., 2001; Landschutze et al., 1995), and disruptions in tapetum and pollen development leading to, for example, a CMS phenotype (reviewed e.g. in Mackenzie and McIntosh, 1999). There is evidence from several plant species that the development of sex and floral organs is mitochondrially controlled. For example, an *Arabidopsis* gene, *HUELLENLOS,* is essential for ovule development, and encodes an mt ribosomal protein (Skinner et al., 2002).

Cytoplasmic male sterility (CMS) is the best known example of a phenomenon in which floral organogenesis is influenced by nuclear-mitochondrial interaction. It has been described for about 150 plant species (Linke and Börner, 2005). This naturally occurring plant phenomenon has been widely exploited by plant breeders and applied to numerous cultivated species for decades (Mackenzie, 2005b). In addition to unviable pollen, abnormal development of floral organs is often observed in CMS plant lines, these phenotypes resembling those of *Arabidopsis* floral homeotic gene mutants (Leino et al., 2003; Linke et al., 2003; Murai et al., 2002; Linke and Börner, 2005). Actually, in several CMS plant lines, reduced levels of transcription of class B MADS genes was found, as reviewed by Linke and Börner (2005). An example of this are the floral homeotic genes *APETALA3* (*AP3*) and *PISTILLATA,* which were found to be down regulated in CMS lines of *Brassica napus* (Teixera et al., 2005). Mitochondria have been shown to directly influence the function of floral homeotic MADS box genes *GLOBOSA* and *DEFICIENS* (Linke et al., 2003) in CMS carrots. Overexpression of the floral homeotic gene *SUPERMAN* (*SUP*) restores the phenotypic abnormalities in CMS tobacco lines and restores fertility in alloplasmic male sterile tobacco lines (Bereterbide et al., 2002). On the other hand, aberrant mt originated proteins in CMS plant lines are expressed only in reproductive tissues (e.g. Sarria et al., 1998). Although the genomic control of mitochondrial processes during development is still mostly uncharacterized, these studies suggest that mitochondrial defects in CMS plants alter the expression of nuclear transcription factors. These in turn activate or repress the transcription of genes specifying organ identity during flower development (Linke and Börner, 2005).

2. AIMS OF THE STUDY

Since the long generation times severely hinder tree breeding and research of flowering genes in trees, the acceleration of flower initiation would be extremely useful. In the first part of this study, the main focus has been on the genes behind the initiation of flowering and flower development in silver birch. The aim is to find practical applications for accelerating the initiation of flowering. Another part of this research concentrates on cellular processes unique to plants. The mitochondrion has numerous roles in many processes of the life and development of plants. However, the regulatory pathways behind mitochondrial activity and regulation during development are still mostly uncharacterized. The specific aims of this study have therefore been:

1. Characterizing the birch homologs of *LEAFY*, *AP1* and *FULL* genes known to control floral initiation and the early phases of flower development in other species. The aim was to compare their similarities and functional homology to previously characterized, related genes from other plant species.

2. In depth study of the function of *BpMADS4*, *BpFULL* and *BpFLO* and testing their suitability in manipulating, accelerating and inhibiting, flowering in birch and other species.

3. Characterizing the mitochondrial genome instability and nuclear-mitochondrial interaction, and to study how the nuclear gene locus *CHM* controls mt genome structure in *Arabidopsis*. A further aim was to specify the

gene locus behind the *chm* mutant phenotype in *Arabidopsis*.

4. Characterizing the putative genes involved in mitochondrial DNA maintenance and evolution and to study the integration and evolution of the mitochondrially originated sequences in the plant genome specifically focusing on the evolution and acquirement of targeting presequences of mitochondrial proteins.

3. MATERIALS AND METHODS

The detailed descriptions of the materials and techniques used in this study can be found in the original publications I-IV. This chapter gives a brief summary.

3.1. The plant material and transformations

The plant material used in the expression analysis and in the construction of the libraries was collected from the various tissues of adult wild silver birch trees (*Betula pendula* Roth., I, II) and *Arabidopsis thaliana* (Col-0, III)

Gene transfer to tobacco (*Nicotiana tabacum* L. cv. Petit Havana SR1) was performed as described in (I). The birch transformations were performed in selected, genetically early flowering birch clones, BPM2 and BPM5 (Lemmetyinen et al. 1998), as well as in two normally flowering birch clones (JR1/4 and K1898, II). The birches were transformed using the *Agrobacterium*-mediated gene transfer method as described previously (Keinonen 1999).

The *Arabidopsis* F2 mapping population in paper III was derived from a cross between the *chm1-1* mutant line in a Col-0 background (pollen donor) and the Landsberg erecta ecotype. A segregating sub-population of 172 variegated plants was analyzed (III). The seeds for the *Arabidopsis* ecotypes (Dijon-G, Aa-O, En-T, Hodja, Shahdara, Cvi-O and WI-O) were obtained from the Arabidopsis Biological Resource Center (Ohio State University, OH). These were used to analyse *chm1-3* induced mitochondrial rearrangements in different genetic backgrounds. The seed stock for the mutant line *chm1-3* was obtained from the laboratory of Chris Somerville (Carnegie Institute, Palo Alto, CA, Martinéz-Zapater et al., 1992). All the *Arabidopsis* plants were grown in soil at 20°C in a growth chamber with 16 hr light/8 hr dark. The variegation and other possible visible phenotypic traits, as well as the mt genotypes of the progenies of the crosses were followed up to five generations. The two sequence-indexed T-DNA insertion mutants were identified at SiGnAL database (Salk Institute Genomic Analysis Laboratory). Seeds for these as well as for the mutant lines *chm1-1* and *chm1-2* were obtained from the Arabidopsis Biological Resource Center (ABRC) (III). Transient transformation experiments were performed on 4 week old leaves of *Arabidopsis thaliana* (Col-0) using the Biolistic PDS-1000/He system (Bio-Rad) as described (III, IV).

3.2 Isolation of cDNAs, sequencing and DNA analysis

The cDNA clones corresponding to *BpMADS3* and *5* were isolated from the library which was prepared from almost mature female catkins. The cDNA clone of *BpMADS4* was obtained from a library made from young female inflorescences (I). A cDNA corresponding to *BpFLO* was isolated from a library which was constructed using poly(A)+RNA isolated from both young female catkins (about 2-4 mm in length) and from early developing male catkins (about 3-10 mm in length). A heterologous probe consisting of a 950 bp fragment of *Antirrhinum FLO* cDNA was used as a probe in library screenings. Two identical cDNA clones were obtained by screening 150 000 λ clones from the ZAPII library. A birch genomic λ ZAPII library was screened by using this cDNA as a probe and two clones containing the 5' and 3' ends of the *BpFLO* gene were isolated.

DNA sequencing of the candidate loci in chm1-1, chm1-2 and chm1-3 mutants was conducted as described in (III). PCR analysis of the mitochondrial shifting in *Arabidopsis* was done using total genomic DNA and the three primer assay described in Sakamoto et al. (1996) and in paper III. The total *Arabidopsis* DNA for the PCR and Southern blot analysis was extracted using the method of Li and Chory (1998). For the southern blot hybridization, the *Arabidopsis* total genomic DNA was digested with BamHI, separated and

blotted in the conditions recommended by the membrane manufacturer Amersham Corp. The 649 nt probe was generated using PCR and was selected to span the mitochondrial genes *atp9* and *rpl16* (Sakamoto et al 1996).

3.3. Gene constructs

The *sense* constructs of *BpMADS3, 4* and *5* were used in transformations as described (I). An antisense gene construct *of BpMADS4*, was constructed by inserting a full-length cDNA of *BpMADS4* under the control of the CaMV 35S promoter in reverse orientation (II). Sense and antisense transformation constructs for *BpFLO* were done similarly (unpublished).

PCR derived fragments of *AtMSH1* were ligated to the pCAMBIA 1302 vector at the start of gfp under the control of the *CaMV 35S* promoter (III). The PCR amplified targeting presequence fragments of *AtPOLγ1* and *AtPOLγ2* were ligated to the pCAMBIA vector as described (IV).

3.4 Computer analysis

Nucleic and amino acid sequences in I were analyzed using the GCG program package release 10.0 (Genetics Computer Group, Inc, Madison, WI, USA) and the distance calculations were performed as described (I). Phylogenetic and molecular evolutionary analyses of *BpFLO, BpMADS3, BpMADS4* and *BpMADS5* were conducted using MEGA version 3.1 (Kumar et al 2004). Homologous sequences from other plant species were obtained through BLASTX and BLASTP searches in the GeneBank database. The database search resulted in numerous (>100) homologous sequences and only a limited amount of sequences were taken for further phylogenetic analysis. The sequences selected for further analysis were either ones from other tree species or the most similar or ones that are well known and documented. The sequences were aligned using the CLUSTAL W program and these alignments were visually refined.

The strategy for the database search for putative mitochondrial targeting sequences from the *Arabidopsis* and rice genomes is described in the original paper IV. Transit peptide predictions were performed using the available prediction programs: Predotar version 0.5 (Mall et al., 2004), TargetP (Emanuelsson et al 2000) and MitoProt (Claros and Vincens 1996). The phylogenetic analysis of targeting presequences was conducted using the PHYLIP software package (Felsenstein, 2002, IV).

3.5. Microscopy

The plant tissues were fixed and *in situ* hybridizations in II were performed as described previously (Lemmetyinen et al. 2004a). Localization of GFP expression was conducted by confocal laser scanning microscopy with Bio-Rad 1024 MRC-ES using 488 nm excitation and a two-channel measurement of emission; 522 nm (green/GFP) and 680 nm (red/chlorophyll) as described (III, IV).

4. RESULTS

4.1. Analysis of the genes regulating birch flower development

4.1.1. Isolation of *BpMADS3*, *BpMADS4* and *BpMADS5* (I)

The isolation of MADS box genes was the most feasible choice for studying genetic regulation of birch flower development for several reasons. At the time, the vast majority of already characterized genes regulating flower development in the model plants *Arabidopsis* and *Antirrhinum*, were members of the MADS family. Members of the MADS gene family share a high homology in their conserved elements or boxes. This facilitated their isolation even from genetically distant species. It was possible to exploit this conservation to design degenerate primer sets for RT-PCR amplification. These amplified and sequenced fragments were used for screening cDNA libraries in order to isolate corresponding full-length cDNA clones. The genes corresponding to isolated cDNA fragments were named *BpMADS3*, *BpMADS4* and *BpMADS5* (*Betula pendula* MADS, I). Further studies gave reason to rename *BpMADS5* on the bases of its homology and function, and it is now called *BpFULL1* (Lännenpää et al., 2005). The relationships of these isolated genes were established by

conducting a phylogenetic analysis (I). On the basis of the sequence similarity, all of these genes fell in to the same MADS box subfamily with *Arabidopsis* genes such as *APETALA1* (*AP1*), *CAULIFLOWER* (*CAL*) and *FUL*. *BpMADS4* and *5* were more homologous to each other than was *BpMADS3*. *BpMADS4* and *5* shared homology with *FUL* whilst the latter gene grouped together with *AP1* and *CAL1* (I).

4.1.2. Isolation and characterization of *BpFLO* (unpublished results)

A cDNA clone corresponding to the *BpFLO* gene was isolated using a heterologous probe consisting of a 950 bp fragment of *Antirrhinum FLO* cDNA. This probe was used for screening a cDNA library constructed using RNA which was isolated from both young female catkins and from early developing male catkins. Two identical cDNA clones were obtained containing the 5' and the 3' ends of the coding sequence. The length of cDNA was 1406 bp, including a 1209 bp open reading frame which codes for a 405 aa protein that has an mw of 45107,97. The database search found a group of highly similar genes from various plant species including *Arabidopsis* (*LEAFY,* Weigel et al. 1992*)*, *Antirrhinum majus* (*FLORICAULA,* Coen et al. 1990), *Pisum sativum* (*UNIFOLIATA*, Hofer et al. 1997), and *Populus* (Rottmann et al., 2000). Thus the gene corresponding to the isolated cDNA was designated *BpFLO* (*Betula pendula FLO*). Overall, BpFLO was identical with homologous gene products from other species to a degree of 70.1 % to 89.0 %. The phylogenetic tree of selected homologous sequences obtained from the database was made using the Neighbor-Joining method (Fig 4). All the putative LEAFY/FLO -like proteins from different species are quite well conserved and the topology of the tree seems to merely reflect the phylogenetic distances between the species.

The southern blot analysis indicates that *BpFLO* exists as a single copy in the birch genome. The intron positions of the *BpFLO* gene were verified using PCR amplification. The *BpFLO* gene contains two introns: 440 nt and 1360 nt in length. The number and positions of the introns seems to be strictly conserved between *BpFLO, FLO* and *LFY,* judging by database comparisons of the published sequences.

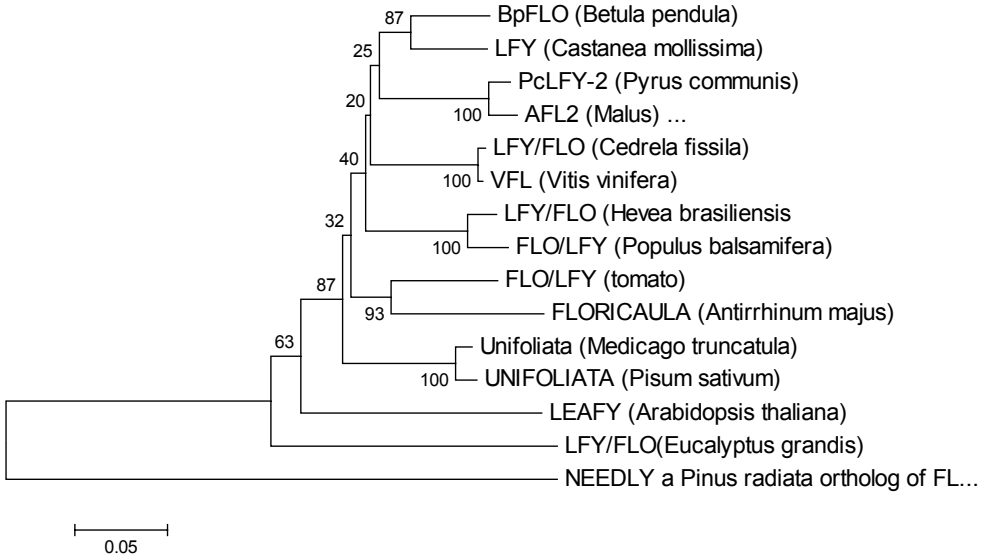

Figure 4. Neighbor-Joining analysis of the putative proteins similar to LEAFY and FLO together with BpFLO. The numbers indicate the bootstrap values for each node. NEEDLY, a monocot orthologue was used as an outgroup

4.1.3. Expression of BpMADS3, BpMADS4, BpFULL1 and BpFLO (I, II, unpublished)

The first expression analyses were carried out using an RNA gel blot analysis. Transcripts corresponding to *BpMADS3, 4* and *BpFULL1* were detected in the birch inflorescences from the earliest developmental phases and throughout inflorescence development, on to seed maturation (I). From these genes, *BpFULL1* seems to be the only one with flower specific expression. *BpMADS3* is expressed in the vegetative shoot tips and *BpMADS4* is expressed in all the vegetative tissues tested, including the roots (I). Expression of *BpMADS3* started later than that of the others in developing male inflorescences. It was concluded that *BpMADS4* would have the highest level of expression seeing as the same exposure conditions and the same RNA blot were used with all the hybridizations. However, neither the exact concentrations nor the T_m of the probes were tested. The *in situ* hybridizations showed a high level of expression of *BpMADS4* already at the earliest stage of when an inflorescence meristem could be detected. The expression pattern was similar in both types of inflorescences (II). In the female inflorescences, the expression continued past flowering and was concentrated in the ovule during seed maturation, whereas in the mature male flowers, the expression was weak and diffuse.

According to the RNA gel blot analysis, *BpFLO* has a low level of expression in developing male inflorescences (Fig. 5). In female inflorescences, levels of expression are higher whereas the highest level of expression is detected in mature female inflorescences. Transcripts are also seen in female inflorescences during dormancy (Fig 5). RT-PCR revealed that *BpFLO* is expressed in all the vegetative tissues excluding the roots. The size of the transcript, as aproximated on RNA gel, is about 1600 nt which is in the same range as the transcript sizes of *LFY* and *FLO* (Anthony et al., 1993).

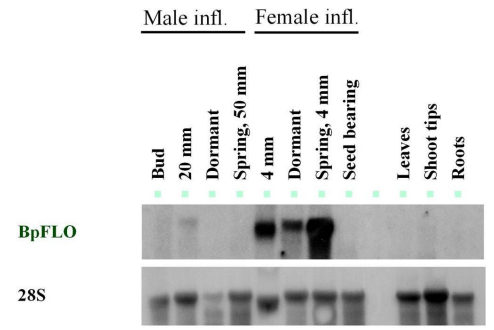

Figure 5. Northern blot analysis of *BpFLO* expression in different tissues of birch. The lower line represents the hybridisation with a 28S ribosomal RNA probe as a loading control

4.1.4. Modification of the expression of the birch genes (I, II, unpublished)

More light on the function of these genes was shed by experiments in which these genes were either over-expressed or suppressed in various plants. In order to form a preliminary sketch of the functions *of BpMADS3, BpMADS4, BpFULL1* and *BpFLO,* they were ectopically expressed in *Arabidopsis*, tobacco and birch plants under the control of the CaMV 35S promoter (I, II, unpublished). The expression of *BpMADS3* in *Arabidopsis* (unpublished data) and tobacco resulted in early flowering. The inflorescence stem of the *Arabidopsis* plants which expressed *BpMADS3* grew in an abnormal manner. The main inflorescence axis branched instead of forming secondary shoots, which would be easily identified by the supporting cauline leaves (fig. 6). In *Arabidopsis,* the growth of the plants terminated with the production of a terminal flower. Tobacco plants expressing *BpMADS3* flowered early and vigorously and remained very small, apparently producing inflorescences from every axillary bud (I). The research on the effects of the expression or suppression of this gene in birch is continuing.

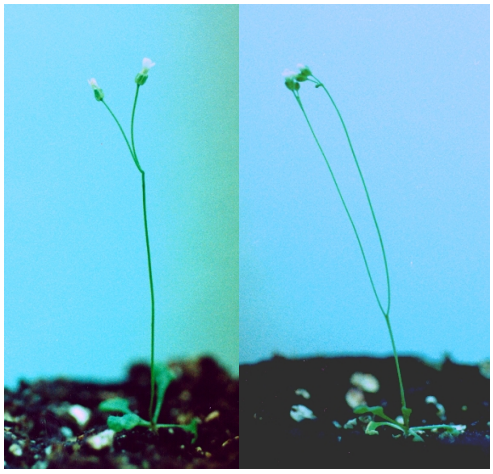

Figure 6. Transgenic Arabidopsis plants expressing *35S::BpMADS3*. Plants were early flowering and showed an abnormal branching pattern, and the growth ended with the production of terminal flowers.

The overexpression of *BpMADS4* resulted in early flowering in all the species tested. The *Arabidopsis* plants expressing *BpMADS4* developed a few flowers after forming a few rosette leaves and died soon after the emergence of the flowers. The whole tobacco plants expressing *BpMADS4* resembled an inflorescence of the WT plant. It seemed that these transgenic plants omitted the vegetative growth phase altogether (I).

The early flowering BPM2 birch lines transformed with *35S::BpMADS4* were predominantly early flowering. In the earliest line (35S::BpMADS4_1), the first inflorescences emerged in only 11 days after planting. The plants grew on peat and were 2-3 cm high at the time of flowering. In all the nine non-transgenic control plants, the inflorescences emerged 85 to 107 days after planting. In the earliest flowering lines, all the emerged flowers were female ones, whereas normally an overwintering treatment is needed in order for female flowers to emerge. The earliest flowering plants died soon after the emergence of the inflorescences. This was obviously due to their incapability of vegetative growth. All the early flowering lines showed a degree of phenotypic abnormalities, for example changes in their vegetative growth pattern, which was typically branched and bushy with either deeply lobed or curled leaves. A typical leaf phenotype is shown in fig. 7. Some individual plants in these overexpression lines did not show any signs of flowering at all during the course of the two years our study. Other plants produced abnormal inflorescences later (after more than 100 days). These consisted only of scales, having no normal floral structures. These phenotypes were likely to be due to co-suppression of a high expressing transgene, as we observed similar phenotypes in some antisense lines as well (see below).

Figure 7. Typical leaf shape in *35S::BpMADS4* birch lines. A secondary shoot of the line F1 3L ends in an inflorescence and the leaves have typical single lobed edges (Picture by A. Novak).

In addition to the early-flowering birch, the same construct was transferred into two normally flowering, wild type birch clones (JR1/4 and K1898). The results prove that the overexpression of *BpMADS4* is capable of inducing early flowering in normally flowering genotypes of birch (II) also.

The *BpMADS4-antisense* (*as*) construct delayed or even completely inhibited flowering in several lines when transferred into the early flowering birch clone, BPM2 (II). The flowering *BpMADS4*-antisense lines produced apparently normal male inflorescences in most cases, but in two lines, abnormal inflorescences developed with leaves instead of flowers (Fig 8). Unlike the *BpMADS4*-sense construct, the *BpMADS4*-antisense construct did not seem to have major effects on vegetative growth. Only in two lines, *35S::BpMADS4as_2* (non-flowering) and *35S::BpMADS4as* 6 (partially flowering), plants were smaller with thick leaves and short nodes on the shoots (II).

Figure 8. An abnormal inflorescence in the *35S::BpMADS4-as* birch line. Leaves developed instead of flowers (Picture by J. Lemmetyinen).

Similarly to *BpMADS3* and *BpMADS4,* the overexpression of *BPFULL1* (*BpMADS5*) resulted in early flowering in *Arabidopsis* and tobacco but without other apparent phenotypic changes (I). In the early flowering birch line BPM5, however, the effect on flowering time was not as clear. Some lines flowered slightly earlier and in a smaller size than the controls but others showed even delay in flowering time (Table 1, unpublished). The clearest phenotypic changes were seen in the structures of the inflorescences and flowers (Fig. 9 A-G). Sometimes the inflorescence axis was elongated as seen in Fig. 9A. In some inflorescences, the base supporting the bracts and the tepals of the flowers was similarly elongated (Fig. 9B). In lines showing strong phenotypes, the inflorescences were branched (Figs. 9 C-E). In one line, branches of new inflorescences developed instead of the flowers resulting in a rather striking, almost Christmas tree-shaped inflorescence (Figs. 9 F-G). A more detailed analysis of the abnormal looking inflorescences and flowers showed that numerous extra male organs developed either on the inflorescence or outside the bracts (Fig. 9H). Alternatively, the individual flowers developed numerous abnormal extra stamens instead of the two normal ones (Fig. 9I).

Two lines of plants expressing the *BpFULL1* antisense, both of which were in a BPM5 background, were more slender with shorter secondary shoots than the controls (Fig. 10). Several lines showed abnormally sized and shaped leaves. Generally, however, phenotypic effects were quite rare and unnoticeable. Effects in flowering time or in inflorescence or flower structures were not seen in comparison with controls. The average flowering times are presented in Table 1.

Table 1. The effects of *BpFULL1*-sense and -antisense constructs on inflorescence emergence in the early-flowering birch clone, BPM5.

Construct	Line	Emergence of the first inflorescence[a]	Number of individuals	Plant size (cm) [b]
AKAT6 (sense)	*C3*	81-106	9	41,5-111
	F6	85-113	8	42,5-102,5
AKAT5 (antisense)	*H1*	81-113	7	40,5-93
	D1	85-131	12	43,5-123
Non-transformed control		92-102	5	40,0-94,0

[a] indicates time in days, from the transfer of the plants onto soil to the emergence of the first inflorescences
[b] size of the plants at the time of initiation of the first inflorescence

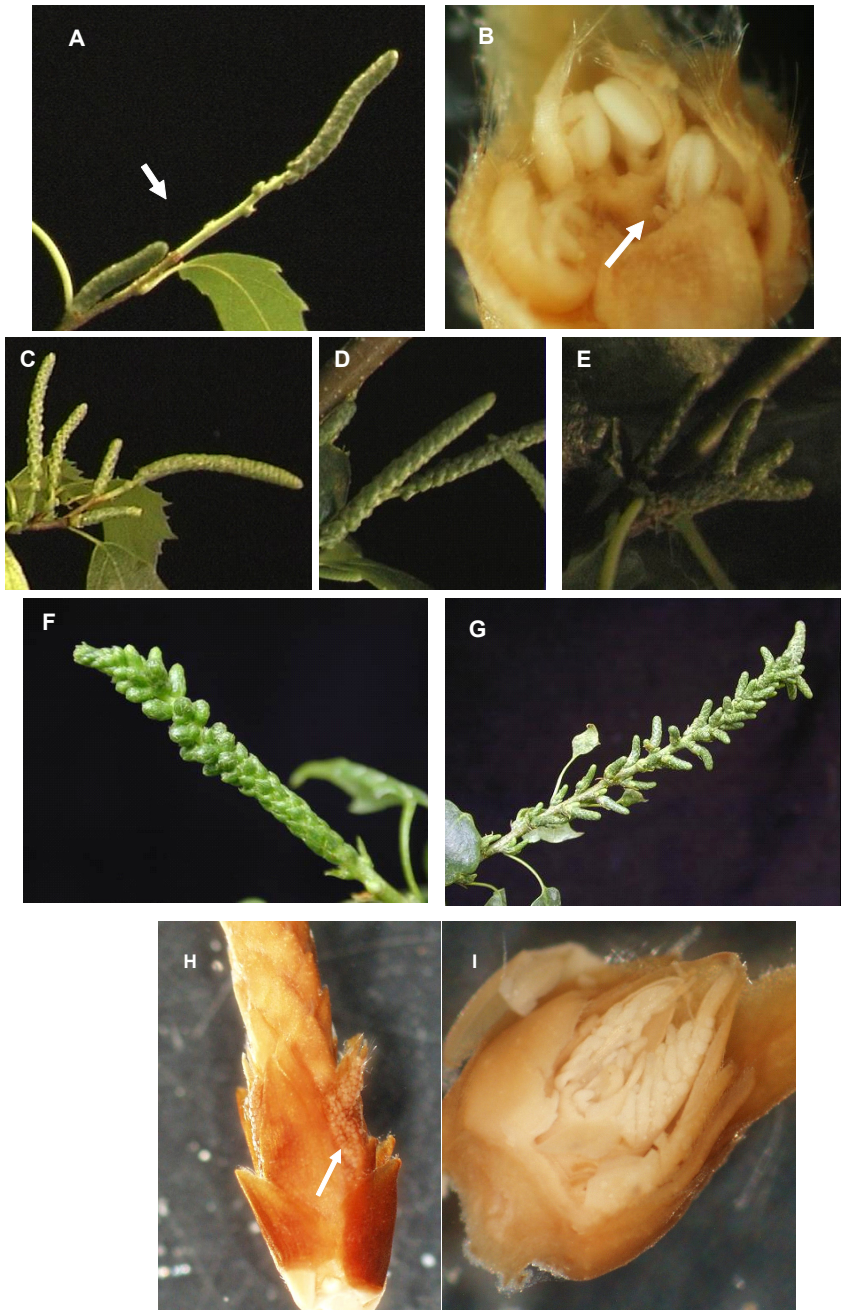

Figure 9. Effects of the ectopic expression of *BpFULL1* in the early flowering birch line BPM5. (A) An abnormally elongated inflorescence axil. (B) An elongated floral axil (base) in a male flower. (C-E) Inflorescences showing various branching patterns. (F-G) An inflorescence with new inflorescences developing instead of flowers. (H) Extra male organs developing on the outer surface of the bract. (I) An individual flower with numerous extra stamens instead of the normal two.

Figure 10. Effect of the *35S::BpFULL1-as* on the growth pattern of birch. On the right, the *BpFULL1-as* line *H1*, with the typical short side shoots. On the left, the control, untransformed BPM5 birch clone (Picture by A. Novak)

Arabidopsis ecotype Wassilevskaja plants expressing *35S::BpFLO* (construct AKAT3) were early flowering compared to controls. The phenotypes varied from severe to mild as follows. Plants with severe phenotypes produced very few (2-4) rosette leaves and were extremely small sized (data not shown). Several primary transformants also showed lowered viability and died without flowering after development of a few cauline leaves. Despite the severe effects in *Arabidopsis*, the tobacco plants transformed with the *35S::BpFLO* did not show any visible phenotypic changes in any of the 16 independent transgenic lines grown. The proper expression of the transgene in these lines was verified by RNA blotting and hybridization.

The effects of *35S::BpFLO* on flowering time in birch seemed to depend on the genetic background used in the transformation. In clone BPM2, the average flowering time was reduced compared to controls (table 2a), although it was delayed in two lines. This was possibly due to a co-suppression effect. In this clone, the overexpression of *BpFLO* seemed to affect vegetative growth also, since the plant height in the transgenic lines was reduced by almost half compared to the controls (table 2a). Viability of the plants was also reduced with several plants dying before production of inflorescences. In the second clone, BPM5, the average flowering time was delayed from 113 days to 182.5 days in lines expressing *35S::BpFLO* (table 2b). The transgenic plants in this clone also grew somewhat higher than the controls did.

Only a few phenotypic changes were observed in the lines overexpressing *35S::BpFLO*. Most of the transformed plants of clone BPM2 were branched, bushy and small in size. The occasional large sized and abnormally shaped leaf was also seen. Only a few cases of phenotypic changes in inflorescences or flowers were observed. A few inflorescences developed in a bunch or their shape was abnormally rough or uneven (Fig 11a-b). Flowers in the abnormal looking inflorescences had an increased number of scales and tepals which were irregularly organized (Fig. 11c).

The results from the birch lines expressing antisense *BpFLO* are only indicative. Only two transgenic lines, both in a BPM5 background, were obtained and grown in this experiment. Overall, the visible effects were minor, and the flowering time, for example did not seem to be affected. Some individual plants had abnormally shaped and sized leaves and several plants had short secondary shoots. These phenotypes looked very similar to the ones that overexpress *BpFULL1*.

Table 2a. The effects of *BpFLO*-sense constructs (AKAT3) on inflorescence emergence in the early-flowering birch clone, BPM2.

Line	Nr of individuals	Emergence of the first inflorescences[a]	Aver.	Plant size (cm)[b]	Average size (cm)
1L D1	2	212-245	228,5	47-76	61,5
1L P1	6	135-212	173,5	32-49	36,2
3L N1	5	212-285	226,6	42-72	58
4HS F1	5	135-212	173,5	63-99	75,4
4HS K1	6	285-491	421	95-142	111,8
2L J1	3	135-491	279,3	38-83	56
3L K2	4	334-491	425	66-148	120,8
2L L1	3	212	212	27-40	35,6
1L O1	2	135-446	290,5	39-51	45
2L G1	4	135-342	154,3	44-61	53,25
1L F1	1	342	342	132	132
1L Y1	2	212-342	277	46-50	48
2L O1	1	135	135	28	28
3L D1	2	212-254	233	48-83	65,5
2L Y1	5	212-496	306,2	60-90	77,6
3L C2	3	135	135	45-64	53
Average			**254,6**		**68,0**
Control BPM2	8	245-491	338	101-167	122,5

[a] indicates time in days, from the transfer of the plants onto soil to the emergence of the first inflorescences
[b] size of the plants at the time of initiation of the first inflorescences

Table 2b. The effects of *BpFLO*-sense constructs (AKAT3) on inflorescence emergence in the early-flowering birch clone, BPM5.

Line	Nr of individuals	Emergence of the first inflorescences[a]	Aver.	Plant size (cm)[b]	Average size (cm)
G2	6	146-202	186,3	63-146	119,7
G1	9	122-390	269,1	90-172	132,6
L1	6	167-202	172,8	90-104	98,7
C6	4	167	167	85-123	111
C7	6	146-220	174,7	112-157	132
C8	6	107-220	158,8	80-146	118,7
F2	6	107-202	162,8	64-139	112,5
D1	6	146-202	169,3	86-126	111,2
M1	5	146-220	165	31-55	43,5
L1	5	167-220	205,8	113-180	152,8
H3	6	76-167	107,5	39-180	66,8
J1	6	146-297	202,2	104-139	125,3
Average			**182,45**		**111,7**
Control BPM5	10	69-167	113,1	36-121	75,3

[a] indicates time in days, from the transfer of the plants onto soil to the emergence of the first inflorescences
[b] size of the plants at the time of initiation of the first inflorescences

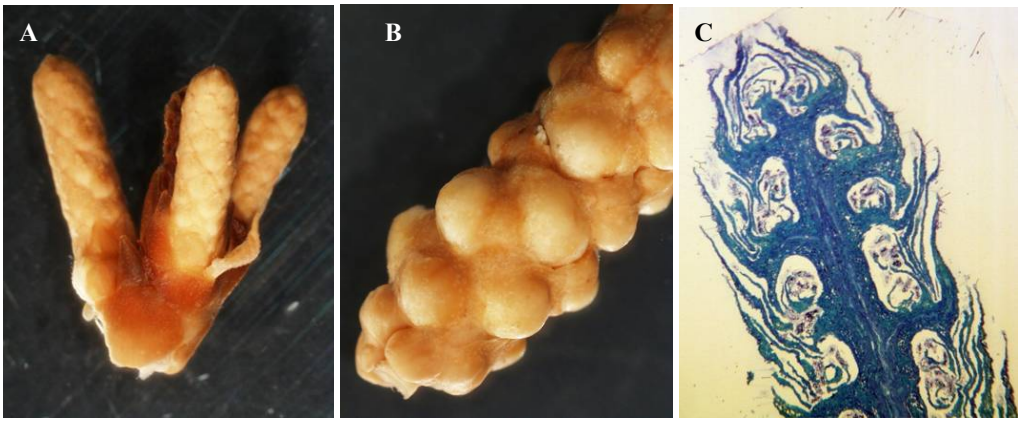

Figure 11. The phenotypes caused by the ectopic expression of *BpFLO* in birch line BPM2. (A) A bunch of inflorescences. (B) A "rough" inflorescence, typical for this line. (C) A section image of a "rough" inflorescence, flowers have an abnormal number of scales and tepals (section image by H. Mäkelä).

4.2. Introduction of mitochondrial substoichiometric shifting into different genetic backgrounds (partially unpublished)

A Chm1-3 nuclear mutation was introduced into seven different ecotypes of *Arabidopsis* by crossing. The aim of this study was to determine how mitochondrial DNA shifting behaves in different genetic backgrounds over the course of five generations. The visible phenotypes of the subsequent generations were followed by Neemann (2002) and the variegated phenotype appeared in the F_2 populations in all seven crosses with different ecotypes as expected. Usually the degree of variegation was low and was restricted to a few white or yellow stripes on leaves. PCR testing revealed that the variegation was associated with the introduction of a *chm1-3* mutation in these lines (Neemann, 2002). Usually the degree of variegation was more severe in subsequent generations with all the ecotypes; even albino plants (a non-vital phenotype) appeared. Distorted leaf genotypes (wrinkled, curved leaves) were commonly seen in variegated plants as described earlier by Sakamoto et al. (1996) as well as a reduction in plant size (Neemann, 2002). In addition, several individual plants of subsequent generations in all the ecotypic lines displayed floral abnormalities. Most commonly, the stamens but often also the carpels, were completely missing in the affected flowers. This indicates that the proper functioning of mitochondria is needed for flower development. This floral effect seems to be dependent on a nuclear or cytoplasmic background since the flowers in *chm* mutant lines with a Col-0 background seem to be unaffected, with the exception that the flowers developing in completely white sectors do not produce pollen.

The mitochondrial shifting associated with variegation and supposedly induced by *chm1-3* was studied using a PCR-based diagnostic assay previously described by Sakamoto et al. (1996). However, the test which was informative with the Columbia ecotype proved to be inadequate in evaluating changes in mitochondrial genomes with the other ecotypes. Consequently, a mitochondrial genome configuration was tested using DNA gel blot analysis with a mitochondrial LMB56 fragment as a probe (Sakamoto et al., 1996). Results prove that mitochondrial shifting was evident in all ecotypes and it showed up as novel bands in DNA blot assays. In some individuals, additional new bands appeared, which were apparently of mitochondrial origin. Nevertheless, these bands were not seen in all of the individuals as one might expect as a result of the maternal origin of the mitochondrial genome. The novel bands that appeared in the F_5 progeny Aa-O are presented

in Fig 12. In F_5 progenies of Aa-O, Dijon-G, Hodja and Shahdara, the *chm1-3* induced, additional mitochondrial bands seem to be lost or reverted back to the wild type, although all of these plants had a variegated phenotype. Some of the faint bands seen in DNA gel blots are very likely to result from a hybridization of the nuclear copy of the mitochondrial genome, since the *Arabidopsis* chromosome II contains two copies of the mitochondrial genome (Stupar et al., 2001) and the total DNA was used in this study. Usually the nuclear derived fragments show weaker hybridization than mitochondrial ones due to much lower copy numbers. Also, their polymorphism should remain the same in subsequent generations. These preliminary results indicate that either the cytoplasmic or the nuclear background has an effect on either DNA recombination or on mismatch repair of the mitochondrial genome. Further, the mitochondrial genomes might in some cases revert or shift back to a wt like conformation.

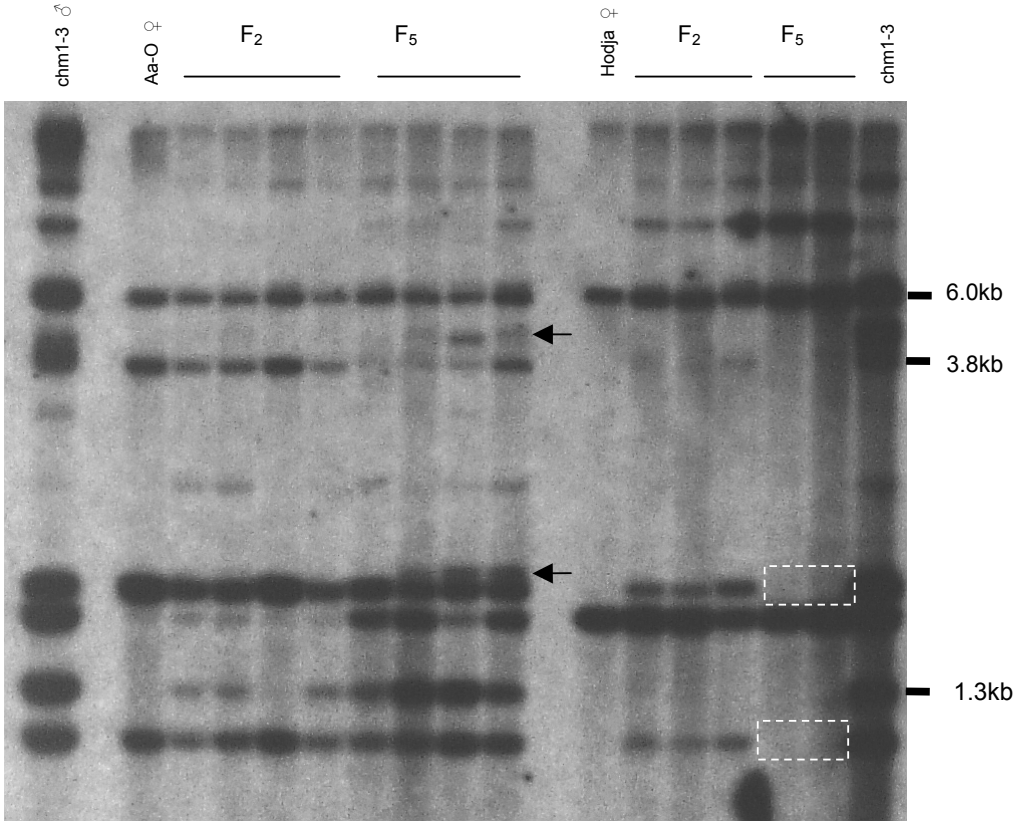

Figure 12. An example of mitochondrial genome shifting caused by the introduction of *chm1-3* nuclear mutation in Arabidopsis wt ecotypes Aa-O and Hodja. A Southern analysis was conducted using *Arabidopsis* total DNA and a mitochondrially derived LMB56-fragment (Sakamoto et al., 1996) as a probe. Additional bands appearing in later generations are marked with arrows, and "disappearing" or reverted bands are marked with dashed boxes. All the plants used in this array showed a variegated phenotype.

4.3. Mapping of *chm* (III)

The *CHM* locus in which a recessive mutation produces a variegated phenotype in *Arabidopsis* was originally located on chromosome III, in a 5-Mb region between loci *glabra* (*gl1*) and *long hypocotyl2* (Redei, 1973; Martínez-Zapater et al., 1992). The PCR-based codominant markers (Jander et al., 2002) from the Cereon Arabidopsis Collection were developed to span this region (III). Since mutation line *chm1-1,* which was used to produce the mapping population, was in a Col-0 background, these markers were designed to distinguish between Col-0 and Landsberg erecta phenotypes. The *Glabra* (*gl1*) locus was used as a visible marker in a cross. Using the mutant line *chm1-1* as a pollen donor and Landsberg erecta as the maternal line, an F_2 population of approximately 1000 individuals was obtained. Of these, a segregating subpopulation of 172 variegated plants was analyzed. On the basis of evidence from recombinants, it was possible to delimit the CHM locus to an 80-kb interval on *Arabidopsis* chromosome III. A single bacterial artificial clone (K7M2) contained this interval and a gene candidate was identified based on predicted mitochondrial target features. The candidate CHM locus contained 22 exons with two MuTS like conserved elements. The transcript derived from this gene was 3.5 kb and encoded a protein with a length of 1,118 aa, including the predicted 43-aa mitochondrial presequence (III). This region was sequenced from all of the known mutant alleles, *chm1-1*, *chm1-2* and *chm1-3* (Martínez-Zapater et al., 1992) which were all found to contain mutations in the coding region of the candidate locus (III). To finally prove that the identified region was the *CHM* two *Arabidopsis* lines (Col-0 ecotype), T-DNA insertions on the candidate gene were performed (III). The phenotypes of these T-DNA lines were identical to those observed in mutant lines, having green-white leaf variegation. In addition, a three primer PCR assay as well as a southern blot expreriment revealed a mitochondrial substoichiometric shifting in all the lines homozygous for T-DNA insertion (III).

The candidate gene had several characteristics for a mismatch repair component (III). To confirm the mitochondrial targeting, the 5' end of the transcript was amplified by RACE-PCR. This confirmed a new start site for the transcript start site, 578 residues upstream of those previously annotated in databases (MIPS and Gene Bank). This added 102 aa to the predicted protein (III). A mitochondrial targeting presequence was identified from this site and consequently a transgene construction was developed to confirm the localization of the protein. A confocal microscopy analysis revealed the mitochondrial localization but the possible dual localization to include plastids could not be confirmed in this experiment. EST database searches within various plant families identified several plant sequences with high (>60%) sequence homology in soybean, tomato, potato, rice and barley. The identified locus seems to represent a plant counterpart to the yeast mitochondrial MutS homolog (*MSH1*) and was designated *AtMSH1*.

4.4. Clustering of mt genes in *Arabidopsis* (IV)

Whilst mapping the *CHM* locus on *Arabidopsis* Chromosome III, a more detailed analysis of the region was carried out. It was found to be unusually rich in genes likely to be involved in mitochondrial DNA and RNA maintenance. A second, smaller gene interval was found on Chromosome V. A complete listing of the >50 loci that have been identified is found in publication IV. A statistically significant cluster, also similar to the CHM linkage group on Arabidopsis Chromosome III, was found in rice (Oryza sativa) Chromosomes I and IV, albeit the rice sequences were only partially annotated at that time. This survey focused on proteins that were predicted to have either a mitochondrial targeting capacity or a high procaryotic sequence homology (IV). Before this study, almost none of the plant genes for mitochondrial DNA replication and repair had been identified, and this survey has revealed several candidates putatively involved in these functions. These include the *mitochondrial DNA polymerase-γ* (At3g20540), *DNA gyrase α* (At3g10690) and *β* (At3g10270) subunits, *RecA* (At3g10140), *DNA helicase*

(At3g24340), a *single-stranded binding protein* (SSB, At3g18580) and a homolog to yeast *MMF1*(At3g20390).

Several of these identified genes appeared to be dual targeted to both plastids and mitochondria, suggesting a close coevolution of these two organelles. A detailed survey of this chromosomal region also revealed several loci with a high homology to genes known to be involved in mt maintenance in yeast or that have rickettsial or cyanobacterial features but lack organellar targeting capacity. No linearity in gene order between *Arabidopsis* and *Rickettsia* genomes was found. Evidently, an extensive interchange of organellar targeting presequences, between unrelated proteins, has occurred. This would suggest that intergenic recombination has been the major mechanism in the acquisition of organelle-targeting capacity for the genes in this region. Duplication of gene loci and even larger chromosome regions seems to be a common event giving rise to gene duplicates. In several instances, gene duplications have resulted in mitochondrial, plastid, and dual targeting forms of nearly identical proteins (Christensen et al., 2005).

One of the most interesting pairs of apparently duplicated proteins was chosen for further analysis. The gene homologous to DNA polymerase I, which was identified on Chromosome III (At3g20540) and designated *AtPolγ1,* and it's duplicate on Chromosome I (At1g50840), which was designated *AtPolγ2,* contain features similar to the gamma type polymerase found in the mitochondria of other species (Lecrenier et al., 1997). The Chromosome III encoded protein was predicted to be targeted to mitochondria (MitoProt value of 0.80) or to be dual targeted (Predotar values of mt 0.78, cp 0.64; TargetP values mt 0.74, cp 0.59). The Chromosome I version was predicted to target either mitochondria (MitoProt value of 0.90) or chloroplasts (Predotar value of 0.96, TargetP value of 0.93). In vivo targeting properties of both genes were confirmed by developing gene constructions that fused the predicted targeting presequence information from either locus. These gene constructions were used for particle bombardment of *Arabidopsis* leaf tissues. Subsequent confocal analysis suggested that the gene product from the Chromosome I locus, *AtPolγ1* (At1g50840), was targeted to plastids while the gene coded by Chromosome III, locus *AtPolγ2* (At3g20540), was dual targeted to mitochondria and plastids (IV).

5. DISCUSSION

5.1. The role of *AP1/SQUA* like genes and *BpFLO* in birch

5.1.1. The AP1/SQUA subfamily in birch

BpMADS3, *BpFULL1* (formerly *BpMADS5*) and *BpMADS4* are all members of the AP1/SQUA subfamily of MADS box genes. This family of proteins is further divided into three clades, one being eudicot AP1-like genes and two separate clades of *FUL*-like genes, as a consequence of duplication during core eudicot evolution (Litt and Irish, 2003; Calonje et al., 2004; Fig. 13). The birch gene *BpMADS3* associates with the genes in the *AP1* clade, and *BpMADS4* and *BpFULL1* with the *FUL*-like genes. They are in separate clades but have close sequence similarity as shown in Fig. 13. *BpMADS4* is associated with the same clade of FUL-like genes as, for example, *VFUL-F* from *Vitis vinifera* and *DEFH28* from *Antirrhinum* (Müller et al., 2001, Calonje et al., 2004). *BpFULL1* is associated with the same euFUL subclade as, for example, *EAP1* from *Eucalyptus*, *MdMADS2* from *Malus*, *SLM5* from *Silene, FUL* from *Arabidopsis*, *AMFUL* from *Antirrhinum*, *NAP1-1* from *Nicotiana* and *FBP26* from *Petunia*. More complete phylogenetic analyses of AP1/SQUA like genes including the birch homologs have been published by Litt and Irish (2003) and Calonje et al. (2004).

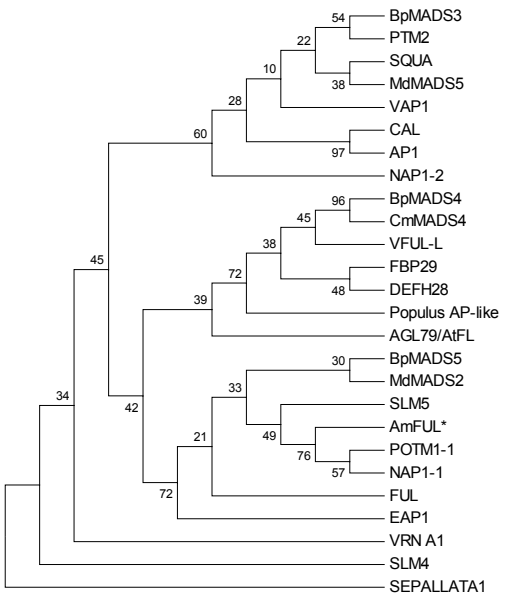

Figure 13. Phylogenetic, tree of AP1/FULL –like proteins from different plant species: *Antirrhinum* SQUA, DEFH28, AmFUL (*incomplete sequence); *Arabidopsis* AP1, CAL, FUL, AGL79/AtFL; Apple MdMADS2, MdMADS5; *Betula* BpMADS3, BpMADS4, BpMADS5 (BpFULL1); *Castanea* CmMADS4; *Eucalyptus* EAP1; *Nicotiana* NAP1-1, NAP1-2; Petunia FBP29; Populus AP-like; Potato POTM1-1; Silene SLM4, SLM5; *Vitis* VAP1, VFUL-L; wheat VRN A1. *Arabidopsis* SEPALLATA1 was used as an outgroup.

The birch gene *BpMADS3* is phylogenetically related to eu*AP1* like genes, a group that includes genes such as *Arabidopsis AP1* and *CAL*, *Antirrhinum SQUA* and *Vitis VAP1* (Litt and Irish, 2003; Calonje et al., 2004; Fig. 13). The members of this gene group are expressed in the early phases of flower initiation and thus have been implicated in the specification of the floral meristem as well as having a homeotic A-function in sepal and petal development (e.g. Irish and Sussex, 1990; Theissen, 2001). In contrast, *BpMADS3* is strongly expressed in the late phases of flower development and even during seed maturation (I). Expression in carpels has also been detected, at least with *Antirrhinum SQUA* (Hujser et al., 1992), the *Silene AP1*-like gene (Hardenack et al., 1994) and the *Vitis VAP1* (Calonje et al., 2004). The overexpression of *BpMADS3* in *Arabidopsis* and tobacco caused early flowering, and, especially in tobacco, *BpMADS3* seemed to promote the growth and division of the inflorescence meristem, causing plants to produce inflorescence shoots from every axillary shoot. The preliminary results in birch show that *BpMADS3* seems to have a similar effect in promoting the formation of the inflorescence meristem (J. Lemmetyinen, personal communication), and its overexpression induces flowering in birch (Lemmetyinen, 2003).

BpMADS4 is expressed in the earliest stages of developing inflorescences, as soon as the developing meristem can be identified, but also has clear vegetative expression in roots (I). In situ hybridization was used to show that the expression was highest in the apex of the meristem before the appearance of the floral meristems (II). *BpMADS4* seems to be an ortholog of *Antirrhinum DEFH28* (Fig. 13), and it is also expressed in the apex of the inflorescence meristem, suggesting an important function in the formation of the inflorescence (Müller et al., 2001). Ectopic expression of *DEFH28* in *Arabidopsis* caused early flowering similar to the ectopic expression of *BpMADS4* in all the species studied. Also, a homologous gene from *Vitis, VFUL-L,* has a very similar role in the floral transition and in carpel and fruit development as well as in the development of tendrils (Calonje et al., 2004). The orthologous gene in *Arabidopsis* seems to be *AGL79* (also referred to as *AtFL* in Calonje et al., 2004 and Litt and Irish, 2003). It has some expression in the roots (Pařenicová et al., 2003). However, *AGL79* seems to have lost its floral function and does not have any expression in the inflorescences. The *Arabidopsis* gene *FUL* associates in different subclades of *FUL*-like genes than *BpMADS4*, *DEFH28* or *AGL79* do, but is involved in cauline leaf, carpel and fruit development. Its pattern of expression is very similar to that of *BpMADS4* and *DEFH28* (I, II; Gu et al., 1998; Ferrándiz et al., 2000). However, unlike *BpMADS4*, neither *DEFH28* nor *FUL* seem to have any detectable expression in the roots. Judging from similarities in expression pattern and the overexpression phenotypes, *BpMADS4* seems to have similar functions in birch flower development as, for example, *DEFH28*, *VFUL-L* or *FUL*, but its possible role in the vegetative development or roots of birch is still unclear.

Our results prove that *BpMADS4* is one of the major genes in birch flower initiation and inflorescence development. Our results suggest that *BpMADS4* alone is capable of turning the vegetative apical meristem into an inflorescence meristem. In tobacco, the whole transgenic plant looked like the top of the shoot or "inflorescence" of a flowering tobacco control plant (I). The inhibition of flowering when the expression of *BpMADS4* was suppressed further supports the inference that *BpMADS4* is important in birch inflorescence initiation (II). A high level of *BpMADS4* expression is needed for flower initiation, since *BpMADS4* is expressed at low levels in vegetative growth. It seems that *BpMADS4* is directly under the control of the birch homolog of the *CONSTANS* gene (M. Aalto, unpublished result). Further on in the development, *BpMADS4* promotes the initiation of female flowers in birch since the earliest flowering birch lines over-expressing *BpMADS4* bore female inflorescences. Normally, an over-wintering treatment is needed for the emergence of female inflorescences. Silencing of *BpMADS4* by an RNAi construct delayed, but did not prevent, either female or male inflorescence development in birch, suggesting a redundancy among the genes controlling the inflorescence initiation. Thus floral initiation could not be totally prevented by silencing only one gene (Lännenpää, 2005). RNAi inhibition also caused some vegetative defects and problems in rooting suggesting a functional role in birch root development also (Lännenpää, 2005).

Phylogenetically, *BpFULL1* (formerly known as *BpMADS5*) is more similar to *FUL* than to *BpMADS4* (Litt and Irish 2003; Calonje et al., 2004; Fig. 13). The expression pattern also has similarities to *FUL*, suggesting similar roles in inflorescence and flower development (I). *BpFULL1*, however, did not have any detectable vegetative expression. The expression of *BpFULL1* was detected in young male inflorescence primordials by in situ hybridizations. Later in the inflorescence development, the expression was strongest in stamen primordia and in the developing carpels but also in the central axis of the inflorescences (Lännenpää, 2005). Over-expression of *BpFULL1* resulted in accelerated flowering in *Arabidopsis* and tobacco but in birch, no significant effect on flowering time was detected (I, unpublished). *BpFULL1as* produced no major phenotypic changes either in the time of flowering or in the development of the inflorescences or flowers. Notably, the *BpFULL1::BARNASE* construct efficiently inhibited flowering in *Arabidopsis*, tobacco and birch with some effects on vegetative growth (Lännenpää et al., 2005). The elongation of the inflorescence axil and the abnormal branching of the inflorescences in the birch lines overexpressing *BpFULL1* suggest that *BpFULL1* is needed for the determination of the inflorescence meristem and the development and growth of the inflorescence axil (Figs 9A-G). This inference is in agreement with the expression pattern and the analysis of the *BpFULL1-promoter-GUS* construct in *Arabidopsis* (Lännenpää et al., 2005). Additionally, *BpFULL1* is likely to promote the development of male organs in birch since the overexpression of *BpFULL1* causes growth of stamen like organs instead of tepals or even scales, which are the birch equivalents of bracts (Figs 9 H-I).

According to these results, the birch genes *BpMADS4* and *BpFULL1* share a functional similarity with *FUL*, and these genes have at least some degree of redundancy in their function. In *Arabidopsis,* an apparent *BpMADS4* ortholog, *AGL79* does not seem to be involved in floral induction or development. It is thus likely that *FUL* performs the whole function that, in several eudicot plant species like birch, is shared between two related genes. *Antirrhinum,* like birch, has two copies of *FUL*-like genes: *DEFH28* and *AmFUL*. The predicted function of *DEFH28* is discussed above but the function of *AmFUL,* which shows more sequence similarity to *BpFULL1,* is not yet known (Litt and Irish, 2003).

5.1.2. The role of *BpFLO* in birch

FLO/LEAFY -like genes are phylogenetically well conserved group. In *Arabidopsis, LFY* plays a role in the transition to flowering and controlling whorl 1 and 2 development in the flower (Blázquez et al., 1997; Ferrándiz et al., 2000a). It has been suggested that *LEAFY* interacts with *AP1* to specify meristem identity in *Arabidopsis*, and that *LEAFY* has a crucial role in activating *AP1* and other homeotic genes (e.g. Nilsson et al., 1998, Ferrándiz et

al., 2000a). In birch, the expression of *BpFLO* is not restricted to the early phases of inflorescence or flower induction. Its expression continues and is strongest in the fully differentiated, mature female inflorescences, suggesting another role in addition to flower initiation.

The results obtained from functional experiments in birch suggested that *BpFLO* has some role in the control of flowering time, inflorescence formation and possibly in whorl 1 and 2 development, similar to *LFY*. However, the role that this gene has in floral initiation or in flower development has not been distinctly characterized and is likely to be redundant. In birch clone BPM2, overexpression of *BpFLO* accelerated flowering compared to controls (Table 2a). In the second birch clone, BPM5, flowering was delayed (Table 2b). In the clone BPM5, the delay of flowering might be caused by co-suppression. These results are only indicative since we have not analyzed the natural expression level or pattern of *BpFLO* in either of the birch clones used in the transformation experiments. In most transgenic lines, neither overexpression nor suppression of the function of this gene produced any change in the development of inflorescences or flowers. Occasionally, bunches of inflorescences formed, indicating some function in regulating the development of the inflorescence meristem. At least in one birch line, overexpressing *BpFLO* caused numerous extra scales and tepals to form in the male inflorescences, indicating some level of homeotic function. Most of the phenotypes in the transgenic birch lines were vegetative, such as the short growth with shorter shoots and abnormal leaf shape. This indicates that *BpFLO* might regulate the growth of the shoots and leaf shape in birch. Similarly, the homologous genes *UNIFOLIATA* (*UNI*) and *FALSIFLORA,* in pea and tomato respectively, regulate the development of the leaves (Gourlay et al., 2000, Molinero-Rosales et al., 1999).

5.1.3. The homeotic roles of *AP1/FULL* like genes and *BpFLO*

Predicting the possible homeotic function of the birch genes *BpMADS3, 4, BpFULL1* and *BpFLO* is difficult because birch flowers are structurally very different from the model species *Arabidopsis* or *Antirrhinum.* Firstly, birch flowers are unisexual. Secondly, the flowers have a much reduced structure; the female flowers completely lack outer whorl organs (sepals and petals). The simple tepals in the birch male flower are likely to represent both sepals and petals. The genetic mechanism behind the organization and gender development of birch flowers is still unclear and difficult to predict on the basis of the results obtained so far. The reduced structure and even lack of outer whorl organs in the female flowers cannot be explained by the suppression of the genes homologous to *LFY*, *AP1*, or *FUL*, since all of the birch homologs seem to be expressed in proper places and times during inflorescence development. On the other hand, *Arabidopsis* is the only species so far, in which a mutation in a *AP1/FUL* like gene results in changed organ identity. This suggests that in most plants, the members of this gene group specify the floral meristem identity rather than the A-function (Litt and Irish, 2003).

The ectopic expression of the *BpFULL1* caused the growth of male organs on the surface of scales and tepals. Nevertheless, the lack of stamens in wt birch flowers is not likely to be due to the suppression of *BpFULL1* in this whorl, since *BpFULL1* is expressed uniformly in developing male inflorescences in wild type trees. All the first emerged inflorescences in the transgenic early flowering birch lines were female ones, so it can be deduced that *BpMADS4* seems to especially promote the development of female inflorescences. Normally, in wt trees, the female inflorescences develop slightly later than the male ones and emerge only after over-wintering. Overexpression of *BpFLO* promoted the growth of scales and tepals in their right positions suggesting a homeotic A-function and that the supporting scales are merely equivalents to sepals rather than cauline leaves. A peculiarity is that a very similar phenotype was obtained by suppressing the function of the birch *SEPALLATA3* homologue, *BpMADS1* (Lemmetyinen et al 2004a). This suggests that *BpFLO* and *BpMADS1* might control each others' functions. Fig.14 presents a summary of birch genes isolated by our group that appear to regulate flower development. The figure

includes their detected expression and predicted function in inflorescence and flower development.

5.1.4. *BpMADS4* can be used to either accelerate or inhibit flowering

The BpMADS4 antisense construct significanlty delayed the flowering of the early flowering birch, suggesting its applicability in the inhibition of flowering. However, the more specific RNAi technique used to silence *BpMADS4,* seemed to only delay the inflorescence development (Lännenpää et al., 2005). In addition, some vegetative effects like weaker growth and rooting were observed in this experiment. The more efficient inhibition of flower development using an antisense construct might be explained by the lower specificity of this construct. The other explanation might lie in the genetic differences between the birch clones BPM2 and BPM5 used in the experiments. In this experiment, a high homology between MADS-box genes like *BpMADS4*, *BpMADS3* and *BpFULL1* (*BpMADS5*) might have resulted in the silencing of all of these genes simultaneously. On the other hand, the fact that the suppression of the function of *BpFULL1* by antisense did not have any effect on the time of flowering, would suggest otherwise. As concluded earlier by Lännenpää (2005) the best and most reliable prevention of birch flowering might be achieved using either one RNAi construct aimed to block several flowering genes or by transformation of the plant with several different constructs. It is also possible to use a promoter-cytotoxin construct with those genes that are strictly expressed in either the inflorescences or the flowers (Lemmetyinen, 2003).

The acceleration of flowering by overexpressing *BpMADS4* looks promising for research and breeding of forest trees. The results suggest that by overexpressing *BpMADS4, it* is possible to accelerate flowering in a variety of plant species. The over-expression of *BpMADS4* in apple caused precocious flowering only 13 weeks after transformation (Flachowsky et al., 2007). On the other hand, this result is not surprising since *Betula* and *Malus* are both genetically related Rosidae species, phylogenetically more closely related than birch and tobacco or *Arabidopsis*.

All of the birch genes in this study accelerated flowering when overexpressed in *Arabidopsis*. This suggests that flower initiation is more sensitive to the expression levels of the inflorescence genes in an annual herb like *Arabidopsis* than it is in a perennial such as birch.

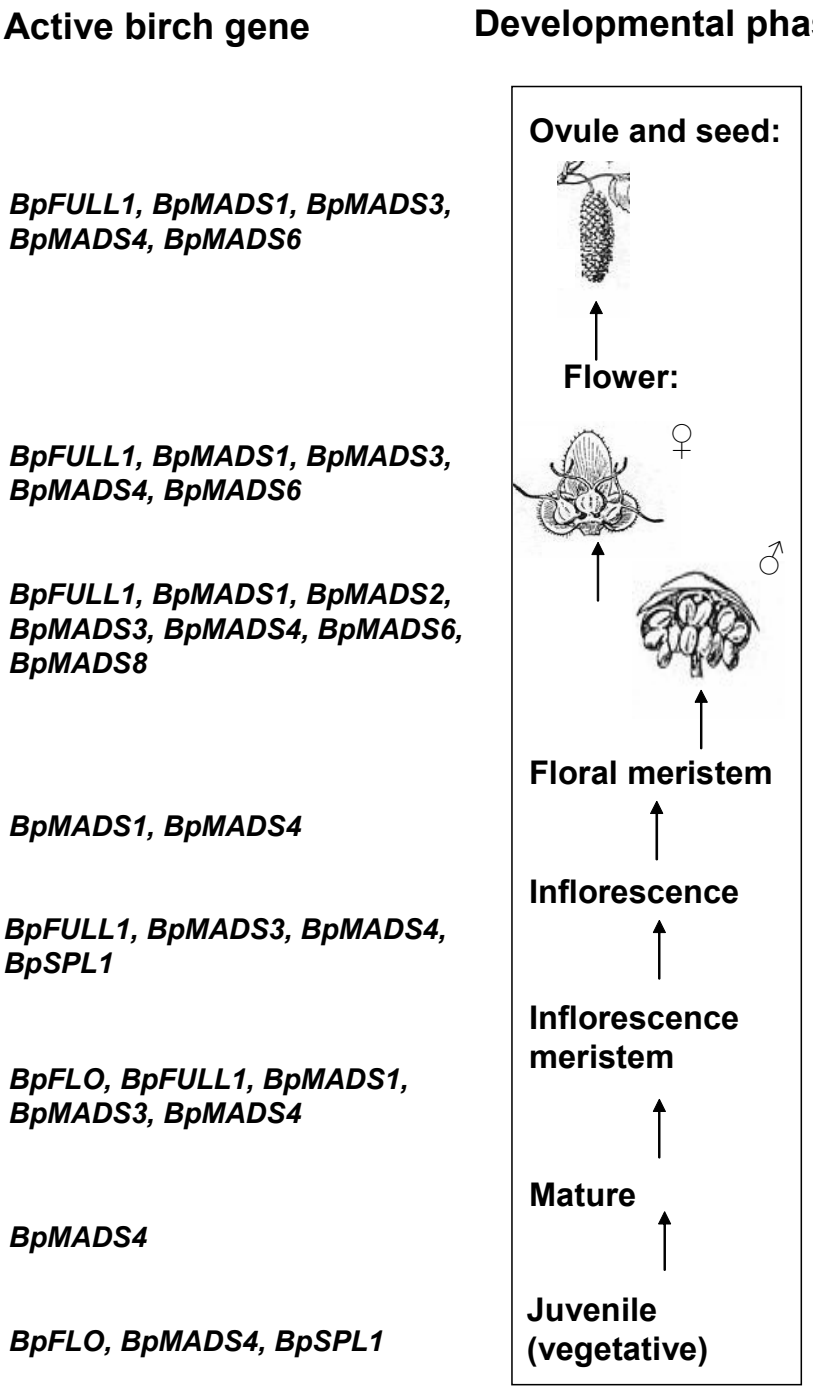

Figure 14. The summary of the isolated genes that are active during flower development in birch. This model is based on the expression and experimental data gathered so far. The phase of development or organ is presented on the right and the respective, active genes on the left. *BpMADS1* and *BpMADS6* were described by Lemmetyinen et al., 2004a; *BpMADS2* by Järvinen et al., 2003; *BpMADS3, 4* and *BpFULL* in papers I, II, Lännenpää et al., 2005 and unpublished data; by Lemmetyinen et al., 2004a; *BpMADS7* and *8* by Lemmetyinen, 2003; *BpSPL1* by Lännenpää et al., 2004, and the data on *BpFLO* is unpublished

5.2. Plant mitochondria

5.2.1. *CHM* and the recombination processes in mitochondrial genome

The cloned nuclear *CHM* gene in *Arabidopsis* that effects mitochondrial substoichiometric shifting was shown to encode a protein that is targeted to mitochondria and that has a high homology with a MutS protein. The identified locus appears to represent a plant counterpart to the yeast mitochondrial *MutS* homolog (*MSH1*) and was designated *AtMSH1*. Mutation of the *MSH1* locus in yeast results in rapid accumulation of mitochondrial genomic rearrangements leading to the disruption of mitochondrial function and the so called petite phenotype (Reenan and Kolodner, 1992). Further studies revealed mitochondrial *MSH1* to be well conserved throughout higher plant families (Abdelnoor et al., 2006).

In known *Arabidopsis chm* mutant lines no signs of accumulation of subsequent point mutations in the mitochondrial genome was noted in a DNA gel blot analysis; not even in lines *chm1-1* and *chm1-2* which have been established in 1973 (Martínez-Zapater et al., 1992, Sakamoto et al., 1996, this research). If the *MSH1* product was involved in mitochondrial mismatch repair functions, we would anticipate that at least one of these mutants would display a random pattern of novel fragment polymorphisms as a consequence of increased accumulation of mutations. These results imply that mismatch repair of the mitochondrion is unaffected in these lines and that *AtMSH1* is no longer predominantly active in mitochondrial DNA mismatch repair. In the light of the results obtained so far, it is conceivable that the primary role of *AtMSH1* in the plant mitochondrial genome is to regulate nonhomologous recombination. This is further supported by the consideration that yeast mitochondrial *MSH1* does not seem to be involved in mismatch repair in yeast (Sia and Kirkpatrick, 2005).

However, preliminary results from the introduction of the *chm* mutation in different nuclear and cytoplasmic backgrounds points to at least some degree of new polymorfism in subsequent generations (Fig. 12). This might indicate that mismatch repair is indeed impaired in these lines and it is important to study this further. It is also important to rule out the possibility that the additional polymorphism seen in the progenies of some of the ecotypes is not simply due to an error in the data, like mislabelling of the lines or mixing the seed stocks. Another possible explanation is that recombinational activity is different in different nuclear and cytoplasmic backgrounds.

It is a significant observation that the observation that *CHM*, a gene noted for its ability to affect substoichiometric shifting of the *Arabidopsis* mitochondrial genome, encodes a *MutS* homolog. This observation indicates that the process of mitochondrial genomic shifting is likely to involve a direct influence on the replication or recombination activity of the mitochondrial genome, as opposed to a population segregation process at the whole mitochondrion level. The physical organization of the mitochondrial genome upon substoichiometric shifting is not known. We can only predict that if *MSH1* influences mitochondrial genome configuration in a molecule-specific manner, physical mapping of the shifted mitochondrial genome will reveal the selective amplification of a single molecular form. Previous analysis of the mitochondrial genome of *chm* mutants indicated the amplification of a chimeric region of the genome that shows evidence of ectopic recombination (Martinez-Zapater et al., 1992; Sakamoto et al., 1996). However, these studies relied on DNA gel blot hybridization analysis, so it was not possible to assess the full extent of the genome change that occurred. Nevertheless, results from these previous studies demonstrated that the genomic alterations evident within the shifted mitochondrial genome were few, which is consistent with the copy number modulation of a single molecular form. In yeast, the nuclear mismatch repair apparatus is involved not only in mismatch repair functions, but in the suppression of ectopic recombination activity (Chen and Jinks-Robertson, 1999; Harfe and Jinks-Robertson, 2000).

The explanation and the mechanism behind the plastid phenotype caused by a mutant allele of *CHM/AtMSH1* are also interesting. Originally, the *CHM* locus was named *chloroplast mutator* because the color and shape of the plastids was affected in the white sectors of variegated plants. However,

extensive research using chloroplast probes did not reveal any changes in their genomes (Martínez-Zapater et al., 1992). Similarly, the variegated phenotype, nonchromosomal stripe (NCS), in maize is correlated with specific alterations in the mitochondrial genome and identical changes in chloroplast structure (e.g. Roussel et al., 1991). Original data from targeting presequence-*gfp* constructs of *AtMSH1* using the annotated ATG as the start of the construct, confirmed the mitochondrial targeting. However, the possible dual targeting to include plastids could not be verified (III). Interestingly, an inclusion of the predicted 5' UTR did result in clear dual targeting of the protein (Christensen et al., 2005). This experiment proved that the *AtMSH1* product is indeed capable of plastid targeting but its possible function there remains unresolved. White-green leaf variegation was also demonstrated when Msh1 expression was suppressed by RNAi in tomato. Here, the dual targeting of the MSH1 protein was confirmed (Sandhu et al., 2007).

The evolution and role of *MSH1* in mitochondrial genome behavior might be linked with a phenotypic plasticity that serves an adaptative role in sessile organisms. This is supported by the finding of a parallel gene in soft corals (Abdelnoor et al., 2006). Increasing evidence suggests a direct relationship between mitochondrial activity and sex determination in both animals and plants (Bazinet, 2004; Mittwoch, 2004; Abdelnoor et al., 2006). Suppression of *Msh1* expression by RNAi caused mitochondrial DNA rearrangements associated with male sterility in tobacco and tomato plants (Sandhu et al. 2007). So it is feasible to think that the interconversion of cytoplasmic male sterility and the hermaphrodite condition in gynodioecious plant populations can be regulated, at least in part, by the action of *MSH1* (Mackenzie, 2005c, Sandhu et al. 2007).

5.1.2. Clustering of the genes involved in mitochondrial genome maintenance (IV)

A Survey was carried out of the *Arabidopsis* nuclear genes that encode mitochondrial proteins involved in mitochondrial genome maintenance. It revealed a number of gene candidates on chromosome III, several of which had never been characterized in higher plants. This study revealed that this cluster was probably not due to preferential integration of the genes transferred from the mitochondrion and that present day gene linkages are likely to be maintained by selection. A comparison of *Arabidopsis* and rice genomes revealed the existence of a similar linkage group of mitochondrial genes in rice. Until recently, possible genetic clustering of genes with related functions was considered unusual in higher plant genomes. More examples of functional gene clusters have been revealed, however. This has been made possible by large scale sequencing of entire plant genomes and the development of more efficient systems, such as microarray technologies, for scanning the simultaneous expression of several genes. Also recently, a study of several genomes, including that of *Arabidopsis*, has used the KEGG database to demonstrate that genes functioning in the same pathway are, in fact, often clustered in the genome (Lee and Sonnhammer, 2003). An example of this is provided by recent studies of *Arabidopsis thaliana* which have identified clustered genes in relation to root development (Birnbaum et al., 2003). Those studies have also found that clustering of nuclear genes encoding organellar proteins occurs above expectation (Alexeyenko et al., 2006). The selective advantage of a single linkage group of genes involved in organellar DNA and RNA metabolism is not clear. Since mitochondria in *Arabidopsis* and the majority of other plant species are strictly maternally inherited, it is possible that these genes are co-ordinately regulated through gene imprinting, for example. The maternal transmission of the mt population into the next generation takes place during the development of female gametophytes and possible changes in mt genome structure and substoichiometric shifting takes place in this phase of development. The mechanisms of wider genetic control in plants are still unclear but might involve changes in chromatin structure and methylation. The *Arabidopsis* floral homeotic gene *SUPERMAN* (*SUP*) restores phenotype abnormalities and even restores fertility in alloplasmic CMS tobacco lines (Bereterbide et al., 2002). Interestingly, the *SUPERMAN* locus is known to be regulated through methylation and is located on

Chromosome III in the same position as the mt DNA and RNA metabolism gene cluster (Jacobsen and Meyerowitz, 1997; IV). Chromatin remodelling is thought to be a major effector in activating the genes involved in inflorescence initiation and in determining floral organ identity (reviewed e.g. by Sung et al., 2003). Co-ordinated gene regulation is thought to be facilitated by a physical linkage of the co-regulated genes (Boutanaev et al., 2002; Oliver et al., 2002; Spellman and Rubin, 2002). Several large-scale analyses of expression data in higher eukaryotes have shown that neighbouring genes tend to have similar expression patterns as reviewed by Williams and Bowles, 2004. Even in the absence of coordinate regulation, the close proximity of neighboring genes in eukaryotic genomes could lead to sharing of cis-regulatory elements such as enhancers or insulators, leading to a similarity in their expression patterns (Williams and Bowles, 2004). However, there are several cases where physical clustering is not required for co-expression or regulation of genes for organellar proteins and the clustering merely results from continuous selection that favors chromosomal proximity of genes acting on the same organelle (Alexeyenko et al., 2006).

5.2.3. The evolution of organellar targeting, using *AtPolγ1* and *2* as examples

During eukaryotic evolution, the mitochondrial genome has relinquished most of its genes to the nucleus by a process of interorganellar gene transfer. This transfer of organellar genes to the nucleus had to be accompanied by the acquisition of mitochondrial or plastid protein-targeting information to allow the gene to become functional (e.g. Martin and Herrman, 1998). After this integration, an extensive rearrangement and duplication of the genome has taken place. Intergenic recombination has facilitated the acquisition of targeting information by integrated mitochondrial sequences. .Duplication of the gene loci may have facilitated functional specialization and organellar co-evolution. This study suggests that many of the components of plant mitochondrial DNA metabolism are dual targeted to mitochondria and chloroplasts, suggesting that the mitochondria and chloroplasts share their genome maintenance apparatus. It is known that dual targeting to mitochondria and plastids can be a feature of the targeting presequence itself or can arise by alternate translation start sites within a gene (Small et al., 1998; Peeters et al., 2001). Two duplicate *Arabidopsis* genes were used In this study: *AtPolγ1* and *AtPolγ2* which are dual and plastid only targeted respectively (IV). However, the alignment of these two sequences revealed an unusually high degree of nucleotide, amino acid, and reading frame conservation within the predicted, untranslated leader region (IV; Christensen et al., 2005). Surprisingly, similar sequence conservation was noted on two homologous genes identified from rice (Christensen et al., 2005). Later experiments confirmed these regions to be translationally active and so proper targeting of these proteins was required (Christensen et al., 2005). With the DNA polymerase, *AtPolγ2* (At1g50840), the targeting presequence conferred plastid targeting capacity only (IV). A more detailed analysis revealed that an additional mitochondrial targeting feature of the protein is apparently located within the 5' untranslated leader sequence immediately adjacent to the translation start codon. Results of later experiments suggested that 5' UTRs and non AUG translation start sites might have a significant role in the targeting of several identified loci in the Chromosome III region, as is case with the *CHM/AtMSH1* gene product (IV, Christensen et al., 2005, unpublished results). Obviously, a combination of intergenic recombination and relaxation of the constraints on translation initiation has occurred in those proteins capable of functioning in both plastids and mitochondria (this study, Christensen et al., 2005; Mackenzie 2005a).

6. CONCLUSIONS

1. Initiation of flowering in birch is under strong genetic control in addition to being influenced by environmental factors. *BpMADS3*, *BpMADS4* and *BpFULL1*, despite their high sequence homology, they have differentiated but partly redundant roles in birch flower initiation and development. Their roles in birch flower development are generally similar to those of homologous genes in other species. *BpMADS4* and *BpFULL1* seem to share the function which *FUL* performs alone in *Arabidopsis*. Despite a rather distinct flower structure, generally the same genes are found to be controlling reproductive development in birch as in the other plant species studied. Thus the differences in floral initiation and flower structures might simply result from the differential expression of these genes.

2. By overexpressing or suppressing a single birch gene, *BpMADS4*, it is possible to either accelerate or suppress initiation of flowering in birch. *BpMADS4* is a very promising candidate for accelerating flowering in various plant species. However, this gene might not be as useful in preventing flowering because it has vegetative expression and therefore its suppression is likely to be followed by growth defects.

3. The plant mitochondrial genome is a dynamic, nuclearly controlled system. This study confirms that *CHM* (*AtMSH1*) maintains mitochondrial genome stability in *Arabidopsis*. Despite the homology to mismatch repair proteins, the main function of *AtMSH1* is likely to be in controlling recombinational activity in the mitochondrial genome with a likely role in maintaining phenotypic plasticity and sex determination in plants.

4. Several of the genes apparently involved in mitochondrial DNA maintenance and recombination functions are found in the same linkage group in *Arabidopsis* Chromosome III. Thus the genes behind mtDNA metabolism are likely to be co-regulated (e.g. through imprinting).

5. Protein targeting in the plant organelles is flexible and under constant evolution. This study reveals the tight and ongoing evolution between the genomes of the nucleus, mitochondria and plastids. In several examples, two cell organelles share the genes coding for proteins involved in their genome maintenance functions.

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REFERENCES:

Abad A, Mehrtens B, and Mackenzie S. 1995. Specific expression and fate of a sterility-associated mitochondrial protein in cms common bean. Plant Cell 7:271-285.

Abdelnoor RV, Christensen AC, Mohammed S, Munoz-Castillo B, Moriyama H, and Mackenzie SA. 2006. Mitochondrial genome dynamics in plants and animals: convergent gene fusions of a *MutS* homologue. Journal of Molecular Evolution 63:165-173.

Adams KL, Song K, Roessler PG, Nugent JM, Doyle JL, Doyle JJ, and Palmer JD. 1999. Intracellular gene transfer in action: Dual transcription and multiple silencings of nuclear and mitochondrial cox2 genes in legumes. Proceedings of the National Academy of Science of the United States of America 96:13863-13868.

Alexeyenko A, Millar AH, Whelan J, and Sonnhammer ELL. 2006. Chromosomal clustering of nuclear genes encoding mitochondrial and chloroplast proteins in *Arabidopsis*. Trends in Genetics 22:589-593.

Andersson SGE, Zomorodipour A, Andersson JO, Sicheritz-Ponten T, Alsmark UCM, Podowski RM, Naslund, AK, Eriksson AS, Winkler HH, and Kurland CJ. 1998. The genome sequence of Rickettsia prowazekii and the origin of mitochondria. Nature 396:133-143.

Andre C, Levy A, and Walbot V. 1992. Small repeated sequences and the structure of plant mitochondrial genomes Trends in Genetics. 8:128-132.

Anthony RG, James PE, and Jordan BR. 1993. Cloning and sequence analysis of a *flo/lfy* homologue isolated from cauliflower (*Brassica oleracea* L. var. *botrytis*). Plant Molecular Biology 22: 1163-1166.

Arrieta-Montiel M, Lyznik A, Woloszynska M, Janska H, Tohme J, and Mackenzie S. 2001.Tracing evolutionary and developmental implications of mitochondrial genomic shifting in the common bean. Genetics 158:851-864.

Backert S and Börner T. 2000. Phage T4-like intermediates of DNA replication and recombination in the mitochondria of the higher plant *Chenopodium album* (L.), Current Genetics 37:304–314.

Bazinet C. 2004. Endosymbiotic origins of sex. BioEssays 26: 558-566.

Bereterbide A, Harnoud M, Farbos I, Glimelius K, and Mouras A. 2002. Restoration of stamen development and production of functional pollen in an alloplasmic CMS tobacco line by ectopic expression of the *Arabidopsis thaliana SUPERMAN* gene. Plant Journal 29:607-615.

Bergstrom CT and Pritchard J. 1998. Germline bottlenecks and the evolutionary maintenance of mitochondrial genomes. Genetics 149:2135-2146.

Birnbaum K, Shasha DE, Wang JY, Jung JW, Lambert GM, Galbraith DW, and Benfey PN. 2003. A Gene Expression Map of the *Arabidopsis* Root. Science 302:1956-1960.

Blázquez MA. 2000. Flower development pathways. Journal of Cell Science 113:3547-3548.

Blázquez MA, Soowal LN, Lee I, and Weigel D. 1997. *LEAFY* expression and flower initiation in *Arabidopsis*. Development 124:3835-3844.

Boutanaev AM, Kalmykova AI, Shevelyov YY, and Nurminsky DI. 2002. Large clusters of co-expressed genes in the *Drosophila* genome. Nature 420:666-669.

Brennicke A, Grohmann L, Hiesel R, Knoop V, and Schuster W. 1993. The mitochondrial genome on its way to the nucleus: different stages of gene transfer in higher plants. FEBS Letters 325:140-145.

Böhlenius H, Huang T, Charbonnel-Campaa L, Brunner AM, Jansson S, Strauss SH, and Nilsson O. 2006. *CO/FT* regulatory module controls timing of flowering and seasonal growth cessation in trees. Science 19:1040-1043.

Calonje M, Cubas P, Martínez-Zapater JM, and Carmona MJ. 2004. Floral meristem identity genes are expressed during tendril development in grapevine. Plant Physiology 135:1-11.

Chen W and Jinks-Robertson S. 1999. The role of the mismatch repair machinery in regulating mitotic and meiotic recombination between diverged sequences in yeast. Genetics. 151:1299-313.

Christensen AC, Lyznik A, Mohammed S, Elowsky CG, Elo A, Yule R, and

Mackenzie SA. 2005. Dual-domain, dual-targeting organellar protein presequences in *Arabidopsis* can use non-AUG start codons. Plant Cell 2005 10:2805-16.

Claros MG and Vincens P. 1996. Computational method to predict mitochondrially imported proteins and their targeting sequences. European Journal of Biochemistry 241:779-786.

Coen ES, Romero JM, Doyle S, Elliot R, Murphy G, and Carpenter R. 1990. *floricaula*: a homeotic gene required for flower development in *Antirrhinum majus*. Cell 63:1311-1322.

Emanuelsson O, Nielsen H, Brunak S, and von Heijne G. 2000. Predicting subcellular localization of proteins based on their N-terminal amino acid sequence. Journal of Molecular Biology 300: 1005-1016.

Emelyanov VV. 2001. Rickettsiaceae, richettsia-like endosymbionts, and the origin of mitochondria. Bioscience Reports 21:1-17.

Fauron C, Casper M, Gao Y, and Moore B. 1995. The maize mitochondrial genome: Dynamic, yet functional. Trends in Genetics 11:228-235.

Felsenstein J. 2002. PHYLIP, Phylogeny Inference Package version 3.6a3. Department of Genome Sciences, University of Washington, Seattle.

Ferrándiz C, Gu Q, Martienssen R, and Yanofsky MF. 2000a. Redundant regulation of meristem identity and plant architechture by *FRUITFULL*, *APETALA1* and *CAULIFLOWER*. Development 127:725-34.

Ferrándiz C, Liljegren SJ, and Yanofsky MF. 2000b. Negative regulation of the *SHATTERPROOF* genes by *FRUITFULL* during Arabidopisis fruit development. Science 289:436-438.

Flachowsky H, Peil A, Sopanen T, Elo A, and Hanke V. 2007. Overexpression of BpMADS4 from silver birch (*Betula pendula* Roth.) induces early-flowering in apple (*Malus* x *domestica* Borkh.). Plant Breeding 126:137-145.

Gallois J-L, Achard P, Green G, and Mache R. 2001. The *Arabidopsis* chloroplast ribosomal protein L21 is encoded by a nuclear gene of mitochondrial origin. Gene 274:179-185.

Gourlay CW, Hofer JMI, and Ellis THN. 2000. Pea compound leaf architecture is regulated by interactions among the genes *UNIFOLIATA, COCHLEATA, AFILA,* and *TENDRIL-LESS*. Plant Cell 12: 1279-1294.

Gray MW, Burger G, and Lang BF. 1999. Mitochondrial evolution. Science 283:1476-1481.

Gu Q, Ferrándiz C, Yanofsky MF, and Martienssen R. 1998. The *FRUITFULL* MADS-box gene mediates cell differentiation during *Arabidopsis* fruit development. Development 125:1509-1517.

Hardenack S, Ye D, Saedler H, and Grant S. 1994. Comparisons of MADS box gene expression in developing male and female flowers of the dioecious plant white campion. Plant Cell 6:1775-1787.

Hanson MR and Bentolila S. 2004. Interactions of mitochondrial and nuclear genes that affect male gametophyte development. Plant Cell 16:S154-S169.

Harfe BD and Jinks-Robertson S. 2000. DNA mismatch repair and genetic instability. Annual Review of Genetics 34:359-399.

Hofer J, Turner L, Hellens R, Ambrose M, Matthews P, Michael A, and Ellis N. 1997. *UNIFOLIATA* regulates leaf and flower morphogenesis in pea. Current Biology 7:581-587.

Huang J, Struck F, Matzinger DF, and Levings CJ. 1994. Flower-enchanced expression of a nuclear-encoded mitochondrial respiratory protein is associated with changes in mitochondrion number. Plant Cell 6:439-448.

Huijser P, Klein J, Lonnig WE, Meijer H, Saedler H, and Sommer H. 1992. Bracteomania, an inflorescence anomaly, is caused by the loss of function of the MADS-box gene *squamosa* in *Antirrhinum majus*. EMBO Journal 11:1239-1249.

Jacobsen SE and Meyerowitz EM. 1997. Hypermethylated SUPERMAN epigenetic alleles in *Arabidopsis*. Science 277: 1100-3.

Jack, T. 2001a. Plant development going MADS. Plant Mol. Biol. 46:515-520.

Jack, T. 2001b. Relearning our ABCs: New twists on an old model. Trends in Plant Science 6:310-316.

Jander G, Norris SR, Rounsley SD, Bush DF, Levin IM, and Last RL. 2002. *Arabidopsis* Map-Based Cloning in the Post-Genome Era. Plant Physiology 129:440-450.

Janska H and Mackenzie S. 1993. Unusual mitochondrial genome organization in cytoplasmic male sterile common bean and the nature of cytoplasmic reversion to fertility. Genetics 135:869-879.

Janska H, Sarria R, Woloszynska M, Arrieta-Montiel M, and Mackenzie S. 1998. Stoichiometric shifts in the common bean mitochondrial genome leading to male sterility and spontaneous reversion to fertility. Plant Cell 10:1163-1180.

Johns C, Lu M, Lyznik A, and Mackenzie S. 1992. A mitochondrial DNA sequence is associated with abnormal pollen development in cytoplasmic male sterile common bean. Plant Cell 4:435-449.

Joyce PB and Gray MW. 1989. Chloroplast-like transfer RNA genes expressed in wheat mitochondria. Nuclear Acids Research 17:5461-5476.

Järvinen P, Lemmetyinen J, Savolainen O, and Sopanen T. 2003. DNA sequence variation in *BpMADS2* gene in two populations of Betula pendula. Molecular Ecology 12:369-384.

Kadowaki K, Kubo N, Ozawa K, and Hirai A. 1996. Targeting presequence acquisition after mitochondrial gene transfer to the nucleus occurs by duplication of existing targeting signals. EMBO Journal 15:6652-6661.

Kalanditis K, Wilson ZA, and Mulligan BJ. 2002. Mitochondrial gene expression is differentially regulated during male gametogenesis in *Arabidopsis*. Sexual Plant Reproduction 14: 299-304.

Kanazawa A, Tsutsumi N, and Hirai K. 1994. Reversible changes in the composition of the population of mtDNAs during dedifferentiation and regeneration in tobacco. Genetics 138: 865-870.

Kmiec B, Woloszynska M, and Janska H. 2006. Heteroplasmy as a common state of mitochondrial genetic information in plants and animals. Current Genetics 50: 149-159.

Krepinsky K, Plaumann M, MartinW, and Schnarrenberger C. 2001. Purification and cloning of chloroplast and cytosolic isoenzymes encoded in eukaryotic chromosomes. European Journal of Biochemistry 268: 2678–2686.

Kumar S, Tamura K, and Nei M. 2004. MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. Briefings in Bioinformatics 5:150-163.

Landschutze V, Wilmitzer L, and Muller-Rober B. 1995. Inhibition of flower formation by antisense repression of mitochondrial citrate synthase in transgenic potato leads to a specific disintegration of the ovary tissues of flowers. EMBO Journal 14:660-666.

Lawson, EJ and Poethig RS. 1995. Shoot development in plants: time for a change. Trends in Genetics 11:519-550.

Lee JM and Sonnhammer EL. 2003. Genomic gene clustering analysis of pathways in eukaryotes. Genome Research 13:875-882.

Leino M, Teixera R, Landgren M, and Glimelius K.2003. Brassica napus lines with rearranged Arabidopsis mitochondria display CMS and range of developmental aberrations. Theoretical and Applied Genetics 106:1156-1163.

Lemmetyinen J. 2003. The birch genes BpMADS1 and BpMADS6 and their use in the modification of flowering. University of Joensuu, PhD Dissertations in Biology, No.23. 85 pp.

Lemmetyinen J and Sopanen T. 2005. Modification of flowering in forest trees. In: Kumar S, and Fladung M (eds), Molecular genetics and breeding of forest trees. The Haworth Press, Inc., NY, pp. 263-291.

Lemmetyinen J, Keinonen-Mettälä K, Lännenpää M, von Weissenberg K, and Sopanen T. 1998. Activity of the CaMV 35S promoter in various parts of transgenic early flowering birch clones. Plant Cell Reports 18:243-248.

Lemmetyinen J, Pennanen T, Lännenpää M, and Sopanen T. 2001. Prevention of flower formation in dicotyledons. Molecular Breeding 7:341-350.

Lemmetyinen J, Hassinen M, Elo A, Porali I, Keinonen K, Mäkelä H, and Sopanen T. 2004a. Functional characterization of *SEPALLATA3* and *AGAMOUS* orthologues in silver birch. - Physiologia Plantarum 121:149-162.

Lemmetyinen J, Keinonen K, and Sopanen T. 2004b. Prevention of flowering of a tree, silver birch. Molecular Breeding 13:243-249.

Linke B and Börner T. 2005. Mitochondrial effects on flower and pollen development. Mitochondria 5:389-402.

Linke B, Nothnagel T, and Börner T. 2003. Flower development in carrot CMS plants: mitochondria affect the expression of MADS box genes homologous to GLOBOSA and DEFICIENS. Plant Journal 34:27-37.

Litt A and Irish VF. 2003. Duplication and diversification in the APETALA1/FRUITFULL floral homeotic gene lineage: implications for the evolution of floral development. Genetics 165:821-833.

Lännenpää M. 2005. Genetic regulation of birch flower development and potential biotechnological applications. University of Joensuu, PhD Dissertations in Biology, No.37. 94 pp.

Lännenpää M, Jänönen I, Hölttä-Vuori M, Gardemaister M, Porali I, and Sopanen T. 2004. A new SBP-box gene BpSPL1 in silver birch (Betula pendula). Physiologia Plantarum 120:491-500.

Lännenpää M, Hassinen M, Ranki A, Hölttä-Vuori M, Lemmetyinen J, Keinonen K, and Sopanen T. 2005. Prevention of flower development in birch and other plants using a *BpFULL::BARNASE* construct. Plant Cell Reports 24:69-78.

Macdonald AD and Mothersill DH. 1987. Shoot development in Betula papyrifera. VI. Development of the reproductive structures. Canadian Journal of Botany 65:466-475.

Mackenzie SA. 2005a. Plant organellar protein targeting: a traffic plan still under construction. Trends in Cell Biology 10:548-54.

Mackenzie SA. 2005b. The influence of mitochondrial genetics on crop breeding strategies. Plant Breeding Reviews 25:115-138. John Wiley and Sons.

Mackenzie SA. 2005c. The mitochondrial genome of higher plants: A target for natural adaptation. In: Henry RJ (ed), Diversity and evolution of plants. CABI Publishers, Oxon, UK, pp. 69-80.

Mackenzie S and McIntosh L. 1999. Higher plant mitochondria. Plant Cell 11:571-585.

Martin W and Herrmann RG. 1998. Gene transfer from the organelles to the nucleus: How much, what happens and why? Plant Physiology 118:9-17.

Martín-Trillo M and Martínez-Zapater J. 2002. Growing up fast: manipulating the generation time of trees. Current Opinion in Biotechnology 13:151-155.

Martínez-Zapater J, Gil P, Capel J, and Somerville C. 1992. Mutations at the Arabidopsis *CHM3* locus promote rearrangements of the mitochondrial genome. Plant Cell 4:889-899.

Meilan R. 1997. Floral induction in woody angiosperms. New Forests 14:179-202.

Mireau H, Lancelin D, and Small ID. 1996. The same Arabidopsis gene encodes both cytosolic and mitochondrial alanyl-tRNA synthetases. Plant Cell 8:1027-1039.

Mittwoch U. 2004. The elusive action of sex-determining genes: mitochondria to rescue? Journal of Theoretical Biology 228:359-365.

Molinero-Rosales N, Jamilena M, Zurita S, Gómez P, Capel J, and Lozano R. 1999. *FALSIFLORA*, the tomato orthologue of *FLORICAULA* and *LEAFY*, controls flowering time and floral meristem identity. Plant Journal 20:685–693.

Mouradov A, Cremer F and Coupland G. 2002. Control of flowering time: interacting pathways as a basis for diversity. Plant Cell 14:S111-S130.

Müller BM, Saedler H, and Zachgo S. 2001. The MADS-box gene DEFH28 from Antirrhinum is involved in the regulation of floral meristem identity and fruit development. Plant Journal 28:169-179

Neemann K. 2002. Investigations of nuclear mitochondrial genetic interaction in several ecotypes of Arabidopsis thaliana. BSc thesis. University of Nebraska-Lincoln, Department of Agronomy and School of Biological Sciences. 27 pp.

Nilsson O, Lee I, Blázquez MA, and Weigel D. 1998: Flowering time genes modulate the response to *LEAFY* activity. Genetics 150:403-410.

Oliver B, Parisi M and Clark D. 2002. Gene expression neighborhoods. Journal of Biology 1:4.

Palmer JD, Adams KL, Cho Y, Parkinson CL, Qiu YL, and Song K. 2000. Dynamic evolution of plant mitochondrial genomes: Mobile genes and introns and highly variable mutation rates. Proceedings of the National Academy of Science of the United States of America 97:6960-6966.

Pařenicová L, de Folter S, Kieffer M, Horner DS, Favalli C, Busscher J, Cook HE, Ingram RM, Kater MM, Davies B, Angenent GC, and Colombo L. 2003. Molecular and phylogenetic analyses of the complete MADS-box transcription factor family in Arabidopsis: New openings to the MADS world. Plant Cell 15:1538-1551.

Peña L, Martín-Trillo M, Juárez J, Pina JA, Navarro L, and Martínez-Zapater JM. 2001. Constitutive expression of Arabidopsis LEAFY and APETALA1 genes in citrus reduces their generation time. Nature Biotechnology 19:263-267.

Peeters N and Small I. 2001. Dual Targeting to mitochondria and chloroplasts. Biochimica et Biophysica Acta 1541:54-63.

Redei GP. 1973. Extra-chromosomal mutability determined by a nuclear gene locus in Arabidopsis. Mutation Research 18:149-162.

Reenan RAG and Kolodner RD. 1992. Isolation and characterization of two Saccharomyces cerevisiae genes encoding homologs of the bacterial HexA and MutS mismatch repair proteins. Genetics 132:963-973.

Rottmann WH, Meilan R, Sheppard LA, Brunner AM, Skinner JS, Ma C, Cheng S, Jouanin L, Pilate G, and Strauss SH. 2000. Diverse effects of overexpression of *LEAFY* and *PTLF*, a poplar (*Populus*) homolog of *LEAFY/FLORICAULA*, in transgenic poplar and *Arabidopsis*. Plant Journal 22: 235-245.

Roussell DL, Thompson DL, Pallardy, SG, Miles D, and Newton KJ. 1991. Chloroplast structure and function is altered in the NCS2 maize mitochondrial mutant. Plant Physiology 96:232-238.

Sakamoto W, Kondo H, Murata M, and Motoyoshi F. 1996. Altered mitochondrial genome expression in a maternal distorted leaf mutant of Arabidopsis induced by chloroplast mutator. Plant Cell 8:1377-1390.

Sandhu APS, Abdelnoor RV, and Mackenzie SA. 2007. Transgenic induction of mitochondrial rearrangements for cytoplasmic male sterility in crop plants. Proceedings of the National Academy of Science of the United States of America 104:1766-1770.

Sarria R, Lyznik A, Vallejos E, and Mackenzie SA. 1998. A Cytoplasmic male sterility-associated mitochondrial peptide in common bean is post-tranlationally regulated. Plant Cell 10:1217-1228.

Sia EA and Kirkpatrick DT. 2005. The yeast MSH1 gene is not involved in DNA repair aor recombination during meiosis. DNA repair 4:1581-1590.

Skinner DJ, Baker SC, Meister RJ, Broadhvest J, and Schneitz K. 2001: The Arabidopsis HUELLONLOS gene, which is essential for normal ovule development, encodes a mitochondrial ribosomal protein. Plant Cell 13:2719-2730.

Small ID, Isaac PG, and Leaver CJ. 1987. Stoichiometric differences in DNA molecules containing the atpA gene suggest mechanisms for the generation of mitochondrial genome diversity in maize. EMBO Journal 6:865-869.

Small ID, Suffolk R, and Leaver CJ. 1989. Evolution of plant mitochondrial genomes via substoichiometric intermediates. Cell 58:69-76.

Small I, Wintz H, Akashi K, and Mireau H. 1998. Two birds with one stone: genes that encode products targeted to two or more compartments. Plant Molecular Biology 38:265-277.

Small ID, Peeters N, Legeai F, and Lurin C. 2004. Predotar: A tool for rapidly screening proteomics for N-terminal targeting sequences. Proteomics 4:1581-1590.

Spellman PT and Rubin GM. 2002. Evidence for large domains of similarly expressed genes in the Drosophila genome. Journal of Biology 1:5.

Stern K. 1961. Über den Erfolg einer über drei Generationen geführten Auslese auf frühes Blühen bei Betula verrucosa. Silvae genetica 10:48-51.

Strassburger E, Noll F, Schenck H, and Schimper AFW. 1971. Lehrbuch der Botanik. –842 s. G. Fischer Verlag. Stuttgart.

Stupar RM, Lilly JW, Town CD, Cheng Z, Kaul S, Buell CR, and Jiang J. 2001. Complex mtDNA constitutes an approximate 620 kb insertion on Arabidopsis thaliana chromosome 2: Implication of potential sequencing errors caused by large unit repeats. Proceedings of the National Academy of Science of the United States of America 98:5099-5103.

Sung ZR, Chen L, Moon YH and Lertpiriyapong K. 2003. Mechanisms of floral repression in Arabidopsis. Current Opinion in Plant Biology 6:29-35.

Teixera RT, Farbos I and Glimelius K. 2005: Expression levels of meristem identity and homeotic genes are modified by nuclear-mitochondrial interactions in alloplasmic male-sterile lines of Brassica napus. Plant Journal 42:731-742.

Telfer A, Bollman KM, and Poethig RS. 1997. Phase change and the regulation of thricome distribution in Arabidopsis thaliana. Nature 408:796-815.

Telfer A and Poethig RS. 1998. HASTY: A gene that regulates the timing of shoot maturation in Arabidopsis thaliana. Development 125: 1889-1898.

Theissen G. 2001. Development of floral organ identity: stories from the MADS house. Current Opinion in Plant Biology 4:75-85.

Theissen G, Becker A, Di Rosa A, Kanno A, Kim JT, Münster T, Winter K-U, and Saedler H. (2000). A short history of MADS-box genes in plants. Plant Molecular Biology 42:115-149.

Unseld M, Marienfeld JR, Brandt P, and Brennicke A. 1997. The mitochondrial genome of Arabidopsis thaliana contains 57 genes in 366,924 nucleotides. Nature Genetics 15:57-61.

Weigel D and Meyerowitz EM. 1994. The ABCs of floral homeotic genes. Cell 78:203-209.

Weigel D and Nilsson O. 1995. A developmental switch sufficient for floral initiation in diverse plants. Nature 377:495-500.

Weigel D, Alvarez J, Smyth DR, Yanofsky MF, and Meyerowitz EM. 1992. LEAFY controls floral meristem identity in Arabidopsis. Cell 69:843-859.

Williams EJB and Bowles DJ. 2004. Coexpression of Neighboring Genes in the Genome of Arabidopsis thaliana. Genome Research 14:1060-1067.

Zaegel V, Guermann B, Le Ret M, Andrés C, Meyer D, Erhard M, Canaday J, Gualberto JM, and Imbault P. 2006. The Plant-specific ssDNA binding protein OSB1 is involved in the stoichiometric transmission of mitochondrial DNA in Arabidopsis. Plant Cell 18:3548-3563.