Meiosis in flowering plants and other green organisms

C. Jill Harrison*, Elizabeth Alvey and Ian R. Henderson*
Department of Plant Sciences, University of Cambridge, Downing Street, Cambridge CB2 3EA, UK
* To whom correspondence should be addressed: E-mail: cjh97@cam.ac.uk and irh25@cam.ac.uk

Received 17 February 2010; Revised 21 May 2010; Accepted 25 May 2010

Abstract

Sexual eukaryotes generate gametes using a specialized cell division called meiosis that serves both to halve the number of chromosomes and to reshuffle genetic variation present in the parent. The nature and mechanism of the meiotic cell division in plants and its effect on genetic variation are reviewed here. As flowers are the site of meiosis and fertilization in angiosperms, meiotic control will be considered within this developmental context. Finally, we review what is known about the control of meiosis in green algae and non-flowering land plants and discuss evolutionary transitions relating to meiosis that have occurred in the lineages giving rise to the angiosperms.

Key words: Meiosis, organogenesis, recombination, sex, sporangia.

Introduction

Man has selected from natural genetic variation to breed crop species useful for agriculture. Considerable genetic diversity remains to be fully exploited, including variation capable of increasing yield and broadening crop range. Sustainable increases in agricultural yield in the range of 50% will be required to feed the 9 billion people estimated to exist by 2050 (The Royal Society, 2009). To increase the efficiency of crop breeding, it is important to understand the mechanism by which variation is generated and transmitted. Variation is generated in a specialized, reductive cell division termed meiosis during which recombination between homologous chromosomes, termed crossover (CO), occurs. CO frequency varies within and between species and can be limiting such that useful variation is not accessible for breeding. Although the majority of crops are angiosperms in which meiosis occurs within floral organs, non-flowering plants have provided novel insights into the control of plant meiosis. The nature of the meiotic cell division in plants, the genetic mechanisms that promote variation, and the developmental context in which meiosis occurs in land plants and their closest sister groups are reviewed here.

Meiotic cell divisions

Meiosis is a mode of cell division specific to eukaryotic organisms whereby four haploid daughter cells are produced from a single diploid parent cell (Villeneuve and Hillers, 2001; Hamant et al., 2006; Mezard et al., 2007). This reductive division is achieved by a single round of DNA replication followed by two rounds of chromosome segregation and cell division (meiosis-I and meiosis-II) (Fig. 1). Meiosis appears to be an ancestral trait within eukaryotes and is speculated to have arisen close to the group’s origin (Villeneuve and Hillers, 2001; Cavalier-Smith, 2002). This idea is strengthened by observations that many unicellular eukaryotes, once presumed to be asexual, have since been found to possess conserved meiosis-specific genes (Ramesh et al., 2005; Malik et al., 2007). The first meiotic division differs dramatically from mitosis as homologous chromosomes pair before segregation. During the second division sister chromatids segregate to opposite poles in the same manner as during mitosis. While homologues are paired during meiosis-I they are tightly associated via the synaptonemal complex that forms along their length (Page and Hawley, 2004). Meiosis-specific expression of an endonuclease, SPO11, causes a large number of double strand breaks (DSBs) along the paired chromosomes (Villeneuve and Hillers, 2001). A subset of these break sites are repaired through recombination pathways that lead to physical exchange between the paired chromosomes (CO) (Villeneuve and Hillers, 2001; Hamant et al., 2006; Mezard et al., 2007).
can be visualized cytologically as chiasmata (Armstrong et al., 2009). Independent segregation of maternal and paternal chromosome sets during meiosis in combination with CO between chromosomes means that gametes are likely to possess novel combinations of genetic variation (Fig. 1).

**Programmed DNA breakage and repair**

An essential first step in achieving CO is the generation of DSBs throughout the genome by SPO11. SPO11 is related to the A subunit of archaeabacterial topoisomerase IV, which acts to relieve torsion in DNA by generating transient DSBs (Villeneuve and Hillers, 2001; Cavalier-Smith, 2002). Hence, components of the meiotic recombination machinery may have been recruited from prokaryotic DNA repair mechanisms. Two related orthologues SPO11-1 and SPO11-2 are non-redundantly required for meiotic DSBs in Arabidopsis thaliana (Grelon et al., 2001; Stacey et al., 2006; Hartung et al., 2007). The spo11-1 and spo11-2 mutants lack meiotic DSBs and show an absence of homologue pairing and synapsis, meaning that univalent chromosomes segregate at meiosis-I (Grelon et al., 2001; Stacey et al., 2006; Hartung et al., 2007). As univalent chromosomes segregate independently from their homologous partner, spo11 mutants show a high incidence of chromosomally unbalanced gametes (Grelon et al., 2001; Stacey et al., 2006; Hartung et al., 2007). The PUTATIVE RECOMBINATION INITIATION DEFECTS1 (PRD1), PRD2 and PRD3 genes are also necessary for SPO11-dependent DSB formation and cause univalent segregation when mutated (De Muyt et al., 2007, 2009). PRD1 and PRD3 share sequence similarity with known meiotic proteins, mammalian Mei1 and rice PAIR1, respectively, but perform unknown functions during DSB formation (Libby et al., 2002; Nonomura et al., 2004; De Muyt et al., 2007, 2009).

A second functional class of genes, including MND1, AHP2, RAD50, RAD51C, XRCC3, MRE11, and COM1/SAE2, share a mutant phenotype of meiotic chromosome fragmentation without synapsis, which is suppressed when combined with spo11 or prd3. (Schommer et al., 2003; Bleuyard et al., 2004; Bleuyard and White, 2004; Puizina et al., 2004; Li et al., 2004; Kerzendorfer et al., 2006; Panoli et al., 2006; Uanschou et al., 2007; Vignard et al., 2007). This indicates that these proteins act to process DSBs during meiotic recombination, and unrepaired DSBs cause chromosome fragmentation. Meiotic DSB processing proceeds via break site resection to form 3' single-stranded DNA, which is used to invade the intact duplex of a paired homologous chromosome (Bhatt et al., 2001; Villeneuve and Hillers, 2001; Hamant et al., 2006; Mezard et al., 2007). Invasion of DNA duplexes by ssDNA requires the DMC1 and RAD51 recombinases, which are related to prokaryotic RecA DNA strand exchange proteins (Couteau et al., 1999; Masson and West, 2001; Villeneuve and Hillers, 2001; Cavalier-Smith, 2002; Li et al., 2004). Though RAD51...
and DMC1 are both thought to act at the step of ssDNA invasion, rad51 mutants show SPO11-dependent chromosome fragmentation, whereas dmc1 mutants show univalent segregation (Couto et al., 1999; Li et al., 2004). One explanation for this difference may be that DSBs are repaired using sister chromatids in dmc1, but not in rad51 (Couto et al., 1999; Li et al., 2004).

DSBs lead to CO via formation of a double Holliday junction (dHJ) between paired homologous DNA duplexes (Villeneuve and Hillers, 2001). Subsequent to strand invasion, a series of events are required for dHJ formation, including second end capture and DNA synthesis and ligation (Villeneuve and Hillers, 2001). These junctions are ultimately resolved into CO events via an unknown dHJ resolvase in plants, although the structure-specific endonucleases GEN1/Yen1 and SLX4 serve this function in animals (Svensen and Harper, 2010). An excess of DSBs are generated by SPO11 relative to the number of CO events ultimately observed. The remaining DSBs are repaired via a gene conversion (also known as a non-crossover, NCO) pathway, which does not involve the exchange of flanking genetic markers (Villeneuve and Hillers, 2001). The synthesis-dependent strand annealing pathway appears to be a major mechanism for NCO formation in Saccharomyces cerevisiae, which acts independently of dHJ formation (McMahill et al., 2007). In S. cerevisiae the decision to repair DSB sites as CO or NCO events is made early in prophase-I, prior to dHJ formation, so it is unlikely that the CO/NCO choice represents alternative processing of dHJs (Allers and Lichten, 2001; Borner et al., 2004).

Control of meiotic crossover frequency

The position of COs can be influenced by other COs on the same chromosome via a phenomenon known as interference. In interference one event inhibits the formation of adjacent events in a distance-dependent manner meaning that they are more widely distributed than expected at random (Sturtevant, 1915; Muller, 1916; Copenhaver et al., 2002). However, non-interfering COs that have a random distribution also occur (Copenhaver et al., 2002). In A. thaliana class I interfering COs are the majority (75–85%) and class II non-interfering COs the minority (15–25%) (Copenhaver et al., 2002; Higgins et al., 2004; Mercier et al., 2005; Berchwitz et al., 2007; Higgins et al., 2008a). Class I COs in A. thaliana require a set of genes including MSH4, MSH5, MLH3, MER3/ROCK-N-ROLLERS, PARTING DANCERS, ZIP4/SPO22, RPA1a and SHOC1 (Higgins et al., 2004, 2008b; Chen et al., 2005; Mercier et al., 2005; Jackson et al., 2006; Wijeratne et al., 2006; Che lysheva et al., 2007; Macaisne et al., 2008; Osman et al., 2009). Knockout of these genes results in a dramatic reduction in CO frequency and the events that remain are randomly distributed. The ancestry of meiotic proteins in prokaroytic DNA repair is again evident as MSH4 and MSH5 proteins are related to bacterial MutS mismatch repair proteins (Villeneuve and Hillers, 2001; Cavalier-Smith, 2002). Class II non-interfering COs require the MUS81 gene, which encodes a protein similar to structure-specific endonucleases (Berchowitz et al., 2007; Higgins et al., 2008a).

Paired chromosomes generally show at least one CO event, the obligate CO, that is required for proper patterns of chromosome segregation in A. thaliana (Grelen et al., 2001). Each A. thaliana chromosome shows approximately 1.8 CO per meiosis and very few chromosomes show no CO (Copenhaver et al., 1998, 2002; Higgins et al., 2004; Drouaud et al., 2006, 2007). CO position is highly variable and hot- and cold-spots of CO frequency exist along the chromosomes (Copenhaver et al., 1998, 1999; Drouaud et al., 2006, 2007). Pronounced increases in CO frequency and gene density are observed towards the telomeres of wheat and maize chromosomes (Liu et al., 2009; Saintenac et al., 2009; Schnable et al., 2009). A recombination hotspot at the maize Bronze (Bz) locus lies in a gene-rich region close to the telomere. This region shows 40–80-fold higher CO rates than the genome average and is flanked by large stretches of nested retrotransposon insertions that infrequently crossover (Dooner and Martinez-Ferez, 1997; Fu et al., 2001, 2002). Repetitive regions flanking centromeres are also suppressed for CO, and centromere-proximal events associate with chromosome mis-segregation (Koehler et al., 1996; Lamb et al., 1997; Deng and Lin, 2002; Rockmill et al., 2006).

Differences in crossover frequency may be accounted for by epigenetic information. For example in mouse and S. cerevisiae H3 K4 trimethylation marks DSB hotspots, and disruption of this modification reduces DSBs (Borde et al., 2009; Buard et al., 2009). Conversely, in A. thaliana the CO-cold repetitive sequences flanking the centromere are transcriptionally silenced using epigenetic information including DNA cytosine methylation and targeted DNA methylation in Ascobolous immersus is sufficient to repress CO frequency several hundred fold (Maloisel and Rognan, 1998; Zhang et al., 2006; Zilberman et al., 2007). Repetitive insertions and inversions can also locally suppress CO, and play an important functional role in genome organization (Dooner, 1986; Nary et al., 1998). This is illustrated by CO suppression at sex chromosomes, mating-type loci and self-incompatibility loci, where it is important to maintain linkage between genes required for opposite mating/incompatibility types (Ferris and Goodenough, 1994; Casselman et al., 2000; Ming and Moore, 2007; Bergero and Charlesworth, 2009). Hence, CO frequency is likely to be determined by a combination of local DNA sequence, trans-factors, and epigenetic information.

Control of meiotic cell cycle progression

Meiosis requires the modification of mitotic cell cycle control, such that a single S-phase is followed by two sequential rounds of chromosome segregation. Progression through the cell cycle is controlled by cyclins that interact with and activate cyclin-dependent kinases (CDKs) which mediate stepwise transitions through the cycle via phosphorylation (Huntley and Murray, 1999). Several genes
implicated in the regulation of meiotic progression have been identified in *A. thaliana*. A novel, cyclin-like gene **SOLO DANCERS (SDS)** is specifically expressed during meiosis and sds mutants show defects in chromosome pairing, segregation, and CO (Azumi et al., 2002). Recently, sds mutants have been observed to form DSBs but repair them efficiently, most likely via RAD51-mediated inter-sister repair (De Muyt et al., 2009). The novel gene **OMISSION OF SECOND DIVISION** (*OSD1*) is required for meiosis-II and *osd1* produces diploid dyad products of meiosis instead of haploid tetrads (d’Erfurth et al., 2009). A temperature-sensitive substitution allele of **CYCLIN A1:2**, termed **tardy asynchronous meiosis** (*taml*) causes a delay in meiotic progression, which also leads to dyad formation (Magnard et al., 2001; Wang et al., 2004). Regulation of protein stability is critical for cell cycle control and SKP1-like F-box proteins act to promote ubiquitination and destruction of target proteins, including cyclins (Bai et al., 1996). Consistently, mutations in the SKP1-related gene **ASK1** show defects in meiotic chromosome segregation (Yang et al., 1999a; Zhao et al., 2006). In animals, cell cycle checkpoints cause later events to depend upon the completion of earlier events (Murakami and Nurse, 1999). Meiotic checkpoint mechanisms have not been genetically defined in *A. thaliana*, although *msb4* and *asy1* show a significant delay in meiosis, suggesting feedback on the regulation of meiotic progression (Higgins et al., 2004; Sanchez-Moran et al., 2007). Together these results demonstrate that defects in cell cycle progression can disrupt meiosis.

Key early events in meiosis are the identification of homologous chromosome partners, pairing, and formation of the synaptonemal complex (SC) (Page and Hawley, 2004). Homologous partner identification occurs by unknown mechanisms during prophase-I. In polyplody species pairing is complex as homologues must also avoid pairing with related homeologues (Martinez-Perez et al., 1999). For instance, in hexaploid wheat *Phl* affects the stringency of homologue/homeologue discrimination by influencing chromatin remodelling associated with pairing and localization of the SC component **ASY1** (Martinez-Perez et al., 1999; Prieto et al., 2004, 2005; Boden et al., 2009). *Phl* maps to a repetitive locus containing cell cycle-dependent kinase genes, which causes down-regulation of unlinked **CDK** genes (Griffiths et al., 2006; Al-Kaff et al., 2008). As related **CDK** genes in mammals influence meiotic progression, **trans**-silencing of **CDKs** by *Phl* could lead to changes in homologue pairing (Ortega et al., 2003). Pairing may depend on DSB formation as in *A. thaliana* or may occur via achiasmate mechanisms as in female *Drosophila melanogaster* (Grelo et al., 2001; Page and Hawley, 2004). **SWITCH/DYAD** encodes a novel protein expressed during prophase-I, necessary for chromosome pairing, synopsis, and recombination and in *swi1/dyad* mutants univalents segregate at meiosis-I. Studies of a maize homologue, **AMEIOTICI** indicate that these functions are conserved within angiosperms (Golubovskaya et al., 1993; Mercier et al., 2001, 2003; Agashe et al., 2002; Ravi et al., 2008; Pawlowski et al., 2009). **POOR HOMOLOGOUS SYNAPSISI** encodes a second novel protein required for chromosome pairing and synopsis, which causes high levels of non-homologous pairing when mutated (Pawlowski et al., 2004; Ronceret et al., 2009).

Co-incident with pairing, the SC forms between homologous chromosomes (Page and Hawley, 2004) and SC components identified in *A. thaliana* include **ASYNAPTICI** (*ASY1*), **ZYP1A, ZYP1B, SCC3**, and **REC8/DIF1/SYN1** (Bai et al., 1999; Bhatt et al., 1999; Cai et al., 2003; Chelysheva et al., 2005; Higgins et al., 2005). **ASY1** and **ZYP1** show distant identity with the animal **HOP1** and **ZIP1** SC proteins, respectively (Caryl et al., 2000; Armstrong et al., 2002; Higgins et al., 2005). Loss of SC proteins causes failures in synopsis and CO formation, and can lead to univalent segregation and chromosome fragmentation (Bai et al., 1999; Bhatt et al., 1999; Caryl et al., 2000; Armstrong et al., 2002; Cai et al., 2003; Chelysheva et al., 2005; Higgins et al., 2005). Interestingly, increases in CO frequency are associated with increases in total SC length via an unknown mechanism (Lynn et al., 2002; Drouaud et al., 2007). These genetic mechanisms provide novel insights into the interrelated processes of homologue recognition, pairing, and synopsis in plants.

Correct patterns of chromosome segregation are required to generate balanced gametes and depend on regulation of the SC and chromosome cohesion. During mitosis the cohesion complex holds sister chromatids together until the SCC1 subunit is cleaved by **SEPERASE1** at anaphase, allowing chromosome segregation (Uhlmann et al., 1999). **REC8/DIF1/SYN1** is a meiosis-specific orthologue of SCC1 and the **rec8/dif1/syn1** mutant disrupts normal SC localization of **SCC3** (a cohesin subunit shared with mitosis) (Bhatt et al., 1999; Cai et al., 2003; Chelysheva et al., 2005). As in animal systems, *A. thaliana* **REC8** is cleaved by the cysteine protease **SEPERASE1** (ESPI) (Liu and Makaroff, 2006). Cohesion in the chromosome arms is released by **ESPI** at anaphase-I, but maintained at the centromeres until anaphase-II (Liu and Makaroff, 2006). In maize, centromeric **REC8/AFD1** is protected from **ESPI** destruction by the conserved Shugoshin protein (**SGO1**) during anaphase-I, but maintained at the centromeres until anaphase-II (Liu and Makaroff, 2006). **SGO1** is then removed and **REC8** is destroyed at the centromere during anaphase-II to allow chromatid segregation. Step-wise formation and removal of connections between homologues is thus required for correct patterns of meiotic chromosome segregation and recombination.

**The developmental context for meiosis**

Successful completion of meiosis and sexual life cycles depends on activation of the meiotic cell cycle at the correct developmental time and place. In many animals separation of a dedicated germ cell lineage from somatic cell types occurs during embryogenesis, a distinction not observed in some early diverging animal lineages and plants (Gilbert, 1994; Dickinson and Grant-Downton, 2009). In the algal sister groups to land plants single-celled zygotes undergo...
meiosis immediately following fertilization (Fig. 2A). By contrast, in all land plants mitotic divisions intercede fertilization and meiosis, and meiosis occurs after a period of diploid development in specialized structures termed sporangia that produce numerous spores (Bower, 1935; Becker and Marin, 2009). The initiation of sporangium development pathways often follows a switch in meristem identity from a vegetative to a reproductive fate (Steeves and Sussex, 1989). The location and structure of sporangia vary by plant group and are associated with their secondary functions, which are spore dispersal and nutrition. Heteromorphic sporangia and spores have evolved convergently in vascular plants and associate with specialized functions (Fig. 2). Female megasporangia have fewer, larger spores (megaspores) that may be retained within the parent plant after fertilization, whereas male microsporangia develop numerous small spores (microspores) that have a dispersal function (Bower, 1935). Gender can also influence patterns of CO frequency (Drouaud et al., 2007). Thus the initiation and progression of meiosis depend on the developmental identity of the tissue in which it is activated, discussed by plant group below.

**Meiosis in chlorophyte and charophyte algae**

In both chlorophyte and charophyte algal sister groups to the land plants meiosis occurs immediately following fertilization (Becker and Marin, 2009). In the single-celled chlorophyte alga, *Chlamydomonas reinhardtii* two haploid mating types, *plus* and *minus*, differentiate into gametes which fuse during fertilization to form a single-celled zygote.
meiosis in charophytes is via an unknown mechanism. Currently no charophyte genetic models, potential roles for constitutive expression of that do not initiate meiosis can also be generated, and zygote development in the absence of fertilization (Zhao in the opposite gamete type is sufficient to trigger by plant group (Lewis and McCourt, 2004; McCourt et al. 2008). Following fertilization GSP1 and GSM1 heterodimerize, translocate to the nucleus, and initiate zygotic gene expression patterns (Lee et al. 2008). Constitutive expression of either GSP1 or GSM1 in the opposite gamete type is sufficient to trigger zygote development in the absence of fertilization (Zhao et al., 2001; Lee et al., 2008). Stable C. reinhardtii diploids that do not initiate meiosis can also be generated, and constitutive expression of GSP1/GSM1 together in these cells is sufficient to induce meiosis with normal patterns of recombination (Lee et al., 2008). This indicates that GSP1/ GSM1 homoeodomain proteins are potential triggers of meiosis in a chlorophyte alga. In contrast to chlorophytes, charophytes have a multicellular haploid body that generates free-swimming sperm in antheridia and egg cells that are retained within an oogonium on the parent plant (Fig. 2B). Egg retention (oogamy) is an innovation shared between algae and bryophytes that has recently accelerated due to the establishment of moss (Physcomitrella patens) and liverwort (Marchantia polymor-

**Protracheophytes and seed plant sister groups**

Key features that distinguish vascular plants from bryophytes are the elaboration of an indeterminately growing and branching diploid body (Mishler and Churchill, 1985; Donoghue, 2005; Langdale and Harrison, 2008). Fossil plants whose form is not represented in living plants, such as Cooksonia, have low orders of branching and may have amplified spore numbers by increasing numbers of terminal sporangia (Fig. 2B) (Edwards and Feehan, 1980; Graham et al., 2000; Donoghue, 2005; Gerrienne et al., 2006). These fossils raise interesting questions about the developmental nature of the association between axis development, sporangium number, initiation, and development are perturbed. These defects may arise as a consequence of abnormal sporophytic development, although spore number and germination are also highly variable in the mutants, suggesting meiotic defects (Tanahashi et al., 2005). Similarly Class I KNOX mutants in P. patens have abnormal sporangia and reduced spore numbers (Sano et al., 2005; Sakakibara et al., 2008). Interestingly KNOX expression is sporophyte specific in P. patens (Champagne and Ashton, 2001; Sakakibara et al., 2008), and a potential role of KNOX and BEL genes in diploid development conserved between algae and bryophytes remains to be explored.

**Sporophyte development and meiosis in bryophytes**

In contrast to their algal sisters, all land plants have a period of multicellular diploid growth, the extent of which varies by plant group (Lewis and McCourt, 2004; McCourt et al., 2004; Becker and Marin, 2009). The bryophyte sister groups to the vascular plants exhibit limited post-embryonic development with no indeterminate apical growth (Mishler and Churchill, 1985; Shaw and Renzaglia, 2004; Donoghue, 2005). Sporophytes comprise a small single stem with a terminal sporangium that represents the simplest basal land plant body plan (Kenrick, 2002; Donoghue, 2005; Qiu et al., 2006; Boyce, 2008) (Fig. 2). In liverworts and mosses sporangium development arrests diploid growth, whereas hornwort sporophytes contribute to their own nutrition and have sporangia that grow indeterminately from a basal meristem (Boyce, 2008; Kato and Akiyama, 2005). A superficial epidermal archesporial cell layer is specified during sporangium development and divides either by meiosis to generate spores (mosses) or spore mother cells and interspersed elater cells that perform nutritive or dispersal functions (liverworts and hornworts). The tissues surrounding the archesporial cell layer perform dispersal functions specific to each bryophyte group (Bower, 1935). The genetic and developmental mechanisms that regulate bryophyte sporophyte development are currently poorly understood, but interest has recently accelerated due to the establishment of moss (Physcomitrella patens) and liverwort (Marchantia polymor-

---

(Fig. 2B) (Lee et al., 2008). Plus and minus gamete identity is specified from the MATING-TYPE locus and requires cytoplasmic accumulation of BEL [GAMETE-SPECIFIC PLUS1 (GSP1)] and KNOX [GAMETE-SPECIFIC MINUS1 (GSM1)] class homeodomain proteins, respectively (Ferris and Goodenough, 1994; Lee et al., 2008). Following fertilization GSP1 and GSM1 heterodimerize, translocate to the nucleus, and initiate zygotic gene expression patterns (Lee et al., 2008). Constitutive expression of either GSP1 or GSM1 in the opposite gamete type is sufficient to trigger zygote development in the absence of fertilization (Zhao et al., 2001; Lee et al., 2008). Stable C. reinhardtii diploids that do not initiate meiosis can also be generated, and constitutive expression of GSP1/GSM1 together in these cells is sufficient to induce meiosis with normal patterns of recombination (Lee et al., 2008). This indicates that GSP1/ GSM1 homoeodomain proteins are potential triggers of meiosis in a chlorophyte alga. In contrast to chlorophytes, charophytes have a multicellular haploid body that generates free-swimming sperm in antheridia and egg cells that are retained within an oogonium on the parent plant (Fig. 2B). Egg retention (oogamy) is an innovation shared with land plants thought to have been a key adaptation in their evolution (McCourt et al., 2004). As there are currently no charophyte genetic models, potential roles for KNOX/BEL genes are unexplored, and the initiation of meiosis in charophytes is via an unknown mechanism.
on leaves (Fig. 2B). With the exception of the leptosporangiate and whisk ferns, nutritive tapetal tissues arise from non-sporogenous tissue (Bower, 1935; Parkinson, 1987). As in bryophytes, the genetic basis of diploid development is poorly characterized in lycophytes and monilophytes. Notably sporophytic KNOX expression is conserved, and meristematic expression domains suggest likely roles in indeterminate growth (Bharathan et al., 2002; Harrison et al., 2005; Sano et al., 2005), although reproductive roles have not yet been explored. Thus the structure and dispersal functions of sporangia vary broadly across the land plants and the developmental context for the initiation of meiosis is lineage specific. An evolutionary trend towards the amplification of spore numbers by alterations in body plan, sporangium size, and the number of sporangia is apparent (Bower, 1935).

**Sporophyte development in seed plants**

In seed plants (gymnosperms and angiosperms) a prolonged period of vegetative growth is followed by the reproductive transition. This transition involves a change in meristem identity and leads to the development of cones or flowers (Steeves and Sussex, 1989). Seeds develop in the context of the ovule following fertilization of the female egg cell by a male sperm cell transferred in pollen, thus dispersal functions are provided both by haploid pollen and diploid seed. Ovules are the site of megasporangium (nucellus) development, which precedes meiosis. Whilst in gymnosperms one to several nucellar cells enter meiosis, in angiosperms a single megaspore mother cell undergoes meiosis to form a tetrad, three members of which degenerate to form a single functional megaspore, which divides mitotically to form the embryo sac (Campbell, 1940; Colombo et al., 2008). Pollen sac (microsporangium) development occurs from a microsporophyll or in the anther in gymnosperms and angiosperms respectively. In both, sub-epidermal cells are specified as archesporial cells that divide periclinally to form a layer of parietal cells surrounding the sporogenous cells (Campbell, 1940; Feng and Dickinson, 2007). Sporogenous cells may then either directly enter meiosis or continue to proliferate. Parietal cells divide further to form a variable number of concentrically arranged cell layers, the innermost of which differentiates into the nutritive tapetum (Campbell, 1940; Feng and Dickinson, 2007). During meiosis sporogenous cells become encased in an impermeable callose ((1-3)-β-D-glucan) wall, which later breaks down when members of the microspore tetrads are released (Gifford and Foster, 1988). Callose appears to play an important role in sporogenesis, as tapetal expression of callase causes male sterility in tobacco (Worrall et al., 1992). Following meiosis, the resulting haploid microspores undergo mitosis and differentiate into pollen grains.

The genetic control of vegetative development, the reproductive transition, and sporangium formation are well studied in the angiosperm *A. thaliana*. Activity of the class I KNOX gene SHOOTMERISTEMLESS (STM) is necessary for the establishment of an indeterminate meristem (Long et al., 1996), and STM and BREVIPEDICELLUS (BP) act redundantly to maintain indeterminacy and repress determinate leaf development (Byrne et al., 2002). Class I KNOX proteins promote indeterminacy by dimerizing with BEL transcription factors and triple berringer, poundfoolish, Arabidopsis thaliana homeobox1 BEL mutants phenocopy <em>stm</em> mutants (Rutjen et al., 2009). Thus, in *A. thaliana* KNOX and BEL genes play key roles in elaboration of the diploid body, providing the context for later reproductive development and meiosis. Flower development follows conversion of indeterminate, vegetative shoot meristems to reproductive fates. This switch is controlled by a large network of genes that ensure reproduction is co-ordinated with environmental and developmental conditions (Baurle and Dean, 2006). These signalling pathways converge on a key set of transcription factors required for floral meristem identity, including LEAFY and APETALA1 (Baurle and Dean, 2006). Floral organ identity genes encode three functional classes of MADS-box transcription factors (A, B, and C) that are expressed in overlapping domains to specify the four floral organ types (Coen and Meyerowitz, 1991). Stamens identity requires overlapping expression of the B and C class MADS genes <em>PISTILLATA</em> and <em>APETALA3</em>, and <em>AGAMOUS</em> (AG) (Yano’sky et al., 1999; Jack et al., 1992; Goto and Meyerowitz, 1994). Carpel identity requires activity of the C class gene AG, which acts in conjunction with three additional MADS proteins, SEEDSTICK, SHATTERPROOF1, and SHATTERPROOF2 to specify ovule identity (Yano’sky et al., 1999; Pinyopich et al., 2003). The KNOX genes STM and KNAT2, and BEL1 genes also play roles in the specification of carpel and ovule identity (Modrusan et al., 1994; Pautot et al., 2001; Scofield et al., 2007). Thus genes involved in meristem identity also play roles in the specification of reproductive fate.

In both micro- and megasporangial archesporial cell specification precedes sporangium formation (Gifford and Foster, 1988). The mechanisms of archesporial cell specification are poorly understood, but one gene, SPOROCYTELESS/NOZZLE (SPL/NZZ), functions directly downstream of the C class organ identity gene AG to promote sporogenesis (Schieflhaler et al., 1999; Yang et al., 1999b; Ito et al., 2004). SPL is a nuclear protein with distant homology to MADS box transcription factors that performs an unknown function (Schieflhaler et al., 1999; Yang et al., 1999b). spl mutants differentiate microsporangial archesporial cells that divide once, but fail to form microsporocytes or tapetal cells, causing sterility (Schieflhaler et al., 1999; Yang et al., 1999b). The <em>spl</em> mutants are also female sterile due to nucellar defects, meaning that the archesporial cells do not differentiate and meiosis fails to initiate (Schieflhaler et al., 1999; Yang et al., 1999b). Ectopic activation of SPL in <em>agamous</em> mutants is sufficient to induce staminoid development and pollen formation (Ito et al., 2004). Together this indicates that <em>SPL/NZZ</em> performs an upstream meiotic function in both male and
female development. Male sporocyte identity in *A. thaliana* is not yet clear. It is regulated by the leucine-rich repeat (LRR) receptor kinase EXTRA SPOROCYTES CELLS/EXCESS MICROSPOROCYTES1 (EXS/EMS1) in conjunction with its small protein ligand TAPETUM DETERMINANT1 (TPD1) (Yang et al., 1999b; 2003; Canales et al., 2002; Zhao et al., 2002). The first archesporial cell division normally separates reproductive sporocyte fate from non-reproductive wall and tapetal fates. Microsporangial development is altered in *exs/ems1* and *tpd1* mutants such that sporogenous cells develop at the expense of tapetal cells (Yang et al., 1999b; 2003; Canales et al., 2002; Zhao et al., 2002). This implies that EXS/EMS1 kinase signalling is important either to promote tapetal or to repress sporogenous cell identity. Although *exs/ems1* mutations in *A. thaliana* show normal meiasporangial development, mutations in the rice homologue *MULTIPLE SPOROCYTES1 (MSP1)* show supernumerary sporocytes in both the anther and ovule, as does the *multiple archesporial cells1 (mac1)* mutant in maize (Sheridan et al., 1996; Nonomura et al., 2003). Interestingly, additional LRR receptor kinases have also been implicated in proper differentiation of the anther cell layers (Albrecht et al., 2005; Colcombet et al., 2005; Mizuno et al., 2007; Hord et al., 2008). How these signalling processes are organized between the cell types within the developing anther is not yet clear.

**Future perspectives**

Developmental genetic studies in *A. thaliana* have significantly advanced our understanding of the context in which meiosis arises in plants. Future goals will be to tie together our understanding of the context, initiation, and progress of meiosis in diverse plant groups so that potential variation can be released to breeding. During plant diversification, genes that may have originally been involved in reproductive development have been co-opted to vegetative development pathways. Whilst KNOX/BEL proteins may trigger meiosis in a chlorophyte alga, their role is unknown in charophytes and most bryophytes and thus the point of functional diversification remains to be identified. The mechanisms for archesporial cell development are not yet fully characterized in flowering plants and are unknown in non-flowering plants. The derivation of nutritive tapetal tissues in different plant groups may or may not be independent of archesporial lineages. It will be interesting to test homology between archesporial and tapetal cell types by testing the function of *SPL, EXS*, and *TPD1* homologues in different lineages. A detailed picture is emerging of the mechanisms that control plant meiotic chromosome pairing, synopsis, recombination, and segregation in *A. thaliana*. Understanding how these mechanisms integrate during progression of the meiotic cell cycle will be a major challenge. Equally, the pattern of CO hot- and cold-spots is complex and the mechanisms that determine plant CO frequency remain to be determined. Knowledge of these mechanisms may allow CO to be targeted during crop breeding and facilitate the generation of novel high-yielding agricultural strains.

**Acknowledgements**

We thank the Royal Society and the Gatsby Charitable Foundation for funding, Karen Van Winkle-Swift, Vasily Kantsler, Li Zhang, Jenny Morris, and Dianne Edwards for photographs, and two anonymous reviewers for helpful comments on the manuscript.

**References**


Higgins JD, Buckling EF, Franklin FC, Jones GH. 2008a. Expression and functional analysis of AtMUS81 in Arabidopsis meiosis reveals a role in the second pathway of crossing-over. The Plant Journal 54, 152–162.


Higgins JD, Vignard J, Mercier R, Pugh AG, Franklin FC, Jones GH. 2008b. AtMSH5 partners AtMSH4 in the class I meiotic crossover pathway in Arabidopsis thaliana, but is not required for synopsis. The Plant Journal 55, 28–39.


Sano R, Juarez CM, Hass B, Sakakibara K, Itô M, Banks JA, Hasebe M. 2005. KNOX homeobox genes potentially have similar...
function in both diploid unicellular and multicellular meristems, but not in haploid meristems. *Evolution and Development* 7, 69–78.


Zhao D, Yang X, Quan L, Timofejeva L, Rigel NW, Ma H, Makaroff CA. 2006. ASK1, a SKP1 homolog, is required for nuclear reorganization, presynaptic homolog juxtaposition and the proper distribution of cohesin during meiosis in Arabidopsis. *Plant Molecular Biology* 62, 99–110.

