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P4C1–PLANT REPRODUCTIVE BIOLOGY

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P4/C1.1–Meiosis, recombination and chromosomes

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Meiosis, a process including reciprocal recombination and halving of the chromosome number, leading to gamete production, is an essential part of the life cycle of sexually reproducing higher organisms. Meiosis normally involves causally linked recognition, alignment and synapsis of homologous chromosomes, synaptonemal complex (SC) formation, recombination, and the regular disjunction of homologues during metaphase I. Most molecular genetics, including cloning and characterization of meiotic genes, has been in yeast, where there are an estimated 200–300 meiotic genes of which 50 are specific to recombination alone. In yeast, based on extensive genetics, sequence data and mutants, valuable models have been advanced for meiotic control, chromosome pairing, recombination and segregation. More limited work has shown parallels in *Drosophila* and mammals, but only now do we have the data and methodology to start to show that only parts of the yeast model, based on a genome size of 14 Mbp, can be applied to plants. In particular, pairing models and timing and distribution of meiotic recombination need to be reconsidered. Using molecular cytogenetics methods, we have examined the behavior of chromosomes and the physical organization of specific DNA sequences during meiosis showing that genome organization and meiosis are tightly linked having both consequences for genetic mapping, species evolution, hybrid or polyploidy reproduction, generating variation and seed production.

P4/C1.2–Characterization of Asy1, a meiotic protein associated with the synaptonemal complex in *Arabidopsis*

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We are currently dissecting the events that occur prior to and during chromosome synapsis in prophase I of meiosis in *Arabidopsis thaliana*. *ASY1* is one of several genes under investigation that is required for synapsis. Antibody raised against recombinant *Asy1* expressed in *E. coli* has been used to investigate the expression and localization of the protein in male and female meiocytes in *Arabidopsis* and the closely related crop species *Brassica oleracea*. This has revealed that *Asy1* and its *Brassica* orthologue *bAsy1* begin to accumulate during premeiotic interphase. Expression increases, peaking at leptotene before gradually decreasing towards the later stages of meiosis. Immunolocalization of *Asy1/bAsy1* on meiotic chromosome spreads using light microscopy indicates that in both species the protein accumulates as punctate foci. As meiosis progresses labeling is clearly associated with chromosome axes as an extended signal. This is maintained throughout pachytene but begins to disappear as the homologues desynapse. This suggests that *Asy1* is closely associated with an integral component of the synaptonemal complex (SC). To further clarify this, EM studies using immunogold labeled Ab were undertaken on anthers of *Brassica* from the pre-leptotene to pachytene stages. These studies reveal that *Asy1* is a SC associated protein rather than an integral component of the SC complex.

P4/C1.3–Analysis of meiotic telomere functions in maize

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During meiotic prophase, telomeres actively attach to the nuclear envelope and cluster in a chromosome end arrangement called the bouquet. The bouquet is unique to meiosis, conserved among most eukaryotes, and thought to facilitate homologous chromosome synapsis and recombination. To better understand the role of telomeres during meiosis, we have used 3-dimensional molecular cytology to characterize the nuclear architecture of pollen mother cells fixed at different stages of meiotic prophase. Telomere FISH and chromosome painting studies in maize and oats defined the onset of telomere clustering as just prior to homolog synapsis at the zygotene stage. In a search for bouquet mutants, we examined previously isolated maize meiotic mutants

using 3-D telomere FISH as a phenotypic assay. We report our new findings here. Two mutants, *desynaptic* (*dy*) and *desynaptic1* (*dysl*), exhibit aberrant telomere behavior. The *dy* mutation is associated with premature detachment of telomeres from the nuclear envelope at pachytene. Alleles of *dysl* are associated with a subtle but reproducible partial bouquet phenotype. However, the *desynaptic2* mutation does not disrupt telomere distribution. The absence-of-first-division mutants appear to lack all aspects of synapsis, including the bouquet stage. The *ameiotic1-pral* mutants lack a bouquet, because they arrest prior to meiotic prophase. In summary, the analysis of 3-D FISH data yielded new information regarding the phenotypes of several meiotic mutants. Specifically, *dy* and *dysl* mutants may be disrupted for gene products that play a direct role in meiotic telomere functions.

P4/C1.4—Isolation and characterization of genes essential for *Arabidopsis thaliana* meiosis

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In order to elucidate the mechanisms and the regulation of meiosis in higher plants, *Arabidopsis thaliana* mutants obtained after T-DNA transformation were screened for defects in fertility. An average of one meiotic mutant per 1000 lines was recovered. Mutants selected so far show defects in various aspects of the meiotic program, and two of them will be presented here.

The *Saccharomyces cerevisiae* SPO11 protein catalyses DNA double strand breaks (DSBs) that initiate meiotic recombination. In plants in which the *AtSPO11-1* (one of the three *Arabidopsis SPO11* homologue) gene is disrupted, both male and female meiocytes show an asynaptic phenotype that has been associated with a drastic reduction of meiotic recombination. Our data show that initiation of meiotic recombination by SPO11-induced DSBs is a mechanism conserved in plants and that SPO11 is necessary for normal synapsis in plants.

We have characterized a new gene *SWI1*, involved in sister chromatid cohesion during both male and female meiosis in *Arabidopsis thaliana*. In *swi1.2* male meiosis, chromatid arms and centromeres lose their cohesion in a stepwise manner before metaphase I and 20 chromatids instead of 5 bivalents are seen at the metaphase I plate. In contrast, *swi1.2* female meiocytes perform a mitotic-like division instead of meiosis, indicating a different effect of the loss of SWI1 function in both processes. SWI1-GFP fusion indicates that SWI1 protein is present in meiocyte nuclei, before meiosis and at very early stage of prophase suggesting a role of SWI1 in the sister chromatid cohesion establishment.

P4/C1.5—Early meiosis in *Arabidopsis*

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The timing and progression of early meiosis in *Arabidopsis* has been analysed with a combination of immunological and fluorescence in situ hybridisation (FISH) techniques.

The duration of meiotic stages was established using a 2 h pulse of the thymidine analogue BrdU to label the meiotic S-phase. Hourly sampling of inflorescences after the pulse and detection of BrdU in pollen mother cells with an antibody to BrdU, enabled us to establish a time course for meiosis in *Arabidopsis*. Labelling in early leptotene first appeared 16 h after the pulse, suggesting that there is a long G2 stage of up to 16 h. Zygotene and pachytene were labelled from 20 h and up to 24 h after the pulse. In an earlier study, (Armstrong et al, 2001) we demonstrated that telomere pairing preceded chromosome synapsis. We have extended this study to look at chromosome behaviour with a series of single copy probes along specific chromosomes, particularly in the S/G2 phase and early prophase, by using BrdU to establish the cell stage and FISH to mark the chromosome. In addition, we have included in our analysis detection of specific antibodies, particularly against Asy1 and Rad 51, in relation to both cell stage and chromosome behaviour, in order to develop a comprehensive description of early meiosis in *Arabidopsis*.

Reference:

Armstrong SJ, Franklin FCH, Jones GH. 2001. Nucleolus-associated telomere clustering and pairing precede meiotic chromosome synapsis in *Arabidopsis thaliana*. *J. Cell Science*. 114, 407–4217.

P4/C1.6—Genetic analysis of asymmetric division during male gametophyte development

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The first post-meiotic division of the microspore is highly asymmetric and produces two unequal daughter cells, the vegetative and generative cells that have very different structures and developmental fates. The larger vegetative cell produces the pollen tube, whereas the smaller generative cell divides once to produce the two sperm cells. Several gametophytic mutants that affect the onset and symmetry of this division have been isolated using morphological screens, including: *sidecar pollen* (*scp*), *gemin pollen1* (*gem1*) and *two-in-one* (*tio*) (1). In the *gem1* mutant ultrastructural analysis has revealed that microspore cytokinesis and karyokinesis are uncoupled and that complex internal wall profiles occur as a result of aberrant cytokinesis (2). Positional cloning and complementation has revealed that *GEMINI POLLEN1* is identical to the recently described *MICROTUBULE ORGANISATION 1* gene in *Arabidopsis* which

is required for the maintenance of cortical microtubule arrays (3). GEM1/MOR1 belongs to the ch-TOG/XMAP215 family of microtubule-associated proteins that play an important role in spindle microtubule dynamics. Our phenotypic studies reveal functions for GEM1/MOR1 in the control and organisation of both male and female gametophytic cytokinesis and in patterns of cell division during early embryogenesis.

References:

1. Twell et al., (1998) Trends Pl. Sci. 3, 305–310.
2. Park and Twell (2001), Plant Physiol. 126, 899–909.
3. Whittington et al., (2001) Nature, 411, 610–613.

P4/C1.7—Role of the pistil in pollen tube guidance

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The topic of pollen tube guidance is a controversial one. A recent finding supports the role of the synergids in the crucial guidance cue for the pollen tube tip at the micropyle but experimental evidence for other single ‘guidepost’ cells in the pistil is still lacking. The fact that the pollen tube must first travel through the matrices of the stigma and style before it can respond to the cue from the ovule makes it likely that there is a hierarchy of signaling events in pollen/pistil interactions starting at the stigma and ending at the micropyle. Recent work on the pollen tube side provides evidence for the signal transduction machinery necessary for this interaction but we know little about the specific receptors in the pollen tube that could be responding to signals in compatible pistils. On the pistil side several model systems have emerged where specific molecules are implicated in either physical or chemical guidance. On the stigma, physical cues involving lipids have been proposed to orient germination of pollen in tobacco. A gradient of glycosylation of the TTS protein in tobacco styles plays a role in guidance. In lily, which has a hollow style, adhesion molecules (pectin and SCA) are implicated in guidance. SCA alone is capable as well of inducing pollen chemotropism in an in vitro assay suggesting that this peptide plays a dual role in lily pollination, chemotactic in the stigma and haptotactic in the style.

P4/C1.8—Small GTPases in pollen tube vesicular transport

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Pollen tube elongation depends on the secretion of large amounts of membrane and cell wall materials at the pollen tube tip in order to sustain rapid growth. A large family of RAS-related small GTPases, Rabs or Ypts, is known to regulate both antero- and retrograde trafficking of transport vesicles between different endomembrane compartments and the plasma membrane in mammalian

and yeast cells. Studies on the functional roles of analogous plant proteins are emerging. We are working on a number of tobacco pollen Rab GTPases, each presumably functioning at a different step in vesicle trafficking. GFP fusions to each of these Rabs are being used for localization studies to have an initial assessment of the functional location for these Rabs. The functions of these small G proteins are being probed by expressing their dominant negative forms in transformed pollen tubes. We will discuss mainly our work on two different Rabs—NtRab2 and NtRab11. NtRab2 regulates trafficking between ER and Golgi while NtRab11 has a primary role in post-Golgi trafficking. We will discuss how dominant negative forms of these two small G-proteins affect the trafficking of model and native pollen tube proteins and pollen tube growth in vitro and in vivo within the pistil.

P4/C1.9—Pollen-pistil interaction and pollen tube growth in Solanaceae

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Two major types of pollen-pistil interactions have been well characterized, one that occurs in most Solanaceae and one that is typical of Brassicaceae. In the former, when the pollen lands on the stigma, it comes in contact with the fluid exudate secreted by the specialized cells of the secretory zone. This type of stigma is known as ‘wet’ stigma. By contrast, in the latter type of interaction, the pollen lands on a ‘dry’ stigma devoid of fluid secretion but provided with very pronounced papillae. In spite of the morphological differences, pollen-pistil interactions on ‘wet’ as well as on ‘dry’ stigmas rely on the presence of lipids. These are the major component of the fluid exudate on ‘wet’ stigmas, whereas are carried on the coat of the pollen grain in species with ‘dry’ stigmas. To further investigate the possible analogies in these two types of pollen pistil interactions, we are presently characterizing the proteins secreted in the fluid exudate of *Nicotiana tabacum* and are analyzing their putative role in pollination.

P4/C1.10—Extracellular calcium, pH and boron alter growth oscillations in *Lilium formosanum* pollen tubes

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Pollen tubes exhibit pronounced oscillations in their rate of growth, which may be due to periodic changes in the yielding properties of the cell wall. Calcium (Ca^{2+}), hydrogen (H^+) and boron are three growth essential elements that may achieve their effects by modifying cell wall structure. Ca^{2+} and boron, by forming cross-links

between pectin residues, would stiffen the wall, while H^+ , through the down regulation of pectin methyl-esterase, would soften the wall. Here we demonstrate that extracellular concentrations of Ca^{2+} , H^+ and H_3BO_4 affect not only the average growth rate of lily pollen tubes, but also influence the period, and in some instances the amplitude, of growth rate oscillations. Ca^{2+} produces maximal average growth ($0.27 \mu m s^{-1} = 16.2 \mu m min^{-1}$) and the shortest periods of oscillation (17.55 s) at 0.05 mM, while H_3BO_4 has a similar effect at concentrations between 1.6 and 3.2 mM. With H^+ , optimal growth occurs at pH 5.5–6.0; increasing extracellular pH decreases the period of oscillation. The amplitude of oscillations is most influenced by changes in extracellular H_3BO_4 and pH, while extracellular Ca^{2+} has no significant effect on this parameter. Our results indicate that Ca^{2+} and boron may act similarly to alter the yield threshold of the wall, wherein the optimal concentrations are high enough to impart rigidity, which is sufficient to prevent bursting in the face of cell turgor, but low enough to allow the wall to stretch quickly during periods of accelerating growth.

P4/C1.11—The pollen tube growth and reorientation. A model to study network signalling pathways in plant cells

R. Malhó, L. Camacho, University of Lisbon

The role of cytosolic free calcium ($[Ca^{2+}]_c$) in pollen tube growth has received a great deal of attention in recent years. It is however, only one of the components of an intricate signalling network, which controls this tip-growing cell (Annals of Botany. 85 (suppl A): 59–68). Other molecules such as InsP3, calmodulin, CDPKs and, more recently, cAMP have all been found to play an important role in growth and guidance. In this talk we will analyse our data which suggest that Ca^{2+} and cAMP signalling pathway crosstalk. We will also discuss the implications of our finding that the pollen adenylate cyclase-PSiP (Proc. Natl. Acad. Sci. USA 98: 10481–10486)-shows strong homologies to defence signalling proteins.

In our search of the different targets for these signalling pathways, we obtained also data that indicates a crosstalk between $[Ca^{2+}]_c$ and GTPase activity which seems to mediate exo and endocytosis activity. These findings will be discussed and incorporated into a more general model of pollen tube growth and reorientation.

P4/C1.12—Pollen profilin: a complex regulator of actin dynamics

C.J. Staiger, D.R. Kovar, F. Chaudhry and T. Matsumoto, Purdue University, USA

A remarkable example of cellular morphogenesis, pollen germination and tube growth, is essential for plant reproduction. In order to deliver non-motile sperm to the embryo sac, the vegetative cell of the pollen grain forms a tip-growing protuberance that extends at rates up to 1 cm/h. A dynamic network of filaments and associated proteins, the actin cytoskeleton, organizes the pollen cytoplasm, provides the molecular railroad tracks for cytoplasmic streaming, and orchestrates the delivery of secretory vesicles to the growing apex. To understand the molecular mechanism of tip growth, we have focused attention on a gene family for the actin monomer-binding protein, profilin, from the crop plant *Zea mays*. Our central hypothesis is that profilin is a fundamental regulator of actin polymerization and its cellular function is mediated through interactions with a diverse set of binding partners. We have characterized the interaction of recombinant and native maize profilins with three major ligands (G-actin, PtdIns(4,5)P₂ and polyproline) and discovered several important differences between plant and non-plant profilins and among plant profilin isoforms. Live cell and in vitro mutagenesis studies demonstrate that binding to both actin and proline-rich sequences is important for profilin's cellular function. Furthermore, we show that the sequestering activity of profilin is regulated by calcium. Our results are consistent with two distinct populations of profilin playing different roles during pollen tube growth.

P4/C1.13—Stimulus-responsive cytoskeletal proteins

P.J. Hussey, Biological Sciences, University of Durham, UK

The plant cytoskeleton is a highly dynamic, fibrous structure crucial to many cellular processes including cell division, cell expansion, cytoplasmic streaming and the alignment of cellulose microfibrils in the plant cell wall. This structure is stimulus responsive being effected by internal stimuli, by biotic and abiotic stresses in signal transduction pathways still to be fully assembled. The completion of the Arabidopsis genome sequence has allowed for a comparative identification of many cytoskeletal proteins and components of signal transduction cascades. In many cases the pathways may be similar to their animal and fungal counterparts but the signals in plants are quite unique. Precise control of the cytoskeleton is essential for plant cell morphogenesis and my group has concentrated on the identification of proteins that bind and regulate the structure of the actin and microtubule networks. Understanding the mechanisms by which these proteins respond to internal and external signals is the main goal of this research.

P4/C1.14—Control of pollen tube growth by a Rop GTPase signaling network

Z. Yang, Y. Gu, Y. Fu, V. Vernoud and G. Wu, University of California, Riverside, USA

Pollen tubes extend by tip growth—an extreme form of polar growth. Using an integrated approach in *Arabidopsis* and tobacco pollen tubes, we have demonstrated that a plant-specific Rho-family GTPase, Rop1, acts as a critical switch to activate polar growth in pollen tubes. Our evidence suggests that this switch is controlled by a positive feedback loop of Rop activation and recruitment at the plasma membrane (PM) that is initiated locally and amplified laterally by unknown mechanisms. This feedback loop is inhibited globally by a co-ordinate action of Rop GTPase-activating proteins and guanine nucleotide dissociation inhibitors to generate a tip-high gradient of active Rop proteins at the apical PM domain. Our results suggest that the active Rop is to specify the apical PM domain for polar growth as well as to activate polar growth via the regulation of both dynamic tip F-actin and tip-focused cytosolic calcium gradients. Using green fluorescence protein-tagged mouse talin, we have shown that pollen tubes contain short actin bundles at the apex and that the dynamics of these actin bundles is critical for polar growth in pollen tubes. We are focusing on further understanding of the interplay among Rop GTPase, tip calcium and actin dynamics and identifying molecules involved in this interplay.

P4/C1.15—The pollen SI response involves actin depolymerization

B.N. Snowman, V.E. Franklin-Tong, Birmingham, C.J. Staiger, D.R. Kovar, Purdue University, West Lafayette, USA

Signal perception and the integration of signals into networks effecting cellular changes is essential for all cells. The self-incompatibility (SI) response in *Papaver rhoeas* pollen triggers a Ca^{2+} -dependent signaling cascade resulting in inhibition of pollen germination and tube growth. I will review the signalling components identified and focus on studies relating to a recently identified target of these signals: the actin cytoskeleton. We have observed SI-induced alterations in the actin cytoskeleton of both the pollen grains and pollen tubes. By measuring the amount of F-actin in pollen grains and tubes before and during the SI response, we demonstrate that SI induction results in a 56–74% reduction in F-actin levels. To our knowledge these provide the first quantitative evidence for a specific stimulus-mediated depolymerization of actin filaments in plant cells. Actin depolymerization was also achieved by treatments that raise $[\text{Ca}^{2+}]_i$ artificially. This provides a link between these two SI-induced events, suggesting that they are on the same signalling pathway. We suggest Ca^{2+} -mediated depolymerization of F-actin as a molecular mechanism

whereby SI-induced tip growth inhibition may be achieved. We have determined the cellular concentrations and binding constants for profilin, and examine its potential role during SI *in vitro* since it is currently the best candidate for mediating actin depolymerization.

P4/C1.16—S-RNase Mediated Self-Incompatibility

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Solanaceae type self-incompatibility is controlled by the multiallelic *S*-locus. To date, the only *S*-locus gene whose function has been determined is the S-RNase gene, which controls pistil function in self-incompatibility. We have used PCR differential display and subtractive hybridization to identify 13 pollen-expressed genes of *Petunia inflata* that show *S*-linked polymorphism. A genetic map of the *S*-locus has been constructed based on relative rates of recombination between each of these 13 genes and the S-RNase gene. Nine are very tightly linked to the *S*-locus, whereas the other four showed varying rates of recombination among 1,120 F₂ plants analyzed. BAC libraries have been constructed for S₁S₁ and S₂S₂ genotypes, and BAC clones for all the 13 genes have been isolated for both genotypes. To construct a BAC contig of the *S*-locus of the S₂-haplotype, we have initiated chromosome walking from different *S*-locus regions each containing one of the nine tightly linked genes. So far, we have isolated BAC clones that collectively span approximately 2 Mb; however, gaps of unknown sizes remain between contigs, suggesting a large physical size of the *S*-locus. A 328-kb contig containing the S₂-RNase gene has been completely sequenced. Sequence analysis has revealed that this region is rich in retroelements (Gypsy-type or Copia-type LTR elements) and other types of repetitive sequences. Approximately 60 putative protein-coding genes, most of which encode polyproteins and retrotransposons, have been identified by several gene prediction programs. Some of the remaining putative genes are being investigated for any possible role in self-incompatibility.

P4/C1.17—S-RNase complexes and pollen rejection

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In *Nicotiana*, S-RNases contribute to both inter- and intraspecific pollen rejection. At the intraspecific level, pollen rejection is exquisitely specific. Each allelic S-RNase causes rejection of only a single pollen-S genotype. Interspecific pollen rejection displays less specificity. Many S-RNases cause rejection of pollen

from a single species. By examining the pollen rejection phenotypes of transgenic plants expressing S-RNase we found that several pollen rejection mechanisms require non-S-RNase factors as well as S-RNase. Currently, we are attempting to identify these factors. One hypothesis is that some non-S-RNase factors may be S-RNase binding proteins. Using immobilized S-RNase as an affinity matrix we identified four style proteins from SI *Nicotiana glauca* that bind S-RNase. One protein corresponds to a small copper binding protein. At least three non-classical AGPs also occur in the bound fraction. These three glycoproteins have previously been shown to interact directly with pollen tubes and may be involved in supporting compatible pollen tube growth. Our speculation is that S-RNase dependent pollen rejection systems may also rely, at least in part, on these glycoproteins.

P4/C1.18—Ligand-receptor interaction in self-incompatibility of Brassica

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In *Brassica* self-incompatibility, recognition between pollen and the stigma is controlled by the *S* locus, which contains three highly polymorphic genes: S-receptor kinase (SRK), *S*-locus protein 11 (SP11) (or *S*-locus cysteine-rich protein, SCR) and *S*-locus glycoprotein (SLG). SRK encodes a membrane-spanning serine/threonine kinase which is expressed in the stigmatic papilla cell, and SP11 encodes a small cysteine-rich protein which is expressed in the tapetum of anther and in pollen. Functional analyses in transgenic plants have shown that SRK and SP11 control stigma and pollen S-haplotype specificity, respectively; however, it has not been determined whether SP11 interacts directly with its cognate SRK to elicit the self-incompatibility response. SLG is a secreted stigma protein that is highly homologous to the SRK extracellular domain. Although it is not required for S-haplotype specificity of the stigma, SLG enhances the self-incompatibility response; however, how this is accomplished remains controversial. In this work, we purified SP11 of S_8 haplotype, S_8 -SP11, of *B. rapa* from pollen grain, as well as chemically synthesized S_8 -SP11; immunolocalized S_8 -SP11 to the pollen coat of the mature pollen grain; determined the four intramolecular disulfide linkages of an oxidized form of S_8 -SP11; and showed that this form of S_8 -SP11 alone functioned as the pollen S-haplotype determinant in a pollination bioassay system and induced autophosphorylation of SRK₈ in vitro. Furthermore, using chemical cross-linking we showed that SRK and SLG together formed a high-affinity receptor complex for SP11 on the stigma membrane.

P4C1.19—Interacting partners of SRK in the self-incompatibility response in Brassica

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In the Brassicaceae, the self-incompatibility (SI) response-preventing self-pollination is genetically controlled by a single polymorphic locus, the *S* locus. Self-pollen recognition by the epidermal cells (papillae) of the stigma implicates a ligand-receptor interaction between the SCR (*S* locus Cysteine-Rich protein) and SRK (*S* locus Receptor Kinase) proteins. SCR is a highly polymorphic peptide carried by the pollen. SCR specifically binds to the extracellular domain of the transmembrane receptor serine-threonine kinase SRK, which functions in the stigma (Kachroo et al., Science (2001) 293, 1824–1826). Recently, we have demonstrated that SRK proteins are always found as oligomeric complexes in planta (Giranton et al., PNAS (2000) 97, 3759–3764), and that following self-pollen perception by the stigma papillae, SRK autophosphorylates (Cabrillac et al., Nature (2001) 410, 220–223). In vitro, we have also shown that the kinase activity of SRK is negatively regulated by stigma thioredoxins (THL), and that this inhibition is released by the addition of pollen coat proteins containing SCR (Cabrillac et al., Nature (2001) 410, 220–223). Here we present new interactors of the kinase domain of SRK.

P4/C1.20—Dissection of the SI reaction in Papaver rhoeas

M.J. Wheeler and F.C.H. Franklin, Biosciences, University of Birmingham

Self-incompatibility (SI) is a recognition mechanism enabling flowering plants to distinguish between self and non-self pollen enabling the prevention of self-fertilisation. The SI response of *Papaver rhoeas* (poppy) is under gametophytic control. It is proposed that genes situated at a polymorphic *S*-locus encode a pollen component and a corresponding stylar component and that the interaction between these two gene products leads to the SI response. The response in poppy depends upon the interaction of S-proteins secreted by the stigma with an as yet unidentified S-receptor on the pollen surface. Sequencing of a part of the S_1 locus has yet to reveal any evidence of pollen expressed genes within the immediate vicinity of the S_1 gene. An S_1S_3 cosmid library is currently being screened to isolate larger sections of the *S*-locus that will enable both a larger part of the *S*-locus to be screened for pollen-S candidates and a comparison of the genetic structure of two *S*-loci. It is now apparent that the *Arabidopsis* genome contains a family of genes (termed *Sph*) with homology to poppy S-genes. As *Arabidopsis* is self-compatible this gene

family is likely to be involved in other processes requiring cell-cell communication. All of the *Sph* genes encode proteins predicted to be secreted and thus are candidate ligands. As yet none of these genes has been assigned a function in *Arabidopsis* although there is evidence for their expression. We are currently investigating the function of the *Sph* genes using a combination of a genetic and immunocytological strategies.

P4/C1.21—Just how complex is the S-receptor complex?

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The last few years have seen great advances in our understanding of the molecular control of self-incompatibility (SI) in *Brassica*, a system that permits the recognition and rejection of 'self' pollen. In particular, the long-awaited identification of the pollen-borne determinant of SI has signalled the start of a new phase of research into gaining a detailed understanding of pollen recognition in this species. This ligand, termed SCR (*S*-locus cysteine-rich protein), has been shown to bind the stigmatic *S*-determinant SRK (*S*-receptor kinase) in a haplotype specific manner resulting in its activation. Activation of SRK ultimately leads to pollen rejection. Although SCR and SRK have been shown to be the sole determinants of *S*-specificity it is clear that other proteins play a role in establishing the 'active' receptor complex. SLG (*S*-locus glycoprotein), another stigmatic product of the *S*-locus, although not essential for SI has been found in some instances to complex with SRK and SCR. Weak interactions between SCR and SLG have also been reported. To add yet further complexity, SLG is known to bind another pollen coat protein, PCP-A1, which has similarities to SCR. Preliminary data suggests that PCP-A1 also binds an as yet uncharacterised stigmatic membrane protein. Although the significance of such molecular interactions is at present unclear it is tempting to speculate that they may provide insight into phenomena such as the strength of the SI phenotype, pollen adhesion and perhaps interspecific pollination relationships.

P4/C1.22—Sporophytic self-incompatibility in *Senecio squalidus* (Asteraceae)

S.J. Hiscock, S.M. McInnis, C. Henderson, D.A. Tabah, Biological Sciences, University of Bristol

We are using *Senecio squalidus* as a model species to study the genetics and molecular genetics of self-incompatibility (SI) in the Asteraceae. *S. squalidus* has a strong system of sporophytic SI (SSI) and populations within the UK contain very few *S* alleles probably on account of it being an introduced species. The genetic control of SSI in *S. squalidus* is complex and may involve a second locus epistatic to *S*. Here we report

progress towards identifying the female determinant of SSI in *S. squalidus*. Research is focussed on plants carrying two defined *S* alleles, *S*1 and *S*2. *S*2 is dominant to *S*1 in pollen and stigma. RT-PCR was used to amplify three *SRK*-like transcripts from stigmas of *S*1*S*2 heterozygotes but the expression patterns of these transcripts suggest that they are not directly involved in SI. Stigma-specific proteins associated with the *S*1 allele and the *S*2 allele have been identified using isoelectric focussing and their cDNAs cloned by 3' and 5' RACE. The proteins, designated SSP1 (Stigma *S*-associated Protein 1) and SSP2, share 95% amino acid sequence identity and their genomic clones have identical intron positions. SSP is expressed exclusively in stigmas and is developmentally regulated, with maximal expression occurring at and just before anthesis, when SI is fully functional. SSP is not expressed in small buds. We are currently investigating the linkage of SSP to the *S* locus using an RFLP marker and SNPs.

P4/C1.23—Interactions between gametophytes and between gametes in flowering plants

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We have been using two strategies to understand interactions between gametophytes and between gametes in flowering plants. First, we used an *in vitro* fertilization system in maize. We took advantage of the accessibility to the gametes to study egg activation and the initiation of development. We could show that gamete fusion triggers a cytosolic calcium increase as well as a calcium influx. Here we will present data suggesting that the influx proceeds the cytosolic increase and that these two events may be involved in two distinct signaling cascades at fertilization. As a second strategy, we defined steps in *Arabidopsis thaliana* wild type fertilization using Confocal Laser Scanning Microscopy. We could detect (a) Synergid degeneration and penetration by the pollen tube 7 h after pollination in the Columbia ecotype, (b) A fast change in egg cell polarity, (c) Karyogamy 8–9 h after pollination as detected by the transient presence of two nucleoli in the zygote nucleus as well as the endosperm nucleus. We isolated mutants corresponding to these different steps. Here we will report on the identification of two mutants affected in the penetration of the embryo sac. The 'timide' mutant shows pollen tubes reaching the embryo sac but not fertilizing it and is under male gametophytic control. The 'sirene' mutant has pollen tubes entering the embryo sac but overgrowing in the micropylar region without fertilizing the female gametophyte. The phenotype of this second mutant is under female gametophytic control.

P4/C1.24—Biparental regulation of early maize endosperm development through imprinting

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Evidence is accumulating that the male and female haploid gametes of flowering plants can differ dramatically in the epigenetic imprints they carry, and that these imprints play an important role in regulating early development. In maize (*Zea mays*) imprinting has been clearly demonstrated to control the expression of the *R*-locus, and a number of zein sequences in the endosperm, but the dramatic consequences of reciprocal interploidy crosses suggest that a significant number of genes regulating development are also under epigenetic control. To identify these genes and estimate the total number of loci expressed during early maize development that are affected by imprinting, we have used a system of allelic message display (AMD) to reveal sequences expressed in the early endosperm which are transcribed solely from the maternal, or the paternal gametic genomes. New technical strategies also now permit the expression of these sequences to be monitored during the central cell/zygote transition and early endosperm development. Furthermore, it is proving possible to use microarrays to explore the number and classes of genes affected when development is disrupted through genomic imbalance. Data from these studies, together with those from *Arabidopsis*, will hopefully throw light on the role of parental imprinting in flowering plant development, and the selective pressures responsible for its evolution.

P4/C1.25—Apomixis—the gender issue

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In the vast majority of sexually reproducing flowering plants and mammals, a specific ratio of maternally derived to paternally derived genomes is essential for normal development of the endosperm and embryo, respectively. The 2maternal:1paternal requirement in endosperm is due to epigenetic differences in the parental genomes: in either gamete genome, a complementary set of genes is silenced in a sex-specific way by genomic imprinting. This non-equivalence of gamete genomes reveals a level of sexual identity that we describe as 'epigender'. In natural pseudogamous and autonomous apomicts the endosperm develops successfully despite deviations from the usual 2m:1p ratio that would prove lethal in sexual plants. We have developed a model which explains how this so-called 'endosperm problem' is circumvented by proposing that apomicts modify the epigender of the polar nuclei. In autonomous apomicts, for example, removal of the imprints from the maternally derived endosperm genome leads to extensive paternalisation of the polar nuclei allowing the expres-

sion of normally maternally silenced genes, and effectively supplies the missing paternal genome. As a proof of concept, we have shown that in *Arabidopsis*, a combination of the *fertilisation independent endosperm (fie)* mutation and hypomethylation of the genome (which inhibits imprinting) creates such a situation in the endosperm genome. As a result, in a *fie* mutant, hypomethylated ovules undergo complete autonomous endosperm in the absence of fertilisation. More subtle changes to epigender may account for the success of endosperm development in pseudogamous apomicts.

P4/C1.26—Applications of Apomixis

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The development of apomixis as a practical breeding tool for the major crops is a major technical challenge which will test the combined skills of the enlightened plant science disciplines. Whether the goal is achieved via introgression of traits from natural apomicts or by the reconfiguration of reproductive mechanisms through gene manipulation and mutation, the foreseen benefits lie in the enhancement of FI hybrid breeding strategies through the stabilisation of F1 progenies, especially those derived from diverse or exotic germplasm and non-inbred parents. Formal breeding approaches as well as strategies for the deployment of plant varieties will need to be reconsidered to take account of an unprecedented bounty of rapidly accessible variation and of locally adapted hybrid lines. Much will depend upon the design and fine-tuning of a set of tools for switching the apomictic habit on or off without interference in other aspects of plant performance, in particular the specifics of endosperm yield and composition. A further key design parameter will be compatibility with both large and small-scale breeding activities. There is an opportunity in this regard, via participatory tool development, to reinforce local and small scale breeding enterprise and to empower local agronomic and land-race knowledge in varietal selection.

P4/C1.27—Functional analysis of Arabidopsis Cohesins

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The *Arabidopsis* genome encodes most components of the cohesin complex, critical for chromosome segregation in eukaryotes. In order to dissect the role of cohesins in plant meiosis, we have identified and characterised components of the cohesin complex in *Arabidopsis* and are generating loss of function mutants for specific cohesin subunits. Unlike yeast, *Arabidopsis* has four different *REC8/RAD21* homologs [1,2]-*DIF1*, *AtRAD21-1*, *AtRAD21-2* and *AtRAD21-3*, all with con-

served N and C terminal domains, but of different sizes (617, 809, 693 and 1031 amino acids, respectively). The functional analysis of these, *DIF1* (*determinate, infertile 1*), with a function essential for meiosis, and *AtRAD21-1*, *AtRAD21-2*, *AtRAD21-3*, with functions that are currently unknown, will be presented. In *dif1* mutants the defects in meiosis, are first evident by metaphase I, when *dif1* pollen mother cells show extensive chromosome fragmentation and univalents. DIF1 specific antibodies and GFP fusions are being used to analyse the localisation of DIF1. Reducing the expression of *AtRAD21-1* through anti-sense expression has no effect on transgenic plants, suggesting that *AtRAD21-1* is not essential for mitosis or meiosis. Other putative components of the *Arabidopsis* cohesin complex have also been identified and cloned; these include *Arabidopsis* homologs of the *SMC1* and *SMC3* gene. The functions and interactions of these putative cohesin genes is being analysed through the isolation of T-DNA and transposon insertions, and RNAi mediated silencing.

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P4/C1.28—Chiasma frequencies in different ecotypes of *Arabidopsis thaliana* (L.)

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Chiasma frequencies were analysed in metaphase I pollen mother cells (PMCs) of eight different accessions of *Arabidopsis thaliana* (L.) to look for differences in total recombination levels genome-wide and the distribution of recombination events through the genome. Fluorescence in situ hybridisation (FISH) with 45S rDNA and 5S rDNA as probes was used to identify the five chromosome pairs in the different ecotypes analysed. Ring bivalents predominated in the metacentric/submetacentric chromosomes (1,3 and 5), whereas acrocentric chromosomes (2 and 4) formed mostly rod bivalents and of these the majority were bound via their long arms. The mean chiasma frequencies of the different bivalents are proportional to chromosome size. A statistical analysis of mean cell chiasma frequencies confirmed that accessions included in this study differ significantly for chiasma frequency and that the pattern of chiasma distribution between arms and among chromosomes is not consistent over accessions. When the analysis of variance was carried out omitting accessions Cvi and Ler (both with lower chiasma frequencies), the remaining accessions did not differ significantly for chiasma frequency. We concluded that both Cvi and Ler have significantly lower chiasma frequencies than the other six accessions included in this study.

P4/C1.29—Characterization of meiotic proteins associated with synapsis and recombination in *Arabidopsis*

N. Jackson, S.A. Armstrong, G.H. Jones and F.C.H. Franklin, University of Birmingham, UK

The synapsis and recombination of homologous chromosomes during prophase I of meiosis involves a highly complex and closely integrated set of events. Historically plants have proved particularly amenable to cytogenetic approaches that have enabled extensive studies of these meiotic processes. However, in contrast to yeast, comparatively little progress has been made in higher plants towards characterising the molecular and biochemical events associated with synapsis and recombination. The emergence of *Arabidopsis* as a model plant system for molecular studies has presented an opportunity to rectify this situation, hence, several groups are now engaged in studies aimed at the isolation and characterisation of plant meiotic genes. Using a range of experimental approaches we have identified and cloned several meiotic genes (eg *RAD51*, *DMC1*, *MLH1*, *ASY1* etc). These genes have been expressed in *E. coli* to obtain recombinant proteins that have in turn been used to raise antibodies. The expression profile of the proteins has been investigated by western analysis. More detailed studies are now in progress using immunolocalisation techniques in conjunction with a modified spreading procedure for meiotic chromosomes isolated from *Arabidopsis* and *Brassica oleracea*. Progress in establishing the spatial and temporal distribution of these proteins from pre-leptotene to the end of meiosis I will be presented.

P4/C1.30—Identification of transposon-tagged progametic phase genes in *Arabidopsis*

E.B. Lalanne, J.A. Johnson, R. Patel and D. Twell, University of Leicester

The progametic phase of reproductive development involves post-pollination events from pollen germination to gamete fusion which are not easily accessible. As a strategy for the identification of progametic mutations, which act gametophytically, we have used transposon insertional mutagenesis based on screening for distorted segregation of an antibiotic resistance marker. Screening of 3,616 transposon lines allowed the identification of 19 potential gametophytic mutations. Cosegregation tests indicated that these transposon insertions are tightly linked to the reduced transmission phenotype. Three male-specific mutants, *pgp1*, *pgp2* and *pgp3*, produce pollen with normal cellular morphology, but fail to transmit the insertion during the progametic phase. No failed ovules or aborted seeds were observed suggesting no effect on fertilisation events. *PGP1* codes for a putative

plasma membrane protein containing a protein-protein interaction domain. PGP2 shows significant similarity with a sugar phosphate isomerase involved in capsule synthesis in bacteria. PGP2 could modify surface-associated polysaccharides and play a role in cell surface interactions. PGP3 presents significant similarity with RPT2 and NPH3, two signal transducers of the phototropic response. These proteins may function as scaffold proteins to bring together the enzymatic components of signalling pathways. Progress on genetic, phenotypic and functional analysis of these mutants will be presented.

P4/C1.31—Characterisation of two genes acting during the progamic phase in *Arabidopsis thaliana*

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The progamic phase of reproductive development involves a complex pathway of extracellular and intracellular processes from pollen germination to gamete fusion. In order to identify essential gametophytic genes, which function in this pathway a screen was performed involving segregation ratio distortion for an antibiotic resistance marker (Howden et al., *Genetics* 149,621). We screened a population of 3,616 Ds transposon insertion lines (Sundaresan et al., *Genes Dev* 9,1797) generated in the laboratory of Ueli Grossniklaus. Among 19 selected gametophytic mutants 8 produce pollen with normal cellular morphology and were classed as progamic phase mutants (pgp). For two of these mutants, pgp5 and pgp6, cosegregation tests indicated that their insertions are tightly linked to the reduced transmission phenotype. Reciprocal crosses show that pgp6 does not transmit the antibiotic resistance marker through the male whereas only 0.8% male transmission is observed for pgp5. Both mutants show normal transmission through female. The insertion in pgp5 is present within the 3' UTR of a gene encoding a putative serine/threonine protein kinase of 39 kDa. The insertion in pgp6 disrupts the coding region of a gene encoding a putative protein of 73 kDa with similarity to importin alpha, a protein involved in nuclear import. Complementation studies to date have confirmed that the pgp6 mutation is responsible for the reduced transmission phenotype by transformation with a JAtY clone (GARNet) and a gene-specific PCR-amplified fragment. Progress on the phenotypic and functional analysis of two of these mutants will be presented.

P4/C1.32—Gametophytic mutations affecting cytokinesis at pollen mitosis I

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To improve our understanding of molecular components and mechanisms involved in pollen cytokinesis, two EMS-derived gametophytic cell division mutants are being characterized. The mutants, named two-in-one (tio) produce ~35% aberrant pollen grains containing either two free nuclei within the same cytoplasm, a single fusion nucleus, or collapsed pollen at the mature pollen stage. Reciprocal testcrosses revealed that genetic transmission of both tio mutations was completely blocked through the male and very limited (~10%) through the female. Developmental analysis revealed that binucleate pollen in tio results from the failure of cytokinesis at pollen mitosis I. In common with sporophytic mutants that affect cytokinesis during embryogenesis (keule, knolle, and cyt1), tio mutants produce incomplete dividing walls attached to the parental cell wall. Developmental defects were also observed during megagametogenesis by cytological analysis of cleared whole-mount ovules indicating a lack of cellularisation at the micropylar pole of the embryo sac. Both tio mutations were mapped to the same location (~72 cM) on chromosome 1, suggesting that they are allelic mutations. The molecular analysis of TIO would help to define its precise role in cytokinesis and provide valuable insight into the molecular mechanisms of gametophytic cytokinesis.

P4/C1.33—Genetic analysis of sperm cell formation in *Arabidopsis*

Durberry and D. Twell, University of Leicester, UK

In flowering plants microgametogenesis leads to sperm cell formation and it is a key process for sexual reproduction and plant fertility. To understand the mechanisms that govern sperm cell formation six independent gametophytic division mutants were isolated from an EMS-mutagenised pool. The mutants, termed duo pollen mutant (duo), specifically affect the generative cell division. The duo mutants produce pollen that contains a vegetative nucleus and a single generative-like cell. In duo1, duo2 and duo3 mutants the generative-like cell is round and highly condensed whereas in duo4, duo5 and duo6 the generative cell is frequently elongated. Detailed studies on duo2 revealed that the duo pollen mutant never enter mitosis. Map based cloning showed that these mutations mapped to different chromosomal locations. Analysis of genetic transmission performed by reciprocal test cross reveal normal transmission through the female and limited or no transmission through the male. Studies on these mutants will provide valuable insight into the mechanisms controlling the generative cell division.

P4/C1.34—Identification and characterization of novel genes involved in Arabidopsis pollen tube growth

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In order to study male gametophyte development in *Arabidopsis*, pollen defective mutations have been scored from the Versailles T-DNA transformants collection, on the basis of a T-DNA transmission defect to the selfed progeny (Bonhomme et al, 1998, MGG 260:444; Procissi et al, 2001, Genetics 158: 1773). Two types of mutations have been isolated which affect pollen tube growth: 1/ *pok* (*poky pollen tube*), leading to reduced and/or slowed pollen tube elongation, and for which only one mutant is available, at the hemizygous state, and 2/ *kip* (*kinky pollen*), which leads to twisted (kinky) pollen tubes and misshapen root hairs (in homozygous lines), and for which 3 allelic forms have been found. *POK* and *KIP* genes expression pattern is unexpectedly large since transcripts of both genes are found in pollen grains, but also in almost all sporophytic tissues. Overall expression level is low for both genes, however, higher in roots and flower buds. Encoded *POK* and *KIP* protein sequences count, respectively, 695 and 2587 aa, and although putative orthologs are found in most organisms, their functions remain enigmatic. However, both *POKY POLLEN TUBE* and *KINKY POLLEN* novel proteins are for sure crucial for tip growth process.

P4/C1.35—Characterization of expressed genes in the SLL2 region of self-compatible Arabidopsis thaliana

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Self-incompatibility in Brassica species is regulated by a set of *S*-locus genes, *SLG*, *SRK*, and *SP11/SCR*. In the vicinity of the *S*-locus genes, several expressed genes, *SLL2* and *SP2/ClpP*, etc., were identified in *B. campestris*. *Arabidopsis thaliana* is self-compatible Brassica relative, and its genome sequence was completed. From comparison of the genomic sequences between *B. campestris* and *A. thaliana*, microsynteny between gene clusters of *Arabidopsis* and Brassica *SLL2* regions was observed, though the *S*-locus genes, *SLG*,

SRK, and *SP11/SCR* were not found in the region of *Arabidopsis*. Almost predicted genes in this region of *Arabidopsis* were expressed in both vegetative and reproductive organs, suggesting that the genes in the *SLL2* region might not related to self-incompatibility. Considering the recent speculation that the *S*-locus genes was translocated as a unit between *Arabidopsis* and Brassica, the translocation might have occurred in the region between *SLL2* and *SP7* genes.

P4/C1.36—The molecular basis of self-compatibility in Petunia hybrida

P. Wright, K. Clarke and T.P. Robbins, University of Nottingham

Self-incompatibility is a widespread genetic mechanism by which hermaphrodite plants prevent self-fertilisation and promote outbreeding. *Petunia hybrida* has a gametophytic self-incompatibility system typical of the Solanaceae family. The maternal component, a protein that has ribonuclease (RNase) activity, is only produced in the stigma and style. The self-incompatibility RNase (*S*-RNase) gene is located within a complex of genes (the *S*-locus) that includes an unidentified pollen recognition gene. Two of these proteins (*Sb* and *So*) have previously been shown to have N-terminus similarity and on further DNA sequence analysis the two alleles have been shown to be identical. In spite of this, *SoSo* homozygotes are self-compatible whilst *SbSb* homozygotes are self-incompatible. When the *So* (compatible) gene is crossed into an *Sb* background a restoration of function can be shown. Conversely, when the *Sb* gene is crossed into the *So* background a loss of function is observed. The differential response is primarily due to a difference in the pollens response to *S*-RNases present. The difference between the two lines is genetic in origin and can be shown to segregate independently from the *S*-locus. There is preliminary evidence for these modifying genes affecting the methylation of the *S*-locus. The significance of these findings and the possible implication for a role of imprinting in the breakdown of SI systems will be discussed.

P4/C1.37—Transgenic analysis of gametophytic self-incompatibility in Petunia Hybrida

C. Moore, K. Clarke, and T. P. Robbins, University of Nottingham

Self-incompatibility causes the rejection of self-pollen in preference to cross pollen. *Petunia hybrida* is one of the many species in the Solanaceae having the gametophytic system of self-incompatibility. A single multi-allelic *S*-locus encodes a pistil specific ribonuclease (*S*-RNase) that is secreted into the extracellular matrix of the transmitting tract. It is proposed that the *S*-RNase degrades the ribosomal RNA of the extending pollen tube, thus

halting the incompatible pollen. The aim of this project is to distinguish between two models, which propose that either the S-RNase is taken into the pollen tube in an allele specific manner, or allele specific inhibition takes place within the pollen tube itself. This will be achieved by expressing an S-RNase gene in pollen under the control of a tomato pollen specific promoter (LAT52).

As a preliminary investigation the expression pattern of the LAT52 promoter will be determined in petunia, this will test whether the pollen specificity originally observed in tobacco and tomato is retained. This is being achieved with a LAT52:GUS binary vector, using transformation of both self-compatible (V26 and W115) and self-incompatible (S_vS_v and S_3S_3) lines. Full-length genomic S-RNase sequences will be used to prepare complete LAT52:S-RNase constructs for both S_v and S_3 alleles that will be transformed into the self-compatible and self-incompatible petunia lines. The ectopic expression of S-RNase in the pollen of regenerated plants in self-incompatible lines with matching and non-matching S-alleles will allow a test of the inhibitor model of gametophytic self-incompatibility.

P4/C1.38—Incompatibility studies in plum

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This project aims to deepen understanding of self-incompatibility in two species of Rosaceae, diploid myrobalan plum (*Prunus cerasifera*) and hexaploid domestic plum (*P. domestica*). Although domestic plum is of much greater economic importance, its incompatibility relationships are complicated greatly by polyploidy. Self-incompatible myrobalan plum was included as a simple preliminary model. Our aim is to test whether genotypes revealed by molecular methods can be correlated with known incompatibility relationships.

Total pistil RNA was isolated from five myrobalan accessions and RT-PCR performed using degenerate primers based upon conserved peptide S-RNase sequences in sweet cherry (*P. avium*) and almond (*P. dulcis*). Products of ~600bp were cloned and six different products were identified from the myrobalans with close nucleotide and deduced peptide identity with published almond and cherry S-RNases. Consensus primers based on conserved regions in almond, sweet cherry and Japanese apricot (*P. mume*) were used on genomic DNA of 22 plum cultivars and three myrobalan accessions. So far another four alleles have been identified from myrobalan plum and five from plum.

Sequence comparisons show these 15 sequences share features common to other *Prunus S* sequences. Alleles of both species have five conserved regions (C1–C5) and a hypervariable region containing an intron of variable length. Aligning partial sequences (C2–C5) with other *Prunus* revealed that plums and myrobalans do not

form distinct clusters but overlap with sweet cherry, almond and Japanese apricot. These new sequences confirm that *Prunus S* alleles form a group distinct from the *Malus/Pyrus* group in the Rosaceae.

P4/C1.39—cDNA microarray is powerful tool for identification of reproductive organ-specific genes in model legume, Lotus japonicus

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To gain understanding of the molecular mechanisms of the sexual organ development, cDNA microarray was used to isolate cDNA clones, which specifically expressed during the development of the sexual organs (anther and pistil) in model legume *Lotus japonicus*. We compared the expression pattern among anthers (stages 1, 2 and 3), pistils (stages 1, 2 and 3) and leaves using cDNA microarray, which was fabricated from 4048 cDNA clones derived from the flower bud library. After cluster analysis with microarray data, we found 22 independent cDNA clones, which were specifically or predominantly expressed in immature anther, and 111 independent cDNA clones, which were specifically or predominantly expressed mature anther. Approximately half of these cDNA clones showed no significant similarity to known sequences or fall in the category of proteins with unidentified function. In the case of the pistil, 23 independent cDNA clones were specifically expressed in pistil. To determine the accuracy of the DNA microarray data, RT-PCR experiments were performed with several anther specific clones, as a representative. Results of RT-PCR provided a direct assessment of the accuracy and reproducibility of our approach. This cDNA microarray technology is an effective tool for identification of novel reproductive organ-specific genes.

P4/C1.40—Floral development in *Allium ampeloprasum* var. *babingtonii*

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This Cornish member of the *Alliaceae* produces typical *Allium* umbels. However, the flowers are male and female sterile, the plant reproducing clonally through the production of daughter bulbs after flowering, bulblets (offshoots) and sessile bulbils (topsets), the latter being most significant in terms of numbers. Secondary umbels are often produced. Doubled florets or bulbils may be produced, with or without scapes. One or more bulbils may be produced within a bract on the inflorescence,

often with one or more florets. Individual florets are produced without bracts.

It is suggested that this highly variable morphology reflects changes in meristem identity, between the floral/inflorescence/vegetative states.

By charting the physiological development of this plant, and identifying and monitoring the expression of a putative *LEAFY* homologue, the meristem condition can be monitored and related to the developing floral physiology, in particular, the production of bulbils.

P4/C1.41—Glucose signalling and floral gene expression in *Pharbitis nil*

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Shoot apical meristems (SAMs) are defined as florally determined if, following removal from induced plants, they form flowers when cultured in non-inductive conditions. In the qualitative short day plant *Pharbitis nil*, the minimum determination time of carpels in florally induced SAMs is longer when cultured on medium containing sucrose than when cultured on medium contain-

ing glucose. Detailed dissection of the response reveals that each of the four whorls has a different minimum determination time when SAMs are cultured on sucrose, that carpels have a longer minimum determination time than other whorls when SAMs are cultured on glucose. Moreover, minimum determination times are shorter on glucose than on sucrose. Hence, glucose may be involved in a signalling mechanism leading to expression of homeotic floral genes. When we cultured SAMs on medium containing the glucose analogue 3-*O*-methyl glucose, a reduction in carpel minimum determination time occurred similar to that for SAMs cultured on glucose. This analogue is transported through cell membranes but apparently is not further metabolized, or is metabolized very slowly. Hence, the putative glucose signal may be sensed when glucose binds to, or is transported by, a membrane glucose transporter.

To find possible target genes for the putative glucose signal, RT-PCR was performed on *P.nil* SAMs using degenerate primers designed from floral homeotic genes known in other species. Structural homologues of the *Arabidopsis thaliana* genes *AGAMOUS*, *CRABS CLAW* and *LEAFY* have been identified.