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The Regulation of Sexual Development in Plants

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SUMMARY

Plant reproduction comprises an interlocking array of developmental pathways which include the formation of the sexual organs, the generation of germ lines *de novo*, and the operation of the mechanisms which regulate epigenetic imprinting and the system of self-incompatibility found in many angiosperms. Little is known of how these processes are regulated at a molecular level, with the exception of the floral organs which are determined by families of homeotic genes operating in a heterochronic fashion. In dioecious and monoecious plants the expression of these 'floral' genes must be modulated by sex-determination sequences, situated in some circumstances on sex chromosomes. Older, physiological data indicate that sex can be determined by growth regulators, particularly gibberellic acid (GA) and cytokinins, and it is possible that sex-determination genes establish local concentrations of growth regulators at the apex, which in turn influence the expression of the homeotic floral genes. Evidence from anther development indicates genes involved in differentiation of the male and female germ lines to be regulated by defined promoter, enhancer, and silencer regions, but few data are available on the sequences directing the initiation and regulation of meiosis; certainly parallels can be drawn with similar events in microorganisms, and useful complementation strategies may be devised, but significant differences do exist between yeasts and higher plants suggesting that more appropriate parallels should be drawn with multicellular eukaryotes such as nematodes. The loci involved in epigenetic imprinting and self-incompatibility are important because they affect both male and female developmental pathways. Nothing is known of the regulatory sequences which direct the epigenetic imprinting of the sperm and central cell genomes, but information is becoming available on the promoter regions of the *S*(incompatibility)-locus. Interestingly, sequences directing expression in male and female tissues are contained within a single 5′ stretch within the locus, and these promoters also induce expression in different cell types in the anther and pistil depending on the type of self-incompatibility involved. Regulation of reproductive development in plants is apparently not very stringent, for there are examples in both male and female germ lines of reversion to an embryonic condition (apomixis and microspore embryogenesis); whether this reflects the highly dedifferentiated state of these cells or differences in the regulation of somatic and reproductive development remains to be determined.

1. INTRODUCTION

Plants and animals differ conspicuously in their sexual strategies, but there are tantalizing similarities in the way sexual differentiation is initiated and regulated in the two kingdoms. However, while key sequences involved in animal sex determination have become focused on identifiable sex chromosomes, the hermaphrodite nature of many flowering plants has not generated the selective pressure for evolution of sexual inheritance mechanisms. The dioecious angiosperms, however, exhibit a range of chromosomally based sexual inheritance systems to rival animals. Even in plants with clear-cut sex chromosomes, explaining sexual determination in terms of gene expression has not proved easy. Certainly dramatic progress is currently being made in the understanding of genes carried by mammalian sex chromosomes (Goodfellow *et al.*, this symposium), but the difficulties inherent in transforming many of the plants involved and the apparent scatter of many of the key genes throughout the genome has resulted in little useful progress to date, although new cloning strategies are beginning to provide encouraging results (Ciupercescu *et al.* 1990; Veuskens *et al.* 1992).

More is known of the molecular basis of sexual differentiation. After induction of flowering, the floral organs themselves have been shown to result from the combinatorial action of a gene family common to the angiosperms (Coen & Meyerowitz 1991; Bradley *et al.*, this symposium). Genes directing the differentiation of plant male germ-line cells have also been characterized (McCormick 1991), and information concerning their promoter and enhancer regions is accumulating (Twell *et al.* 1991). However, at this level, regulation of sexual and somatic development appears identical and the plethora of studies in reproductive development probably reflects the strong commercial interest in modifying plant reproductive systems (Dickinson 1989), and the fact that the anther represents an excellent experimental paradigm, rather than the presence of features unique to reproductive systems.

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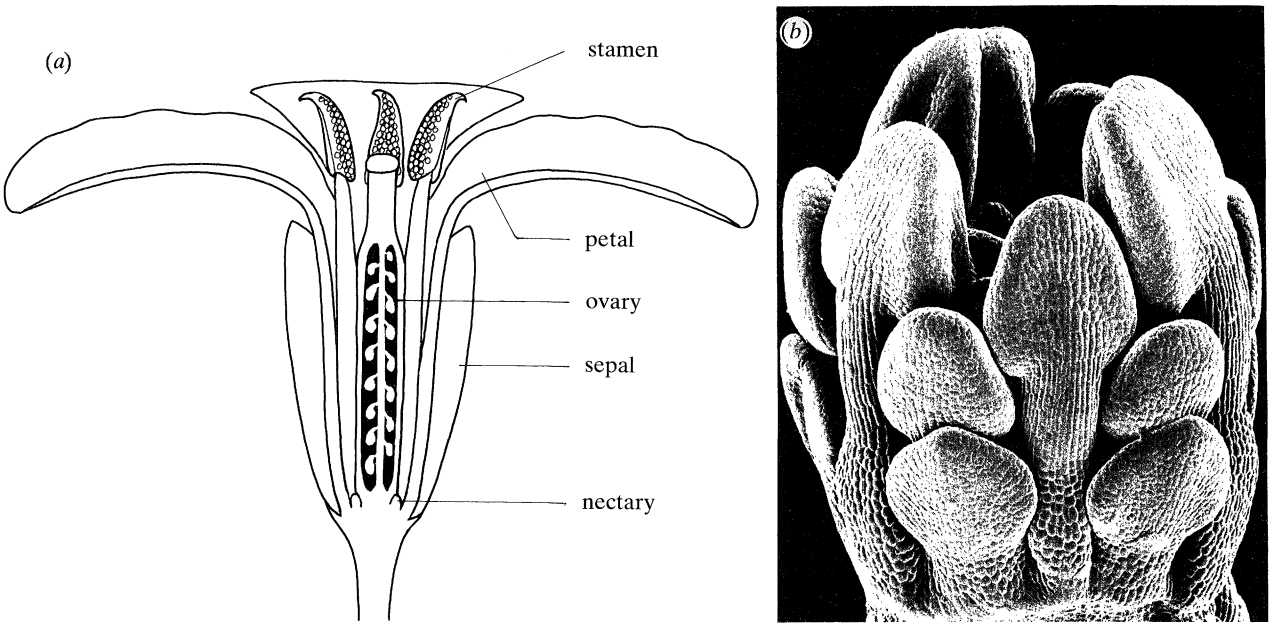

Figure 1. (*a*) Diagrammatic representation of a mature *Brassica* flower showing the principal organs (×8). (*b*) Developing flower of *Silene coeli-rosa*, viewed using low temperature scanning electron microscopy. One whorl of stamens is well advanced whereas another is at an earlier stage of development. The young petals are in register with these smaller stamens (micrograph courtesy of C. Jeffree) (×133).

Interestingly, reproductive development has proved to be surprisingly labile; for example, differentiation of pollen grains may easily be switched to embryogenic development (Guha & Maheshwari 1964; Zaki & Dickinson 1991).

There are, however, two systems that are unique to sexual development and which have different molecular consequences depending upon the sex of the individual: these are epigenetic imprinting and self-incompatibility. Epigenetic, or genomic, imprinting in flowering plants favours sexual reproduction, not by inducing abortion of young embryos in plants attempting parthenogenesis, but by ensuring the failure of the nutritive endosperm tissue (Håkansson 1953). A convincing body of data now exists indicating that the pollen and endosperm genomes receive different epigenetic imprints late in anther and ovary development (Lin 1982; Haig & Westoby 1989).

Ever since the proposition by Lewis (1954) of a tripartite structure for the *S*(incompatibility)-locus, with pollen and stigmatic domains directing the expression of similar products in male cells and the pistil, strenuous attempts have been made to identify such *S*-locus products (Scutt *et al.* 1990). More recent data have dramatically revealed that *S*-locus promoters direct the expression of different products in pollen and stigmas (Sato *et al.* 1991), implying a level of genetic regulation so far unsuspected.

2. SEXUAL DETERMINATION IN HIGHER PLANTS

Sexual expression in flowering plants takes a variety of forms, and this variety is reflected in its genetic control. Most angiosperms carry hermaphrodite flowers, but some 7% are dioecious and 6% monoecious (Irish & Nelson 1989). Selection pressure for the separation of the sexes must have lessened early in the evolution of the angiosperms when the first systems of self-incompatibility evolved (Whitehouse 1950), and thus it is not surprising that sex-chromosome systems have failed to become as prevalent as in animals. Certainly, sex chromosomes are found in dioecious plants, but only in five genera (Parker 1990) of which *Melandrium*, *Rumex*, and *Humulus* have been investigated in some depth. Sex determination in *Melandrium* is truly heterogametic, with XY individuals being male and XX female (Westergaard 1958). The Y chromosome is active, promoting maleness in addition to suppressing female development. In a classical series of experiments involving segregation of parts of the large Y chromosome through triploids, Westergaard was able to demonstrate different domains of the chromosome to regulate enhancement of male development and the suppression of femaleness. Interestingly, as in *Drosophila*, while maleness and femaleness are principally controlled by the Y chromosome, the dosage of X chromosomes or autosomes can modify the maleness expressed (Frankel & Galun 1977). Detailed molecular data are not available either for the Y or X chromosomes of *Melandrium*, but RAPDs have been used to generate a range of Y-specific probes and a number of enrichment strategies have been adopted in attempts to clone Y-specific cDNAs (Veuskens *et al.* 1992).

Both *Rumex* and *Humulus* possess X:autosome balance systems determining sex, but there is considerable variety in each group (Parker & Clarke 1991) with *Rumex hastatulus* displaying elements of both X:autosome and active Y systems. In *Rumex acetosa*, where females are XX and males XY_1Y_2, the X chromosomes are uniform while some considerable variation occurs in Ys, particularly in the centromeric region. Preliminary molecular analysis of the Y chro-

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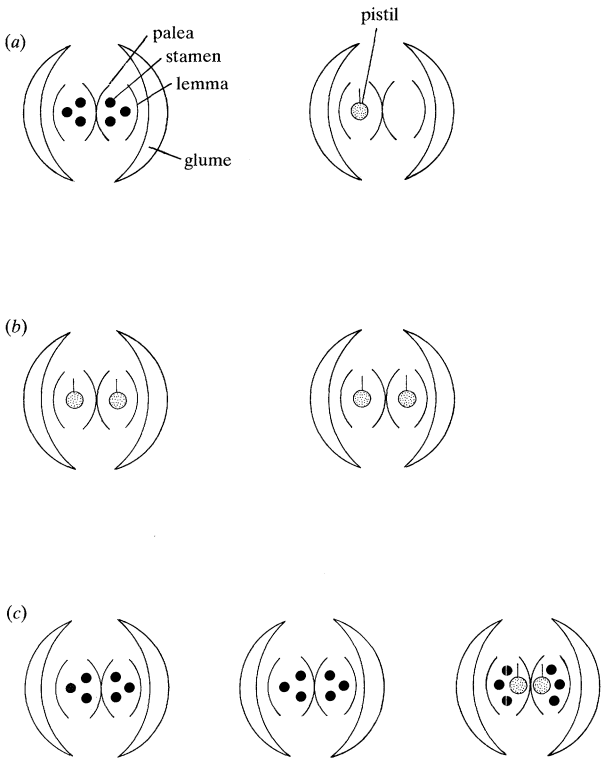

Figure 2. (*a–c*) Diagrammatic representation of floral structure in *Zea mays*, redrawn from Irish & Nelson (1989). (*a*) Wild-type tassel (male; left) and ear (female; right) flowers showing stamens and pistils. (*b*) Tassel seed mutant (*ts-1*, *ts-2*) where stamens have been replaced by pistils. (*c*) Dwarf mutant (*dw*) where pistils have been partially or fully replaced by stamens.

mosomes of *Rumex acetosa* reveals the presence of highly repetitive heterochromatin domains; however, these sequences may also be found in the X chromosomes and scattered throughout the autosomes (Parker & Clarke 1991). Further, the frequency of these sequences in the Y chromosomes does not exceed that found either in the autosomes or X chromosomes, suggesting that the high frequency of heterochromatic sequences seen in the sex chromosomes of mammals (Jones & Singh 1981) is not paralleled in plants. Sex determination in *Rumex acetosa* is via a classical X:autosome balance system, with ratios between X chromosomes and autosome sets higher than 1.0 forming females while ratios lower than 0.5 induce maleness. Intermediate ratios result in 'intersex' plants, as is the case with *Drosophila*. Recent data suggest that the Y chromosome in *Rumex acetosa* is not required for male development *per se*, but affects sporophytic development in such a way as to influence the latter stages of pollen mother-cell differentiation (Parker & Clarke 1991).

Humulus, like *Rumex*, features two types of sex determination system. In *Humulus japonicus* sex is determined by an X:autosome balance system, while *Humulus lupulus* (wild hops), and most cultivated relatives, have a complex, multiple sex-chromosome system (Parker & Clarke 1991). In hops, like *Rumex*, the Y chromosome is not necessary for the development of male flowers, but is required for the maturation of pollen.

Whether determined by genes carried on sex chromosomes or autosomes, sexual determination is achieved by the suppression of development of one set of sex organs. Evidence from work on *Zea* (see Heslop-Harrison (1972), for review) and *Mercurialis* (Durand & Durand 1984) also indicates that growth regulators play a central role in this suppression. In *Zea*, female development is inhibited in tassels and male development in ears. Examination of the earliest stages in floral development indicates the initiation of both sets of sex organs in tassels and ears, but also some other characteristics of the mature structure, for example the branch primordia in tassels (Cheng *et al.* 1983). Two mutants (see figure 2), *dwarf* (*Dw*/*dw*) and *tassel seed* (*Ts*/*ts*), have proved useful in unravelling sex determination in *Zea*. *Dw*/*dw* mutants form normal tassels, but develop male organs in the ears. Six unlinked loci – some dominant and some recessive – have been demonstrated to regulate this mutation; interestingly, at least one recessive can be rescued by the application of exogenous GA (Phinney 1961). The involvement of GA in sex determination in *Zea* is supported by the observation that it will feminize normal tassels (Hansen *et al.* 1976) and that the ear shoots contain some 100 × the somatic levels of GA_1. Further, low light and short days – known to increase GA levels – tend to feminize plants (Heslop-Harrison 1972), and many of the recessive *dw* mutations have now been identified as regulating steps in GA synthesis (Phinney 1961).

In *Ts*/*ts* mutants, pistils develop in the normally staminate tassels. Again there are at least five *Ts*/*ts* mutants, regulated by unlinked loci (Coe *et al.* 1989). While *ts-1* and *ts-2* produce clear phenotypes, other mutations generate complex morphology featuring male and female organs. Significantly the product of at least one of the *tassel seed* alleles is non-diffusible (Johri & Coe 1983), indicating it not to be a growth regulator. The analysis of the few *dwarf*/*tassel seed* double mutants that have been studied suggests the two loci are unlinked. There is also circumstantial evidence that once the sex of a particular flower has been determined, the remainder of development is comparatively stable. Cultured tassels and ears will develop satisfactorily in the absence of GA, although the early stages of female and the later stages of male development require cytokinins (Polowick & Greyson 1985; Bommineri & Greyson 1987). Irish & Nelson (1989) propose that, among other signals, local GA levels are perceived by 'pivotal sex determination genes' that activate appropriate sexual differentiation pathways.

Study of the dioecious *Mercurialis* confirms the central role of growth regulators in the determination of sex. Louis & Durand (1978) have reported three genes to be involved in regulating sexuality. These promote maleness to a greater or lesser extent and were identified by their ability to suppress the feminizing influence of exogenous cytokinins. While earlier experiments had already shown (Durand 1967) that males were feminized by cytokinins, females could only be

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transformed to males by *in vitro* culture in the presence of auxins (Champault 1973). However, the accumulated growth regulator data suggest that the genes identified by Durant maintain endogenous growth regulator levels, rather than determine sexual expression *per se*. More recently Hamdi *et al.* (1989) have investigated gene expression in male and female flowers of *Mercurialis* using hybridization kinetics and *in vitro* protein synthesis. While their study also involved male sterile and restored lines, specific poly $(A)^+$ RNAs were identified in male flowers, and a cDNA probe was used to demonstrate that this transcript was downregulated in the presence of feminizing hormones (cytokinins). These authors regard these findings as supporting the existence of 'sexual hormones' controlled by regulator genes, which activate the appropriate developmental pathways in bipotential meristems.

The current position with regard to sex determination in plants thus remains highly unsatisfactory. It is not surprising that the adoption of dioecy by groups of plants has led to the evolution of sex chromosomes, but their study, and the search for sequences regulating sex determination in other plants, has resulted in little useful data. Certainly growth regulators appear to be involved in the formation of the sexual organs themselves, but identity of the 'pivotal' genes which activate growth regulator controlling sequences such as *dw* remains unknown. More particularly, the genetic steps are missing which link the gross effects of GA, auxins, and cytokinins to the heterochronic and combinatorial action of the homeotic genes – discussed further below – known to control floral morphogenesis in angiosperms (Coen & Meyerowitz 1991; Bradley *et al.*, this symposium).

3. FLORAL INDUCTION AND INITIATION

The switch to reproductive development in flowering plants is not well understood. Floral initiation normally commences in response to single or multiple environmental cues, and is first detected as cell-cycle changes at the apex (Bernier 1992). Although the switch from vegetative to reproductive development has tacitly been assumed to be regulated by a number of genes, recent studies of natural and transposon-induced mutants suggest otherwise. For example, mutation of the *floricaula* locus in *Antirrhinum* results in indeterminate shoot formation in the sites normally occupied by determinate flowers (Bradley *et al.*, this symposium). Data are not yet available concerning the cytological changes, if any, that take place at the apex of *floricaula* mutants.

Studies of floral mutants have also enabled identification of the genes regulating the development of the induced apex into the complete hermaphrodite flower. This work, which has been carried out simultaneously in *Arabidopsis* and *Antirrhinum* (Coen & Meyerowitz 1991), strongly indicates that – within these groups – floral development is directed by similar sets of genes, and whereas these are expressed sequentially during the generation of the floral organs, they operate in a combinatorial manner in certain phases of development. The formation of individual floral organs is directed by three classes of homeotic genes, encoding functions termed *a*, *b*, and *c*. The *a* function alone results in the formation of sepals; *a* and *b* combined, the production of petals; *b* and *c*, stamens; and *c* alone, carpels. Interestingly, function *c* – revealed, for example, by mutations at the *agamous* locus in *Arabidopsis* – also regulates floral determinacy. While *Arabidopsis* is radially symmetrical *Antirrhinum* is zygomorphic, indicating the presence of genes which modulate the *a*, *b* and *c* functions. Mutations at the *cycloidia* locus in *Antirrhinum* result in the formation of radially symmetrical flowers and the products of this locus are considered to interact with the organ-generating functions in particular domains of the developing apex (Carpenter & Coen 1990).

Although all of these genes have yet to be fully characterized, some (e.g. *deficiens* from *Antirrhinum*) have been shown to encode transcription factors with homology to sequences in yeast and mammals (Coen & Meyerowitz 1991). Of particular interest is the role

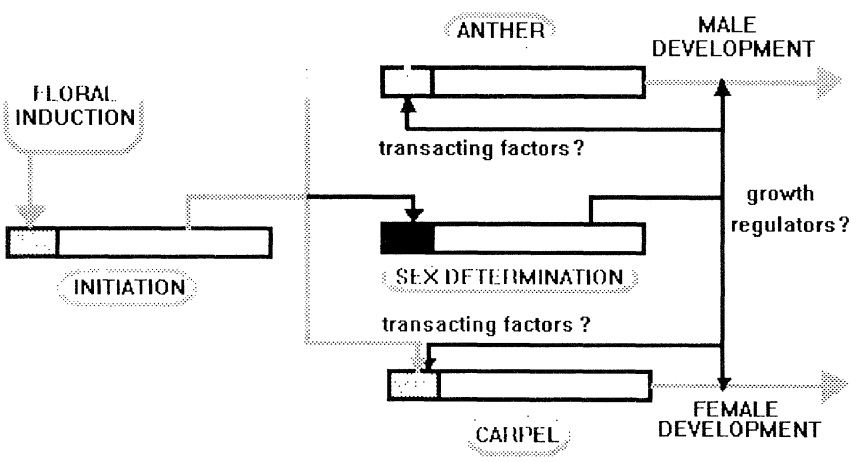

Figure 3. Regulation of sex determination in higher plants. Sex determination genes, carried on sex chromosomes or scattered throughout the genome, are activated following floral initiation. The products of these genes modulate floral organogenesis either directly by gene regulation, or indirectly through altering local concentrations of growth regulators.

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played by these genes in monoecy and dioecy. The formation of single-sex flowers has always been regarded as involving the suppression of either stamens or carpels; conditions which can be mimicked in the mutants described above. Further, both hormonal treatments and environmental stress can induce changes in sex expression in many plants. Partial answers to these questions about sex determination may be provided by *in situ* hybridization, which enables the expression of the gene products of many of these sequences to be followed at a cellular level (Jones *et al.* 1990), but it remains unclear whether 'sex-determination' sequences (on sex chromosomes or otherwise) modulate the expression of the organ-forming homeotic genes such as *agamous* and *sepaloidia* directly by *trans*-acting factors, or through local changes in the levels of growth regulators (see figure 3). For this reason, the nature of the sequences carried on the sex determining domains of active Y chromosomes, such as those of *Melandrium*, remains of considerable interest.

4. MALE AND FEMALE GERM-LINE DEVELOPMENT

Development of the male germ line and its investing tapetal tissue in the anther has been the focus of much recent molecular research. Although development of the embryo sac and its nutritive nucellus is of equal significance, a combination of the small numbers of cells involved, their inaccessibility, and a lack of commercial interest has resulted in female gametogenesis remaining largely unstudied. However, the use of *in situ* and immunolocalization methods is permitting the investigation of molecular events in the differentiating ovule (Dow & Mascarenhas 1991). A striking difference between the germ lines of animals and plants is that the latter are formed *de novo*. Gametes in higher animals are normally derived from a distinct cell line, determined in the embryo and giving rise only to cells involved in reproduction. In plants, following floral induction, vegetative meristems alter in their organization, forming flowers. Within the flowers, gametes develop in the ovaries and anthers.

Conservative estimates indicate the presence of 10 000 anther-specific genes (Kultnow *et al.* 1990) and thus it is not surprising that differential screening and the screening of subtractive libraries have resulted in the characterization of anther-specific cDNA, ranging in time of expression from meiosis to late in pollen development (see McCormick (1991) for review). A number of genes have also been cloned from mature and germinating pollen (Mascarenhas 1992). Interestingly, most sequences have been cloned from pollen mitosis I onwards, presumably reflecting the low levels of poly $(A)^+$ RNA in meiotic cells (Porter *et al.* 1984). Further, the intense synthetic activity of the tapetum is reflected in the large number of tapetal-specific cDNAs now reported (McCormick 1991), although there has been commercial pressure to obtain the promoters for sequences of this type for use in engineered systems of male sterility (Mariani *et al.* 1990). More recent data suggest that a small but significant number of anther-specific sequences, of which the *Bcp1* gene from *Brassica* is an example (Therakulpisut *et al.* 1991), are expressed in both the sporogenous cells and the tapetum, generally at different developmental stages. Not surprisingly, a large proportion of the sequences expressed in the anther, such as *LAT52* from *Lycopersicum* (McCormick 1991), are also transcribed in other organs and tissues. Despite this plethora of anther-specific genes, those directing the initiation and progression of meiosis remain elusive. Apart from the *pLEC* sequences cloned by Bouchard (1990), which appear to belong to a heat-shock family, no meiosis-specific genes have been identified, although their homologues in yeast are well characterized (e.g. *HOP1*, Hollingsworth *et al.* 1990). It is thus likely that the genes involved in meiotic initiation and recombination will be cloned by complementation, or through their homology at the DNA or protein level with sequences from organisms such as *Saccharomyces* and *Caenorhabditis*.

The polypeptides encoded by anther-specific genes have been predicted from DNA sequence data and range from ascorbate oxidase, via pectic lyases and polygalacturonases, to lipid transfer proteins and Kunitz trypsin inhibitors (see McCormick (1991) for review). Kopczak *et al.* (1992) have recently reported specific forms of β tubulin to be synthesized in the pollen, and it is possible that there are pollen-specific forms of other cytoskeletal components including actin and intermediate filaments. A surprising number of pollen-specific cDNAs, while encoding putative proteins rich in specific amino acids (particularly glycine, proline and cysteine), have no homologies with sequences on current data bases. The pectin-degrading enzymes and polygalacturonases presumably play a role in the development of pollen on the stigma surface and in the style, but otherwise few specific functions have been assigned to pollen- or tapetum-specific genes.

The most comprehensive analysis of the promoter regions of pollen-specific genes has been carried out by Twell *et al.* (1991) on *LAT52* and *LAT59* from *Lycopersicum*. These genes are coordinately expressed late in anther development, both in the pollen and sporophytic tissue. The expression in transgenic plants of constructs containing deleted promoter sequences linked to the *GUS* reporter gene demonstrated only small stretches of promoter sequence (less than 200 b.p.) to be necessary for expression in pollen and specific sporophytic cells. Twell *et al.* (1991) also used a transient assay system to identify a number of upstream *cis*-acting regulatory regions of these, and other, promoters. Interestingly, one of these regions enhanced the expression of the *CaMV35S* promoter in pollen. Analysis of these *cis* elements from different promoters revealed shared sequences, suggesting that similar *trans*-acting factors may activate a number of anther-specific genes. Most recently, Twell (1992) has used the promoter region of the *LAT52* gene from *Lycopersicum* to investigate gene regulation in the developing microgametophyte. Again using a *LAT52-GUS* construct, but on this occasion fusing a nuclear location signal to the *GUS* gene, he demonstrated the promoter to direct expression of the reporter gene in

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the vegetative cell in transgenic *Nicotiana*. GUS was detected neither in the nucleus nor in the cytoplasm of the generative cell. This dramatic difference in gene expression in adjacent cells, so soon after their formation from a common parent, underlines the significance of pollen mitosis I in male germ-line development (Dickinson 1992*a*). As long ago as 1964 Guha & Maheshwari demonstrated that disruption of this division could 'reset' development and result in the production of embryos. More recent data (Pechan & Keller 1988; Zaki & Dickinson 1990, 1991) suggest that the asymmetry of this division is crucial to the correct development of both vegetative and generative cells. Treatment with colchicine immediately prior to pollen mitosis I, which induces division symmetry, results in a higher rate of embryogenesis (Zaki & Dickinson 1991), suggesting that simple factors such as nuclear:cytoplasmic volume ratios may determine the subsequent fates of these cells. The molecular basis of this developmental switch has yet to be determined, but Pechan *et al.* (1991) have demonstrated heat-shock proteins to be synthesized in microspores after inductive treatments. These polypeptides, which can interact with the cytoskeleton, may act directly to disrupt the symmetry of division, or operate via gene expression to switch development from a gametophytic to a sporophytic pathway.

Developmental flexibility, of which microspore embryogenesis is an example, is a characteristic of reproductive development in higher plants. It is particularly evident in apomictic development in females, where embryos can arise spontaneously from either the nucellar tissue or from one of the cells of the embryo sac. Sometimes apomixis is stimulated by pollination, but in most instances it occurs spontaneously. Many angiospermous groups contain facultative or constitutive apomicts and a surprisingly high proportion of higher plant seed is produced by this process. The genetic control of apomixis is poorly understood, but t-DNA mutation of *Arabidopsis* has resulted in apomictic plants (E. Herbele-Bors, personal communication) and thus the way is open for the identification and characterization of genes involved in this process.

5. EPIGENETIC IMPRINTING DURING SEXUAL DIFFERENTIATION

The presence of a mechanism promoting double fertilization in angiosperms by haploid gametic nuclei was first indicated in experiments involving reciprocal pollinations between individuals of different ploidy. Håkansson (1953), working on *Brassica*, demonstrated that pollination with diploid gametes resulted in the inhibition of endosperm development and lack of seed set. Interestingly, matings between individuals of increased ploidy were sometimes successful, especially when a 'normal' gamete:endosperm (polar) nuclear ratio of 1:2 was maintained. Study of the expression of the *R*-locus of *Zea* also produced some puzzling results, for different phenotypes could be produced by the same genotypic combination of dominant and recessive alleles, depending upon whether particular alleles were donated by the male or female parent. As this could be interpreted as a gene dosage effect, Kermicle (1970) used the B chromosome reciprocal exchange system in *Zea* to investigate whether this apparent asymmetry in gene expression resulted from a wrong balance of alleles being present in the endosperm, or from the fact that the maternal:paternal nuclear contributions were incorrect. The results proved conclusively that the *R*-locus phenotype was regulated by the parental origin of the alleles involved. The long arm of chromosome 10 in *Zea* carries a number of genes involved in endosperm development and Lin (1982), again using the B chromosome translocation system, synthesized six interchanges with reduced endosperm development. Interestingly, endosperms of all genetic constitutions, but with appropriate dosages of chromosome 10 donated maternally rather than from both parents, proved to be defective. Thus a proportion of the endosperm-specific sequences on chromosome 10 must be donated paternally for effective development of the tissue.

Although this sex-specific imprinting of the genome undoubtedly favours sexual reproduction, and may even be significant in the apomictic strategies adopted by plants (Haig & Westoby 1989), there is no information as to how it is achieved. The early view that epigenetic imprinting in animals involved differential methylation of key sequences is currently under serious reconsideration (Surani *et al.*, this symposium). Certainly many sequences – including transgenes – demonstrated to be imprinted are heavily methylated, but on the other hand not all methylated sequences are imprinted, at least as far as determining the fate of the young embryo. Methylation of plant DNA is well documented, and changes in the methylation status of particular sequences have been reported to result in alterations in their expression (Heslop-Harrison 1990). Indeed, selective demethylation of specific endosperm genes during reproductive development may well represent the mechanism by which these genes are activated (Bianchi & Viotti 1988). However, as is the case with animals, conclusive data have yet to be obtained showing that differential imprinting during plant sexual development is achieved solely through cytosine methylation. Most recently, families of genes have been identified of which the products determine the supramolecular structure within the chromosome, and thus, indirectly, can regulate gene expression. Perhaps the best characterized is *polycomb* in *Drosophila* (Jürgens 1985), the gene product of which is responsible for the organization of heterochromatin domains, in which gene expression is suppressed. A *polycomb* homologue has been identified in plants (Singh *et al.* 1991), and it remains a distinct possibility that a combination of alterations in chromatin structure, induced by the action of *polycomb*-like genes, and selective methylation results in differential epigenetic imprints on the sperm and central cell genomes.

The juncture at which the male and female genomes are imprinted is not known. The sperms in the pollen (or pollen tube) are derived from the generative cell which is formed, with the vegetative

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cell, by the asymmetrical division of the microspore. Because the microspore, the vegetative cell, and sometimes even the generative cell may be switched directly into an embryogenic pathway (Dickinson 1992*b*), the epigenetic imprint – if at all comprehensive – is most likely applied during the maturation of the highly condensed sperm nuclei. Because the character and fate of the binucleate central cell differs so markedly from that of the other cells of the embryo sac, any epigenetic modifications must take place during the final differentiation of the polar nuclei. The differential epigenetic imprinting involved in higher plant reproductive development thus represents part of the general sequence of sexual differentiation, presumably regulated by promoters – so far undiscovered – directing expression in the sperm cells of the pollen and the central cell of the embryo sac.

6. THE REGULATION OF GENE EXPRESSION IN SELF-INCOMPATIBILITY

The *S*(incompatibility)-locus is of considerable interest because, although specific to reproductive development, it is differentially expressed in the cells of the pollen and pistil. In the majority of angiosperms possessing self-incompatibility (SI) the presence of identical alleles at a single *S*-locus results in the identification and rejection of self-pollen (Dickinson 1990) (see figure 4). There are considerable variations on this theme; the Graminae possess two loci and *Ranunculus* spp. three. Further, heteromorphic SI mechanisms are regulated by complex, single *S*-loci which also encode 'physiological' self-rejection systems.

SI in members of the Cruciferae and Asteraceae is also complicated by the sporophytic inheritance of pollen compatibility (Bateman 1955), where the alleles of the pollen parent, rather than of the haploid microgametophyte itself, determine the fate of the grain on the stigma surface. Although the site of the *S*-allele products in the pollen is so far unknown, their sporophytic origin suggests that they are synthesized prior to meiosis and survive the entire gametophytic phase, or that they are applied to the pollen late in development, perhaps as part of the tapetally derived pollen grain coating (Dickinson & Lewis 1973).

The elegant dimer hypothesis of Lewis (1965) predicts that identical *S*-locus products are formed in the pollen and pistil and that, on pollination, a dimer is formed which inhibits further development. Other domains of the locus are held to direct the expression of messages encoding these products in male and female tissues. The use of then-new cloning strategies by Anderson *et al.* (1986) and Nasrallah *et al.* (1985) resulted in the identification and characterization of *S*-locus sequences encoding glycoproteins (*S*-locus glycoprotein genes: *SLG*s) expressed in the pistils of *Nicotiana* and *Brassica* respectively. More recent work on the *S*-locus of *Brassica oleracea* has revealed it also to contain an *S*-receptor kinase (*SRK*) gene (Stein *et al.* 1991) analogous to a transmembrane serine–threonine kinase gene found in *Zea* (Walker & Zhang 1990). In addition, sequences with strong homologies to the *SLG*s are also present elsewhere in the genome, but

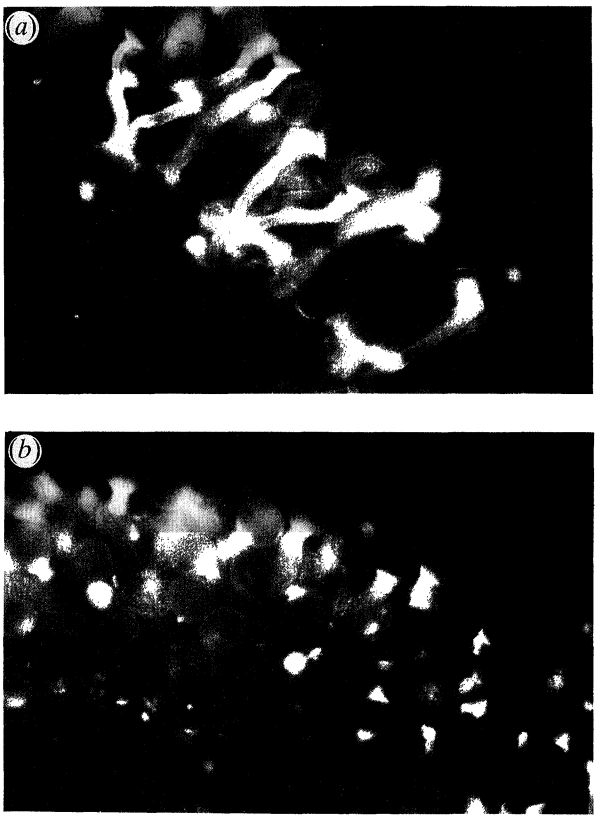

Figure 4. (*a*,*b*) Self-incompatibility in *Brassica oleracea*. (*a*) Compatible cross-pollination stained with aniline blue and viewed in the fluorescence microscope. Fluorescence of the callose in the pollen tube walls shows the pollen to have germinated and the tubes to be entering the pistillar tissue. (*b*) As (*a*), but following a self-pollination. Pollen grains have either failed to germinate, or produced very truncated tubes. (× 660)

unlinked to the *S*-locus (Lalonde *et al.* 1989; Trick & Flavell 1989). While the *SRK* is expressed in both male and female cells, the male determinant of SI in *Brassica* has yet to be unequivocally identified, although peptides derived from the pollen grain coating have been demonstrated to interact specifically with the *SLG* product (Doughty *et al.* 1993*a*,*b*).

The study of promoters within the *S*-locus has proved particularly revealing. Transgenic expression, in *Brassica oleracea*, of constructs containing regions upstream of *SLG* coupled with various reporter genes indicates these sequences to direct expression solely in the superficial papillar cells of the stigma, and to a far lesser extent in the vestigial transmitting tissue of this plant (Sato *et al.* 1991). More dramatically, these promoters also direct expression of the reporter sequences in the anther, both in the tapetal cells and less strongly in the microspores (Sato *et al.* 1991). This observation is significant not only because expression occurs in the tapetum, which may be the source of the *S*-allele products carried by the pollen, but also because – contrary to expectation – a single stretch of 5′ upstream sequences seems to be involved in regulating expression in male and female cells. While it is possible that these promoters respond to different

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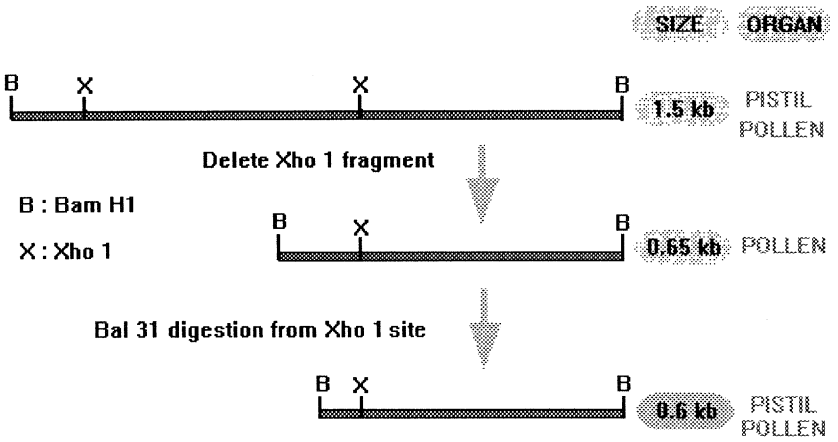

Figure 5. Expression of the *GUS* reporter gene in *Nicotiana*, driven by modified *Brassica SLR* promoters. The full 1.5 kb 5′ region (top) directs expression in the pollen and pistil at appropriate times. Deletions to generate a 0.65 kb promoter (middle) result in pollen expression only. Further truncation of the promoter to 0.6 kb (bottom) resulted in expression in pollen and pistils, but with some loss of temporal regulation (data from Hackett *et al.* 1992).

trans-acting factors in anthers and stigmas, and indeed other sequences are involved in the expression of the male and female *S*-locus products, the effectiveness of reporter constructs in cells of both sexes suggests that SI has evolved as a truly pan-sexual system. Thus although SI may well have evolved from a pathogen defence system located in the pistil (Dickinson *et al.* 1992), gene modification and duplication within the locus, combined with the development of *trans*-acting factors capable of activating it within the pollen, have resulted in the generation of male and female determinants capable of rejecting self-pollen.

Transgenic expression of reporter constructs containing *Brassica SLG* promoters have provided further unexpected data, for in *Nicotiana* (where the inheritance of SI is gametophytic) the reporter gene is expressed in the transmitting tissue of the style, rather than in the stigma, and in the developing pollen instead of the tapetal cells (Thorsness *et al.* 1991). Significantly, in gametophytically regulated SI, the *S*-locus products are expressed in the transmitting tissue, and presumably in the pollen. Thus the *trans*-acting factors responsible for the activation of what appears to be a common SI promoter are distributed in a cell-specific fashion so as to ensure the appropriate expression of the *S*-locus.

Brassica SLG promoters are under study in a number of laboratories, but preliminary results suggest that like many reproductive cell-specific promoters (Twell *et al.* 1991), these sequences contain a

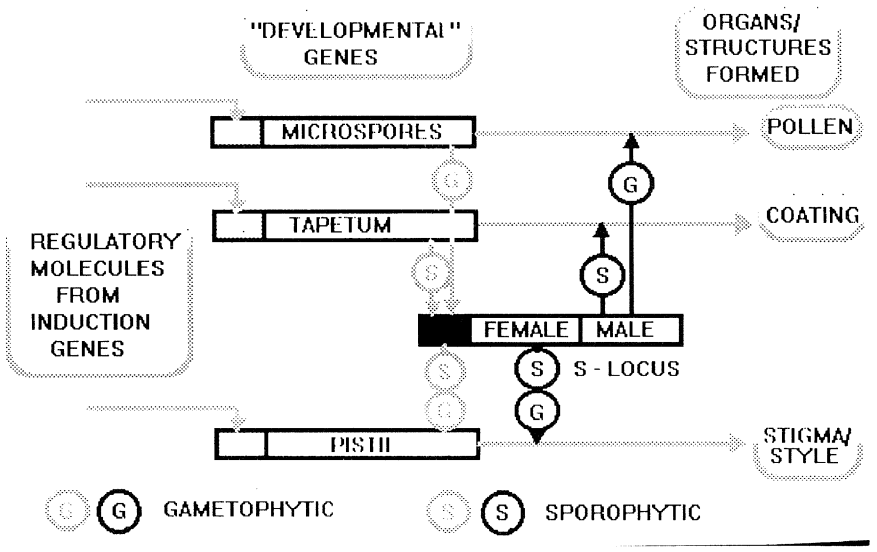

Figure 6. *S*(incompatibility)-locus expression in angiosperms. The locus is differently regulated in sporophytically and gametophytically controlled SI. In sporophytic SI the male *S*-product appears to be synthesized in the tapetum, while in gametophytic SI it is formed in the pollen. Presumably the locus is activated by factors in these different cell types.

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mosaic of enhancers and suppressors ranging from a few to tens of bases in length (Hackett *et al.* 1992). No definitive consensus sequence has yet been identified which will direct the expression of the *S*-locus under all circumstances. However, promoter deletion experiments involving the components of the 1.5 kb upstream region of the *SLR-1* (a sequence coexpressed with *SLG*) *Brassica* gene driving a *GUS* reporter construct in transgenic *Nicotiana* (see figure 5), indicate the presence both of sequences which direct expression in male and female tissues, and of silencing elements (Hackett *et al.* 1992). The fact that the same sequence will direct expression in different tissues in plants with differing systems of SI is interesting (see figure 6), and points either to these promoters having a previously unsuspected complexity, or to differences in the distribution of similar *trans*-acting factors.

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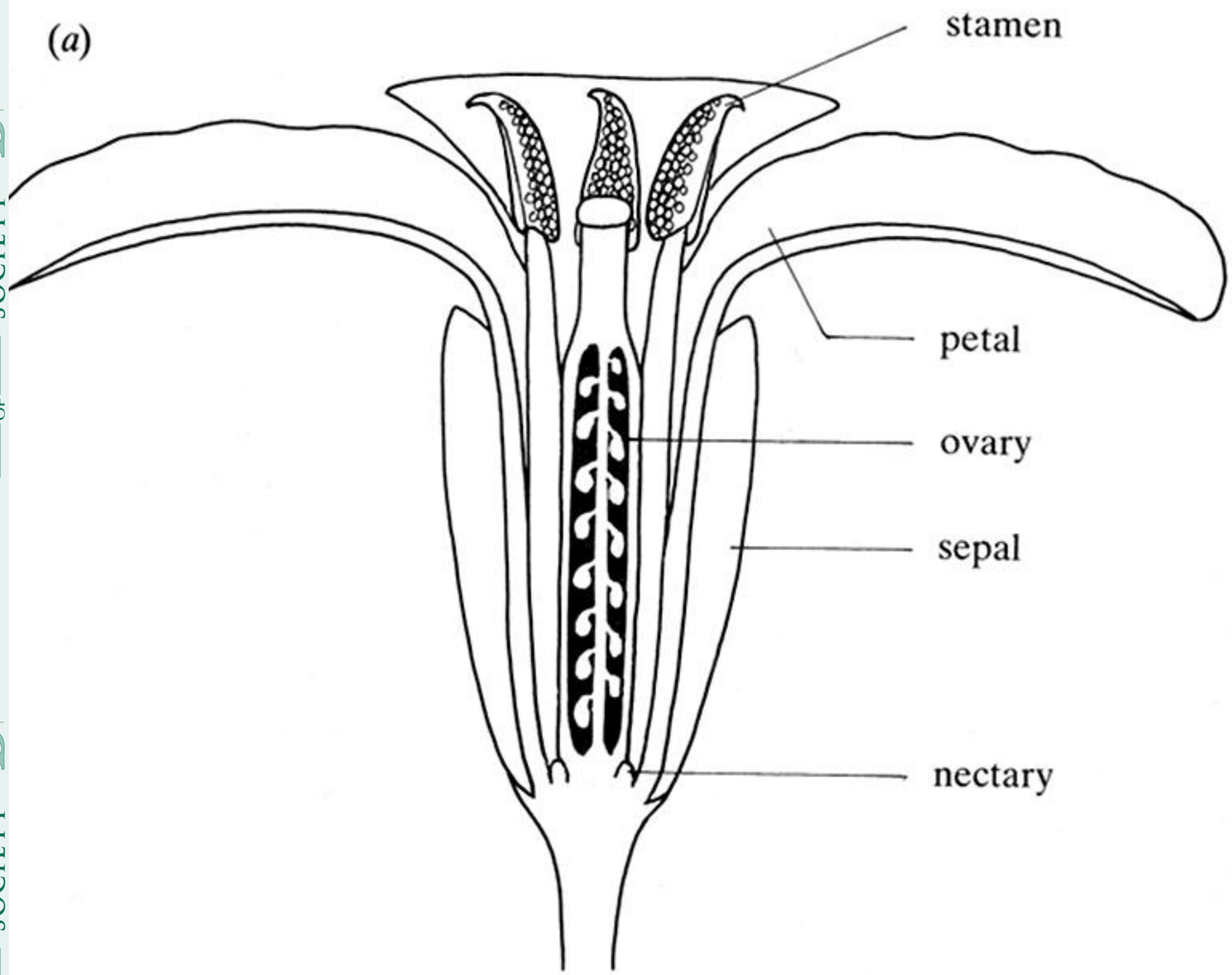

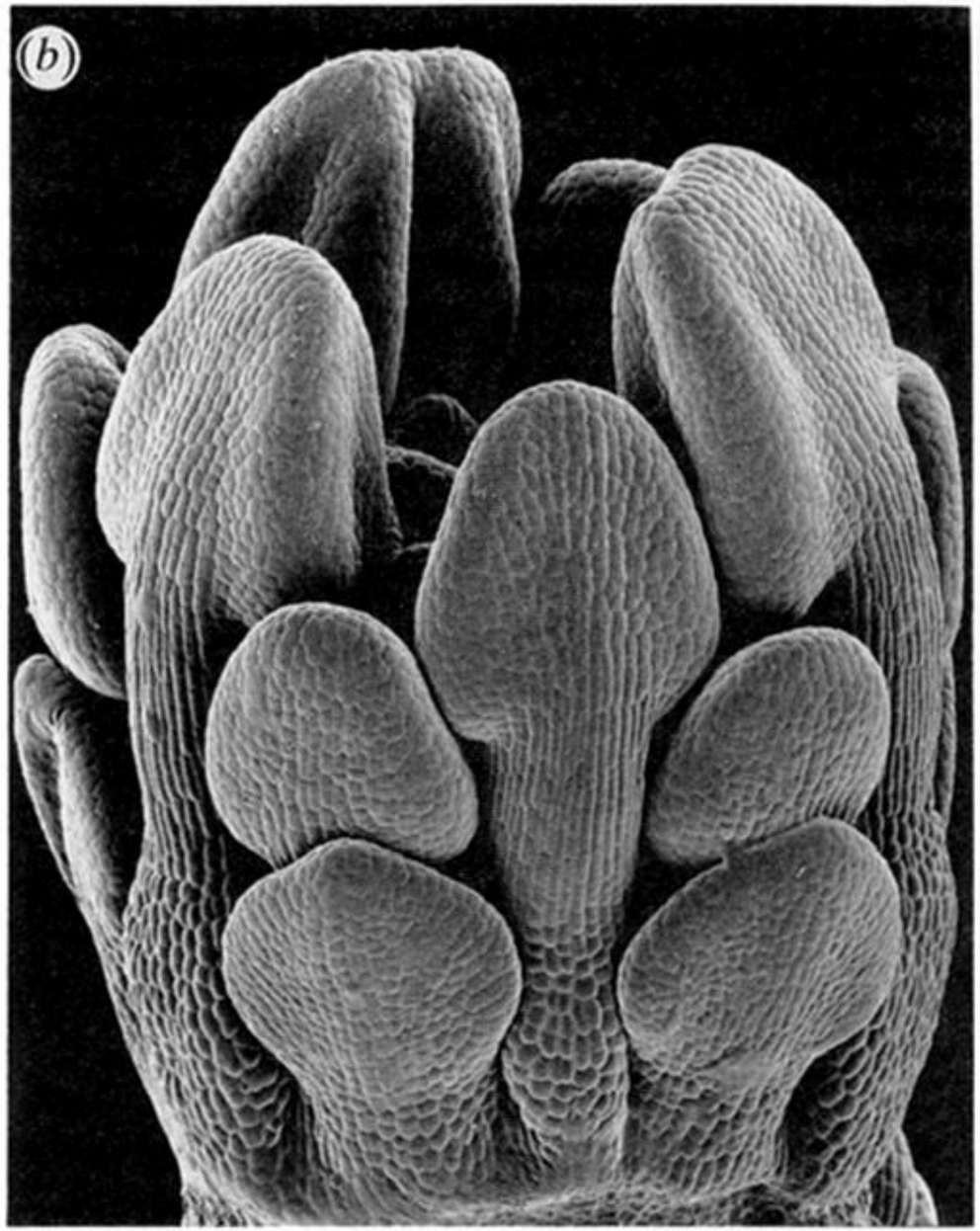

Figure 1. (*a*) Diagrammatic representation of a mature *Brassica* flower showing the principal organs (×8). (*b*) Developing flower of *Silene coeli-rosa*, viewed using low temperature scanning electron microscopy. One whorl of stamens is well advanced whereas another is at an earlier stage of development. The young petals are in register with these smaller stamens (micrograph courtesy of C. Jeffree) (×133).

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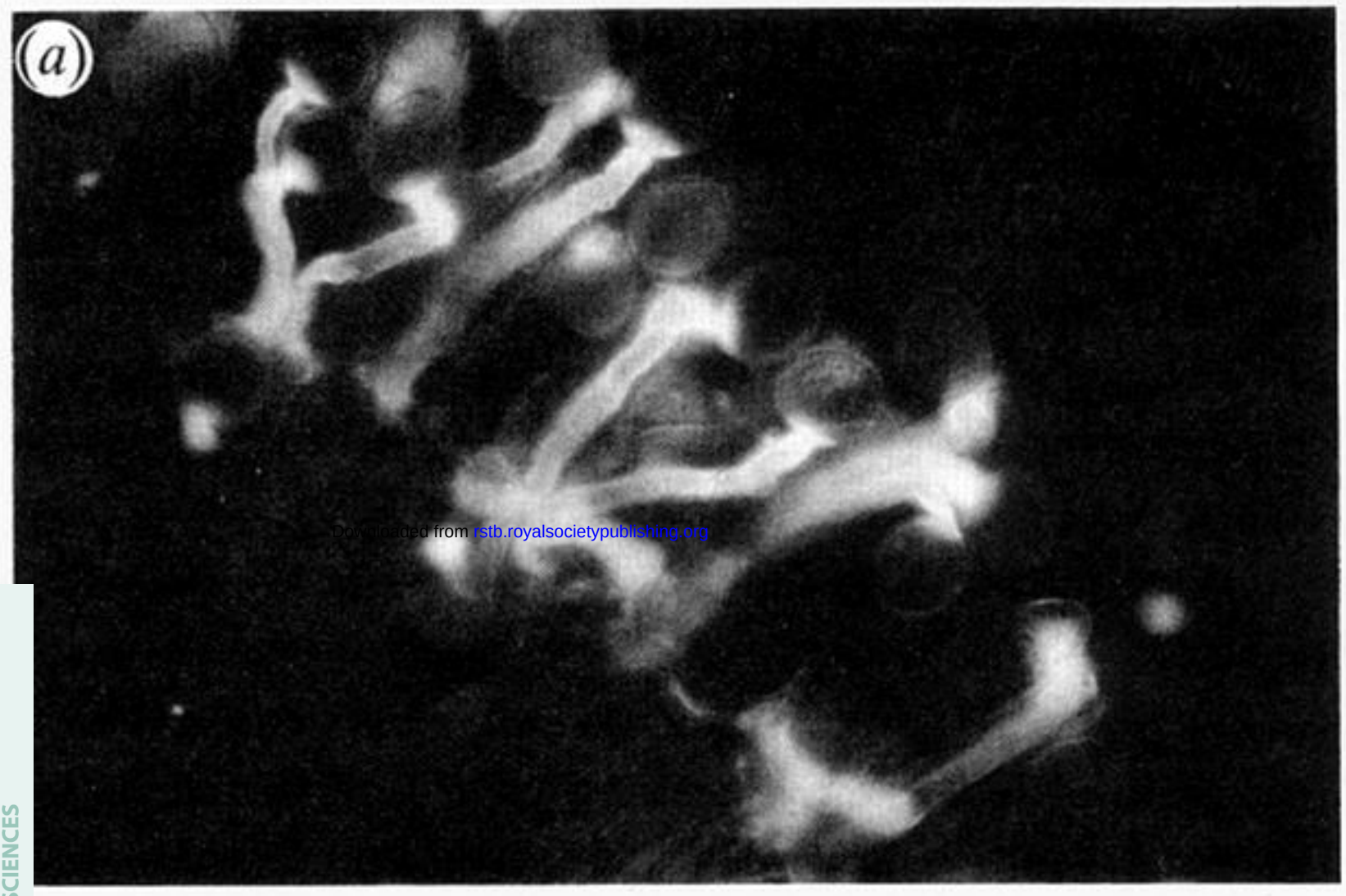

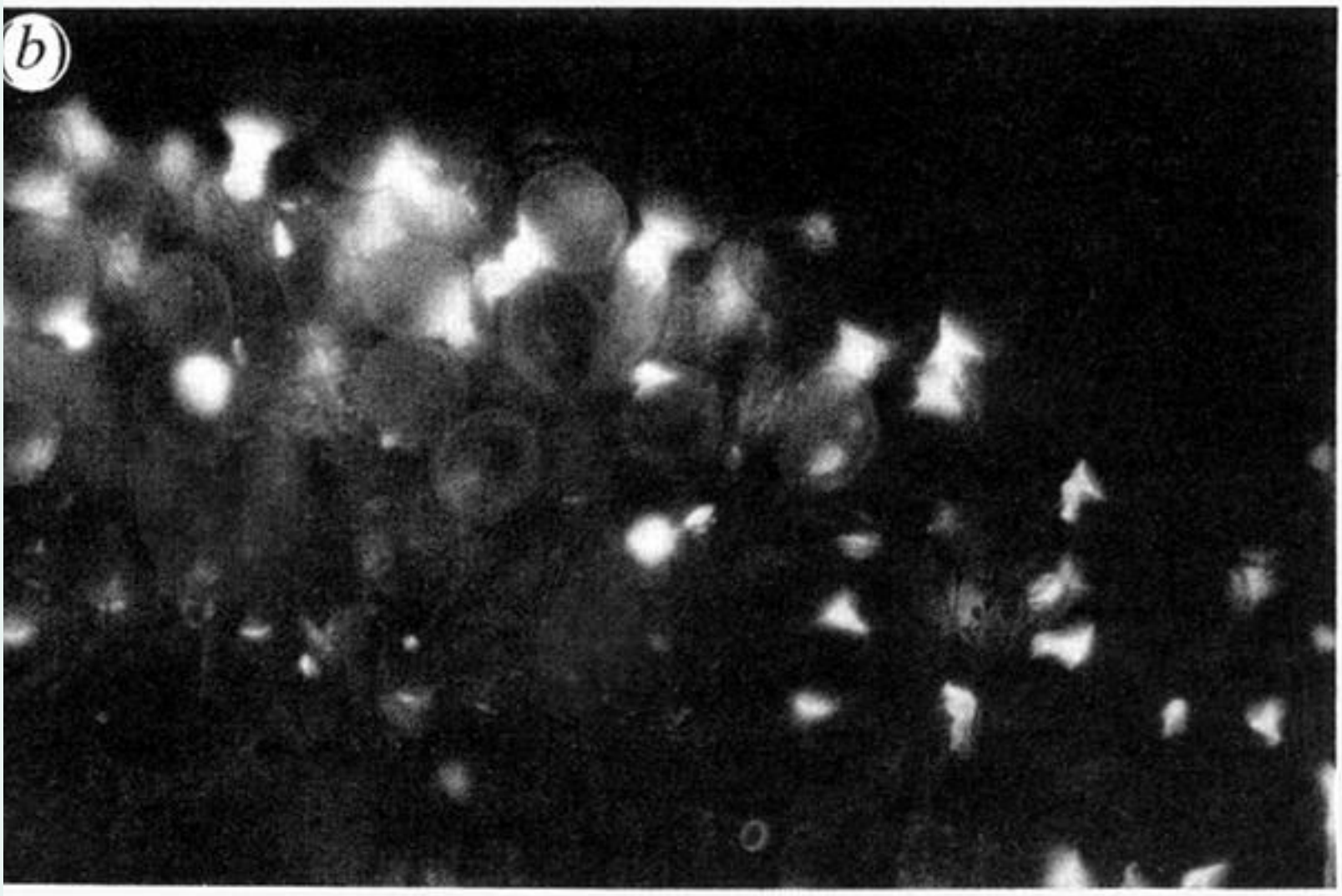

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