Development 125, 71-84 (1998)
Printed in Great Britain © The Company of Biologists Limited 1998
DEV0132

STYLOSA and *FISTULATA*: regulatory components of the homeotic control of *Antirrhinum* floral organogenesis

Patrick Motte*, Heinz Saedler and Zsuzsanna Schwarz-Sommer†

Department of Molecular Plant Genetics, Max-Planck-Institut für Züchtungsforschung, Carl-von Linné-Weg 10, D-50829 KÖLN, Germany
*Present address: Botany Department, University of Liège, B-4000 Liège, Sart-Tilman, Belgium
†Author for correspondence (e-mail: schwarzs@mpiz-koeln.mpg.de)

Accepted 20 October 1997; published on WWW 8 December 1997

SUMMARY

The identity and developmental pattern of the four organ types constituting the flower is governed by three developmental functions, A, B and C, which are defined by homeotic genes and established in two adjacent whorls. In this report we morphologically and genetically characterise mutants of two genes, *STYLOSA* (*STY*) and *FISTULATA* (*FIS*) which control floral homeotic meristem- and organ-identity genes and developmental events in all floral whorls. The morphology of the reproductive organs in the first and second whorls of *sty fis* double mutant flowers indicate that the two genes are part of the mechanism to prevent ectopic expression of the C-function in the perianth of wild-type flowers. This is verified by the detection of the expansion of the expression domain of the class C gene *PLENA* (*PLE*) towards the perianth. Interestingly, in the second whorl of *sty* and *fis* mutants, spatial differences in stamenoid features and in the pattern of ectopic expression of the *PLE* gene were observed. This suggests that, with respect to the negative control of *PLE*, petals are composed of two regions, a lateral and a central one. Mutation in *ple* is epistatic to most of the *sty*/*fis*-related homeotic defects. *PLE*, however, is not the primary target of *STY*/*FIS* control, because dramatic reduction of expression of *FIMBRIATA*, meristem identity genes (*FLORICAULA* and *SQUAMOSA*) and of class B organ identity genes (*GLOBOSA*) occur before changes in the *PLE* expression pattern. We propose that *STY*/*FIS* are hierarchically high-ranking genes that control cadastral component(s) of the A-function. *SQUAMOSA* as a potential target of this control is discussed. Retarded growth of second whorl organs, subdivision of third whorl primordia and the failure to initiate them in *sty*/*fis* mutants may be mediated by the *FIMBRIATA* gene.

Key words: Flower development, *Antirrhinum*, Homeotic genes, Double mutants, *STYLOSA*, *FISTULATA*

INTRODUCTION

The identity of the four floral organs, sepals, petals, stamens and carpels, is governed by homeotic selector genes (Haughn and Somerville, 1988; Schwarz-Sommer et al., 1990; Coen and Meyerowitz, 1991). In the currently accepted model these genes fall into three classes (Okamuro et al., 1993; Weigel and Meyerowitz, 1994; Haughn et al., 1995), defining three developmental functions, A, B and C that control alone and in combinations the developmental fate of organs. The A-function in the first whorl controls sepal identity and combined with B that of petals in the second; the C-function alone controls carpel identity of fourth whorl organs and in combination with B the developmental fate of stamens in the third. The A, B and C functions in *Arabidopsis* were defined by loss-of-function mutants in homeotic genes, but only B- and C-type mutants were found in *Antirrhinum* (Schwarz-Sommer et al., 1990).

The control of floral organ identity in different species is similar in many respects. In particular, the class B and class C genes display structural and functional similarity (reviewed by Davies and Schwarz-Sommer, 1994). Their precise function and transcriptional control during flower development, however, may differ as shown by comparing class B genes of *Antirrhinum* and *Arabidopsis* (Samach et al., 1997).

The A-function in *Arabidopsis* includes two different levels of developmental control: that of organ identity by selection of the appropriate fate and the spatial (cadastral) restriction of the C-function to whorl 3 and 4. Mutants in class A genes may reveal both or only one of these functions. In addition, class A genes like *AP1* and *AP2* are involved in the control of the inflorescence to flower transition (Bowman et al., 1993; Jofuku et al., 1994). Recently, Okamuro et al. (1997) showed that the control of identity of perianth organs in *ap1* and *ap2* mutant flowers is likely to be linked to the role of these genes in the control of floral meristem identity rather than to a floral homeotic selector function.

There is little information concerning the A-function in *Antirrhinum*. The semidominant mutants *Ovulata/Macho* (Carpenter and Coen, 1990; Schwarz-Sommer et al., 1990; Coen and Meyerowitz, 1991) exhibit some of the morphological features of A-function mutants, indicating loss of a cadastral function that restricts C to whorls 3 and 4.

Ectopic expression of *PLENA* (*PLE*), a class C gene in this species, confirms this (Bradley et al., 1993). *Ovulata*/*Macho* are gain-of-function mutants; a transposon insert within an intron of the semidominant *Ple* allele allows *PLE* transcription in the perianth, without altering the structure of the mature *PLE* mRNA (Bradley et al., 1993). A recessive loss-of-function mutant, representing the negative regulator of *PLE*, whose *cis*-acting binding site may be corrupted in the *Ple* allele was not found. Here we describe recessive mutants of two genes, *STYLOSA* and *FISTULATA*, which together control the restriction of the C function to the inner whorls of the flower. Genetic, morphological and expression studies show, however, that *STY*/*FIS* are more general regulators of homeotic functions in flower development.

MATERIALS AND METHODS

Plant material and genetic stocks

Antirrhinum majus plants were grown in the greenhouse at 18-25°C with additional light during winter. Some mutants and double mutants were transferred into climate chambers where they were grown at 15°C or 26°C.

The wild-type line Sippe 50 and the *stylosa* (*sty*), *fistulata* (*fis*), *fimbriata* (*fim*) and *deficiens*-globifera (*def*-gli) mutants were obtained from the Gatersleben seed collection. The *squamosa*-347 (Huijser et al., 1992), *plena*-237 and *Plena*-888 (*Macho*; Schwarz-Sommer et al., 1990; Lönnig and Saedler, 1994) alleles were isolated in a transposon mutagenesis programme. The lines 165E (niv-98::Tam3) and T53 (niv-53::Tam1) were provided by Rosemary Carpenter (Norwich, UK).

The *stylosa* mutant (Stubbe, 1974) was crossed to different wild-type lines in order to analyse the extent of phenotypic variation. The F_2 progeny consisted of both wild-type and *sty* plants that segregated in an approximately 3:1 ratio suggesting that the mutation is a single recessive trait. However, mutant plants were underrepresented in some populations. In the genetic background of Sippe 50 or T53, the *sty* phenotype was homogeneous. In the 165E background the floral morphology was variable and even a single inflorescence exhibited flowers with both weak and strong phenotypes. Variation in the floral phenotypes was sometimes acropetal, with old *sty* flowers showing a weak, sometimes almost wild-type phenotype, and young flowers displaying strong morphological abnormalities. Since we found no heritable traits influencing the *sty* phenotype, it is likely that this variability reflects changes in environmental conditions and/or a gradient of some internal factors within the inflorescence.

Double mutants

Double mutants between *sty* and other floral homeotic mutants were identified in F_2 populations as plants displaying floral features not observed in the single mutants. Genetic constitution of *sty fis*, *sty Ple-888* and *sty fim* double mutant plants was confirmed by test crosses. *Sty def* double mutants were identified by analysing the segregation ratio.

Double and triple mutant combinations of *sty* and *fis* with *ple* display subtle morphological differences compared to *ple*. Reduced fertility of *sty* and sterility of *ple* made it impossible to obtain larger number of double and triple mutants by selfing plants homozygous for one trait and heterozygous for the other. The *ple*-237 allele, in addition, showed increased somatic instability in the background of the *fis* mutation providing us with easily selectable *fis ple* double mutants, or *sty fis ple* triple mutants (after selfing of *fis* plants heterozygous for *ple* and *sty*). The occurrence of such double mutants indicates that the *STY*, *FIS* and *PLE* loci are not tightly linked genetically.

Microscopy and in situ hybridisation

For scanning electron microscopy (SEM), inflorescences were processed as described previously (Sommer et al., 1990). Tissue preparation and in situ hybridisation experiments were carried out

Table 1. Homeotic morphology displayed by different mutants

			Whorl 1[p]			Whorl 2[p]		Whorl 3	Whorl 4
Wild type	Upper leaves	Bracts	Abaxial sepals (2)	Lateral sepals (2)	Adaxial sepal (1)	Abaxial petals (3)	Adaxial petals (2)	Stamens	Gynoecium
stylosa	–	–	{petaloid}	{petaloid}	–	stamenoid	retarded*	stamenoid/carpelloid, subdivided	+
fistulata	–	–	–	–	–	stamenoid	retarded *	–	–
sty fis	–	–	carpelloid	carpelloid	–	stamenoid/ carpelloid, arrested	arrested[u]	stamenoid/carpelloid, missing/subdivided	+
Macho	–	carpelloid*	carpelloid	carpelloid	carpelloid	stamenoid	retarded	–	–
sty Macho	carpelloid	carpelloid[u]	carpelloid[u]	carpelloid[u]	carpelloid[u]	stamenoid/ carpelloid, arrested*	arrested[u]	stamenoid/carpelloid, subdivided	+
ple	–	–	–	–	–	–	–	petaloid	carpelloid/sepaloid/ petaloid, indeterminate
sty fis ple	–	–	–	–	–	retarded	retarded[u]	petaloid, subdivided	*ple*-like
def	–	–	–	–	–	sepaloid	sepaloid	carpelloid	absent
sty def	–	–	–	–	–	carpelloid, +	carpelloid, +	carpelloid[u], +	+, locules
fim	–	–	–	–	–	petaloid/ sepaloid	petaloid/ sepaloid	{petaloid/carpelloid}	short style
sty fim	–	–	–	–	–	stamenoid/ carpelloid	retarded*	carpelloid[u]	+

[p]Differences observed in features of abaxial and adaxial organs may be due to the function of genes determining the symmetry of the flower and are not discussed in this report; – wild type ; *weak ; [u]strong ; {} rare; + extra styles.

according to published procedures (Coen et al., 1990; Huijser et al., 1992). As a control, sections of wild-type buds were placed side by side with mutant sections.

RESULTS

Wild-type flowers have previously been described in detail (Schwarz-Sommer et al., 1992). Briefly, the perianth consists of two abaxial, two lateral and one adaxial sepals in the first whorl forming the calyx and five petals in the second whorl forming the corolla with two adaxial and three abaxial lobes. The third whorl is composed of four stamens and the stamenodium. The gynoecium is formed by fusion of two carpels in the centre of the flower.

In most mutants and mutant combinations described below, morphological anomalies differ in abaxial and adaxial organs (Table 1), perhaps due to combinatorial effects with genes involved in the control of the symmetry of the flower. Detailed description and discussion of these features are not the subject of this report.

Morphology of *sty* flowers

The *stylosa* mutation does not affect vegetative parts of the plant but causes complex floral homeotic organ alterations. The morphology of sepals is usually normal, except that sometimes they appear petaloid along their margins (Fig. 1A and Table 1).

In mature *sty* flowers petal morphology is distorted. The organs are reduced in size, or retarded in growth and are sometimes twisted (Fig. 1A,B). The marginal region of petals may display morphological features of stamens with filament-like structure at their base and along the tube and an anther-like structure at the upper part (Fig. 1A,B). Complete transformation of petals into stamens was not observed.

The third whorl organs are feminised. However, both the number of feminised stamens and the degree of feminisation are variable. In the most frequent intermediate phenotype, individual organs display combined stamenoid and carpelloid identities. Some of the feminised stamens fuse to the fourth whorl, resulting in a complex chimeric structure in the centre of the flower (Figs 1C and 2G,H). The number of third whorl organs seems to be increased, although the number of third whorl primordia is not affected (see below). In the strongest phenotype, third whorl organs are almost completely carpelloid and show little stamen identity. In the weakest phenotype only abaxial third whorl organ number seems to be increased (Fig. 1D). The shape of anthers is abnormal and a short style sometimes develops at the tip (not shown). Due to feminisation of stamens the male fertility of *sty* flowers is reduced.

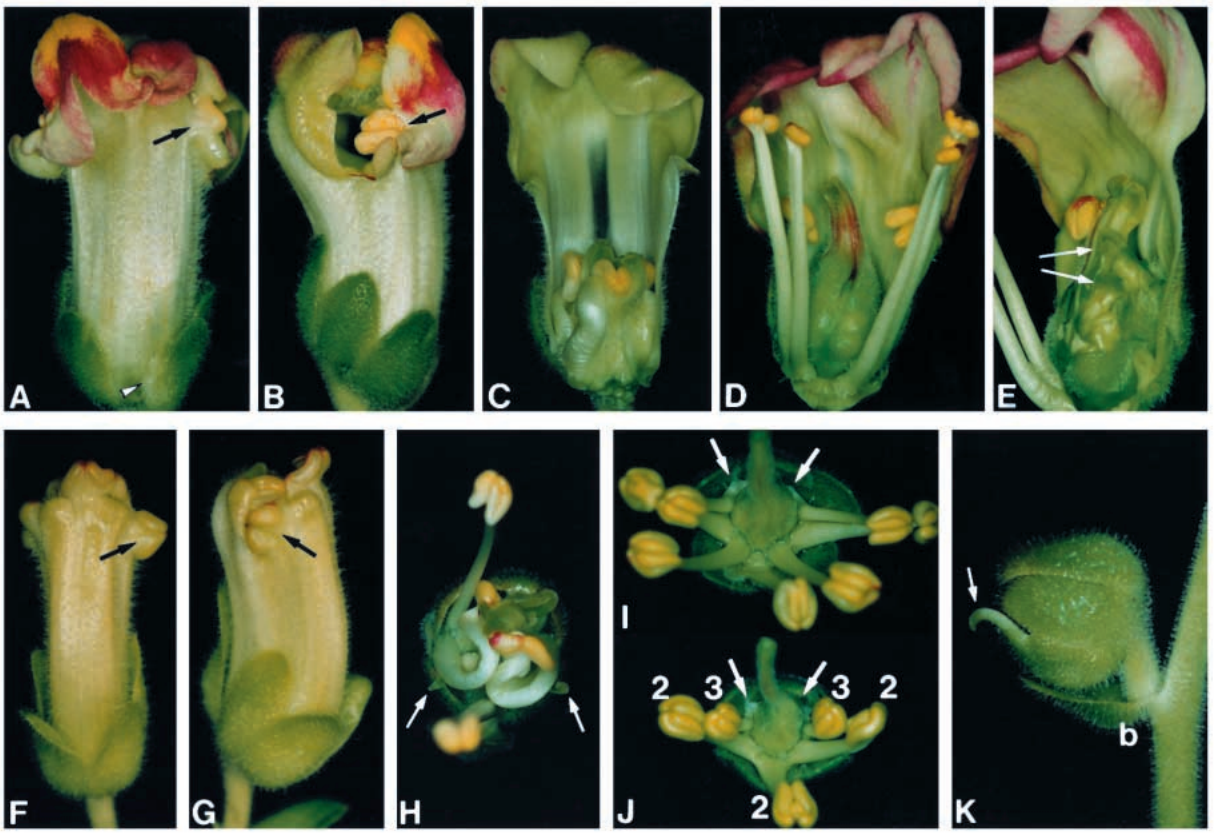

Fig. 1. Phenotypes of mature *sty* (A-E), *fis* (F,G) and *sty fis* mutant (H-K) *Antirrhinum* flowers. A and C-F show flowers in a ventral view; B and G, lateral view and in H-J, top view. Abaxial sepals and petals are removed to show the complex chimeric structure due to fusion of feminised stamens with the gynoecium (C,D) and the style-like outgrowths inside the carpels (arrows in E). Black arrows in A,B,F and G point to stamenoid second whorl organs and the arrowhead in A points to a petaloid sector of the abaxial sepal. The white arrows in H and K show first whorl organs with carpelloid features and indicate positions of the arrested second whorl organs in I and J. In J the numbers indicate the position of the stamens in whorl 2 and 3.

The *sty* mutation most severely affects development of the gynoecium. The carpel and the style may be shorter and broader than in the wild type (Fig. 1D). The two carpels sometimes fail to fuse. In all types of *sty* flowers, thin or broad style-like structures with stigmatic papillae are visible inside the fourth whorl (Fig. 1E). The origin of these structures is not clear, but it is likely that they develop from the placenta or from the receptacle rather than from homeotically altered ovules. The number of these style-like structures and the extent of their growth are variable. In extreme cases, style-like outgrowths confer such a high physical tension to the inner surface of the carpel that it bursts. Although carpels contain well-differentiated ovules the female fertility of *sty* flowers is reduced or completely abolished.

Ontogeny of *sty* flowers

Inflorescences were examined by SEM to determine at which stage floral organogenesis of *sty* mutants deviates from wild type. The developmental pattern of *sty* floral meristems does not differ from wild type until after stage 5 (petal mound; Carpenter et al., 1995) when the five sepal primordia are well separated and start to overgrow the floral meristem. At this stage, as in the wild type, five petal primordia initiate at alternate positions with respect to the sepals and four stamen primordia start to initiate at positions alternate to petal primordia (compare Fig. 2A with Fig. 2E). Retardation of second whorl organ development is visible after stage 6 (Fig. 2G,H).

Third whorl primordia subdivide and grow as two instead of one organ soon after initiation (Fig. 2F). Thus, the increased number of third whorl organs in mature flowers correlates to subdivisions of primordia and not to initiation of additional primordia. In flowers with intermediate phenotype all third whorl primordia become subdivided, resulting in several filamentous outgrowths. Concomitantly, stamens undergo homeotic alterations and differentiate into stamen-carpel chimeric structures. Feminised third whorl primordia invade the centre of the meristem where they fuse with the developing gynoecium (Fig. 2G).

Morphology and ontogeny of *fis* flowers

Fis mutant plants and double mutant combinations with *fis*

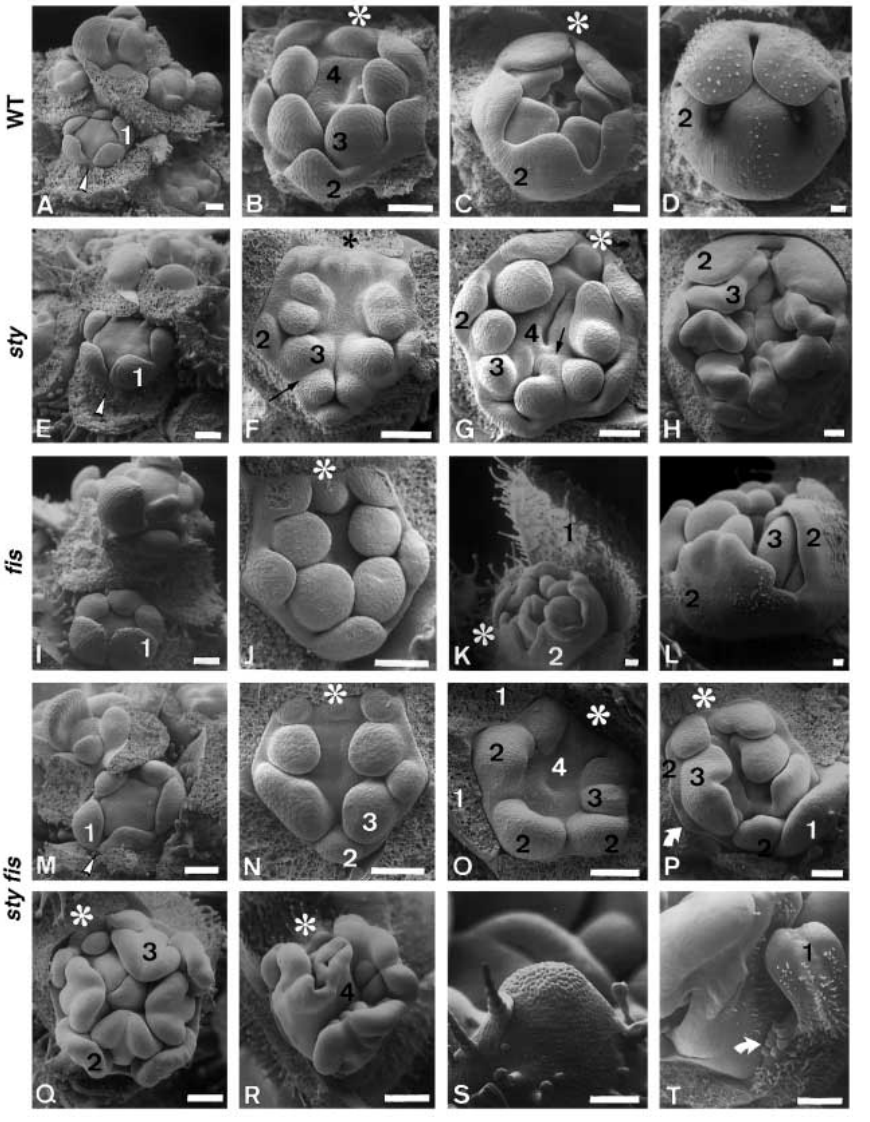

Fig. 2. SEM showing the ontogeny of wild-type (A-D), *sty* (E-H), *fis* (I-L) and *sty fis* (M-T) mutant flowers. (A,E,I,M) Inflorescences with developing young flowers at different stages of development. Arrowheads point to flowers at stage 5 (Carpenter et al., 1995). (B,F,J,N) Flowers at stage 6. The genotype of plants is indicated at the left of the rows. Asterisks show the adaxial position within the flower and numbers indicate the position of organs in the floral whorls. The arrow in F points to a subdivision characteristic for third whorl organs of *sty* and the arrow in G shows the fusion of the subdivided third whorl organs to the fourth whorl. The flower in H reveals this fusion together with suppression of petals and abnormal anthers. (J) *fis* flowers are morphologically similar to wild type until stage 6 and morphological differentiation of lower petal lobes into antheroid structures is apparent after stage 6 (K). (L) Suppression of adaxial lobes and fusion of the corolla tube. (M) a *sty fis* flower displays irregularly formed sepals at early stage 5. (N) Notice initiation of five second whorl organs and further development of second whorl organs with fusion between second and third whorl organs. O and P demonstrate the absence of third whorl organ initiation (empty space between the two second whorl organs designated by black 2s) and arrested development of lateral second whorl organs (white arrow), respectively. Q shows a less extreme phenotype with suppression of adaxial petals, complete homeotic transformation of abaxial petals into stamens and absence of a corolla. If they develop, second whorl organs can become carpelloid and fuse to the gynoecium (R). S and T show the degree of homeotic sepal transformations of plants grown at 15°C (S) or at 25°C (T, arrow points to ovules). The bars represent 100 μm except in Q, R and T where they are 500 μm.

were only analysed as far as was necessary to understand the interaction of *FIS* with *STY*. *Fis* petals are frequently transformed into stamenoid structures or their development is retarded (Fig. 1F,G). The lower part of the petals still forms a corolla tube but their upper part does not display typical lobes. Instead, the tips of the 3 abaxial petals often bear anther-like and male fertile structures. SEM analysis showed that homeotic transformation of petals occurs late, after stage 6 of development (Fig. 2J,K). It also confirmed that sepals, stamens and the gynoecium are not affected homeotically (Fig. 2I,K). Homeotic transformation of *fis* second whorl petals strongly resembles the *blind* mutant in petunia in that mainly the lobes are transformed into a stamenoid structure (Tsuchimoto et al., 1993). *Blind* mutants display weak carpelloidy of sepals, a feature occasionally apparent in the first whorl of *fis*-2, a newly isolated *fis* allele (Z. Schwarz-Sommer and E. de Andrade Silva, unpublished).

The stamenoid features of second whorl organs can increase or decrease in different backgrounds, depending on the presence or absence of modifying factors (I. Heidmann and Z. Schwarz-Sommer, unpublished data). *Fis* sister plants in the F_2 population (necessary to obtain double mutants with *sty*) displayed the uniform intermediate phenotype shown in Figs 1 and 2.

Double mutant analysis

Double mutants were constructed to uncover interactions between *STY* and genes controlling flower development. Phenotypic differences between wild-type and mutant flowers are summarised in Table 1.

stylosa/fistulata

The *sty fis* double mutant combination severely affects all four whorls (Figs 1H-K, 2M-T and Table 1). The sepals of *sty fis* flowers are carpelloid with some variability depending on growth conditions. Plants grown at 26°C display strong carpelloidy of sepals with ovules developing along their margins (Fig. 2T). Carpelloidy of sepals is less pronounced at 15°C, where stigmatic papillae may develop at the tips of the organs (Fig. 2S).

The second whorl of *sty fis* flowers shows transformation of the abaxial petals into stamens. The organs are either composed of carpelloid stamens fused to the third whorl organs (Fig. 1H) or of narrow filaments and anthers resembling wild-type stamens (Figs 1I,J and 2Q). Adaxial petals do not develop (arrows in Fig. 1I,J) or, at 26°C, develop into aborted stamens or into short and narrow petaloid filaments.

Similar to *sty* single mutants, third whorl organs of *sty fis* flowers exhibit different phenotypes under greenhouse conditions. In one type of *sty fis* flowers, carpelloid stamens that may be fused to the gynoecium are present (Figs 1H and 2Q) and in the other type the organs show no severe feminisation (Fig. 1I,J). However, growth at 26°C facilitates feminisation of second and third whorl organs and their fusion to the fourth whorl. In both phenotypes, the total number of second and third whorl stamens is often reduced, but in the mature flower it is not always clear which stamen, in which whorl, is absent. SEM analysis showed that arrest of second whorl organ development occurs subsequent to organ initiation while sometimes third whorl organs are not initiated (Fig. 2O,P). SEM studies also revealed complex fusion between second and third and third and fourth whorl organs (Fig. 2P,R) as well as carpelloidy of the first whorl sepals (Fig. 2S,T).

Features displayed in *sty fis* flowers not observed in the single mutants, such as carpelloidy of sepals or the reduced number of third whorl organs, indicate synergistic interaction between the two genes.

In summary, *sty fis* flower morphology resembles that of the *Arabidopsis* A-function mutants *ap2* (Jofuku et al., 1994) and *ap1* (Schultz and Haughn, 1993), in all homeotic organ type alterations. However, floral identity of organs in the first whorl is not affected and perianth organ number is not reduced. The similarity to mutants like *lug* (Liu and Meyerowitz, 1995) and *clf* (Goodrich et al., 1997) is also incomplete: *sty fis* does not affect leaf morphology, and *lug* and *clf* flowers do not display feminised features in their second or third whorl organs.

The phenotypic similarity of *sty*/*fis* and mutants of the *Arabidopsis* A class gene *AP2*, prompted us to clone *Antirrhinum* cDNAs which display sequence similarity to *AP2* (H. Sommer, P. Motte, H. Meijer, Z. Schwarz-Sommer, unpublished). RT-PCR and northern blot experiments failed to show alteration of expression of these genes in *fis* or *sty* mutants (not shown). Thus the structural similarity of the *STY* or *FIS* genes with *AP2* remains an open question.

stylosa/plena

The morphology of *sty*, *fis* and *sty fis* mutants indicates ectopic expression of the C-function in the two outer whorls. Double mutants were constructed with *ple* to determine if mutation in this class C gene is epistatic to *sty* or *fis*. In the recessive *plena* mutant the first and second whorls appear normal, while the stamens are petaloid narrow organs and often fuse to the second whorl (Fig. 3A,D). The two fourth whorl organs display a combination of sepaloid, carpelloid and petaloid features. Inside, a new flower with petaloid and sepaloid organs develop (Bradley et al., 1993). In the F_2 populations only wild-type, *sty* and *ple* phenotypes appeared, suggesting that *ple* is epistatic to *sty*. On some of the *ple* plants, flowers displayed additional abaxial petaloid organs adjacent to the third whorl organs, resembling subdivision of third whorl organs in the *sty* mutant (Fig. 3E).

In the F_2 population, developed to obtain *fis ple* double mutants, frequent somatic reversion of the genetically unstable *ple*-237 allele occurred, making determination of the *fis ple* phenotype difficult. Among non-reverting *ple* flowers some displayed slightly retarded development of second whorl petals. Interestingly, due to partial somatic reversion of *ple*, some *fis ple* double mutants with partially restored stamens still display indeterminacy (Fig. 3C,F). The petaloid organs internal to the fourth whorl are stamenoid, suggesting that restoration of the *PLE* function in the centre of the flower is sufficient to confer *fis*-dependent stamenoidy to the petaloid organs, while it is insufficient to prevent indeterminacy (Fig. 3F).

The *sty fis ple* triple mutant was obtained in the progeny of a selfed *fis* plant that was heterozygous for *sty* and *ple*. Among 32 *ple* plants, some of which displayed the features of *ple sty* or *ple fis* double mutants mentioned above, two plants with unusual morphology were found. The genetic constitution of the triple mutant was confirmed by examining in situ expression patterns of genes that are severely affected in the *sty fis* double mutant (see below). In their third whorl the triple mutants exhibit slightly increased petaloidy compared to *ple*

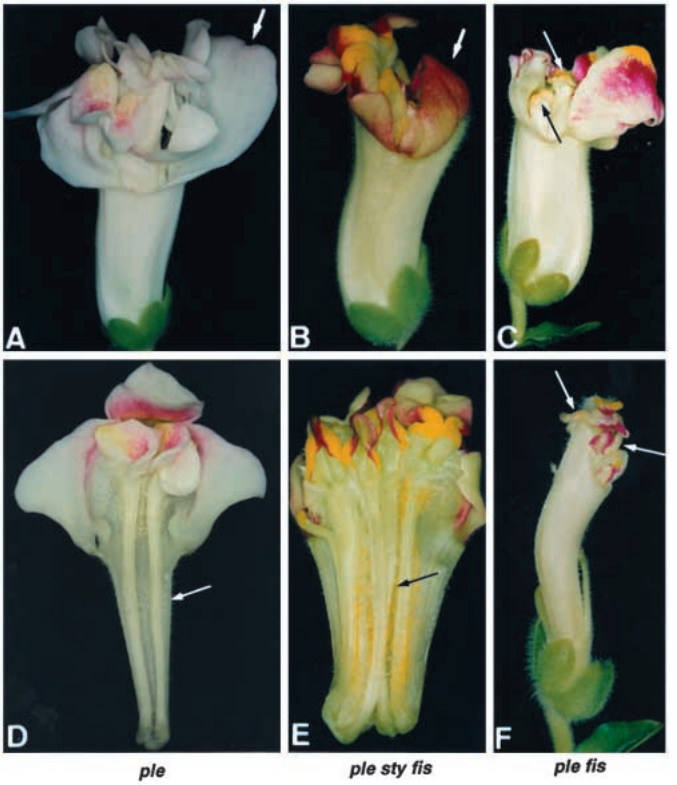

Fig. 3. Phenotype of *ple* (A,D), *sty fis ple* (B,E) and *ple fis* (C,F) flowers obtained in a population segregating for the *ple*, *sty* and *fis* mutants. Intact flowers in the top row are shown in a lateral view. Arrows in A and B point to the adaxial lobe of the corolla which is severely reduced in the triple mutant (B). D and E show the morphology of abaxial petaloid third whorl organs (fused to the abaxial lobe and indicated by arrows) after removing the adaxial part of the flower. Notice the complex, subdivided third whorl organs in E compared to the simple petaloid stamens in D. The *ple fis* flower in C (lateral view) and F (second and third whorl organs removed) displays partial somatic reversion of the *ple*-237 allele, revealed by restoration of stamens (arrows in C) and indeterminacy combined with development of stamenoid petals (arrows in F) in the centre of the flower.

mutants, and split organs similar to subdivisions in *ple sty* flowers (Fig. 3). Second whorl upper and lower lobes are severely suppressed (Fig. 3B). These observations suggest that *ple* is epistatic to *sty* and *fis* with respect to homeotic organ transformations, but the *STY* and *FIS* functions are independent of *PLE* expression with respect to retardation of development of the second whorl and possibly to subdivisions in the third whorl.

The double mutant, *sty Ple-888* (Lönnig and Saedler, 1994) was constructed to determine whether structural alteration of the semidominant *Ple* allele (see Introduction) renders its expression independent of *STY*. In our F_2 populations developed to generate double mutants with *sty*, *Ple-888* sister plants showed five carpelloid sepals with or without ovules. Adaxial second whorl organs appeared as narrow, split petaloid structures, retarded and sometimes arrested in development, similar to the stamenodium in the third whorl of wild-type flowers. The three abaxial petaloid organs often displayed antheroid features. Female fertility of *Ple-888* flowers is frequently reduced. Upper leaves were not affected by the mutation.

Sty in an either homo- or heterozygous *Ple-888* background conditions strong homeotic transformation of the youngest leaves subtending the inflorescence, into carpelloid leaves with stigmatic tissue at their tips (Fig. 4A,B). This homeotic transformation of the upper leaves facilitated selection of double mutants. The bracts subtending the floral meristems also display strong carpelloidy. In double mutants, in contrast to wild-type and *Ple-888* inflorescences, the carpelloid bracts do not overgrow the apical meristem which remains visible for a long period of development (Figs 4B and 5A). The aberrant morphology of bracts and upper vegetative organs uncovers functions of the *STY* gene outside the flower.

Within the flower, first whorl organs may fuse laterally due to increased carpelloidy of the sepals. The second whorl organs either develop as (carpelloid) stamens without petaloid sectors (Fig. 4C,D) or are arrested in development and abort (Fig. 5C and Table 1). The stamenoid-carpelloid third whorl primordia subdivide as in *sty* flowers (Fig. 5B) and can fuse to the gynoecium and to the sometimes feminised second whorl stamens. Because of such fusions it is difficult to determine the origin of organs in mature flowers. Complete transformation of the stamenodium into a small individual ovule-containing carpel may also occur. In extreme cases, floral organs in all whorls display strong carpelloidy and little or no stamenoid features Fig. 5D). Increased severity of the *Ple-888* mutant phenotype including carpelloidy of non-floral organs by combination with *sty* indicates that the structural alteration of the *PLE* gene in the *Ple-888* allele does not prevent control events governed by *STY*.

stylosa/deficiens

The null allele *def*-gli confers sepaloidy to petals and carpelloidy to stamens (Sommer et al., 1990; Schwarz-Sommer et al., 1992). *Sty def* double mutant flowers exhibit a first whorl of five wild-type-like sepals with no morphological alterations. The sepaloid second whorl organs are feminised. Their margins are carpelloid and may bear ovules and their tips are stigmatic (Figs 4E,F and 5H). Carpelloidy of second whorl organs in the absence of *DEF* function confirms stamenoidy of petals in the *sty* mutant. The margins of second whorl organs can fuse to the third whorl. Sometimes second whorl carpelloid sepals become incorporated into the third whorl and grow as a broad individual organ. Such a fusion is most frequent between one pair of organs but several fusions are also possible (Fig. 5G). SEM analysis showed that the four carpelloid third whorl organs initiate at the correct position and correct time, although early fusion between the margins of the second and third whorl organs may result from a slight displacement of primordia conditioning spiral rather than whorled arrangement of organs (Fig. 5G). Style-like outgrowths, characteristic of the fourth whorl carpels of *sty* flowers can differentiate in all carpelloid organs of *sty def* flowers (Fig. 4F). Subdivision of third whorl primordia similar to that of *sty* flowers cannot be observed (Fig. 5E,F). Interestingly, additional locules develop inside the third whorl of *sty def* flowers (Fig. 4I), where no organs develop in *def*-gli flowers, reminiscent of *fim def* double mutant morphology (Tröbner et al., 1992). The reason for this feature is not clear.

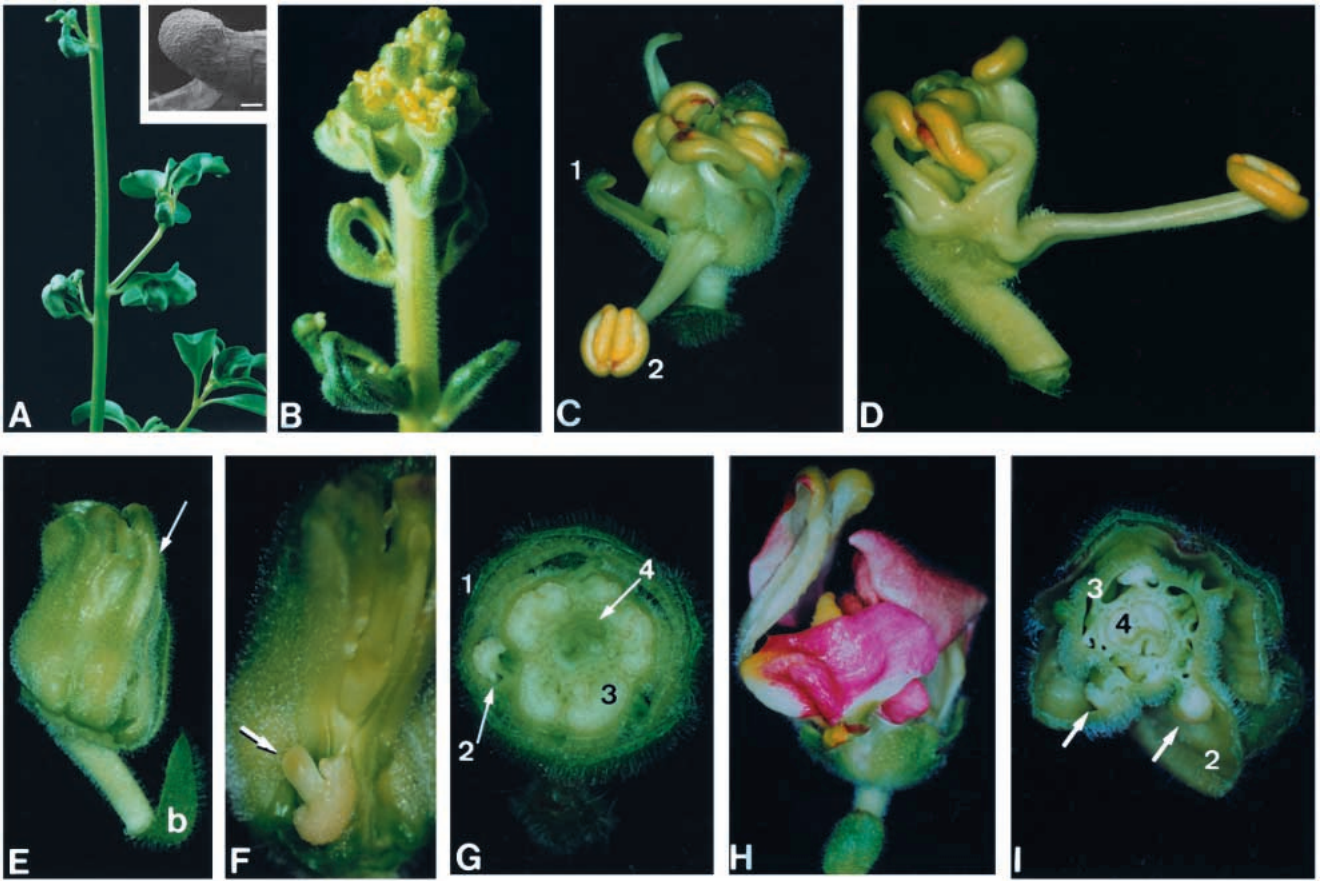

Fig. 4. Phenotypes of *sty Ple-888* (A-D), *sty def* (E-G) and *sty fim* (H,I) double mutants. The upper leaves below the inflorescence of *sty Ple-888* mutants are curly and the SEM demonstrates stigmatic papillae at their tips (A; bar = 100 μm). The bracts are strongly carpelloid (B). (C) Frontal and (D) lateral views of a mature floral bud with carpelloid sepals (1) in the first whorl and morphologically wild-type or carpelloid stamens (2) in the second. (E) Lateral view of the interior of a *sty def* bud with carpelloid second whorl margins (arrow; b, bract). Ovules adjacent to a style-like structure (arrow) can develop at the fusion between carpelloid second whorl sepals and third whorl organs (F). This is also visible in the cross-section in G (2) as well as additional locules internal to third whorl organs (4). (H) A *sty fim* flower in frontal view. Complete homeotic transformation of stamens into carpels and their fusion to the fourth whorl is clear in the cross-section in I. Carpelloidy of the margins of petaloid organs and their fusion to the third whorl where ovules differentiate is also visible (arrows). Floral whorls are indicated by numbers.

stylosa /fimbriata

Fim flowers display homeotic organ alterations in whorls 2, 3 and 4 and the extent of these alterations differs in different *fim* alleles (Simon et al., 1994). In the analysed F_2 population the petals of *fim* flowers showed streaks of green sepaloid tissue, the stamens were wild-type-like, or slightly petaloid to carpelloid and the organs sometimes fused to the gynoecium. The gynoecium was either wild-type-like or displayed a short style and reduced female fertility. The *fim* mutation in this population most strongly affects establishment of the B-function.

In *sty fim* double mutant flowers (Figs 4H,I and 5I-L) organ differentiation in the second whorl is complex. In the mature flower, petals contain sepaloid sectors, they are not fused and the abaxial organs display stamenoid and/or carpelloid features. A filament and an antheroid structure can develop at the margin of some of the second whorl organs (Fig. 5L). The four stamens and the stamenodium in the third whorl are transformed into carpelloid organs (Figs 4I and 5J,K). Second and third whorl organs can fuse and along this fusion ovules develop (arrows in Fig. 4I). SEM uncovers early fusion of second whorl organs with a third whorl carpel (Fig. 5J) similar to the spiral pattern described for stronger *fim* alleles (Ingram et al., 1997) and *sty def* flowers. The subdivision of third whorl organs, typical for *sty* flowers, does not occur. In this respect *fim* seems to be epistatic to *sty*, while the two genes act synergistically concerning homeotic effects related to decrease of the B-function.

In situ hybridisation

In situ mRNA hybridisation experiments were carried out using probes for meristem identity genes, *FIM* and organ identity genes to determine more precisely the role of *STY* and *FIS* in the spatial and temporal control of homeotic gene expression.

Expression of meristem identity genes

In the bracts and in very young floral meristems of wild type, *sty*, and *fis* mutants *SQUA* (Huijser et al., 1992) is uniformly expressed at high level (Fig. 6A,B,D). During organ initiation *SQUA* expression in the wild type and in the mutants becomes restricted to first and second whorl organs. At stages 4 to 5 *SQUA* transcription in *sty* sepals and petals starts to be slightly

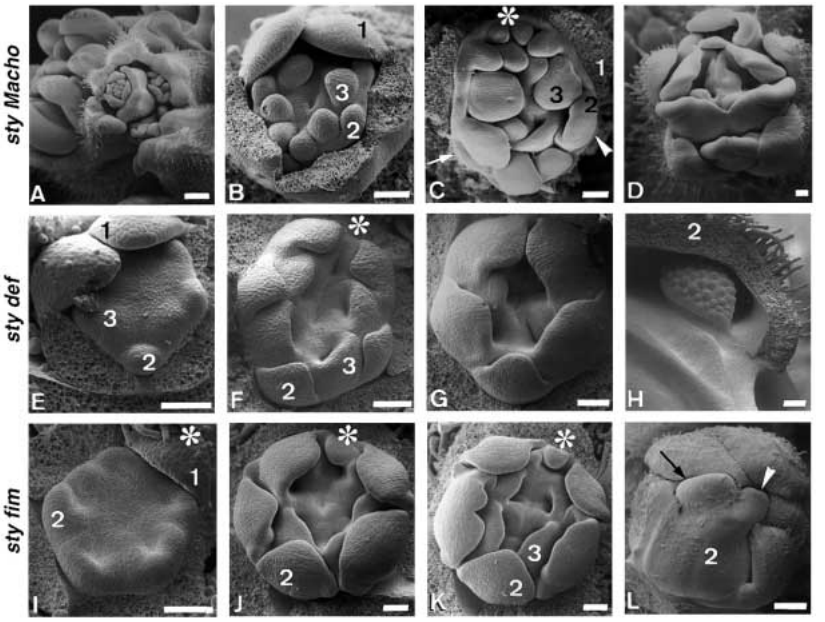

Fig. 5. SEM of developing flowers of *sty Ple-888* (A-D), *sty def* (E-H) and *sty fim* (I-L) double mutants. The *sty Ple-888* inflorescence in A shows strong carpelloidy of bracts that fuse to each other and do not cover the apical meristem. In B a stage 6 flower is shown with subdivisions of abaxial stamens. Extreme phenotypes are depicted in C and D, with retarded second whorl development (arrow) or homeotic transformation of petals into carpelloid organs (arrowhead in C). E and F show initiation of third whorl primordia at the correct position and the absence of their subdivision in *sty def* mutants. In G fusion of the margins of some second whorl organs with carpelloid third whorl organs confers spiral arrangement of second and third whorl organs. (H) A close-up of differentiating ovules at the fusion between second and third whorl organs. *Sty fim* flowers display homeotic transformation at the margin of second whorl organs at early stage 5 (I) and 6 (J), but subdivision of third whorl organs is absent (J,K). (L) The margins of a *sty fim* second whorl organ differentiate into stamenoid (arrow) and carpelloid (arrowhead) tissues. Numbers indicate the respective floral whorls. Bars represent 100 μm; except in A and L, 500 μm.

reduced as compared to wild type (Fig. 6B,E,F) and decreases more strongly during later stages (not shown). This observation suggests that *STY* is necessary to maintain *SQUA* transcription.

In *sty fis* double mutants *SQUA* expression is similar to wild type until stage 3 (Fig. 6C). Subsequently the transcript level is severely reduced in the entire young flower including sepals and in initiating second whorl organs (Fig. 6G). *SQUA* transcripts are present until stages 4 to 5 in sepals and in the cells giving rise to second whorl organs, but the amount is low such that undiluted emulsion and longer exposure time had to be used for detection (not shown). Later *SQUA* transcripts disappear in sepals and in arrested second whorl organs (Fig. 6H). During late stages of development *SQUA* expression reappears in the fourth whorl of wild-type, *sty* and *fis* flowers within the carpel wall facing the placenta, and also at reduced levels in the *sty fis* double mutant (Fig. 6H). Decrease in early *SQUA* expression in *sty fis ple* flowers is similar to *sty fis* flowers, confirming the genetic constitution of the triple mutant (not shown).

Interestingly, in the second whorl organs of *fis* flowers at stage 6, and later, *SQUA* expression is only detectable in those regions that do not undergo homeotic organ transformation (Fig. 6I). This resembles the reduction of *SQUA* expression in wild-type stamens (Huijser et al., 1992) and suggests that late reduction of *SQUA* transcription is concomitant with stamen development, irrespective of the position of the organ.

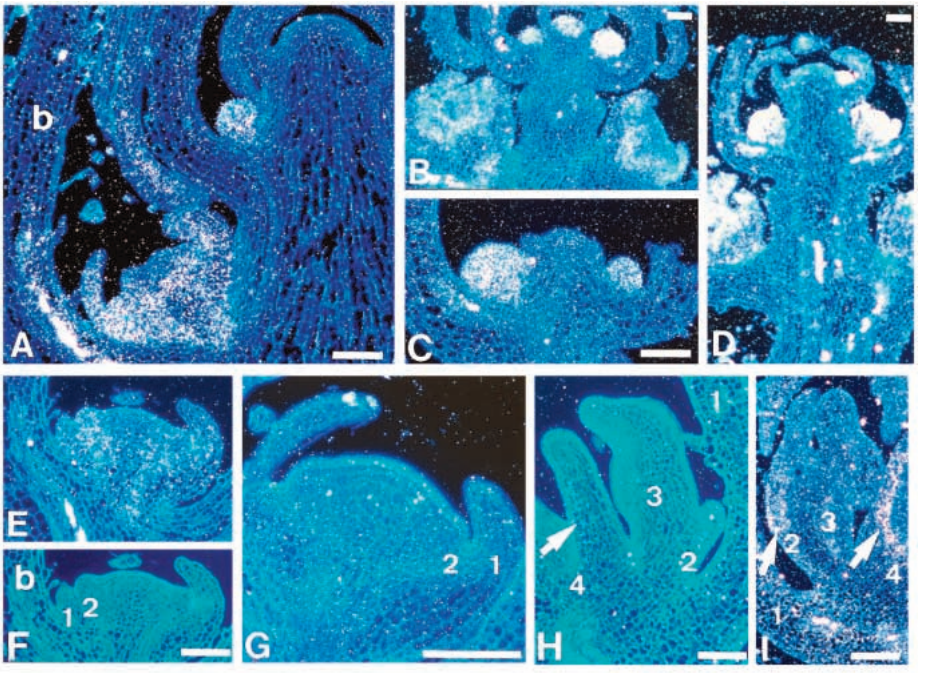

Fig. 6. In situ expression pattern of *SQUA* in wild-type (A), *sty* (B,E), *fis* (D,I) and *sty fis* (C,G,H) mutants. Longitudinal sections of inflorescences (A-D) and flowers (E-I) were hybridised with the ^{35}S-labelled antisense RNA probe. The hybridisation signal was photographed in dark-field, superimposed on the epifluorescence exposure. The oldest flower shown in A is at about stage 5; those in B and D are at stage 4 (right) and 6 (left) and those in D at stage 4 (right) and about 6 (left). (F) An epifluorescence photograph of E to demonstrate uniform decrease in *SQUA* expression in stage 5 *sty* flowers in the entire floral meristem including initiating sepals and petals. The *sty fis* flower in G reveals severe overall reduction of *SQUA* expression at stage 5. The arrow in H points to the hybridisation signal within the fourth whorl. (I) *SQUA* expression (arrows) is restricted to homeotically non-transformed regions of *fis* second whorl organs. Numbers indicate the respective floral whorls. b, bract. Bars represent 100 μm.

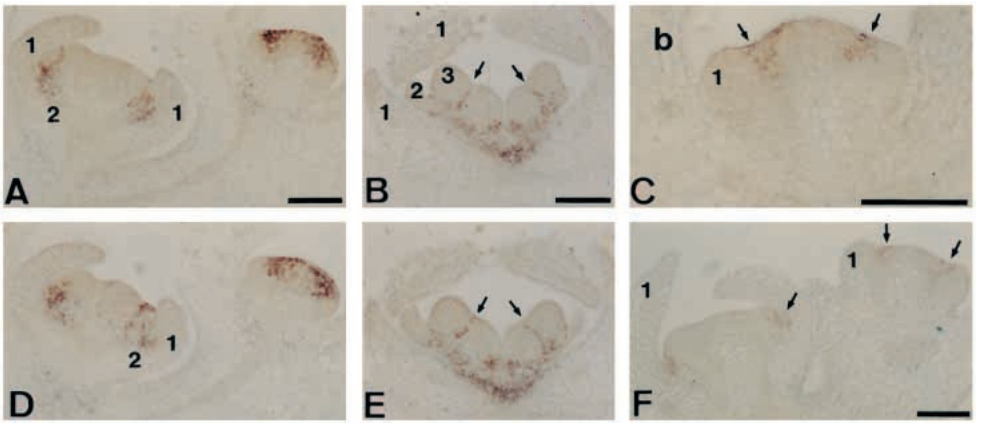

Fig. 7. In situ expression patterns of *FIM* in *sty* (A-E) and *sty fis* (F) mutant flowers. Longitudinal sections through floral meristems were hybridised with DIG-labelled antisense RNA probes, immunodetected with anti-DIG antibody coupled with alkaline phosphatase. In the bright-field photographs the hybridisation signal appears as a purple/brown precipitate. (A,B,D,E) Serial sections of floral buds of an intermediate *sty* mutant at stages 3 (A,D right), stage 5 (A,D left) and stage 6 (B,E). The serial sections in B and E demonstrate ectopic expression of *FIM* within third whorl organ subdivisions (arrows). In strong *sty* flowers the *FIM* hybridisation signal is severely reduced at stage 3 (C) and it is absent in the central dome of the meristem. In the *sty fis* double mutant (F), *FIM* transcripts are hardly detectable in stage 3 and older floral meristems (arrows). b, bract; floral whorls are indicated by numbers. Bars represent 100 μm.

The expression pattern of *FLORICAULA* (*FLO*; Coen et al., 1990) was also determined and was found to be similar to that described above for *SQUA* (not shown).

Expression of *FIMBRIATA*

The spatial pattern of early *FIM* expression in wild-type, *sty* and *fis* flowers is similar except that the hybridisation signal seems to be patchy in *sty* (Fig. 7A,D). At stage 3 *FIM* transcripts appear in a ring internal to wild-type or mutant sepal primordia (Fig. 7A,C,D) with reduced signal intensity in strong *sty* flowers (Fig. 7C). Interestingly, *FIM* is expressed ectopically around the subdivisions of the third whorl organs, which are characteristic of *sty* flowers (Fig. 7B,E).

The level of *FIM* expression is markedly reduced in the *sty fis* double mutant. Already by late stage 3 *FIM* transcripts are hardly detectable and are only present at the boundary of the first and the second whorl and in a few cells, which may later separate the second and third whorls (Fig. 7F). At later stages only cells between sepals and the second whorl show weak *FIM* expression (Fig. 7F).

Expression of the B organ identity genes *DEF* and *GLO*

Early *DEF* transcription (Hantke et al., 1995; Zachgo et al., 1995) is not affected in *sty* flowers, except that it is slightly increased in the sepals and in the fourth whorl until stage 6 (Fig. 8F,G). In contrast, *GLO* expression (Tröbner et al., 1992) in *sty* flowers is reduced and patchy by stage 5 (Fig. 8C), and heterogeneity of the hybridisation signal by stage 4 indicates an early effect (Fig. 8B). Ectopic expression of the *GLO* gene is sometimes observed in the sepals (not shown). Reduction of *GLO* transcription in the second and third whorl organs of the *sty* mutant continues during later stages of development until it becomes restricted to petals and to the stamenoid tissue of the chimeric structure developing by the fusion of feminised stamens and the gynoecium (Fig. 8D). *DEF* transcripts after stage 6 are only detected in the tissues expressing *GLO* (not shown). These observations suggest that early expression of *GLO* is controlled directly or indirectly by *STY* before the onset of the autoregulatory control in the second and third whorls that also affects *DEF* transcription (Tröbner et al., 1992; Zachgo et al., 1995).

Similarly, in *sty fis* double mutant flowers, the *GLO* hybridisation signal is heterogeneous at stage 4 (not shown). By late stage 5, the signal is weak within regions facing the centre of the meristem and somewhat stronger in the region towards the first whorl. During later stages *GLO* expression becomes further reduced and hardly detectable in the rudimentary second whorl organs (Fig. 8E).

Expression of the C organ identity gene *PLENA*

In the *sty* mutant early *PLE* expression in the centre of the flower, spanning the region that gives rise to stamens and carpels, is comparable to the pattern in wild type (Bradley et al., 1993; not shown). The intensity of the hybridisation signal does not seem to differ significantly from that in the wild type, but it is difficult to compare in situ expression patterns for subtle quantitative differences. Ectopic *PLE* expression in the initiating second whorl primordia by stage 5 is slightly above background and detectable by stage 6 (Fig. 9A). First whorl organs do not express *PLE*: undiluted emulsion combined with long exposure time did not reveal a signal above background (not shown). The spatial pattern of ectopic *PLE* expression in the second whorl is variable, but *PLE* transcripts were usually detected in sections through the marginal regions of differentiating second whorl organs even without stamenoid features (Fig. 9G,I). In the fourth whorl of *sty* flowers *PLE* is expressed in the style-like structures, which emerge from the placenta (Fig. 9I) and, similar to wild type, in the placenta, in developing ovules and in the tissue giving rise to the stigma (not shown).

PLE expression was observed within the tip of upper leaves and in the carpelloid bracts of *sty Ple-888* double mutants where it is stronger than in *Ple-888* plants (not shown).

In the *fis* mutant *PLE* is ectopically expressed in the abaxial second whorl organs at stage 6 (Fig. 9B). When antheroid structures develop, *PLE* transcripts are detectable in the same differentiated tissue as in wild-type third whorl organs, such as the vascular tissue, the epidermis of the style facing the gynoecium and the cells located between two antheroid locules. Epidermal cells of the adaxial corolla tube, facing the centre of the meristem and corresponding to the middle of second whorl organs, also show *PLE* transcription (Fig. 9).

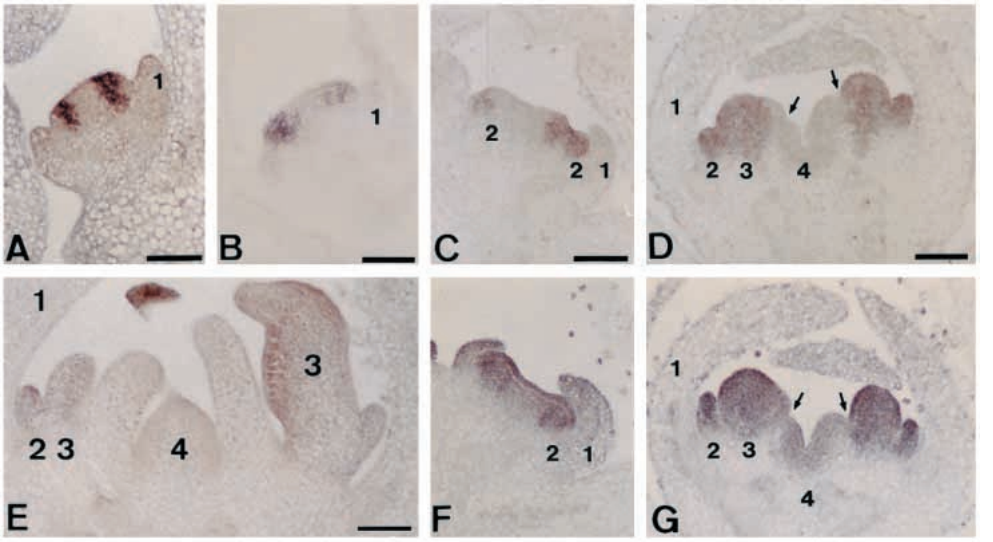

Fig. 8. In situ expression pattern of class B genes in wild-type (A) *sty* (B-D and F-G) and *sty fis* (E) flowers. Longitudinal sections of flowers were hybridised with antisense glo (A-E) and def (F-G) probes as indicated in the legend to Fig. 7. Wild-type (A) and *sty* (B) buds at stage 4 are shown side by side to demonstrate early reduction of *GLO* expression in the mutant. Serial sections from *sty* buds at stage 5 (C,F) and later (D,G) were probed with glo (C,D) and def (D,G) antisense probes for comparison of expression patterns. Arrows in D and G point to fusion of carpelloid second and third whorl organs. Weak ectopic *DEF* expression in the first whorl is visible in F. The developmental stage of the *sty fis* flower in E is comparable to the *sty* flowers in D,G. Floral whorls are indicated by numbers. Bars represent 100 μm.

Thus in the second whorl of *sty* and *fis* flowers the spatial pattern of ectopic *PLE* expression differs, corresponding to distinct regions that undergo homeotic transformation in mature organs of the mutants (Fig. 1A,F).

Ectopic *PLE* expression in the second whorl and in the epidermal cells of sepals in the first whorl is detectable in the *sty fis* double mutant by early stage 6 (Fig. 9C,D). In the second whorl *PLE* is either expressed in the entire initiating primordium or only in the cells facing the centre of the meristem. *PLE* transcripts are absent in those regions of the second whorl that appear as a rudimentary ridge in older flowers (Fig. 9E). *PLE* expression increases later in abaxial and lateral sepals and in developing second or third whorl stamens.

In summary, mutation in the *STY* and *FIS* genes leads to expansion of the expression domain of *PLE* towards the perianth organs of the flower. However, several lines of

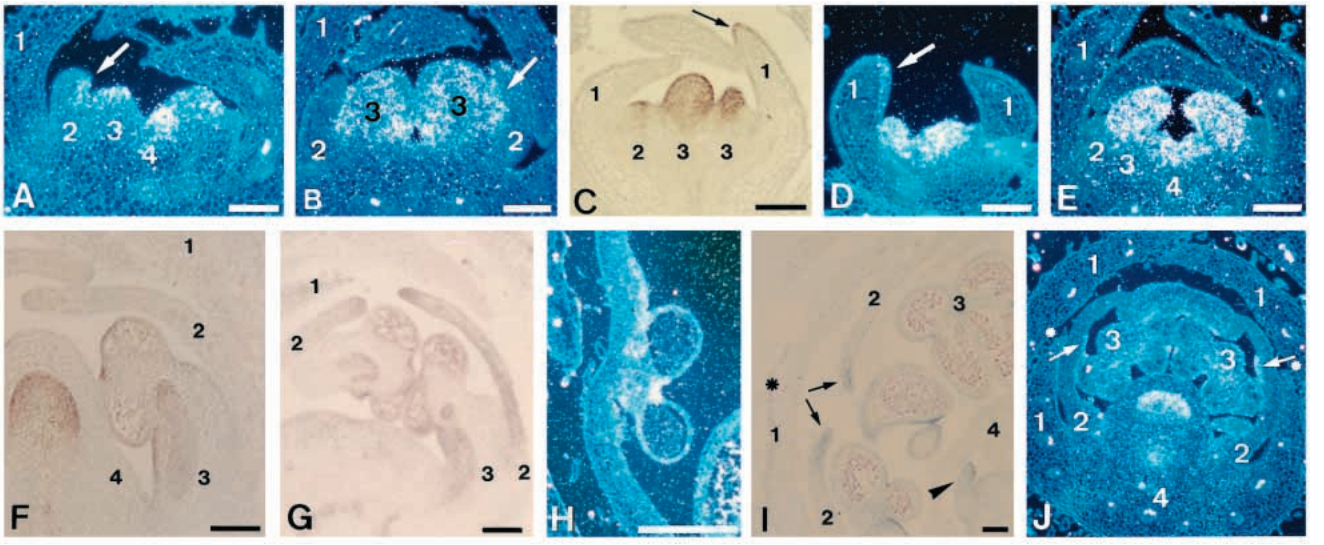

Fig. 9. In situ expression pattern of *PLE* in *sty* (A,G-I), *fis* (B,J) and *sty fis* (C-E) mutants. Longitudinal (A-H) and cross sections (I,J) of flowers were hybridised with the ^{35}S- or DIG-labelled antisense ple probe and photographed as stated in the legends to Figs 6 and 7. The section in I was in addition stained with fuchsin (red-coloured pollen). Arrows indicate ectopic *PLE* expression in the second whorl of stage 6 *sty* (A) and *fis* (B) buds as well as in the sepals of early stage 6 (C,D) *sty fis* flowers. (E) *sty fis* flower, stage 6. The spatial pattern of *PLE* expression in stamenoid organs of older mutant flowers is shown in the bottom row in comparison to that of a wild-type stamen (F). In G the *PLE*-expressing margin of the second whorl organ at the right is defined by sectioning through the filament of the neighbouring stamen. (H) The stamenoid second whorl organ of a strong *sty* flower. (I,J) Cross sections demonstrating the complementary pattern of hybridisation signals (arrows) obtained in the second whorl of *sty* (I) and *fis* (J) flowers. For orientation, the star in I marks the middle of a lateral sepal corresponding to the position of the margins of the second whorl organs, while the stars in J mark the margins of the lateral sepals corresponding to the middle position of the second whorl organs. The arrowhead in I points to a style like outgrowth within the fourth whorl, expressing *PLE* at its tip. Floral whorls are indicated by numbers. Bars represent 100 μm.

evidence, in part summarised in Figure 10, suggest that *PLE* is not the primary target of *STY*/*FIS* control. Firstly, early alteration of *SQUA* expression is independent of *PLE* in the *sty fis ple* triple mutant. Secondly, the ectopic *PLE* pattern appears later than changes in *SQUA*, *FLO*, *FIM* and *GLO* expression during *sty*/*fis* flower development. Alterations in the spatial expression patterns of *SQUA* (and *FLO*) are also not correlated with ectopic expression of *PLE*, because overall reduction of *SQUA* transcripts in *sty* and *sty fis* mutant flowers occurs in the absence of *PLE* expression in the first whorl (Fig. 10D,F). In contrast, initiating *Ple-888* first whorl organs already display ectopic *PLE* expression at stage 4 (Fig. 10C) without changes in the expression of *SQUA* (Fig. 10E), *GLO* (Fig. 10K) or *FIM* and *FLO* (not shown). In older *Ple-888* flowers transcription of *SQUA* is reduced in restricted regions within the first whorl (Fig. 10I) that coincide with the ectopic presence of *PLE* transcripts (Fig. 10G). Thus, during later stages of organ differentiation *PLE* negatively regulates *SQUA*.

DISCUSSION

This work provides the first evidence that in *Antirrhinum* two genes, *STYLOSA* and *FISTULATA*, control homeotic gene expression, including the negative control of the C-function gene *PLE* in the two outer whorls of the flower. In this feature and in the epistatic relation of *ple* to their mutants, *STY* and *FIS* resemble *Arabidopsis* A-function genes. However, several observations discussed below suggest that *PLE* is not the immediate target of *STY* /*FIS* control. Although the synergistic effect of *sty* and *fis* indicates that the two genes control similar developmental event(s), it does not exclude the possibility that the target(s) are different. For simplicity the effects of *STY*/*FIS* are considered together in the discussion; the evidence for differences in *STY* and *FIS* target genes is beyond the scope of this report.

The role of *STY/FIS* in the spatial control of perianth organ identity

The C-function, represented by *PLE*, is necessary in the two inner whorls of the flower for the development of reproductive organs (Bradley et al., 1993). Reproductive organs in the perianth of *sty fis* mutants and the ectopically modified expression pattern of *PLE* indicate that in the wild type the *STY* and *FIS* genes together are responsible for the absence of *PLE* expression in the two outer whorls.

The influence of *STY*/*FIS* on *PLE* is possibly mediated by the meristem identity genes *FLO* (Coen et al., 1990) and *SQUA* (Huijser et al., 1992). Dramatic changes in expression of *FLO* and *SQUA* during early *sty fis* flower development show that, in the wild type, maintenance of early *SQUA* and *FLO* expression is controlled by *STY*/*FIS*. In the *sty fis* mutant the loss of this (positive) control event precedes alterations of the *PLE* expression pattern. Thus, in the wild type, meristem identity genes may control exclusion of *PLE* from the outer whorls. This resembles the control relationships between class A and class C genes in *Arabidopsis*.

Interestingly, *SQUA* is similar to *AP1* (Mandel et al., 1992), one of the class A genes in *Arabidopsis* that is necessary to negatively control the class C gene *AGAMOUS* (*AG*; Yanofsky et al., 1990) in the first and second floral whorls (Bowman et

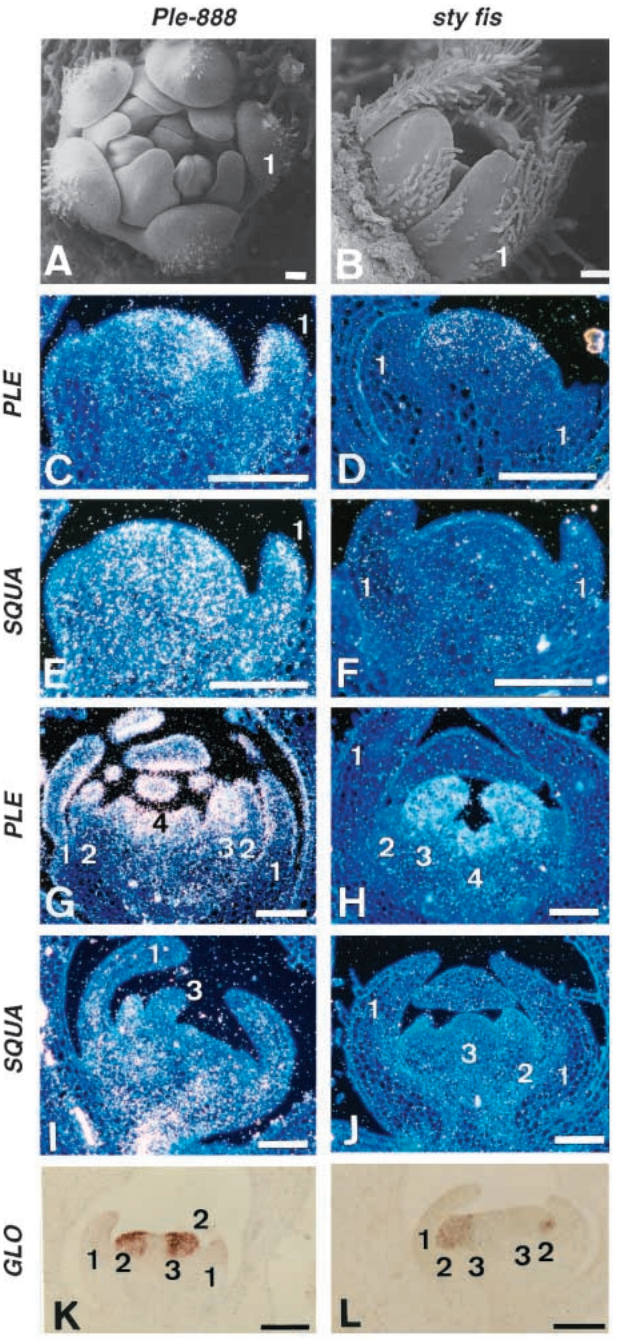

Fig. 10. Comparison of the in situ expression patterns of *PLE*, *SQUA* and *GLO* in *Ple-888* and *sty fis* mutants. Genotypes are indicated above the panels and the probes used for hybridisation are shown at the left. (A,B) SEMs displaying severe organ transformation in the perianth of a *Ple-888* flower (A) compared to the mild carpelloidy in the first whorl of a *sty fis* double mutant (B). The serial longitudinal sections for in situ hybridisation were prepared from flowers at stage 4 (C-F), stage 6 (G-J) and stage 5 (K,L) of development. Notice early ectopic *PLE* transcripts and strong *SQUA* expression in the *Ple-888* flowers (C and G, respectively), compared to severe reduction of *SQUA* in the young *sty fis* flower (H) in the absence of altered *PLE* expression pattern (D). Reduction of *SQUA* in the first whorl of *Ple-888* flowers (I) is detectable later in first whorl regions expressing *PLE* (G). Numbers indicate the floral whorls. Bars represent 100 μm.

al., 1993; Schultz and Haughn, 1993). The function of *SQUA* in the negative control of *PLE* is not manifested in the phenotype of *squa* mutants: its severe effect on floral meristem identity (revealed by indeterminate production of inflorescence shoots in the axils of bracts) and the fact that the *SQUA* function is occasionally by-passed in *squa* null mutants (revealed by development of more or less complete flowers; Huijser et al., 1992) could mask its role as a (cadastral) class A gene. Similarly, the strong *ap1-1* mutant, in spite of phenotypic differences to *squa*, displays inflorescence-like traits but does not reveal the cadastral role of *AP1* (Gustafson-Brown et al., 1994) in the control of *AG*.

AG negatively controls *AP1*. This is demonstrated in the wild-type third and fourth whorls and by decreased *AP1* transcription due to ectopic *AG* expression in the perianth of transgenic plants and *ap2* mutants (Jack et al., 1997). Similarly, *SQUA* transcription gradually decreases in the C expression domain during early stages of development in the wild type and is reduced during late events of C-dependent organogenesis in *sty* and *fis* mutants. Furthermore, in *ple* mutants *SQUA* transcription is maintained internal to the second whorl (Motte, unpublished). *SQUA* thus behaves like *AP1*. However, during early development, ectopic expression of *PLE* does not severely affect *SQUA* transcription in *Ple-888* mutants reflecting, as shown in *sty fis ple* flowers, that *SQUA* is controlled by *STY*/*FIS* and not by *PLE*. The negative relation between *SQUA* as a class A gene and *PLE* as a single C class gene is apparent only during later stages of development.

Control of floral organ specification

STY is involved in suppression of the C-function in upper parts of the plant as revealed by carpelloid features of upper leaves and bracts of the *Ple-888 sty* double mutant. The semidominant *Ple*-888 allele is only sufficient to confer carpelloidy to sepals and sometimes bracts and rarely affects upper leaf morphology (Bradley et al., 1993). This indicates that *STY* already functions during the vegetative to floral transition.

However, initiation and specification of perianth organ development are not affected in *sty* and *fis* mutants, and prior to organ initiation early expression levels of *FLO* and *SQUA* in *fis*, *sty* and *sty fis* mutants are similar to wild type. Assuming that the A-function is comparable between species and considering *STY* and *FIS* as its upstream regulators this could indicate that, along with *FIS* and *STY*, other not yet identified components govern organogenesis in the perianth, or that residual functionality of the *sty*/*fis* alleles prevents detection of interference in this process. But it is also possible that in *Antirrhinum* the establishment of floral meristem identity is not easily separable from organ specification in the first whorl. If so, then meristem identity genes, as homeotic selectors, inevitably control the floral identity of organs in the flower. Consequently, a non-cadastral A function either does not exist or will escape detection in *Antirrhinum*. This agrees with observations suggesting that in *Arabidopsis* the role of *AP1* and *AP2* in the specification of floral organ identity is inherent to their role in the establishment of floral meristem identity (Okamuro et al., 1997).

STY/*FIS* control organogenesis in all floral whorls

Once floral identity is established *STY*/*FIS* are necessary to maintain expression of *SQUA* and *FLO* in the perianth. Enhancement of the *sty* phenotype in the background of a weak *flo* mutation (which displays mild floral abnormalities without affecting floral transition; kindly provided by R. Carpenter) supports this notion (not shown). Alteration of B and C class organ identity gene expression in *sty*/*fis* mutants appears to be the consequence of this early event.

Retarded development of *sty fis ple* petals and feminisation of second and third whorl stamenoid organs in *sty*/*fis* mutants, relate to decreased expression of *FIM* and of class B homeotic genes (discussed below), where primarily *GLO* and later *DEF*, are affected. The complex and temporally and spatially variable regulatory relationship between meristem identity genes, *FIM* and class B genes makes it difficult to identify the primary target(s) of *STY*/*FIS*. *FLO* is a candidate, because of its early control of both *FIM* and class B transcription (Hantke et al., 1995).

Style-like structures inside the carpels of *sty* and *sty fis* mutant flowers suggest a function of *STY* in wild-type reproductive organ development. Mutation in *ple* is epistatic to *sty* with respect to the style-like structures, but overall enhanced expression of *PLE* in *Ple-888* has no effect on stamen and little on carpel development (Bradley et al., 1993). Therefore, *STY* and *FIS* may control genes that interact with *PLE*, which may or may not belong to class C, during reproductive organ development. Residual carpelloidy of fourth whorl organs and aberrant morphology of third whorl organs of *ple* mutant flowers point to additional class C gene functions.

Control of initiation and growth of organs by *STY* is possibly mediated by *FIMBRIATA*

Displacement of organ primordia causing fusion between floral organs, or a spiral rather than whorled organisation of mutant flowers indicate *STY*/*FIS*-dependent control of *FIM* as *fim* mutants display similar features. The control of organ growth by *STY*, as seen in subdivisions of third whorl primordia of *sty* mutants, arrested growth of second whorl primordia and reduction of organ number in the third whorl of the *sty fis* double mutant may also relate to early changes in the expression pattern of *FIM* in the mutants. *FIM* may be involved in the negative control of cell division (Ingram et al., 1997) and also in establishment of homeotic B and C class gene expression (Simon et al., 1994; Hantke et al., 1995; Ingram et al., 1997). The positive regulatory effect of *FIM* on class C genes is apparently circumvented in *sty fis* mutants.

Localised *FIM* expression represses growth, as evident, for example, from the presence of its transcript around second whorl primordia but not within the developing primordia. Subdivision of third whorl primordia in *sty* flowers may be promoted by this negative control of cell divisions in cells ectopically expressing *FIM*. In agreement with the proposed role of *FIM*, *sty fim* double mutant flowers do not display subdivisions of third whorl organs. Expression of B-function genes is necessary for this type of control, because subdivisions are absent in the *sty def* double mutant.

Reduced *FIM* expression in the *sty fis* double mutant affects expression of B-function genes, which also contribute to the control of cell division and/or elongation subsequent to initiation of organ primordia (Perbal et al., 1996). This could

account, along with homeotic effects, for the severe arrest of organ development in the second whorl of *sty fis* flowers and also for reduced third whorl organ number. However, down-regulation of *FIM* (or B) alone cannot be responsible for the failure to initiate organ primordia, because *fim*, *def* or *fim def* mutants do not display such features. We assume, therefore, that reduction of the *FIM* and B-functions combined with elevated C-function in *sty* and *sty fis* mutants cause these anomalies. Indeed, organ number in the third whorl is not affected in *sty fis ple* flowers, or in *Ple-888* flowers (with ectopic *PLE* expression and with wild-type *STY* function).

STY and *FIS* control the C-function in different regions of wild-type petals

The domains within wild-type petals where *STY* and *FIS* negatively control *PLE*, differ. Petals of *sty* mutants exhibit stamenoid features at their margins where they sometimes do not fuse and ectopic *PLE* expression is restricted to this marginal area. In contrast, *fis* second whorl organs display stamenoidy within the lobe and lateral fusions resulting in formation of the corolla tube. Ectopic *PLE* expression in *fis* petals is predominant in the lobe and within the middle of the tube-forming region. Concomitant loss of negative regulation of the C-function in the *sty fis* double mutant results in formation of morphologically nearly normal stamens in the second whorl or, as discussed above, in retarded growth of second whorl primordia. These observations suggest that, with regard to the developmental control of the C-function, wild-type second whorl organs are composed of two 'compartments': (1) a central (and in the mature flower, upper) region, controlled by the *FIS* gene and (2) a more lateral region controlled by *STY*. Such differences in the control of the central and lateral identity of organs are also documented in maize, based on studies with the recessive *narrow sheath* (*ns*) mutant (Scanlon et al., 1996). Developmental events (negatively) regulated by *FIS* and *STY* occur later in organogenesis than that controlled by *NS*, but both mechanisms reveal some similarity to compartmentalised wing formation in *Drosophila*, as discussed by Scanlon et al. (1996).

We are grateful to Drs Brendan Davies, George Haughn and Mark Wilkinson for discussions during preparation of this manuscript and for correction of the English. The criticism of the unknown reviewers that helped us to clarify our point of view is greatly acknowledged. We also thank Iris Heidmann and Heiner Meyer z.A. for their help in genetic experiments and maintenance of plants. This work was supported by grants from the Deutsche Forschungsgemeinschaft (SFB 243) and from the EEC/Biotechnology Programme (PTP Theme A1) to Z. Sch.-S.

REFERENCES

Bowman, J. L., Alvarez, J., Weigel, D., Meyerowitz, E. and Smyth, D. R. (1993). Control of flower development in *Arabidopsis thaliana* by *APETALA1* and interacting genes. *Development* **119**, 721-743.

Bradley, D., Carpenter, R., Sommer, H., Hartley, N. and Coen, E. (1993). Complementary floral homeotic phenotypes result from opposite orientations of a transposon at the *plena* locus of *Antirrhinum*. *Cell* **72**, 85-95.

Carpenter, R. and Coen, E. S. (1990). Floral homeotic mutations produced by transposon-mutagenesis in *Antirrhinum majus*. *Genes Dev.* **4**, 1483-1493.

Carpenter, R., Copsey, L., Vincent, C., Doyle, S., Magrath, R. and Coen, E. (1995). Control of flower development and phyllotaxy by meristem identity genes in *Antirrhinum*. *Plant Cell* **7**, 2001-2011.

Coen, E. S. and Meyerowitz, E. M. (1991). The war of the whorls: genetic interactions controlling flower development. *Nature* **353**, 31-37.

Coen, E. S., Romero, J. M., Doyle, S., Elliott, R., Murphy, G. and Carpenter, R. (1990). *floricaula*: a homeotic gene required for flower development in *Antirrhinum majus*. *Cell* **63**, 1311-1322.

Davies, B. and Schwarz-Sommer, Z. (1994). Control of floral organ identity by homeotic MADS-box transcription factors. Results and Problems in Cell Differentiation. In *Plant Promoters and Transcription Factors* **20**, 235-258.

Goodrich, J., Puangsomlee, P., Martin, M., Long, D., Meyerowitz, E. M. and Coupland, G. (1997). A Polycomb-group gene regulates homeotic gene expression in *Arabidopsis*. *Nature* **386**, 44-51.

Gustafson-Brown, C., Savidge, B. and Yanofsky, M. F. (1994). Regulation of the *Arabidopsis* floral homeotic gene *APETALA1*. *Cell* **76**, 131-143.

Hantke, S. S., Carpenter, R. and Coen, E. S. (1995). Expression of *floricaula* in single cell layers of periclinal chimeras activates downstream homeotic genes in all layers of floral meristems. *Development* **121**, 27-35.

Haughn, G. W., Schultz, E. A. and Martinez-Zapater, J. M. (1995). The regulation of flowering in *Arabidopsis thaliana* – meristems, morphogenesis, and mutants. *Can. J. Bot.* **73**, 959-981.

Haughn, G. W. and Somerville, C. R. (1988). Genetic control of morphogenesis in *Arabidopsis*. *Dev. Genet.* **9**, 73-89.

Huijser, P., Klein, J., Lönnig, W. -E., Meijer, H., Saedler, H. and Sommer, H. (1992). Bractomania, an inflorescence anomaly, is caused by the loss of function of the MADS-box gene *squamosa* in *Antirrhinum majus*. *EMBO J.* **11**, 1239-1249.

Ingram, C. G., Doyle, S. D., Carpenter, R., Schultz, E. A., Simon, R. and Coen, E. S. (1997). Dual role for *fimbriata* in regulating floral homeotic genes and cell division in *Antirrhinum*. *EMBO J.* (in press).

Jack, T., Sieburth, L. and Meyerowitz, M. (1997). Targeted misexpression of *AGAMOUS* in whorl 2 of *Arabidopsis* flowers. *Plant J.* **11**, 825-839.

Jofuku, K. D., den Boer, B. G. W., Van Montagu, M. and Okamuro, J. K. (1994). Control of *Arabidopsis* flower and seed development by the homeotic gene *APETALA2*. *Plant Cell* **6**, 1211-1225.

Liu, Z. and Meyerowitz, E. M. (1995). *LEUNIG* regulates *AGAMOUS* expression in *Arabidopsis* flowers. *Development* **121**, 975-991.

Lönnig, W. E. and Saedler, H. (1994). The homeotic *Macho* mutant of *Antirrhinum majus* reverts to wild-type or mutates to the homeotic *plena* phenotype. *Mol. Gen. Genet.* **245**, 636-643.

Mandel, M. A., Gustafson-Brown, C., Savidge, B. and Yanofsky, M. F. (1992). Molecular characterization of the *Arabidopsis* floral homeotic gene *APETALA1*. *Nature* **360**, 273-277.

Okamuro, J. K., den Boer, B. G. W. and Jofuku, K. D. (1993). Regulation of *Arabidopsis* flower development. *Plant Cell* **5**, 1183-1193.

Okamuro, J. K., Szeto, W., Lotys-Prass, C. and Jofuku, K. D. (1997). Photo and hormonal control of meristem identity in the *Arabidopsis* flower mutants *apetala2* and *apetala1*. *Plant Cell* **9**, 37-47.

Perbal, M. -C., Haughn, G., Saedler, H. and Schwarz-Sommer, Z. (1996). Non-cell-autonomous function of the *Antirrhinum* floral homeotic proteins *DEFICIENS* and *GLOBOSA* is exerted by their polar cell-to-cell trafficking. *Development* **122**, 3433-3441.

Samach, A., Kohalmi, S. E., Motte, P., Datla, R. and Haughn, G. W. (1997). Divergence of function and regulation of class B floral organ identity genes. *Plant Cell* **9**, 559-570.

Scanlon, M. J., Scheeberger, R. G. and Freeling, M. (1996). The maize mutant *narrow sheath* fails to establish leaf margin identity in a meristematic domain. *Development* **122**, 1683-1691.

Schultz, E. A. and Haughn, G. W. (1993). Genetic analysis of the floral initiation process (FLIP) in *Arabidopsis*. *Development* **119**, 745-765.

Schwarz-Sommer, Z., Hue, I., Huijser, P., Flor, P. J., Hansen, R., Tetens, F., Lönnig, W. -E., Saedler, H. and Sommer, H. (1992). Characterization of the *Antirrhinum* floral homeotic MADS-box gene *deficiens*: evidence for DNA binding and autoregulation of its persistent expression throughout flower development. *EMBO J.* **11**, 251-263.

Schwarz-Sommer, Z., Huijser, P., Nacken, W., Saedler, H. and Sommer, H. (1990). Genetic control of flower development by homeotic genes in *Antirrhinum majus*. *Science* **250**, 931-936.

Simon, R., Carpenter, R., Doyle, S. and Coen, E. (1994). *Fimbriata* controls flower development by mediating between meristem and organ identity genes. *Cell* **78**, 99-107.

Sommer, H., Beltran, J. P., Huijser, P., Pape, H., Lönnig, W. E., Saedler, H. and Schwarz-Sommer, Z. (1990). *Deficiens*, a homeotic gene involved in the control of flower morphogenesis in *Antirrhinum majus*: the protein shows homology to transcription factors. *EMBO J.* **9**, 605-613.

Stubbe, H. (1974). Neue Mutanten von *Antirrhinum majus* L. *Kulturpflanze.* **XXII**, 189-213.

Tröbner, W., Ramirez, L., Motte, P., Hue, I., Huijser, P., Lönnig, W. -E., Saedler, H., Sommer, H. and Schwarz-Sommer, Z. (1992). *GLOBOSA*: a homeotic gene which interacts with *DEFICIENS* in the control of *Antirrhinum* floral organogenesis. *EMBO J.* **11**, 4693-4704.

Tsuchimoto, S., van der Krol, A. R. and Chua, N. H. (1993). Ectopic expression of pMADS3 in transgenic petunia phenocopies the petunia blind mutant. *Plant Cell* **5**, 843-853.

Weigel, D. and Meyerowitz, E. M. (1994). The ABCs of floral homeotic genes. *Cell* **78**, 203-209.

Yanofsky, M. F., Ma, H., Bowman, J. L., Drews, G. N., Feldmann, K. A. and Meyerowitz, E. M. (1990). The protein encoded by the *Arabidopsis* homeotic gene *agamous* resembles transcription factors. *Nature* **346**, 35-39.

Zachgo, S., de Andrade Silva, E., Motte, P., Tröbner, W., Saedler, H. and Schwarz-Sommer, Z. (1995). Functional analysis of the *Antirrhinum* floral homeotic *DEFICIENS* gene in vivo and in vitro by using a temperature-sensitive mutant. *Development* **121**, 2861-2875.